FACULDADE DE
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DEPARTAMENT OF
EARTH SCIENCES

DESIGN AND IMPLEMENTATION OF A TECHNICAL ITINERARY FOR BIOFORTIFICATION OF CALCIUM IN *SOLANUM TUBEROSUM* L. TUBERS OF AGRIA, PICASSO AND ROSSI VARIETIES

ANA RITA FONSECA COELHO

Master in Production Technologies and Agro-industrial Transformation

DOCTORATE IN AGROINDUSTRIAL TECHNOLOGIES
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Design and Implementation of a Technical Itinerary for Biofortification of Calcium in *Solanum tuberosum* L. tubers of Agria, Picasso and Rossi Varieties

À minha família / To my family

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ABSTRACT

Calcium (Ca) is an essential mineral with several crucial functions in the human body, such as bone formation and tooth mineralization. However, Ca deficiency can lead to conditions like osteoporosis and rickets, characterized by low bone density and fragility. In this context, biofortification can provide a sustainable and long-term solution to combat the rising of mineral deficiencies, namely Ca, by enhancing nutrient contents in staple crops. As such, this study aimed to develop, design, implement and optimize a technical itinerary for Ca biofortification in three varieties of *Solanum tuberosum* L. (Agria, Picasso, and Rossi) through foliar applications during the plants' productive cycle in three experimental fields located in Lourinhã (Portugal). This study further aimed to innovate by creating processed products derived from Ca biofortified potatoes, such as starch and dehydrated mashed potatoes. To achieve these goals, edaphoclimatic characteristics were analyzed, namely soil, irrigation water and climate in a three-year experimental period. Also, remote sensing, photosynthetic parameters, mineral monitoring of the different organs of *Solanum tuberosum* L. plant (roots, stems, and leaves), the mineral quantification and Ca location, colorimetric parameters, protein, fatty acids, starch, soluble solids, morphometry parameters, dry weight content, total solids soluble, texture and sensory analysis were measured. Moreover, Ca increased with foliar applications in the three years of experiments, suggesting that through foliar applications Ca is redistributed in phloem and complements xylem mass flow, resulting in Ca increase in tubers. In the third year it was possible to optimize the Ca biofortification itinerary for the three varieties. As such, seven foliar applications of 12 $kg.ha^{-1}$ of Ca-EDTA showed the highest Ca content in tubers with skin - with a Ca biofortification Index of 8.2 %, 88 % and 78 % for Agria, Picasso and Rossi, respectively - as well as the lowest yields and negative impacts on photosynthesis apparatus. Indeed, overall, Ca biofortification process during the three years of the experiment did not seem to influence the morphological, physical, and organoleptic characteristics of potatoes. It was further concluded that through Ca biofortification it is possible to obtain a functional food and/or food product based on potato tubers, which is a value-added product with functional characteristics.

Keywords: Calcium biofortification; Mineral quantification and location; Physical, chemical, and organoleptic characteristics; *Solanum tuberosum* L..

Resumo

O cálcio (Ca) é um mineral essencial com diversas funções cruciais no corpo humano, como a formação óssea e a mineralização dos dentes. No entanto, a deficiência de Ca pode levar a condições como osteoporose e raquitismo, caracterizadas por baixa densidade óssea e fragilidade. Nesse contexto, a biofortificação fornece uma solução sustentável e de longo prazo para combater o aumento das deficiências minerais, incluindo o Ca, ao aumentar os teores de nutrientes nas culturas básicas. Assim, este trabalho visa desenvolver, projetar, implementar e otimizar um itinerário técnico para biofortificação de Ca em três variedades de *Solanum tuberosum* L. (Agria, Picasso e Rossi), através de aplicações foliares durante o ciclo produtivo das plantas em três campos experimentais localizados na Lourinhã (Portugal). Além disso, o estudo pretende inovar ao criar produtos processados derivados de batatas biofortificadas com Ca, como a fécula e o puré de batata desidratado. Para atingir esses objetivos foram analisadas algumas características edafoclimáticas como, solo, água de irrigação e clima nos três anos de estudo. Além disso, consideraram-se análises envolvendo a detenção remota, parâmetros fotossintéticos, monitorização mineral dos diferentes órgãos da planta *Solanum tuberosum* L. (raízes, caules e folhas). Adicionalmente, nos tubérculos efetuou-se ainda a quantificação e localização mineral ao nível tecidular, assim como a determinação de parâmetros colorimétricos, proteína, ácidos gordos, amido, sólidos solúveis, parâmetros morfométricos, teor de peso seco, sólidos totais solúveis, textura e análise sensorial. Verificou-se que o teor de Ca aumentou com as aplicações foliares nos três anos deste estudo, concluindo-se que através das aplicações foliares o Ca é redistribuído no floema e complementa o fluxo de massa do xilema, induzindo o aumento de Ca nos tubérculos. No terceiro ano foi ainda possível otimizar o itinerário de biofortificação em Ca para as três variedades. Como tal, constatou-se que sete aplicações foliares de 12 $kg.ha^{-1}$ de Ca-EDTA apresentaram os teores mais elevados de Ca, com um índice de biofortificação correspondendo a 8,2 %, 88 % e 78 %, para as variedades Agria, Picasso e Rossi, respetivamente. A nível geral, o processo de biofortificação de Ca durante os três anos de estudo, não pareceu influenciar as características morfológicas, físicas e organoléticas das batatas. Adicionalmente, considerando a análise sensorial, o tratamento com 12 $kg.ha^{-1}$ de Ca-EDTA parece influenciar positivamente na maioria das características sensoriais avaliadas nas três variedades. Aponte-se, contudo, que através do processo de biofortificação em Ca, é possível obter um alimento funcional e/ou um produto alimentício processado de batata, obtendo-se produtos com valor económico adicional agregado e com características funcionais.

Palavas chave: Biofortificação em cálcio; Características físicas, químicas e organoléticas; Quantificação e localização mineral; *Solanum tuberosum* L.

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ABBREVIATIONS

0.5N	0.5 kg/ha $Ca(NO_3)_2$
12A	12 kg/ha $CaCl_2$
12B	12 kg/ha Ca-EDTA
1A	1 kg/ha $CaCl_2$
1N	1 kg/ha $Ca(NO_3)_2$
24A	24 kg/ha $CaCl_2$
24B	24 kg/ha Ca-EDTA
2N	2 kg/ha $Ca(NO_3)_2$
3A	3 kg/ha $CaCl_2$
4N	4 kg/ha $Ca(NO_3)_2$
6A	6 kg/ha $CaCl_2$
AAS	Atomic absorption spectrophotometry
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
Ca-EDTA	Calcium EDTA
CEC	Cations exchange capacity
C_i	Internal [CO_2]
Ctr	Control
DACCs	Depolarization-activated channels
DAP	Days after plantation
DBI	Double bound index

DW	Dry weight content
E	Transpiration rate
EC	Electrical conductivity
EFSA	European food safety authority
EU	European union
F_0	Measurements of minimal fluorescence
FA	Foliar applications
FAO	Food and Agriculture Organization
F_m	Maximal fluorescence
F_v/F_m	Maximum PSII photochemical efficiency
F_v'/F_m'	Actual PSII photochemical efficiency
g_s	Stomatal conductance to H_2O vapor
HACCs	Hyperpolarization-activated channels
IOM	Institute of medicine
IoT	Internet of the Things
IPMA	Instituto Português do Mar e da Atmosfera
ISL	Langelier saturation index
iWUE	Instantaneous water use efficiency
LSI	Langelier saturation index
M	Months of cold conservation
NDVI	Normalized difference vegetation index
OM	Organic matter
PCA	Principal component analysis
pH	Potential hydrogen
pHe	Equilibrium ph
pHs	Saturation ph
P_n	Net photosynthesis rate
PS	Maximal photochemical efficiency of photosystem
q_L	Photochemical quenching coefficient
q_N	Non-photochemical quenching
RGB	Red, green and blue
RH	Relative humidity
SACN	Scientific Advisory Committee on Nutrition
SAR	Sodium absorption rate

T.	Treatments
TFA	Total fatty acid
TSS	Total soluble solids content
UAVs	Unmanned aerial vehicles
WHO	World health organization
XRF	X-ray fluorescence spectrometry
$Y_{(II)}$	Estimate of quantum yields of non-cyclic electron transport
$Y_{(NO)}$	Non-regulated energy dissipation in PSII
$Y_{(NPQ)}$	Regulated energy dissipation in PSII

SYMBOLS

r	Pearson index
ρ	Spearman index

1

INTRODUCTION

1.1 General Introduction

Currently there is an enormous pressure on the maintenance of the world's food, given the prospect of an increase in population growth, from 7.6 billion in 2017 to 8.6 billion in 2030, and more than 9 billion in 2050 (FAO, 2009a; Lockyer et al., 2018). Yet, to feed the worldwide future population, food production must increase about 70 % by 2050 (FAO, 2009a). As such, the demand for food and other crop products will continually increase (Thornton et al., 2020). Additionally, most of the growth in world's population will take place in urban areas, with the perspective of an increase of vegetables, roots, and tubers (**Figure 1.1**) and a rising demand for semi-processed and ready-to-eat foods (FAO, 2009a).

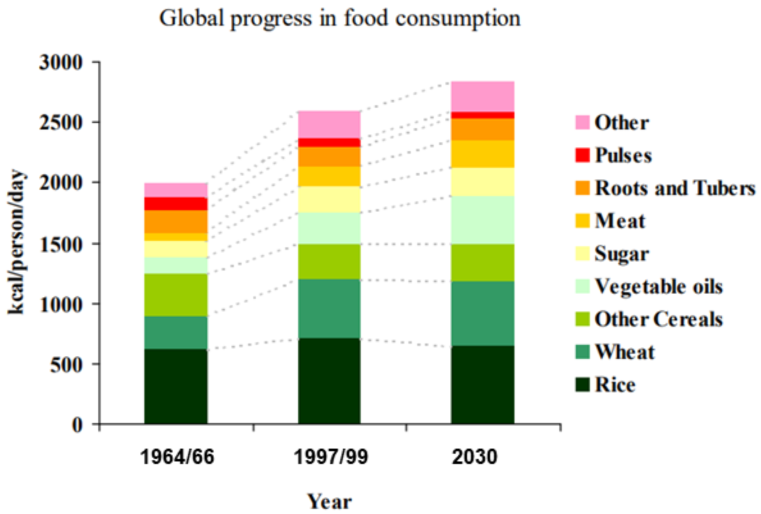


Figure 1.1 Global progress in food consumption and 2030 perspective. Source: FAO (2009a).

Additionally, not only is important the increase of food productivity and the confirmation that we will be able to feed worldwide population in 2050, but it is also necessary to have adequate and sustainable supplies with proper nutritional composition (mainly on staple foods), namely in terms of nutrients and proteins (FAO, 2009a; Lidon et al., 2018). Accordingly, food quality needs to be considered, providing all the key nutrients to maintain human health (Morales et al., 2020). Even though agriculture has become more efficient over the years, there is competition for natural resources due to consumption patterns (dietary habits, industrial development and even climate change). Nevertheless, with the technological development and innovation there is a hope of meeting future food needs in a sustainable way. Yet, it will only be possible through the increase of investments and public-private partnerships, to increase yields in a sustainable way, reducing poverty and increasing food security (FAO, 2017). In this context, a solution to some of the key challenges in agriculture can come from the *Internet of the Things* (IoT) and the use of *Unmanned Aerial Vehicle* (UAV) (Radoglou-Grammatikis et al., 2020). As such, precision agriculture and smart farming can be a useful tool to deal with future food needs (Ghalazman et al., 2022). Evidencing the use of UAVs in crop monitoring and/or spraying applications and in creating learning models intended to estimate vegetation health (Radoglou-Grammatikis et al., 2020). In fact, to maintain human's well-being and health it is known that, at least, 22 mineral elements (macro elements: Na, K, Ca, Mg, S, P and Cl, and micro elements: Fe, Zn, Cu, Mn, I, F, B, Se, Mo, Ni, Cr, V, Si, Sn and Co (Welch & Graham, 2004; Gomez-Coronado et al., 2019)) are necessary. These can be provided through the practice of a proper and balanced diet (White & Broadley, 2009; Gomez-Coronado et al., 2019), though supplementation, food fortification or biofortification (increasing the mineral element content during food production) (White & Broadley, 2009). However, nutritional deficiencies are already widespread around the globe causing a serious global health problem (Akhtar et al., 2011). Yet, even in richer countries, a reduction in dietary diversity occurs that leads to different pathologies, namely diabetes, obesity, and heart disease, due to preference for less complex high-energy diets (Johns & Eyzaguirre, 2007). In this context, it is estimated that most of world's population has Fe, Zn, I and Se deficiencies, being Ca, Mg and Cu deficiencies also common in a lot of countries (White & Broadley, 2009). Indeed, in Africa the risk of Ca, Zn, I and Fe deficiency is estimated to be around 54 %, 40 %, 19 % and 5 %, respectively (Joy et al., 2014). Therefore, biofortification of different crops can be a long-term solution to provide nutrient-rich food crops to consumers (Garg et al., 2018) and fight mineral deficiencies in individuals.

1.2 Theoretical framework

1.2.1 Potato

1.2.1.1 Relevance in the world and in Portugal

Potato (*Solanum tuberosum* L.) is one of the most important staple food crops worldwide, after wheat, maize, and rice (Bradshaw & Ramsay, 2009). In fact, these four crops supply 50 % of the world's food energy needs (FAO, 2014). Also, potatoes are considered the third most consumed staple food, after rice and wheat (CIP, 2018). It is considered a successful culture worldwide, mainly due to its ability to grow under a wide range of environments (Woolfe, 1987), thrive in high altitudes and arid climates, where crops like rice, wheat, and maize, don't have the capacity to develop (Smith, 2011). Besides, also due to its ability to produce more nutritious food in harsher climates, more quickly and on less land than any other major crop (FAO, 2008a). Thus, potatoes are considered one of the most important foods in the human diet (Yang et al., 2016), having a major role in feeding the world (Smith, 2011), due to its high nutritional value (Woolfe, 1987) and to the huge percentage of edible part for human consumption (about 85 %) compared to cereals (around 50 %) (FAO, 2008a). Thus, it is a culture to consider in fighting hunger, malnutrition, and poverty, since their prospective role in the development of agriculture (Wijesinha-Bettoni & Mouillé, 2019). Considering its adaptability, yielding capacity and nutrition, it has a long history of helping and contributing to improving household incomes in times of crisis beyond the expansion of population worldwide (Devaux et al., 2021). Nevertheless, one hectare of potato can yield 2 to 4 times more food compared to grain crops and can produce more food per unit of water than any other staple food crop (CIP, 2018).

Potato originated from Andes mountains of South America, and was domesticated there (Smith, 2011; FAO, 2022). According to molecular and archaeological evidences, it has been pointed that domestication of potato occurred around 1.800 a.C. in Peru (Garzóm, 2007). In fact, with the domestication of potato, resulted progressively in wider food production, mainly due to the constant population growth and development of technology, sciences, and cities (De Jong, 2016). In the 16th century potato was introduced by Spain in Europe and rapidly spread around the world (FAO, 2008a). In fact, before 1990, most of potatoes were cultivated and consumed in North America, Europe, and countries from the old Soviet Union. After that, there occurred a huge increase in potato production and demand in countries, situated in Asia, Africa, and Latin America (FAO, 2008b). In 2005, Asia consumption per capita was 24 kg, being Belarus, the country which consumes more kg per capita (181 kg per capita). In 2007, according to FAO, China, Russia, and India were the top three countries that produced more potatoes (FAO, 2008b). In 2020, in EU (European Union), potatoes were cultivated on 1.7 million hectares (ha) and harvested around 55.3 million tons of potatoes, with Germany sharing the first position of potato production (with *c.a.,* 21.2 %), being Portugal the 14th country with a share of 0.9 % (EUROSTAT, 2022).

In Portugal, potato plays a very important role at the social and economic levels in the region's producers (mainly in the North and Center), for their contribution in job creation and for being one of the crops produced for self-consumption (GPP, 2015). However, according to the data from 2019/2020, despite the consumption per capita was 93.1 kg in Portugal, (**Figure 1.2A**) (INE, 2021a), there were some oscillations in the consumption, with a decreased in 2019/2020 relatively to 2006/2007, where the consumption was 95.2 kg/inhabitant/year (GPP, 2020). Moreover, the country only has the capacity to produce about 50 % of its food needs in potatoes (**Figure 1.2B**). Also, potato is considered the most purchased vegetable abroad in Portugal, in which preserved potatoes and seed potatoes, represented 23.6 % and 17.9 %, respectively, of the total value of entries (GPP, 2020).

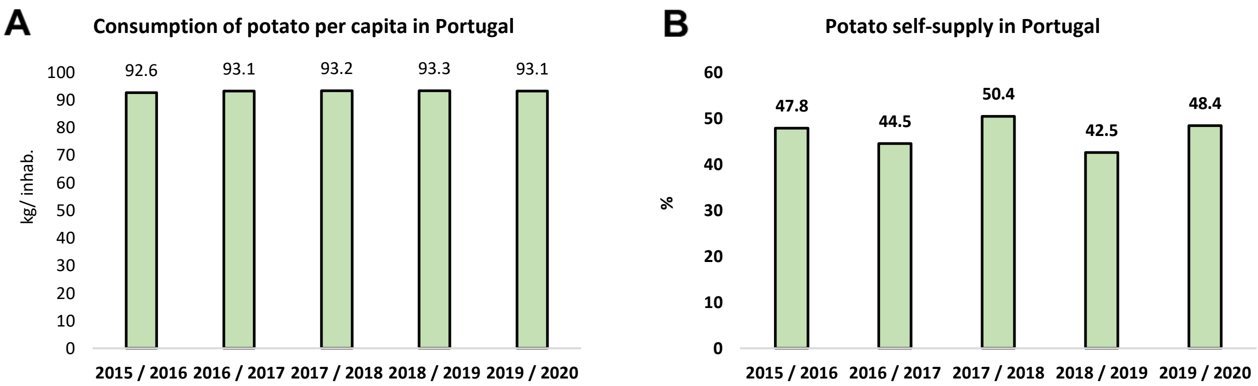


Figure 1.2 A: Human consumption of Potato in Portugal per capita. Source: INE (2021a). B: Portugal self-provisioning of potato. Source: INE (2021b).

Moreover, in 2017, potato production in Portugal was about 515 030 tonnes, having increased 25 % relatively to 2016, and being much lower than 2007 and 2008 (**Figure 1.3**). Nevertheless, the availability of tubers in Portugal increased by 0.8 % (reaching 222.7 g/inhabitant/day), however due to the decrease of potatoes supply for consumption in 2016-2020 there were a negative annual variation of 1.5 % (INE, 2021c).

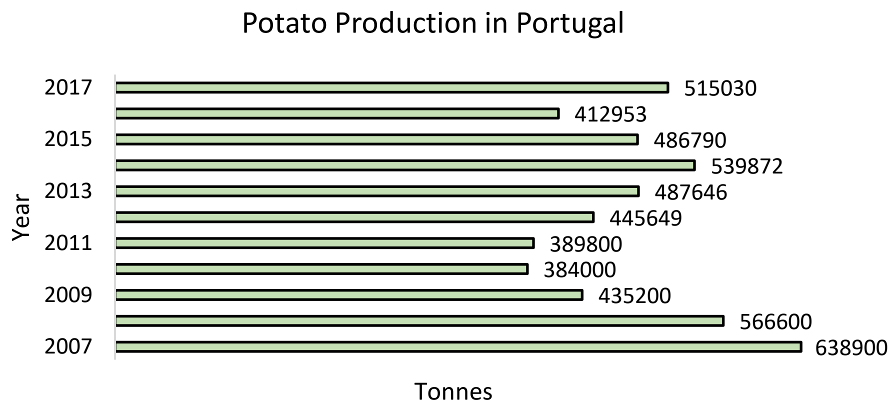


Figure 1.3 Portugal potato production. Source: POTATOPRO (2022).

The choice of varieties is also very important and, in Portugal, some of the most suited potato varieties for Vale do Tejo region are Monalisa, Agria, Asterix and Vivaldi (**Figure 1.4**) (AGRO-MAIS, 2013).

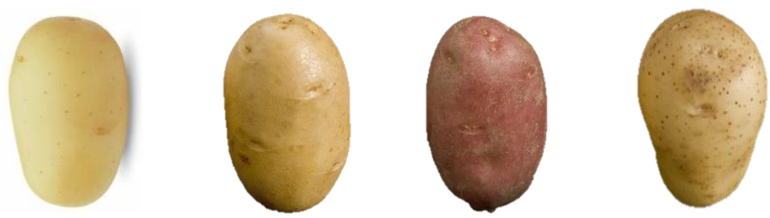


Figure 1.4 Potato varieties: Monalisa, Agria, Asterix and Vivaldi, respectively. Source: [1]

In this context, it is important to understand, not only the adequate variety considering the region intended to implement the culture, as well as the botanical aspects and the development of the *Solanum tuberosum* L. plants.

1.2.1.2 Botanical aspects and tubers development

The potato plant (*Solanum tuberosum*) is an annual herbaceous dicotyledon plant (Gould, 1999), producing tubers rich in starch (FAO, 2009b), being the most cultivated tuber of the *Solanum* species (EFSA, 2020). Belongs to the Solanaceae family which includes at least 1000 other species (*i.e.*, tomato, tobacco, eggplant, and pepper) (Horton, 1987; FAO, 2009b; CIP, 2018). The potato plant can grow up to 100 cm and produce both flowers and berries

(CIP,2018) and has shallow roots which normally do not exceed 40 - 50 centimeters (Horton, 1987) (**Figure 1.5**).

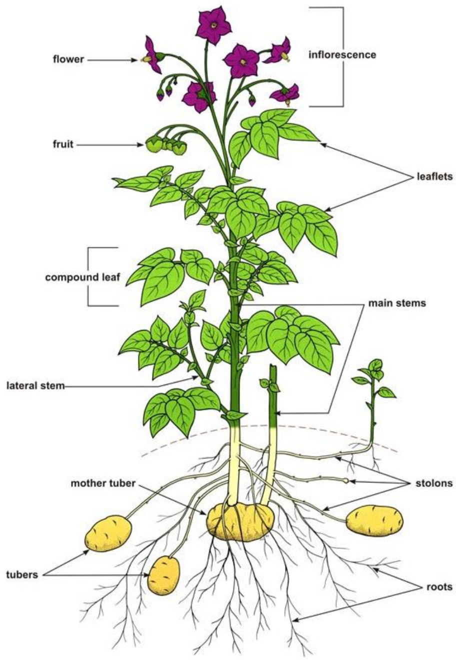


Figure 1.5 Potato plant components. Source: CIP (2018).

As plants grow and develop, their leaves produce starch that is transferred to the ends of its underground steams also known as stolons. After that, the stems stated to thicken to form tubers (up to 20) close to the soil surface. Yet, the number of tubers that reach maturity depends on soil nutrients and of the available moisture and they can vary in shape and size (CIP, 2018). After the growing season, leaves and stems of the potato plant start to die down to the soil level and tubers detach from their stolons. Each tuber has from two to up 10 buds (also known as "eyes"), being arranged in a spiral pattern (CIP, 2018). Potato is a vegetatively propagated plant, meaning that a new plant can grow from a seed (a cut piece of potato) or a potato (in which the buds generate shoots that can develop a new potato plant) (CIP, 2018; Thornton, 2020). The new plant will be genetic clones from the piece of potato (propagated asexually) or potato, unlike the botanical seeds (*i.e.*, "true seeds" - that can be reproduced sexually) that will produce new tubers, genetically different from the mother plant (Horton, 1987; CIP, 2018), being each botanical seed genetically unique (Thornton, 2020). The seed (potato piece) should have a consistent size and weight for regular standing and should be previously treated to prevent diseases (Gould, 1999). Moreover, due to the vegetative propagation through potato seed, potato crops usually emerge slower compared to the seeded crops, but the development is faster due to the energy reserves (in carbohydrates form) in the seed (Thornton, 2020).

Tubers are an extended portion of an underground stem designed to store photosynthates and for reproduction of the plant (Horton, 1987), developing adventitious roots in the bottom of each sprout (**Figure 1.6**) and afterwards, above the nodes of the underground part of each stem (Huaman, 1986).

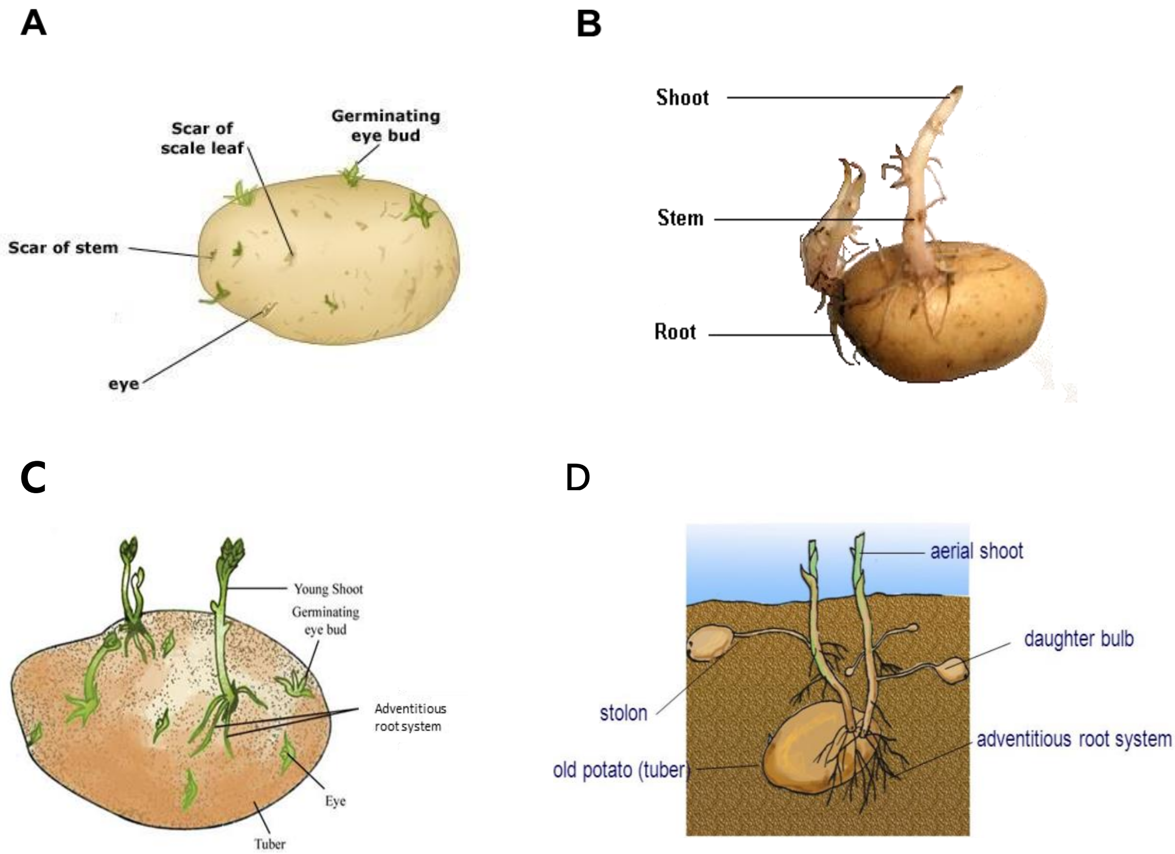


Figure 1.6 Development from a potato. A: first stage of development; B: development of steam and shoot from a potato. C: Young shoot from vegetative germination; D: Aerial shoot and adventitious root system. Source: A-[2]; B-[3]; C-[4]; D-[5].

The outer layer of the tuber's cells is known as the periderm (consisting of several layers of corky cells), forming the tuber's skin (Horton, 1987; Troncoso et al., 2009) (**Figure 1.7**). The skin of a mature tuber gives good protection against microorganisms, is practically impermeable to chemicals, gases, and liquids, and is resistant to water loss (Horton, 1987). However, tubers should be harvested before they are fully mature to meet consumers demands (Gould, 1999). Thus, tubers harvested without a mature skin can be easily damaged, microorganism can also enter and will lose moisture rapidly during storage (Horton, 1987).

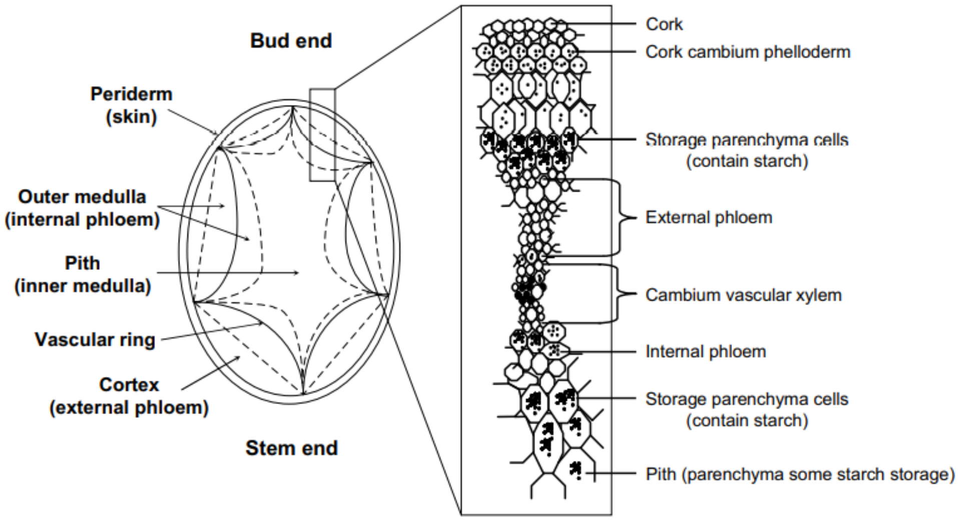


Figure 1.7 Cross section and the different structures or elements that compose the potato tuber. Source: Troncoso et al. (2009).

The immature tuber has an epidermis that is replaced by a layer of cork cells (periderm – known as "skin") in the fully matured potato (Fedec et al., 1977). Additionally, cork cells are dead cells and do not contain starch or protein grains (Fedec et al., 1977). The cells of the periderm ("skin") are oriented in a circumferential direction, and in a depth of 10-11 cells, reach the phellogen (cork cambium). After that, there's a zone of external phloem (cortex), the cambium vascular xylem (being a ring of disconnected vascular bundle regions), the internal phloem, and the center of the tuber – the pith (which the parenchyma cells storage starch) (**Figure 1.7** and **Figure 1.8**). Inside the cortex there is a vascular storage parenchyma, high in starch content and the xylem and phloem can be found in tiny strands or bundles within the boundary between the cortex and the vascular area. Additionally, the storage parenchyma cells contain starch granules (small, round, and oval-shaped) (Troncoso et al., 2009). The internal phloem occupies about 75 % of the total volume of the tuber, having the presence of cells with starch granules of similar size to those of the cortex ones (Fedec et al., 1977). The cells in the tissues of potatoes are bound together by a pectin-rich region – commonly known as the middle lamella (*i.e.*, outer layer of the cell wall), being dissolved during heating processes. Regarding raw potatoes, cells contain several partially crystalline starch granules, with a great variability regarding their composition and distribution, being altered during potato processing (Troncoso et al., 2009). Additionally, in potato tubers, the bud end is richer in the eyes compared to the stem end (Troncoso et al., 2009).

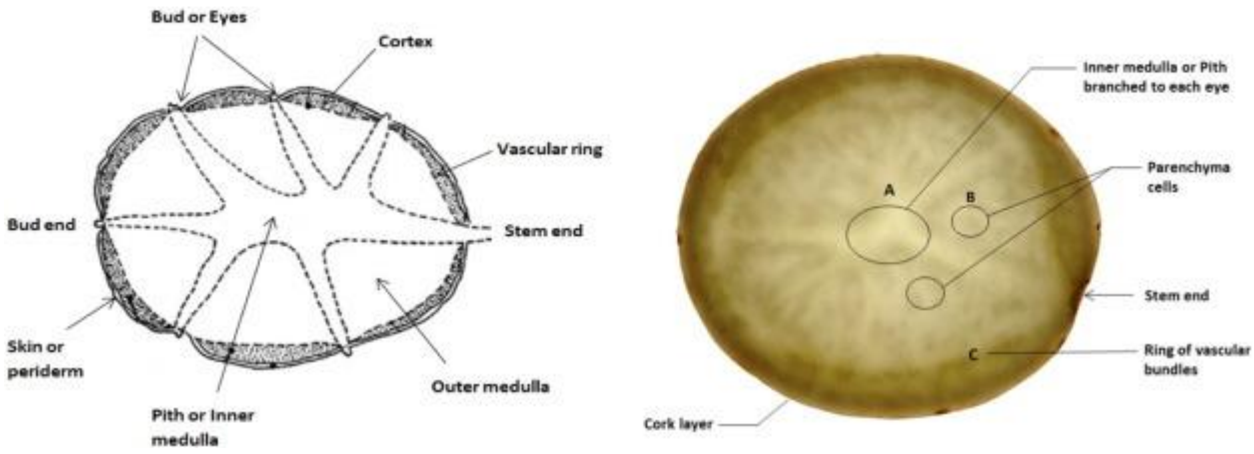


Figure 1.8 Cross section showing internal structure of potato tuber. Source: Faridnia et al. (2015).

Therefore, potato plants can be considered as a set of variable number of main stems, which display different levels of branching being reliant on variety, environmental conditions and on the physiological age of the mother tuber (Gamboa-Celis, 2002). Additionally, there are many factors that may affect the formation, development and even the number of tubers, such as, temperature, water supply, the formation of carbohydrates, period of light and photoperiod (length of the dark period), age of the mother tuber or nitrogen supply (Gould, 1999; Gamboa-Celis, 2002).

There are more than 4000 varieties grown around the world, mostly found in Andes with different sizes, shapes (CIP, 2018), color, texture, and nutritional content (**Figure 1.9**) (Wijesinha-Bettoni & Mouillé, 2019), but only few cultivars have been commercialized due to their feasibility to be marketed and stored and due to high production ratio and consumer acceptance preferences (Yang et al., 2016). Nevertheless, new findings indicate that there are two different cultivar groups of *Solanum tuberosum*: *andigenum* (Andean) (mainly grown in the Andes and adapted to short days) and *chilatanum* (Chilean) (cultivated around the world and developed from Andean cultivars) (FAO, 2009b).

Figure 1.9 Andean potato tubers. Source: [6]

Considering the different types of potatoes worldwide, it is important to understand the different growth stages of the potato phenological cycle.

1.2.1.3 Development stages

In potato tubers, nutrient absorption varies along the different development stages (Zhang et al., 2019). Besides, also the variety of potatoes, geographical location, fertility of the soil, soil type, as well as other factors related to the environment have an effect in growth and nutrient uptake of tubers (Jackson & Haddock, 1959). However, tubers formation is considered an especially complex set of development stages that includes sequential phases, leading to the production of tubers that can be harvest (Gamboa-Celis, 2002). Nevertheless, potato phenological cycle can be divided into five distinct growth stages (**Figure 1.10**):

1) **Sprout Development (I)**: this growth stage consists of a period that lasts from 3 to 6 days and starts when the environmental conditions are ideal. The sprouts develop from the eyes of the seed tuber and begin to emerge from the soil, while the roots begin to develop. The seed piece is the only energy source for growth during this stage of development (EMBRAPA, 2015; Patil et al., 2016; Thornton, 2020);

2) **Vegetative growth/Plant Establishment (II)**: this stage includes the growth period from sprout emergence until new tubers start to develop (including roots and shoot development). It is commonly referred to "vegetative growth" stage since all vegetative parts of the plants (leaves, branches, roots, and shoots) are formed. This phase lasts from 15 to 40 days depending on different factors namely: the variety, environmental conditions, planting date, soil temperature and moisture conditions of the soil (EMBRAPA, 2015; Patil et al., 2016; Thornton, 2020). Additionally, soil nutrient content can impact growth at this phase of plant development, due to the start of the transition from relying on nutrients and energy stored in potato seeds to nutrient uptake from soil by roots and energy produced through photosynthesis in leaves (Thornton, 2020);

3) **Tuber initiation (III)**: in this growth stage occurs the first visual manifestation of tuber initiation, in which the stolon stops growing and the ends swell. When the swelling reaches about twice the diameter of the stolon, it is considered a tuber incipient and it is usually said that tuber initiation has begun (Gamboa-Celis, 2002; Thornton, 2020). This stage will last around 2 weeks (EMBRAPA, 2015; Patil et al., 2016; Thornton, 2020), however it is very influenced by different factors (namely, temperature, soil moisture and day length, nitrogen available in soil) and tubers need certain nitrogen levels and chilly nights for good growth (Thornton, 2020);

4) **Tuber Bulking (IV)**: is considered the process of dry matter accumulation in tubers, being a result of translocation of carbohydrates produced in photosynthesis. In fact, in harvest, it is estimated that around 90% of the dry matter in tubers

is correlated to the output from photosynthesis occurred in this growth phase (Thornton, 2020). In this growth stage, tubers increase in size and weight because of cell division, cell elongation and the deposition of starch and protein (Gamboa-Celis, 2002), and foliage development is completed (EMBRAPA, 2015). In tuber initiation, tuber bulking rate and duration can be affected by many environmental factors (namely, temperature, soil moisture and nutrient availability) and the deficit and excess of fertilizer applications can decrease tuber bulking rates (the imbalance of nutrients can delay or slow tuber growth) (Thornton, 2020). Additionally, this stage has the longest duration and is dependent on the planting date and variety which, as such, can last up to three months (Patil et al., 2016);

5) **Maturation (V)**: the vines start to turn yellow (senesce starts) and lose leaves, physical and chemical characteristics occur to the tubers and photosynthesis gradually decreases along to the slowing down of tuber growth (Patil et al., 2016; Thornton, 2020). Tuber skin or periderm thickens and suberize (providing more protection to the tuber during harvest and post-harvest handling and against pathogens) (Gamboa-Celis, 2002; Thornton, 2020). Additionally, during the maturation of tubers, dry matter increases, leading to the improvement of quality for fresh and processing market consumption. Also, proper mature tubers, when stored, have lower respiration rates and have a longer dormancy (sprouting later), as well as a better resistance to pathogens (Thornton, 2020).

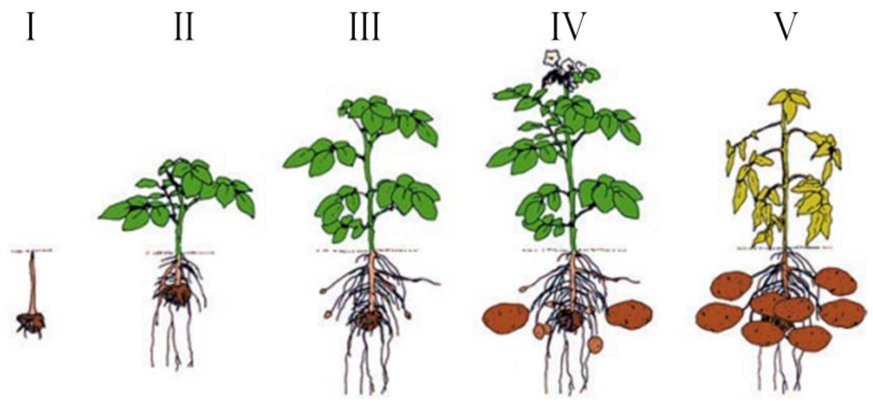
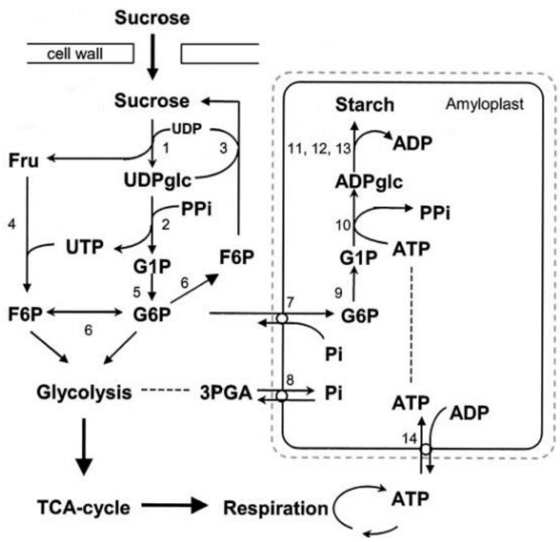


Figure 1.10 Potato phenological cycle: I- Sprout Development; II- Vegetative Growth/Plant Establishment; III-Tuber Initiation; IV- Tuber Bulking and V-Maturation. Source: Thornton, 2020.

During the growth of potato crops, in the photosynthetic metabolism, simple sugars (glucose and fructose – reducing sugars) are synthesized (Gould, 1999; Thornton, 2020), releasing O_2 and water as byproducts (Thornton, 2020). The simple sugars (glucose and fructose) through glycosidic reactions synthesizes sucrose, which is translocated from the above ground stem to the underground stem (tuber). In tubers, through polymerization sucrose is converted into starch, which is stored in tubers and keeps accumulating following the continuous synthesis of sugars (Gould, 1999). Additionally, growth and health of potato plants determines the accumulation of total solids (starch and other carbohydrates, amino acids, proteins, fats, minerals, and vitamins) (Gould, 1999). Nevertheless, the process of reducing sugars to sucrose and subsequently to starch can be reversed or hydrolyzation throughout respiration (**Figure 1.11**) under stress conditions (namely, extremely high or low temperatures, lack or excess of water, inadequate fertilization) (Gould, 1999). Moreover, proper, and appropriate management decisions can be done knowing that what happens in each stage of tuber growth in the plant, determines the harvest yield and quality (Thornton, 2020).



1 - sucrose synthase
2 - UDP-glucose pyrophosphorylase
3 - sucrose-phosphate synthase
4 - fructokinase
5 - cytosolic phosphoglucomutase
6 - phosphoglucoisomerase
7 - hexose phosphate translocator
8 - triose-phosphate translocator
9 - plastidial phosphoglucomutase
10 - ADP-glucose pyrophosphorylase
11 - soluble starch synthase
12 - granule-bound starch synthase
13 - branching enzyme
14 - adenylate translocator

Figure 1.11 Conversion of sucrose to starch mechanism in potato tubers. Source: Geigenberger, 2003.

One of the most delicate stages in potato growth is the period between planting and emergence (stage I) (Horton, 1987). Considering stage I and II of plant development (**Figure 1.10**), when the sprout development and plant establishment occur, it is essential to have healthy potato seeds and adequate soil needs for sprouts growth. As such, to avoid slow sprout development, or seed decay that will reduce the plant growth rate, the proper use of fungicide treatments to control dry rot and rhizoctonia stem canker must be applied, whereas the soil must have moisture and the temperature must range between 7.2 - 18.3 ºC. After that, when plants emerge, is essential to provide ideal soil moisture and nutrients (Thornton, 2020). Regarding, stage III (the beginning of tuber initiation) (**Figure 1.10**), the most sensitive stage for the development of quality problems, it is important to maintain adequate moisture and nutrient availability. In stage IV (longest phase of the potato phenological cycle) (**Figure 1.10**), any type of condition that can limit growth or healthy foliage (namely, seed age, plant spacing, fertilization, irrigation, or pests), interfere with tuber growth, or shifts the dry matter from the tuber to the foliage (limiting the number of days and thus, potential yield will decrease) (Thornton, 2020).

1.2.1.4 Growth cycle

Potatoes can grow in different parts of the world, but the culture can vary in different producing areas (Gould, 1999). For instance, in Portugal, planting, growth and harvest of potatoes take place in different months according to different regions of the country (**Figure 1.12**) (PORBATATA, 2017).

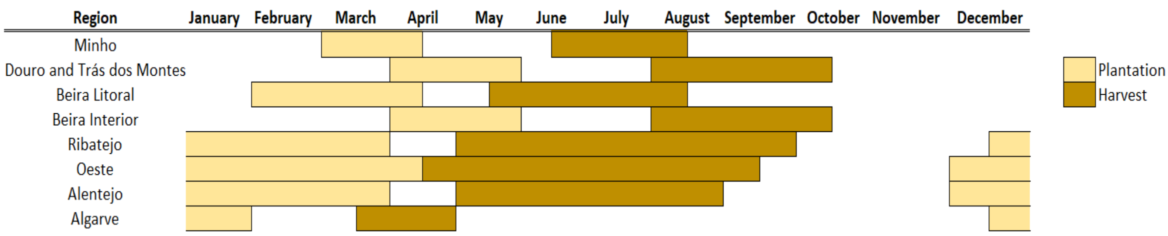


Figure 1.12 Plantation and harvest of potato in Portugal regions. Adapted from: PORBATATA, 2017.

Additionally, there is a difference in the length of time needed to reach maturity according to the variety of potato (Horton, 1987). In fact, subspecies of *tuberosum* mature quicker than subspecies *andigena* (having a growing period between 4 to 6 months) and producers/farmers usually choose varieties according to the length of cropping season in their region of the country. Nevertheless, in regions where the growing season is short, they typically produce early potatoes and where it is longer, they prefer later maturing varieties (Horton, 1987). In fact, the duration of the growth cycle of potato varieties can be classified into four types: early maturing, semi-early maturing, semi-late maturing, and late maturing (**Figure 1.13**) according to the days of the growth development (PORBATATA, 2017).

Figure 1.13 Duration of the growth cycle of potato varieties. Adapted from: PORBATATA, 2017.

Early maturing varieties can produce a reasonably high yield in a short period of time and present an early emergence and a moderate growth of foliage. On the other hand, late varieties can have higher yields than early maturing varieties (when harvested later in the season) and present a later emergence and more foliage (Horton, 1987). Yet, it is important to point out that late varieties have a disadvantage, as have prolonged exposure to pests, drought and other unfavorable conditions that can lead to lower yields or tuber quality (Horton, 1987).

1.2.1.5 Nutritional characterization

Potatoes are a healthy food with a high nutritional value (Alexopoulos & Petropoulos, 2021) and provides, for a large population worldwide, the nutritional and energy requirements (Singh et al., 2020). Hence, potato is considered a carbohydrate-rich food (Raigond et al., 2020) and a crop that can contribute to limited malnutrition (Singh et al., 2020). It is also considered a good source of important minerals (Singh et al., 2020) and antioxidants (Burgos et al., 2023), having a high protein-calorie ratio and producing more edible energy and protein, compared to other major food crops (Singh et al., 2020).

Potatoes are rich in carbohydrates and are an excellent source of dietary fiber, vitamin C, thiamine, niacin, and riboflavin (Singh et al., 2020). Also, potatoes containing low fat and no cholesterol, are a good source of minerals (namely, iron, zinc, and potassium), with a low level of sodium (Smith, 2011), whereas it has various health-promoting compounds that include carotenoids, flavonoids and caffeic acid (Singh et al., 2020).

The potato tuber contains between 63 - 87 % water (Singh et al., 2020) and 13 to 37 % of dry matter (Gould, 1999) (being approximately 60 – 80 % starch) (Singh et al., 2020; Alexopoulos & Petropoulos, 2021). The protein content of potatoes (on a dry weight basis) is, like cereals, higher than other roots and tubers (Singh et al., 2020). Hence, protein is composed of all essential amino acids in potatoes (Gould, 1999) and usually oscillates between 1.7 - 2.1 % of tuber fresh weight (Alexopoulos & Petropoulos, 2021).

Potatoes are a good source of complex carbohydrates (they take longer to break down into glucose), having longer carbon chains (namely starches and fibers). In potatoes, the main sugars include glucose, fructose, and sucrose (Singh et al., 2020). Starch is the predominating carbohydrate (Mu et al., 2017; Burgos et al., 2023) (ranging between 16.5 and 20 g/100 fresh weight) (Burgos et al., 2023) and is a polysaccharide composed of two biopolymers: amylose and amylopectin (Liu et al., 2007), ranging between 21-25 % and 75 - 95 %, respectively (Gould, 1999). Moreover, starch is an enzyme-resistant starch which after gelatinization becomes susceptible to hydrolysis by α-amylase (Liu et al., 2007).

In **Figure 1.14.** it is shown that the mineral composition and the energy in raw potatoes per 100 g edible portion.

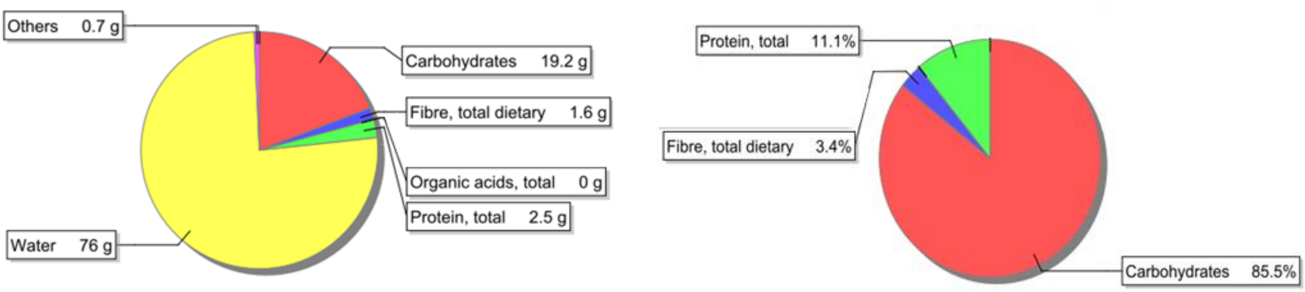


Figure 1.14 Composition and energy distribution in raw potatoes per 100g of edible portion. Source: INSA, 2022.

In raw potatoes, around 87 % constitutes the edible part (INSA, 2022), and the dietary fiber content, oscillates between 1 to 2 g/100 g fresh weigh, being obtained mainly from its cell walls (about 1.2% of the fresh weight of the tubers) (Singh et al., 2020). Additionally, due to the presence in potatoes of a small percentage of phytic acid, it enhances phosphorus bioavailability to the human body and helps the bioavailability of Ca, Fe and Zn (Singh et al.,2020).

In **Table 1.1** is described according to INSA (2022) the nutritive content of raw potatoes per 100 g edible portion.

Table 1.1 Nutritive content of raw potatoes per 100 g edible portion. Source: INSA, 2022.

Name	Content
Energy	90 kcal
Macronutrients	
Carbohydrates	
- Total sugars	1.2 g
- Sucrose	0.6 g
- Total starch	18 g
Total dietary fiber	1.6 g
Total protein	2.5 g
Water	76 g
Vitamins	
Alpha-tocopherol	0.06 mg
Thiamin	0.21 mg
Riboflavin	0.02 mg
Performed niacin	1.4 mg
Total equivalents niacin	2 mg
Niacin equivalents from tryptophan	0.6 mg
Total vitamin B6	0.44 mg
Vitamin C	14 mg
Total folate	35 µg
Minerals	
Ash	0.74 g
Na	9 mg
Ca	9 mg
K	450 mg
P	42 mg
Mg	13 mg
Fe	0.2 mg
Zn	0.2 mg

Additionally, raw potatoes, compared to cereals, have lower protein content, but in cocked potatoes crude protein (total N x 6.25) is comparable to cocked rice and other cocked cereals (Horton, 1987).

The nutritional composition of potatoes (as in other plant foods) is affected by several factors in pre-harvest and post-harvest, such as environmental conditions, cultural practices, biotic and abiotic stress (Burgos et al., 2023), the incidence of pests and diseases (Horton, 1987), processing, storage, and transport (Burgos et al., 2023). In fact, storage, freezing, drying, and cooking processes tend to decrease nutrient content in foods (Horton, 1987). Moreover, the composition of potatoes varies (Gould, 1999; Liu et al., 2007) with the cultivar, growing region, and fertilization management (Liu et al., 2007).

1.2.1.6 Cultivation requirements conditions

Environmental conditions during plant development affect yield levels and potato quality. As such, climate, soil, fertilization (Horton, 1987), irrigation, pest management (Thornton, 2020), topography (Stark & Thornton, 2020), temperature or solar radiation (Haverkort & Struik, 2015), need to be suitable for the development of the culture. Additionally, potato can be produced around the world under different growth conditions of water, temperature, or even nutrients supply, which does not mean that these conditions are optimal for potato growth and development (Haverkort & Struik, 2015).

1.2.1.6.1 Climate

Climate is perhaps the most significant factor "controlling" the overall productivity (Dean, 2018), and can impact yield and the nutritional needs of plants (Thornton, 2020). In this context, air and soil temperatures are considered the main environmental factors (Thornton, 2020), but the daylength can also affect the growth of potato crops (Horton, 1987). The best productions of potatoes have been reported in regions with long daylength and mild temperatures (15-20 ^{o}C) during the development stages (EMBRAPA, 2015). In fact, different temperatures have been reported considering the different development stages of *Solanum tuberosum* L. (Lizana, 2021) and the varieties of *tuberosum* subspecies (Horton, 1987). There is a stimulation of tuber initiation with short days and moderate temperatures (being lower at night) (Horton, 1987; Dean, 2018) and the tuberization is fastened when the photoperiod is below 12h and 16h for S. *tuberosum* spp. *andigena* and for S. *tuberosum* spp. *tuberosum*, respectively (Lizana, 2021). Additionally, most commercial varieties tuberize better with average temperatures above 15 ^{o}C, while night temperatures above 22 ^{o}C, significantly reduce tuber production (EMBRAPA, 2015). Nevertheless, for most potato varieties, soil temperatures between 15 and 18 ^{o}C are the most favorable (Horton, 1987). Soil temperatures influence sprout growth, affecting growth when temperatures are below 12 ^{o}C, or above 28 ^{o}C (Horton, 1987), and high soil temperatures also affect tuber physiology and inhibit starch deposition (which is high soil temperatures are even more damaging than high air temperatures to tuber quality) (Thornton, 2020). The base temperature for sprouting is between 2 and 5 °C and the optimum temperature for leaf development and stem elongation is between 21 and 25 °C and around 25 °C, respectively. Temperatures above 25 °C stimulates the development of rhizomes and rhizome branching, which can increase the number of tubers per plant. In different phases of tuber development, the optimal temperatures are 15, 22 and 15 °C for induction, initiation, and setting, respectively (Lizana, 2021). Moreover, the high daytime and the minimum nighttime temperature are very important and critical in potato production. The high daytime temperatures are associated with a high rate of respiration and transpiration (which can lead to plant moisture stress) (Horton, 1987) and when the temperature is above 25 °C, the net photosynthesis decrease with the increase of temperature (Horton, 1987; Thornton, 2020), leading to the reduction of carbohydrate that can be transported to tubers and lately could be converted into starch (Thornton, 2020). In fact, the optimum temperature for starch synthesis in tubers is 25 °C and the relative partitioning rate is higher in tubers with temperatures ranging between 12 and 20 °C (Lizana,2021). Also, high night temperatures increase plant respiration (draining the carbohydrate reserves and further leading to a lower tuber growth rate) and if the temperature is above 20 °C, tubers will not start to form (Horton, 1987). High wind speed or low relative humidity can also lead to high evaporative requirement that can diminish photosynthesis by leading stomate to close, following with a restriction in CO_2 uptake. (Thornton, 2020). Conversely, stresses caused by weather and climate cannot be eliminated, however through proper management, the damage can be minimized (Thornton, 2020).

1.2.1.6.2 Topography and soil

Soil properties (chemical, physical and biological) have an impact on nutrient availability (Thornton, 2020) and potato crop development is grater on deep, friable soils that have good retention of water (Horton, 1987; Thornton, 2020), well drained and with low sodium concentrations and soluble salt (Thornton, 2020). It is important to have soil chemical characteristics that improve nutrient availability and uptake, and suitable levels of organic matter to promote good soil structure, tilth and minimize soil compaction (providing aeration and drainage) (Thornton, 2020).

Soil pH, salinity, and cation exchange capacity (CEC) are important chemical properties (Thornton, 2020). Regarding pH, potatoes can grow under a wide range of pH levels (pH between 5.7 – 8.4, being frequently grown on pH >7.5), but pH between 6.5 and 7.5 provide maximum nutrient availability (Thornton, 2020). The availability of nutrients is strongly affected by soil pH (Power & Prasad, 1997) (**Figure 1.15**). and soil pH can increase the availability of certain nutrients in particular soil conditions (Neina, 2019). In fact, when pH is high (>7.5) the availability of zinc, iron, phosphorus, and manganese are generally reduced. Also, nutrient availability is reduced when pH is acid (<6.0) (Thornton, 2020), especially phosphorus [7].

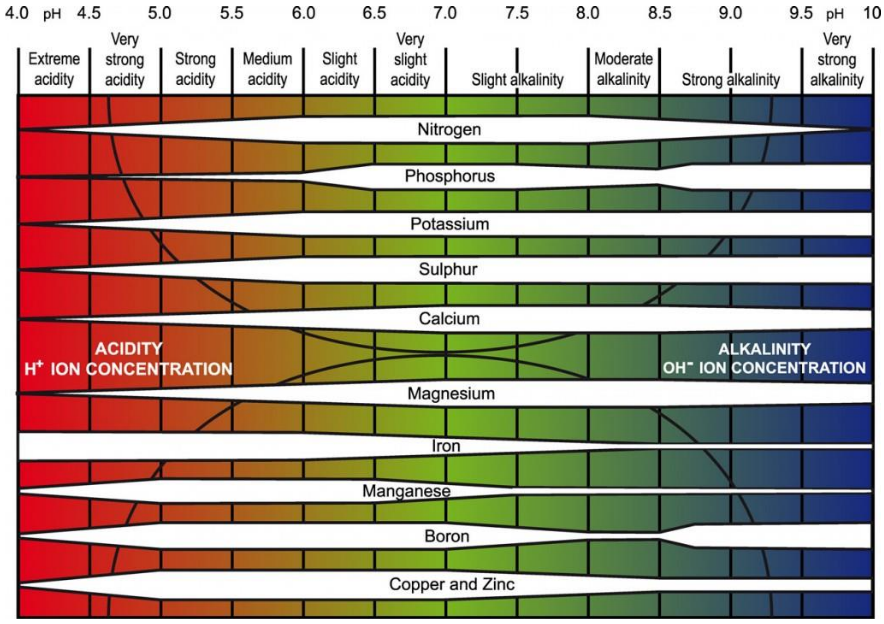


Figure 1.15 Nutrient availability at different pH levels (wider the white bar, the more available is the nutrient). Source: [7]

Cation exchange capacity (CEC) is proportional to the clay content in soils and influences the availability of cations (*i.e.*, K, Ca, Mg, Fe, Zn and Mn). Yet, despite CEC is usually not a limitation to potato production, when soils have low CECs, they generally have a higher need for nutrients being applied (Thornton, 2020). Besides, soils with high soluble salt concentrations can reduce the ability of plants to absorb water from soil, leading to a reduction in potato growth and yield, affecting the physiological processes implicated in dry matter production and distribution within plants, causing a reduction in tuber growth. As such, the electrical conductivity of the soil, to avoid yield loss, should not exceed 1.7 $dS.m^{-1}$ (Thornton, 2020).

Regarding physical properties, soil texture influences potato growth and development, despite potatoes being able to grow from sand to clay loam soils (Gould, 1999; Thornton, 2020). In fact, is vital for proper potato growth a well textured soil (Gould, 1999). Moreover, soils with fine textures (namely, clay loams and silty clay loams) have lower water infiltration rates and can lead to a problem of runoff and unequal soil water distribution in fields with slopes higher than 5 %. Yet, considering the lower infiltration rates in fine textured soils, they are suitable for sprinkler irrigation systems or solid-set systems, and for surface irrigation. On the other hand, sandy soils generally have a lower water-hold capacity and a higher infiltration rate, being suitable for center-pivot or linear-move irrigation systems (Thornton, 2020).

Compared to fine textured soils, sandy soils are more prone to nutrient leaching due to their lower water-hold capacity (Thornton, 2020). Soil water-holding capacity, infiltration and drainage can be decreased in zones of high soil bulk density (Thornton, 2020). Potato plants can be grown on most soil types if there is no compaction of the soil (Gould, 1999), since the efficiency of potato production can be reduced, due to the increase of mechanical resistance of the soil, which interferes in roots functioning and tubers growth, triggering the occurrence of less vigorous plants and more vulnerability to tubers defects. Also, there is an increase in the energy required for the emergence of the plant and for root extension, leading to a reduction of the plant's ability to absorb water and nutrients (Thornton, 2020). Commonly, sandy soils compact less than clay soils, and wet soils are more susceptible to compaction than dry soils (it is important to have dry to moderate soil moisture before field and tillage operations being carried out) (Thornton, 2020). Additionally, for potato production, moisture of the soils should be above 50 % (Djaman et al., 2021) and should range between 60 – 80 % for loam and sandy soils (Gould, 1999).

Slope percentage in a potato growing field should be considered due to the severe limitations in planting, cultivation, and harvest operations. Also, higher slopes increase the potential for soil erosion and unequal water distribution. Slopes greater than 10 % leads to problems in slippage and alignment of planting, cultivation and harvesting equipment. Moreover, in a field with different slopes, the lower areas tend to accumulate excess moisture (increasing the risk of diseases), while the upper regions tend to be too dry, leading to a reduction in yield and quality of the potatoes (Thornton, 2020).

1.2.1.6.3 Fertilization

Fertilization in potato cultures is used to attend best yields and quality (Gould, 1999), as *Solanum tuberosum* L. is a plant that grows and develops fast and has a high demand for nutrients (EMBRAPA, 2015). Nevertheless, all plants must require 17 essential elements for their normal growth and development (Uchida, 2000; Thornton, 2020; Kumar et al., 2021), being available in the soil (Kumar et al, 2021). Carbon (C), hydrogen (H) and oxygen (O) are obtained from the atmosphere and from soil water (Uchida, 2000), while N, P, K, Ca, S, Fe, Zn, Mg, Mn, Cu, B, Mo, Cl and Ni (Thornton, 2020) are obtained either from soil minerals and organic matter or by organic or inorganic fertilizers (Uchida, 2000). Nitrogen (taken up by plant as nitrate (NO_3^-) or ammonium (NH_4^+)), phosphorus (taken up as phosphate ($H_2PO_4^-$ and HPO_4^-) and potassium (taken up as K^+) are considered the primary nutrients, while Ca^{2+}, Mg^{2+} and SO_4^- are considered the secondary nutrients (Thornton, 2020). The remain essential nutrients are considered micro-nutrients (being required in small quantity) (Thornton, 2020; Kumar et al 2021). In this context, considering that some nutrients often limit potato productions, there is a need to be added as fertilizers (Thornton, 2020). Thus, the quantities of fertilizers should be adequate to allow a good growth and development of potatoes, considering that doses below the necessary will limit the plant development, whereas an excess might cause an abnormal plant development (due to toxicity, salinity, inhibition of absorption of a specific nutrient by the excessive presence of another) (EMBRAPA, 2015). Yet, the efficiency of nutrients by the potato plant can vary due to the influence of factors such as the variety/cultivar, soil, soil water or climate (EMPRAPA, 2015).

Potato plants required optimal nutrient content during the development season, but nutrient uptake is slow during the initial growth, thereafter, improving (having high uptake rates) during the rapid vegetative growth and during the early stages of tuber development. Thereafter it stabilizes during tuber maturation and at the end of plant development (Thornton, 2020), as it can be seen for N, P, K in **Figure 1.16**.

The molecular form of the fertilizers applied can influence the growth of the plants, namely if subsists a lack of N, P, and K. Considering N, there is less uptake of NO_3^- at temperatures below 13 ^oC, relatively to NH_4^+ (being more easily taken up by the plants) (Dean, 2018). In fact, the most important nutrient that must be available throughout the growth and development of potatoes is N. Moreover, excess supply of N will result in smaller tubers, cracks in the tubers and the harvest will be delayed (Gould, 1999). Nitrogen is required for vegetative growth (namely, in photosynthesis being a component in chlorophyll molecule) (Uchida, 2000), synthesis of starch in leaf, production of amino acids and yield (Kumar et al, 2021). On the other hand, N deficiency can cause yellowish color in the older leaves and in all the plant under severe stress (Dean, 2018). In sandy soils with poor organic matter content, which did not receive any N fertilization, the deficiency will be more severe and marked (EMBRAPA, 2015). Additionally, at harvest, around 30 % of N remains in the vines, while 60 to 65 % is contained in the tubers (Thornton, 2020).

Phosphorus is crucial to stimulate the seed piece and for the normal development of roots, the growth of stem and for the initiation blooming (Gould, 1999). Adequate availability of P in the soil is essential to early crop development, tuber initiation (Horton, 1987; Thornton, 2020) and enhances tuber maturity (Thornton, 2020). In the presence of P deficiency, leaves of the potato plants start to turn dark green, foliage can decrease (Dean, 2018), and there is a reduction in tuber shape, size, and specific gravity (Thornton, 2020), as well as a decrease in potato production and yield (EMBRAPA, 2015). *Solanum tuberosum* L., responds well to P application in soils (EMBRAPA, 2015).

Potassium is essential in the translocation of sugars, promotes root growth (helping the plant maintain the upright position) (Gould, 1999), plays a major role in energy storage and transfer in photosynthesis and respiration (Uchida, 2000). Potassium is further required in greater amounts by potato plants (EMBRAPA, 2015). Thus, when K in soils is low, it is necessary to apply high doses to obtain high yields (EMBRAPA, 2015). Potassium deficiency can lead to darker green color and can turn a bronzing color in the tips and margins of the leaves (Dean, 2018) and in a severe deficit may present necrosis (EMBRAPA, 2015). The availability of K affects the number of tubers, tuber size, yield, specific gravity, blackspot bruise predisposition and storage quality (Thornton, 2020).

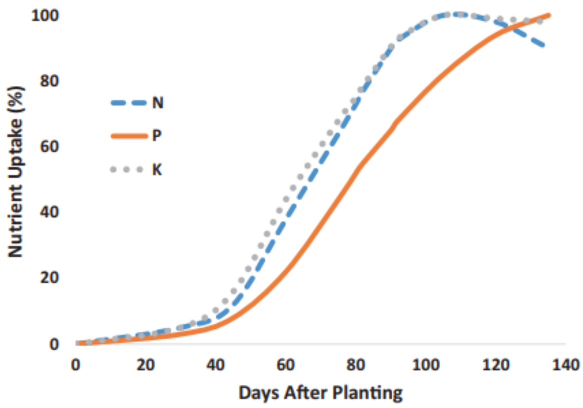


Figure 1.16 Example of total N, P and K uptake by potato (variety Russet Burbank). Source: Thornton, 2020.

The remaining nutrients tend to follow the pattern of uptake of P (Thornton, 2020). Additionally, it may be necessary to carry out soil corrections, namely liming. Liming promotes an important modification in the root environment, reducing soil acidity and providing Ca and Mg, which increases the availability and efficiency of different nutrients. Thus, potato plants respond positively to the application of acidity correctives despite being relatively tolerant to soil acidity (EMBRAPA, 2015). In fact, Ca indirectly improves yield by reducing the acidity of soils and the first symptoms of deficiency occurs on the younger leaves and leaf tips, and the growing tips of leaves and roots can die (Uchida, 2000). Hence, some quality tuber disorders are associated with Ca deficiencies (namely, brown spot), being necessary to supplement Ca in soils when the concentration of Ca is less than 400 and 700 ppm in sandy and heavier textured soils, respectively (Thornton, 2020). As such, Ca should be applied (namely as calcium sulfate, calcium nitrate or calcium- ammonium nitrate) near the tuber growth zone to help Ca uptake by the stolon of the roots (Thornton, 2020). Additionally, potatoes benefit from nutrients obtained from manure and from the organic matter added to the soil (Horton,1987). In fact, to fit potato varieties in their crop system, farmers frequently adapt their agronomic practices (Horton, 1987). However, different factors, such as climate or pests, will impact nutrient needs and potential yield. In this case, fertilizers should be applied (particularly the primary macronutrients) to increase potential yield. Nevertheless, between varieties there can be huge differences regarding nutrient needs (Thornton, 2020).

Regarding pests and diseases, potato plants are susceptible to more than 300, however farmers only try to control those that lead to extensive crop losses or that "live" in the soil for an extended time period (Horton, 1987). In fact, pests and diseases can reduce nutrient uptake and transport within the plant because they attack both roots and stem tissues of the potato plant (Thornton, 2020). To prevent different fungal diseases and insects and nematodes, fungicide and pesticides must be applied to the culture (Horton, 1987).

1.2.1.6.4 Irrigation

Solanum tuberosum L., is more sensitive to drought relatively to other cultures (Horton, 1987) and water management is vital due to the water-sensitivity of the potato culture (as it has a shallow root system) (Djaman et al., 2021). As such, it is important to irrigate properly during the development/growing season to optimize potato production (Dean, 2018). In fact, not only the timing, but the amount of water, needs to be considered (Dean, 2018) to produce tubers with quality (Gould, 1999). Thus, it is important to have a sustainable and efficient water management (Djaman et al., 2021).

During the planting time, a substantial amount of water should be presented in the soil to promote a rapid emergence of the plant and, after the emergence, the availably of water affects the yield (due to the shallow root system and the negative effect of water stress on photosynthesis) (Horton, 1987). As such, adequate irrigation water management, before and during tuber initiation, will increase the number of tubers per plant and total yield (Djaman et al., 2021). The two most stress-sensitive stages of potato growth that are influenced by irrigation methods and regimes are bulking and ripening (Djaman et al., 2021).

Water plays a key role for cooling plants by evapotranspiration and providing a way for transport minerals and organic compounds within tissues. Moreover, only a small part of the water taken up by the plant will be used in the photosynthesis directly (Horton, 1987), but water stress reduces photosynthetic efficiency (Onder et al., 2005). Throughout cool periods, potatoes can tolerate brief droughts, however, they can reduce potato yields due to low oxygen levels that can damage roots and rot new tubers (due to saturated soil). Additionally, supplying water irregularly can grow cracks or malformations in the tubers (Horton, 1987). Nevertheless, potato water uses and evapotranspiration, vary with diverse factors, such as irrigation regimes, methods, technology used, fertilizer management, and local climates (Djaman et al., 2021). In fact, water demand varies from 380 to 800 mm, according to the climatic conditions of the region (Djaman et al., 2021).

Different irrigation methods can be used in potato production, depending on local climate and soil conditions, namely, sprinkler, furrow, surface drip, drip irrigation or subsurface (Djaman et al., 2021). Sprinkler and furrow are two methods of irrigation widely used in the Mediterranean region, but nowadays the use of drip irrigation increased due to the improvement in water use efficiency in crop systems (Onder et al., 2005). Thus, the electrical conductivity of the irrigation water should not exceed 1.1 $dS.m^{-1}$ to avoid yield losses (Thornton, 2020). Nevertheless, irrigation management is not only important for yield in potato production, since it is also required for the management of fungal and bacterial diseases (such as late blight early blight, while mold and bacterial ring rot) (Djaman et al., 2021). Thus, sprinkler irrigation can create high humidity on the potato leaves and within the canopy, generating favorable conditions to the development of potato foliage diseases, being important to let foliage dry between irrigation and the potato crop should be irrigated early evening or late afternoon (Djaman et al., 2021). Nonetheless, sprinkler irrigation (together with chemical pesticides) helps to restrict mite damage due to the increase of the humidity on plant leaves (up to more than 60 %) (Djaman et al., 2021).

1.2.1.7 Harvest and storage

1.2.1.7.1 Harvest

Potatoes are usually harvested through a mechanical process where they are lifted from the soil with a mechanical digger. However, not only potatoes are lifted from the soil but also vines, soil, rocks, and other materials, being necessary to separate the potatoes from the other materials by shaking (as they are transported through the machine) (**Figure 1.17**). Thereafter, potatoes are stored in boxes and transported to the storage facility (Gould, 1999).

Figure 1.17 Harvest in field A (2018). Source: Louricoop.

One of the most serious problems during harvesting, handling and storage of the tubers is the development of bruises in the potatoes, which leads to a great loss of potatoes (Gould, 1999) and thus economic losses (Kaundal et al., 2022). Bruising affects potato quality (Kaundal et al., 2022) and there are four reasons that can induce bruises in potatoes: soil and tuber conditions at harvest, temperature, and the way the harvest is carried out (**Figure 1.18**). For instance, soil moisture should be 60 to 80 % for loam and sandy soils, yet heavy and wet soils or heavy and dry soils make the separation of the tubers from the soil very difficult. Tuber condition is another aspect to considered, due to the need for the tubers to set their skin, however, they are one of the easiest types of bruises (skinning) to avoid by only handling the tubers when they are fully mature. Regarding temperature, to prevent the development of shatter bruises (cracks on tuber surface that go through tuber's flesh) and black spots (dark spots in the tuber's flesh), the tubers should be handled when temperature is above 12.8 °C and never should be handled when the temperature is below 7.2 °C. The mechanized process used during harvest generates a lot of bruises in potatoes (Kaundal et al., 2022). Thus, the way the harvest is carried out has a huge importance to avoid bruises in potatoes, and it is important to adjust the speed of chains (preventing tubers roll back and tuber drops) in a process that depends on the field conditions (Gould, 1999). Additionally, another type of bruise that can occur is called "pressure bruise", occurring due to storage when the humidity is low (below 90 %) and results from continuous pressure. Also, when processing, those potatoes may cause a discolor of the flesh – showing grey zones (Gould, 1999).

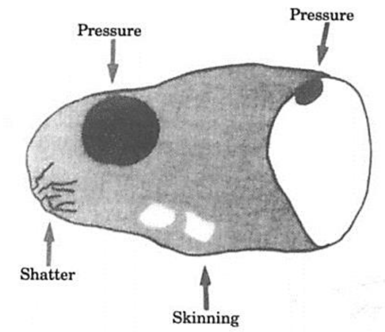


Figure 1.18 Different types of potato bruises. Source: Gould, 1999.

Considering the importance of harvest to avoid potato losses, storage conditions played an important role in the maintenance of potato quality.

1.2.1.7.2 Storage conditions

Storage is essential to ensure continuous supply for both fresh markets and post-harvest processing industries (Wang et al., 2020). Indeed, proper storage conditions are necessary to maintain the quality of potatoes, as they are living, respiring, and biologically active organisms (Olsen & Kleinkopf, 2020). Yet, to have optimal and proper storage conditions, it is important to consider the high amounts of water that potato tubers have in the edible portion (Alexopoulos & Petropoulos, 2021). In fact, potato tubers contain more water in the edible portion compared to other staple food crops (namely, rice, wheat, and maize) (Alexopoulos & Petropoulos, 2021). Nevertheless, the main goal of storage is to minimize any losses in quality (Olsen & Kleinkopf, 2020). Potatoes should be matured to be stored (Gould, 1999; Olsen & Kleinkopf, 2020), and tuber maturity is considered when the skin (periderm) becomes set and growth stops. Hence, maturity can be measured through sucrose content (Gould, 1999). Additionally, there are some basic criteria for storage potatoes, such as: being a dark and ventilated facility, and temperature and relative humidity should be maintained. Yet, considering that potatoes are constantly respiring, they must have oxygen (Olsen & Kleinkopf, 2020).

Freshy harvested potatoes typically go through a period of wound healing (curing period) prior to when storage holding temperatures are achieved. This process takes between 2 to 3 weeks at 10 – 12 °C (depending on the variety, sugar content, and other factors) and promotes the healing of cuts, bruises, and skin damage, being very important in reducing disease development and in decreasing weight loss during storage period. After the wound healing, the ideal is to reduce the temperature gradually to the chosen holding temperature of storage (Olsen & Kleinkopf, 2020). During storage low light conditions should be maintained.

During potato storage, the sucrose content should be monitored, and the potatoes for the chip market must contain 1 % or less sucrose (Gould, 1999). Additionally, if the sucrose content increases, the potatoes must be used immediately to avoid the occurrence of dark chips (Gould, 1999), because starch is converted to reducing sugars and can lead to dark discoloration after being fried (called "browning") (Horton, 1987). Nonetheless, storage temperatures vary according to its intended end use, being usually stored between 7 and 12 °C (to limit the content of reducing sugars in the potato) (Olsen & Kleinkopf, 2020; Murigi et al., 2021). Regarding the potato processing sector, tubers must be maintained at 8 - 10 ºC to avoid unwanted cold-induced changes (Wiltshire & Cobb, 1996). Despite the storage lower temperatures (2 – 4 ºC) being indicated for potatoes intended to be stored for a long term due to limited sprout development (Paul et al., 2016), for potatoes intended for fresh consumption, or post-harvest processing, these are not indicated because of the accumulation of reduced sugars (glucose and fructose) (Sonnewald, 2001). As such, temperatures ranging between 7 and 12 °C are usually used for storing potatoes, yet the use of sprout inhibitors is important since they are favorable temperatures for sprouting (Murigi et al., 2021). Moreover, despite cooler storage temperatures and sprout inhibitors being able to slow down sprout development, sprouting can happen during extended periods of storage and leads to a slightly increase of the free amino acid content (Horton, 1987). The type of sprout inhibitor products will depend on the intended future use and on the variety, considering that each variety reacts differently to sprout inhibitors (Olsen & Kleinkopf, 2020). Nevertheless, as soon as tubers have exited from the dormancy period in storage, the sprouting of buds can occur despite of the relative humidity (Alexopoulos & Petropoulos, 2021).

During cold storage nitrogen content is stable, however ascorbic acid (between 40 – 75 % of content can become lost after 8 months of cold storage) and folic acid (can be lost up to 40 % of content) are adversely affected by storage (Horton, 1987). Additionally, for 8 to 10 months of storage, the typical total weight loss is 5-8 %, being 1.5 % due to respiration and the remainder due to evaporation and decay. Yet, weight loss increases with storage time and lower relative humidity (RH), which can stimulate a bigger weight loss. Thus, the recommended RH must be situated between 90 – 98 %. Nevertheless, carbohydrates in tubers can be reduced due to high rates of respiration, which might have implications for texture and quality of potato products (Olsen & Kleinkopf, 2020). Furthermore, cold temperatures reduce the tuber respiration rate, whereas high temperatures allow for carbohydrate conversions, leading to a decrease in reducing sugar content (which is a requirement in the industry). Hence, in potatoes for processing, it is essential to have minimal sugar accumulation (Olsen & Kleinkopf, 2020).

Potato is considered a bulky and semi-perishable food, being bruising is one of the major problems for the industry of potato (Kaundal et al., 2022), since bruising creates access points for diseases and enhances respiration and evaporation rates in potatoes (Olsen & Kleinkopf, 2020). Additionally, the higher moisture content increased the susceptibility to metabolic activity and physical damage (Abewoy, 2021). In fact, potatoes that are damaged or bruised could require further consideration for appropriate storage management (Olsen & Kleinkopf, 2020). In this context, it should be noted that storage conditions may vary with variety, location of growth, field conditions, harvest date, and other factors associated with the variability of potatoes Olsen & Kleinkopf, 2020).

1.2.1.8 Processed potato products

Potatoes are worldwide crop that has the capacity and adaptability to be processed and transformed into different products (Kaudal et al., 2022). In fact, potatoes turned out to be the first modern "convenience" food mainly because it's cheap, ready to cook, easy to grow, nutritious and high-rich energy food (FAO,2009b). Potatoes can be used in several dishes, either used fresh or after processing (Alexopoulos & Petropoulos, 2021). Nevertheless, only less than half of the potatoes produced worldwide are consumed fresh, the remain is processed in different products namely, as seed tubers, in potato food products, namely, french fries, chips/crisps, flakes and granules, flour, starch (FAO, 2009b), chilled-peeled and canned potatoes (Kirkman, 2007) or in livestock feed (Bond, 2014). Additionally, potatoes can be used in the extraction of pharmaceutical compounds for the food and beverage industry (Alexopoulos & Petropoulos, 2021) or converted through processing into vodka or schnapps (Pavek, 2014).

Global potato production reached about 373 787 000 tonnes, being *ca.* 9 322 000 tonnes the amount of processed potatoes (EUROSTAT, 2022). In the European Union, despite commercializing potatoes as a raw commodity, the main four types of processed potato products are frozen, dried, prepared, or preserved potatoes, and potato starch (EUROSTAT, 2022). In this context, in 2019 processed potato products reached 9.1 billion (€), corresponding to 1.6 % of the value of production of the entire food industry in EU (EUROSTAT, 2022). Nevertheless, in 2019, in terms of the production value of processed potatoes, frozen potatoes are considered the most significant product in EU (EUROSTAT, 2022) (**Table 1.2**).

Table 1.2 Production value of processed potatoes (in billions of €) in 2019, in European Union. Source: EUROSTAT, 2022.

Product	Production value (billion €)
Frozen, prepared, or preserved potatoes	3.8
Prepared or preserved potatoes, including crips (excluding frozen, dried, flakes or in the form of flour)	3.6
Potato starch	0.7
Dried potatoes (in form of flour, flakes, granules, pellets or meals)	0.6
Others	0.3

1.2.1.8.1 Incorporation of food additives

Food additives are natural or synthetic substances, that are added to food products to increase the shelf life, intensify, or even modify their properties, without interfering negatively with their nutritious value (Lidon & Silvestre, 2007; Silva & Lidon, 2016). In Europe, their use is regulated by EU laws, considering the foods in which the food additives can be applied, the maximum amounts allowed, their purity, and chemical characterization (Silva & Lidon, 2016). Considering the food additives, each one corresponds to a code consisting of the letter 'E' followed by three or four digits, being this system valid in all EU countries. Within this framework, preservatives are classified from E200 to E299 and are substances that extend the shelf life of food products, protecting them from deterioration caused by microorganisms (Lidon & Silvestre, 2007) and antioxidants are classified from E300 to E399 and extend the shelf life of food products, as well as protect them against oxidation (Lidon & Silvestre, 2007). The use of antioxidants is highly used in agri-food industry, being applied to a wide range of foods (Silva & Lidon, 2016). Accordingly, starch and potato puree are two products obtained by processing potatoes, intended for human consumption (Lidon & Silvestre, 2007), in which food additives are incorporated in their processing. Considering starch, additives are added after the dehydration stage, while in mashed potatoes, preservatives are added after cutting the potatoes (after being brushed and washed) and in the final phase after the cooking and crushing stage (with the consistency of puree), and/or the additives can be incorporated prior to dehydration and packaging (Lidon & Silvestre, 2007).

Moreover, the addition of food additives varies according to processed potato products, being regulated by the European Commission Regulation Nº. 1129/2011, of 11 November.

1.2.1.9 Varieties used in the study: Agria, Picasso, and Rossi

In Portugal, Agria, Picasso, and Rossi are three of the most commercialized varieties. Regarding Agria, is considered a medium early-ware potato with multiple processing possibilities (EUROPLANT, 2022), namely for fresh consumption, chips/crips, french fries (PORTATOPRO, 2022; EUROPLANT, 2022) or granular and convenience products (EUROPLANT, 2022), being suitable for growth in Europe and Middle East (POTATOPRO, 2022). Agria has yellow skin and flesh, is big and oval (**Figure 1.19**), presents a high dry matter content, is susceptible to common scab, bruising, and fusarium (dry rot), is resistant to virus X and potato cyst nematodes (PCN) 1 and 4, and presents a good resistance to virus A and Yn (POTATOPRO, 2022).

Figure 1.19 Agria variety at harvest in field C (2020). Source: Louricoop.

Picasso is a maincrop ware variety, having a good tolerance for drought and bruising and damage, and being suitable for long-term storage (**Figure 1.20**). Picasso has an oval shape, light yellow color, yellow skin, and prominent red eyes (POTATOPRO, 2022). Additionally, is it susceptible to common scab, bruising, fusarium and to mechanical damage. Also, is slightly susceptible to virus X, Yn and susceptible to virus Yntn, being resistant to nematodes Ro1/4 (AGRICO, 2023).

Figure 1.20 Picasso variety at harvest in field B (2020). Source: Louricoop

Rossi is a later maturity variety, being suitable for retail or for traditional markets and presenting a good resistance to common scab and good storability (HZPC, 2022) (**Figure 1.21**). The tuber presented oval and a cream color flesh, having shallow and yellow eyes and red skin (GOVERNMENTOFCANADA, 2022). Additionally, Rossi variety has a high dry matter content, and it is not sensitive to Yntn (HZPC, 2022).

Figure 1.21 Rossi variety at harvest in field C (2020). Source: Louricoop

1.2.2 Calcium

Calcium is an essential mineral element for both humans and plants (Peacock, 2010; White & Broadley, 2003). In humans, it is acquired through dietary sources (Peacock, 2010) and in plants from the soil solution by the root system (White & Broadley, 2003).

1.2.2.1 On soil and in plants

Calcium has one of the highest concentrations in the earth's crust (around 3.64 %) (Power & Prasad, 1997) and is considered one of the most predominate cations in soil solutions (Kabata-Pendias, 2011; Pandey & Sanyal, 2020), being present in nonexchangeable and exchangeable forms (Power & Prasad, 1997). In fact, calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), and gypsum ($CaSO_4H_2O$) are the primary source of Ca (Kabata-Pendias, 2011), being calcite the most important source of this essential mineral in calcareous soils (Power & Prasad, 1997). Additionally, Ca in soil solution can range from 68 to 778 $mg.kg^{-1}$ however, Ca availability to plants is dependent on different soil conditions (namely, soil type, pH, CEC, and Ca supply) (Power & Prasad, 1997), climatic factors (González-Fontes et al., 2017) and the presence of competing ions (such as Na^+, K^+ and Mg^{2+}) (Sharma et al., 2017; Jadon et al., 2019), which may result in Ca^{2+} deficiency (Pandey & Sanyal, 2020). As seen in **Figure 1.15**, in neutral or even slightly basic soil pH, plants can readily uptake Ca^{2+}, however in more acidic soils the absorption is hindered (Hawkesford et al., 2012; Pandey & Sanyal, 2020). Calcium (as Ca^{2+}) uptake from the soil solution occurs through ion channels, located in root cells (White et al., 2002; Pandey & Sanyal, 2020) and these channels which are normally nonselective, can be taken in Ca^{2+} and from different mineral elements (Pandey & Sanyal, 2020). Also, they can be classified as depolarization-activated channels (DACCs) and hyperpolarization-activated channels (HACCs) (Thor, 2019; Pandey & Sanyal, 2020). Nevertheless, after being taken up, Ca is distributed within the plant (Thor, 2019) from the roots to the shoot through the xylem due to the transpiration stream (Hanger, 1979; White & Broadley, 2003; Jadon et al., 2016; Pandey & Sanyal, 2020), being the speed of water flow a key factor of Ca^{2+} transport (Yang & Jie, 2005). In the roots, Ca^{2+} to reach the xylem follows either the apoplastic (though the space between cells) or symplastic (through the cytoplasma of cells linked by plasmodesmata) movement (González-Fontes et al., 2017; Pandey & Sanyal, 2020), being this movement well balanced to regulate Ca rate to the xylem and avoid the accumulation of toxic cations in the shoot (White &Broadley, 2003; González-Fontes et al., 2017). Besides, the epidermis and cortex are outer cells layers of the roots, being responsible for loading, unloading, and long-distance transport of solutes both in the xylem and phloem, and are implicated in the acquisition of water and minerals (Yang & Jie, 2005). As such, calcium movement stats in the epidermis, traverses the cortex and stops at the casparian stips of the endodermis (being a transverse and radial longitudinal structure, functioning as a barrier) and enters the cytosol via channel proteins, and is exported to the xylem by Ca^{2+}ATPases or Ca^{2+}/H^{+} antiporters (Yang & Jie, 2005; Thor, 2019; Pandey & Sanyal, 2020). Once is loaded into the xylem, Ca^{2+} is transported (in chelate form such as calcium citrate or calcium malate) to the shoot through mass flow of water and ion exchange (Yang & Jie, 2005) and distributed within the leaf cells (Thor, 2019) and storage in the cells (preferential inside the vacuole) (Pandey & Sanyal, 2020). In fact, the calcium stored in the vacuole is essential for maintaining the cellular ion-balance (Pandey & Sanyal, 2020). Moreover, transpiration is the main factor of Ca^{2+} movement, as such, low rates of transpiration can lead to low rates of Ca^{2+} accumulation into aerial organs (Yang & Jie, 2005). In this context, within the plant, the uptake and transport of Ca^{2+} require several cells and cross through symplastic or/and apoplastic pathways, having three key steps: traversing the root cortex to xylem, transport Ca^{2+} in the xylem and distributing it to leaves (Yang & Jie, 2005). Nevertheless, in the shoot, with plants developing with adequate Ca contents, the amount of Ca ranges between 0.1 and 5 % of the dry weight (Marschner, 1995; White & Broadley, 2003; Thor, 2019). Moreover, Ca cannot be redistributed via the phloem (Welch, 1999; White & Broadley, 2003; Busse & Palta, 2006), or mobilized from older tissues to the actively growing parts of plants (White & Broadley, 2003; Dayod et al., 2010). As such, the developing/growing tissues can only rely on the immediate supply of Ca into the xylem, which is dependent on the transpiration rate (White & Broadley, 2003; Thor, 2019; Singh, 2020). Indeed, some controversy prevails about Ca redistribution through the phloem, since It has been pointed out that low Ca limits the kinetics rate (Taiz & Zeiger, 2002; Yang & Jie, 2005; Dayod et al., 2010; Subramanian et al., 2011; Jadon et al., 2016; Hocking et al., 2016; Thor, 2019), namely in potato tubers (Davies & Millard, 1985; Oparka & Davies, 1988; Nelson et al. 1990; Coelho et al., 2021; Coelho et al., 2022). Moreover, Ca has a dual function in plants: a structural role and serves as a second messenger. Thus, plants must strictly regulate the uptake, distribution, and storage of Ca to fulfill both functions (Thor, 2019). Calcium is a crucial component of the cell wall (maintaining the integrity and providing rigidity) (Pandey & Sanyal, 2020; Singh, 2020), in cell elongation (Pandey & Sanyal, 2020) and it is necessary for the formation of Ca pectates (holding plant cells together) (Sharma et al., 2017). Thus, Ca^{2+} has an important role in counteract the cytotoxic effect of other ions and in reducing the toxic effects of exogenous NaCl (Sharma et al., 2017). Calcium also has a central role in stress response signaling (Hocking et al., 2016), being required as a cofactor by enzymes involved in the catabolism of ATP (Taiz & Zeiger, 2002), and also involved in multiple photosynthetic pathways, such as stomatal closure or photosystems functioning (Hochmal et al., 2015; Wang et al., 2019). Nonetheless, Ca deficiency in plants usually occurs due to low Ca availability or to water stress (leading to low transpiration rates) (Jadon et al., 2016). In fact, due to the low remobilization from old to young or growing tissues via the phloem (Thor, 2019), deficiency symptoms of Ca usually occur in young leaves or fruits (Jadon et al., 2016; Thor, 2019; Singh, 2020). Usually, the symptoms include the curling of young leaves or shoots scorching (Jadon et al., 2016), but can also lead to tipburn or brown heart in leafy vegetables, blossom end rot in tomatoes (Marschner, 1995; Yang & Jie, 2005; Dayod et al., 2010), pepper and watermelon, to black heart in celery (Yang & Jie, 2005), bitter pit in apples (Marschner, 1995; Yang & Jie, 2005), increase of the susceptibility of pathogens (Hocking et al., 2016), reducing root growth and loss of cell membrane integrity (Sharma et al., 2017). Hence, this type of disorders is commonly triggered by slow absorption of Ca^{2+} and due to the poor distribution after transport (Yang & Jie, 2005). Nevertheless, Ca deficiency can affect crop plants leading to a decrease in quality and yield (Dayod et al., 2010). The opposite, excess of Ca, also can occur when there is excessive Ca in the rhizosphere solution, causing toxicity to plants and leading to reduced plant growth rate (White & Broadley, 2003).

1.2.2.2 Importance in humans

Calcium is an essential element to the human organism, namely implicated in bone formation (Weaver & Heaney, 2006; IOM, 2011), additionally is one of the most abundant mineral elements in the human body (Peacock, 2010). In the human body, 99 % of total Ca is found in bones and teeth as calcium hydroxyapatite ($Ca_{10}(PO_4)_6)(OH)_2$) (IOM, 2011), providing skeletal strength (Peacock, 2010; IOM, 2011) and, simultaneously, keeps a reserve that allows the maintenance of intra and extracellular calcium pools (Peacock, 2010). In fact, bones are a reservoir for calcium and other inorganic nutrients (EFSA, 2015). The remaining total calcium in the human body (around 1 %) acts as a crucial intracellular messenger in cells and tissues (EFSA, 2015) and is responsible for several vital functions, namely, nerve impulse transmission, muscle contraction (Peacock, 2010) and relaxation, in hormones, nerves, blood pressure regulation (Pravina et al., 2013), and in the circulatory system (IOM, 2011).

Calcium is only obtained through dietary sources (Peacock, 2010; Pravina et al., 2013; EFSA, 2015) and supplements (IOM, 2011). Thus, Ca must be daily ingested by eating a healthy and balanced diet, in which the consumption of food naturally rich in Ca prevails (Pravina et al., 2013) (namely, milk, leafy vegetables, nuts, yogurt, and cheese) (Cormick & Belizán, 2019), followed by a successful absorption by the human body (Pravina et al., 2013). In fact, the majority of Ca comes from dairy products, followed by vegetables, grains, legumes, fruits, meat, and others (IOM, 2011).

The requirement of Ca is dependent on age and physical conditions (*i.e.*, toddlers, pregnant women and women in menopause need a higher Ca intake) (Pravina et al., 2013) (**Table 1.3**). However, Ca intake is also dependent on the state of the individual's Ca metabolism (mainly regulated by intestinal absorption, renal reabsorption, and bone turnover) (Peacock, 2010). Nevertheless, the average dietary values for an adult range between 1000 and 1300 mg according to different governmental sources (SACN, IOM, FAO/WHO, and EFSA - *cf.* Cormick & Belizán, 2019), being reported that Ca intake of 1000 to 1200 mg/day is sufficient for fracture prevention (Ströhle et al., 2015).

Table 1.3 Recommended Dietary Allowance/Adequate intake for Calcium (in mg). Source: NIH, 2022.

<table>
<tr><th>Age</th><th>Male</th><th>Female</th><th>Pregnant and Lactating</th></tr>
<tr><td>0-6 months</td><td colspan="2">200</td><td>-</td></tr>
<tr><td>7-12 months</td><td colspan="2">260</td><td>-</td></tr>
<tr><td>1-3 years</td><td colspan="2">700</td><td>-</td></tr>
<tr><td>4-8 years</td><td colspan="2">1000</td><td>-</td></tr>
<tr><td>14-18 years</td><td colspan="3">1300</td></tr>
<tr><td>19-50 years</td><td colspan="3">1300</td></tr>
<tr><td>51-70 years</td><td>1000</td><td>1200</td><td>-</td></tr>
<tr><td>Over 70 years</td><td colspan="2">1200</td><td>-</td></tr>
</table>

Moreover, Ca is absorbed in the intestinal mucosa by active transport and passive diffusion (IOM, 2011). Thus, about 30 % of the ingested dietary Ca is absorbed in the small intestine, most of which is absorbed by the upper intestine (Peacock, 2010). Around 8 to 23 % of Ca absorption occurred by passive diffusion (Peacock, 2010). Calcium absorption is controlled by various aspects that affect the concentration, absorption rate, and in the intestine the transit time (Shkembi & Huppertz, 2022). Additionally, Ca absorption is conditioned by this nutrient need in the body and by its availability in the foods eaten, also being dependent on vitamin D content (*i.e.,* Ca absorption increases with vitamin D) and by the phosphorus and magnesium (as excess of both hinders Ca absorption) (Pravina et al., 2013). However, dietary Ca bioavailability can be enhanced but can also be lowered due to calcium-binding agents (namely, oxalate, phosphate, or cellulose) (Peacock, 2010). Emphasizing that, in root vegetables, Ca bioavailability is reduced due to some of the Ca being bound by oxalate (Bourassa et al., 2022). Nevertheless, Ca deficiency appears when there is inadequate amount of Ca in the human body (Pravina et al., 2013) that is not able to meet the physiological requirements (EFSA, 2015). Thus, when there is a low intake and/or an inadequate absorption by the gastrointestinal system, Ca is resorbed from the skeleton to keep the blood concentrations in the required range for the normal functions of cells and tissues (EFSA, 2015). However, this can lead to osteopenia and therefore osteoporosis. In fact, at different extents both pathologies are associated with low bone mineral density, increasing the risk of fractures in the bones (EFSA, 2015). In fact, bone balance changes depending on age due to the rate of bone formation and resorption. Hence, children have a positive bone balance, and the elderly usually have a negative bone balance causing bone loss (Peacock, 2010). Bone loss and osteoporosis are commonly associated with menopause (IOM, 2011), since the formation of new bone resorption (Peacock, 2010; IOM, 2011). However, men can also have bone loss and osteoporosis (IOM, 2011). Moreover, due to medical treatments, renal failure and diuretics, Ca deficiency can be due to hypocalcemia (*i.e.,* low content of Ca in blood) (Pravina et al., 2013). Additionally, Ca deficiency can lead to other pathologies rather than osteopenia and osteoporosis, namely rickets (as bones cartilage do not mature and do not mineralize as normal) and osteomalacia (adult rickets) (IOM, 2011, EFSA, 2015). Thus, Ca can reduce the risk not only of these pathologies, but additionally hypertension and colon cancer (Miller et al., 2001), and can function as a natural tranquilizer and manages to calm the nerves and can lower cholesterol (Pravina et al., 2013). Additional, early Ca supplementation can prevent arthritis, rheumatism (Pravina et al., 2013), and cardiovascular complications (Cormick & Belizán, 2019). Nevertheless, excess of Ca can lead to hypercalcacemia due to a high intake of Ca (from diet or supplements), in which the supplementation with Vitamin D has a higher impact (considering that vitamin D increases Ca absorption) (EFSA, 2015). Usually, this pathology occurs in individuals with cancer (breast, prostate, thyroid, colon, or lung) (Pravina et al., 2013). However, according to EFSA (2015) a daily Ca intake of 2 500 mg (from diet or/and supplements) do not cause adverse effects on human health.

1.2.3 Biofortification

The agricultural system was designed only to increase yield and crop productivity, leading to a quick rise in nutrient deficiency in crops, causing nutrient malnutrition among consumers (Garg et al., 2018). Hence, agriculture is recently experiencing a change from producing more quantities to nutrient-rich food crops (Garg et al., 2018; Sheoran et al., 2022). As such, the biofortification of different crops can be a long-term solution to supply nutrient-rich food crops to consumers (Garg et al., 2018).

1.2.3.1 Types of biofortification

Biofortification or "biological fortification" (Garg et al., 2018), stated to originate around the Green Revolution period (1966 -1985), and in the early 90s, Howarth Bouis took the first step to solve nutrient deficiencies through biofortification (Lockyer et al., 2018; Sheoran et al., 2022). But the term "biofortification" was coined only in 2001 by Steve Beebe (Lockyer et al., 2018; Sheoran et al., 2022). Indeed, the first "wave" of biofortified crops was bred and approved between 2009 and 2013, and since 2014 the distribution of biofortified crops has been scaled up in multiple countries (Lockyer et al., 2018). Moreover, biofortification is considered the process of breeding nutrients in food crops (Saltzman et al., 2013; Singh et al., 2016). It is considered a cost-effective, sustainable, and long-term strategy (Singh et al., 2016), being a viable way of reaching malnourished rural populations, which have little diversity of foods in their diet and limited access to supplements and fortified foods (Saltzman et al., 2013). Besides, biofortification can have advantages and benefits at marginal cost (Kumar et al., 2022). In fact, in developing countries, these population relies on staple food crops (Mishra et al., 2022). Nevertheless, there are three common approaches in biofortification: agronomic (using fertilization strategies), conventional (crop breeding - parent lines with high mineral content are crossed over various generations to produce plants that have agronomic traits and the nutrient or nutrients required), and transgenic (using biotechnology by incorporating the target nutrient which naturally does not exist in the crop) (**Figure 1.22**) (Saltzman et al., 2013; Bouis & Saltzman et al., 2017; Garg et al., 2018; Jha & Warkentin,2020).

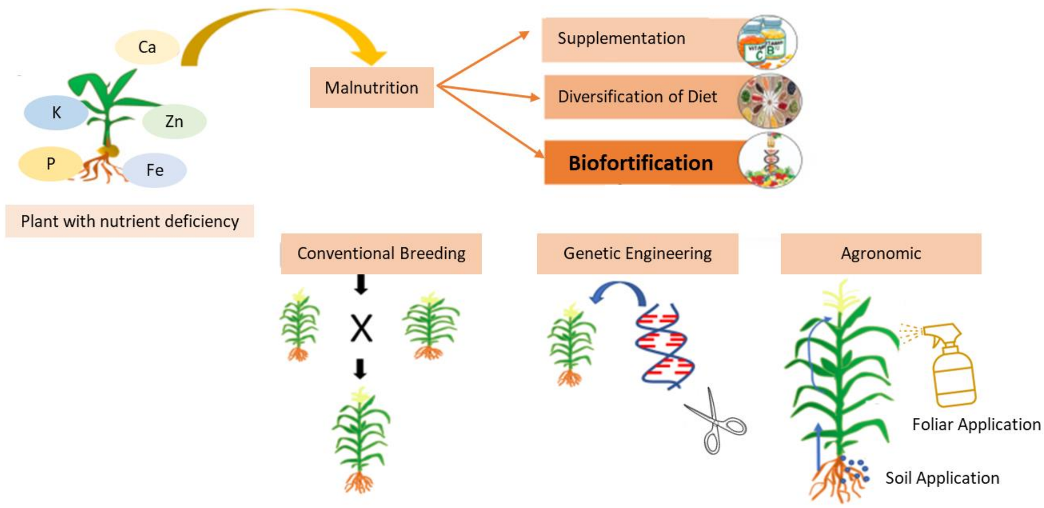


Figure 1.22 Types of biofortification. Adapted from: Sheoran et al. (2022).

Indeed, the choice of the type of biofortification requires data, namely on the targeted population, intervention practices, and nutrient bioavailability in the biofortified crops (Rao & Annadana, 2017). Moreover, biofortification aims to improve the nutrient content of some specific food crops during their growth and not during processing (inside plant cells), which is different from fortification (Díaz-Gómez et al., 2017; Mishra et al., 2022). However, the bioavailability of the nutrient biofortified is dependent on each nutrient and the food matrix (Díaz-Gómez et al., 2017). Some of the most targeted crops for biofortification are rice, wheat, maize, potato, sweet potato, and tomato (**Figure 1.23**) (Garg et al., 2018). Nevertheless, it is important to consider that the majority of biofortified foods before consumption are subject to processing, packaging, storage and even cooking, which is vital to assess the absorption of nutrients by the human body regarding biofortified crops (Kumar et al., 2022). Moreover, biofortified crop varieties once optimized and developed can be adapted for different environments and geographies around the world (Kumar et al., 2022).

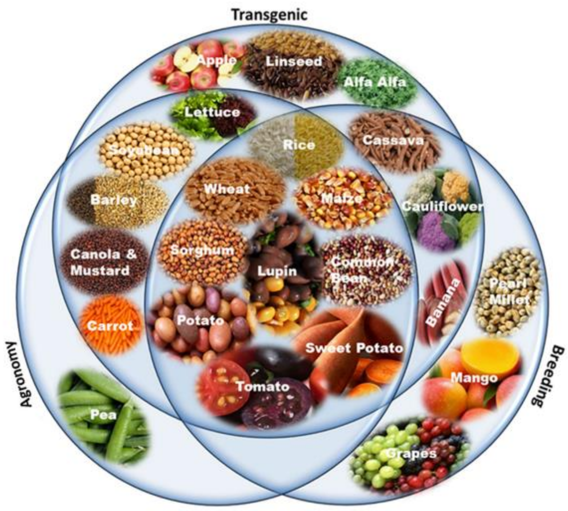


Figure 1.23 Some of the biofortified crops generated by the three most common biofortification approaches: agronomic, transgenic, and conventional breeding. Source: Garg et al. (2018).

Conventional breeding is considered one of the most accepted (Garg et al., 2018; Sheoran et al., 2022) and reliable approaches for biofortification, resulting over time in the development of various varieties of different staple food crops, which showed improvements in different essential nutrients (Sheoran et al., 2022). In fact, HarvestPlus considered conventional breeding to be the fastest way to get more nutritious crops into the hands of both farmers and consumers (Bouis & Saltzman, 2017). However, there are some limitations in this type of approach, namely regarding the longtime development in generating cultivars with the desired trait or traits, the limited genetic change in the plant gene pool, and the dependence on the phytoavailability of the mineral nutrients in the soil (Carvalho & Vasconcelos, 2013; Jha & Warkentin, 2020). Hence, for conventional plant breeding being feasible, is necessary for sufficient genotypic variation in the characteristics of interest (Garg et al., 2018). As such, conventional plant breeding is considered a very successful approach for minerals and vitamins, one-off cost, and a long-term strategy (Carvalho & Vasconcelos, 2013), in which parent lines with high content of nutrients are crossed with the recipient line, with the desired agronomic characteristics over various generations, in order to produce plants with the chosen nutrient and agronomic traits (Garg et al., 2018).

Genetic engineering or transgenic approaches are usually utilized when crops have low or no genetic diversity for the desired nutrients among plant varieties (Garg et al., 2018; Kumar et al., 2022; Sheoran et al., 2022), making the production of biofortified varieties with the desired nutrient content and agronomic traits possible (Kumar et al., 2022). Although compared to conventional breeding, transgenic development requires considerably higher efforts in research, with a lower success rate regarding a specific variety (Sheoran et al., 2022). However, can speed up the process of conventional breeding (Carvalho & Vasconcelos, 2013). Nevertheless, the transgenic approach has advantages considering that when a useful gene is discovered, it can be used for targeting multiple crops (Garg et al., 2018) and is more precise considering that only a certain gene can be introduced in the target plant (Singh et al., 2021). Yet, despite this type of approach, it might be cost-effective in the long term and successful for minerals and vitamins enhance (Carvalho & Vasconcelos, 2013; Buturi et al., 2021). Besides, it is the least employed approach considered, not only the long research phase, but also the very slow and expensive development of a certain biofortified food (Buturi et al., 2021). Hence, although the release of transgenic varieties might require several years, it is also dependent on consent by national biosafety and regulatory processes (Bouis & Saltzman, 2017) and has low public acceptance, especially in Europe (Carvalho & Vasconcelos, 2013).

Agronomic biofortification of crops is considered a promising method to improve nutrient content in the edible parts of plants, being considered the cheapest approach to alleviate nutrient deficiency around the world (Szerement et al., 2022). This type of strategy can provide the increase of nutrients that can be directly absorbed by the plant through fertilizers (applied in soil and/or in leaves), which leads to the increase of mineral content in the edible tissues of the crop (White & Broadley, 2009; Szerement et al., 2022). Additionally, this approach is dependent upon management practices (namely, water management and nutrient interactions), soil conditions (namely, pH and nutrient content and interactions) and plant factors (namely, root characteristics, translocation within plant, and nutrient accumulation in plant organs) (Praharaj et al., 2016). Thus, mineral elements with a good mobility both in soil and in plants are considered the easiest to show a successful agronomic biofortification (Carvalho & Vasconcelos, 2013) and unlike transgenic approach, agronomic one can be applied on existing crops and varieties being the products easily accepted by the consumers (Chaudhary et al., 2022). Nevertheless, the main advantages of agronomic biofortification are that can be done on crops already cultivated (Rahal & Shivay, 2016), shows immediate results, is quite a simple method, and can be utilized as a complement to other strategies (Carvalho & Vasconcelos, 2013). However, the main weaknesses of this approach regarding Its success depends on minerals and crop varieties, the need to have frequent applications, and can lead to negative environmental impacts (Carvalho & Vasconcelos, 2013). Indeed, agronomic biofortification has been studied around the world and with different nutrients (namely, Fe, Zn, and Se), and different crops (namely, rice, wheat, maize, barley, chickpea, potato, sweet potato, and tomato) (Garg et al., 2018).

1.2.3.2 Agronomic biofortification with Calcium

During the last decades the awareness of the need to enhance mineral content (namely, Ca, Fe, and Zn) in foods has increased (Rao & Annadana, 2017). Besides, one of the major contributors to Ca insufficiency in human populations is due to the consumption of staple foods with insufficient Ca content and bioavailability. Hence, due to the major source of dietary, Ca in milk and dairy products, as for vegetarians and vegans, there is a constant need for alternative food sources to meet this nutrient needs (Knez & Stangoulis, 2021). As such, Ca biofortification can be an approach to consider since different studies were already carried out in different crops (**Table 1.4**) to increase Ca content and improve quality characteristics.

Table 1.4 External application of calcium and their beneficial effects in different crops.

Biofortified crop	Country	Intervention	Intervention effect	Reference
Grapes	China	Chelated Ca sugar alcohol – four foliar sprays with 1.2, 2.4, 3.6, 4.8 or 6.0 L/hm^2	Improve the photosynthetic characteristics, yield and quality of wine grapes, the content of soluble solids and tannins	Ma et al. (2023)
Pineapple	Indonesia	Ca-EDTA, $Ca(NO_3)_2$ or $CaCl_2$ – four foliar sprays with 75 kg/ha and four foliar sprays with Calcibor 10L/ha in 2000L water	$CaCl_2$ and Calcibor increased Ca content and decreased electrolyte leaking significantly. $CaCl_2$ generated more rigidity in the cell wall.	Loekito et al. (2022)
Apples	Turkey	$CaCl_2$ – two foliar sprays 0.5%	Fruit weight, width and height increased, the respiration rate and ethylene production decreased	Küçükyumuk & Erdal (2022)
Grapes	China	$CaCl_2$ – four foliar sprays with 5g/L	Reduced grape cracking due to the increased of Ca content in the peels which increased the peel stability	Shi et al. (2022)

Sweet Cherry	Chile	$CaCl_2$ – foliar spray with 0.8%	Improvement in fruit textural properties	Matteo et al. (2022)
Chickpeas	-	$Ca(NO_3)_2$ – 4 mM	foliar application with $Ca(NO_3)_2$ may alleviate toxic effects of Cd through enhanced plant growth, high content of photosynthetic pigments and low oxidative stress	Parveen et al. (2022)
Rocha Pears	Portugal	Two foliar sprays with $CaCl_2$ (0.4, 0.8 and 1.6 kg/ha) or $Ca(NO_3)_2$ (0.1, 0.3 and 0.6 kg/ha) and the last two foliar sprays were carried out with 4 and 8 kg/ha $CaCl_2$, respectively	Increased of Ca content and prevailed in the epidermal region of the fruits	Pessoa et al. (2021)
Strawberry	Iraq	$CaCl_2$ - 1 or 2 g/L	$CaCl_2$ showed a higher percentage of soluble solids after storage	Lateef et al. (2021)
Blueberries	Chile	$CaCl_2$ – 2.5 and 5.0 L/ha	Increased fruit Ca levels, total phenolic content and reduced the percentage of dehydrated and decayed berries after storage, reducing deterioration in cold storage	Lobos et al. (2021)
Peanuts	China	Sorbitol non-chelated calcium (sorbitol + calcium nitrate + calcium acetate) with 0.4, 0.8, 1.6 or 2.4 g/L or sorbitol-chelated calcium with 1.6 or 2.4 g/L	Improved yield, quality, dry matter accumulation and Ca content. Sorbitol chelated calcium treatments showed better results compared to non-chelated treatments	Liu et al. (2021)
Lettuce	China	$CaCl_2$ – foliar sprays with 60, 120 or 180 mg/L	Ca content increased having variability among the different cultivars	Yuan et al. (2018)
Pears	China	$CaCl_2$ – foliar sprays with 2%	Inhibited the incidence of rate of hard end disorder and decreased lignin content and fruit firmness during storage	Wang et al. (2018)
Basil, Mizuma, Tatsoi and Endive	Italy	$Ca(H_2PO_4)_2$ and $CaCl_2$ – 200 mg/L in nutrient solution	Increased Ca content significantly in leaves of basil (15%) and mizuna (12%), but not in tatsoi or endive	D'Imperio et al. (2016)
Pomegranate	Turkey	$Ca(NO_3)_2$ – two foliar sprays with 2 or 4%	Ca application with 2% showed the maximum yield and increased N, P, K, Ca, Mg, Fe, Zn, Cu and Mn content. $Ca(NO_3)_2$ 4% only increased K, Ca and Fe content	Korkmaz & Askin (2015)

Tomato	Pakistan	$CaCl_2$ – foliar sprays with 0.3 or 0.6%	Increased the plant height and fruits per plant and decreased the incidence of blossom end rot	Rab & Haq (2012)
Apples	Iran	$CaCl_2$ – four foliar sprays with 0.5%	Increased Ca content of apples, texture, and brix index as well as the yield	Asgharzade et al. (2012)
Grapes	Chile	$CaCl_2$ - foliar sprays (solution 1% w/w), or/and soil application (300 kg/ha)	Did not affect Ca content but showed more firmness in berries at harvest	Bonomelli & Ruiz (2010)
Sweet corn	Egypt	Ca-EDTA – eight foliar sprays with 500 or 1000 ppm	Increased the vegetative growth (namely, plant height, fresh and dry weight), total chlorophyll and Ca, N, P and K content	El-Yazied et al. (2007)
Peaches	New Zealand	$CaCl_2$ – foliar sprays with 50 or 100 mg/10L	Ca content of peach epidermis increase significantly by at least 50% and reduced the number of brown rot infected fruit	Elmer et al. (2007)
Pears	U.S.	$CaCl_2$ – foliar sprays with 34%	Ca content and yield increase and reduced the incidence of cork spot	Raese & Drake (1995)

Several studies on different crops have shown that the effects of Ca applications are dependent on different factors, such as types of compounds, types of application, rates, and timing as well as the type of crops linked to its variety (Dayod et al., 2010; Lara, 2013).

1.2.3.3 Agronomic biofortification in *Solanum tuberosum* L.

Most of the crops targeted to be biofortified are staple crops (Garg et al., 2018). As such, considering that potato is widely grown worldwide and are progressively turning out to be the main staple food, different centers (namely, the International Potato Center - CIP) are working to enhance human nutrition through the biofortification of potatoes (Kromann et al., 2017). In fact, Singh et al. (2021a) refers to potato as "an ideal crop for biofortication", mainly due to the enormous industrial demand and to the fact that it can be grown in different climatic conditions. Also, can provide in a short time high yield, nutrition, and dry matter (Singh et al. (2021a) (**Figure 1.24**).

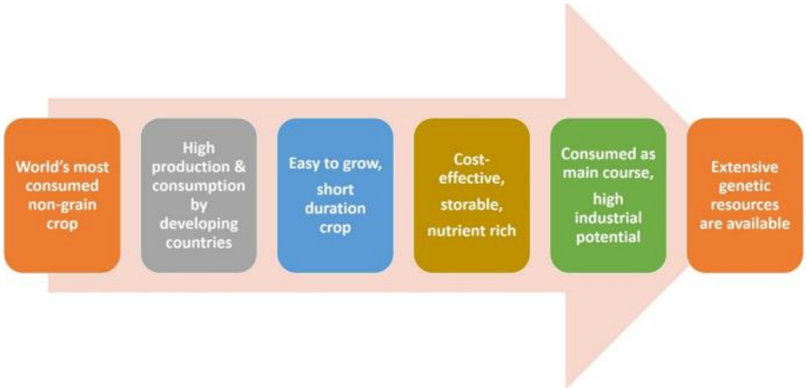


Figure 1.24 Some of the reasons for potato being considered as "an ideal crop for biofortification". Source: Singh et al. (2021a).

However, until today, a limited data regarding the development of a workflow for agronomic biofortification of potatoes (Kromann et al., 2017) subsists. In **Table 1.5** it is presented a summary of some of the agronomic biofortification workflows in *Solanum tuberosum* L. carried out over the years around the world. Hence, foliar sprays with Ca (namely, $CaCl_2$ and/or $Ca(NO_3)_2$), Zn, and I represent a large part of the majority of the studies carried out. In general, it can be seen that there is an accumulation response that is very dependent on the cultivar chosen or the establishment in the itinerary applied.

Table 1.5 External application of different mineral elements in *Solanum tuberosum* L..

Potato variety	Country	Intervention	Intervention effect	Reference
cv. Longshu Nº.7	China	Five different Fe fertilizers - foliar sprays	Plants were sprayed after potato emerged, four times, once in a 7-day cycle	Zhang et al. (2022a)
cv. Fandango and Sifra	Iraq	Ca (500 or 100 mg/L) and K (2.5 or 5 mg/L) – three foliar sprays	Plants treated with 500 and 100 mg/L of Ca achieved a significant increase of different parameters (such as the plant height, number of stems and plant area). Plants treated with K showed an increase in relative chlorophyll content of leaves	Ibraheem & Al-Dulaimi (2022)
cv. Beleny	Egypt	B – foliar sprays with 50 or 100 mg/L associated with organic fertilizers tea	Increase in tuber potato tuber yield and the highest values in vegetive growth parameters with 100 m/L of B and different organic fertilizers tea	ELsadany et al. (2021)

cv. Kufri Jyothi	India	Different mixes with gypsum (soil application) and B (foliar or/and soil application). gypsum application- 75 or 150 kg/ha; soil application of B-2.5 or 5 kg/ha and foliar application of B- 0.25 or 0.5%	Increased of N, P and K in tubers with a mix with 150 kg/ha of gypsum + 0.5% foliar sprays with B and with 150 kg/ha of gypsum + soil application of 5 kg/ha B. The highest content of protein and starch in tuber was in RDF + 150 kg/ha gypsum + 0.5% foliar sprays with B.	Shirur et al. (2021)
cv. Catania	Poland	Silicon – product containing 200g SiO2 + 24g Fe in 1 dm^3 in the form of Na_2SiO_3 and Fe-EDTA – different foliar applications with 0.25 dm^3/ha or 0.50 dm^3/ha carried out at different times	Higher tuber weight per plant under periodic water deficits and drought conditions	Wadas (2021)
cv. Lady Rosetta	Egypt	K – foliar application with potassium citrate (2.64 g/L), potassium silicate (10 g/L) or monopotassium phosphate (2.94 g/L) with soil application of different doses of soil potassium fertilizers	The application of foliar monopotassium or potassium citrate increased most of the plant growth and tuber chemical composition-related parameters (namely, N, protein, amino acid, P, K and starch content)	Ali et al. (2021)
cv. Arosa and Neveske	Russia	$CaCl_2$ – two foliar sprays with 2 or 4 kg/ha	Enhanced plant growth and tuber yield under infection of *Phoma* dry rot pathogen	Mohammed et al. (2020)
cv. Irga	Poland	KI (soil) and four foliar sprays with KIO_3- KI with 0.5, 1.0 or 2.0 kg/ha and $KI0_3$ with 0.02, 0.2 or 2 kg/ha	Increased of iodine content in potato tubers without no decrease of starch or sugar content. Highest biofortification of iodine with 2 kg/ha KIO_3 treatment	Ledwożyw-Smoleń et al. (2020)
cv. Balatoni rózsa	Hungary	KI (using irrigation) – 0.1 and 0.5 mg/L	Higher content of Fe and lower Mg and P concentration. Mn, Cu, Zn and B was not influenced by the iodine treatment. Iodine content did not increase significantly	Dobosy et al. (2020)

cv. Bellini	Italy	Three foliar sprays with a mix of B (0.9%), Cu (0.6%), Fe (13.6%), Mn (5.2%), Mo (0.2%) and Zn (2.2%)	Increased of Fe and Zn content in raw potatoes	Ierna et al. (2020)
-	Poland	Soil supply of Mg: 20,40,60,80 or 100 kg/ha of MgO using kieserite ($MgSO_4$ 15-7% Mg)	Mg fertilization applied during potato growth increased the content of Mg in tubers. Also, after 3 and 6 months of tuber storage reduce the loss of ascorbic acid	Wszelaczyńska et al. (2020)
cv. Kufri Joyti	India	Different mixes with $ZnSO_4$ (0.2%), $MnSO_4$ (0.2%) and Borox (0.1%)	Different mixes showed best attributes namely in growth parameters, yield attributes and quality parameters	Miyu et al. (2019)
cv. E-potato 10	China	Na_2SeO_3 or Na_2SeO_4 – foliar sprays	Selenite foliar application during the tuber bulking stage increased Se content in tubers	Zhang et al. (2019)
cv. Kufri Joyti	India	Different mixes of Mg, S, Zn and B – foliar sprays	Higher dry matter content, starch content and sugar of tubers	Ramesh et al. (2019)
cv. Lord and Milek	Poland	Ti – three foliar sprays with 0.2 or 0.4 L/ha	Increased Fe and Mn content and did not affect Zn content. Titanium content was higher at 0.2 L/ha with foliar sprays carried out at the leaf development and tuber formation stages	Wadas & Kalinowski (2019)
cv. Kufri Pukhraj	India	Zn – foliar sprays with 5, 10, 15, 20, 25, 30, or 35 ppm	Treatment with 30 ppm showed the highest tuber yield, carbohydrate and TSS content.	Singh et al. (2018)
cv. Saxo, Golden Millennium, Vales Everest and 12601ab1	UK	ZO or $ZnSO_4$ – up to four foliar sprays	Increase in tuber Zn concentration in both flesh and skin of tubers, however excessive applications can reduce tuber yield. Fe, Mn, Cu, Ca, Mg or K in tubers was not influenced by Zn biofortification	White et al. (2017)
cv. INIAP-Natividad, INIAP- Puca shungo, Chaucha amarilla, Chaucha roja	Ecuador	Zn (soil) applied as an amino acid-based Zn nutrient – 0,8,16,24 or 32 mg.kg-1 and Zn (foliar sprays) applied as EDTA-chelated Zn – 3.06 mM	Increase in Zn content in tubers with soil and foliar biofortification. Tuber Fe content did not increase with Fe fertilization	Kromann et al. (2017)

and Coneja negra		Fe (soil) applied as an amino acid-based Fe complex – 0,25,50,75 or 100 mg.kg-1 and Fe (foliar sprays) applied as EDTA-chelated Fe - 6.71 mM		
cv. Salany	Egypt	$Ca(NO_3)_2$ – foliar sprays with 0.6 or 0.8%	Plant tuber yield and average tuber weight increased with increasing foliar application of Ca but the number of tubers plant^{-1} decreased. Increased in the number of aerial stems, leaf area, chlorophyll content in leaves and percentage of dry matter in leaves	El-Hadidi et al. (2017)
-	India	Foliar sprays with Zn (1g/L) or B (1.5 g/L) or Fe (10g/L) or Mn (5g/L) or NPK (10 g/L) or mixture of Zn, B, Fe and Mn or mixture of NPK, Zn, B, Fe and Mn	Mixture of NPK, Zn, B, Fe and Mn showed the highest tuber yield, tuber weight and tuber diameter	Moinuddin et al. (2017)
-	Iraq	Mix of 60 ppm Zn and 30 ppm Mn - three foliar sprays	Increased total yield, yield per plant and weight of potato tuber	Al-Fadhly (2016)
cv. Pinta Boca and Waych'a	Bolivia	Sixteen levels of Fe and Zn (soil and foliar applications) – combination of four levels of Fe and four of Zn. In the soil – 10 or 20 kg/ha of Fe or 5 or 10 kg/ha of Zn	Fe and Zn content in tubers was greater in cv. Pinta Boca compared to cv. Waych'a. There was a significant differences between for location, crop and levels in Zn.	Gabriel et al. (2015)
-	Tunisia	$Ca(NO_3)_2$- seven foliar applications with 20,40 60,80,100 or 120 kg/ha	Ca content in tubers and leaves increased and tuber quality improved (namely, tuber size). However, reduced the number of tubers per plant.	Hamdi et al. (2015)
cv. Spounta	Egypt	$Ca(NO_3)_2$ – by irrigation with 10 or 20 kg/feddan of net Ca and by	Ca content in tubers improved as well as plant growth and enhanced tuber yield and quality	Helal & AbdEl-hady (2015)

		gypsum form ($CaSO_4$) with 500 kg/feddan		
-	Iraq	Fe, Mn, Cu and Zn – foliar applications with a mixture contain (330g $ZnSO_4$ + 330g $MnSO_4$ + 150g $FeSO_4$ + 80g $CuSO_4$)	Zn, Mn, Fe and Cu increased yield and dry matter percentage of potato crop	AL-Jobori & Al-Hadithy (2014)
cv. Mila	Poland	S (applied in soil) – ammonium sulphate or potassium sulphate or elemental S, with 20 or 40 kg/ha treatments each fertilizer	Increase in potato tuber yield and in the protein content. No significant effect on dry matter content and starch in the potato tubers	Barczak et al. (2013)
cv. Draga, Aladin, Elpaso, Kurado, Diseree, Provento and Red Brown	Iraq	Fertilizer high in potash (Alaska foliar fertilizer with 36% of K_2O) – two or four foliar applications	Potato cvs differ in all the parameters analyzed (namely, plant height, tuber weight and yield). High yields were obtained in Red Brown, Provento and Draga cvs.	Jasim et al. (2013)
cv. Ditta and Cambisol	Czech Republic	Na_2SeO_3 – one foliar spray with 200 or two foliar sprays with 400 g/ha (split dose 200 + 200 g/ha with 7 days between doses)	Increased the relative contente of total essential (EAA) and non-essential (NEAA) AMINO ACIDS. The highest dose showed toxic effects	Ježek et al. (2011)
Combination of four varieties: Kufri Chipsona-1, Kufri Chipsona-2, Kufri Joyoti and Kufri Pushkar	India	S (soil) – 10,15,30,45 or 60 kg/ha	S application showed significant influence on quality (namely, dry weight, sugar and starch content) and yield, increased with the increasing dose of S up to 45 kg/ha	Sharma et al. (2011)
cv. Gera and Shenkola	Ethiopia	$CaCl_2$ and/or $Ca(NO_3)_2$ – foliar sprays with 5, 10 or 10 g/L per plant	Both alone or combined application of $CaCl_2$ and $Ca(NO_3)_2$ enhanced potato plant growth and improves tuber yield	Seifu & Deneke (2007)
-	Iran	$ZnSO_4$ or $MnSO_4$ – two foliar sprays 2,4 and 8 ppt solution	Zn and Mn applications increased yield and quality of potato tubers,	Mousavi et al. (2007)

			namely their content in tubers. However, there was a decrease in P.	
cv. Russet Burbank	U.S.	$CaCl_2$ and $Ca(NO_3)_2$ –42 kg/ha - dissolve in irrigation water	Ca content in tubers increased and reduced the incidence of internal brown spot	Ozgen et al. (2006)
cv. Primura	Italy	Na_2SeO_3 or Na_2SeO_4 – foliar sprays with 50 or 150 g/ha	Increased in tuber Se content	Poggi et al. (2000)
cv. La Chipper, Superior and Atlantic	U.S.	Ca application from gypsum with 450 or 900 kg/ha	Ca contents were higher in petiole, medulla and periderm in tubers and higher yield	Locascio et al. (1992)

Foliar application compared to soil application is a more efficient approach to enhance mineral content in the edible parts of a certain crop (Kromann et al., 2017; Singh et al., 2021a). Although agronomic biofortification has huge potential to enhance the nutrient content in potatoes, it has limitations such as soil composition and pH, mineral accumulation and mobility, environmental conditions, and the stage of development of the plants when the biofortification workflow is applied (Garg et al., 2018; Singh et al., 2021a)

1.3 Aims of the work

The main aim of this study was the development and implementation of a technical itinerary for Ca biofortification in *Solanum tuberosum* L. tubers of three commercial varieties in Portugal (Agria, Picasso, and Rossi) in three different locations in the same region of Portugal (Lourinhã). Considering the pathologies associated with deficiency in Ca intake, it is intended to create an auxiliary approach that contributes to solve this problem in the human population. The main goal was to obtain tubers naturally enriched with Ca in the three varieties with interest in Portugal. As such, the present work seeks to answer certain technical and scientific questions, namely: How can be optimize the production of biofortified tubers in Ca? What type and form of fertilization should be applied? Which foliar fertilizers enriched in Ca are best suited to the different varieties? Considering that the varieties are different, what are the effects of foliar application on the physiology of plants and tubers? What are the effects of synergistic and antagonistic relationships on tissue accumulation? In terms of quality control and conservation, would there be changes? What are the effects on chemical, physical, and organoleptic characteristics of the tubers? Also, assessing the nutritional profile of processed potato products, will they be following industrial requirements?

2

MATERIALS AND METHODS

2.1 Fields location

During the years, the study was carried out in three experimental fields (A, B and C) located in the western part of Portugal (Lourinhã) (GPS coordinates: 39.281101, -9.253018 (Field A), 39.275734, -9.229710 (Field B) and 39.270123, -9.237315 (Field C), intended for potato (*Solanum tuberosum* L.) production. The locations of the three fields are shown in **Figure 2.1**.

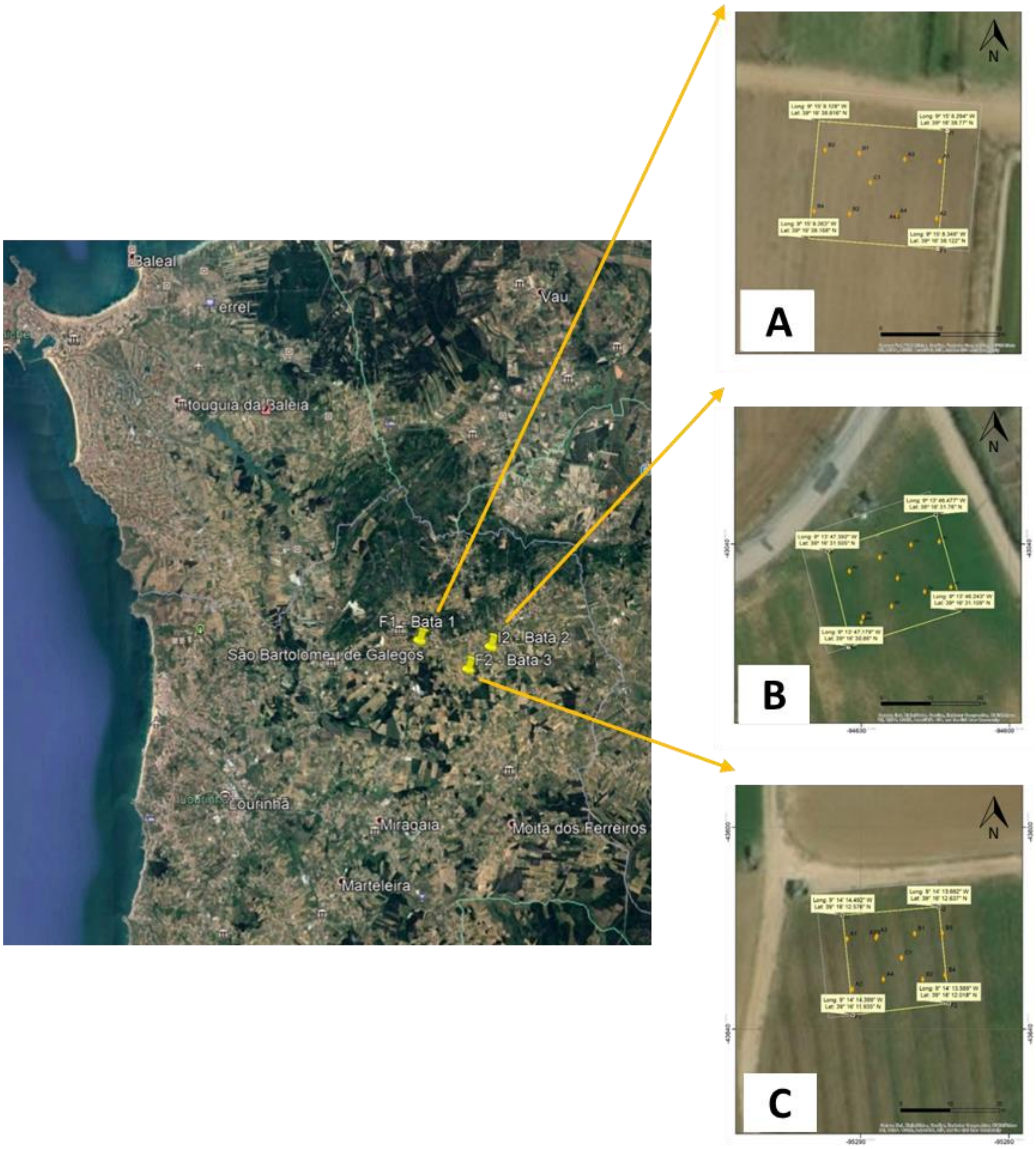


Figure 2.1 Geographic location of the three experimental fields (A, B and C). Indication (in yellow) of the limits of soil sample collection in the fields and indication (in orange) of the region of soil sample collection.

2.2 Technical itinerary

The workflow for Ca biofortification was carried out using three varieties (Agria, Picasso, and Rossi), being annually its implementation rotated through the three fields. Over three years, and in each field, control plants were not sprayed at any time with biofortification fertilizers. In the first year four foliar applications were carried out after the beginning of tuberization, with 8 to 12 days interval, applying four concentrations of $CaCl_2$ (1,3,6 and 12 kg/ha) or $Ca(NO_3)_2$ (0.5, 1, 2 and 4 kg/ha) (**Table 2.1**). The commercial name of products applied for $CaCl_2$ treatments was "Tecnifol Cálcio" and for $Ca(NO_3)_2$ was "Nitrato Cálcio Rega". The duration of the cycle of Agria, Picasso, and Rossi were 123, 136, and 133 days, respectively.

Table 2.1 Itinerary of calcium biofortification of the first year of study.

Field	Variety	Planting date	Foliar applications				Harvest date	Fertilizers
			1º	2º	3º	4º		
A	Agria	4/5/2018	6/7/2018	16/7/2018	26/7/2018	3/8/2018	4/9/2018	$CaCl_2$ (1,3,6 and 12 kg/ha) or $Ca(NO_3)_2$ (0.5, 1, 2 and 4 kg/ha)
B	Rossi	11/5/2018	25/7/2018	3/8/2018	14/8/2018	24/8/2018	24/9/2018	
C	Picasso	15/5/2018	24/7/2018	2/8/2018	14/8/2018	24/8/2018	25/9/2018	

In the second year seven foliar applications were carried out after the beginning of tuberization, with 6 to 8 days interval, applying two concentrations (12 and 24 kg/ha) of $CaCl_2$ or Ca-EDTA (**Table 2.2**). Treatment of 24 kg/ha Ca-EDTA was only applied once due to the occurrence of toxicity symptoms. The commercial name of products applied for the $CaCl_2$ treatments was "Tecnifol Calcio" and for the Ca-EDTA was "Tradecorp Ca". The duration of the cycle of Agria, Picasso, and Rossi were 141, 136, and 125 days, respectively.

Table 2.2 Itinerary of calcium biofortification of the second year of study.

Field	Variety	Planting date	Foliar applications							Harvest date	Fertilizers
			1º	2º	3º	4º	5º	6º	7º		
A	Picasso	21/3/19	30/5/19	7/6/19	14/6/19	21/6/19	28/6/19	4/7/19	12/7/19	9/8/19	$CaCl_2$ (12 and 24 kg/ha) or Ca-EDTA (12 and 24 kg/ha)
B	Agria	15/3/19	30/5/19	7/6/19	14/6/19	21/6/19	28/6/19	4/7/19	12/7/19	29/7/19	
C	Rossi	15/5/19	17/7/19	22/7/19	29/7/19	5/8/19	12/8/19	19/8/19	26/8/19	17/9/19	

In the third year seven foliar applications were carried out after the beginning of tuberization, with 6 to 8 days interval, applying only one concentration (12 kg/ha) of $CaCl_2$ or Ca-EDTA (**Table 2.3**). The commercial name of products applied for the $CaCl_2$ treatments was "Tecnifol Calcio" and for Ca-EDTA was "Tradecorp Ca". The duration of the cycle of Agria, Picasso, and Rossi were 153, 132, and 177 days, respectively.

Table 2.3 Calcium biofortification itinerary of the third year of study.

Field	Variety	Planting date	Foliar applications							Harvest date	Fertilizers
			1º	2º	3º	4º	5º	6º	7º		
A	Rossi	16/3/20	19/5/20	27/5/20	3/6/20	9/6/20	16/6/20	23/6/20	30/6/20	11/8/20	$CaCl_2$ (12 kg/ha) or Ca-EDTA (12 kg/ha)
B	Picasso	12/3/20	19/5/20	27/5/20	3/6/20	9/6/20	16/6/20	23/6/20	30/6/20	22/7/20	
C	Agria	7/5/20	1/7/20	8/7/20	15/7/20	22/7/20	29/7/20	4/8/20	12/8/20	31/8/20	

During the three years of the experiment, each treatment was carried out in quadruplicate in 20 x 20 m plots (in A and C fields) and 20 x 24 m (in B field), with a 60 – 80 cm compass. Also, about 57 plants were used per treatment in the three fields.

2.3 Meteorological conditions

In the first year, the meteorological conditions during the production cycle of the tubers in the three fields were monitored and recorded by the national Portuguese meteorological network station located at Monte Real Air Base (39°49'41.293"N; 8°52'52.427"O). The test period, which took place between May 4 (date of the 1st plantation) and September 25 (date of the last harvest), was characterized by a maximum average temperature of 23 °C and a minimum average of 15 °C, with the maximum and minimum values recorded were 41 °C and 6 °C, respectively. During the experimental period, the maximum air humidity was 100 % and the minimum was 8 %, with the average of the minimum and maximum daily values being 53% and 96 %, respectively. According to the IPMA meteorological bulletin, the accumulated precipitation in the months of March to May was 429 mm, which corresponds to about 200 % of the average value. In the experimental period, total accumulated precipitation was 60.4 mm (with a daily maximum of 18.03 mm), which corresponds to a daily average of 0.41 mm. Additionally, there was no rain after any of the foliar applications in the three experimental fields.

In the second year, the meteorological conditions were recorded by the weather station of Lourinhã – ILISBOAL7 (39.23°N, 9.31°W, with 54 m of elevation), during the production cycle of the tubers in the three fields. Between March 15 (date of the 1st plantation) and September 17 (date of the last harvest) the maximum and minimum average temperature were 21.9 °C and 13.8 °C, respectively. The maximum and minimum values recorded were 34.8 °C and 4.7 °C, respectively. Regarding air humidity, the minimum registered was 12 % and the maximum was 96 %, the average maximum and minimum were 90 % and 63 % respectively. In the course of the experimental period, the average registered precipitation was 0.51 mm and the average maximum values daily was 10.41 mm.

As in the second year, in the three fields, the meteorological conditions in the third year of the study were recorded by the weather station of Lourinhã – ILISBOAL7 during the production cycle of the tubers. Between Mach 12 (date of the 1st plantation) and August 31 (date of the last harvest), the period was characterized by a maximum average temperature of 22.0 °C and a minimum average of 14.9 °C, with the maximum and minimum values recorded of 36.6 °C and 4.8 °C, respectively. The maximum and minimum air humidity were 100 % and 27 % and the average of the maximum and minimum daily values were 91 % and 65.6 %, respectively. Regarding the rainfall, the average was 1.21 mm and the average of the maximum daily values was 34.29 mm.

2.4 Fertilization and plant health.

During the three years, for the three varieties (Agria, Picasso, and Rossi), the workflow for Ca biofortification adopted the usual set of actions in potato production (but additionally being the monitoring of plant health and production cycle carried). The application dates of the phytopharmaceutical and fertilizers products during the first year of the experiment are present in **Table 2.4** and **Table 2.5**, respectively. Additionally, the monitoring of plant health and production cycle also in the first year is shown in

Table 2.6.

Table 2.4 Dates of application of phytopharmaceutical products during the first year of experiment.

Commercial name	AV/APV	Safety interval (days)	Intention	Date of application		
				Field A	Field B	Field C
Monceren	3851	-	Rhizoctonia	4/5/2018	11/5/2018	15/5/2018
Pyrinex 5G	1001	-	Pin	4/5/2018	11/5/2018	15/5/2018
Artist	0701	-	Infests	22/5/2018	15/6/2018	29/5/2018
Spirit pro	0799	21	Mildew	19/6/2018 and 12/7/2018	26/6/2018 and 20/7/2018	25/6/2018 and 11/7/2018
Calypso	0071	21	Epitrix or scarab	19/6/2018 and 20/7/2018	26/6/2018 and 11/8/2018	25/6/2018 and 20/7/2018
Carial flex	0994	21	Mildew	4/7/2018 and 20/7/2018	11/7/2018 and 31/7/2018	3/7/2018 and 20/7/2018
Coragen	4020	14	Scarab	12/7/2018 and 30/7/2018	20/7/2018 and 22/8/2018	11/7/2018 and 30/7/2018
Cabrio duo	0196	7	Mildew or early blight	30/7/2018 and 8/8/2018	22/8/2018 and 3/9/2018	30/7/2018 and 10/8/2018
Decis Evo	0813	7	Moth	8/8/2018	3/9/2018	10/8/2018
Basta S	0521	-	Desiccant	17/8/2018	8/9/2018	7/9/2018

Table 2.5 Dates of application of fertilizers products during the first year of experiment.

Commercial name	Type of application	Date of application		
		Field A	Field B	Field C
Adubo orgânico folhadouro 4-3-3	Soil	2/5/2018	8/5/2018	11/5/2018
Nergetic 10-10-22	Soil	2/5/2018	8/5/2018	11/5/2018
Nutricomplex 13-30-13	Foliar	1/6/2018	22/6/2018	22/6/2018
Fortan	Foliar	22/6/2018 and 31/7/2018	11/7/2018 and 22/8/2018	10/7/2018
Delfan	Foliar	-	20/7/2018	-
Aton Az	Foliar	-	20/7/2018	-
Nitrato Calcio (Calcium Nitrate)	Soil	4/7/2018	24/7/2018	23/7/2018

Fitoalgas Green	Foliar	20/7/2018	11/8/2018	11/8/2018
Sprint plus	Foliar	-	-	17/8/2018

Table 2.6 Visualization of some aspects of the production cycle of the three potato varieties during the first year of experiment.

Field	**Soil preparation**	**Vegetative development**	**Harvest or preparation for har-vest**
A			
	18/5/2018	23/6/2018	4/9/2018
B			
	4/6/2018	6/8/2018	24/9/2018
C			
	18/5/2018	9/7/2018	18/9/2018

dates of application of phytopharmaceutical and fertilizers products during the second year of experiment are present in **Table 2.7** and **Table 2.8**, respectively.

Additionally, the monitoring of plant health and production cycle also in the second year is shown in **Table 2.9**.

Table 2.7 Dates of application of phytopharmaceutical products during the second year of experiment.

Commercial name	AV/APV	Safety interval (days)	Intention	Date of application		
				Field A	Field B	Field C
Pison	1001	-	Pin	21/3/2019	15/3/2019	-
Artist	0701	-	Infests	13/4/2019	8/4/2019	-
Proman	0802	-	Infests	-	-	2/6/2019
Ekyp Mz	3878	14	Mildew	-	-	9/6/2019
Armetil-M	3883	14	Mildew	29/4/2019	-	-
Torero	3308	7	Mildew	6/5/2019, 15/6/2019 and 5/7/2019	26/4/2019	-
Spyrit Pro	0799	21	Mildew	13/5/2019	15/5/2019	-
Karate Zeon	0020	7	*Agrotis spp.*	-	26/4/2019	-
Carial Top	0716	3	early blight	5/6/2019	8/6/2019	7/7/2019 and 21/7/2019
Calypso	0071	21	Scarab	26/6/2019	-	7/7/2019
Vendetta	1172	14	Early blight	-	-	14/7/2019
Carial Flex	0994	21	Mildew	26/6/2019	-	2/8/2019
Coragen	4020	14	Scarab	-	8/6/2019	2/8/2019 and 20/8/2019
Cabrio Duo	0196	7	Early blight	-	19/6/2019	-
Epik SL	0717	14	Aphidoidea	29/5/2019		

Table 2.8 Dates of application of fertilizers products during the second year of experiment.

Commercial name	Type of application	Date of application		
		Field A	Field B	Field C
Adubo orgânico folhadouro 4-3-3	Soil	21/3/2019	15/3/2019	-
Soil set	Soil	21/3/2019	15/3/2019	21/3/2019
Pedrin	Soil	21/3/2019	15/3/2019	21/3/2019
Amicote 8-12-12	Soil	-	-	15/5/2019
Fortan	Foliar	20/5/2019	20/5/2019	14/7/2019 and 2/8/2019
Fitroleader	Foliar	20/5/2019	-	-
Sulfato Potassio (Potassium Sulfate)	Foliar	-	20/6/2019	-

Table 2.9 Visualization of some aspects of the production cycle of the three potato varieties during the second year of experiment.

Field	Soil preparation	Vegetative development	Harvest or pre-harvest
A			
	12/4/2019	17/7/2019	9/8/2019
B			
	13/3/2019	13/5/2019	29/7/2019
C			
	21/5/2019	17/7/2019	9/9/2019

Dates of application of phytopharmaceutical and fertilizers products during the third year of experiment are presented in **Table 2.10** and **Table 2.11**, respectively. Additionally, the monitoring of plant health and production cycle also in the third year is shown in **Table 2.12.**

Table 2.10 Dates of application of phytopharmaceutical products during the third year of experiment.

Commercial name	AV/APV	Safety interval (days)	Intention	Date of application		
				Field A	Field B	Field C
Belem Pro 0.8 mg	122	-	Pin	16/3/2020	12/3/2020	7/5/2020
Artist	0701	-	Infests	-	-	-
Proman	0802	-	Infests	9/4/2020	-	14/5/2020
Ekyp Mz	3878	14	Mildew	-	-	31/5/2020
Armetil-M	3883	14	Mildew	-	-	7/6/2020
Torero	3308	7	Mildew	22/4/2020, 1/6/2020, 13/7/2020 and 22/7/2020	24/4/2020 and 30/4/2020	-
Spyrit Pro	0799	21	Mildew	3/7/2020	8/5/2020	23/6/2020
Karate Zeon	0020	7	*Agrotis spp.*	-	24/4/2020	-
Carial Top	0716	3	early blight	22/5/2020	16/6/2020	11/8/2020
Nando 500SC	1120	7	early blight	-	9/6/2020 and 23/6/2020	-
Carnadine	1175	7	Scarab	22/5/2020	20/5/2020	31/5/2020
Vendetta	1172	14	Early blight	17/6/2020	1/7/2020	18/7/2020 and 4/8/2020
Cythrin 10 EC	0524	3	Aphidoidea	17/6/2020	-	8/7/2020
Carial Flex	0994	21	Mildew	12/5/2020	20/5/2020	8/7/2020
Mancozebe Sa-pec	0600	7	early blight	3/7/2020	-	-
Coragen	4020	14	Scarab	-	-	4/8/2020

Table 2.11 Dates of application of fertilizers products during the third year of experiment.

Commercial name	Type of application	Date of application		
		Field A	Field B	Field C
Plusmaster 8-12-12	Soil	16/3/2020	12/3/2020	7/5/2020
Soil set	Soil	16/3/2020	12/3/2020	7/5/2020
Pedrin	Soil	16/3/2020	12/3/2020	7/5/2020
Fitoalgas		-	-	31/5/2020
Patenkali	Soil	-	18/3/2020	-
Sierra	Foliar	22/4/2020	8/5/2020	-
Fitoleader	Foliar	12/5/2020	24/4/2020	7/6/2020
Nutrileaf 12-48-8	Foliar	-	24/4/2020	-
Fortan	Foliar	26/6/2020, 3/7/2020,13/7/2020 and 22/7/2020	1/7/2020	4/8/2020
M 10 AD	Foliar	-	9/6/2020	-

Table 2.12 Visualization of some aspects of the production cycle of the three potato varieties during the third year of experiment.

Field	Early vegetative development	Pré-harvest
A		
	19/5/2020	8/7/2020
B		
	19/5/2020	8/7/2020
C		
	3/6/2020	12/8/2020

2.5 Soil sampling and analysis

2.5.1 Soil sampling

In the first year, before the implementation of the workflow for Ca biofortification, a regular hexagonal grid (4.50 m × 6.60 m) was applied in the experimental fields of the growing potato varieties, and 9 soil samples of each field (100 g, picked up at 30 cm depth) were collected for physical and chemical analysis (**Figure 2.1**).

2.5.2 pH, EC, and organic matter content

Soil samples of each experimental field (*n* = 9) were passed through a 2 mm nylon sieve to remove major debris before analysis. After drying at 105 °C, for 24 h, until constant weight, soil moisture was determined. Organic matter (OM) was then estimated after combustion for 4 h at 550 °C, according to Margesin & Schinner (2005). Electrical conductivity (EC) and pH were measured with a multiparameter analyzer (C 6030) and SP21 (pH) and SK20T (CE) electrodes, in a soil mixture with water (1:2.5 g soil mL^{-1} water milli-q) under stirring after a thermal bath (25 °C) for 30 min, according to Pessoa et al. (2021).

2.6 Water sampling and analysis

In each year, the quality of the irrigation water of each field was analyzed, considering physical and chemical parameters (pH, temperature, electrical conductivity, bicarbonate, sulfate, chloride, sodium, calcium, magnesium, potassium, nitrate, and phosphate). Electrical conductivity and pH were determined using a Consort Multiparameter Analyzer (C 6030) and SP21 (pH) and SK20T (CE) electrodes. Calcium, Na, K and Mg ions were quantified with a Metrohm (Model 761 Compact IC) chromatograph, equipped with column and pre-column (Metrosep cation 1–2, 6.1010.000), using an eluent mixture (4 mM tartaric acid/1 mM dipicolinic acid) at a flow rate of 1.00 mL/minute and a sample injection of 10.0 µL. Alkalinity/bicarbonate was determined by titration, in 100 mL of water samples, using 0.1 N hydrochloric acid as titrant, in the presence of 0.1 % methyl orange Rodier et al. (2009). Chloride, sulphate, nitrate, and phosphate ions were quantified by photometry, using specific kits from Spectroquant NOVA 60, Merck (1.14897, 1.14779, 1.14773 and 1.14842). Water classification in the soils of the three fields, considering dominant ions, followed Piper (1994). The Langelier saturation index (ISL) was also estimated (at 20 °C) from the pHe (equilibrium pH), to determine the fouling or aggressiveness of the water relatively to calcium carbonate (**Annex I**). Sodium adsorption index (SAR) was determined and related to the electrical conductivity, in classes C and S (**Annex I**). Additionally, the Wilcox and Piper diagram was carried out for each water irrigation analyzed with Grapher program.

2.7 Monitoring through remote detection

Orthophotomaps of the three experimental fields, were produced using a high-definition and multi-sector RGB camera (with three electromagnetic spectra bands—red, green, and blue) and a parrot sequoia camera (with five electromagnetic spectra bands—NIR (near infrared), REG green, red and RGB) installed in an unmanned aerial vehicle (UAV). The calibration of the camera (parrot sequoia) considered the environmental brightness conditions. The images were processed with Workstation (AORUS, GIGA-BYTE Technology Co., Ltd. 2019, Croatia) in order to obtain information regarding the morphology of the fields (namely, digital elevation model and drainage lines or the normalized difference vegetation index (NDVI) for plant vigor). The NDVI allows to identify the absence or presence of vegetation due to vegetation has its maximum reflectance in the NIR and the minimum in the red region (**Annex II**). The NDVI is index ranges between 1 to 1 (values close to 0 – soil or leafless vegetation (low vigor) and close to 1 – higher vigor/healthy vegetation).

The drainage patterns of surface water and the geomorphology of the field was studied with an Agisoft PhotoScan Professional (Version 1.2.6, Software of 2016 and the ESRI of 2011 and ArcGIS Desktop-Release 10 from Redlands, CA: Environmental Systems Research Institute). The classification of surface water drainage areas followed Direcção Geral de Agricultura Desenvolvimento Rural (1972) and is shown in **Table 2.13.** The lower class corresponded to flattened surfaces, because of the accumulation of surface water, representing potential infiltration areas. The highest class represented zones that, due to its morphology, promote the surface water runoff, having a reduced amount of water infiltration.

Table 2.13 Slope classes for drainage surfaces and their suitability for accumulating or draining surface. Adapted from: Direcção Geral da Agricultura Desenvolvimento Rural (1972).

Class	Slope Class	Class Description	Drainage Surface
1	[0 – 5 % [	Flat or soft slope	Low surface water accumulation/drainage zones
2	[5 – 20 % [	Moderate slope	Medium to high surface drainage
3	≥ 20 %	High slope	High surface drainage

The geometry of the flights in the UAV program, before the flights have been carried out in the three experimental fields, are shown in **Figure 2.2**.

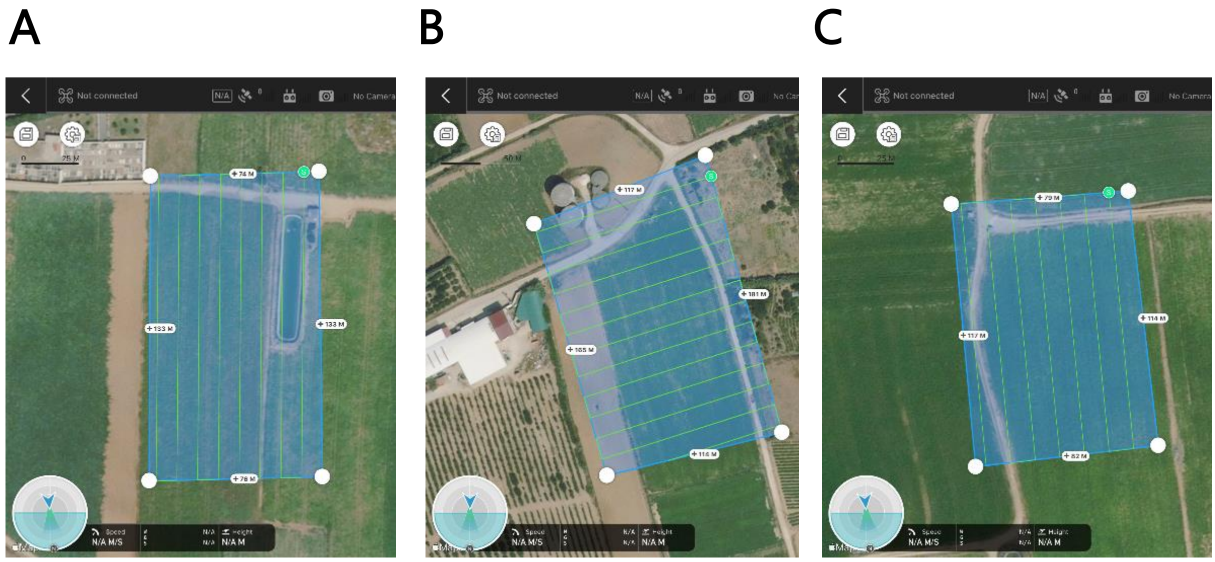


Figure 2.2 Geometry of the flights of the UAV in the three experimental fields A, B and C, respectively.

2.8 Monitoring of the photosynthetic functioning

The photosynthetic metabolism is an indicator of the ability of plants to acclimatize to certain environmental conditions, namely, periods of extreme conditions as temperature (cold or hot), water availability (drought or flooding) and mineral (deficiency or toxicity). In this context, leaf gas exchange parameters and chlorophyll a fluorescence parameters were determined during the three years of the Ca biofortification workflow. In the first year Agria varieties were used as a test system, whereas in the second and third years, the determination of leaf gas exchange parameters and chlorophyll a fluorescence parameters were carried out in the three varieties. In **Table 2.14,** the dates of monitoring of the photosynthetic functioning , considering the three varieties is present.

Table 2.14 Dates of the monitoring of the photosynthetic functioning (leaf gas exchange parameters and chlorophyll a fluorescence parameters) in the 3 years, in the three varieties.

Variety	1st year	2nd year	3rd year
Agria	2/8/2018 (only for leaf gas exchange parameters) and 14/8/2018	21/6/2019, 11/7/2019 and 24/7/2019	22/6/2020 and 30/7/2020
Picasso	-		
Rossi	-		

2.8.1 Infrared gas analysis (IRGA) at leaf level

The determination of the leaf gas exchange parameters occurred in the three years, according to Rodrigues et al. (2016), being used 4 to 6 randomized plants per treatment. Net photosynthesis rate (Pn), stomatal conductance to water vapor (gs), CO_2 internal concentration (Ci), and transpiration rate (E) were measured under photosynthetic steady-state conditions after 2 h of illumination. A portable open-system infrared gas analyzer (Li-Cor 6400, LiCor, Lincoln, NE, USA) was used under environmental conditions, with a photosynthetic photon flux density (PPFD) ranging between 1200–1400 μmol m^{-2} s^{-1}, air temperature of 33.8 ± 0.4 °C, and external CO^2 (*ca.* 400 ppm). The ratio of Pn-to-E (representing the units of assimilated CO_2 per unit of water lost through transpiration) allowed the calculation of leaf instantaneous water-use efficiency (iWUE).

2.8.2 Chlorophyll a fluorescence parameters

The determination of chlorophyll a fluorescence parameters was carried out using a PAM-2000 system (H. Walz, Germany), as previously described in Rodrigues et al. (2016), following the formulae discussed in other studies for calculations (Schreiber, 2004; Kramer et al., 2004; Krause & Jahns, 2004). Measurements were carried out in 5 independent leaves of 5 different plants per treatment. Measurements of minimal fluorescence (F_0), maximal fluorescence (F_m), and maximal photochemical efficiency of photosystem (PS), PSII (F_v/F_m), were performed on overnight dark-adapted leaves. F_0 was assessed using a weak light (<0.5 μmol m^{-2} s^{-1}) beam, while F_m was obtained using a saturation flash of ca. 7500 μmol m^{-2} s^{-1} of actinic light for 0.8 s.

Another group of parameters were determined under photosynthetic steady-state conditions (with at least 3 to 4 h of light exposure), under natural irradiance (ca. 1200–1400 μmol m^{-2} s^{-1}) and superimposed saturating flashes: F_v'/F_m', q_L, q_N, $Y_{(II)}$, $Y_{(NPQ)}$, $Y_{(NO)}$ (Schreiber, 2004; Klughammer & Schreiber, 2008; Huang et al., 2011). F_0', which was required for the quenching calculations, was measured in the dark, immediately after the actinic light was switched off and before the first fast phase of the fluorescence relaxation kinetics. F_v'/F_m' expresses the PSII photochemical efficiency of energy conversion under light exposure. q_L is the photochemical quenching based on the concept of interconnected PSII antennae, and represents the proportion of energy captured by open PSII centers and driven to photochemical events. Estimates of photosynthetic quantum yields of non-cyclic electron transfer ($Y_{(II)}$), photoprotective regulated energy dissipation of PSII ($Y_{(NPQ)}$), and non-regulated energy dissipation (heat and fluorescence) of PSII ($Y_{(NO)}$) were also calculated, where $Y_{(II)} + Y_{(NPQ)} + Y_{(NO)} = 1$.

2.9 Monitoring during biofortification process and storage conservation conditions after harvest

During the biofortification process, monitoring of different organs of *Solanum tuberosum* L. was carried out in the three varieties, as well as tubers, at harvest and after storage conservation conditions (cold chambers between 6 and 8 °C) (**Table 2.15**). The first sample collection after harvest of potatoes stored in cold chambers occurred in the first and second year in the first days of January, and in April in the last year. The second sample collection occurred in April (in the first year) and in May (of the second and third year). Yet, it should be noted that there were delays due to COVID-19.

Table 2.15 Sample collection and type of sample during the three years of experiment. Foliar applications (FA); M (months of cold conservation).

Sample collection	Type of sample	1st year	2nd year	3rd year
After 3 FA	Roots, tubers, stems and leaves	-	✓	✓
After 5 FA	Roots, tubers, stems and leaves	-	-	✓
After 6 FA	Roots, tubers, stems and leaves	-	✓	-
After 7 FA	Roots, tubers, stems and leaves	-	✓	✓
Harvest	Tubers	✓	✓	✓
3 M	Tubers	✓	-	-
4 M	Tubers	-	✓	-
6 M	Tubers	✓	-	-
7 M	Tubers	-	-	✓
8 M	Tubers	-	✓	✓

2.10 Quantification of mineral elements and Ca location

During the Ca biofortification workflow, mineral quantification was carried out by X-ray fluorescence spectrometry (XRF) and Ca location was assessed by µ-EDXRF system.

2.10.1 X-ray fluorescence spectrometry (XRF)

Quantification of minerals in soil, tubers, roots, stems, and leaves samples was carried out in four samples (n = 4) by X-ray fluorescence, using a Thermo Scientific Niton XL3t 950 He GOLDD ± XRF under He atmosphere (after dried at 60 °C, until constant weight and grounded), according to the methodology of Pelica et al. (2018). The detection limits of the method for the elements analyzed are expressed in Table **2.16**.

Table 2.16 Detection limits of some mineral elements using a Thermo Scientific Niton XL3t 950 He GOLDD ± XRF.

Mineral element	Detection limits in ppm (mg.kg-1)
As	5
Pb	4
Cu	12
Zn	6
Fe	25
Mn	30
Ca	65
K	200
Cl	75
S	90
P	450
Al	750
Mg	0.25 %

2.10.2 µ-EDXRF system - Calcium location

Through µ-EDXRF system (M4 Tornado™, Bruker, Germany), as previously described in detail for food matrixes (Cardoso et al., 2018; Mangueze et al., 2018), Ca location was analyzed in the first and third year. The X-ray generator was operated at 50 kV and 100 µA without the use of filters, to enhance the ionization of low-Z elements. For a better quantification, a set of filters between the X-ray tube and the sample, composed of three foils of Al/Ti/Cu (with a thickness of 100/50/25 µm, respectively) was used. All the measurements with filters were performed with 600 µA current. Detection of fluorescence radiation was performed by an energy-dispersive silicon drift detector, XFlash™, with 30 mm^2 sensitive area and energy resolution of 142 eV for Mn Kα. To better measure the distribution mapping of minerals, the tubers were cut at the equatorial region, into slices with a stainless-steel surgical blade (**Figure 2.3**). Measurements were carried out under 20 mbar vacuum conditions. These point spectra were acquired during 200 s. The values of the content of the mineral elements analyzed were obtained through the average of four readings taken by the device.

Figure 2.3 Sample preparation of Rossi variety of the third year and the different locations analyzed (from epidermis/skin (1) to center (5)).

2.11 Kinetic of accumulation

During the Ca biofortification workflow, monitoring of mineral accumulation in different organs of *Solanum tuberosum* L. (tubers, roots, stems, and leaves) was carried out by X-ray fluorescence spectrometry (XRF) (**section 2.10.1**), to understand the accumulation through the plant and during the life cycle. Determination of the kinetic of accumulation was carried out following Lidon & Henriques (1992, 1993). As such, the determination of the absorption rate in terms of time (by the different organs of the plant), of the mass of analyte present in the sample (in mg), the absorption rate by mineral element and the translocation rate was calculated through the equations present in **Annex V** in dry weight.

2.12 Morphological, physical, and organoleptic parameters

2.12.1 Height, diameter, dry weight, total soluble solids content, weight, and number of tubers per plant and yield

Height and diameter were measured considering 10 randomized tubers per treatment during the Ca biofortification workflow, at harvest and after storage. Dry weight was performed considering four randomized tubers per treatment (n = 4) (**Annex IV**) and was carried out during the Ca biofortification workflow, at harvest and after storage. Total soluble solids (an indication of sucrose, glucose, and fructose) were also measured in the juice of four randomized tubers per treatment (*n* = 4), using a digital refractometer Atago (Atago, Tokyo, Japan) and was carried out at harvest and after storage. During the Ca biofortification process, monitoring of weight and number of tubers per plant was carried after foliar applications in the third year. Productivity at harvest was carried out for each variety, considering 57 plants for each treatment, during the three years of the experiment.

2.12.2 Colorimetric parameters

Colorimetric analysis was performed through two different techniques: through the CieLab system and by scanning spectrophotometric.

2.12.2.1 CieLab system

The measurement of colorimetric parameters, using fixed wavelength, was carried out according to Pessoa et al. (2021). Brightness (L) and chromaticity parameters (a* and b* coordinates) were obtained with a Minolta CR 300 colorimeter (Minolta Corp., Ramsey, NJ, USA) coupled to a sample vessel (CR-A504). The system of the Commission Internationale d' Eclaire (CIE) was applied using the illuminant D_{65}. Parameter L represents the brightness of the sample, indicating the variation in the tonality between dark and light (range between 0—black and 100—white). Parameters a* and b* indicate color variations between red (+60) and green (−60), and between yellow (+60) and blue (−60), respectively. The null value approximation of these coordinates indicates neutral colors such as white, grey, and black. Chroma is the relationship between the values of a* and b*, where the real color of the analyzed object is obtained. Hue is the angle formed between a* and b*, indicating the saturation of the object's color. The equations to calculate Chroma (C) and Hue-Angle (H) are indicated in **Annex III**. Colorimetric analyses were carried out in soil samples (with and without organic matter), fresh tubers (at harvest and after storage) and during the process of heat treatment of the tuber pulp. Additionally, regarding soil samples, the colorimetric analysis was carried out in samples with organic matter and these samples had no moisture, considering that previously the analysis was 24h in a stove at 105 °C and subsequently were maintained in a desiccator for 1h until reaching room temperature.

2.12.2.2 Scanning spectrophotometric colorimeter

Colorimetric parameters were also determined with a scanning spectrophotometric colorimeter (Agrosta, European Union). The sensor provides a 40 nm full-width half-max detection, covering the visible region of the electromagnetic spectrum. This sensor has 6 phototransistors with sensibility in a specific region of the spectrum (380 nm—violet; 450 nm—blue; 500 nm—green; 570 nm—yellow; 600 nm—orange; 670 nm—red). Light was furnished by a white light-emitting diode (LED) covering all the visible region. Colorimetric analyses were carried out in fresh tubers at harvest in quadruplicate.

2.12.3 Fatty acid content

Quantitative and qualitative analyzes of fatty acids in tubers (at harvest and after storage) was performed in 5 peeled tubers (*ca.* 5 g of fresh weight) per sample, according to Daccak et al. (2022). The fatty acids composition was determined following the methodology of Vidigal et al. (2018), by direct acidic transesterification using a methanol:sulfuric acid solution (39:1, v:v), after addition of an internal standard (heptadecanoic acid). Additionally, the lipid unsaturation index (DBI – 'double bond index') was calculated according to Mazliak (1983).

2.12.4 Sugar content

Sugar extraction was performed in 40 g of tubers without skin per sample (n=4) at harvest and after storage, according to Medlicott & Thompson (1985), with minor modifications according to Daccak et al. (2022), in an HPLC (Waters, USA) system.

2.12.5 Protein content

Determinations of soluble protein content of tubers, at harvest and after storage, was carried out on composite samples (*ca.* 5 g fresh weight) from 5 peeled tubers, being 4 samples prepared per treatment, which were frozen at -20 ^{o}C. Proteins were extracted and quantified by the biuret method, according to Gornall et al. (1949). These analyzes were carried out on the tubers at harvest and after different periods of cold storage.

2.12.6 Starch content

Starch content in tubers at harvest and after storage in the third year of experiment was carried out in quadruplicate, according to Nielson (1943), with minor alterations. In each sample, 1 g of fresh and peeled tubers was mixed with 5 mL of distilled water and 5 mL of I + KI solution. After mixed in a vortex for 5 minutes, the absorbance of the reaction system was measured at 620 nm, and starch content was expressed in units of mg/g $_{FW}$.

2.12.7 Heat treatment of tuber pulp

Each sample was washed, peeled, rinsed, and cut into 3 x 3 cm cubes. For each replica, 11 cubes were made as shown in the **Figure 2.4.**

1 0 min	2 0 min	3 5 min	4 7.5 min	5 10 min	6 12.5 min
7 15 min	8 17.5 min	9 20 min	10 20 min	11 T	

Figure 2.4 Graphical example of the different cubes and temperatures used in the heat treatment of tuber pulp.

Nine of the cubes were placed in a pot of boiling water (100 °C) (**Figure 2.5**). After 5 minutes, the first cube was taken out, placed in cold water for 1 minutes and then allowed to cool to room temperature. From that moment on, every 2.5 minutes, a cube was removed and handled in the same way. Two cubes out of this set - one uncooked (1) and one after 20 minutes of cooking (2) - were weighed, placed inside the plastic jars and frozen at -80 °C. The last sample (11) was used to measure the temperature at the geometric center of the cube, with a Fluke precision thermocouple (±1 °C). The color and texture were analyzed for each sample.
The method used to determine the temperature profile of the potatoes consisted of measuring the temperature during the heat treatment of each sample. During the measurement the Fluke thermocouple was placed at the geometric center of the sample cube and values were collected every 2.5 min. in a total processing time of 20 min.

A B C

Figure 2.5 Analytical procedure. A- sample section; B - controlled cooking with Fluke thermocouple precision ±1 °C and C- color determination with CieLab system.

In the second- and third-year, treatments with $CaCl_2$ and Ca-EDTA were analyzed considering the binomial 20 minutes/100 °C, corresponding to the samples being boiled at 100 °C for 20 minutes.

2.12.7.1 Color analysis

Colorimetric analysis was carried out as Indicated in **section 2.11.2.1,** during the process of heat treatment of the tuber pulp, with a Minolta CR-300 JAPAN previously calibrated according with the pre-stablish standards.

2.12.7.2 Texture

Texture was measured with a texturometer (Stable Micro System TAHDi, USA). For each sample the texture was measured with a punch test (three repetitions), using a 3 mm probe up to a 15 mm of distance. The load cell force used was 5 Kg, the test was carried out at a speed of 1 mm/s. The analysis was performed at 23°C and each sample were analyzed in quintuplicate. From the texture profiles (texturogram), the following parameters were determined: fractability (N), which represents the resistance exerted by the surface of the food to penetration and is registered by the force registered in the first peak; the work of the force (Nxs) that represents the energy exerted by the probe in the penetration and is determined by the positive area of the texturogram; the number of peaks (dimensionless quantity), which is an indirect measure of crispness); the hardness (N), is determined by the maximum strength of the texture, being a measure of the firmness of the material; and the adhesiveness (-N*s) which is the work required to overcome the forces of attraction between the material and the surface of the probe. It is obtained by the value of the area corresponding to the negative strength of the texturegram. In **Figure 2.6** is shown an example of texturograms.

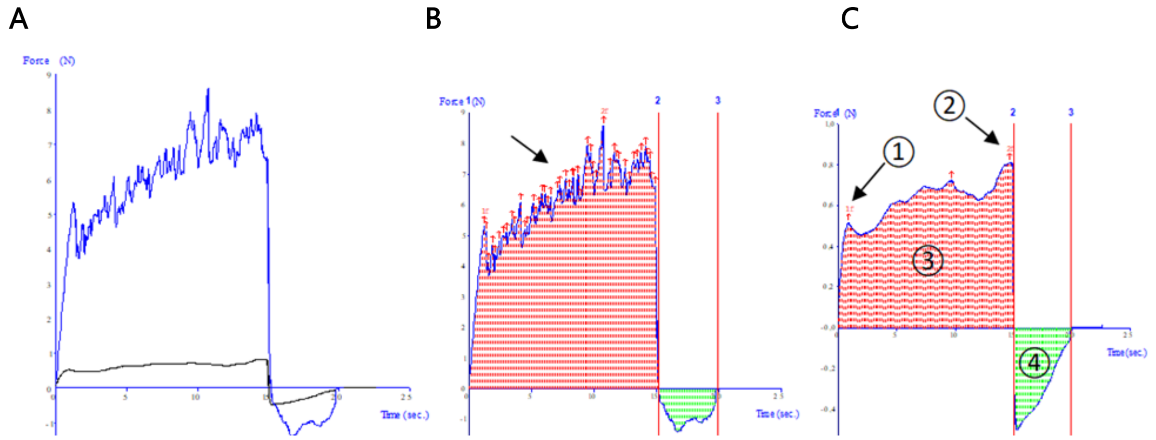


Figure 2.6 Texturograms examples. A- difference in profile between a sample of raw potato (in blue) and cooked potato (in black); B- example of counting the number of peaks and C- Determination of fracturability (①), Hardness (②), penetration work (③) and adhesiveness (④).

The softening (%) of the potato tubers after cooking was reported based on the texture profile analysis of maximum force (N). As such, softening was calculated as described by Chiavaro et al. (2006), in the following equation:

$$\text{Softening (\%)} = 1 - \frac{\textit{Shear force of cooked sample}}{\textit{Shear force of raw sample}} \times 100$$

2.12.7.3 Sensory analysis

The sensory analysis was carried out in the second and third year, in the Technology and Innovation Unit of the INIAV, according to NP 4258:1993 (ISO 8589:1988). In the second samples of each variety of control and 24 kg ha^{-1} $CaCl_2$ or Ca-EDTA were presented to 18 semi-trained adult tasters. In the third year, samples of each variety of control, 12 kg ha^{-1} of $CaCl_2$ and Ca-EDTA were presented to 12 semi-trained adult tasters (66 % women and 33 % men). Each taster subjectively evaluated potato cubes, of the same size and cooked under the same temperature (100 °C) and time conditions (20 minutes). In **Figure 2.7** , the samples of cocked potatoes prepared for sensory analysis are shown.

A B C

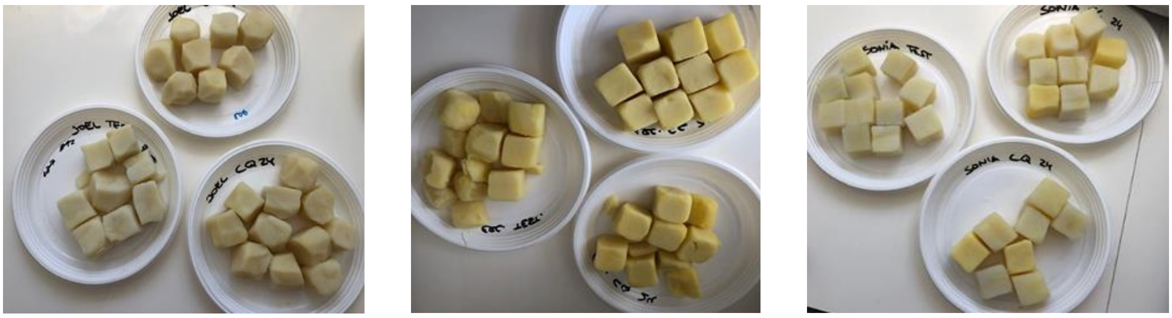


Figure 2.7 Samples for the sensory analysis of the third year. A- Agria, B – Picasso and C- Rossi variety.

The tasters expressed their opinion regarding the attributes of appearance, pulp color, consistency, flavor, and general appreciation based on a 5-point hedonic scale:

(5) - I liked it a lot;

(4) - I liked it;

(3) - I didn’t like it/Neither it disliked it;

(2) - Disliked;

(1)- Disliked a lot.

The samples (samples treated with different treatments and samples without - control samples) arranged in properly coded disposable plates, were provided to the panelists in individual booths.

2.13 Production of starch and dehydrated mashed potatoes

In the third year, the production of starch and dehydrated mashed potatoes were carried out in all treatments (control included) of the three varieties with “harvest potatoes” frozen at -20 ^{o}C.

For starch, 1 kg of sample (washed, cut, and peeled potatoes) was grounded with 1 L of water, and therefore the preparation was filtered and rested for 1h in a glass. The liquid was decanted and the white substance (starch) that remained at the bottom of the glass was dried at 40 – 50 ^{o}C for about 8h (**Figure 2.8**).

Figure 2.8 Different stages of potato starch production.

For the dehydrated mashed potatoes (**Figure 2.9**), 1 kg of sample (washed, cut, and peeled potatoes) was cooked for 25-30 minutes. After being cooked, the potatoes were drained and placed to crush together with about 50 mL of water. The mixture was evenly placed on glass plates and placed to dry at 50 – 57 °C with ventilation. After the mixture was completely dry (about 12h) it was grounded.

Figure 2.9 Different stages of dehydrated mashed potatoes.

2.13.1 Incoporation of food additives

Food additives are natural or synthetic substances that are added to food to increase its durability (increasing the shelf life of the product), intensify or modify its properties, as long as they do not negatively interfere with its nutritional value (Silva & Lidon, 2016a; Lidon & Silvestre, 2007). Its use is regulated by European Union (EU) laws, considering the foods in which it can be applied, the maximum amounts allowed, its chemical characterization and purity (Silva & Lidon, 2016a). Potato starch and mashed potatoes are two of the products obtained by processing potatoes, intended for human consumption (Lidon & Silvestre, 2007), in which food additives are incorporated in their processing. In the case of starch and mashed potatoes, both of which are dehydrated processed products, few food additives can be used (**Annex VI**). As such, in the third year was carried out the incorporation of food additives (E223 - Sodium metabisulfite – $Na_2S_2O_5$, and E320 - Butil-Hidroxianisolo, BHA) after both processed potato products were dried, in the maximum concentration allowed by law (**Annex VI**). E223 inhibits the development of yeasts, fungi, and bacteria (Silva & Lidon, 2016b) and E320 is an artificial antioxidant, insoluble in water (Silva & Lidon, 2016a), and its addition is only possible at the final stage of processing. This additive is widely used in foods to minimize oxidation rates by reacting with free radicals and thus reducing the rate of auto-oxidation (Silva & Lidon, 2016a). To assess if microorganisms occurred in the different samples of both processed potato products, samples were visualized, after 41 days and 82 days (the samples were hermetically sealed and at a temperature of between 22-25 ºC), through the microscope's software and the imaging system Leica M165 C – (Leica Microsystems).

2.14 Statistical analysis

Data were statistically analyzed using one-way or two-way ANOVA ($p \leq 0.05$) through IBM SPSS software and R (R Project for Statistical Computing). Using ANOVA statistics through IBM SPSS, to assess differences between treatments among different fields, and based on the results, a Tukey's test for mean comparison was performed, considering a 95 % confidence level. Moreover, Pearson and Spearman correlation coefficients were calculated to evaluate the strength of linear relationships between mineral elements concentration.

Data normality and homogeneity of variance were carried out. The principal component analysis (PCA) was performed on the correlation matrix and the first two components were retained and rotated using varimax rotation.

3

RESULTS

3.1 First year

During the first year, the workflow of Ca biofortification was implemented with Agria, Rossi and, Picasso varieties in field A, B and C, respectively. Four foliar applications were conducted after the beginning of tuberization, applying four concentrations of $CaCl_2$ (1,3,6, and 12 $kg.ha^{-1}$) or $Ca(NO_3)_2$ (0.5, 1, 2, and 4 $kg.ha^{-1}$). The selection of $CaCl_2$ and $Ca(NO_3)_2$ was made based on extensive research encompassing both types of Ca. Specifically, the choice of $Ca(NO_3)_2$ was based on El-Hadidi et al. (2017), who observed an increase in tuber yield in plants, and by Hamdi et al. (2015), who verified an increase in Ca content in tubers. Nevertheless, prior studies performed with $CaCl_2$ and $Ca(NO_3)_2$, have demonstrated an increase in Ca content in tubers and an enhancement in tubers yield (Seifu & Deneke, 2017; Ozgen et al., 2006). However, none of these studies were carried out in Portugal and there has never been a biofortification study undertaken with Agria, Rossi, and Picasso varieties. The selected concentrations and foliar applications were determined to test the plant response in this initial approach of Ca biofortification.

3.1.1 Soil

The physical and chemical parameters of soils across of the three experimental field are detailed in **Table 3.1.** The pH within these experimental fields ranged from 7.3 to 7.41, revealing a slightly alkaline nature (**Table 3.1**). Field A exhibited a significantly lowest EC (205 $\mu S.cm^{-1}$, corresponding to 0.205 $dS.m^{-1}$), and field B and C showed a similar EC of 332 and 349 $\mu S.cm^{-1}$, respectively (**Table 3.1**) (corresponding to 0.332 and 0.349 $dS.m^{-1}$, respectively).

Table 3.1 Physical and chemical parameters of the soil (*n = 9*) of the experimental fields selected for Ca biofortification of Agria, Picasso and Rossi varieties, respectively. Electrical conductivity (EC); Organic matter (OM).

Fiel	pH	EC	OM	Ca	K	Mg	P	Fe	S	Zn	Mn	Pb	As
		$\mu S.cm^{-1}$	%						mg.kg^{-1}				
A	7.41 ± 0.03a	205 ± 17.5b	1.88 ± 0.12c	0.39 ± 0.03b	2.20 ± 0.03b	0.15 ± 0.01b	0.23 ± 0.01b	1.19 ± 0.07b	55.9 ± 4.8b	19.6 ± 1.5c	318 ± 27b	11.0 ± 0.57c	12.5± 0.29a
B	7.30 ± 0.06a	332 ± 13.7a	4.13 ± 0.14b	0.71 ± 0.07a	2.64 ± 0.02a	0.24 ± 0.01a	0.19 ± 0.01c	0.50 ± 0.07c	66.6 ± 1.2b	41.7 ± 1.6b	270 ± 31b	13.7 ± 0.75b	13.1 ± 0.09a
C	7.40 ± 0.05a	349 ± 24.2a	4.59 ± 0.10a	0.65 ± 0.03a	2.23 ± 0.07b	0.19 ± 0.02b	0.29 ± 0.01a	2.59 ± 0.06a	77.6 ± 1.1a	62.7 ± 2.8a	703 ± 57a	19.7 ± 0.32a	13.0± 0.15a

Means in the same column, not followed by a common letter, are significantly different ($p \leq 0.05$)

Our data revealed significant differences between the three experimental fields in terms of all analyzed mineral elements, with the exception of As (**Table 3.1**). Within this context, P content in the soil ranged from 0.19 to 0.29 % in the three experimental fields. Concerning K, values oscillated between 2.20 - 2.64 % in all the three fields. Iron was the second most abundant element in the soils, with field C showing a significantly higher content (2.59 %), followed by field A (1.19 %) and with a significantly lower content in field B (0.50 %). Upon analyzing Ca data from the three experimental fields (0.39 – 0.71 %) it was observed that Ca content in field B and C was significantly higher than in field A. Magnesium displayed a significantly higher content in field B compared to the other fields, varying between 0.15 - 0.24 %. Moreover, S content ranged from 55.9 to 77.6 mg.kg^{-1}, with field C exhibiting a significantly higher S content relatively to fields A and B. Zinc and Mn contents exhibited significant differences across the experimental fields, varying between 19.6 – 62.7 mg.kg^{-1} and 270 – 703 mg.kg^{-1}, respectively. Additionally, Mn was identified as the third most abundant mineral element in the soils, following Fe and Mg. Nevertheless, field C demonstrated a significantly higher content of Pb and As, followed by field B and A.

To facilitate the interpretation of data, bivariate and multivariate statistics was carried out. In this framework, the correlations between the macro and microelements were carried out according to the Pearson and Spearman indexes (**Table 3.2 - Table 3.4**) and following the Hierarchical Ascending Sort (**Figure 3.1**), as well as the Principal Component Analysis (PCA) (**Figure 3.2**) methods to the mineral elements of the soil samples and to the mineral elements, pH, EC, MO of soil samples of the three fields, respectively. Regarding the correlation between minerals in the soil of field A, a negative correlation among them could not be found (**Table 3.2**). According to Pearson (r), Fe and Zn, S and Zn, and S and Fe showed a very strong correlation (r >0.90) and As and Pb, Zn and As, Fe and As, Ca and Zn, Ca and Fe, K and Pb, K and As, K and Z, K and Fe, S and As, S and Ca, S and K, P and As, P and Zn, P and Fe, P and Ca, P and K and P and S, Mg and As, and Mg and Zn, showed a strong correlation (r ranging between 0.70 and 0.89).

Moreover, K and Mn showed a very weak correlation (r ranging between 0 and 0.19). According to Spearman (ρ), Zn and K, Zn and S and Fe and K, showed a very strong correlation, Pb and As, Pb and Fe, Pb and K, As and Fe, Zn and Fe, Zn and Ca, Zn and P, Fe and Ca, Fe and S, Ca and K, Ca and S, Ca and P, K and S, K and P and S and P, showed a strong correlation, and only Pb and Mn showed a very weak correlation.

Table 3.2 Correlation matrix indices of minerals of soil samples from field A. Pearson index (blue) and Spearman (green).

	Pb	As	Zn	Fe	Mn	Ca	K	S	P	Mg
Pb	1.000	0.749	0.679	0.722	0.227*	0.538*	0.796	0.584*	0.592*	0.521*
As	0.807	1.000	0.820	0.801	0.219	0.621	0.877	0.853	0.776	0.769
Zn	0.653	0.456	1.000	0.927	0.630	0.891	0.826	0.933	0.880	0.743
Fe	0.883	0.731	0.862	1.000	0.541*	0.821	0.797	0.934	0.807	0.673
Mn	0.167*	0.084	0.594	0.400	1.000	0.648	0.169	0.553	0.545	0.501*
Ca	0.600	0.353*	0.879	0.767	0.417*	1.000	0.682	0.768	0.859	0.699
K	0.850	0.529*	0.904	0.900	0.417*	0.767	1.000	0.741	0.774	0.676
S	0.617	0.513*	0.912	0.833	0.400*	0.833	0.800	1.000	0.855	0.670
P	0.683	0.479*	0.762	0.667	0.467*	0.700	0.800	0.733*	1.000	0.648*
Mg	0.633*	0.639*	0.577*	0.667	0.383*	0.517*	0.583*	0.333*	0.417*	1.00

*Correlation isn't significant at the 0.05 level ($p \leq 0.05$)

Regarding the correlation between minerals of soil samples from field B, a negative correlation was found in both correlation indexes (**Table 3.3**). According to Pearson (r), only Ca and Mn showed a very strong correlation (r >0.90), Mn and As, and Ca and As showed a strong correlation (r varying between 0.70 and 0.89) and Fe and Pb, Mn and Pb, Mn and Zn, K and Pb, K and Zn, K and Fe, S and Pb, S and Zn, S and Ca, S and K, P and Mn, P and S, Mn and Pb, Mg and S, showed a negative correlation. According to Spearman (ρ), only K and P, and Ca and Mg showed a strong correlation between each other, however Pb and Fe, Pb and Mn, Pb and S, Pb and Mg, Zn and Mn, Zn and S, Fe and K, Fe and P, Mn and P, Ca and S, K and S, S and P, S and Mg, and P and Mg were correlated negatively.

Table 3.3 Correlation matrix indices of macro and microelements of soil samples from field B. Pearson index (blue) and Spearman (green).

	Pb	As	Zn	Fe	Mn	Ca	K	S	P	Mg
Pb	1.000	0.229	0.618	-0.377	-0.093	0.038	-0.276	-0.529	0.610	-0.222
As	0.152	1.000	0.405	0.175	0.755**	0.729	0.085	0.163	0.357	0.330
Zn	0.580	0.422	1.000	0.170	-0.104	0.194	-0.010	-0.265	0.510	0.124
Fe	-0.167	0.151	0.251	1.000	0.217	0.321	-0.109	0.571	-0.644	0.570
Mn	-0.192	0.605	-0.276	0.167	1.000	0.854**	0.199	0.148	-0.038	0.528
Ca	0.226	0.471	0.343	0.283	0.200	1.000	0.338	-0.055	0.036	0.696**
K	0.310	0.496	0.075	-0.167	0.350	0.667	1.000	-0.508	0.346	0.642
S	-0.569	0.025	-0.251	0.500	0.167	-0.483	-0.583	1.000	-0.663	-0.135
P	0.360	0.294	0.209	-0.700**	-0.017	0.283	0.700**	-0.767**	1.000	-0.133
Mg	-0.184	0.118	0.000	0.650	0.267	0.750**	0.300	-0.100	-0.267	1.000

**Correlation is significant at the 0.05 level ($p \leq 0.05$)

Regarding the correlation among minerals in soil samples from field C, a negative correlation was also found in both correlation indexes (**Table 3.4**). According to Pearson (r), Fe and Zn showed a very strong correlation (r >0.90), Fe and As, Ca and As, Ca and Fe, K and As, K and Fe, Mg and Pb, Mg and As, Mg and Zn, Mg and Fe, and Mg and K showed a strong correlation (r varying between 0.70 and 0.89), Mn and As, Mn and Fe, Ca and Mn, K and Mn, S and Pb, S and As, S and Fe, S and Mn, P with the remaining mineral elements, and Mg and P showed a negative correlation. According to Spearman (ρ), there was only a very strong correlation between K and Mg, the pairs with strong correlation were As and Fe, As and Ca, As and K, As and Mg, Zn and Fe, Zn and K, Zn and Mg, Fe and Ca, Fe and K, and Fe and Mg. However, there were mineral elements which correlate negatively: Pb and S, Pb and P, As and Mn, As and S, As and P, Zn and P, Fe and Mn, Fe and S, Fe and P, Mn and Ca, Mn and P, Ca and S, Ca and P, K and P, S and P, and P and Mg.

Table 3.4 Correlation matrix indices of minerals of soil samples from field C. Pearson index (blue) and Spearman (green).

	Pb	As	Zn	Fe	Mn	Ca	K	S	P	Mg
Pb	1.000	0.568	0.516	0.466	0.613	0.123	0.412	-0.035	-0.301	0.734**
As	0.567	1.000	0.677**	0.776**	-0.053	0.734**	0.725**	-0.053	-0.371	0.882**
Zn	0.477	0.586	1.000	0.910**	0.148	0.645	0.687**	0.165	-0.485	0.786**
Fe	0.536	0.887**	0.833**	1.000	-0.073	0.792**	0.706**	-0.075	-0.540	0.851**
Mn	0.393	-0.184	0.167	-0.100	1.000	-0.493	-0.125	0.526	-0.241	0.189
Ca	0.134	0.778**	0.633	0.800**	-0.400	1.000	0.669**	-0.214	-0.446	0.676**
K	0.561	0.795**	0.767**	0.833**	0.100	0.567	1.000	0.181	-0.498	0.784**
S	-0.310	-0.234	0.117	-0.217	0.433	-0.183	0.200	1.000	-0.433	0.079
P	-0.184	-0.410	-0.567	-0.367	-0.150	-0.517	-0.633	-0.567	1.000	-0.683**
Mg	0.678**	0.879**	0.767**	0.850**	0.083	0.683**	0.917**	0.017	-0.667**	1.000

**Correlation is significant at the 0.05 level ($p \leq 0.05$)

Figure 3.1 illustrates the Hierarchical Ascending Sort through a graphical representation know as a "dendogram", resembling a "tree" in which the representation resists in sequences of object associations or differentiations based on similar values. The numbers between 1 to 9, 10 to 18, 19 to 27, corresponds to soil samples of fields A, B and C, respectively. As such, the dendogram effectively categorizes the soil samples based on their geomorphological characteristics, revealing distinctive traits in each of the three fields. Notably, soil samples between 1 to 9 (Field A) are more heterogeneous compared to fields B and C. Field C, in particular, stands out for its homogeneity and distinctiveness, contrasting with the other fields.

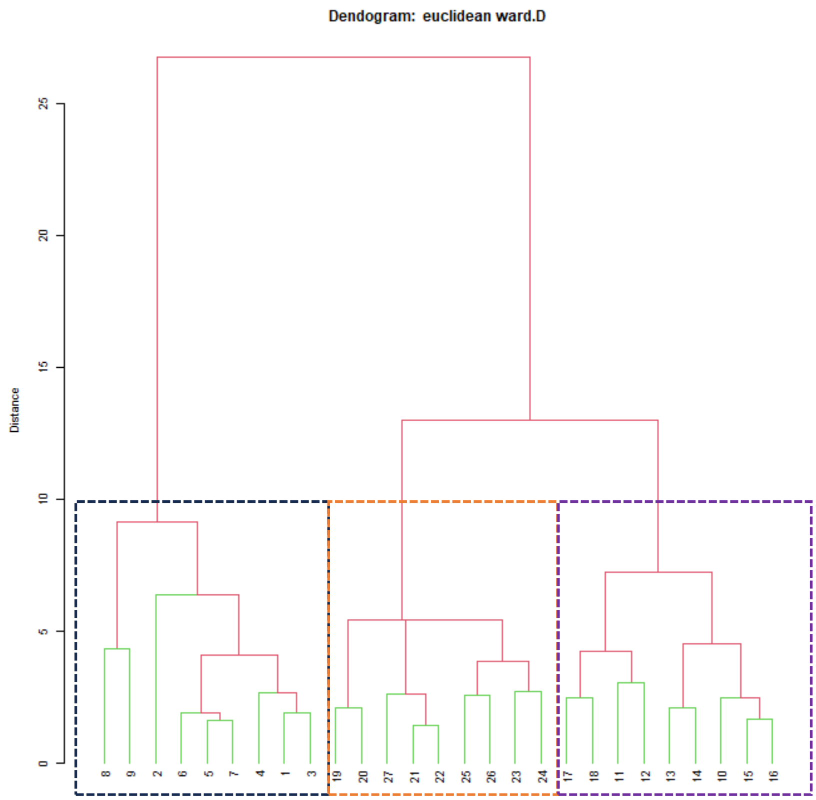


Figure 3.1 Dendogram (obtained with samples of soil from the experimental fields (A - blue lines, B - purple lines and C - orange lines)), using the Euclidean distance according, using the grouping method Ward.D2.

Figure 3.2 and **3.3** showed the contribution of the variables of the principal components and the projection of the variables (mineral elements and pH, EC, and MO), in the factorial plane considering the three experimental fields (A, B and C), defined by Factor 1 (F1, component 1 or Dim1) and Factor 2 (F2, component 2 or Dim2) axes, which explain 72.5 % of the initial data variability. Considering the contribution of the variables of the principal components, OM, EC, Pb, As, Zn, Fe, Mn, Ca, S, and Mg showed a greater contribution in Factor 1. Variables as P and pH revealed a greater contribution in Factor 2 and K in Factor 5. Considering F1 of the projection in the factorial plane (**Figure 3.3**), variables are projected on the positive semi-axis (except for pH) and in F2 variables are divided between the positive (pH, P, Mn, Pb, S, EC, and Zn) and the negative semi-axis (OM, Fe, Ca, As, Mg and K). The proximity between variables defined the similarity between them. In fact, there was a greater similarity when the distance between their projections is smaller. Accordingly, Ca and As, or S, Zn, and EC, showed a great affinity due to their position in F1 with a higher coordinate. Soil from field A was poorer in OM than the other two soils and had lower levels of contaminating elements such as heavy metals and P and S (**Table 3.1**) Soil from field B was more clayey (thus, with more K and Mg) and soil from field C had more Zn, Pb, S and P (**Table 3.1**).

Additionally, there were three groups in both components (Dim1 and Dim2). The first group was composed by samples from field A, in which the individuals are projected in F1 on the negative semi-axis and in F2 on the positive semi-axis (except for soil sample number 1). The second group was from field B, in which the individuals are projected in F1 on the positive semi-axis (except for soil samples number 10 and 18) and in F2 on the negative semi-axis. The third group was from field C, in which the individuals are projected in F1 and F2 on the positive semi-axis.

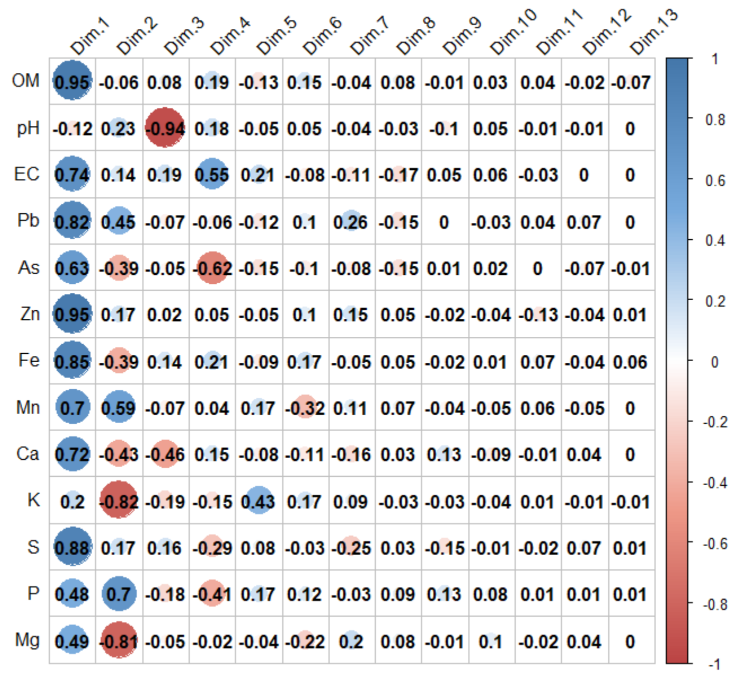


Figure 3.2 Contribution of the variables of the principal components of mineral and pH, EC, and MO of soil samples of the three fields (A, B and C).

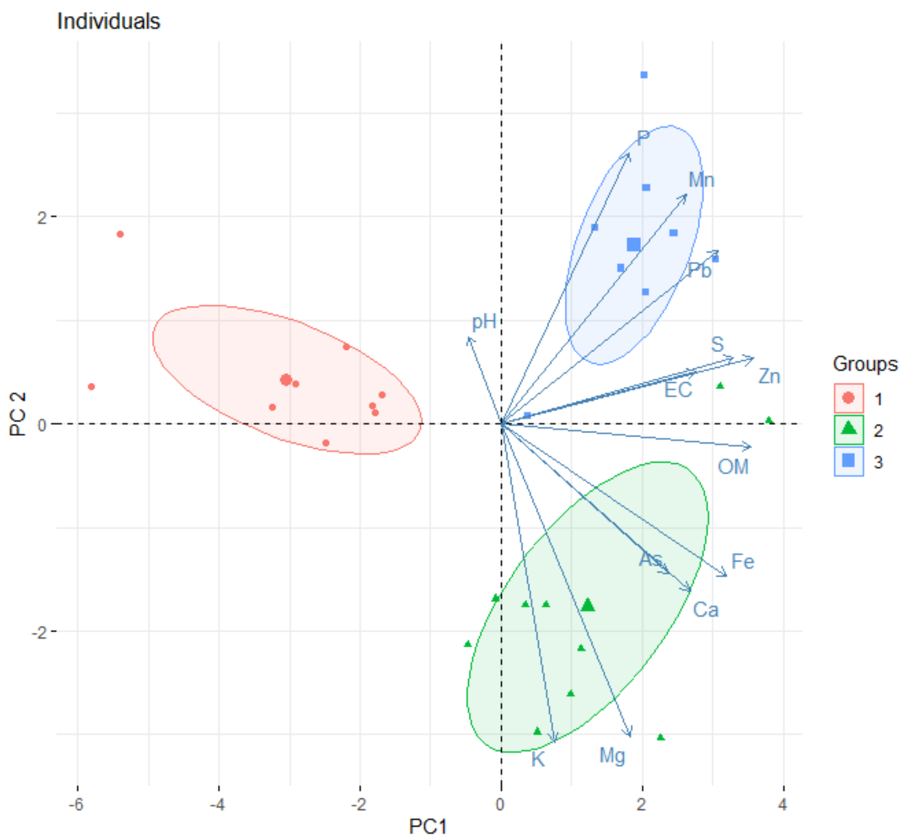


Figure 3.3 Projection of the factorial plane of individuals created with component 1 (or F1 or Dim1) (49.6 % variance) and com-ponent 2 (or F2 or Dim2) (22.9 % variance) axes of the mineral elements and pH, EC, and MO of soil samples of the three Fields (A, B and C)

Considering the various statistical analysis conducted for the three experimental fields - bivariate statistical analysis (correlation of Pearson and Spearman) and multivariate statistical analysis (Hierarchical Ascending Sort and Principal Component Analysis (PCA)) – distinct correlations among minerals were identified. This divergence is due from variations in mineral contents and soil types across the three fields.

The colorimetric assessment of soil parameters, both with and without organic matter (OM), were carried out using the CieLab system (L – brightness, a* - red (+) and green (-) and b* - yellow (+) and blue (-)) across samples from the three experimental fields (**Table 3.5**). Consequently, both sample types (with OM and without OM) showed dissimilar values for each parameter within each field. In contrast, soil samples with OM revealed significant differences between each field and in each parameter (L, a* and b*) and, in soil samples without OM, only the a* parameter did not show significant differences between the three fields. A comparative analysis between the two soil types revealed an overall increase in all the parameters for samples without OM, evidencing a brighter color, as well as more red and yellow, compared to the samples with OM.

Table 3.5 Colorimetric parameters of soils, with organic matter (OM) and without organic matter, through CieLab system of each field. Mean values (*n = 4*) ± SE (standard error).

Field	With OM			Without OM		
	L	a*	b*	L	a*	b*
A	38.9 ± 1.29b	3.8 ± 0.07a	13.2 ± 0.26b	49.1 ± 0.46a	15.9 ± 0.24a	32.4 ± 0.37c
B	43.0 ± 0.37a	2.6 ± 0.16b	14.7 ± 0.27a	48.4 ± 0.30a	16.8 ± 0.30a	34.9 ± 0.34b
C	40.4 ± 0.36b	2.7 ± 0.06b	13.7 ± 0.17b	49.8 ± 0.62a	16.6 ± 0.20a	36.6 ± 0.32a

Different letters indicate significant different between fields in each parameter ($p \leq 0.05$).

3.1.2 Geological and geomorphological characterization

The experimental fields are situated in the "Planalto das Cesaredas", located in the terminal zone positioned at the extreme south to southeast. From a geological perspective, these fields are divided into two different sheets of the Geological Map of Portugal. Field A falls within sheet 30A (Lourinhã) (Manupella et al., 1999), while fields B and C are positioned in sheet 30B (Bombarral) (Zbyszewski et al., 1966). Field A, occupies the J^3Ca unit (Castelhanos marls and sandstones – Kimmeridgian), being predominantly composed by fine to coarse quartz sandstones with frequent calcareous clays (Manupella et al., 1999). Moreover, fields B and C are situated on J^3C (Abadia beds – Kimmeridgian), being constituted by marls and thin intercalations of marly limestones, with ferruginous and limonitic concretions (Zbyszewski et al., 1966). Specifically, field A is composed of fine clays leached from the highest part of the ground, a result of significant surface runoff, and exhibits a strong furrow in the ground (indicated by the red arrow represented in **Figure 3.4**).

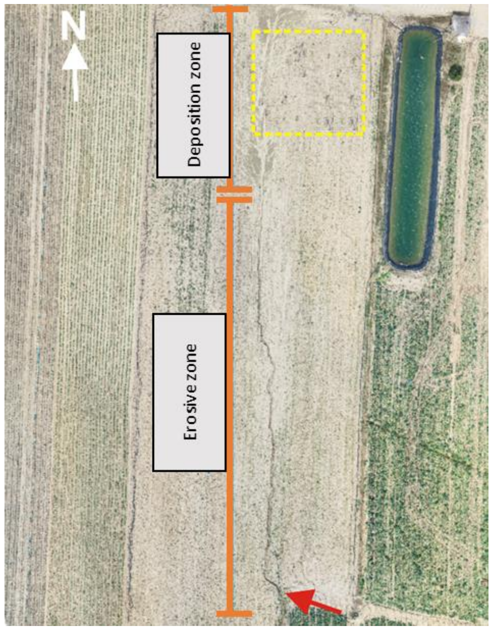


Figure 3.4 Field A (in yellow) and the identification of the deposition zone and erosive zone. Image obtained by UAV in 2018 before the implementation of the culture.

3.1.3 Irrigation water

The assessment of the physical and chemical composition of irrigation water is crucial, considering its potential impact on soil (waterproofing and/or alkalization) and on crops (toxicity), as well as the equipment used in irrigation (incrustations and corrosion). Accordingly, the classification of the irrigation water of the three experimental fields was carried out, being detailed in **Table 3.6**.

Table 3.6 Physical and chemical parameters of irrigation water of the experimental fields selected for Ca biofortification of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, respectively. Electrical conductivity (EC) at 20 ºC.

Field	pH	EC	Ca^{2+}	K^+	Mg^{2+}	Na^+	Cl^-	HCO_3^-	SO_4^{2-}	NO_3^-	PO_4^{3-}
		$\mu S\ cm^{-1}$	mg L^{-1} (meq L^{-1})								
A	7.2	1322	156.4 (7.8)	2.1 (0.05)	22.5 (1.8)	22.8 (1.4)	56.6 (1.6)	297.6 (4.8)	166 (3.4)	90.3 (1.4)	<1.5 (< 0.04)
B	6.9	1381	169.6 (8.4)	2.2 (0.06)	20.6 (1.7)	41.2 (1.7)	69 (1.9)	330.6 (5.4)	234 (4.8)	26.7 (0.4)	< 1.5 (<0.04)
C	6.9	1340	119.2 (5.9)	4.6 (0.1)	37.1 (3.0)	56.3 (2.4)	89 (2.5)	374.5 (6.1)	164 (3.4)	1.2 (0.01)	< 1.5 (< 0.04)

Water classification can be effectively established through the Piper Diagram (**Figure 3.5**; **Annex VII**). This diagram shows the projection of the anions and cations of the different samples, where the proximity of the samples in the diagram indicates their relational proximity. In this context, it was found that irrigation water is from underground origin in the three fields and the hydrochemical facies are characterized by calcium bicarbonate.

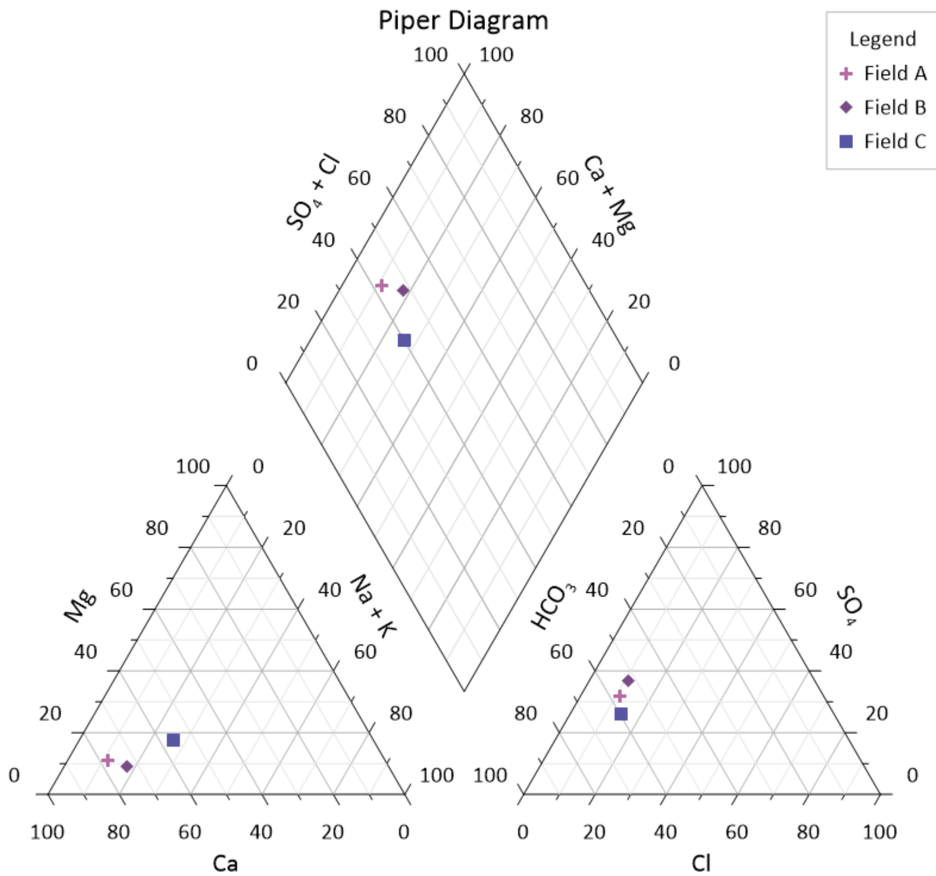


Figure 3.5 Piper Diagram of the three experimental fields (A, B and C).

The three fields showed a high salinity in terms of EC (between 750 and 2250 µS.cm^{-1} at 20 ºC) belonging to class C3S1 (**Figure 3.6**; **Annex VII**), considering the SAR index (**Table 3.7**).

Table 3.7 SAR index, pHe and LSI of the irrigation water of the experimental fields selected for Ca biofortification of Agria, Picasso and Rossi varieties, respectively. Sodium Adsorption Ration (SAR); Saturation pH (pHs); Langelier Saturation Index (LSI).

Field	SAR	pHs	LSI
A	0.64	7.3	-0.41
B	0.75	7.4	-0.18
C	1.19	7.4	-0.5

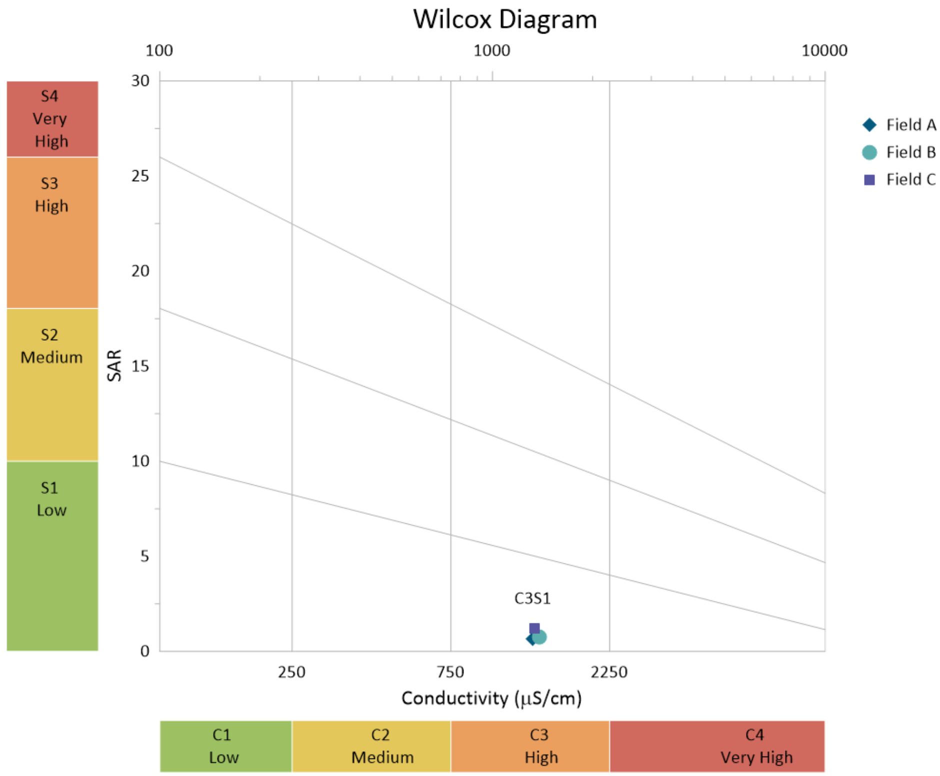


Figure 3.6 Wilcox Diagram of the three experimental fields (A, B and C).

3.1.4 Remote detection

Remote detection was performed by UAVs in the three experimental fields to assess the initial land state of the fields, as their geomorphology strongly affects water surface drainage. Consequently, by processing the images collected before the implementation of the culture it was possible to calculate the slopes of each plot, the drainage or surface drainage zones, and the NDVI (Normalized Difference Vegetation Index), as represented in **Figure 3.7**.

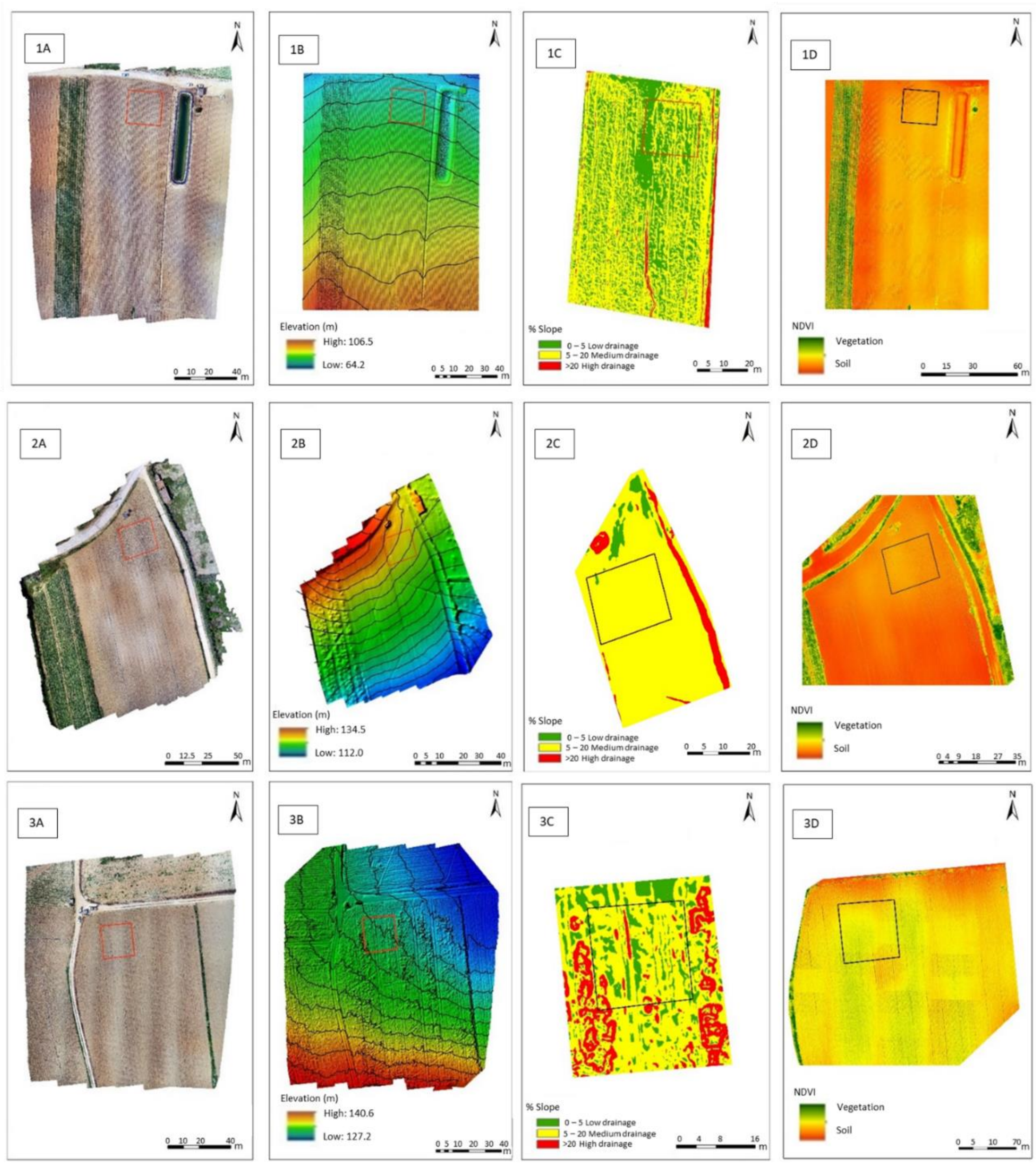


Figure 3.7 Orthophotomaps of the three experimental fields (1-A, 2-B and 3- C). Indication (in red) of limits of the three fields (1A, 2A and 3A); Digital elevation model of the fields (1B, 2B and 3B); Digital map of slopes of the fields (1C, 2C and 3C); NDVI model of the fields (1D, 2D and 3D). Information collected before tubers harvesting and biofortification treatments (18 May for the three fields).

The NDVI index of the three experimental fields (**Figure 3.7**), confirmed the anticipated observation that the soils across all three fields lacked vegetation, represented by yellow and orange colors, except for the green areas, where the presented vegetation did not match the crops in this study. Regarding **Table 3.8**, field A demonstrated a greater aptitude/propensity for accumulation of surface water, and/or infiltration, with an approximately 54 % of the total area of the field with flat or smooth slope morphology. This condition was previously highlighted in **section 3.1.2.** (**Figure 3.4**). Conversely, field C exhibited a lower percentage of area with low drainage capabilities (about 21 %). About 99.5 % of field B revealed moderate drainage capabilities, thus with poor conditions for accumulation and/or water infiltration, promoting runoff.

Table 3.8 Slope class of the fields and the ability to accumulate or drain surface water of the three experimental fields.

Field	Slop Class (%)	Surface Drainage	Area (m^2)	Area (%)
A	1– [0–5%]	Low	238.0	53.7
	2– [5–20%]	Moderate	212.9	47.2
	3– >20%	High	0.5	0.1
B	1– [0–5%]	Low	2.5	0.5
	2– [5–20%]	Moderate	475.4	99.5
	3– >20%	High	0.1	-
C	1– [0–5%]	Low	76.8	21.1
	2– [5–20%]	Moderate	265.5	72.9
	3– > 20%	High	21.7	6.0

3.1.5 Photosynthetic functioning

Photosynthesis plays a crucial role in determining the yield of crops, as it influences the efficiency of light capture, converting it into biomass. In this context, to assess the potential phytotoxicity, field A was chosen as a test field. In this regard, foliar gas exchange analyzes were carried out, as well as the quantification of chlorophyll a fluorescence parameters. The analysis excluded the lowest treatments of $CaCl_2$ and $Ca(NO_3)_2$, 1 kg/ha and 0.5 kg/ha, respectively. This omission was intentional, as the focus was directed toward the higher treatments, where the most substantial impact on photosynthetic functioning s could occur.

3.1.5.1 Infrared foliar gas exchange

In field A, foliar gas exchange analyses were conducted after the 3rd foliar application (2 August 2018) (**Figure 3.8**), considering that the synthesis of photoassimilates would be impaired if the threshold of toxicity is reached. In this context, leaf gas exchange parameters were monitored to assess potential stress (net photosynthesis rate (P_n); stomatal conductance to H_2O vapor (g_s); internal [CO_2] (C_i); transpiration rate (E) and instantaneous water use efficiency (iWUE)). The Pn parameter exhibited a significant decrease relative to the control in both $CaCl_2$ or $Ca(NO_3)_2$ treatments (between 15.2 and 22.4 %), showing the lowest value in the 12A treatment. Moreover, stomatal conductance (g_s) followed a similar pattern of variation as P_n, but with an even more pronounced reduction, showing a significant decrease for all the biofortification treatments, ranging between 40.3 - 53.3 %, with the lowest value obtained in 3A treatment. Internal CO_2 (C_i) also showed a significant reduction, between 9.5 - 41.7 %, with 2N showing the highest decrease and 4N the lowest decrease, relatively to control. In context, iWUE increased in all the biofortification treatments (between 11.2 – 38.3 %), presenting a significantly higher value in 3A and 6A treatments.

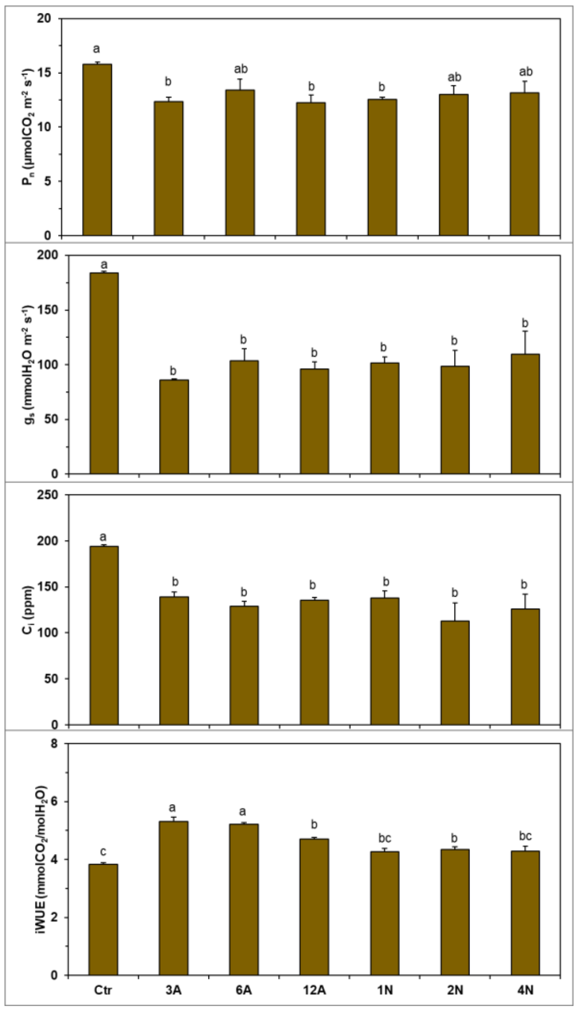


Figure 3.8 Mean values (*n = 4-6*) ± SE (Standard Error) of the variation of net photosynthesis rates (Pn) and stomatal conductance (gs), as well as the instantaneous efficiency of water use (iWUE = Pn/E), in the different treatments applied for Agria variety after the 3rd foliar application. $CaCl_2$ 3 kg/ha (3A), $CaCl_2$ 6 kg/ha (6A), $CaCl_2$ 12 kg/ha (12A), $Ca(NO_3)_2$ 1 kg/ha, $Ca(NO_3)_2$ 2 kg/ha and $Ca(NO_3)_2$ 4 kg/ha. For each parameter the different letters express significant differences be-tween treatments (a,b) ($p \leq 0.05$).

3.1.5.2 Chlorophyll a fluorescence parameters

In field A, following the 3rd and 4th foliar applications, on 2 August 2018 and 14 August 2018, respectively, the chlorophyll a fluorescence parameters were monitored (**Table 3.9 -Table 3.11**). This monitoring aimed to assess the functioning of the photosynthetic apparatus. During the initial assessment, conducted after the 3rd foliar application, Agria variety showed no significant differences in all the parameters, regardless of the biofortification treatments compared to the control (**Table 3.9 - Table 3.11**). However, the 4N treatment demonstrated a significantly lower in the Y(NPQ) compared to the other treatments (**Table 3.10**). Also, in the first monitoring, the use of energy through photochemistry (q_L and $Y_{(II)}$) was not affected, compared to control. Regarding the second monitoring (after the 4th foliar application), the PSII photochemical efficiency (F_v/F_m and F_v'/F_m') showed a significant reduction in 2N treatment (relatively to the control of F_v/F_m), and no significant differences were found among treatments regarding the F_v'/F_m' (Table 3.9). Moreover, considering $Y_{(II)}$ (**Table 3.10**), 3A treatment showed the highest value and 12A, 1N and 4N treatment revealed significantly lower values compared to the remaining treatments. Considering q_L (**Table 3.11**), the significantly lower value was obtained in the control.

Table 3.9 Leaf chlorophyll a fluorescence parameters in Agria variety after the 3rd foliar application (2 August 2018) and after the 4th foliar application (14 August). Mean values (*n = 5*) ± SE (Standard Error) of the maximum (F_v/F_m) and the actual (F_v'/F_m') PSII photochemical efficiency.

Treatments	F_v/F_m		F_v'/F_m'	
	2 August	14 August	2 August	14 August
Ctr	0.817 ± 0.006aA	0.823 ± 0.006aA	0.510 ± 0.025aA	0.505 ± 0.03aA
3A	0.789 ± 0.009aA	0.795 ± 0.008abA	0.489 ± 0.03aA	0.474 ± 0.033aAB
6A	0.813 ± 0.004aA	0.800 ± 0.008abA	0.487 ± 0.023aA	0.508 ± 0.019aA
12A	0.818 ± 0.004aA	0.795 ± 0.006abA	0.468 ± 0.018aA	0.371 ± 0.058aB
1N	0.812 ± 0.007aA	0.792 ± 0.009abA	0.525 ± 0.013aA	0.446 ± 0.039aAB
2N	0.821 ± 0.007aA	0.784 ± 0.007bA	0.446 ± 0.022aA	0.445 ± 0.017aAB
4N	0.818 ± 0.003aA	0.794 ± 0.006abA	0.462 ± 0.011aA	0.390 ± 0.025aB

For each parameter the different letters express significant differences between treatments (a,b) or between treatments in each date (A,B) ($p \leq 0.05$).

Table 3.10 Leaf chlorophyll a fluorescence parameters in Agria variety after the 3rd foliar application (2 August 2018) and after the 4th foliar application (14 August). Mean values (*n = 5*) ± SE (Standard Error) of the estimate of quantum yields of non-cyclic electron transport ($Y_{(II)}$) of regulated energy dissipation in PSII ($Y_{(NPQ)}$) and of non-regulated energy dissipation in PSII ($Y_{(NO)}$).

Treatments	$Y_{(II)}$		$Y_{(NPQ)}$		$Y_{(NO)}$	
	2 August	14 August	2 August	14 August	2 August	14 August
Ctr	0.418 ± 0.023aA	0.331 ± 0.036aAB	0.372 ± 0.026aA	0.479 ± 0.042aAb	0.211 ± 0.006aA	0.190 ± 0.009aA
3A	0.407 ± 0.019aA	0.379 ± 0.04aA	0.381 ± 0.024aA	0.419 ± 0.04aB	0.212 ± 0.007aA	0.201 ± 0.008aA
6A	0.391 ± 0.017aA	0.363 ± 0.009aAB	0.397 ± 0.021aA	0.413 ± 0.009aB	0.212 ± 0.009aA	0.223 ± 0.008aA
12A	0.384 ± 0.015aA	0.244 ± 0.034bB	0.397 ± 0.019aA	0.563 ± 0.043aA	0.219 ± 0.006aA	0.193 ± 0.014aA
1N	0.437 ± 0.025aA	0.303 ± 0.042bAB	0.336 ± 0.026aA	0.485 ± 0.041aAB	0.227 ± 0.005aA	0.211 ± 0.01aA
2N	0.335 ± 0.025aA	0.309 ± 0.018aAB	0.450 ± 0.026aA	0.477 ± 0.021aAB	0.215 ± 0.006aA	0.213 ± 0.009aA
4N	0.348 ± 0.006aA	0.237 ± 0.026bB	0.427 ± 0.011bA	0.554 ± 0.028aA	0.225 ± 0.005aA	0.208 ± 0.008aA

For each parameter the different letters express significant differences between treatments (a,b) or between treatments in each date (A,B) (*$p \leq 0.05$*).

Table 3.11 Leaf chlorophyll a fluorescence parameters in Agria variety after the 3rd foliar application (2 August 2018) and after the 4th foliar application (14 August). Mean values (*$n = 5$*) ± SE (Standard Error) of the photochemical quenching coefficient (q_L) and non-photochemical quenching (q_N).

Treatments	q_N		q_L	
	2 August	14 August	2 August	14 August
Ctr	0.768 ± 0.026aA	0.820 ± 0.027aA	0.690 ± 0.032aA	0.483 ± 0.038bB
3A	0.775 ± 0.026aA	0.800 ± 0.031aA	0.720 ± 0.038aA	0.687 ± 0.077aA
6A	0.787 ± 0.021aA	0.777 ± 0.015aA	0.679 ± 0.038aA	0.558 ± 0.047aAB
12A	0.793 ± 0.016aA	0.872 ± 0.029aA	0.708 ± 0.016aA	0.580 ± 0.088aAB
1N	0.734 ± 0.019aB	0.822 ± 0.026aA	0.710 ± 0.058aA	0.541 ± 0.065aAB
2N	0.821 ± 0.018aA	0.823 ± 0.014aA	0.629 ± 0.043aA	0.560 ± 0.03aAB
4N	0.804 ± 0.011aA	0.861 ± 0.016aA	0.622 ± 0.014aA	0.480 ± 0.037aB

For each parameter the different letters express significant differences between treatments (a,b) or between treatments in each date (A,B) (*$p \leq 0.05$*).

3.1.6 Quantification of mineral elements and Ca location

Aiming the agronomic biofortification of *Solanum tuberosum* L. with Ca, thus, to increase the concentration of this nutrient in the tubers, through foliar sprays during the production cycle, the quantification in tubers of Ca, K, S and P and tissues localization of Ca were carried out.

3.1.6.1 Tubers at harvest

Calcium, K, S and P, and Ca tissue location were conducted at harvest in the three potato varieties - Agria, Picasso, and Rossi. Additionally, an assessment of Ca biofortification index was further carried out using the X-ray fluorescence spectrometry technique.

3.1.6.1.1 X-ray fluorescence spectrometry (XRF)

The accumulation of mineral elements in the tubers was carried out at harvest (**Table 3.12**). In all the biofortification treatments, Ca and S contents, in Agria, did not vary significantly relatively to the control.

Regarding K and P, 12A showed a significant increase relatively to the control. In Picasso, only K did not show significant differences regarding control. Moreover, 0.5N, 1N and 2N treatments showed significantly higher contents of Ca, relatively to control. Additionally, P and S contents were significantly higher in 12A treatment. Regarding Rossi, only Ca and S showed significant differences between treatments. Hence, relatively to control, Ca and S contents were significantly higher in 0.5N and 4N treatments, respectively. Considering Ca biofortification index, Rossi and Picasso varieties significantly increased Ca content. Rossi variety showed a Ca content of about 0.23 % in the 0.5N treatment (*i.e.*, an increase of 2.5 fold, relatively to the control). Although there were no significantly higher increases, Agria showed the highest Ca content in the 0.5N treatment, as well as Picasso with 1N treatment (showing relatively to the control an increase of 27.4 % and 57 %, respectively).

Table 3.12 Calcium, K, S and P content (on a dry weight basis) in tubers with skin of the three varieties of *Solanum tuberosum* L. (Agria, Picasso and Rossi) at harvest. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria				Picasso				Rossi			
	Ca	K	S	P	Ca	K	S	P	Ca	K	S	P
	%				%				%			
Ctr	0.106 ± 0.015a	2.483 ± 0.369b	0.154 ± 0.027a	0.152 ± 0.024b	0.072 ± 0.002b	3.064 ± 0.096a	0.198 ± 0.018ab	0.227 ± 0.018ab	0.092 ± 0.006b	2.44 ± 0.034a	0.162 ± 0.018b	0.134 ± 0.015a
1A	0.106 ± 0.006a	2.673 ± 0.126ab	0.166 ± 0.006a	0.179 ± 0.018ab	0.047 ± 0.004c	2.667 ± 0.142a	0.151 ± 0.004ab	0.173 ± 0.016b	0.043 ± 0.005b	2.089 ± 0.071a	0.20 ± 0.01a	0.142 ± 0.004a
3A	0.108 ± 0.017a	2.782 ± 0.054ab	0.182 ± 0.003a	0.225 ± 0.011ab	0.058 ± 0.003bc	2.825 ± 0.242a	0.192 ± 0.024ab	0.209 ± 0.04b	0.072 ± 0.004b	2.364 ± 0.225a	0.199 ± 0.031ab	0.154 ± 0.003a
6A	0.111 ± 0.010a	2.462 ± 0.262b	0.144 ± 0.027a	0.206 ± 0.008ab	0.068 ± 0.001b	2.715 ± 0.179a	0.15 ± 0.011b	0.173 ± 0.013b	0.072 ± 0.014b	2.518 ± 0.424a	0.198 ± 0.027ab	0.176 ± 0.038a
12A	0.115 ± 0.001a	3.506 ± 0.232a	0.205 ± 0.005a	0.267 ± 0.028a	0.069 ± 0.001b	3.364 ± 0.168a	0.241 ± 0.021a	0.433 ± 0.074a	0.115 ± 0.011b	2.437 ± 0.148a	0.166 ± 0.008b	0.120 ± 0.011a
0.5 N	0.135 ± 0.012a	3.47 ± 0.159ab	0.236 ± 0.016a	0.212 ± 0.008ab	0.097 ± 0.009a	2.649 ± 0.103a	0.15 ± 0.008b	0.21 ± 0.045b	0.230 ± 0.041a	2.604 ± 0.331a	0.215 ± 0.019ab	0.24 ± 0.017a
1N	0.122 ± 0.008a	3.025 ± 0.092ab	0.217 ± 0.018a	0.225 ± 0.02ab	0.113 ± 0a	3.438 ± 0.487a	0.227 ± 0.016ab	0.27 ± 0.038ab	0.077 ± 0.015b	2.315 ± 0.04a	0.140 ± 0.009b	0.093 ± 0.003a
2N	0.114 ± 0.007a	3.058 ± 0.154ab	0.226 ± 0.008a	0.227 ± 0.023ab	0.097 ± 0.003a	2.603 ± 0.119a	0.212 ± 0.019ab	0.265 ± 0.067ab	0.118 ± 0.02b	2.783 ± 0.221a	0.163 ± 0.006b	0.185 ± 0.02a
4N	0.087 ± 0.014a	3.27 ± 0.206ab	0.229 ± 0.03a	0.207 ± 0.011ab	0.07 ± 0.006b	2.778 ± 0.081a	0.218 ± 0.03ab	0.256 ± 0.042ab	0.095 ± 0.009b	2.819 ± 0.203a	0.276 ± 0.023b	0.207 ± 0.017a

Different letters indicate significant different between treatments in each mineral element ($p \leq 0.05$).

Regarding the correlation between macro and microelements, only a negative correlation between the macro and microelements in the Pearson correlation in Agria variety was found (**Table 3.13**). According to Pearson (r), in Agria, P and K showed a strong correlation (r ranging between 0.70 and 0.89), whereas K and Ca, as well as S and Ca showed a negative correlation. In Picasso, S and P showed a very strong correlation (r >0.90), but P and S, as well as S and K revealed a strong correlation. In Rossi, there was no pair with a very strong or strong correlation. According to Spearman (ρ), the only strong correlation occurred between K and P (in Agria), as well as in P and S (in Picasso and Rossi).

Table 3.13 Correlation matrix of Ca, K, S and P content (on a dry weight basis) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Pearson (blue) and Spearman (green) indexes.

Agria

	Ca	K	P	S
Ca	1.000	-0.052	0.101	-0.040
K	0.045	1.000	0.794	0.676
P	0.165	0.790	1.000	0.474
S	0.042	0.501	0.446	1.000

Picasso

	Ca	K	P	S
Ca	1.000	0.273	0.342	0.510
K	0.095	1.000	0.700	0.793
P	0.379	0.483	1.000	0.905
S	0.510	0.623	0.888	1.000

Rossi

	Ca	K	P	S
Ca	1.000	0.227	0.152	0.140
K	0.449	1.000	0.507	0.378
P	0.054	0.442	1.000	0.569
S	0.336	0.628	0.781	1.000

3.1.6.1.2 µ-EDXRF system - Calcium location

To evaluate the accumulation of Ca in the different regions of tubers, five distinct zones were defined in the equatorial region of each treatment (from the epidermis/skin (1) to the center (5)) (**Figure 3.9**). This analysis revealed that Ca prevails in the epidermis region, followed by the inner regions of the tuber.

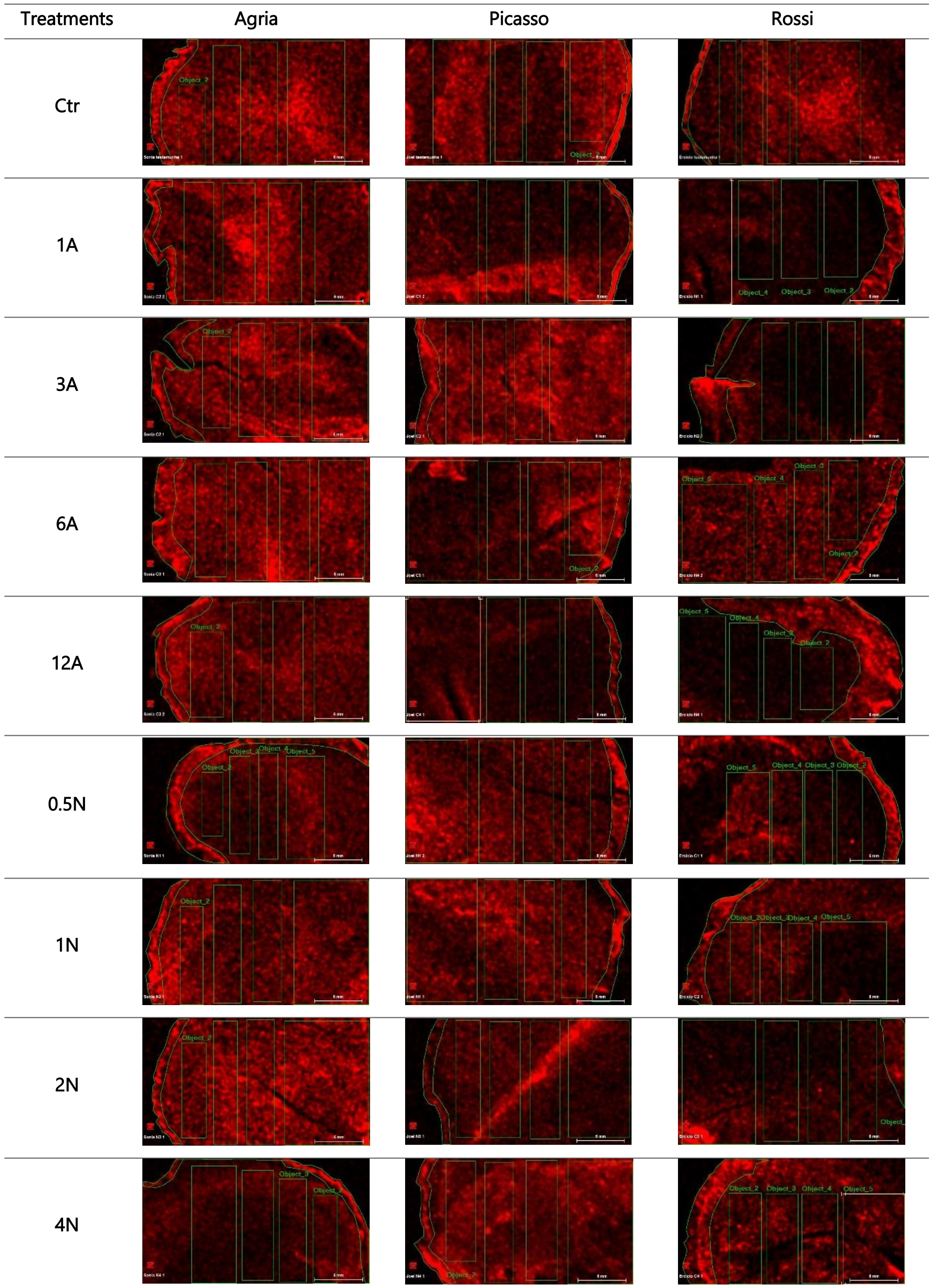


Figure 3.9 Calcium deposition in the 5 regions of the equatorial region of the tubers of *Solanum tuberosum* L. for Agria, Picasso and Rossi varieties.

3.1.7 Morphological, physical, and organoleptic parameters of tubers at harvest and after storage

Following harvest, and a three-month storage period, an evaluation of morphological, physical, and organoleptic parameters of the tubers was performed to verify if there were any discernible differences between the control and the Ca biofortification treatments.

3.1.7.1 Height, diameter, dry weight content and total soluble solids

After harvest, there were no significant differences observed between treatments in terms of height and diameter for each variety (**Table 3.14**).

Table 3.14 Height and diameter (cm) of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, at harvest. Mean values ($n = 4$) ± SE (Standard Error).

T.	Agria		Picasso		Rossi	
	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)
Ctr	9.3 ± 0.32a	5.6 ± 0.15a	8.8 ± 0.6a	5.43 ± 0.26a	9.63 ± 1.64a	6.23 ± 0.56a
1A	11.53 ± 1.44a	5.33 ± 0.18a	8.43 ± 0.93a	5.47 ± 0.32a	9.33 ± 1.14a	6.37 ± 0.64a
3A	9.5 ± 0.32a	5.7 ± 0.21a	8.4 ± 0.4a	5.07 ± 0.27a	9.27 ± 1.04a	5.63 ± 0.52a
6A	9.9 ± 1.17a	4.73 ± 0.49a	7.57 ± 0.64a	5.67 ± 0.48a	8.9 ± 0.61a	5.57 ± 0.38a
12A	10.67 ± 0.45a	4.93 ± 0.15a	8.27 ± 0.62a	5.37 ± 0.09a	9.7 ± 0.56a	5.6 ± 0.47a
0.5N	11 ± 0.83a	5.77 ± 0.71a	9.63 ± 0.3a	5.63 ± 0.3a	11.47 ± 1.98a	5.87 ± 0.94a
1N	10.53 ± 1.02a	5.23 ± 0.38a	9.03 ± 0.27a	5.37 ± 0.5a	8.53 ± 0.3a	5.6 ± 0.51a
2N	11.77 ± 0.53a	5.47 ± 0.22a	9.6 ± 0.76a	6.03 ± 0.24a	6.97 ± 0.75a	4.47 ± 0.03a
4N	11.43 ± 1.58a	4.77 ± 0.26a	9 ± 0.26a	5.3 ± 0.21a	9.27 ± 1.58a	5.53 ± 0.19a

Different letters indicate significant different between treatments in each parameter ($p \leq 0.05$).

Considering the dry weight content (DW) at harvest, significant differences were observed (varying between 20.01 – 25.59 %, 17.64 – 22.99 %, and 18.06 – 26.1 % for Agria, Picasso, and Rossi, respectively) (**Table 3.15**). Also, at harvest, Agria and Rossi exhibited higher values in the control, while Picasso showed a higher value in the 6A treatment. Moreover, 0.5N treatment showed the lowest content of DW in Picasso and Rossi varieties at harvest. After three months of storage (3M) (**Table 3.15**), significant differences among treatments were not found in the three varieties, varying DW between 18.28 - 21.48 %, 19.58 - 23.24 % and 18.71 - 22.16 % for Agria, Picasso, and Rossi, respectively. Overall, in the three varieties, most treatments revealed a decrease of DW, relative to the harvest data.

At harvest, total soluble solids content (TSS) (**Table 3.15**) showed significant differences between treatments in the three varieties. For Agria, Picasso, and Rossi, TSS varied, respectively, between 5

– 6.17 °Brix, 4.83 - 6.5 °Brix and 5.17 - 7.67 °Brix, and the highest contents were found in the control, 3A and 1N treatments. Picasso was the only variety that showed significant differences in TSS after 3M (**Table 3.15**), with 2N treatment showing the highest content and 1N the lowest. Comparing the data obtained for the same treatment at harvest and after 3M, there was a significant decrease after 3M in all the treatments in Picasso (except in 2N treatment) and Rossi (except in 12A and 2N treatment), and only in the control of Agria variety.

Table 3.15 Dry weight (%) and total soluble solids (°Brix) content at harvest (H) and after three months of storage (3M) of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria				Picasso				Rossi			
	DW (%)		TSS		DW (%)		TSS		DW (%)		TSS	
	H	3M	H	3M	H	3M	H	3M	H	3M	H	3M
Ctr	25.59 ± 0.62aA	18.28 ± 0.75aB	6.17 ± 0.14aA	5.17 ± 0.14aB	20.93 ± 0.49abA	22.68 ± 0.73aB	7.5 ± 0.41aA	6.33 ± 0.14aB	26.1 ± 0.96aA	22.16 ± 0.11aA	4.83 ± 0.14bB	5.83 ± 0.14abA
1A	21.08 ± 0.18bA	21.48 ± 0.58aA	5.93 ± 0.05abA	5.5 ± 0.24aA	19.74 ± 0.63bcB	20.66 ± 0.69aB	7 ± 0abA	5.83 ± 0.27aB	23.98 ± 0.75abA	21.61 ± 0.12aA	5.5 ± 0.24abA	5.33 ± 0.27abA
3A	22.65 ± 0.32abA	20.61 ± 0.34aB	5.07 ± 0.05abA	5.33 ± 0.14aA	19.66 ± 0.13bcB	23.05 ± 0.29aA	7.33 ± 0.27abA	6.17 ± 0.14aB	23.17 ± 0.62abA	22.38 ± 0.43aA	6.5 ± 0.24aA	5.17 ± 0.14abB
6A	22.43 ± 0.65abA	20.32 ± 0.3aB	5.3 ± 0.24abA	5.67 ± 0.36aA	22.99 ± 0.58aA	23.22 ± 0.7aA	7 ± 0.24abA	6.33 ± 0.14aB	23.24 ± 0.07abA	20.42 ± 1.78aB	5.5 ± 0.24abA	5 ± 0abB
12A	21.06 ± 0.69bA	19.75 ± 0.57aA	5.17 ± 0.14abA	5.17 ± 0.14aA	22.94 ± 0.61aA	24.43 ± 0.13a	6.5 ± 0.24abA	5.67 ± 0.14aB	20.65 ± 0.7bcB	19.86 ± 1.03aB	5.33 ± 0.27abA	5.83 ± 0.14abB
0.5N	21.04 ± 0.29bA	19.8 ± 0.39aA	5.33 ± 0.27abA	5.17 ± 0.14aA	17.64 ± 0.33cB	20.93 ± 2.04aA	6.17 ± 0.14bcA	5.83 ± 0.36aA	18.06 ± 0.56cB	19.95 ± 0.08aA	4.93 ± 0.05bB	5.5 ± 0.24abA
1N	20.01 ± 1.5bA	21.38 ± 0.82aA	5.67 ± 0.27abA	5.67 ± 0.27aA	21.86 ± 0.32abA	19.58 ± 1.83aB	7.67 ± 0.14aA	5.83 ± 0.36aB	23.24 ± 1.08abA	21.36 ± 0.45aA	5.33 ± 0.14abA	4.83 ± 0.14bB
2N	20.04 ± 0.2bA	20.76 ± 0.6bA	5.4 ± 0.25abA	5.17 ± 0.14aA	20.93 ± 0.44abA	20.48 ± 0.47aB	5.17 ± 0.14cB	5.83 ± 0.14aB	21.55 ± 0.34bcA	20.97 ± 0.28aA	5.17 ± 0.14bB	6 ± 0.24aA
4N	20.19 ± 0.54bA	19.78 ± 0.84aA	5 ± 0bA	5.33 ± 0.27aA	21.57 ± 0.33abA	22.31 ± 0.4aA	6.67 ± 0.14abA	5.5 ± 0.24aB	22.8 ± 0.33abA	18.71 ± 0.99aB	5.5 ± 0.24abA	5.33 ± 0.14abA

Different letters indicate significant different between treatments at harvest or after 3 months of storage for each variety (a,b) or between harvest or after 3 months of storage in the same treatment of biofortification (A,B) ($p<0.05$).

3.1.7.2 Total fatty acid content

Considering that lipid degradation in food matrixes, such as in potatoes, can cause off-flavor and rancidity problems (Galliard, 1973), the analysis of total fatty acid content was conducted at harvest, after three (3M) and six months (6M) of storage in tubers of the three varieties. Notable differences were observed among the three periods of analysis (**Table 3.16 - 3.18**).

In the case of Agria variety at harvest (**Table 3.16**), significant differences between treatments were evident for total fatty acid content (TFA), fatty acid profiles (<C16:0, C16:0, C18:0, C18:1, C18:2, and C18:3), and Double Bond Index (DBI). Also, Agria exhibited an increase in TFA in all biofortified treatments (despite not being significant), along with the increasing unsaturation in fatty acid

profiles as evidenced by the increase in DBI. However, after 3M and 6M of storage, significant differences could not be found among treatments in C16:0, C18:0, and C18:2, and in TFA, <C16:0, C18:0, C18:1, and C18:3, respectively. Considering TFA, there was an increase of the content: 6M > 3M > Harvest. Relatively to the harvest period, <C16:0, C18:3 and DBI increased relatively to 3M, but the remaining total fatty acid decreased or maintained their content, considering most of the treatments. Considering DBI, comparing 3M to 6M, it was possible to observe a significant decrease, persisting lower values than the ones obtained at harvest. Furthermore, at harvest it was possible to verify that the control showed a content of TFA similar in the three varieties (around 0.4 g/100g_{FW})(**Table 3.16 - 3.18**), being total fatty acid profile similar in the three varieties and being characterized by the highest abundance of linoleic acid (C18:2), followed by palmitic acid (C16:0), linolenic acid (C18:3), stearic acid (C18:0) and oleic acid (C18:1).

Table 3.16 Total fatty acid content TFA (g/100g on a fresh weight (FW) basis), fatty acids profiles (relative abundance, mol %) and double bound index (DBI) of lipids at harvest (H) and after three (3M) and six months of storage (6M) from tubers of *Solanum tuberosum* L., Agria variety. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
				Harvest				
Ctr	0.43 ± 0.05bC	1.18 ± 0.09cB	21.29 ± 1.02abB	4.62 ± 0.43aB	0.71 ± 0.04abB	51.92 ± 1.52abB	20.28 ± 0.75cB	6.33 ± 0.43bB
1A	0.77 ± 0.1aC	1.27 ± 0.02cC	21.7 ± 0.75aA	4 ± 0.2aB	0.86 ± 0.08aB	51.38 ± 0.82bA	20.79 ± 0.31bcB	6.42 ± 0.2bB
3A	0.83 ± 0.04aC	1.44 ± 0.21bcC	19.58 ± 1.12abcB	2.01 ± 0.3bB	0.55 ± 0.03bB	52.11 ± 0.61abA	24.31 ± 0.91abcA	8.17 ± 0.61abA
6A	0.93 ± 0.09aC	1.18 ± 0.15cC	15.42 ± 1.36bcB	2.25 ± 0.46bB	0.59 ± 0.09abC	56.75 ± 1.52aA	23.82 ± 1.62abcB	10.8 ± 1.24aB
12A	0.95 ± 0.09aC	2.21 ± 0.33abcC	16.43 ± 1.18abcB	2.09 ± 0.4bC	0.64 ± 0.03abB	52.61 ± 0.63abB	26.02 ± 1.73aA	10.09 ± 1.01abA
0.5N	0.92 ± 0.08aC	2.06 ± 0.56abcC	18.11 ± 2.09abcB	2.11 ± 0.45bC	0.6 ± 0.08abB	54.06 ± 1.48abA	23.06 ± 1.64abcB	9.16 ± 1.3abB
1N	0.92 ± 0.04aC	1.77 ± 0.32abcC	15.23 ± 1.11cB	1.65 ± 0.06bB	0.53 ± 0.02bB	56.75 ± 1.11aA	24.07 ± 0.39abcB	11.08 ± 0.99aB
2N	0.89 ± 0.05aC	2.91 ± 0.28aB	17.65 ± 1.33abcB	1.58 ± 0.18bC	0.45 ± 0.05bB	51.64 ± 1.26abA	25.78 ± 0.53abB	9.4 ± 0.82abB
4N	1.03 ± 0.06aB	2.72 ± 0.25abC	14.48 ± 0.78cB	1.57 ± 0.2bC	0.54 ± 0.02bB	54.3 ± 0.55abA	26.4 ± 0.75aB	11.71 ± 0.92aB
				3M				
Ctr	1.84 ± 0.15aB	3.82 ± 0.58bA	11.21 ± 0.31aC	1.76 ± 0.2aC	0.5 ± 0.09bcC	55.58 ± 2.32aA	27.12 ± 2.28abA	15 ± 0.54abA
1A	1.18 ± 0.15abcB	15.68 ± 4.59aA	8.49 ± 0.47aC	2.76 ± 0.23aC	2.48 ± 0.28aA	44.91 ± 3.26aC	25.68 ± 1.77bA	15.72 ± 0.55abA
3A	1.26 ± 0.12abcB	5.77 ± 0.88bA	11.58 ± 1.27aC	2.58 ± 0.31aB	1.18 ± 0.26bcA	52.9 ± 1.17aA	26 ± 2.31abA	13.17 ± 1.4abA
6A	1.51 ± 0.09abB	6.12 ± 2.52bA	8.59 ± 0.28aC	1.63 ± 0.18aB	1.3 ± 0.4bA	55.29 ± 1.46aA	27.07 ± 1.26abA	18.93 ± 0.65aA
12A	0.73 ± 0.15cB	8.94 ± 1.86abA	14.42 ± 0.9aC	3.47 ± 0.87aB	2.77 ± 0.05aA	55.38 ± 0.71aA	15.01 ± 1.1bC	8.91 ± 0.52bB
0.5N	1.06 ± 0.14bcB	6.33 ± 0.53bA	8.64 ± 0.4aC	2.75 ± 0.25aB	0.65 ± 0.07bcB	53.82 ± 1.06aA	27.79 ± 1.02abA	15.87 ± 0.87abA
1N	1.58 ± 0.19abB	4.94 ± 0.61bA	8.62 ± 0.46aC	1.26 ± 0.22aC	0.36 ± 0.07bcC	55.87 ± 1.3aA	28.94 ± 1.51abA	19.75 ± 1.32aA
2N	1.3 ± 0.12abcB	7.51 ± 0.54abA	9.28 ± 0.28aC	3.18 ± 0.46aB	0.32 ± 0.04cC	51.38 ± 2.05aA	28.33 ± 2.04abA	15.21 ± 0.71abA
4N	1.11 ± 0.24abcB	8.03 ± 1.11abA	11.37 ± 3.34aC	2.84 ± 1.09aB	0.6 ± 0.21bcB	37.12 ± 12.41aC	40.04 ± 7.42aA	15.55 ± 4.16abA
				6M				
Ctr	1.99 ± 0.09aA	2.6 ± 0.42aA	22.01 ± 0.47bcA	6.29 ± 0.47aA	0.8 ± 0.16aA	47.75 ± 0.7abC	20.55 ± 1.06aC	5.32 ± 0.17abC
1A	1.89 ± 0.13aA	2.93 ± 0.3aB	21.56 ± 0.37cB	5.23 ± 0.48aA	0.81 ± 0.15aB	48.63 ± 0.68abB	20.85 ± 1.64aC	5.62 ± 0.31abC
3A	1.77 ± 0.13aA	2.85 ± 0.39aB	23.73 ± 0.65abcA	5.32 ± 0.23aA	1.22 ± 0.11aA	47.27 ± 1.29abB	19.6 ± 0.97aB	5.06 ± 0.24abB
6A	1.66 ± 0.08aA	2 ± 0.22aB	20.98 ± 0.8cA	5.11 ± 0.26aA	0.98 ± 0.17aB	50.59 ± 1.75aB	20.34 ± 1.61aC	6.09 ± 0.21aC
12A	1.6 ± 0.17aA	2.74 ± 0.08aB	25.17 ± 0.72abcA	5.35 ± 0.13aA	0.66 ± 0.1aB	46.29 ± 1.45abC	19.8 ± 1.6aB	4.71 ± 0.18abC
0.5N	1.74 ± 0.06aA	3.73 ± 0.98aB	27.21 ± 1.58aA	5.73 ± 0.42aA	0.88 ± 0.18aA	45.35 ± 1.38abB	17.09 ± 1.7aC	4.22 ± 0.32bC
1N	1.47 ± 0.22aA	3.21 ± 0.71aB	24.67 ± 1.94abcA	6.05 ± 0.71aA	1.39 ± 0.35aA	45.55 ± 0.81abB	19.12 ± 2.39aC	4.79 ± 0.59abC
2N	1.75 ± 0.05aA	2.4 ± 0.16aC	27.03 ± 0.88abA	5.86 ± 0.33aA	0.71 ± 0.12aA	42.99 ± 0.75bB	21.02 ± 0.59aC	4.38 ± 0.22bC
4N	1.46 ± 0.1aA	3.82 ± 0.43aB	24.53 ± 1.32abcA	5.92 ± 0.6aA	0.91 ± 0.02aA	46.71 ± 1.77abB	18.12 ± 1.12aC	4.67 ± 0.4abC

Different letters indicate significant different between treatments (a,b) and between different times of analysis (Harvest, 3M and 6M) within each treatment (A,B) ($p \leq 0.05$).

In Picasso, at harvest and after 3M of storage, there were significant differences among treatments (**Table 3.17**) for TFA, fatty acid profiles (<C16:0, C16:0, C18:0, C18:1, C18:2 and C18:3) and DBI. But, after 6M of storage, significant differences could not be found among treatments in TFA and <C16:0. Additionally, relatively to the control, it was possible to observe a decrease of the unsaturation degree of the fatty acid profiles (reduction of DBI) in all the biofortification treatments, except in 1A (at harvest and 3M), 0.5N (3M), 2N (6M) and 4N (6M).

Table 3.17 Total fatty acid content TFA (g/100g on a fresh weight (FW) basis), fatty acids profiles (relative abundance, mol %) and double bound index (DBI) of lipids at harvest (H) and after three (3M) and six months of storage (6M) from tubers of *Solanum tuberosum* L., Picasso variety. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
				Harvest				
Ctr	0.47 ± 0.03aC	1.65 ± 0.09bcdB	30.84 ± 0.61abA	5.29 ± 0.26abA	1.75 ± 0.11bcA	49.47 ± 0.22abB	11 ± 0.61aB	3.64 ± 0.14abC
1A	0.44 ± 0.02abC	3.23 ± 0.23aB	23.92 ± 1.26bA	4.71 ± 0.42abA	1.51 ± 0.24cA	56.27 ± 1.15aB	10.35 ± 1.13abB	5.08 ± 0.28aC
3A	0.36 ± 0.04abC	1.63 ± 0.47bcdB	33.75 ± 3.38aA	4.31 ± 0.41bB	2.39 ± 0.31abcA	48.6 ± 2.72abC	9.32 ± 1.57abB	3.46 ± 0.64bC
6A	0.32 ± 0.01abC	1.34 ± 0.11cdB	37.36 ± 1.45aA	7.37 ± 0.48aA	1.76 ± 0.1bcA	42.74 ± 1.49bC	9.43 ± 0.6abC	2.59 ± 0.22bC
12A	0.28 ± 0.01bC	1.52 ± 0.06bcdB	33.83 ± 2.76aA	6.95 ± 1.1abA	2.7 ± 0.2aB	42.63 ± 1.44bC	12.37 ± 1.38aB	3.11 ± 0.42bC
0.5N	0.43 ± 0.05abC	0.97 ± 0.04bcB	37.2 ± 3.64aA	6.54 ± 0.15abA	2.45 ± 0.08abA	45.91 ± 3.89bC	6.92 ± 0.41bB	2.68 ± 0.4bB
1N	0.46 ± 0.04aC	2.11 ± 0.24bcB	30.83 ± 1.51abB	5.59 ± 0.76abB	2.22 ± 0.31abcA	49.04 ± 1.66abB	10.21 ± 0.65abB	3.62 ± 0.34abB
2N	0.47 ± 0.07aC	2.51 ± 0.21abB	37.77 ± 1.37aB	6.81 ± 0.54abB	1.87 ± 0.36abcA	42.16 ± 1.68bB	8.88 ± 0.22abB	2.52 ± 0.16bB
4N	0.47 ± 0.06aC	2.04 ± 0.37bcB	34.3 ± 1.97aB	6.94 ± 1.39abB	1.62 ± 0.11bcB	45.32 ± 2.22bB	9.78 ± 0.93abB	2.97 ± 0.37bB
				3M				
Ctr	1 ± 0.07abA	4.31 ± 0.35bA	26.6 ± 1.77bcA	4.66 ± 0.36cA	1.34 ± 0.19bA	51.52 ± 1.57aA	11.56 ± 0.93abB	4.49 ± 0.45abB
1A	0.93 ± 0.03abcB	4.58 ± 0.34bA	21.17 ± 1.45cA	3.64 ± 0.17cB	0.78 ± 0.09bB	55.81 ± 2.45aB	14.02 ± 0.74aA	6.22 ± 0.49aB
3A	0.67 ± 0.04bcB	5.98 ± 1.1abA	22 ± 1.72cA	4.91 ± 0.76cA	0.96 ± 0.2bB	53.95 ± 1.38aB	12.2 ± 0.89abA	5.39 ± 0.5aB
6A	0.77 ± 0.11abcB	4.8 ± 0.64bA	19.4 ± 1.23cB	5.21 ± 0.83bcB	0.93 ± 0.44bB	56.92 ± 1.8aB	12.74 ± 0.45abB	6.19 ± 0.4aB
12A	1.01 ± 0.17aA	6.23 ± 1.25abA	23.46 ± 1.03cB	5.12 ± 1.3cB	3.53 ± 0.6aA	50.14 ± 0.73aB	11.51 ± 0.33abB	4.75 ± 0.07abB
0.5N	0.83 ± 0.06abcA	6.17 ± 1.47abA	21.41 ± 2.33cB	3.07 ± 0.47cC	0.72 ± 0.03bB	54.81 ± 2.17aB	13.81 ± 1.13abA	6.31 ± 0.75aA
1N	0.98 ± 0.05abA	8.8 ± 1.32aA	33.71 ± 5.21bA	6.07 ± 1.06abcA	0.96 ± 0.08bB	39.57 ± 5.9bC	10.9 ± 0.57bB	3.09 ± 0.83bcC
2N	0.63 ± 0.03cB	5.01 ± 0.52abA	46.26 ± 1.81aA	8.57 ± 1.24abA	1.59 ± 0.47bA	32.97 ± 0.71bC	5.59 ± 0.43cC	1.5 ± 0.04cC
4N	0.71 ± 0.07abcB	6.2 ± 0.37abA	45.84 ± 1.49aA	8.62 ± 0.35aA	3.11 ± 0.48aA	31.21 ± 1.15bC	5.03 ± 0.52cC	1.48 ± 0.09cC
				6M				
Ctr	0.86 ± 0.04aB	0.81 ± 0.13aC	15.88 ± 1.15abcB	2.04 ± 0.09bB	0.23 ± 0.03bB	66.4 ± 1.29abA	14.63 ± 0.53abA	9.75 ± 0.86abA
1A	0.98 ± 0.2aA	0.88 ± 0.08aC	19.19 ± 1.69abB	2.84 ± 0.6abC	0.25 ± 0.04bC	62.74 ± 1.67abcA	14.1 ± 0.77abA	7.62 ± 0.89abA
3A	0.73 ± 0.13aA	1.16 ± 0.19aC	22.33 ± 2.49aB	3.47 ± 0.87abC	0.28 ± 0.04bC	59.91 ± 1.74cAA	12.85 ± 1.52bA	6.3 ± 1.04bA
6A	0.88 ± 0.26aA	0.85 ± 0.17aC	16.21 ± 2.04abcC	4.72 ± 0.55aC	0.54 ± 0.04bC	62.45 ± 1.16bcA	15.23 ± 1.44abA	8.42 ± 1.6abA
12A	0.74 ± 0.09aB	0.71 ± 0.11aC	16.66 ± 1.81abcC	2.43 ± 0.24abC	0.21 ± 0.02bC	65.92 ± 1.14abA	14.07 ± 1.23abA	9.19 ± 1.27abA
0.5N	0.7 ± 0.05aB	0.82 ± 0.09aC	18.31 ± 1.86abcC	3.68 ± 0.76abB	0.49 ± 0.16bC	63.74 ± 2.17abcA	12.96 ± 0.57bA	7.72 ± 1.28abA
1N	0.94 ± 0.03aB	0.65 ± 0.02aC	17.18 ± 0.98abcC	3.6 ± 0.21abC	0.62 ± 0.04bC	65.2 ± 0.24abcA	12.75 ± 1.1bA	8.04 ± 0.57ab
2N	1.15 ± 0.13aA	0.93 ± 0.24aC	11.31 ± 1.31cC	3.23 ± 0.83abC	0.58 ± 0.07bB	66.8 ± 1.09abA	17.14 ± 0.65aA	12.6 ± 0.96aA
4N	1.05 ± 0.13aA	0.97 ± 0.19aC	12.7 ± 1.78bcC	2.27 ± 0.59bC	1.11 ± 0.28aC	68.39 ± 1.26aA	14.55 ± 0.65abA	12.55 ± 2.25aA

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 3M and 6M) within each treatment (A,B) ($p \leq 0.05$).

Regarding Rossi, at harvest and after 3M and 6M of storage, there were significant differences among treatments (**Table 3.18**) for fatty acid profiles (<C16:0, C16:0, C18:0, C18:1, C18:2 and C18:3) and DBI. Considering TFA, significant differences could not be found between treatments at

harvest and after 3M, but at 6M, the 4N treatment showed a significantly lower content of TFA. The content of TFA progressively decreased with the increase of $CaCl_2$ concentration at harvest and after 3M.

Table 3.18 Total fatty acid content TFA (g/100g on a fresh weight (FW) basis), fatty acids profiles (relative abundance, mol %) and double bound index (DBI) of lipids at harvest (H) and after three (3M) and six months of storage (6M) from tubers of *Solanum tuberosum* L., Rossi variety. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
				Harvest				
Ctr	0.4 ± 0.06aC	1.14 ± 0.15cB	25.92 ± 1.02bA	5.73 ± 0.87cA	1.53 ± 0.28aA	57.05 ± 1.4aC	8.63 ± 0.73abB	4.5 ± 0.38aC
1A	0.36 ± 0.02aC	2.02 ± 0.51bcB	32.29 ± 2.68abA	5.59 ± 0.39cB	1.63 ± 0.14aA	50.66 ± 2.62abC	7.81 ± 0.44abC	3.33 ± 0.35abC
3A	0.37 ± 0.03aC	2.07 ± 0.04bcB	34.97 ± 1.79abA	7.16 ± 0.89abcA	1.79 ± 0.08aA	46.74 ± 2.31abC	7.27 ± 0.43abC	2.77 ± 0.31bC
6A	0.37 ± 0.04aC	1.83 ± 0.24bcB	33.15 ± 1.63abA	6.69 ± 0.69abcA	1.68 ± 0.18aA	49.22 ± 2.01abC	7.42 ± 0.37abC	3.04 ± 0.28abC
12A	0.47 ± 0.06aC	1.85 ± 0.48bcB	32.4 ± 3.08abA	6.37 ± 1.06bcA	1.57 ± 0.28aA	48.14 ± 4.13abC	9.67 ± 0.67aC	3.31 ± 0.55abC
0.5N	0.5 ± 0.13aC	3.75 ± 0.33aB	30.73 ± 3.43abA	7.42 ± 0.99abcA	1.4 ± 0.17aA	48.07 ± 2.2abC	8.62 ± 1.16abB	3.28 ± 0.47abB
1N	0.48 ± 0.05aC	2.35 ± 0.2bcB	34.48 ± 2.41abA	8.36 ± 1.53abcA	1.93 ± 0.48aA	45.85 ± 3.62bC	7.03 ± 0.8abB	2.72 ± 0.55bB
2N	0.58 ± 0.12aC	2.38 ± 0.09bcB	35.77 ± 0.42aA	9.57 ± 0.63abA	1.63 ± 0.17aA	44.66 ± 0.57bC	6 ± 0.08bC	2.32 ± 0.05bB
4N	0.52 ± 0.08aC	2.61 ± 0.3abB	35.71 ± 1.62aA	10.16 ± 0.77aA	1.6 ± 0.17aA	43.66 ± 2.1bC	6.25 ± 0.46bC	2.3 ± 0.23bB
				3M				
Ctr	0.91 ± 0.1aB	3.99 ± 1.13abA	13.58 ± 0.98aB	3.2 ± 0.93bB	0.5 ± 0.15bB	64.45 ± 1.74abcB	14.29 ± 1.35bA	10.06 ± 0.24bB
1A	0.92 ± 0.1aB	5.49 ± 0.8abA	11.91 ± 0.69aB	7.56 ± 1.1aA	0.69 ± 0.2bB	61.73 ± 0.66bcB	12.62 ± 0.84bB	8.42 ± 0.54bB
3A	1.08 ± 0.24aB	6.84 ± 0.44aA	14.43 ± 2aB	4.96 ± 2.04abB	1.6 ± 0.47aB	61.25 ± 1.39cB	10.92 ± 0.63bB	8.23 ± 0.82bB
6A	1.08 ± 0.17aB	5.01 ± 1.59abA	13.6 ± 0.76aB	3.02 ± 0.81bB	0.66 ± 0.2bB	65.66 ± 1.34abcB	12.05 ± 1.04bB	10.28 ± 0.91bB
12A	1.09 ± 0.08aB	4.59 ± 1.2abA	11.19 ± 0.79aB	1.74 ± 0.29bC	0.43 ± 0.09bB	52.49 ± 1.4dB	29.57 ± 0.92aA	15.16 ± 1.16aB
0.5N	1.1 ± 0.19aB	2.92 ± 0.36bA	12.21 ± 1.33aB	3.06 ± 0.53bB	0.4 ± 0.06bB	68.18 ± 1.29aA	13.23 ± 0.86bA	11.76 ± 1.4abA
1N	1.01 ± 0.09aB	4.33 ± 0.36abA	14.88 ± 1.5aC	3.73 ± 0.45abB	0.69 ± 0.24bB	63.76 ± 1.71abcB	12.61 ± 0.71bA	8.65 ± 0.97bA
2N	1.11 ± 0.27aA	4.9 ± 0.79abA	11.7 ± 1.07aC	2.64 ± 0.41bC	0.58 ± 0.06bB	65.08 ± 1.94abcA	15.11 ± 1.9bA	12.51 ± 1.76abA
4N	0.94 ± 0.17aA	4.06 ± 0.94abA	13.33 ± 1.26aC	1.94 ± 0.71bC	0.31 ± 0.02bB	67.8 ± 1.98abA	12.56 ± 1.05bA	11.11 ± 1.04abA
				6M				
Ctr	1.6 ± 0.17aA	0.25 ± 0.12dC	8.38 ± 0.84cC	2.1 ± 0.39cC	0.36 ± 0.06ab	73.49 ± 0.69aA	15.42 ± 1.22abA	18.7 ± 2.21aA
1A	1.46 ± 0.19aA	0.49 ± 0.13abcdC	10.16 ± 0.75cC	2.12 ± 0.26cC	0.31 ± 0.05ab	69.97 ± 1.48abcA	16.95 ± 1.07aA	15.25 ± 0.83abA
3A	1.19 ± 0.12abcA	0.38 ± 0.09bcdC	11.92 ± 0.81bcC	2.28 ± 0.19bcC	0.4 ± 0.09ab	69.67 ± 0.82abcA	15.34 ± 0.63abA	13.03 ± 1.11abA
6A	1.69 ± 0.15aA	0.38 ± 0.13bcdC	8.17 ± 1.09cC	1.81 ± 0.12cC	0.25 ± 0.04b	72.6 ± 1.39abA	16.79 ± 1.08aA	19.89 ± 2.59aA
12A	1.37 ± 0.09abA	0.31 ± 0.1cdC	11.02 ± 2.73bcB	2.24 ± 0.24bcB	0.27 ± 0.02ab	70.92 ± 2.52abA	15.24 ± 0.71abB	17.41 ± 7.02abA
0.5N	1.46 ± 0.05aA	0.52 ± 0.08abcdC	12.22 ± 0.65bcB	2.74 ± 0.5bcC	0.4 ± 0.07abB	69.66 ± 0.99abcA	14.46 ± 0.86abcA	12.06 ± 1.18abA
1N	1.21 ± 0.21abcA	0.7 ± 0.09abcC	16.76 ± 2.53abB	3.04 ± 0.43abcC	0.63 ± 0.15aB	66.54 ± 2.95bcdA	12.33 ± 0.52bcA	9.09 ± 2.15abA
2N	0.85 ± 0.07bcB	0.79 ± 0.08abC	19.85 ± 0.8aB	3.66 ± 0.37abB	0.51 ± 0.15abB	63.66 ± 0.8cdB	11.54 ± 0.51cB	6.8 ± 0.45bB
4N	0.67 ± 0.07cB	0.86 ± 0.12aC	20.83 ± 0.8aB	4.39 ± 0.43aB	0.27 ± 0.08abC	62.32 ± 0.69dB	11.33 ± 0.85cC	6.18 ± 0.37bB

Different letters indicate significant different between treatments (a,b) and between different times of analysis (Harvest, 3M and 6M) within each treatment (A,B) ($p \leq 0.05$).

3.1.7.3 Soluble sugar content

Soluble sugar contents analysis was conducted on tubers of the three varieties of S*olanum tuberosum* L. - Agria, Picasso, and Rossi (**Table 3.19**). Overall, in most of the treatments, the soluble sugar contents in Agria and Rossi varieties displayed the following tendency: harvest > 6M > 3M, while in Picasso the tendency was different: Harvest> 3M> 6M. Regarding the three periods of analysis, significant differences were observed in Agria, Picasso, and Rossi (**Table 3.19**).

Considering Agria, sucrose contents varied between 0.242 – 0.489, 0.089-0.201, and 0.126-0.303 mg/g_{FW}, respectively for harvest, 3M and 6M. In Picasso, sucrose contents ranged between 0.529-1.072, 0.343-0.564, and 0.223-0.494 mg/g_{FW}, respectively for harvest, 3M and 6M. In Rossi, sucrose contents varied between 0.263-1.552, 0.098-0.452 and 0.367-1.001 mg/g_{FW}, respectively for harvest, 3M and 6M. At harvest, glucose contents varied between 0.077-0.023, 0.003-0.012, and 0.008-0.035 mg/g_{FW} in Agria, Picasso and Rossi, respectively. After 3M, Agria, Picasso and Rossi showed glucose contents that ranged between 0.008-0.130,0.012-0.034, and 0.013-0.039 mg/g_{FW}, respectively. After 6M glucose contents varied between 0.007-0.021, 0.005-0.028, and 0.017-0.044 mg/g_{FW} in Agria, Picasso and Rossi, respectively. Regarding fructose contents, Agria, Picasso, and Rossi showed values ranging between 0.006-0.014, 0.003-0.010 and 0.002-0.013 mg/g_{FW} at harvest. After 3M, the contents of fructose oscillated between 0.003-0116, 0.014-0.026 and 0.010-0.031 mg/g_{FW} for Agria, Picasso and Rossi respectively. After 6M Agria, Picasso and Rossi fructose contents varied between 0.003-0.006, 0.009-0.018 and 0.004-0.018 mg/g of FW, respectively. Considering sorbitol contents, Agria, Picasso and Rossi showed values varying between 0.019-0.247,0.010-0.023 and 0.007-0.034 mg/g_{FW}, respectively. Considering sorbitol contents after 3M, values ranged between 0.001-0.051,0.025-0.045 and 0.017-0.065 mg/g_{FW}, in Agria, Picasso and Rossi, respectively. After 6M sorbitol contents showed values oscillating between 0.028-0.098, 0.019-0.037 and 0.037-0.089 mg/g_{FW} for Agria, Picasso and Rossi, respectively. Considering the amount of total of soluble sugars, significant differences between treatments in the three varieties were observed (except in Agria after 3M and in Picasso after 3M and 6M) (**Table 3.19**). At harvest, for Agria and Picasso, 2N and 4N treatments showed significantly higher contents relatively to the control, respectively, whereas Rossi did not show significantly higher content in 0.5N treatment relatively to the control. After 6M, relatively to the control, Agria revealed a significantly higher content in 3A treatment. In Rossi, after 3M and 6M, a significantly higher content, relatively to the control, was obtained in 1A and 12A, and in 3A treatments, respectively. As such, overall no clear tendency was possible to attain through the data obtained in the three varieties.

Table 3.19 Soluble sugar content (SS) (mg/g_{FW}) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest, after 3 (3M) and 6 months (6M) of storage. Mean values (*n = 4*) ± SE (Standard Error).

SS	**T.**	**Agria**			**Picasso**			**Rossi**		
		Harvest	3M	6M	Harvest	3M	6M	Harvest	3M	**6M**
Sucrose	Ctr	0.431 ± 0.018abA	0.149 ± 0.031aB	0.214 ± 0.022aB	0.790 ± 0.302abA	0.379 ± 0.106aAB	0.247 ± 0.060aB	1.419 ± 0.149abA	0.191 ± 0.032aB	0.590 ± 0.159abB
	1A	0.266 ± 0.048bA	0.106 ± 0.027aA	0.126 ± 0.038aA	0.737 ± 0.110abA	0.430 ± 0.109aAB	0.223 ± 0.051aB	1.001 ± 0.226bA	0.399 ± 0.162aB	0.367 ± 0.098bB
	3A	0.242 ± 0.041bAB	0.089 ± 0.028aB	0.333 ± 0.043aA	0.992 ± 0.141aA	0.529 ± 0.154aAB	0.276 ± 0.045aB	1.277 ± 0.202abA	0.452 ± 0.081aA	1.001 ± 0.100aAB
	6A	0.269 ± 0.068bA	0.104 ± 0.004aA	0.243 ± 0.024aA	1.000 ± 0.170aA	0.499 ± 0.106aB	0.394 ± 0.034aB	0.946 ± 0.072bA	0.221 ± 0.037aB	0.369 ± 0.080bB
	12A	0.469 ± 0.081aA	0.199 ± 0.026aB	0.303 ± 0.103aAB	0.888 ± 0.161abA	0.343 ± 0.092aB	0.434 ± 0.098aAB	0.557 ± 0.331bcA	0.405 ± 0.117aA	0.386 ± 0.130bA
	0.5N	0.491 ± 0.138aA	0.052 ± 0.007aB	0.137 ± 0.022aB	0.780 ± 0.103abA	0.460 ± 0.082aAB	0.282 ± 0.069aB	1.552 ± 0.345aA	0.165 ± 0.019aB	0.530 ± 0.211abB
	1N	0.409 ± 0.035abA	0.201 ± 0.067aB	0.200 ± 0.064aB	0.627 ± 0.184abA	0.564 ± 0.077aA	0.313 ± 0.094aA	0.263 ± 0.076cA	0.180 ± 0.019aA	0.554 ± 0.109abA
	2N	0.489 ± 0.026aA	0.083 ± 0.015aB	0.192 ± 0.026aB	0.529 ± 0.126bA	0.439 ± 0.013aA	0.326 ± 0.108aA	0.654 ± 0.152bcA	0.174 ± 0.024aA	0.457 ± 0.052aAB
	4N	0.338 ± 0.013abA	0.108 ± 0.014aB	0.199 ± 0.030aAB	1.072 ± 0.057aA	0.378 ± 0.074aB	0.494 ± 0.055aB	1.118 ± 0.023abA	0.098 ± 0.034aB	0.462 ± 0.151abB
Glucose	Ctr	0.016 ± 0.003aA	0.027 ± 0.009bA	0.015 ± 0.001aA	0.006 ± 0.003aA	0.013 ± 0.003cA	0.005 ± 0.000cA	0.035 ± 0.003aA	0.021 ± 0.001abA	0.032 ± 0.159abA
	1A	0.007 ± 0.002aB	0.130 ± 0.111aA	0.007 ± 0.001aB	0.008 ± 0.001aB	0.034 ± 0.008aAB	0.017 ± 0.004abA	0.022 ± 0.004abA	0.027 ± 0.005abA	0.017 ± 0.098bA
	3A	0.007 ± 0.002aB	0.027 ± 0.009b A	0.021 ± 0.004aA	0.007 ± 0.001aA	0.012 ± 0.003bcA	0.009 ± 0.001bA	0.028 ± 0.007abA	0.039 ± 0.005aA	0.044 ± 0.100aA
	6A	0.011 ± 0.003aA	0.021 ± 0.013bA	0.014 ± 0.002aA	0.011 ± 0.002aA	0.019 ± 0.004bA	0.018 ± 0.002abA	0.026 ± 0.005abA	0.017 ± 0.003abA	0.019 ± 0.080bA
	12A	0.021 ± 0.003aA	0.014 ± 0.002bA	0.016 ± 0.005aA	0.012 ± 0.003aA	0.014 ± 0.003cbA	0.020 ± 0.003abA	0.018 ± 0.014abA	0.033 ± 0.010abA	0.021 ± 0.130abA
	0.5N	0.023 ± 0.006bA	0.008 ± 0.002bA	0.012 ± 0.002aB	0.006 ± 0.001aA	0.015 ± 0.003cbA	0.005 ± 0.001cA	0.025 ± 0.005abA	0.026 ± 0.002abA	0.021 ± 0.211bA
	1N	0.021 ± 0.001aA	0.024 ± 0.016bA	0.011 ± 0.004aA	0.003 ± 0.001aA	0.012 ± 0.001cA	0.008 ± 0.001bcA	0.008 ± 0.003bA	0.019 ± 0.006abA	0.020 ± 0.109bA
	2N	0.014 ± 0.002aA	0.008 ± 0.002bA	0.016 ± 0.003aA	0.005 ± 0.002aA	0.025 ± 0.001abA	0.017 ± 0.007abA	0.021 ± 0.006abA	0.013 ± 0.003bA	0.024 ± 0.052abA
	4N	0.013 ± 0.002aA	0.017 ± 0.007bA	0.010 ± 0.002aA	0.007 ± 0.001aB	0.017 ± 0.003cbA	0.028 ± 0.004aB	0.028 ± 0.009abA	0.039 ± 0.004bA	0.022 ± 0.151abA
Fructose	Ctr	0.010 ± 0.000aB	0.116 ± 0.056aA	0.003 ± 0.001aB	0.005 ± 0.002aB	0.023 ± 0.005abA	0.009 ± 0.001aB	0.012 ± 0.001aA	0.013 ± 0.001bA	0.007 ± 0.001bA
	1A	0.007 ± 0.001aA	0.013 ± 0.006bB	0.003 ± 0.001aA	0.007 ± 0.001aB	0.026 ± 0.005aA	0.010 ± 0.003aB	0.009 ± 0.001abA	0.028 ± 0.003aB	0.005 ± 0.002bB
	3A	0.006 ± 0.000aA	0.005 ± 0.002bA	0.006 ± 0.001aA	0.007 ± 0.001aA	0.016 ± 0.004abA	0.017 ± 0.003aA	0.010 ± 0.002abA	0.013 ± 0.001bA	0.018 ± 0.001aA
	6A	0.006 ± 0.001aA	0.004 ± 0.000bA	0.004 ± 0.001aA	0.006 ± 0.001aB	0.022 ± 0.004abA	0.015 ± 0.001aAB	0.009 ± 0.002abA	0.015 ± 0.003bA	0.007 ± 0.001bA
	12A	0.009 ± 0.001aA	0.005 ± 0.001bA	0.005 ± 0.002aA	0.010 ± 0.003aB	0.014 ± 0.002bA	0.016 ± 0.004aA	0.005 ± 0.003abA	0.017 ± 0.005bA	0.004 ± 0.002bB
	0.5N	0.008 ± 0.001aA	0.012 ± 0.008bA	0.003 ± 0.000aA	0.006 ± 0.001aB	0.019 ± 0.004abA	0.015 ± 0.003aAB	0.013 ± 0.002aA	0.010 ± 0.001bA	0.010 ± 0.004abA
	1N	0.008 ± 0.000aA	0.008 ± 0.002bA	0.004 ± 0.001aA	0.003 ± 0.001aB	0.018 ± 0.003abA	0.010 ± 0.001aAB	0.002 ± 0.001bB	0.014 ± 0.004bA	0.010 ± 0.001abAB
	2N	0.014 ± 0.002aA	0.030 ± 0.027bA	0.003 ± 0.000aA	0.005 ± 0.001aA	0.017 ± 0.001abA	0.012 ± 0.004aAB	0.006 ± 0.001abA	0.018 ± 0.004bA	0.009 ± 0.002abAB
	4N	0.008 ± 0.000aA	0.003 ± 0.000bA	0.004 ±0.001aA	0.006 ± 0.001aB	0.024 ± 0.004abA	0.018 ± 0.001aA	0.009 ± 0.001abA	0.031 ± 0.004aB	0.009 ± 0.003abB
Sorbitol	Ctr	0.054 ± 0.007bA	0.051 ± 0.006aA	0.085 ± 0.014aA	0.014 ± 0.006aA	0.025 ± 0.005aA	0.020 ± 0.002aA	0.041 ± 0.008aA	0.028 ± 0.011abA	0.056 ± 0.014abA
	1A	0.029 ± 0.008bA	0.005 ± 0.003aA	0.028 ± 0.010aA	0.017 ± 0.001aA	0.033 ± 0.009aA	0.020 ± 0.006aA	0.018 ± 0.002aA	0.058 ± 0.022abA	0.049 ± 0.011abA
	3A	0.021 ±	0.005 ±	0.098 ±	0.018 ±	0.034 ±	0.021 ±	0.024 ±	0.065 ±	0.089 ±

		0.006bA	0.001aA	0.016aA	0.001aA	0.010aA	0.002aA	0.006aB	0.011aAB	0.021aA
	6A	0.019 ± 0.006aA	0.005 ± 0.000aB	0.061 ± 0.006bA	0.022 ± 0.003aA	0.041 ± 0.008aA	0.023 ± 0.001aA	0.024 ± 0.005aA	0.028 ± 0.012abA	0.037 ± 0.008aAB
	12A	0.052 ± 0.013bA	0.025 ± 0.021aA	0.087 ± 0.042aA	0.023 ± 0.008aA	0.035 ± 0.009aA	0.030 ± 0.008aA	0.019 ± 0.014aA	0.049 ± 0.012abA	0.046 ± 0.016abA
	0.5N	0.056 ± 0.019bA	0.013 ± 0.010aA	0.043 ± 0.008aA	0.014 ± 0.001aA	0.032 ± 0.005aA	0.019 ± 0.005aA	0.034 ± 0.007aA	0.031 ± 0.011abA	0.042 ± 0.016bA
	1N	0.048 ± 0.003bA	0.003 ± 0.001aA	0.064 ± 0.022aA	0.010 ± 0.003aB	0.037 ± 0.006aA	0.026 ± 0.007aAB	0.007 ± 0.003aA	0.020 ± 0.004bA	0.045 ± 0.010abA
	2N	0.247 ± 0.148bA	0.001 ± 0.000aA	0.051 ± 0.006aA	0.012 ± 0.004aB	0.045 ± 0.003aA	0.029 ± 0.009aAB	0.018 ± 0.003aA	0.025 ± 0.002abA	0.050 ± 0.007abA
	4N	0.041 ± 0.003bA	0.002 ± 0.000aA	0.068 ± 0.013aA	0.017 ± 0.002aA	0.035 ± 0.006aA	0.037 ± 0.003aA	0.016 ± 0.003aA	0.017 ± 0.002bA	0.040 ± 0.010bA
Total	Ctr	0.510 ± 0.025abA	0.344 ±0.076aA	0.317 ± 0.037abA	0.816 ± 0.312abA	0.439 ± 0.118aAB	0.280 ± 0.060aB	1.507 ± 0.161aA	0.253 ± 0.040bB	0.685 ± 0.183abB
	1A	0.309 ± 0.057bA	0.255 ± 0.092aA	0.163 ± 0.050bA	0.770 ± 0.112abA	0.524 ± 0.129aA	0.270 ± 0.063aA	1.051 ± 0.233abA	0.511 ± 0.191aA	0.439 ± 0.114bA
	3A	0.275 ± 0.048bAB	0.125 ± 0.024aB	0.459 ± 0.062aA	1.024 ± 0.142abA	0.592 ± 0.171aAB	0.324 ± 0.052aB	1.339 ± 0.216aA	0.570 ± 0.096aB	1.151 ± 0.114aAB
	6A	0.305 ± 0.078bA	0.134 ± 0.013aA	0.322 ± 0.031abA	1.039 ± 0.173abA	0.581 ± 0.122aAB	0.450 ± 0.034aB	1.005 ± 0.079abA	0.280 ± 0.056bAB	0.433 ± 0.093bB
	12A	0.552 ± 0.097abA	0.244 ± 0.048aB	0.411 ± 0.152abA	0.932 ± 0.174abA	0.405 ± 0.106aB	0.500 ± 0.114aAB	0.599 ± 0.362bA	0.503 ± 0.144aA	0.457 ± 0.154bA
	0.5N	0.578 ± 0.163abA	0.086 ± 0.019aB	0.196 ± 0.032abA	0.806 ± 0.104abA	0.526 ± 0.094aA	0.321 ± 0.077aA	1.623 ± 0.356aA	0.233 ± 0.014bB	0.603 ± 0.239abA
	1N	0.488 ± 0.033aAB	0.235 ± 0.084aA	0.279 ± 0.090abA	0.643 ± 0.189abA	0.632 ± 0.087aA	0.357 ± 0.103aA	0.280 ± 0.083bA	0.233 ± 0.015bA	0.629 ± 0.123abA
	2N	0.765 ± 0.120aA	0.123 ± 0.013aB	0.263 ± 0.036abA	0.551 ± 0.132bA	0.527 ± 0.018aA	0.384 ± 0.126aA	0.700 ± 0.161bA	0.230 ± 0.028bA	0.540 ± 0.064abA
	4N	0.400 ± 0.018bA	0.130 ± 0.015aA	0.281 ± 0.045abA	1.102 ± 0.055aA	0.454 ± 0.087aB	0.577 ± 0.058aB	1.172 ± 0.036abA	0.185 ± 0.040bAB	0.533 ± 0.170abB

Different letters indicate significant different between treatments in each date (a or b) or between dates for the same treatment (A or B) ($p \leq 0.05$).

3.1.7.4 Protein content

Protein contents in tubers of Agria, Picasso, and Rossi varieties was assessed (**Table 3.20**). At harvest Agria exhibited a higher protein content in most treatments. For Rossi the highest protein content was observed after three months of storage, while for Picasso, all treatments (except 1N) showed the highest protein content after 6 months of storage, being lowest content found after 3 months of storage.

Table 3.20 Protein content (g/100 g_{FW}) at harvest (H), after three months (3M) and six months (6M) of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	**Protein content (g/100g of fresh weight)**		
	Harvest	3M	**6M**
		Agria	
Ctr	1.46 ± 0.2aA	1.39 ± 0.06aA	1.5 ± 0.07abA
1A	1.41 ± 0.05aA	1.21 ± 0.1abB	1.07 ± 0.06bB
3A	1.42 ± 0.04aA	1.29 ± 0.05abB	1.2 ± 0.07abB
6A	1.51 ± 0.21aA	1.21 ± 0.05abB	1.46 ± 0.11abA
12A	1.24 ± 0.05aB	1.02 ± 0.05bC	1.33 ± 0.05abA
0.5N	1.32 ± 0.05aA	1.38 ± 0.06aA	1.25 ± 0.03abB
1N	1.5 ± 0.17aA	1.15 ± 0.08abC	1.32 ± 0.06abB
2N	1.39 ± 0.06aA	1.16 ± 0.11abC	1.25 ± 0.1abB
4N	1.21 ± 0.01aB	1.11 ± 0.03abC	1.66 ± 0.21aA
		Picasso	
Ctr	1.08 ± 0.11abA	0.91 ± 0.06bcB	1.07 ± 0.04bA
1A	1 ± 0.08abA	0.81 ± 0.05cB	1.03 ± 0.07bA
3A	0.95 ± 0.08bB	0.99 ± 0.03abcB	1.15 ± 0.06bA
6A	1.08 ± 0.06abC	1.26 ± 0.06aB	1.36 ± 0.1bA
12A	1.2 ± 0.09abB	1.18 ± 0.04abB	1.34 ± 0.05bA
0.5N	0.9 ± 0.03bB	0.87 ± 0.04cB	1.09 ± 0.12bA
1N	1.29 ± 0.26abA	1.1 ± 0.08abcB	1.18 ± 0.05bB
2N	1.73 ± 0.33aB	0.95 ± 0.09abcC	1.82 ± 0.05aA
4N	1.15 ± 0.09abB	1.04 ± 0.1abcC	1.32 ± 0.11bA
		Rossi	
Ctr	1.27 ± 0.08aA	1.01 ± 0.04bB	1.24 ± 0.07abA
1A	1.16 ± 0.08aB	1.14 ± 0.04abB	1.47 ± 0.08aA
3A	1.38 ± 0.06aA	1.42 ± 0.06aA	0.77 ± 0.04bB
6A	1.21 ± 0.02aA	1.11 ± 0.06bB	0.78 ± 0.09bC
12A	1.29 ± 0.06aB	1.23 ± 0.04abB	1.46 ± 0.14aA
0.5N	1.24 ± 0.06aB	1.19 ± 0.03abB	1.46 ± 0.11aA
1N	1.14 ± 0.1aB	1.26 ± 0.08abB	1.37 ± 0.18aA
2N	1.11 ± 0.04aB	1.18 ± 0.04abA	*Not quantified due to spectrophetometer failure
4N	1.38 ± 0.08aA	1.21 ± 0.11abA	

Different letters indicate significant different between treatments at harvest or after 3 or 6 months of storage for each variety (a,b) or between harvest, after 3 or 6 months of storage in the same treatment (A,B) ($p \leq 0.05$).

3.1.7.5 Colorimetric parameters

Colorimetric parameters were analyzed in tubers at harvest (**Figure 3.10**) and after 3 months under storage conditions (**Table 3.21**). At harvest, scanning colorimetric analysis in the visible spectral region (450 – 650 nm) of the pulp of the tubers from the three varieties, revealed a maximum transmittance at 550 nm, which corresponds to the yellow color.

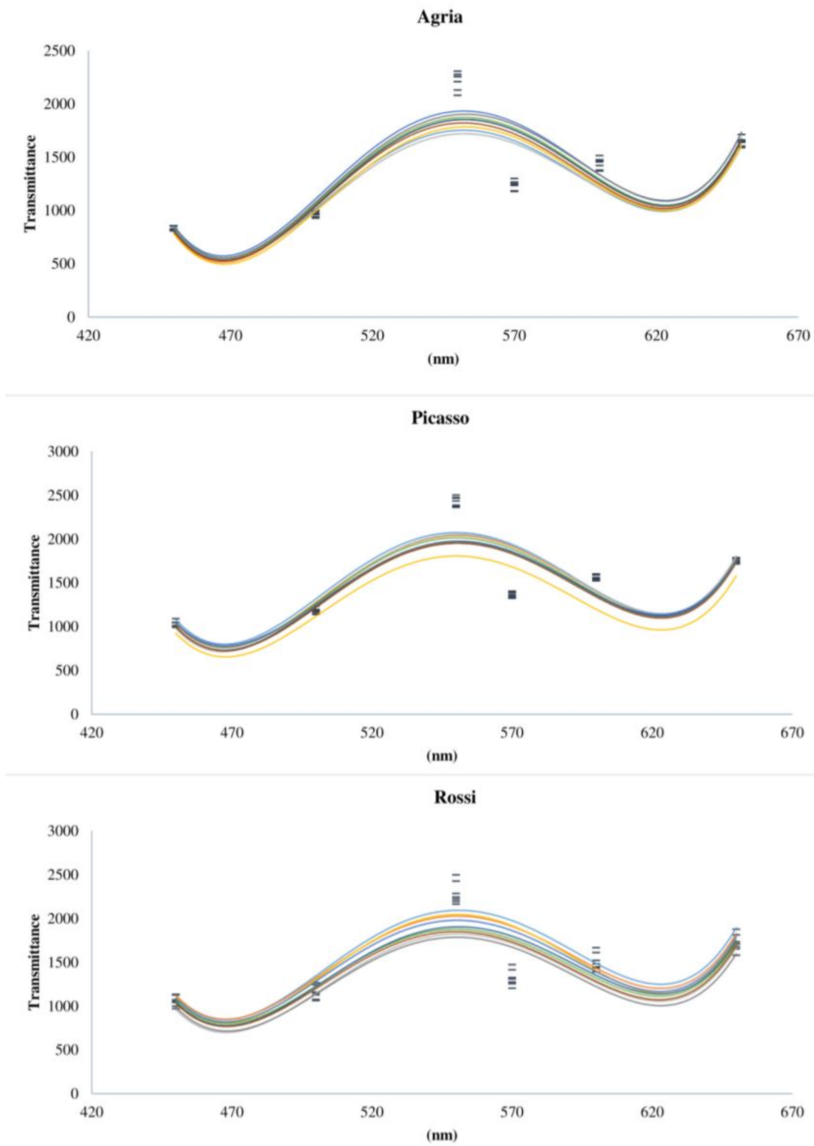


Figure 3.10 Visible spectra showing the average of transmittance *(n = 4)* in tubers of *Solanum tuberosum* L. varieties (Agria, Picasso and Rossi), at harvest, with a degree 4 polynomial (● Control, ● 0.5 kg ha^{-1} $Ca(NO_3)_2$, ● 1 kg ha^{-1} $Ca(NO_3)_2$, ● 2 kg ha^{-1} $Ca(NO_3)_2$, ● 4 kg ha^{-1} $Ca(NO_3)_2$, ● 1 kg ha^{-1} $CaCl_2$, ● 3 kg ha^{-1} $CaCl_2$, ● 6 kg ha^{-1} $CaCl_2$ and ● 12 kg ha^{-1} $CaCl_2$).

Colorimetric parameters of the tuber pulp were analyzed at harvest and after 3 months of storage using the CieLab system (**Table 3.21**). At harvest, significant differences between treatments were not found, except in in b* and Chroma on Picasso and Rossi. After 3 months of storage, significant differences between treatments could not be found in the three varieties.

Table 3.21 Color parameters considering the CieLab scale (L, a*, b*, chroma and hue) at harvest (H) and after three months of storage (3M) of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error) and different letters indicate significant different between treatments at harvest or after 3 months of storage for each variety (a,b) or between harvest and after 3 months of storage in the same treatment (A,B) ($p \leq 0.05$).

T.	L		a*		b*		Chroma		Hue	
	H	3M	H	3M	H	3M	H	3M	H	3M
Agria										
Ctr	54.4 ± 1.07aA	54.4 ± 0.81aA	-2.9 ± 0.14aB	-2.5 ± 0.16aA	20.5 ± 0.18aA	20.5 ± 0.34aA	20.7 ± 0.2aA	20.6 ± 0.32aA	98 ± 0.33aA	97.1 ± 0.54aA
1A	58.9 ± 0.76aA	54.2 ± 0.94aB	-2.9 ± 0.21aB	-2.5 ± 0.05aA	21.1 ± 0.78aA	20.8 ± 0.39aA	21.3 ± 0.8aA	20.9 ± 0.39aA	97.9 ± 0.36aA	97 ± 0.09aA
3A	58.8 ± 0.41aA	54.1 ± 0.02aB	-3 ± 0.17aB	-2.5 ± 0.1aA	22 ± 0.23aA	20.3 ± 0.17aB	22.2 ± 0.22aA	20.5 ± 0.17aA	97.7 ± 0.5aA	97.1 ± 0.29aA
6A	55.8 ± 1.06aA	56.8 ± 0.67aA	-2.8 ± 0.06aA	-3.1 ± 0.07aA	21.7 ± 1.13aA	22.2 ± 0.22aA	21.9 ± 1.13aA	22.4 ± 0.23aA	97.5 ± 0.24aA	97.9 ± 0.1aA
12A	59.6 ± 0.99aA	54.2 ± 1.59aB	-2.9 ± 0.07aB	-2.4 ± 0.15aA	22.6 ± 0.32aA	21.1 ± 0.67aA	22.8 ± 0.32aA	21.3 ± 0.68aA	97.4 ± 0.12aA	96.4 ± 0.23aA
0.5N	56.8 ± 1.96aA	55.7 ± 1.27aA	-2.9 ± 0.22aA	-2.7 ± 0.13aA	22.5 ± 0.69aA	21.5 ± 0.73aA	22.7 ± 0.71aA	21.6 ± 0.74aA	97.4 ± 0.36aA	97.3 ± 0.18aA
1N	55.5 ± 1.03aA	57.1 ± 1.86aA	-1.8 ± 0.81aA	-2.9 ± 0.06aB	21.5 ± 0.78aA	21.9 ± 0.41aA	21.6 ± 0.75aA	22.1 ± 0.41aA	95 ± 2.23aB	97.4 ± 0.16aA
2N	56.8 ± 0.71aA	56.3 ± 1.09aA	-2.9 ± 0.1aA	-3.1 ± 0.19aB	21.3 ± 0.4aA	20.9 ± 0.19aA	21.5 ± 0.41aA	21.1 ± 0.21aA	97.7 ± 0.15aA	98.4 ± 0.46aA
4N	59.5 ± 0.3aA	54.8 ± 0.69aB	-3.3 ± 0.06aB	-2.5 ± 0.18aA	22.3 ± 0.27aA	20.9 ± 0.2aB	22.5 ± 0.27aA	21.3 ± 0.18aA	98.5 ± 0.12aA	97.9 ± 0.52aA
Picasso										
Ctr	64.9 ± 0.28aA	61.7 ± 0.4aB	-2.5 ± 0.19aA	-3 ± 0.22aB	15 ± 0.14bB	17.5 ± 0.63aA	15.2 ± 0.11bB	21.3 ± 0.66aA	99.4 ± 0.78aA	97.3 ± 0.4aA
1A	62.6 ± 3.28aA	57.9 ± 0.14aB	-3.2 ± 0.22aA	-3.2 ± 0.13aA	15.8 ± 0.85bB	18.1 ± 0.46aA	16.2 ± 0.88bB	21 ± 0.47aA	101.3 ± 0.22aA	96.9 ± 0.17aB
3A	64.1 ± 1.79aA	57.2 ± 1.59aB	-3 ± 0.15aA	-2.9 ± 0.07aA	17.1 ± 0.45bA	16.5 ± 0.43aA	17.4 ± 0.46bB	19.7 ± 0.44aA	99.8 ± 0.37aA	98.3 ± 0.08aA
6A	63.2 ± 0.79aA	58.1 ± 1.77aB	-3 ± 0.06aA	-2.9 ± 0.17aA	15.8 ± 0.15bA	16.2 ± 0.65aA	16 ± 0.15bB	19.2 ± 0.67aA	100.7 ± 0.24aA	99.5 ± 0.29aA
12A	61.6 ± 0.98aA	62.3 ± 0.41aA	-3 ± 0.1aB	-2.7 ± 0.06aA	15.3 ± 0.5bA	16.3 ± 0.27aA	15.6 ± 0.51bB	17.8 ± 0.27aA	101.1 ± 0.02aA	99.8 ± 0.24aA
0.5N	63.5 ± 1.99aA	60.3 ± 0.35aA	-3.1 ± 0.16aB	-2.8 ± 0.14aA	16 ± 0.61bA	15.9 ± 0.58aA	16.3 ± 0.63bB	17.7 ± 0.59aA	101 ± 0.17aA	99.7 ± 0.13aA
1N	64.9 ± 2.17aA	58.2 ± 2.3aB	-3.8 ± 0.1bB	-3.2 ± 0.19aA	18.9 ± 0.53aA	17.9 ± 0.85aA	19.3 ± 0.53aA	17.7 ± 0.87aB	101.4 ± 0.21aA	99.6 ± 0.23aA
2N	59.9 ± 1.08aA	58.6 ± 0.81aA	-2.9 ± 0.2aA	-2.9 ± 0.16aA	14.4 ± 0.95bB	16.4 ± 0.57aA	14.7 ± 0.97bB	18.3 ± 0.59aA	101.2 ± 0.05aA	99.9 ± 0.23aA
4N	65.9 ± 0.97aA	58.9 ± 1.23aB	-2.5 ± 0.12aA	-3.3 ± 0.38aB	16.3 ± 0.25bA	17.1 ± 1.47aA	16.5 ± 0.26bB	18.5 ± 1.51aA	98.8 ± 0.27aA	99.9 ± 0.4aA
Rossi										
Ctr	58.8 ± 1.2aA	56.9 ± 0.67aA	-2.5 ± 0.16aA	-2.4 ± 0.12aA	14.4 ± 0.39bA	14 ± 0.63aA	14.6 ± 0.41aB	17.7 ± 0.63aA	99.9 ± 0.44aA	99.9 ± 0.53aA
1A	59.9 ± 2.09aA	57 ± 0.56aA	-2.2 ± 0.07aA	-2.2 ± 0.1aA	15 ± 0.63abA	13.6 ± 0.19aB	15.2 ± 0.63aA	16.7 ± 0.2aA	98.2 ± 0.08aA	100 ± 0.31aA
3A	61.4 ± 1.53aA	57.1 ± 1.03aA	-2.3 ± 0.04aA	-2.2 ± 0.11aA	15 ± 0.53abA	13.9 ± 0.37aB	15.2 ± 0.53aA	16.7 ± 0.38aA	98.8 ± 0.2aA	100 ± 0.24aA
6A	54.4 ± 1.84aB	59 ± 0.66aA	-2.1 ± 0.09aA	-2.3 ± 0.18aA	12.6 ± 0.33abB	14.5 ± 0.4aA	12.8 ± 0.34bB	16.1 ± 0.42aA	99.5 ± 0.17aA	99.9 ± 0.46aA
12A	62.1 ± 2.32aA	58.9 ± 1.38aA	-2.5 ± 0.1aA	-2.3 ± 0.1aA	14.7 ± 0.68bA	14.4 ± 0.25aA	14.9 ± 0.68aB	16.4 ± 0.26aA	99.5 ± 0.07aA	100.2 ± 0.24aA
0.5N	59.3 ± 0.99aA	58.2 ± 0.77aA	-2.8 ± 0.06aB	-2.2 ± 0.09aA	16.7 ± 0.45aA	13.8 ± 0.71aB	16.9 ± 0.44aA	16.1 ± 0.71aA	99.6 ± 0.44aA	99.8 ± 0.09aA
1N	63.3 ± 0.6aA	57.4 ± 1.08aB	-2.6 ± 0.1aA	-2.4 ± 0.07aA	14.2 ± 0.22bA	13.8 ± 0.39aA	14.4 ± 0.23aB	16.4 ± 0.4aA	100.5 ± 0.26aA	99.8 ± 0.1aA
2N	63.3 ± 0.91aA	59.7 ± 0.58aB	-2.7 ± 0.05aA	-2.6 ± 0.05aA	15.7 ± 0.2abA	14.5 ± 0.36aB	15.9 ± 0.2aA	16.5 ± 0.36aA	99.9 ± 0.15aA	99.4 ± 0.08aA
4N	60 ± 1.02aA	59.8 ± 0.69aA	-2.9 ± 0aA	-2.6 ± 0.05aA	15.1 ± 0.29abA	14.7 ± 0.36aA	15.3 ± 0.29aA	15.8 ± 0.36aA	100.8 ± 0.21aA	99.7 ± 0.22aA

3.1.7.6 Heat treatment of tuber pulp

Color analysis and texture were assessed after different cooking times (0, 5, 7.5, 10, 12.5, 15, 17.5, and 20 minutes) on the higher treatments of $CaCl_2$ and $Ca(NO_3)_2$ and in the three varieties (**Table 3.22** and **3.23**).

3.1.7.6.1 Color analysis

To study the color changes before and after cooking, an analysis of color parameters was conducted with the higher treatments of $CaCl_2$ and $Ca(NO_3)_2$, in the three varieties, at different times of cooking (0, 5, 7.5, 10, 12.5, 15, 17.5 and 20 minutes) (**Table 3.22**). In all three varieties, an increase in the cooking time resulted in a decrease in L (brightness) and Chroma (the relationship between parameters a* and b*) values, while the Hue parameter (saturation) exhibited an opposite trend. Consequently, samples with longer cooking times appeared darker, with lower intensity, and more saturated. Indeed, over the different cooking time, there were no significant differences between the two highest treatments with $CaCl_2$ and $Ca(NO_3)_2$.

Table 3.22 Colorimetric changes for each heat treatment of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, considering the control and the two highest treatments carried out with calcium chloride and calcium nitrate. Mean values (*n = 4*) ± SE (Standard Error).

Treatments	Cooking time (min)							
	0	5	7.5	10	12.5	15	17. 5	20
	Agria							
	L							
Ctr	69.1 ± 2.85a	61.4 ± 4.38a	58.5 ± 6.14a	63.0 ± 3.52a	55.4 ± 3.14b	58.9 ± 2.07a	58.7 ± 2.25a	59.5 ± 2.42a
12A	71.0 ± 2.37a	61.6 ± 1.31a	60.4 ± 3.35a	61.6 ± 4.24a	62.0 ± 2.98a	59.0 ± 0.42a	59.7 ± 4.52a	57.7 ± 0.08a
4N	71.0 ± 2.56a	62.1 ± 0.10a	60.5 ± 4.87a	60.8 ± 4.98a	61.9 ± 2.96a	61.0 ± 1.94a	62.8 ± 1.34a	62.0 ± 4.66a
	Chroma							
Ctr	32.4 ± 1.76a	29.4 ± 3.54a	28.0 ± 2.78a	28.8 ± 2.06a	25.5 ± 2.00b	24.1 ± 1.08a	27.7 ± 6.14a	24.4 ± 1.16a
12A	33.6 ± 2.35a	29.5 ± 0.80a	28.1 ± 2.16a	25.6 ± 4.68a	28.2 ± 0.66a	25.4 ± 1.42a	23.7 ± 1.15a	23.5 ± 0.59a
4N	33.0 ± 1.88a	27.4 ± 1.72a	27.7 ± 0.40a	26.4 ± 1.99a	30.5 ± 0.46a	24.5 ± 3.08a	23.6 ± 1.89a	26.2 ± 2.38a
	Hue							
Ctr	97.3 ± 0.64a	108 ± 0.97a	109 ± 1.12a	110 ± 0.74a	111 ± 1.30a	114 ± 1.13a	113 ± 0.38a	113 ± 0.84a
12A	97.4 ± 0.61a	107 ± 0.82a	108 ± 0.97a	110 ± 3.24a	110 ± 0.73a	112 ± 0.64a	113 ± 0.55a	113 ± 0.83a
4N	97.6 ± 0.94a	109 ± 1.51a	109 ± 1.67a	111 ± 0.33a	109 ± 0.90a	114 ± 2.92a	114 ± 2.18a	112 ± 0.96a
	Picasso							
	L							
Ctr	70.3 ± 1.6b	62.67 ± 7.2a	58.8 ± 3.2b	56.9 ± 4.9a	62.0 ± 3.5a	56.3 ± 7.3a	55.6 ± 3.7a	57.9 ± 5.0a
12A	73.4 ± 1.5a	64.2 ± 2.9a	62.1 ± 1.1ab	59.9 ± 7.3a	59.4 ± 1.0a	56.7 ± 4.2a	56.8 ± 3.6a	58.1 ± 4.6a
4N	69.9 ± 0.7b	65.1 ± 2.6a	64.2 ± 1.8a	61.9 ± 2.8a	63.5 ± 4.1a	63.9 ± 2.0a	61.4 ± 2.9a	60.1 ± 1.7a
	Chroma							
Ctr	20.1 ± 1.2a	13.4 ± 1.6a	12.5 ± 6.5a	12.9 ± 8.3a	11.4 ± 2.1a	10.4 ± 0.8ab	9.3 ± 0.2b	8.6 ± 1.6a
12A	20.5 ± 1.7a	14.3 ± 2.3a	14.8 ± 2.2a	9.8 ± 1.3a	10.5 ± 1.0a	9.9 ± 0.7b	10.3 ± 1.3b	9.6 ± 1.0a
4N	20.5 ± 2.0a	15.7 ± 6.7a	14.4 ± 2.7a	13.1 ± 0.5a	10.1 ± 2.1a	12.1 ±1.1a	11.7 ± 1.2a	9.8 ± 0.6a
	Hue							
Ctr	100 ± 0.1a	120 ± 2.8a	124 ± 3.4a	124 ± 1.1a	130 ± 6.6a	131 ± 2.1a	136 ± 0.9a	141 ± 9.1a
12A	101 ± 0.7a	120 ± 4.5a	120 ± 2.8a	130 ± 4.9a	132 ± 3.5a	134 ± 4.1a	132 ± 3.4ab	134 ± 4.4a
4N	100 ± 0.5a	121 ± 7.8a	123 ± 3.4a	126 ± 1.6a	134 ± 7.7a	129 ± 2.3a	129 ± 2.4b	135 ± 1.2a
	Rossi							
	L							
Ctr	71.5 ± 0.71a	63.4 ± 1.49ab	62.6 ± 4.48a	58.4 ± 3.91b	65.1± 1.28a	60.4 ± 4.56a	63.4 ± 5.47a	60.7 ± 3.37a
12A	70.8 ± 2.25a	65.4 ± 0.17a	59.5 ± 2.27a	60.3 ± 2.75ab	62.1 ± 4.06a	58.7 ± 5.18a	60.8 ± 2.29a	59.3 ± 1.37a
4N	71.9 ± 2.74a	61.4 ± 1.77b	60.6 ± 0.56a	64.1 ± 0.57a	60.4 ± 3.82a	61.3 ± 0.04a	59.7 ± 1.95a	63.0 ± 4.41a
	Chroma							
Ctr	19.1 ± 0.26a	14.1 ± 0.52a	12.6 ± 2.30a	10.4 ± 1.93b	13.5 ± 1.04a	10.6 ± 0.46a	11.1 ± 1.66a	10.0 ± 0.95a
12A	19.5 ± 1.09a	16.3 ± 3.51a	11.2 ± 2.08a	11.4 ± 0.11ab	13.3 ± 1.89a	10.4 ± 3.11a	9.7 ± 1.59a	8.53[a] ± 0.33a
4N	19.5 ± 0.37a	12.3 ± 1.23a	11.6 ± 1.76a	13.4 ± 1.02a	10.5 ± 1.73a	10.7 ± 0.70a	15.0 ± 9.71a	11.0 ± 2.46a
	Hue							
Ctr	101 ± 0.40a	116 ± 3.01a	123 ± 4.62a	128 ± 4.56a	122 ± 1.95ab	128 ± 1.75a	128 ± 3.97a	131 ± 4.07a
12A	100 ± 1.19a	116 ± 5.38a	126 ± 5.03a	128 ± 1.18a	124 ± 1.90b	131 ± 7.93a	135 ± 6.21a	138 ± 3.05a
4N	102 ± 1.85a	123 ± 2.37a	125 ± 4.06a	125 ± 2.82a	133 ± 5.23a	131 ± 3.37a	131 ± 7.84a	130 ± 5.08a

Different letters indicate significant differences for each parameter between treatments ($p \leq 0.05$).

The values obtained at 0 min in the three varieties were different compared to the ones obtained in **Table 3.22**, due to the analysis conditions and the equipment being different.

3.1.7.6.2 Texture

Fracturability, work strength, number of peaks, hardness, and adhesiveness were studied in the three varieties submitted to the highest treatments of $CaCl_2$ and $Ca(NO_3)_2$ to assess the textural differences between varieties and Ca biofortification treatments (**Table 3.23**).

After 20 minutes, at 100 °C, Ca applications with the highest concentration applied of $CaCl_2$ and $Ca(NO_3)_2$ showed no depreciative effects in the different texture parameters analyzed (**Table 3.23**). In this context, the range of values obtained in the control, 12A and 4N treatments, after cooking during 20 minutes, for fracturability, work strength, number of peaks, hardness and adhesiveness were, respectively, 0.65-0.90 N, 7.77-10.38 $N*s^{-1}$, 2-3, 0.74-0.94 N, and 0.98-126 -N*s. Although, despite not being significantly different, at 20 minutes, the control showed the lowest values compared to the Ca biofortification treatments in fracturability, work strength, hardness, and adhesiveness. Regarding Agria, significant differences were not found among treatments, considering fracturability and hardness in each time of cooking. Concerning the work of strength, relatively to the Ca biofortification treatments, the control showed a significant lower value at 7.5 minutes, but there was a significantly lower value at 10 and 17.5 minutes for the 12A treatment and at 5 minutes for the 4N treatment. Considering the number of peaks, treatment 12A showed a significantly lower value at 7.5 minutes and treatment 4N at 0, and 7.5 minutes. Regarding adhesiveness, the control presented a significantly lower value at 12.5 min and treatment 12A at 17.5 min. Picasso was the variety with less significant differences among parameters regarding the different times of cooking (in fact, only showed significantly lower values in the number of peaks at 5 minutes in the 12A treatment and, in 4N treatment, in adhesiveness, at 7.5 and 15 minutes. In the other hand, Rossi variety, showed significant differences in fracturability, work strength, number of peaks and hardness. Considering the 12A treatment, Rossi showed a significantly lower values in fracturability at 5 and 10 minutes, in work strength at 10 and 17.5 minutes and in the number of peaks at 5 minutes. Regarding the 4N treatment, Rossi showed a significantly lower values at only 0 minutes in work strength and hardness.

Table 3.23 Fracturability, work strength, number of peaks, hardness, and adhesiveness for each heat treatment of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, considering the control and the two highest treatments carried out with calcium chloride and calcium nitrate at different times of cooking (0, 5, 7.5, 10, 12.5, 15, 17.5 and 20 minutes). Mean values ($n = 4$) ± SE (Standard Error). Fracturability - F. (N); Work strength – WS ($N*s^{-1}$), Number of peaks - NP, Hardness - H (N), Adhesiveness - Ad (-N*s).

T.	Time (min)	F.	WS	NP	H.	Ad.	F.	WS	NP	H.	Ad.	F.	WS	NP	H.	Ad.
		Agria					Picasso					Rossi				
Ctr	0	13.5 ± 1.17a	220 ± 16.66a	48 ± 3ab	20.16 ± 2.12a	9.39 ± 1.60a	12.03 ± 0.81a	220 ± 14.00a	40 ± 4a	20.36 ± 2.65a	6.07 ± 0.49a	14.06 ± 0.30a	271 ± 15.32a	37 ± 4a	26.08 ± 1.16a	10.06 ± 2.51a
	5	8.07 ± 0.75a	127 ± 2.68a	26 ± 2a	12.03 ± 0.61a	2.58 ± 0.38a	6.23 ± 0.75a	107 ± 12.05a	26 ± 4a	9.99 ± 0.97a	2.65 ± 0.33b	9.55 ± 1.29a	118 ± 7.30a	20 ± 4ab	11.40 ± 0.65a	3.39 ± 0.61a
	7.5	3.22 ± 0.47a	57.70 ± 6.39b	23 ± 1a	7.24 ± 3.03a	2.28 ± 0.10a	3.97 ± 0.72a	74.39 ± 1.97a	17 ± 2a	7.34 ± 0.37a	2.23 ± 0.16ab	3.67 ± 0.36a	82.99 ± 9.46a	18 ± 4a	9.21 ± 1.46a	2.43 ± 0.15a
	10	2.27 ± 0.53a	43.15 ± 4.20a	13 ± 2a	4.33 ± 0.51a	1.81 ± 0.44a	1.91 ± 0.20a	35.47 ± 3.26a	13 ± 1a	3.57 ± 0.35a	1.75 ± 0.05a	3.52 ± 0.44a	48.30 ± 4.05a	12 ± 2a	4.01 ± 1.64a	2.00 ± 0.32a
	12.5	1.17 ± 0.24a	16.50 ± 3.69a	9 ± 1a	1.69 ± 0.32a	0.96 ± 0.01b	1.21 ± 0.28a	18.05 ± 2.08a	8 ± 1a	1.79 ± 0.35a	1.46 ± 0.27a	1.54 ± 0.27a	25.05 ± 6.02a	7 ± 2a	2.23 ± 0.52a	2.24 ± 0.62a
	15	1.06 ± 0.02a	12.62 ± 0.31a	7 ± 1a	1.32 ± 0.11a	1.08 ± 0.19a	0.67 ± 0.24a	9.31 ± 2.51a	4 ± 1a	0.92 ± 0.27a	0.92 ± 0.17a	0.75 ± 0.10a	13.42 ± 1.45a	4 ± 1a	1.35 ± 0.17a	1.33 ± 0.21a
	17.5	0.70 ± 0.11a	8.69 ± 1.29b	4 ± 1a	0.83 ± 0.17a	0.77 ± 0.06b	0.66 ± 0.11a	8.44 ± 0.55a	3 ± 1a	0.76 ± 0.08a	1.04 ± 0.23a	1.21 ± 0.71a	16.63 ± 4.60a	3 ± 2a	1.25 ± 0.68a	1.21 ± 0.63a
	20	0.65 ± 0.23a	7.77 ± 0.40a	3 ± 1a	0.74 ± 0.16a	0.98 ± 0.18a	0.72 ± 0.15a	8.16 ± 1.55a	3 ± 1a	0.82 ± 0.15a	1.00 ± 0.22a	0.80 ± 0.30a	7.96 ± 1.02a	2 ± 0a	0.93 ± 0.24a	1.27 ± 0.40a
12A	0	13.5 ± 0.45a	212 ± 11.23a	56 ± 5a	18.42 ± 1.61a	11.83 ± 2.09a	11.6 ± 0.28a	152 ± 85.25a	30 ± 13a	17.76 ± 2.35a	6.01 ± 0.38a	14.22 ± 0.49a	247 ± 24.98ab	39 ± 7a	21.95 ± 1.07ab	8.40 ± 1.27a
	5	6.44 ± 1.72a	116 ± 8.37ab	27 ± 4a	10.93 ± 1.22a	2.38 ± 0.51a	5.43 ± 2.98a	72.84 ± 39.31a	16 ± 6b	8.86 ± 0.21a	2.37 ± 0.18ab	5.36 ± 1.04b	95.52 ± 1.78a	16 ± 2b	9.98 ± 0.58a	3.46 ± 0.70a
	7.5	3.52 ± 0.29a	71.55 ± 3.82ab	19 ± 1b	6.92 ± 0.50a	2.09 ± 0.23a	5.61 ± 3.21a	54.15 ± 31.90a	13 ± 5a	7.10 ± 1.14a	1.81 ± 0.16a	5.65 ± 2.04a	64.96 ± 4.19a	16 ± 2a	7.53 ± 1.56a	2.75 ± 0.69a
	10	1.65 ± 0.44a	36.8 ± 1.33b	14 ± 2a	3.75 ± 0.07a	1.59 ± 0.35a	1.60 ± 0.39a	27.18 ± 8.98a	11 ± 6a	3.05 ± 0.70a	1.48 ± 0.30a	2.21 ± 0.43b	36.55 ± 2.27b	11 ± 2a	3.48 ± 0.17a	2.54 ± 0.36a
	12.5	1.15 ± 0.32a	20.46 ± 3.86a	9 ± 2a	2.16 ± 0.42a	1.34 ± 0.25ab	1.35 ± 0.48a	19.51 ± 6.56a	9 ± 1a	1.65 ± 0.37a	1.50 ± 0.15a	1.52 ± 0.30a	24.28 ± 3.88a	7 ± 0a	2.50 ± 0.39a	2.23 ± 0.28a
	15	0.83 ± 0.18a	13.18 ± 1.29a	6 ± 1a	1.25 ± 0.20a	1.24 ± 0.08a	0.97 ± 0.19a	13.84 ± 3.08a	5 ± 0a	1.23 ± 0.20a	1.43 ± 0.37ab	1.04 ± 0.49a	18.36 ± 3.51a	4 ± 1a	1.44 ± 0.56a	1.38 ± 0.47a
	17.5	0.75 ± 0.15a	8.92 ± 1.78b	3 ± 1b	0.85 ± 0.20a	0.90 ± 0.23b	0.86 ± 0.24a	9.35 ± 2.89a	4 ± 2a	0.90 ± 0.25a	0.94 ± 0.24a	0.82 ± 0.25a	10.49 ± 1.31b	3 ± 1a	0.97 ± 0.23a	1.25 ± 0.11a
	20	0.84 ± 0.15a	9.52 ± 0.67a	2 ± 0a	0.86 ± 0.12a	1.12 ± 0.12a	0.72 ± 0.07a	9.06 ± 0.92a	3 ± 1a	0.78 ± 0.07a	0.90 ± 0.14a	0.76 ± 0.08a	9.83 ± 2.16a	3 ± 1a	0.82 ± 0.08a	1.36 ± 0.37a
4N	0	12.47 ± 0.15a	201 ± 14.14a	43 ± 2b	20.55 ± 5.34a	9.32 ± 2.02a	11.1 ± 0.58a	175 ± 26.71a	40 ± 7a	15.65 ± 2.04a	6.42 ± 0.28a	11.85 ± 0.26b	207 ± 26.40b	35 ± 1a	18.55 ± 3.28b	7.60 ± 0.42a

5	7.84 ± 2.23a	113 ± 2.82b	30 ± 9a	11.24 ± 1.50a	2.29 ± 0.44a	5.10 ± 0.43a	96.99 ±10.11a	23 ± 1a	9.16 ± 1.04a	2.07 ± 0.09a	8.93 ± 0.97a	115.85 ± 19.18a	23 ± 1a	10.84 ± 2.22a	2.80 ± 0.26a
7.5	3.18 ± 0.45a	75.42 ± 9.82a	19 ± 2b	7.80 ± 1.31a	2.26 ± 0.12a	3.32 ± 0.76a	70.86 ± 0.53a	15 ± 2a	6.79 ± 0.30a	2.36 ± 0.29b	3.52 ± 0.74a	77.63 ± 17.64a	18 ± 5a	7.67 ± 2.20a	2.53 ± 0.39a
10	2.15 ± 0.36a	40.26 ± 3.26ab	14 ± 1a	3.47 ± 1.35a	1.41 ± 0.24a	1.76 ± 0.15a	34.61 ± 1.17a	11 ± 1a	3.26 ± 0.17a	1.77 ± 0.18a	2.58 ± 0.27b	44.97 ± 8.36ab	12 ± 4a	4.61 ± 1.12a	2.40 ± 0.59a
12.5	1.22 ± 0.05a	20.70 ± 2.51a	9 ± 0a	2.06 ± 0.26a	1.43 ± 0.29a	1.30 ± 0.62a	18.17 ± 3.94a	8 ± 0a	1.80 ± 0.21a	1.40 ± 0.41a	1.53 ± 0.79a	22.21 ± 6.65a	8 ± 2a	2.16 ± 0.56a	1.88 ± 0.72a
15	0.91 ± 0.14a	12.81 ± 2.08a	4 ± 2a	1.16 ± 0.14a	1.36 ± 0.30a	1.18 ± 0.59a	16.49 ± 6.16a	5 ± 1a	1.47 ± 0.44a	1.66 ± 0.47b	1.36 ± 0.67a	17.56 ± 4.94a	6 ± 3a	1.85 ± 0.78a	1.48 ± 0.25a
17.5	0.87 ± 0.12a	12.99 ± 2.25a	4 ± 1a	1.11 ± 0.25a	1.37 ± 0.33a	0.90 ± 0.26a	10.67 ± 3.10a	5 ± 1a	1.03 ± 0.25a	1.04 ± 0.41a	0.87 ± 0.08a	12.03 ± 1.91ab	4 ± 1a	1.02 ± 0.18a	1.43 ± 0.33a
20	0.90 ± 0.27a	10.38 ± 2.64a	3 ± 1a	0.94 ± 0.23a	1.26 ± 0.41a	0.77 ± 0.14a	8.02 ± 0.92a	2 ± 0a	0.78 ± 0.14a	1.01 ± 0.25a	0.76 ± 0.17a	9.67 ± 2.63a	2 ± 1a	0.83 ± 0.27a	1.02 ± 0.34a

Different letters indicate significant differences for each parameter between treatments considering each time of cooking ($p \leq 0.05$).

3.1.8 Yield

Total yield of the potato tubers submitted to the different treatments was determined at harvest, with 57 plants per treatment of each variety (**Table 3.24**). Tuber's yield varied across each treatment and variety. Considering Agria variety, the highest yield was obtained in the 6A treatment, followed by the control, 0.5N and 1A treatments, being the lowest yield found in the 12A treatment. However, in Picasso the highest yield was found in the 2N treatment, followed by the 2N, 3A, and 6A treatments, whereas the lowest was obtained in tubers submitted to treatment 1A. In Rossi, the highest yield was obtained with the 0.5N treatment followed by 1N, 1A, and 3A treatments, being the lowest yield obtained in the 2N treatment.

Table 3.24 Total yield at harvest of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties.

T.	Total Yield (kgs)		
	Agria	Picasso	Rossi
Ctr	112	106.5	83
1A	104.8	96.1	91.7
3A	97.4	110.1	89
6A	113.5	106.6	84.3
12A	83.6	104.2	76.9
0.5N	106.3	116.6	118.6
1N	104.7	131.2	110
2N	91.9	132.4	71
4N	97.4	109	81.9

Considering the total yield for the three varieties (**Table 3.24**) it was found that each variety showed different patterns relative to the applied Ca treatments, and that the yields varied according to the response of the variety to the different treatments (thus, concentrations of calcium chloride or calcium nitrate).

3.2 Second year

In the second year, the biofortification workflow with Ca was implemented for Agria, Rossi, and Picasso varieties cultivated in fields B, C, and A, respectively. The workflow involved seven foliar applications initiated after the beginning of tuberization with two concentrations of $CaCl_2$ (12 and 24 $kg.ha^{-1}$) or Ca-EDTA (12 and 24 $kg.ha^{-1}$). Considering the insights gained from the results of the first year of the experiment and previous studies (**chapter 3** - **section 3.1**), It was decided to maintain the application of $CaCl_2$ excluding the application of $Ca(NO_3)_2$ (which was replaced by Ca-EDTA). The replacement by Ca-EDTA was considered because the highest application of $Ca(NO_3)_2$ affected the yield in the highest two treatments (**Table 3.24**), being in accordance with previous studies carried out in potatoes that showed a decrease in the number of tubers/plant (Hamdi et al., 2015; El-Hadidi et al., 2017). Also, successful applications of Ca-EDTA via foliar spraying have been documented in other crops, such as in sweet corn (eight foliar sprays showed an increase in Ca content) (El-Yazied et al., 2007) and in Pineapple (four foliar sprays) (Loekito et al., 2022). However, these studies were not conducted in Portugal or did not specifically address the biofortification of Agria, Rossi, and Picasso varieties. The chosen concentrations aimed to evaluate plant responses based on the highest concentration applied of $CaCl_2$ in the first year, with an additional assessment involving the doubling of that concentration. In this context, identical concentrations were applied for both $CaCl_2$ and Ca-EDTA to have a direct comparison between both applied products. Nevertheless, as highlighted in **Chapter 2 (section 2.2)** the 24 $kg.ha^{-1}$ Ca-EDTA was administered only once due to observed toxicity symptoms in *Solanum tuberosum* L. plants.

3.2.1 Irrigation Water

As in the initial experimental year, we conducted an analysis of the physical and chemical composition of the irrigation water to evaluate its effects on soil, crops, and the irrigation equipment. Consequently, was carried out the classification of the physical and chemical parameters of the irrigation water of the three experimental fields (**Table 3.25**).

Table 3.25 Physical and chemical parameters of irrigation water of the experimental fields selected for Ca biofortification of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, respectively. Electrical conductivity (EC) at 20 ºC.

Field	pH	EC	$Ca^{2\pm}$	$K^{\pm}$	$Mg^{2\pm}$	$Na^{\pm}$	Cl^{-}	HCO_3^{-}	SO_4^{2-}	PO_4^{3-}
		$\mu S.cm^{-1}$	mg.L^{-1} (meq.L^{-1})							
A	7.1	1427	176.6 (8.8)	4.1 (0.1)	14.5 (1.2)	38.7 (1.6)	78 (2.2)	297.6 (4.9)	176 (3.7)	<1.5 (< 0.04)
B	7.3	1403	173.7 (8.6)	18.3 (0.4)	15.6 (1.3)	48.2 (2.1)	79 (2.2)	310.4 (5.1)	264 (5.5)	<1.5 (< 0.04)
C	7.1	1495	178.2 (8.7)	4.3 (0.1)	30.2 (2.5)	138.9 (6.0)	80 (2.3)	335.5 (5.6)	283 (5.8)	<1.5 (< 0.04)

The pH of the experimental fields was 7.1, 7.3 and 7,1, respectively for A, B and C (**Table 3.25)**. The electrical conductivity ranged from 1403 to 1495 μS.cm^{-1}. Additionally, water classification was determined using the Piper Diagram (**Figure 3.11** and **Annex VII**). The diagram indicates that the irrigation water from the three experimental fields is from underground origin with calcium sulfated bicarbonate hydrochemical facies considering fields B and C, and calcium bicarbonate in field A.

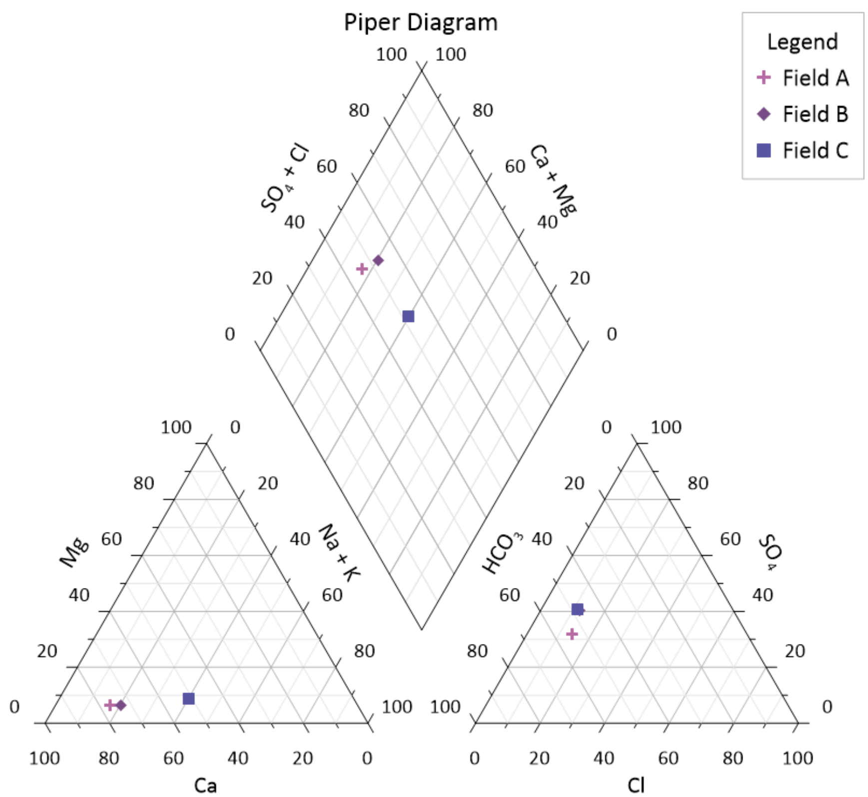


Figure 3.11 Piper Diagram of the three experimental fields (A, B and C).

The three fields showed a high salinity in terms of EC (at 20 ºC, between 750 and 2250 μS.cm^{-1}), and considering the SAR index (**Table 3.26**), belonging to class C3S1 (**Figure 3.12** and **Annex VII**).

Table 3.26 SAR index, pHe and LSI of the irrigation water of the selected experimental fields for Ca biofortification of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, respectively. Sodium Adsorption Ration (SAR); Saturation pH (pHs); Langelier Saturation (LSI).

Field	SAR	pHs	LSI
A	0.71	7.2	-0.074
B	0.94	7.2	0.14
C	2.53	7.1	0.026

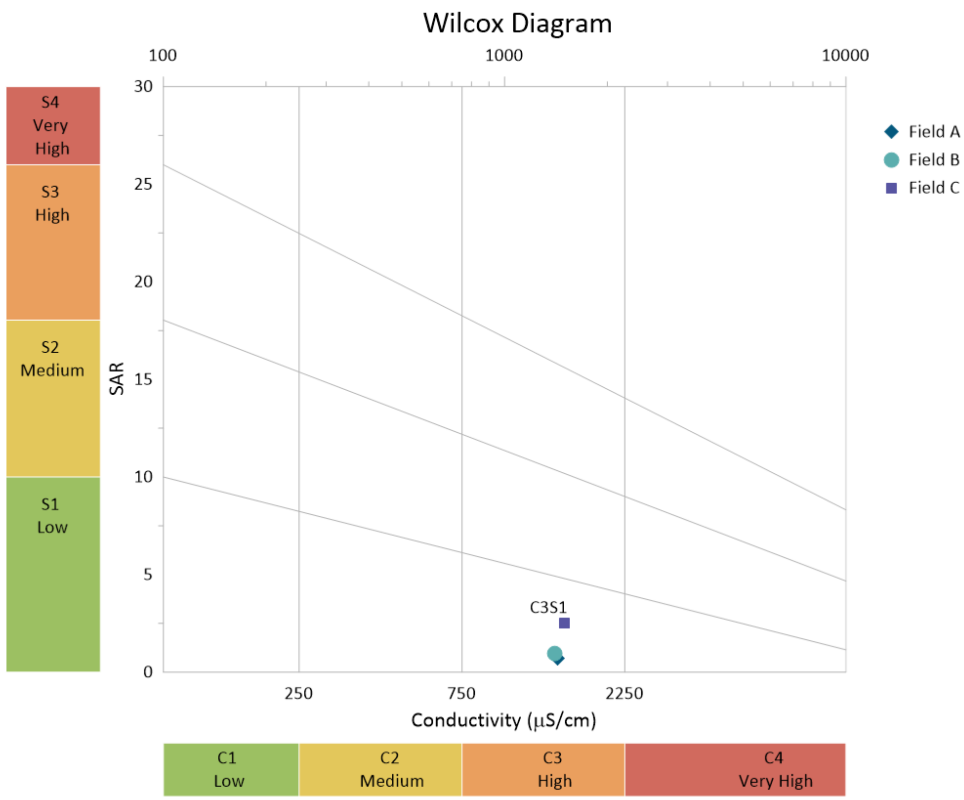


Figure 3.12 Wilcox Diagram of the three experimental fields (A, B and C).

3.2.2 Remote detection

Remote detection provided valuable insights into the distribution, vigor, and productivity of Ca biofortification in *Solanum tuberosum* L. plants. This information was derived from the analysis of vegetation index maps, obtained by processing the multispectral images, collected at different stages of plant development in the three experimental fields. Considering the NDVI model, vegetation information could be obtained, and the maps were interpreted by discerning color variations, being the green color used to illustrate dense and lush vegetation (NDVI values close to 1), the light green to yellow color used to represent values close to zero and orange tones representing the dry vegetation and/or soil, having negatives values. As such, the higher NDVI values are represented in green, while the lower values are in red. Relatively to fields B and C, in field A NDVI is represented in the opposite way (green for lower values and red for higher values) (**Figure 3.13**).

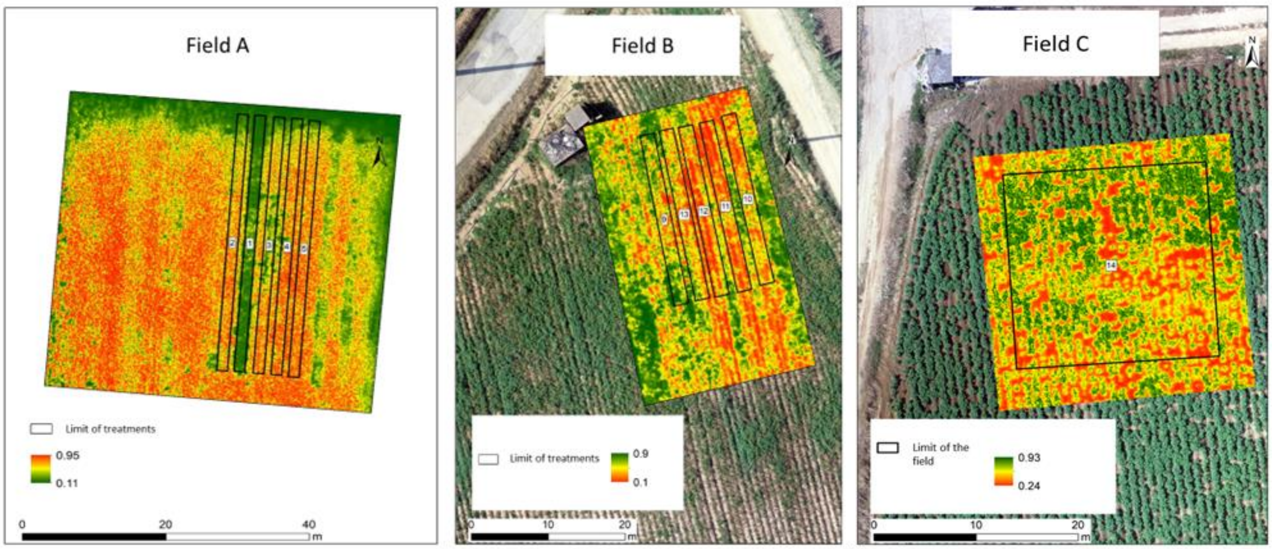


Figure 3.13 NDVI model of the three experimental fields (A, B and C). The NDVI was carried out in field A on June 25 of 2019 (4 days after the 4th foliar application), in field B on July 10 of 2019 (6 days after the 6th foliar application) and in field C on July 10 of 2019 (without any foliar application being carried out).

In **Table 3.27** is represented the NDVI of *Solanum tuberosum* L. plants in the three experimental fields.

Table 3.27 Minimum, maximum and the average of NDVI (± SD) of the different treatments in field A and B and its standard deviation, during the biofortification process. $CaCl_2$ 12 kg/ha (12A), $CaCl_2$ 24 kg/ha (24A), Ca-EDTA 12 kg/ha (12B) and Ca-EDTA 24 kg/ha (24B).

Code	Treatment	Minimum NDVI	Max NDVI	Average NDVI
		Field A		
1	12B	0.25	0.91	0.81 ± 0.11
2	24B	0.48	0.93	0.90 ± 0.03
3	Ctr	0.21	0.93	0.89 ± 0.07
4	24A	0.45	0.94	0.90 ± 0.04
5	12A	0.40	0.94	0.91 ± 0.03
		Field B		
9	Ctr	0.17	0.88	0.65 ± 0.16
10	12A	0.13	0.85	0.50 ± 0.15
11	24A	0.11	0.82	0.40 ± 0.15
12	12B	0.12	0.83	0.44 ± 0.17
13	24B	0.18	0.85	0.54 ± 0.17

Field A showed a positive response after the 4^{th} foliar application with Ca, as all plots have a medium/high NDVI value (> 0.8) very similar to the NDVI of the control plot) **(Figure 3.13** and **Table 3.27)**. Also, treatment 24B (that was only applied once) and both treatments with $CaCl_2$ showed, relatively to the control, a higher NDVI mean/average. In fact, only treatment 12B showed a lower NDVI when compared to the control. In fact, the NDVI map already showed the beginning of toxicity symptoms of Ca-EDTA in the *Solanum tuberosum* L. plants. Nevertheless, regarding field B, after the 6^{th} foliar application, the density of lush foliage decreased, and this effect could be associated with adverse/negative effects on the plant resulting of foliar applications, mainly in the 24A treatment. In fact, all the treatments applied in field B showed a lower NDVI average relatively to the control plot. Moreover, considering **Figure 3.14**, it was possible to verify that, globally potato plants in field A showed a medium/high NDVI value (> 0.8). Also, field A showed the highest general NDVI and field B the lowest NDVI. Yet, it is important to consider that field A and B were in different states of plant development (*i.e.,* plants in field A were less developed). Moreover, field C had not yet implemented the crop, so the general NDVI showed a higher value due to the weeds presented in it.

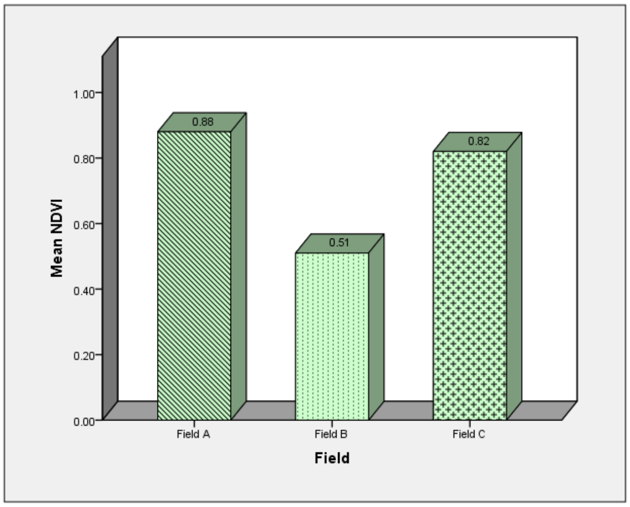


Figure 3.14 General average NDVI for field (A, B and C).

3.2.3 Monitoring of the culture in the fields

Monitoring *in situ* of *Solanum tuberosum* L. development occurred in both fields (**Figure 3.15** and **Figure** 3.16). Regarding field A (**Figure 3.15**), after six foliar applications, Picasso variety showed major negative effects in 12B treatment, despite some minor negative effects in both concentrations of $CaCl_2$. Moreover, 24 kg·ha^{-1} Ca-EDTA (24B) treatment and control plants have a very similar appearance.

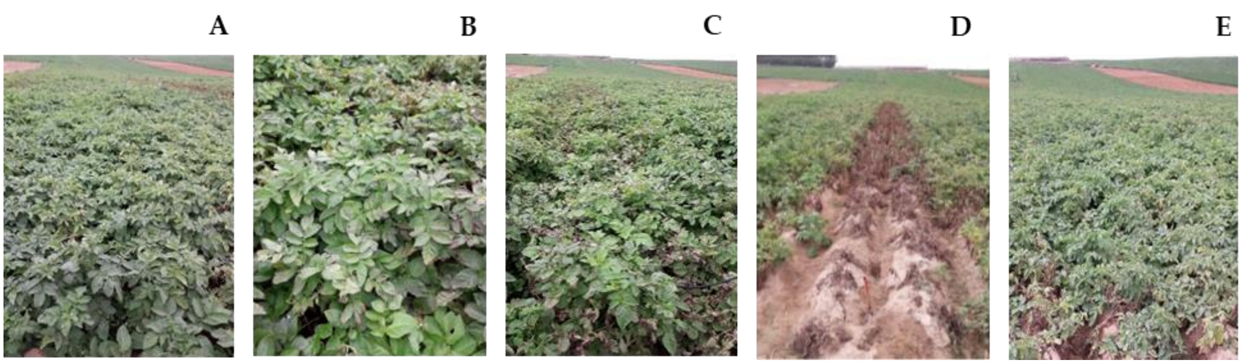


Figure 3.15 Production overview of *Solanum tuberosum* L. in field A (Picasso variety) after six foliar applications: A – Control; B- $CaCl_2$ 12 kg·ha^{-1} (12A); C- $CaCl_2$ 24 kg·ha^{-1} (24A); D- Ca-EDTA 12 kg·ha^{-1} (12B); E- Ca-EDTA 24 kg·ha^{-1} (24B).

In field B (**Figure 3.16**), after six foliar applications, Agria variety showed signs of toxicity with both concentrations of $CaCl_2$ (having more visual damage in the highest concentration) and in the 12B treatment when the toxicity symptoms caused by the Ca-EDTA were more evident.

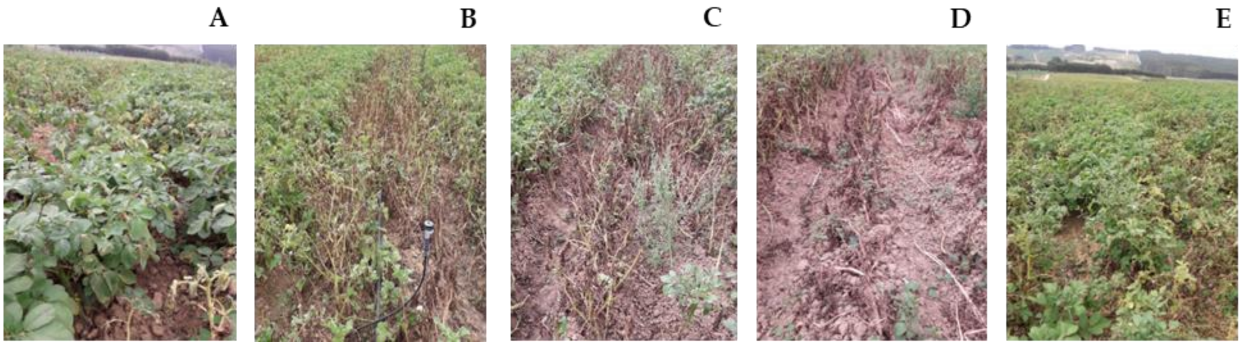


Figure 3.16 Production overview of *Solanum tuberosum* L. in field B (Agria variety) after six foliar applications: A – Control; B- $CaCl_2$ 12 kg·ha^{-1} (12A); C- $CaCl_2$ 24 kg·ha^{-1} (24A); D- Ca-EDTA 12 kg·ha^{-1} (12B); E- Ca-EDTA 24 kg·ha^{-1} (24B).

3.2.4 Photosynthetic functioning

The aim of the evaluation of photosynthetic functioning was to assess whether Ca biofortification has an impact on the synthesis of photoassimilates in *Solanum tuberosum* L. plants. The assessments were conducted after the third (June 21), six (July 11) and seven (July 24) foliar applications in Agria and Picasso varieties. For Rossi variety, measurements were taken before any foliar application and after two foliar applications.

3.2.4.1 Infrared foliar gas exchange

Since Agria and Picasso varieties were analyzed after the same foliar applications, it was possible to observe similar values after the 3rd foliar application in each parameter analyzed (**Table 3.28**).

Moreover, differences between both varieties start to occur after six foliar applications. Additionally, should be pointed out that in the case of the maximum dose of Ca-EDTA (24B), only one application was made as the plants remained immediately affected. Thus, the results presented refer to later developed leaves (reflecting a possible recovery of the plants), which are often better than those presented at the 12B treatment. Therefore, the 24B treatment was discarded. Regarding the net photosynthetic rate (P_n), the 12A treatment had no impact throughout the evaluations in Agria and showed a moderately positive (Picasso) or negative (Rossi) effect, mainly in the last two evaluations. These effects may be directly related to the values in stomatal conductance (g_s) and, consequently in the transpiration rate (E), that were respectively maintained, increase, or decrease, for the three varieties (when compared to their controls). Instantaneous water use efficiency (iWUE) presented some variations, but showed similar values between 12A and the control plants at the end of the life cycle in Agria and Picasso varieties (**Table 3.28**).

Table 3.28 Mean values (*n = 4-6*) ± SE (Standard Error) of the variation of net photosynthesis rates (Pn), stomatal conductance (gs), transpiration rate (E) and the instantaneous efficiency of water use (iWUE = Pn/E), in the different treatments applied for Agria, Picasso and Rossi variety in June 21, July 11 and July 24 of 2019.

	Agria			Picasso			Rossi		
Treatments	21 June	11 July	24 July	21 June	11 July	24 July	21 June	11 July	24 July
P_n (µmol CO_2 m^{-2} s^{-1})									
Ctr	24.2 ± 0.15aA	19.1 ± 0.82bA	15.5 ± 0.51bB	23.3 ± 1.12aA	6.1 ± 0.97cC	12.4 ± 0.52bC	20.3 ± 0.32aA	17.3 ± 1.18aA	17.4 ± 1.34aA
12A	22.4 ± 0.64aAB	17.1 ± 0.8bAB	16.3 ± 1.38bB	24.1 ± 0.17aA	8.7 ± 0.1bC	21 ± 0.22aA	22.3 ± 0.5aA	13.7 ± 0.59bA	13 ± 0.68bA
24A	23 ± 0.45aAB	13.2 ± 1.33bB	11.8 ± 0.48bC	24.5 ± 0.17aA	19.8 ± 0.68bA	17.4 ± 0.91bAB	22.5 ± 0.34aA	9 ± 0.21cB	15.8 ± 2.88bA
12B	19.7 ± 0.72aB	10.6 ± 1.87bBC	21.4 ± 0.6aA	22.4 ± 1.04- A	-	-	20.5 ± 0.98aA	2.3 ± 0.64bC	-
24B	23.2 ± 0.23aA	5.1 ± 0.06cC	13.9 ± 0.3bBC	21.4 ± 0.67aA	11.6 ± 0.43bB	14.5 ± 3bBC	19.5 ± 1.75aA	6.6 ± 0.1bBC	15.8 ± 0.14aA
g_s (mmol H_2O m^{-2} s^{-1})									
Ctr	276.3 ± 5.63aAB	175.1 ± 11.76bA	121.9 ± 6.62bBC	286.5 ± 27.43aAB	35.7 ± 11.13cB	112.5 ± 4.63bC	233.3 ± 19.13aA	79.2 ± 1.93bA	209.3 ± 38.83aA
12A	277 ± 30.31aAB	97.2 ± 2.98cB	156.8 ± 27.87bB	251.5 ± 0.5aB	22.3 ± 5.24bB	281.3 ± 12.89aA	249.8 ± 17.44aA	52.1 ± 5.69bAB	99 ± 7.78bB
24A	330.3 ± 2.81aA	122.2 ± 17bAB	86.7 ± 4.86bC	344.5 ± 3.75aA	122.2 ± 18.23cA	225.5 ± 29.23bAB	228.5 ± 21aA	24.4 ± 2.56bAB	193.7 ± 44.27aA
12B	249 ± 13.28aAB	119.4 ± 16.76bAB	303.4 ± 25.51aA	217.5 ± 2.63- A	-	-	257.3 ± 20.51aA	8.6 ± 1.68bB	-
24B	213.3 ± 13.14aB	13.6 ± 3.31cC	119.8 ± 2.23bBC	205.8 ± 1.31aB	57.7 ± 4.18bB	159.4 ± 53.19aB	197.3 ± 23.58aA	22.5 ± 1.34bAB	173.3 ± 3.33aAB
E (mmol H_2O m^{-2} s^{-1})									
Ctr	2.8 ± 0.24aA	1.9 ± 0.07bA	2.2 ± 0.08aBC	3.1 ± 0.28aA	0.6 ± 0.19cB	2.1 ± 0.06bB	2.8 ± 0.22aA	1 ± 0.01bA	3.5 ± 0.37aA
12A	2.8 ± 0.27aA	1.3 ± 0.02bA	2.5 ± 0.3bB	2.7 ± 0.2bA	0.4 ± 0.1cB	3.9 ± 0.08aA	2.7 ± 0.2aA	0.8 ± 0.07bAB	2.1 ± 0.13aBC
24A	3.1 ± 0.28aA	1.6 ± 0.17bA	1.7 ± 0.07abC	3.1 ± 0.17aA	1.7 ± 0.13bA	3.5 ± 0.24aA	2.8 ± 0.23aA	0.4 ± 0.04bAB	3.1 ± 0.57aAB
12B	2.5 ± 0.16aA	1.5 ± 0.16bA	3.8 ± 0.16aA	2.9 ± 0.3- A	-	-	2.9 ± 0.2aA	0.2 ± 0.03bB	-
24B	2.6 ± 0.24aA	0.4 ± 0bB	2.3 ± 0.02aBC	2.6 ± 0.27aA	0.9 ± 0.05bAB	2.7 ± 0.67aAB	3 ± 0.42aA	0.4 ± 0.03bAB	3.1 ± 0.06aAB
iWUE (mmol CO_2 mol^{-1} H_2O)									
Ctr	9.0 ± 0.8abA	10.0 ± 0.06aB	7.1 ± 0.04bA	7.6 ± 0.34aA	13.3 ± 1.66aB	5.8 ± 0.41aA	7.6 ± 0.56aA	16.5 ± 1bBC	5.1 ± 0.16aA
12A	8.7 ± 1.01bA	13.0 ± 0.52aA	6.8 ± 0.22bA	9.2 ± 0.65bA	41.5 ± 12.83aA	5.5 ± 0.16bA	8.4 ± 0.43aA	17.9 ± 0.93bB	6.2 ± 0.05aA
24A	7.9 ± 0.82aA	8.5 ± 0.35aBC	7 ± 0.1aA	7.9 ± 0.4aA	12.1 ± 0.59aB	5.0 ± 0.09aA	8.4 ± 0.61aA	22.3 ± 2bA	5.1 ± 0.04aA
12B	8.2 ± 0.62aA	6.3 ± 0.81abC	5.6 ± 0.11bA	8.1 ± 0.54- A	-	-	7.4 ± 0.65aA	12.9 ± 1.64bC	-
24B	9.6 ± 0.89bA	14.9 ± 0.8aA	6.1 ± 0.07cA	8.6 ± 0.62aA	13.3 ± 0.83aB	5.5 ± 0.24aA	7 ± 0.54aA	15.1 ± 0.67b	5.1 ± 0.06aA

For each variety, in each parameter and date, the different letters express significant differences between treatments (a,b) or between treatments in each date of each variety (A,B) ($p \leq 0.05$). "-" means that was not perform due to the lack of leaves.

3.2.4.2 Chlorophyll a fluorescence parameters

To conduct a comprehensive assessment of the impact of Ca applications on the photosynthetic performance of *Solanum tuberosum* L. plants across the three varieties, an analysis of several parameters of chlorophyll a fluorescence was carried out (**Table 3.29** - **3.31**). As previously mentioned in **section 3.2.4.1**, the 24B treatment should be discarded to an extended level of toxicity with only one foliar application. Considering Agria and Picasso, in the last evaluation, the 12B treatment caused necrosis in most leaves, so their application should be ruled out. Regarding the maximum photochemical efficiency of PSII (F_v/F_m) (**Table 3.29**), a negative impact of the 12A treatment is noted from the third foliar application onwards in Agria and to a lesser extent in Picasso. The greatest impact was observed in the last evaluation, which suggests a cumulative toxic effect or an anticipation of the end of the plant's life cycle. These impacts were even greater with the 12B treatment, leading to the death of Picasso plants and even in Rossi before the last assessed date. Considering the first (and only) foliar application with the 24B treatment, a partial recovery of plants was observed mainly in Agria. The actual photochemical efficiency of PSII (F_v'/F_m') followed an affectation pattern not dissimilar to what was observed in F_v/F_m. However, the 12A treatment showed only a slightly effect in the three cultivars, whereas the 12B, which caused significant losses in the functioning efficiency of the PSII, was more accentuated in Picasso and Rossi.

Table 3.29 Leaf chlorophyll a fluorescence parameters in *Solanum tuberosum* L. Agria, Picasso and Rossi varieties on 21 June, 11 July and 24 July of 2019. Mean values (*n = 5*) ± SE (Standard Error) of the maximum (F_v/F_m) and the actual (F_v'/F_m') PSII photochemical efficiency.

Treat-ments	F_v/F_m			F_v'/F_m'		
	21 June	11 July	24 July	21 June	11 July	24 July
			Agria			
Ctr	0.78 ± 0.016aA	0.797 ± 0.006aA	0.814 ± 0.011aA	0.579 ± 0.019aA	0.552 ± 0.028aA	0.465 ± 0.027aA
12A	0.748 ± 0.014aA	0.771 ± 0.015aA	0.731 ± 0.02aAB	0.52 ± 0.029aA	0.532 ± 0.008aA	0.481 ± 0.023aA
24A	0.76 ± 0.008aA	0.783 ± 0.01aA	0.78 ± 0.009aAB	0.536 ± 0.039aA	0.516 ± 0.03aA	0.496 ± 0.047aA
12B	0.788 ± 0.006aA	0.73 ± 0.024aA	0.588 ± 0.039bC	0.489 ± 0.035abA	0.35 ± 0.076bB	0.534 ± 0.034aA
24B	0.811 ± 0.003abA	0.785 ± 0.008abA	0.7 ± 0.051bB	0.55 ± 0.043aA	0.474 ± 0.016aAB	0.384 ± 0.058aA
			Picasso			
Ctr	0.761 ± 0.012aAB	0.777 ± 0.016aAB	0.797 ± 0.006aA	0.551 ± 0.032aA	0.593 ± 0.017aA	0.406 ± 0.025bA
12A	0.775 ± 0.016aAB	0.794 ± 0.007aA	0.774 ± 0.01aA	0.561 ± 0.025aA	0.5 ± 0.033aA	0.483 ± 0.04aA
24A	0.812 ± 0.006aA	0.76 ± 0.018aAB	0.77 ± 0.009aA	0.558 ± 0.021aA	0.494 ± 0.032aA	0.435 ± 0.036aA
12B	0.741 ± 0.016aB	0.615 ± 0.039bC	-	0.311 ± 0.028aB	0.233 ± 0.063aB	-
24B	0.791 ± 0.009aAB	0.716 ± 0.031aB	0.755 ± 0.018aA	0.522 ± 0.013abA	0.565 ± 0.04aA	0.402 ± 0.039bA
			Rossi			
Ctr	0.795 ± 0.007bA	0.795 ± 0.007aA	0.788 ± 0.008aA	0.55 ± 0.041aA	0.503 ± 0.009aA	0.451 ± 0.021aA
12A	0.777 ± 0.009bA	0.777 ± 0.009aAB	0.697 ± 0.024aA	0.487 ± 0.021aA	0.405 ± 0.021aA	0.379 ± 0.021aA
24A	0.635 ± 0.047bAB	0.635 ± 0.047abA	0.729 ± 0.019aA	0.508 ± 0.02aA	0.415 ± 0.018aA	0.438 ± 0.016aA
12B	0.708 ± 0.025aA	0.645 ± 0.014aB	-	0.48 ± 0.028aA	0.279 ± 0.052bB	-
24B	0.686 ± 0.025aA	0.788 ± 0.01aA	0.764 ± 0.007aA	0.511 ± 0.01aA	0.411 ± 0.042bA	0.402 ± 0.015bA

For each parameter the different letters express significant differences between treatments in each variety (a,b) or between treatments in each date (A,B) ($p \leq 0.05$). "-" means that there weren't perform due to the lack of leaves.

Estimates of the quantum yields of non-cyclic photosynthetic electron transport ($Y_{(II)}$), regulated dissipation of energy in PSII ($Y_{(NPQ)}$), and unregulated dissipation (heat and fluorescence) in PSII ($Y_{(NO)}$), showed variations dependent on each parameter, but in line with the performance of the photosynthetic machinery (**Table 3.30**). Compared to the control, $Y_{(II)}$ only showed a trend towards lower values (although not always significant) after the first evaluation and until the last one, in the 12A treatment. Moreover, the 12B treatment showed a more pronounced negative effect, except in Agria. Besides, as a complement to $Y_{(II)}$, in general the indicators of dissipation process (and therefore less use) of energy related to photoprotection ($Y_{(NPQ)}$) or problems in the use of energy luminous ($Y_{(NO)}$), as a whole, showed a non-significant increase with the 12A treatment, being in agreement with the change in values of $Y_{(II)}$ and F_v'/F_m'. However, the value of the set of those two parameters, normally showed an increase in the 12B treatment.

Table 3.30 Leaf chlorophyll a fluorescence parameters in *Solanum tuberosum* L., Agria, Picasso and Rossi varieties on 21 June, 11 July and 24 July of 2019. Mean values ($n = 5$) ± SE (Standard Error) of the estimate of quantum yields of non-cyclic electron transport ($Y_{(II)}$) of regulated energy dissipation in PSII ($Y_{(NPQ)}$) and of non-regulated energy dissipation in PSII ($Y_{(NO)}$).

T.	$Y_{(II)}$			$Y_{(NPQ)}$			$Y_{(NO)}$		
	21 June	11 July	24 July	21 June	11 July	24 July	21 June	11 July	24 July
					Agria				
Ctr	0.427 ± 0.032aA	0.429 ± 0.033aA	0.354 ± 0.019aA	0.312 ± 0.026aA	0.407 ± 0.038aB	0.44 ± 0.016aAB	0.261 ± 0.01aAB	0.164 ± 0.006bA	0.206 ± 0.008abB
12A	0.327 ± 0.039aA	0.376 ± 0.01aAB	0.319 ± 0.022aA	0.381 ± 0.038aA	0.437 ± 0.011aAB	0.456 ± 0.026aAB	0.292 ± 0.007aA	0.187 ± 0.003bA	0.226 ± 0.015bB
24A	0.323 ± 0.027aA	0.381 ± 0.027aA	0.298 ± 0.017aA	0.436 ± 0.026aA	0.455 ± 0.031aAB	0.449 ± 0.027aAB	0.242 ± 0.021aAB	0.164 ± 0.004bA	0.254 ± 0.028abB
12B	0.367 ± 0.027aA	0.247 ± 0.06aB	0.389 ± 0.03aA	0.408 ± 0.032aA	0.583 ± 0.065aA	0.281 ± 0.042bB	0.225 ± 0.009bB	0.17 ± 0.007bA	0.33 ± 0.022aA
24B	0.361 ± 0.04aA	0.348 ± 0.017aAB	0.265 ± 0.058aA	0.385 ± 0.044aA	0.471 ± 0.018aAB	0.527 ± 0.064aA	0.254 ± 0.034aAB	0.181 ± 0.009bA	0.208 ± 0.014abB
					Picasso				
Ctr	0.421 ± 0.021aA	0.403 ± 0.032aA	0.259 ± 0.022bA	0.336 ± 0.037bB	0.387 ± 0.025abA	0.526 ± 0.025aAB	0.243 ± 0.017aA	0.21 ± 0.01bA	0.214 ± 0.01abB
12A	0.36 ± 0.02aA	0.378 ± 0.029aA	0.34 ± 0.048aA	0.362 ± 0.019aB	0.441 ± 0.035aB	0.416 ± 0.059aB	0.278 ± 0.024aA	0.181 ± 0.013bA	0.244 ± 0.012abAB
24A	0.359 ± 0.03aA	0.333 ± 0.02aA	0.299 ± 0.037aA	0.352 ± 0.014aB	0.429 ± 0.021aB	0.465 ± 0.042aA	0.289 ± 0.029aA	0.239 ± 0.006bA	0.236 ± 0.012aA
12B	0.159 ± 0.018aB	0.135 ± 0.042aB	-	0.633 ± 0.024aA	0.713 ± 0.036aA	-	0.208 ± 0.008aA	0.153 ± 0.008aA	-
24B	0.308 ± 0.028abA	0.426 ± 0.023aA	0.241 ± 0.04bA	0.433 ± 0.019abB	0.388 ± 0.025bB	0.546 ± 0.042aA	0.259 ± 0.013abA	0.186 ± 0.012bA	0.212 ± 0.013aAB
					Rossi				
Ctr	0.358 ± 0.018aA	0.376 ± 0.009aA	0.299 ± 0.021aA	0.44 ± 0.014aA	0.449 ± 0.008aB	0.491 ± 0.02aA	0.202 ± 0.024aBC	0.175 ± 0.003aAB	0.21 ± 0.003aA
12A	0.346 ± 0.027aA	0.315 ± 0.019aA	0.255 ± 0.005aA	0.477 ± 0.026aA	0.541 ± 0.021aB	0.511 ± 0.01aA	0.177 ± 0.006bC	0.144 ± 0.004bB	0.234 ± 0.008aA
24A	0.34 ± 0.02aA	0.313 ± 0.024aA	0.306 ± 0.018aA	0.426 ± 0.025aA	0.495 ± 0.025aB	0.472 ± 0.02aA	0.234 ± 0.006aAB	0.192 ± 0.008bA	0.222 ± 0.008abA
12B	0.349 ± 0.02aA	0.156 ± 0.056bB	-	0.411 ± 0.028bA	0.662 ± 0.054aA	-	0.24 ± 0.01aAB	0.182 ± 0.013bAB	-
24B	0.357 ± 0.015aA	0.322 ± 0.029aA	0.286 ± 0.007aA	0.399 ± 0.014bA	0.506 ± 0.037aB	0.494 ± 0.011abA	0.244 ± 0.003aA	0.172 ± 0.017bAB	0.22 ± 0.004aA

For each parameter the different letters express significant differences between treatments in each variety (a,b) or between treatments in each date (A,B) ($p \leq 0.05$). "-" means that there weren't perform due to the lack of leaves.

Considering the analysis of qN, which reflects the proportion of energy dissipated as heat by photo-protection metabolism, and qL which refers to the energy captured by open PSII reaction centers and used for photochemical events, were not significantly and consistently affected in *Solanum tuberosum* L. plants in the three varieties, regarding the 12A treatment (**Table 3.31**). Moreover, overall, there was an increase in qN (after the second evaluation in Picasso and Rossi varieties) in the 12B treatment, but it was not possible to record their values in the last evaluation due to the death of the plant leaves

Table 3.31 Leaf chlorophyll a fluorescence parameters in *Solanum tuberosum* L. Agria, Picasso and Rossi varieties on 21 June, 11 July and 24 July of 2019. Mean values (*n = 5*) ± SE (Standard Error) of the photochemical quenching coefficient (qL) and non-photochemical quenching (qN).

T.	q_N			q_L		
	21 June	11 July	24 July	21 June	11 July	24 July
			Agria			
Ctr	0.659 ± 0.023bA	0.792 ± 0.027abA	0.819 ± 0.007aA	0.55 ± 0.057aA	0.608 ± 0.031aA	0.642 ± 0.075aA
12A	0.69 ± 0.033aA	0.794 ± 0.008aA	0.781 ± 0.024aA	0.441 ± 0.034aA	0.531 ± 0.006aA	0.505 ± 0.026aA
24A	0.746 ± 0.038aA	0.821 ± 0.021aA	0.764 ± 0.042aA	0.416 ± 0.043aA	0.577 ± 0.027aA	0.466 ± 0.113aA
12B	0.774 ± 0.033aA	0.872 ± 0.039aA	0.506 ± 0.071bB	0.613 ± 0.063aA	0.594 ± 0.058aA	0.557 ± 0.045aA
24B	0.72 ± 0.055aA	0.832 ± 0.015aA	0.833 ± 0.043aA	0.484 ± 0.092aA	0.596 ± 0.041aA	0.567 ± 0.061aA
			Picasso			
Ctr	0.689 ± 0.052aA	0.729 ± 0.018aA	0.851 ± 0.016aA	0.595 ± 0.041aA	0.468 ± 0.04aA	0.508 ± 0.013aA
12A	0.684 ± 0.036aA	0.811 ± 0.029aA	0.754 ± 0.047aA	0.459 ± 0.067aA	0.609 ± 0.036aA	0.545 ± 0.051aA
24A	0.691 ± 0.028aA	0.768 ± 0.019aA	0.8 ± 0.032aA	0.463 ± 0.065aA	0.54 ± 0.104aA	0.558 ± 0.062aA
12B	0.895 ± 0.016aA	0.933 ± 0.02aA	-	0.425 ± 0.052aA	0.559 ± 0.199aA	-
24B	0.753 ± 0.013aA	0.742 ± 0.033aA	0.845 ± 0.029aA	0.419 ± 0.063aA	0.584 ± 0.081aA	0.477 ± 0.086aA
			Rossi			
Ctr	0.78 ± 0.036aAB	0.823 ± 0.007aA	0.828 ± 0.013aA	0.486 ± 0.098aA	0.596 ± 0.022aA	0.515 ± 0.009aA
12A	0.829 ± 0.015aA	0.89 ± 0.01aA	0.83 ± 0.014aA	0.557 ± 0.036aA	0.675 ± 0.032aA	0.567 ± 0.048aA
24A	0.722 ± 0.025bB	0.821 ± 0.015aA	0.808 ± 0.015aA	0.497 ± 0.005aA	0.642 ± 0.04aA	0.572 ± 0.047aA
12B	0.746 ± 0.031bB	0.902 ± 0.029aA	-	0.584 ± 0.03aA	0.483 ± 0.172aA	-
24B	0.721 ± 0.011bB	0.862 ± 0.023aA	0.837 ± 0.01aA	0.533 ± 0.034aA	0.701 ± 0.076aA	0.597 ± 0.022aA

For each parameter the different letters express significant differences between treatments in each variety (a,b) or between treatments in each date (A,B) ($p \leq 0.05$). "-" means that there weren't perform due to the lack of leaves.

3.2.5 Quantification of mineral elements

The quantification of Ca and other mineral elements were carried out during the biofortification process and after being harvested by X-ray fluorescence spectrometry technique.

3.2.5.1 Monitoring of the different organs of *Solanum tuberosum* L. during the biofortification process

Through X-ray fluorescence spectrometry (XRF), the mineral contents were monitored in the different organs (tubers, roots, stems, and leaves) of the Agria and Picasso varieties of *Solanum tuberosum* L. during the production cycle. Moreover, the monitoring of mineral elements content in the different organs of Rossi variety was not carried out in the second year of the study since foliar applications were quite out of date regarding the other two varieties. Overall, there were significant differences in Ca, K, S and P of tubers (**Figure 3.17**), roots (**Figure 3.18**), stems (**Figure 3.19**) and leaves (**Figure 3.20**), between each treatment after the 4th, 6th and 7th foliar application (4FA, 6FA and 7FA) in each variety. Considering the content of tubers of *Solanum tuberosum* L. (**Figure 3.17**), after the last foliar application (7FA), Ca content was higher in the 24 kg/ha treatments (24B in Picasso and 24A in Agria). In the control tubers from Picasso, Ca content decreased (in treatments 12A and 24A) with the augmentation of foliar sprayings. Additionally, after the 7FA, the highest contents of K and P were obtained with 24A and 12B treatments in Picasso and Agria, respectively. Besides, K showed, in both varieties, a decrease in their content in treatment 24B. Besides, a different tendency in each variety was found regarding 12A and 24A treatments (*i.e.* a decrease of K content in Picasso and an increase in Agria). Sulfur showed a higher content in treatment 12B, relatively to the other treatments in both varieties and, as observes for S, in Picasso P showed a higher content after the 6FA and 7FA in treatment 12B. In general, considering the data obtained after 4FA, 6FA and 7FA, P content in Picasso was higher relatively to Agria. Nevertheless, the tubers of Picasso (**Figure 3.17**) showed the highest Ca content, and the lowest content of K and P, after 7FA, 24B treatment. On the other hand, after the 7FA, in Agria, treatment 12B presented the lowest Ca content, and the highest content of K, S and P. Also, after each analyzed foliar application (4FA, 6FA and 7FA), it was in treatment 12A that the lowest contents of K, S and P were found in Agria. Furthermore, relatively to the control, an increase of Ca content was found after the 7FA, between 5.7 to 95.6 % and 20.7 to 33 %, respectively for Picasso and Agria (**Figure 3.17**). Additionally, after treatments 7FA, 12A, for both varieties, as well as in treatment 12B of Agria, the biofortification of tubers in Ca could not be detected.

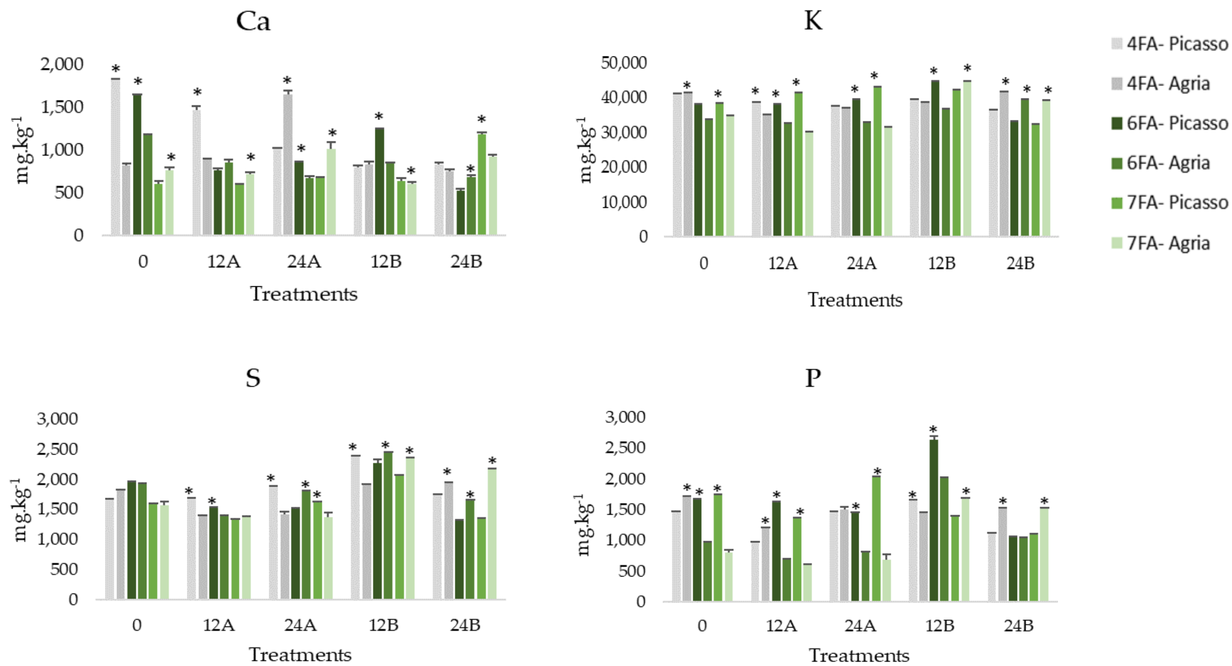


Figure 3.17 Chemical elements in tubers of *Solanum tuberosum* L. Agria and Picasso varieties during the production cycle of plants, after the fourth (4FA), sixth (6FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) with two concentrations (12 and 24 kg ha^{-1}). Control was not sprayed at any time and corresponds to "0". Mean values ($n = 4$) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 4FA, 6FA, and 7FA are identified (*)($p \leq 0.05$).

Regarding roots of *Solanum tuberosum* L. (**Figure 3.18**), Ca contents increased with the application of $CaCl_2$ in both varieties (in 12A treatment of Picasso and with 24A treatment of Agria). Besides, after the last foliar applications (6FA and 7FA), Ca content remained higher with Ca-EDTA treatments in both varieties. Nevertheless, considering treatment 24B, and comparing both varieties, Picasso showed an increase of Ca content with the increase in the number of foliar applications, but Agria presented a contrasting trend. Moreover, roots from *Solanum tuberosum* L. plants, without any foliar applications with Ca (control roots), showed the lowest Ca content in Agria in the different analytical periods (i.e., after 4FA, 6FA and 7FA). A clear tendency regarding the accumulation of K and P in both varieties could not be found (**Figure 3.18**). Yet, in Agria, after the 7FA, the highest content of K and P was obtained in the control. Also, in Agria with treatment 12A, 24A and 24B, a decrease of K content was found, whereas a decrease in Picasso in treatments 12A, 24A and 12B was determined from the 6FA to the 7FA. In both varieties, after 4FA and 6FA, S content was higher with $CaCl_2$ sprayings. However, in Picasso and Agria, S presented the highest content after 7FA with the concentration of 12k/ha of Ca-EDTA and $CaCl_2$, respectively. Moreover, after 7FA, in 12B treatment, Picasso showed the highest Ca and S content, and the lowest content of K and P. Additionally, treatment 24B presented the lowest Ca and S content and the highest content of K. Considering Agria, after treatments 7FA, 24B the highest Ca content and the lowest content of S was found, whereas the control presented the highest content of K and P and the lowest Ca content. Likewise, after the 7FA, and relatively to control, an increase in Ca content was measured (between 9.7 to 41.1% and 16.5 to 55.1% respectively for Picasso and Agria) (**Figure 3.18**).

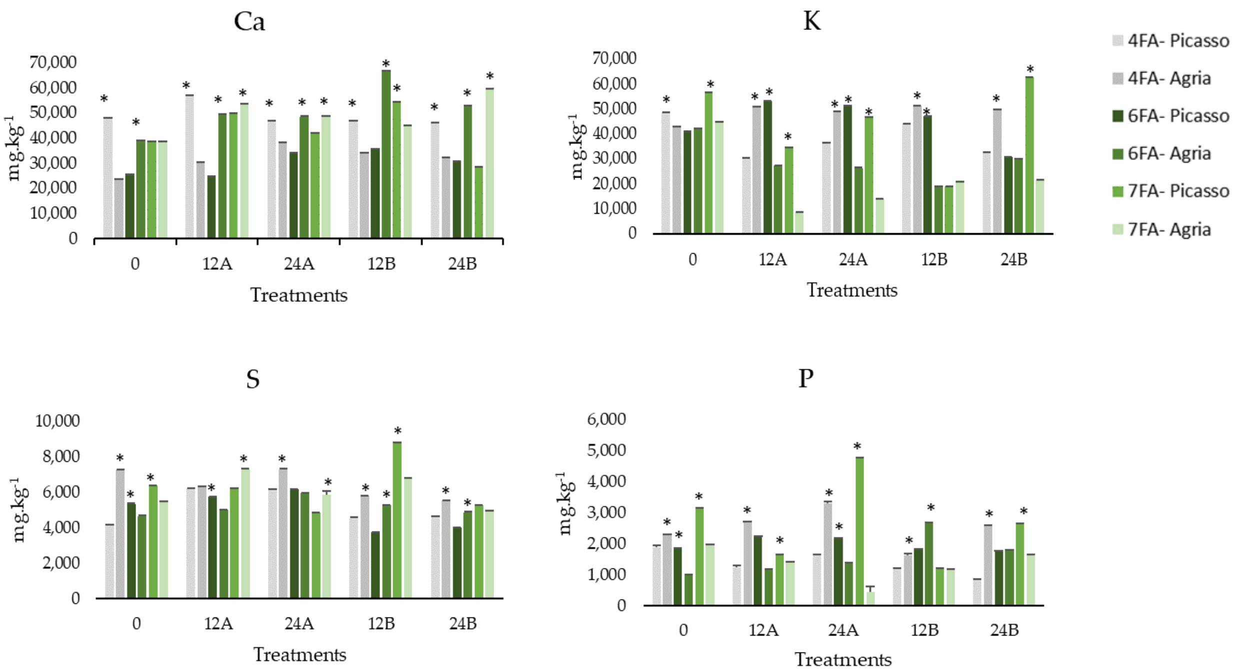


Figure 3.18 Chemical elements in roots of *Solanum tuberosum* L. Agria and Picasso varieties during the production cycle of plants, after the fourth (4FA), sixth (6FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) with two concentrations (12 and 24 kg ha^{-1}). Control was not sprayed at any time and corresponds to "0". Mean values ($n = 4$) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 4FA, 6FA, and 7FA are identified (*) ($p \leq 0.05$).

Considering the stems of *Solanum tuberosum* L. (**Figure 3.19**), the highest Ca content was obtained in 12B treatment after the 4FA, 6FA and 7FA on Picasso variety and in 24A treatment after 7FA on Agria variety. Nevertheless, Ca contents increased with the increase of foliar applications in all the treatments (except in 12A) of Picasso variety. Additionally, as seen in roots of *Solanum tuberosum* L. plants (**Figure 3.18**), there is not a clear tendency regarding the accumulation of K content in both varieties (**Figure 3.19**). Besides, the highest content of K after 7FA was verified in 24A and control on Picasso and Agria, respectively. Considering S and P content, Picasso variety showed the highest content in 24A and 24B treatments, respectively, after 4FA and 6FA. Agria variety showed after 6FA, the highest content of S and P in 12B treatment. After 7FA, the highest content of S was obtained in

12B and 12A treatments on Picasso and Agria, respectively. On the other hand, the highest P content was verified after 7FA in 24A and 12B treatments in Picasso and Agria varieties, respectively. Furthermore, with the increase of foliar applications there was an increase in S content in control, 24A and 12B treatments in Picasso and in 24B treatment in both varieties. Moreover, as observed in roots (**Figure 3.18**), after 7FA, Picasso variety in 12B treatment obtained the highest Ca and S content and the lowest of K and P (**Figure 3.19**). Also, regarding 24A treatment, Ca content was the lowest, and K and P content were the highest. Considering Agria, control obtained the highest content of K and the lowest content of Ca, S and P. Nevertheless, relatively to control data, after 7FA, Picasso and Agria, respectively showed an increase in Ca content that varied between 28.2% to 3-fold and 2 to 3.1. fold.

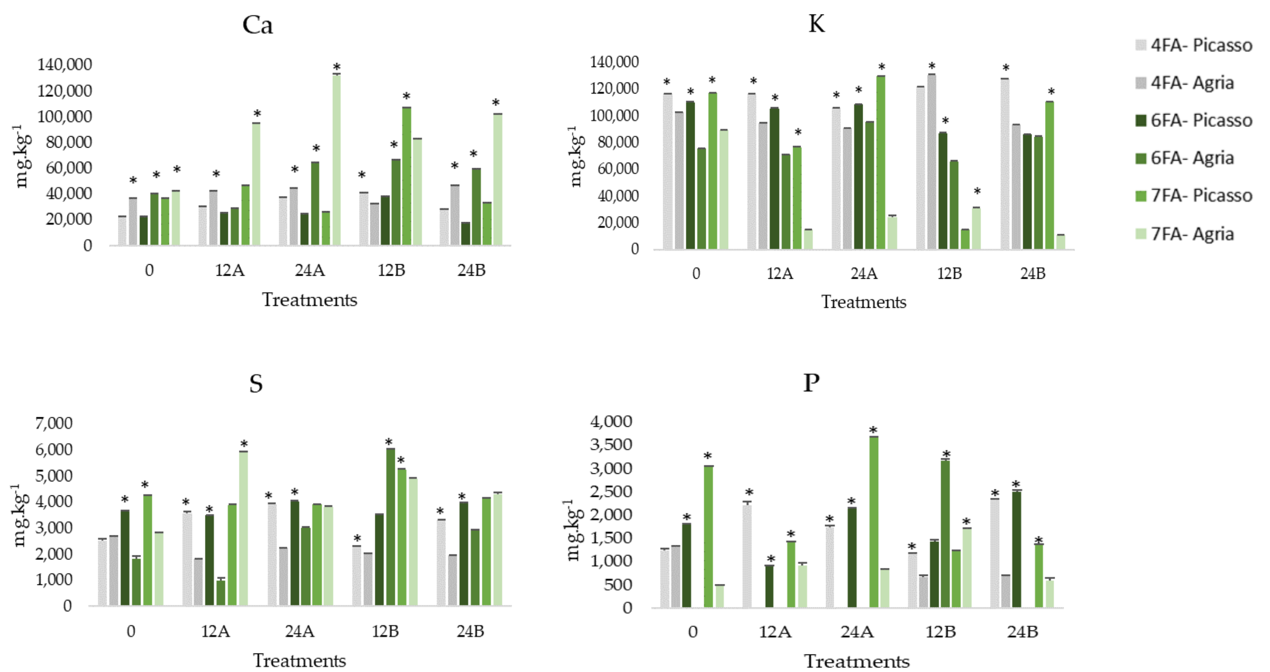


Figure 3.19 Chemical elements in stems of *Solanum tuberosum* L. Agria and Picasso varieties during the production cycle of plants, after the fourth (4FA), sixth (6FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) with two concentrations (12 and 24 kg ha^{-1}). Control was not sprayed at any time and corresponds to "0". Mean values (*n=4*) ± SE (standard error) and significantly higher content between varieties within each treatment after the 4FA, 6FA, and 7FA are identified (*) ($p \leq 0.05$).

Relatively to leaves of *Solanum tuberosum* L. plants (**Figure 3.20**), after 4FA and 7FA with treatment 24B, Ca content showed higher values in Agria, as well as in 12A after 7FA in Picasso. Besides, both varieties showed an increase in Ca content with the increase in foliar applications only with treatment 12A. Considering K content, for both varieties, the highest values were obtained in the control after 7FA. It was also possible to observe a decrease in K content with the increase of foliar applications in all the treatments (except 24B) on Agria and the decrease in its content in all treatments from 6FA to

7FA in Picasso. After the 7FA, in Picasso and Agria, S and P contents were higher in treatments 24A and 24B, respectively. Furthermore, after the 7FA, in Agria, the control presented the lowest contents of Ca, S and P and the highest content of K. In Picasso, after treatments 7FA, 12B, the highest contents of Ca and S and the lowest in K and P were found. In this context, after the 7FA, relatively to control, Picasso and Agria showed an increase of Ca content that ranged between 32.8 to 73.1% and 16.8 to 84.3%, respectively.

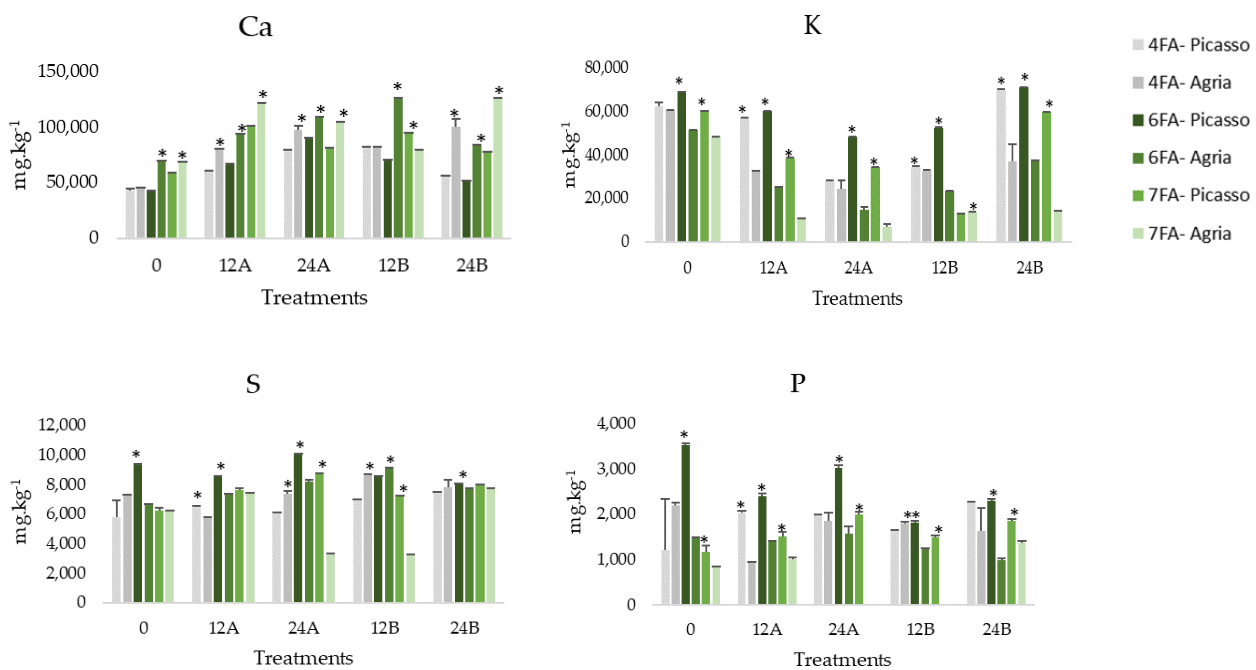


Figure 3.20 Chemical elements in leaves of *Solanum tuberosum* L. Agria and Picasso varieties during the production cycle of plants, after the fourth (4FA), sixth (6FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) with two concentrations (12 and 24 kg ha^{-1}). Control was not sprayed at any time and corresponds to "0". Mean values ($n = 4$) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 4FA, 6FA, and 7FA are identified (*) ($p \leq 0.05$).

3.2.5.2 Kinetic of mineral accumulation during the biofortification process

Kinetic assessment of mineral accumulation during Ca biofortification was carried out for Agria and Picasso varieties. As such, during the plant's life cycle, three periods of analysis occurred for each variety: 102, 116 and 129 days after plantation for Agria, and 96, 110 and 123 days after plantation for Picasso. This approach aimed to unravel the underlying associated to mineral accumulation in the different organs of *Solanum tuberosum* L. throughout the plant's life cycle.

3.2.5.2.1 Each mineral element during the development cycle

Considering each mineral element during the life cycle of Agria and Picasso varieties of *Solanum tuberosum* L., the kinetic of accumulation of Ca, K, S and P were carried in tubers (**Figure 3.21**), roots (**Figure 3.22**), stems (**Figure 3.23**) and leaves (**Figure 3.24**). Regarding the kinetics accumulation in tubers of both varieties (**Figure 3.21**), it was possible to observe different accumulation patterns. Regarding Agria, after 129 DAP the highest content of Ca, K and S was obtained in treatment 12A, however at the same period of analysis, P showed a higher content in treatment 12B. Considering Picasso, after 123 DAP S, K and P showed the highest content in treatment 12A, whereas for Ca in 24B treatment. In both varieties it was possible to observe a decreased tendency (in the second moment of analysis), followed by an increase (in the third and last moment of analysis during the biofortification process and plant development).

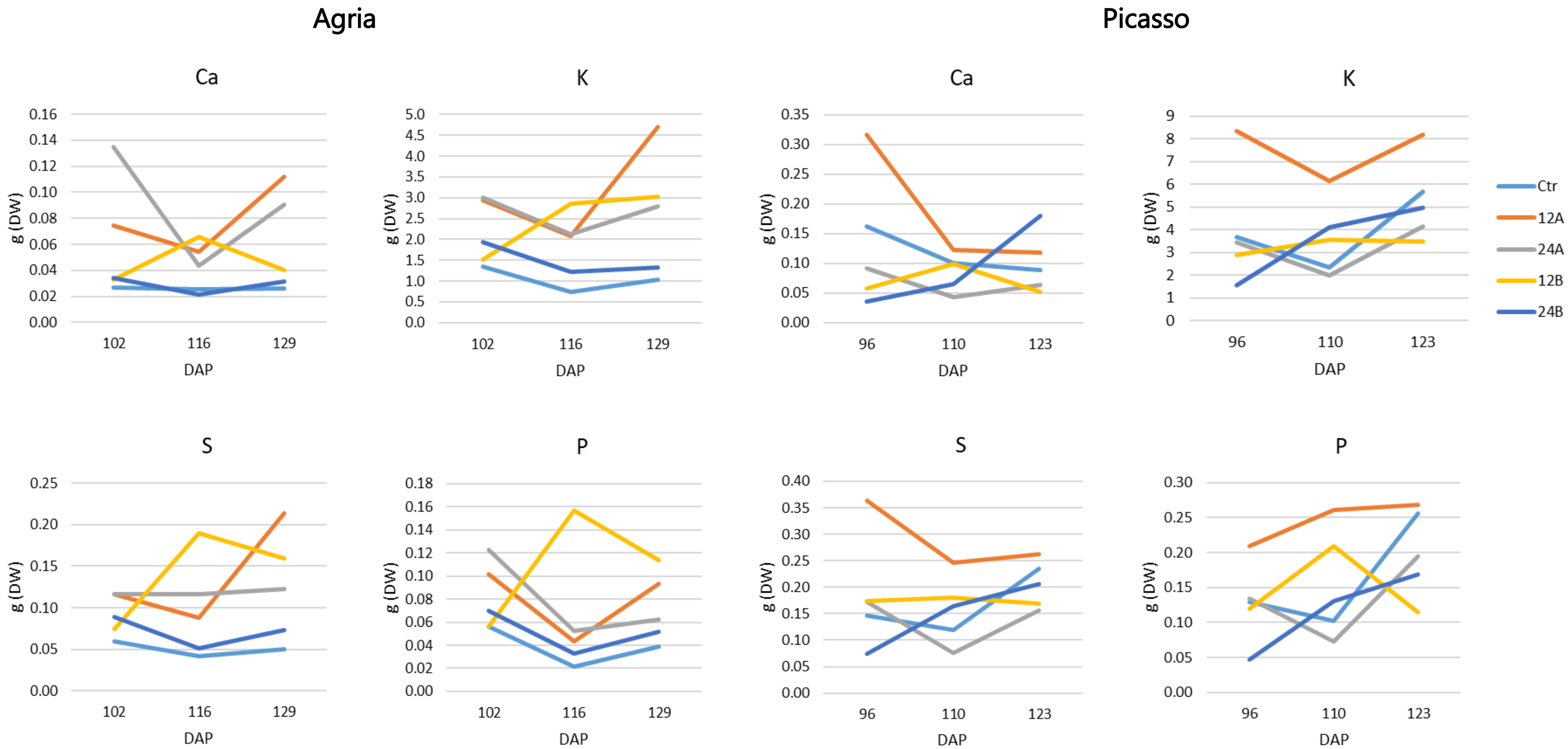


Figure 3.21 Kinetic of minerals accumulation (Ca, K, S and P) in tubers of *Solanum tuberosum* L., during the production cycle of plants after 102, 116 and 129 days after plantation (DAP) in Agria and after 96, 110 and 123 DAP in Picasso varieties.

In both varieties, regarding the kinetics accumulation of Ca, K, S and P, in roots (**Figure 3.22**), it was possible to observe different accumulation trends in tubers (**Figure 3.21**). However, in Picasso variety, Ca showed a similar accumulation in all the treatments regarding the three moments of analysis, with the highest content being obtained in the first moment of analysis, followed by an accentuated decreased in the second moment and followed by an increase in the third and last moment of analysis (123 DAP). For both varieties, in the third moment of analysis, which corresponds to 129 and 123 DAP respectively, control showed the highest content of Ca, K, S and P.

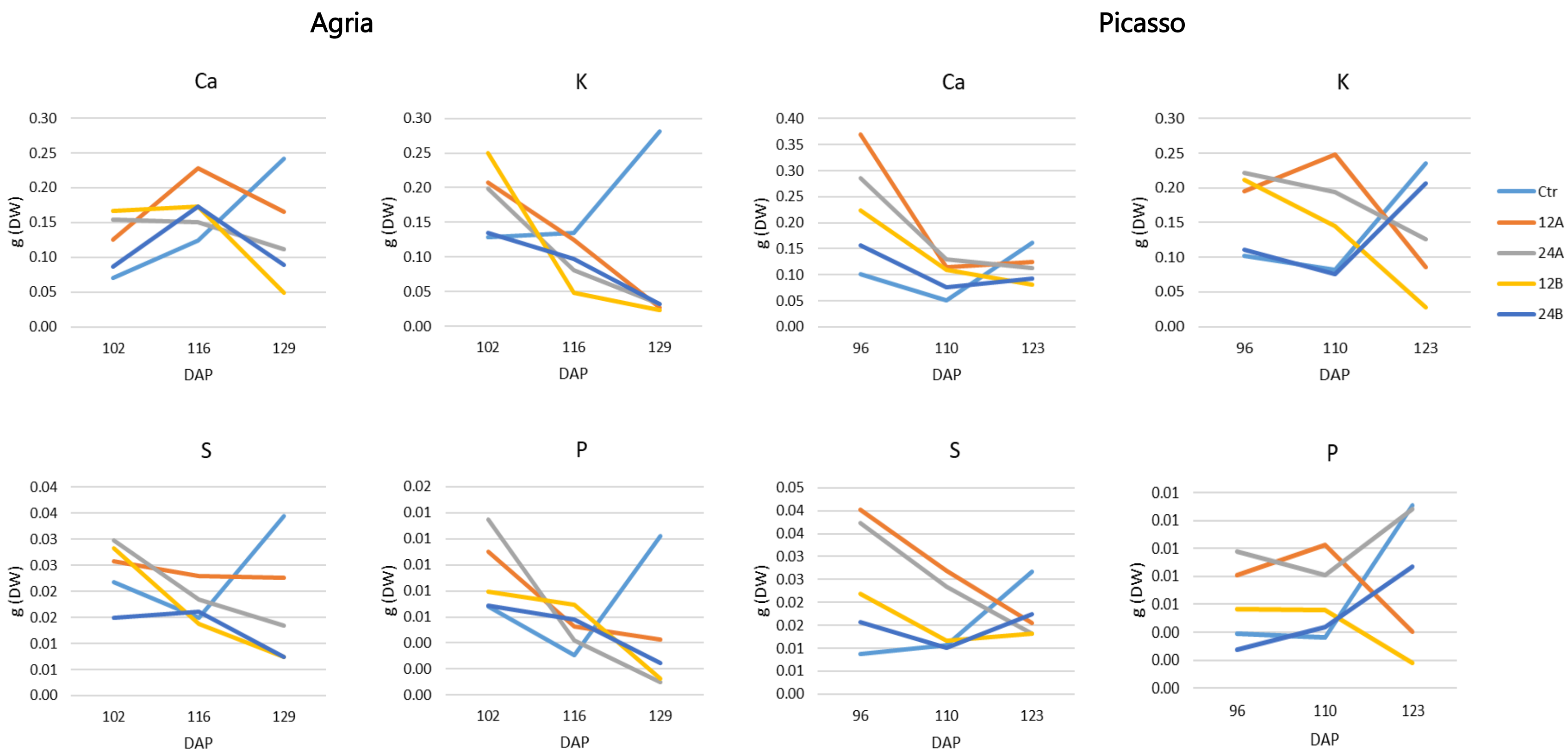


Figure 3.22 Kinetic of minerals accumulation (Ca, K, S and P) in roots of *Solanum tuberosum* L., during the production cycle of plants after 102, 116 and 129 days after plantation (DAP) in Agria and after 96, 110 and 123 DAP in Picasso varieties.

The kinetic accumulation In the stems of both varieties (**Figure 3.23**), showed differences in the accumulation patterns regarding Ca, K, S and P, as previously observed in tubers (**Figure 3.21**) and roots (**Figure 3.22**). In Agria, calcium showed a higher content in treatment

24A at the 129DAP, followed by treatments 12A and 12B. Also, in Agria variety, K, S and P did not reveal an accumulation tendency relatively to the different treatments. Also, considering Picasso, with 24A treatment, it was possible to observe an increase in Ca, K, S and P at the 123DAP.

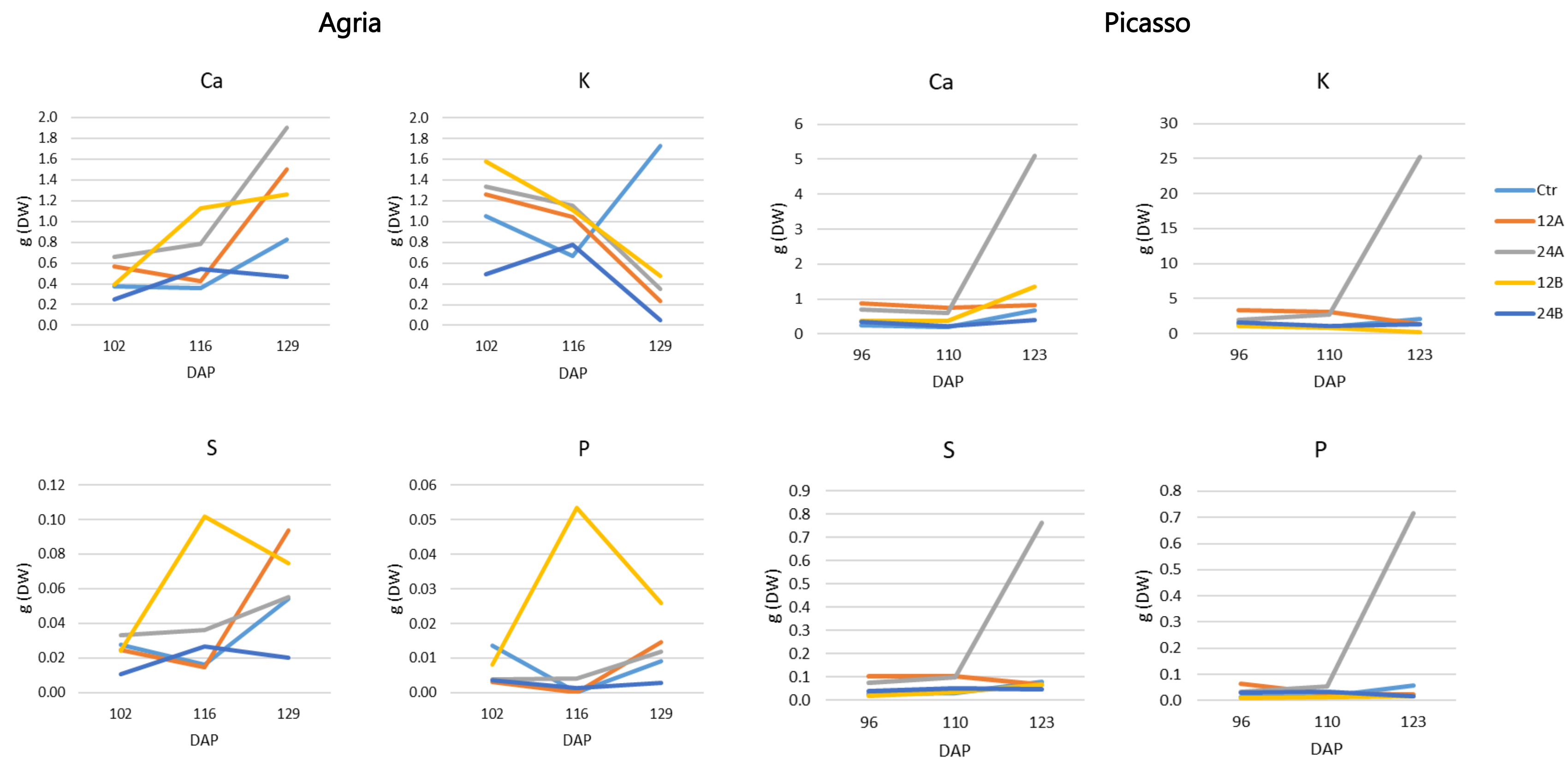


Figure 3.23 Kinetic of minerals accumulation (Ca, K, S and P) in stems of *Solanum tuberosum* L., during the production cycle of plants after 102, 116 and 129 days after plantation (DAP) in Agria and after 96, 110 and 123 DAP in Picasso varieties.

In Agria and Picasso varieties, the accumulation kinetics of Ca, K, S and P in the leaves (**Figure 3.24**), showed different accumulation trends, as previously observed in tubers (**Figure 3.21**), roots (**Figure 3.22**) and stems (**Figure 3.23**). Considering Agria, at the 129DAP, Ca showed a higher content in treatment 12A and K, S and P in the control. In Picasso, at 123 DAP, Ca and S showed the highest content in treatment 12A, whereas K and P prevailed in treatment 24B.

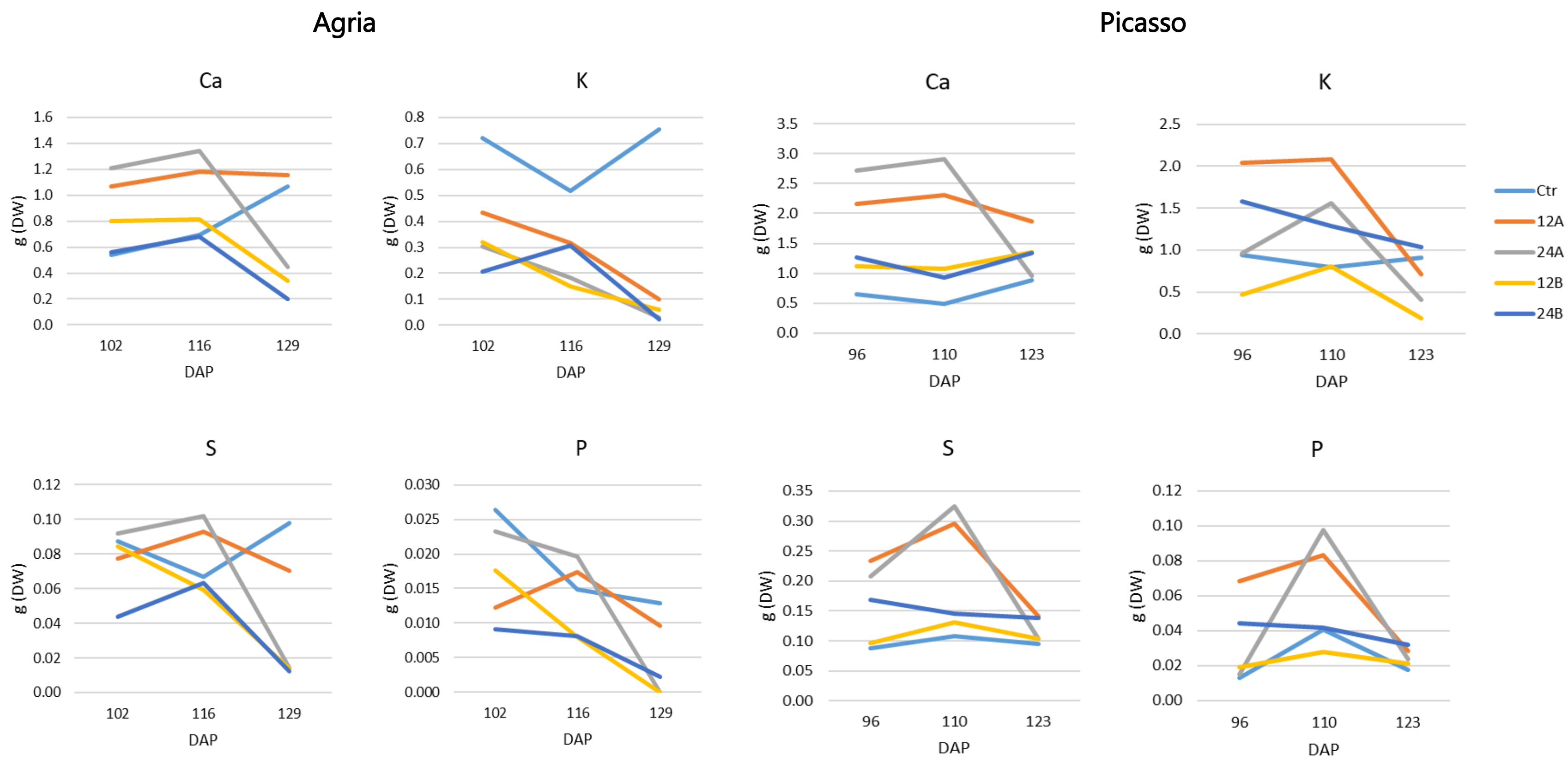


Figure 3.24 Kinetic of minerals accumulation (Ca, K, S and P) in the leaves of *Solanum tuberosum* L., during the production cycle of plants after 102, 116 and 129 days after plantation (DAP) in Agria and after 96, 110 and 123 DAP in Picasso varieties.

3.2.5.2.2 Absorption rate of each mineral element

In **Figure 3.25** and **Figure 3.26**, the daily accumulation rates of Ca, K, S and P in the tubers of both *Solanum tuberosum* L. (considering the dry weight) are represented after 102, 116 and 129 days after plantation for Agria and after 96, 110 and 123 days after plantation for Picasso varieties. It was found that in the last period of analysis (129 DAP in Agria and 123 DAP in Picasso), treatments 12A, 24A and 12B showed higher accumulation rates. Furthermore, Picasso showed a higher accumulation rate of Ca, K and P and a lower accumulation rate of S, relatively to Agria. For Agria (**Figure 3.25**), a discernible trend of decreasing accumulation rates for Ca, K, S and P was observed in treatment 24A with the increase of the days after plantation.

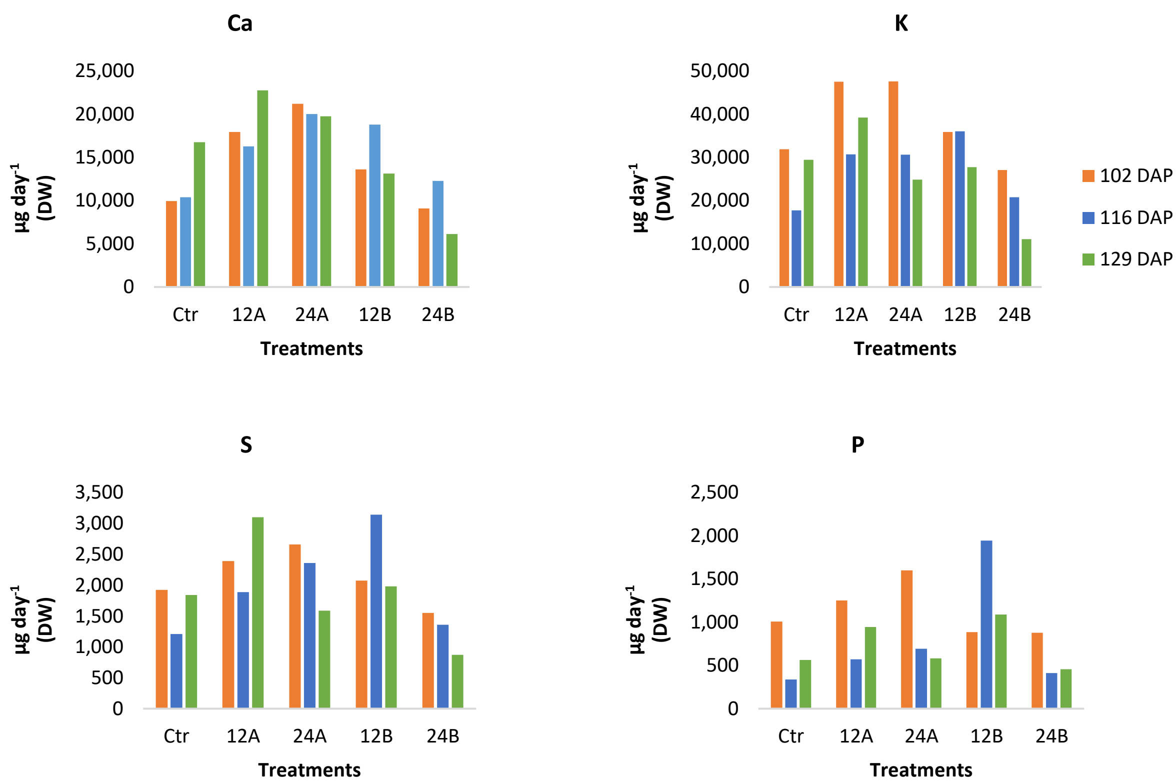


Figure 3.25 Absorption rate (μg by day) of Ca, K, S and P in tubers of *Solanum tuberosum* L. Agria variety.

Considering Picasso variety (**Figure 3.26**), there was a noticeable decreasing trend in the absorption rates of Ca, K, P and S in treatment 12A as the number of days after plantation increased.

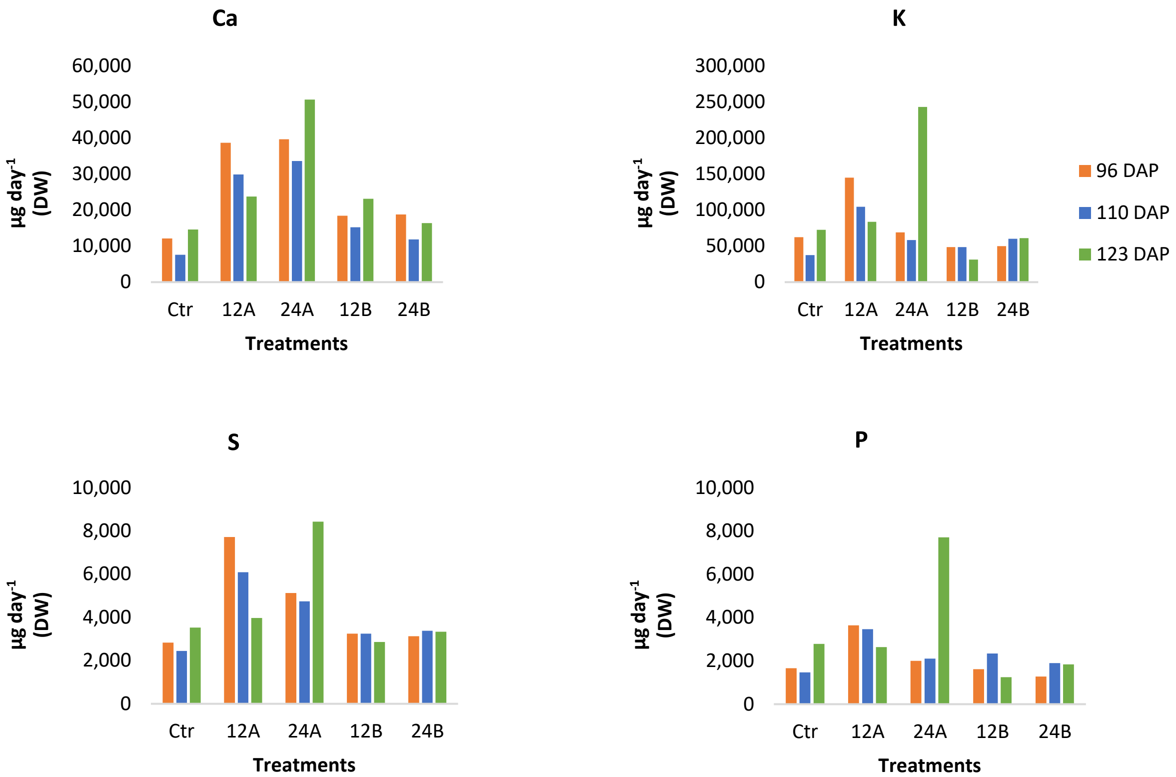
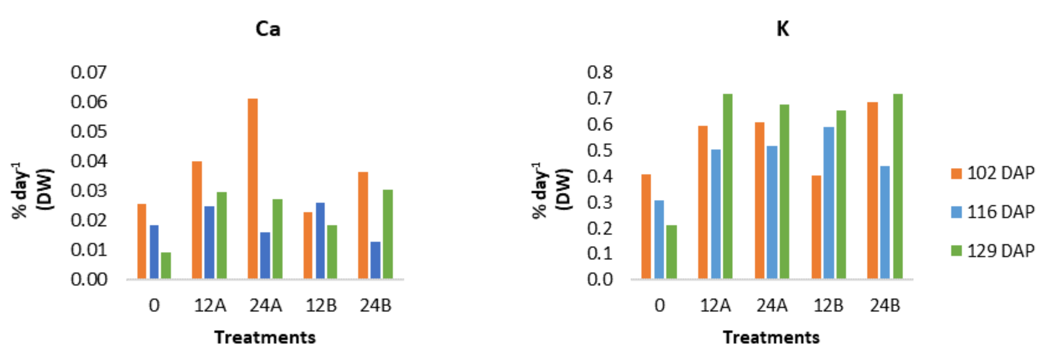


Figure 3.26 Absorption rate (µg by day) of Ca, K, S and P in tubers of *Solanum tuberosum* L. Picasso variety.

3.2.5.2.3 Translocation rate in tubers

Assessing the total translocation rate of Ca, K, S and P in tubers of Agria and Picasso varieties of *Solanum tuberosum* L (**Figure 3.27 - Figure 3.28**), it was observed that Agria exhibited a higher translocation rate for Ca compared to Picasso. However, the translocation rates for K, S, and P are similar between the two varieties. In Agria tubers (**Figure 3.27**), treatment 24B demonstrated a higher translocation rate for Ca, K, S and P after 129 days after plantation.



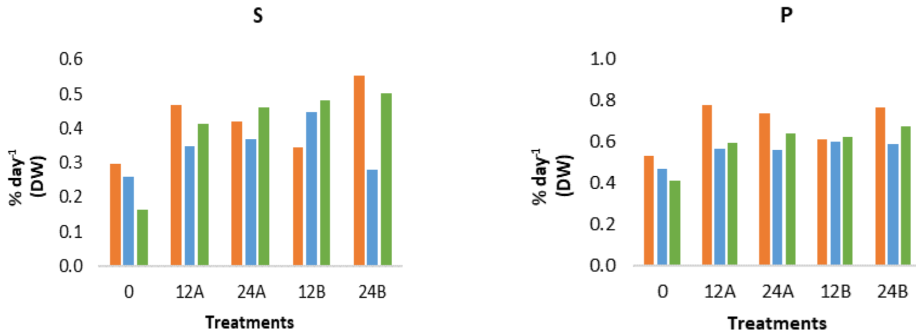


Figure 3.27 Daily translocation rates of Ca, K, S and P in tubers of *Solanum tuberosum* L., variety Agria.

Regarding Picasso variety (**Figure 3.28**), a higher translocation rate was observed for Ca, K, S and P across the different biofortification treatments. Specifically, 123 days after plantation, 12A treatment exhibited a higher translocation rate for S and K, while for Ca, this trend only occurred in treatment 24B (as also seen in Agria variety). Furthermore, 12B treatment demonstrated a higher translocation rate for K.

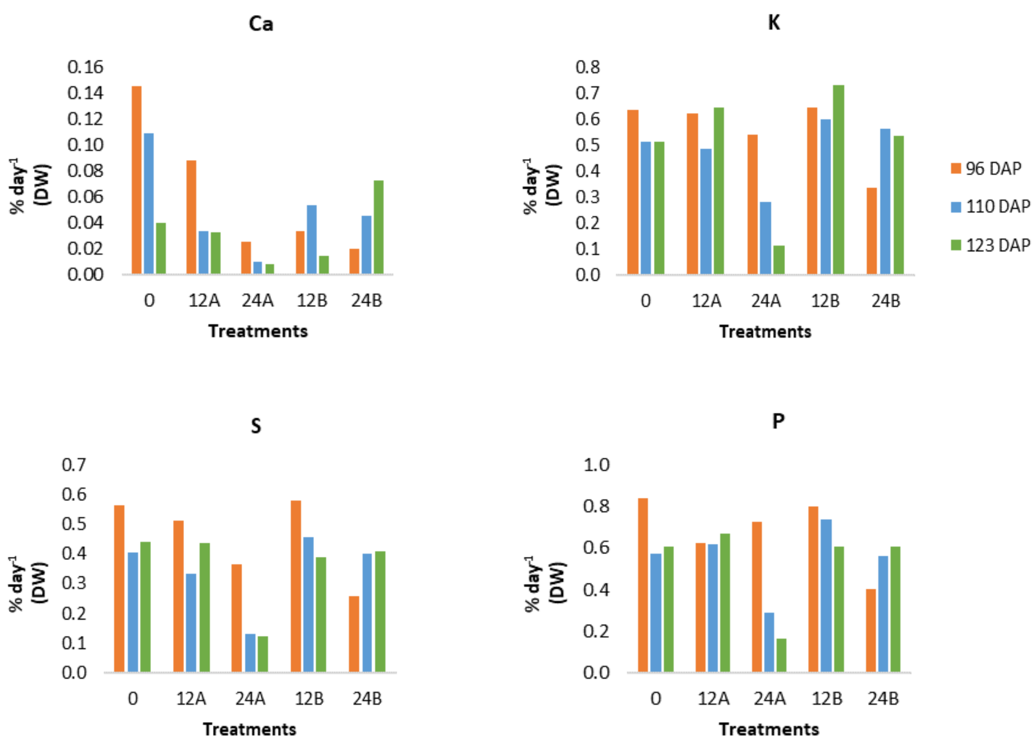


Figure 3.28 Daily translocation rates of Ca, K, S and P in tubers of *Solanum tuberosum* L., variety Picasso.

3.2.5.3 Tubers at harvest

The assessment of mineral element content in potato tubers in the three varieties (Agria, Picasso and Rossi), including the determination of Ca biofortification index, was conducted at harvest utilizing the ray fluorescence spectrometry (XRF) technique.

3.2.5.3.1 X-ray fluorescence spectrometry (XRF)

The evaluation of mineral element accumulation in the tubers was performed at harvest (**Table 3.32**), considering tubers with and without skin, to evaluate disparities in mineral content between these two preparation types. Significant differences were observed among treatments for each mineral element regarding the three varieties. In the case of Ca, Agria variety presented a significantly higher content in tubers, without skin, in the 12A treatment, and with skin, in the 24A treatment. Picasso and Rossi varieties exhibited a notable increase in Ca content in tubers with skin under 12B treatment, whereas without skin, a significant higher content was observed in 24A and 12B treatments, respectively. Hence, only Rossi showed in tubers, with and without skin, a significant higher content in the same treatment (12B). Potassium, S and P contents in Agria tubers, with and without skin, exhibited higher contents in the 12B treatment. Excepting for K in Picasso, 12B treatment consistently exhibited significantly higher values for K, S and P in Picasso and Rossi varieties, with and without skin, relatively to the other treatments. Overall, treatment 12B appears to positively affect the content of K, S and P regardless of the variety. Furthermore, relatively to tubers without skin, in general tubers with skin showed a higher content of Ca, K, S and P.

Table 3.32 Calcium, K, S and P contents (on a dry weight) in tubers of the three varieties of *Solanum tuberosum* L. (Agria, Picasso and Rossi) at harvest. Mean values (*n = 4*) ± SE (Standard Error).

Treatments	Agria				Picasso				Rossi			
	Ca	K	S	P	Ca	K	S	P	Ca	K	S	P
	%				%				%			
Without skin												
Ctr	0.019 ± 0.001b	3.163 ± 0.005b	0.137 ± 0.001b	0.069 ± 0.001bc	0.051 ± 0.001b	4.348 ± 0.012b	0.124 ± 0.008b	0.11 ± 0.006c	0.029 ± 0b	4.073 ± 0.021a	0.160 ± 0.008a	0.143 ± 0.006b
12A	0.031 ± 0.002a	3.432 ± 0.005b	0.137 ± 0.006b	0.075 ± 0.005bc	0.038 ± 0d	3.846 ± 0.005d	0.125 ± 0.003b	0.109 ± 0.001c	0.029 ± 0.001b	3.591 ± 0.002b	0.151 ± 0.002a	0.129 ± 0.002b
24A	0.021 ± 0.001ab	3.379 ± 0.007b	0.146 ± 0.001b	0.059 ± 0.001c	0.053 ± 0.001a	4.472 ± 0.009a	0.117 ± 0.001b	0.126 ± 0.001b	0.018 ± 0c	3.158 ± 0.01d	0.112 ± 0.002b	0.113 ± 0.002c
12B	0.028 ± 0.001ab	4.238 ± 0.004a	0.2 ± 0a	0.124 ± 0.001a	0.042 ± 0c	4.072 ± 0.003c	0.187 ± 0.004a	0.164 ± 0.003a	0.066 ± 0.002a	4.075 ± 0.012a	0.161 ± 0a	0.159 ± 0.001a
24B	0.029 ± 0.002ab	3.371 ± 0.356b	0.161 ± 0.018ab	0.081 ± 0.009b	0.032 ± 0.001d	3.421 ± 0.009e	0.137 ± 0.003b	0.097 ± 0c	0.034 ± 0.001b	3.345 ± 0.014c	0.083 ± 0.003c	0.048 ± 0.002d
With skin												
Ctr	0.078 ± 0.001d	2.676 ± 0.07c	0.082 ± 0.031b	0.034 ± 0.002c	0.057 ± 0.001d	3.092 ± 0.006e	0.113 ± 0.006c	0.08 ± 0.005d	0.058 ± 0.012c	2.369 ± 0.425ab	0.043 ± 0.007d	<LOD
12A	0.079 ± 0d	3.32 ± 0.007a	0.141 ± 0.001ab	0.069 ± 0.001b	0.061 ± 0.002d	3.157 ± 0.008d	0.115 ± 0.001c	0.062 ± 0.001e	0.076 ± 0.001bc	2.899 ± 0.006ab	0.088 ± 0.002c	0.076 ± 0.002c
24A	0.119 ± 0.002a	2.87 ± 0.004b	0.137 ± 0.001ab	0.067 ± 0.001b	0.072 ± 0c	3.54 ± 0.002b	0.124 ± 0c	0.1 ± 0c	0.092 ± 0.001b	2.896 ± 0.007ab	0.111 ± 0.001b	0.079 ± 0.001c
12B	0.097 ± 0.002b	3.415 ± 0.007a	0.166 ± 0.001a	0.124 ± 0.001a	0.127 ± 0.001a	4.123 ± 0.015a	0.207 ± 0.003a	0.172 ± 0.001a	0.226 ± 0.002a	3.039 ± 0.007a	0.14 ± 0.002a	0.123 ± 0.001a
24B	0.091 ± 0.001c	2.77 ± 0.002bc	0.146 ± 0.001ab	0.061 ± 0.001b	0.107 ± 0b	3.228 ± 0.009c	0.149 ± 0.002b	0.134 ± 0.001b	0.059 ± 0.001c	2.056 ± 0.01b	0.105 ± 0.001b	0.099 ± 0.001b

Different letters indicate significant different between treatments in each mineral element (a,b) ($p \leq 0.05$).

Regarding Ca accumulation (**Table 3.32**), all the varieties showed a significantly increased of Ca, relatively to the control tubers. In this context, tubers without skin showed a Ca biofortification index ranging between 10.5% to 63.2 %, and 3.9 %, as well as between17.2 % to 2.3 fold, respectively for Agria, Picasso and Rossi. Considering tuber with skin, Ca biofortification index varied between 1.3% to 52.6 %, 7 % to 2.2-fold and 1.7% to 3.9-fold, respectively. Regarding the correlation between Ca, K, S and P, it was possible to observe a negative correlation of the Pearson and Spearman correlations in Agria (**Table 3.33**). In fact, by comparing tubers without and with skin, a negative correlation was found for Ca. In tubers with and without skin, Ca showed a negative Pearson correlation with all the mineral elements (K, S and P). However, considering the Pearson correlation, in tubers without skin K and S revealed a very strong correlation ($r \geq 0.90$), therefore pointing a close at the absorption level (**Table 3.32**). Besides, P in tubers with skin also showed a strong correlation (r between 0.70 and 0.89) with K, S; additionally, a correlation was detected for P in tubers without skin, and in K and S in tubers with skin, therefore also indicating a close interaction at the absorption level (**Table 3.32**). Moreover, according to the Spearman correlation, in tubers with skin, a very strong correlation only occurred between K and P; other strong correlation was detected between K with skin and without skin, as well as for K without skin and P with skin, and for S without skin and Ca with skin, and for S without skin and P with skin.

Table 3.33 Correlation matrix of Ca, K, S and P content with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Agria variety. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	-0.368	-0.393	-0.437	-0.243	-0.214	-0.097	-0.396
	K	0.129	1.000	0.929	0.883	0.185	0.665	0.520*	0.810
	S	-0.130	0.657	1.000	0.911	0.231	0.455	0.491	0.753
	P	0.041	0.614*	0.464	1.000	-0.069	0.643	0.485	0.781
With skin	Ca	-0.286	0.214	0.725	-0.129	1.000	-0.029	0.345	0.356
	K	0.023	0.789	0.596*	0.471	0.361	1.000	0.646	0.829
	S	0.213	0.607*	0.679	0.686	0.311	0.686	1.000	0.835
	P	0.038	0.832	0.704	0.471	0.454	0.950	0.657	1.000

Correlation is significant at the 0.05 level (*).

Relatively to Picasso, considering the Pearson and Spearman methods, it was possible to observe negative correlations between Ca, K, S and P (**Table 3.34**). According to the Pearson (r) and Spearman (ρ) methods, there was a negative correlation between Ca in tubers without skin and Ca in tubers with skin, as also verified in Agria (**Table 3.33**). In fact, Ca in tubers without skin showed a negative Pearson correlation with all the analyzed minerals. Taking into consideration both Pearson and Spearman correlations, in tubers without skin, K and Ca showed a very strong correlation (> 0.90). This pattern Indicated that the higher K and Ca content were obtained in the same treatment (24A) in tubers without skin (**Table 3.32**). According to the Spearman correlation, in tubers with skin, S and Ca and S and P, showed a very strong correlation, followed by: K and Ca; P and Ca; S and K; P and K. Only for S, in tubers with skin and without skin, a strong correlation was found with the Spearman method. Considering the Pearson correlation, it was possible to observe a very strong correlation between S in tubers with skin and without skin, and for P in tubers without skin and K in tubers with skin, as well as in tubers with skin between Ca and S, Ca and P and S and P. A strong correlation was further found between: S (in tubers without skin) and Ca and K (in tubers with skin); for P (in tubers without skin) and S (in tubers with skin); in tubers with skin between Ca and K as well as between K and S.

Table 3.34 Correlation matrix of Ca, K, S and P content with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Picasso variety. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	0.966	-0.275	0.234	-0.484	0.040	-0.308	-0.272
	K	0.911	1.000	-0.164	0.388	-0.422	0.192	-0.210	-0.225
	S	-0.457	-0.486	1.000	0.809	0.839	0.819	0.953	0.847
	P	0.564*	0.618*	0.243	1.000	0.561*	0.938	0.776	0.653
With skin	Ca	-0.339	-0.268	0.654	0.329	1.000	0.753	0.941	0.968
	K	-0.032	0.104	0.418	0.629*	0.864	1.000	0.877	0.809
	S	-0.250	-0.225	0.736	0.393	0.929	0.829	1.000	0.947
	P	-0.136	-0.107	0.650	0.361	0.875	0.771	0.943	1.000

Correlation is significant at the 0.05 level (*).

Regarding Rossi, considering the Pearson and Spearman methods, it was possible to observe negative correlations between Ca, K, S and P (**Table 3.35**). According to Pearson (r) and Spearman (ρ) methods, in tubers with and without skin, a negative correlation between Ca could not be found, as verified in Agria (**Table 3.33**) and Picasso (**Table 3.34**). In fact, in tubers without skin, Ca showed a negative Pearson correlation with all the minerals. In the framework of the Pearson correlation, in tubers with skin, a very strong correlation (> 0.90) was only found for S and P, whereas with the Spearman method a correlation was detected for P and S, in tubers without skin. Nevertheless, considering the Pearson correlation in tubers without skin, K and S, as well as K and P, showed a strong correlation (r between 0.70 and 0.89). Also, Ca in tubers with and without skin showed a strong correlation, as well as Ca and S in tubers with skin. Moreover, with the Spearman method, only a strong correlation in tubers without skin was found for: S and K; P and K. in tubers with skin a strong correlation was detected for: K and Ca; S and Ca; P and S.

Table 3.35 Correlation matrix of Ca, K, S and P content with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Rossi variety. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	0.676	0.377	0.374	0.783	0.180	0.465	0.471
	K	0.489	1.000	0.837	0.710	0.453	0.119	-0.218	-0.273
	S	0.186	0.846	1.000	0.930	0.356	0.417	-0.297	-0.394
	P	0.354	0.814	0.943	1.000	0.555*	0.595*	-0.043	-0.204
With skin	Ca	0.121	0.068	0.250	0.429	1.000	0.595*	0.742	0.615*
	K	0.171	0.375	0.439	0.532*	0.811	1.000	0.462	0.278
	S	0.332	-0.207	-0.139	0.121	0.782	0.489	1.000	0.952
	P	0.688	-0.082	-0.197	0.054	0.495	0.297	0.842	1.000

Correlation is significant at the 0.05 level (*).

3.2.6 Morphological, physical, and organoleptic parameters

At harvest, as well as after 4 and 8 months of storage, the tubers from Agria, Picasso and Rossi varieties were assessed to evaluate their morphological, physical, and organoleptic characteristics.

3.2.6.1 Height, diameter, dry weight content and total soluble solids

After the harvest of tubers from each variety, height and diameter were measured across the different treatments (**Table 3.36**). No significant differences were observed among treatments for both parameters in the three varieties. The treatments with the highest values for height and diameter were observed in Agria with treatment 12A and Rossi with 12B treatment. Moreover, the height of tubers varied between 8.2 – 10.93 cm, 8.2 – 12.67 cm and 7.27 – 9.5 cm, for Agria, Picasso and Rossi, respectively. On the other hand, diameter varied between, 5.67 – 7.53 cm, 6.6 – 8.03 cm and 5.3 and 6.9 cm, for Agria, Picasso and Rossi, respectively.

Table 3.36 Height and diameter of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, at harvest. Mean values ($n = 4$) ± SE (Standard Error).

T.	Agria		Picasso		Rossi	
	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)
Ctr	9.13 ± 0.7a	6.73 ± 0.79a	8.2 ± 0.49a	7.57 ± 0.48a	8.3 ± 1.18a	6.3 ± 0.68a
12A	10.93 ± 0.74a	7.53 ± 0.23a	10.1 ± 1.01a	8.03 ± 0.52a	8.63 ± 1.09a	5.43 ± 0.81a
24A	9.17 ± 1.68a	6 ± 0.7a	9.2 ± 0.96a	6.63 ± 0.52a	8 ± 1.26a	6.3 ± 0.81a
12B	8.77 ± 1.13a	6.33 ± 0.38a	8.2 ± 0.61a	6.6 ± 0.42a	9.5 ± 1.04a	6.9 ± 0.79a
24B	8.2 ± 0.32a	5.67 ± 0.18a	12.67 ± 2.27a	7.97 ± 0.38a	7.27 ± 0.35a	5.3 ± 0.4a

Different letters (a, b) indicate significant different among treatments of each parameter, in each variety ($p \leq 0.05$).

At harvest, only Rossi showed significant differences among treatments, with the highest dry weight being obtained in control and the lowest with treatment 24A, followed by treatment 12B (**Table 3.37**). Moreover, the dry weight content varied between 22.87 – 26.49 %, 16.77 – 21.89 % and 16.27 – 25.27 %, respectively for Agria, Picasso and Rossi varieties (**Table 3.37**).

Table 3.37 Dry weight at harvest (H), after four (4M) and eight (8M) months of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria			Picasso			Rossi		
	DW (%)								
	H	4M	8M	H	4M	8M	H	4M	8M
Ctr	25.94 ± 0.23aA	22.75 ± 0.78aC	23.54 ± 1.05abB	17.12 ± 0.69aB	15.89 ± 1.61aC	24.36 ± 1.03aA	25.27 ± 1.03aA	19.94 ± 2.62aB	22.88 ± 1.59aA
12A	22.87 ± 0.84aB	17.32 ± 1.87bC	24.73 ± 1.29abA	21.89 ± 0.89aA	19.6 ± 1.09aB	18.77 ± 0.7bcB	23.38 ± 1.87abA	16.91 ± 2.36aB	21.97 ± 1.62aA
24A	24.24 ± 1.03aA	19.6 ± 0.91abB	23.97 ± 0.2abA	16.77 ± 2.52aA	16.49 ± 0.67aA	16.8 ± 0.9cA	16.27 ± 0.89cB	11.76 ± 5.03aC	22.4 ± 1.79aA
12B	26.49 ± 3.91aA	19.58 ± 0.85abB	20.57 ± 0.9bB	20.97 ± 1.87aA	16.72 ± 0.75aC	18.12 ± 0.54bcB	17.43 ± 1.67cA	14.84 ± 1.46aB	18.98 ± 0.7aA
24B	25.87 ± 1.24aA	23.88 ± 0.51aB	23.98 ± 0.37aB	18.99 ± 0.44aB	18.15 ± 0.65aB	21.6 ± 0.66abA	18.48 ± 0.15bcC	20.11 ± 0.84aB	25.44 ± 1.45aA

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 4M and 8M) within each treatment (A, B) ($p \leq 0.05$).

At harvest, the contents of total soluble solids (TSS) In Rossi and Picasso tubers did not show significant difference among treatments (**Table 3.38**). Additionally, it was possible to observed that significant differences occurred in each treatment during the three times of analysis (Harvest, 4M and 8M). At harvest, for Agria, Picasso and Rossi, TSS varied between 5.47 – 6.53 °Brix, 4.67 – 5.1 °Brix and 5.0 – 5.67 °Brix, respectively (**Table 3.38**). After 4M of storage the range of values of TSS in Agria, Picasso and Rossi were 4.97 – 5.4 °Brix, 4.9 – 5.23 °Brix and 4.97 – 5.37 °Brix, respectively. Yet, after 8M of storage, in tubers of Agria, Picasso and Rossi, the values of TSS varied between 5.83 – 8.2 °Brix, 5.33 – 6.37 °Brix and 6.27 – 8.17 °Brix, respectively.

Table 3.38 Total soluble solids at harvest (H), after four (4M) and eight (8M) months of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria			Picasso			Rossi		
	TSS (°Brix)								
	H	4M	8M	H	4M	8M	H	4M	8M
Ctr	6.53 ± 0.12aA	5.13 ± 0.07abB	6.5 ± 0.22bA	5 ± 0.09aB	5.13 ± 0.07abB	6.23 ± 0.05aA	5.33 ± 0.27aB	5.13 ± 0.07abB	8.17 ± 0.12aA
12A	6.3 ± 0.21abA	4.97 ± 0.07bB	6.27 ± 0.12bA	4.67 ± 0.1aB	5.03 ± 0.03abA	5.03 ± 0.07bA	5.67 ± 0.27aB	4.97 ± 0.03bC	7.6 ± 0.12abA
24A	6.03 ± 0.03abA	5.1 ± 0.05abC	5.83 ± 0.07bB	4.83 ± 0.27aB	4.9 ± 0.05bB	6.1 ± 0.12aA	5.0 ± 0aB	5.1 ± 0.05abB	6.27 ± 0.07cA
12B	5.47 ± 0.27bB	5.23 ± 0.05abB	6.27 ± 0.07bA	5.1 ± 0.05aB	5.23 ± 0.07aA	5.33 ± 0.05bA	5.0 ± 0aB	5.07 ± 0.03abB	7.33 ± 0.23bA
24B	6.2 ± 0.09abB	5.4 ± 0.05aC	8.2 ± 0.05aA	4.87 ± 0.05aB	4.97 ± 0.03abB	6.37 ± 0.07aA	5.0 ± 0.47aB	5.37 ± 0.1aB	7.5 ± 0.12abA

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 4M and 8M) within each treatment (A, B) ($p \leq 0.05$).

3.2.6.2 Total fatty acid content

Total fatty acid contents was carried out in tubers of Agria (**Table 3.39**), Picasso (**Table 3.40**) and Rossi (**Table 3.41**), at harvest, after four (4M) and eight (8M) months of storage. At harvest, tubers of Agria (**Table 3.39**) revealed significant differences among treatments in TFA, <C16:0, C16:0, C18:2 and C18:3. Tubers of Agria showed an increase of TFA with $CaCl_2$ treatments and a decreased in Ca-EDTA treatments. Moreover, significant differences were not found in DBI, but the control showed the highest value, which indicated a decrease of unsaturation of the fatty acid profiles in the biofortification treatments. After 4M of storage (**Table 3.39**), only TFA showed significant differences between treatments. In fact, the control showed the significantly lower value of TFA, whereas the Ca-EDTA treatments revealed significantly higher contents. After 8M of storage (**Table 3.39**) there significant differences among treatments in TFA, fatty acid (<C16:0, C16:0, C18:0, C18:1, C18:2 and C18:3) and DBI were not found. Moreover, regarding each treatment at different times of analysis (harvest, 4M and 8M) significant differences were found, but a clear tendency of accumulation was not detected. Overall, the fatty acid content showed a tendency of accumulation of: C18:2 > C18:3 > C16:0 > C18:0 > "<C16:0" > C18:1 at harvest); C18:2 > C18:3 > C16:0 > C18:0 > C18:1 > "<C16:0" (at 4 M); C18:2 > C18:3 > C16:0 > C18:0 > "<C16:0" > C18:1 (at 8 M). In the three times of analysis the fatty acids profile remained similar, being characterized by a highest abundance of linoleic acid (C18:2), followed by linolenic acid (C18:3), DBI, palmitic acid (C16:0) and stearic acid (C18:0). Besides, it was possible to observe an increase in TFA a tendency of 4M>8M>Harvest In Agria and Picasso varieties (**Table 3.39** and **Table 3.40**), but in Rossi the tendency of accumulation of TFA was different: 8M>4M>Harvest (**Table 3.41**).

Table 3.39 Total fatty acid content TFA, fatty acids profiles and double bound index (DBI) of lipids at harvest (H) and after four (4M) and eight (8M) months of storage from tubers of *Solanum tuberosum* L., Agria. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
				Harvest				
Ctr	0.22 ± 0.03abB	0.24 ± 0.03bB	5.03 ± 0.1bB	1.97 ± 0.08aA	0.34 ± 0.09aA	60 ± 1.8abA	32.41 ± 1.81abB	30.69 ± 0.74aA
12A	0.24 ± 0.02ab	0.35 ± 0.02abA	4.89 ± 0.51bC	2.85 ± 0.71aA	0.36 ± 0.09aC	58.8 ± 1.21abA	32.75 ± 1.38aA	29.04 ± 5.01aA
24A	0.24 ± 0.01aC	0.44 ± 0.05abA	6.3 ± 0.22abB	2.97 ± 0.67aA	0.27 ± 0.06aA	56.64 ± 0.86bA	33.39 ± 0.38aA	22.95 ± 2.04aA
12B	0.16 ± 0.02abC	0.32 ± 0.06abA	7.09 ± 0.26aB	2.58 ± 0.98aA	0.32 ± 0.08aA	63.12 ± 1.66aA	26.57 ± 1.4bB	21.63 ± 2.96aA
24B	0.15 ± 0bC	0.46 ± 0.05aA	6.61 ± 0.31aB	1.42 ± 0.05aB	0.2 ± 0aB	56.11 ± 1.03bB	35.19 ± 1.14aA	26.67 ± 1.27aA
				4M				
Ctr	0.9 ± 0.1bA	0.42 ± 0.33aA	9.24 ± 1.27aA	1.43 ± 0.08aB	0.4 ± 0.03aA	58.11 ± 0.65aA	30.41 ± 0.99aC	19.6 ± 3.22aB
12A	1.06 ± 0.13bA	0.29 ± 0.09aB	8.08 ± 0.95aB	1.24 ± 0.37aB	0.62 ± 0.28aA	59 ± 1.65aA	30.77 ± 2.19aB	23.27 ± 2.93aA
24A	1.33 ± 0.1abA	0.17 ± 0.03aC	7.83 ± 1.32aB	1.2 ± 0.23aC	0.26 ± 0.04aA	58.28 ± 2.28aA	32.25 ± 2.74aA	24.48 ± 3.72aA
12B	1.36 ± 0.11ab	0.19 ± 0.09aC	7.03 ± 0.87aB	1.33 ± 0.17aC	0.31 ± 0.16aA	63.09 ± 1.3aA	28.05 ± 0.27aB	26.78 ± 2.21aA
24B	1.57 ± 0.1aA	0.16 ± 0aC	7.21 ± 0.31aB	1.11 ± 0.09aC	0.29 ± 0.08aA	60.74 ± 2.67aA	30.48 ± 2.94aA	25.83 ± 1.39aA
				8M				
Ctr	0.81 ± 0.06aA	0.29 ± 0.02aB	11.01 ± 0.64aA	1.86 ± 0.2aA	0.23 ± 0.01aB	51.05 ± 0.84aB	35.56 ± 0.16aA	16.12 ± 1.05aB
12A	0.72 ± 0.03aB	0.42 ± 0.05aA	11.48 ± 0.23aA	2.25 ± 0.24aA	0.44 ± 0.03aB	52.04 ± 0.75aB	33.37 ± 1.08aA	14.59 ± 0.6aB
24A	0.81 ± 0.03aB	0.28 ± 0.04aB	11.24 ± 0.12aA	2.12 ± 0.1aB	0.22 ± 0.14aA	52.32 ± 1.35aB	33.81 ± 1.54aA	15.3 ± 0.3aB
12B	0.85 ± 0.04aB	0.29 ± 0.05aB	10.95 ± 1.03aA	1.96 ± 0.35aB	0.19 ± 0.05aB	52.9 ± 0.62aB	33.71 ± 0.94aA	16.62 ± 1.64aB
24B	0.92 ± 0.08aB	0.5 ± 0.14aB	11.63 ± 1.6aA	1.76 ± 0.24aA	0.38 ± 0.12aA	50.54 ± 0.7aC	35.19 ± 1.84aA	15.29 ± 1.77aB

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 4M and 8M) within each treatment (A, B) ($p \leq 0.05$).

Regarding Picasso (**Table 3.40**), at harvest significant differences were found among treatments in some fatty acid profiles (<C16:0, C18:0) and DBI, as well as after 4M of storage in TFA, <C16:0, C16:0, C18:1, C18:3 and DBI and, after 8M, in C16:0, C18:0 and DBI. Regarding TFA, at harvest, biofortification treatments showed higher values relatively to the control (although not significantly different). Moreover, after 4M of storage, the biofortification treatments showed significantly lower contents relatively to the control. After 8M of storage, TFA did not show significant differences, however, relatively to the remaining treatments, 24B revealed the highest value and treatment 12A the lowest. At harvest and after 4M of storage, treatment 24B and the control showed a significantly lower value of <C16:0 compared to the other treatments. Moreover, also after 4M of storage, the biofortification treatments showed significantly higher values regarding the control. Still, there was a growing unsaturation of the fatty acid's profiles in the biofortification treatments, as expressed by the increase of DBI at harvest and after 8M (except in treatment 12A) (but the opposite occurred after 4M of storage). After 8M of storage (**Table 3.40**), in treatment 12B, C16:0 and C18:0 fatty acids showed significantly lower values relatively to the remaining treatments, yet treatment 12A revealed significant higher values. Also, treatment 12B showed a significant higher content in DBI, relatively to the remaining biofortified treatments. Overall, at

harvest, after 4M and 8M of storage (**Table 3.40**) the four more abundant fatty acids were: C18:2 (linoleic acid) > C18:3 (linolenic acid) > C16:0 (palmitic acid) > C18:0 (stearic acid).

Table 3.40 Total fatty acid content (TFA), fatty acids profiles and double bound index (DBI) of lipids at harvest (H), after four (4M) and eight months (8M) of storage from tubers of *Solanum tuberosum* L., Picasso variety. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
					Harvest			
Ctr	0.11 ± 0.01aC	0.52 ± 0.05abA	10.49 ± 0.97aA	3.64 ± 0.1abA	0.21 ± 0.06aB	65 ± 1.89aB	20.14 ± 1.91aA	13.41 ± 1.04bB
12A	0.16 ± 0.03aC	0.62 ± 0.04aA	8.15 ± 0.77aC	4.65 ± 0.52aA	0.52 ± 0.1aB	65.52 ± 0.48aA	20.54 ± 1.23aA	14.88 ± 1.28bA
24A	0.19 ± 0.02aC	0.38 ± 0.04bB	9.13 ± 1.85aA	2.73 ± 0.58abA	0.41 ± 0.05aA	69.53 ± 2.08aA	17.81 ± 1.2aB	17.15 ± 3.18abA
12B	0.16 ± 0.02aB	0.38 ± 0.05bcB	10.07 ± 0.79aB	2.07 ± 0.53bA	0.32 ± 0.07aB	69.63 ± 0.94aA	17.53 ± 0.58aB	15.88 ± 1.81bA
24B	0.17 ± 0.03aB	0.3 ± 0.03cB	5.97 ± 0.63aB	1.9 ± 0.24bA	0.46 ± 0.08aA	71.1 ± 0.61aA	20.26 ± 0.86aB	26.06 ± 2.2aA
					4M			
Ctr	1.27 ± 0.07aA	0.15 ± 0.02bC	5.16 ± 0.51bB	1.12 ± 0.1aC	0.23 ± 0.02bB	70.71 ± 0.68aA	22.63 ± 1.3aA	34.04 ± 3.04aA
12A	0.83 ± 0.09bA	0.25 ± 0.04abC	10.56 ± 0.77aB	1.82 ± 0.31aC	0.33 ± 0.04abC	69.65 ± 0.25aA	17.39 ± 1.08abB	15.97 ± 1.74bA
24A	0.95 ± 0.04abA	0.26 ± 0.05abC	10.13 ± 1.79abA	3.21 ± 1.88aA	0.42 ± 0.07abA	67.91 ± 1.78aA	18.08 ± 0.71abB	14.49 ± 1.83bB
12B	0.69 ± 0.07bA	0.44 ± 0.11aB	14.46 ± 1.87aA	2.3 ± 0.23aA	0.24 ± 0.02bB	67.25 ± 1.55aA	15.3 ± 0.78bC	10.9 ± 1.61bA
24B	0.85 ± 0.12bA	0.48 ± 0.06aA	12.95 ± 1.08aA	1.61 ± 0.22aB	0.57 ± 0.13aA	66.74 ± 1.86aB	17.66 ± 3.06abC	13.06 ± 1.84bC
					8M			
Ctr	0.65 ± 0.14aB	0.45 ± 0.09aB	14.24 ± 1.83aA	2.24 ± 0.27abB	0.56 ± 0.05aA	58.2 ± 2.24aC	24.32 ± 1.62aA	11.68 ± 2.05bB
12A	0.58 ± 0.07aB	0.48 ± 0.04aB	16.12 ± 0.9aA	2.77 ± 0.31aB	0.73 ± 0.18aA	58.58 ± 0.47aB	21.31 ± 1.13aA	9.51 ± 0.77bB
24A	0.7 ± 0.03aB	0.55 ± 0.1aA	13.29 ± 1.18abA	1.91 ± 0.03abB	0.5 ± 0.28aA	61.92 ± 0.86aB	21.83 ± 1.19aA	12.39 ± 1.05bB
12B	0.64 ± 0.11aA	0.6 ± 0.15aA	13.05 ± 1.24abA	2.29 ± 0.45abA	0.9 ± 0.28aA	59.64 ± 0.71aB	23.52 ± 1.67aA	12.32 ± 1.7bA
24B	0.83 ± 0.11aA	0.34 ± 0.04aB	9.73 ± 1.12bA	1.48 ± 0.09bB	0.28 ± 0.09aB	61.54 ± 1.73aB	26.64 ± 2.85aA	18.08 ± 2.18aB

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 4M and 8M of storage) within each treatment (A, B, C) ($p \leq 0.05$).

Considering Rossi (**Table 3.41**), significant differences were found among treatments in <C16:0 and C18:1 (at harvest), in C18:1 (after 4M of storage) and in C18:1 and C18:3 (after 8M of storage). At harvest, TFA contents in biofortified tubers with Ca were higher (although not significantly) than the control (except for 12B treatment). Considering DBI, despite in each time of analysis significant differences were not found among treatments, at harvest, the control presented the highest values relatively to the biofortified treatments. However, the control showed the lowest level of DBI, regarding the remain treatments after 4M (except in 12A treatment) and 8M of storage, indicating that occur a growing unsaturation in fatty acid profiles, expressed by the increase in DBI in the biofortification treatments. Furthermore, at harvest the fatty acids content in Rossi tubers was characterized (**Table 3.41**) by the highest abundance of C18:2, followed by C18:3, C16:0, C18:0, C18:1 and <C16:0. After 4M and 8M of storage the highest abundance of the fatty acids in the different treatments were characterized by the following order: C18:2, C18:3, C16:0, C18:0, <C16:0 and C18:1.

Table 3.41 Total fatty acid content TFA, fatty acids profiles (TFA) and double bound index (DBI) of lipids at harvest (H) and after four (4M) and eight months (8M) of storage from tubers of *Solanum tuberosum* L., Rossi variety. Mean values ($n = 4$) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
Harvest								
Ctr	0.13 ± 0.02aB	0.25 ± 0.03bC	11.53 ± 1.65aA	3.02 ± 0.49aA	0.93 ± 0.01abA	66.23 ± 1.81aA	18.04 ± 1.64aB	13.61 ± 2.75aA
12A	0.16 ± 0.01aB	0.44 ± 0.04abB	11.74 ± 2aA	3.63 ± 0.38aA	0.71 ± 0.06abcA	64.54 ± 1aA	18.94 ± 1.33aB	12.54 ± 1.96aA
24A	0.16 ± 0.04aB	0.43 ± 0.06abB	11.44 ± 0.41aA	2.97 ± 0.27aA	0.64 ± 0.11bcA	65.51 ± 0.74aB	19.02 ± 0.2aB	12.97 ± 0.66aB
12B	0.12 ± 0.01aB	0.32 ± 0.04abB	15.35 ± 1.68aA	3.95 ± 0.56aA	0.62 ± 0.06bA	59.15 ± 0.98aB	20.6 ± 1.82aB	9.63 ± 1.62aB
24B	0.16 ± 0.01aB	0.49 ± 0.05aA	10.55 ± 0.61aA	4.12 ± 0.83aA	0.94 ± 0.01aA	58.89 ± 3.11aB	25.01 ± 4.38aB	13.29 ± 1.74aA
4M								
Ctr	0.97 ± 0.11aA	0.54 ± 0.03aA	13.19 ± 1.81aA	1.85 ± 0.2aC	0.37 ± 0.02abC	67.02 ± 1.49aA	17.04 ± 3.46aB	12.61 ± 2.39aA
12A	0.82 ± 0.11aA	0.63 ± 0.12aA	13.87 ± 0.92aA	2.42 ± 0.9aB	0.62 ± 0.19aA	65.35 ± 1.61aA	17.11 ± 0.55aB	11.21 ± 1.35aA
24A	1.24 ± 0.17aA	0.56 ± 0.09aA	9.8 ± 0.59aB	1.2 ± 0.13aB	0.37 ± 0.11abB	66.9 ± 0.56aA	21.17 ± 1aB	17.86 ± 1.48aA
12B	0.95 ± 0.15aA	0.42 ± 0.1aA	13.08 ± 1.52aB	1.56 ± 0.23aB	0.22 ± 0.07bB	63.5 ± 0.51aA	21.21 ± 2.08aB	13.72 ± 2.41aB
24B	1.1 ± 0.09aA	0.49 ± 0.05aA	12.32 ± 1.59aA	1.44 ± 0.31aB	0.41 ± 0.04abB	67.87 ± 1.66aA	17.47 ± 0.29aB	13.79 ± 1.9aA
8M								
Ctr	0.9 ± 0.08aA	0.41 ± 0.04aB	13.79 ± 0.54aA	2.09 ± 0.12aB	0.62 ± 0.08aB	60.91 ± 0.99aB	22.19 ± 1.03cA	12.06 ± 0.43aA
12A	0.93 ± 0.05a	0.39 ± 0.03aB	10.58 ± 0.83aA	1.6 ± 0.08aC	0.51 ± 0.03abB	61.42 ± 1.7aA	25.51 ± 0.84abcA	16.38 ± 1.17aA
24A	1.08 ± 0.06aA	0.42 ± 0.05aB	9.64 ± 0.7aB	1.58 ± 0.27aB	0.38 ± 0.09abB	59.13 ± 1.28aC	28.85 ± 1.3aA	18.55 ± 0.77aA
12B	1.01 ± 0.04aA	0.37 ± 0.06aB	10.08 ± 0.41aC	1.41 ± 0.11aB	0.18 ± 0.1bB	60.69 ± 0.77aA	27.27 ± 0.5abA	17.49 ± 0.66aA
24B	1.01 ± 0.15aA	0.44 ± 0.11aA	11.83 ± 1.5aA	1.96 ± 0.24aB	0.28 ± 0.11abC	60.65 ± 0.77aB	24.84 ± 0.9bcA	14.49 ± 1.73aA

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 4M and 8M of storage) within each treatment (A, B, C) ($p \leq 0.05$).

3.2.6.3 Soluble sugar content

The contents of sugars (sucrose, glucose, fructose, and sorbitol) were analyzed in tubers of Agria, Picasso and Rossi (**Table 3.42**), being found significant differences among these varieties at harvest, 4M and 8M of storage. In Agria, sucrose contents varied between 0.44 – 0.076, 0.006 – 0.067 and 0.017 – 0.553 mg/g_{FW}, respectively for harvest, 4M and 8M of storage. Sucrose contents in Picasso ranged between 0.33 - 0.158, 0.005 – 0.062 and 0.099 – 0.408 mg/g_{FW}, respectively for harvest, 4M and 8M of storage. In Rossi, the values at harvest were significantly higher in all the treatments relatively to the values obtained after 4M and 8M of storage (sucrose contents varied between 0.132 – 0.314, 0.013 – 0.075 and 0.001 – 0.096 mg/g_{FW} at harvest, 4M and 8M, respectively. At harvest, glucose contents varied between 0.064 – 0.089, 0.067 – 0.159 and 0.077 – 0.114 mg/g_{FW}, respectively for Agria, Picasso and Rossi (**Table 3.42**). After 4M of storage, the contents of glucose ranged in between 0.060 – 0.070, 0.254 – 0.327 and 0.096 – 0.308 mg/g_{FW}, for Agria, Picasso and Rossi. Moreover, after 8M of storage the contents of glucose varied between 0.058 – 0.328, 0.053 – 0.445 and 0.362 – 0.594 mg/g_{FW} respectively for Agria, Picasso and Rossi. Fructose contents in Agria, ranged between 0.017 – 0.037, 0.006 – 0.051 and 0.007 – 0.265 mg/g_{FW}, respectively at harvest, 4M and 8M of storage (**Table 3.42**). Considering Picasso, at harvest, 4M and 8M of storage, fructose contents varied respectively between 0.041 – 0.178, 0.140 – 0.188 and 0.013 – 0.394 mg/g_{FW} (**Table 3.42**). In Rossi fructose contents varied between 0.022- 0.054, 0.076 – 0.247 and 0.195 – 0458 mg/g_{FW}, respectively at harvest, 4M and 8M of storage (**Table 3.42**). Furthermore, sorbitol contents showed higher contents than glucose and sucrose in most treatments in the three varieties (**Table 3.42**). In Agria, at harvest, after 4M and 8M of storage, sorbitol contents ranged between 0.077 – 0.099, 0.066 – 0.084 and 0.062 – 0.092 mg/g_{FW}, respectively. Considering Picasso, sorbitol content varied between 0.058 – 0.120, 0.069 – 0.075 and 0.063 – 0.085 mg/g_{FW}, respectively. Moreover, in Rossi sorbitol contents oscillated between 0.063 – 0.082, 0.077 – 0.095 and 0.063 – 0.112 mg/g_{FW}, respectively at harvest, 4M and 8M of storage. Considering the total of soluble sugars, significant differences among treatments in Agria, Picasso and Rossi were observed (**Table 3.42**). Indeed, the highest total soluble sugars were detected in tubers from the three varieties after 8M of cold storage (with treatment 24A of Agria, and with treatment 12B in Picasso and Rossi). As such, in tubers Ca biofortification treatments after 8M of cold storage showed a higher sugar content than the control (except in Agria with treatments 12A and 12B and with treatment Picasso in 23B).

Table 3.42 Soluble sugar content (SS) (mg/g_{Fw}) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest, after 4 (4M) and 8 months (8M) of storage. Mean values (*n = 4*) ± SE (Standard Error).

SS	T.	Agria			Picasso			Rossi		
		Harvest	4M	8M	Harvest	4M	8M	Harvest	4M	**8M**
Sucrose	Ctr	0.096 ± 0.001aB	0.057 ± 0.001aC	0.149 ± 0.003bA	0.172 ± 0bA	0.005 ± 0dC	0.112 ± 0.001aB	0.214 ± 0.002cA	0.013 ± 0dB	0.005 ± 0.0dC
	12A	0.076 ± 0.001aA	0.006 ± 0.003cC	0.017 ± 0.001dB	0.158 ± 0.001bA	0.040 ± 0.001bC	0.220 ± 0.001bB	0.314 ± 0aA	0.047 ± 0.002bC	0.096 ± 0.001aB
	24A	0.067 ± 0aA	0.067 ± 0.004aA	0.053 ± 0.002aB	0.136 ± 0.001cA	0.062 ± 0.001aB	0.150 ± 0cC	0.132 ± 0.003eA	0.072 ± 0.001aB	0.034 ± 0.001bC
	12B	0.044 ± 0.001aB	0.026 ± 0.004bC	0.087 ± 0.002cA	0.320 ± 0.01aB	0.025 ± 0.003cC	0.408 ± 0aA	0.272 ± 0.004bA	0.075 ± 0.001aB	0.010 ± 0cC
	24B	0.062 ± 0.034aB	0.028 ± 0.001bC	0.080 ± 0.001cA	0.330 ± 0.001aC	0.042 ± 0bB	0.099 ± 0.001eA	0.168 ± 0.002dA	0.037 ± 0.003cB	0.001 ± 0.001dC
Glucose	Ctr	0.065 ± 0aB	0.070 ± 0aA	0.063 ± 0dC	0.086 ± 0.001cC	0.295 ± 0cA	0.148 ± 0.004dB	0.077 ± 0.001eC	0.096 ± 0.001bB	0.283 ± 0.005bA
	12A	0.069 ± 0aB	0.060 ± 0bC	0.078 ± 0cA	0.09 ± 0.001cC	0.327 ± 0.002aA	0.226 ± 0.001cB	0.090 ± 0cC	0.097 ± 0.001bB	0.252 ± 0.002cA
	24A	0.064 ± 0aC	0.070 ± 0aB	0.167 ± 0.003bA	0.159 ± 0.003aC	0.254 ± 0.001eB	0.339 ± 0.001bA	0.085 ± 0.001dC	0.264 ± 0.003aB	0.274 ± 0.001bA
	12B	0.082 ± 0aA	0.067 ± 0.002aB	0.058 ± 0dC	0.067 ± 0dC	0.302 ± 0bB	0.445 ± 0.002aA	0.114 ± 0aC	0.308 ± 0.001aB	0.594 ± 0.004aA
	24B	0.089 ± 0.041aB	0.060 ± 0bC	0.328 ± 0.002aA	0.120 ± 0.001bC	0.279 ± 0dA	0.053 ± 0eB	0.096 ± 0bC	0.106 ± 0.021bB	0.283 ± 0.003bA
Fructose	Ctr	0.017 ± 0aB	0.044 ± 0.019aA	0.007 ± 0bC	0.042 ± 0bC	0.159 ± 0cB	0.161 ± 0dA	0.022 ± 0bC	0.121 ± 0.001bB	0.195 ± 0.043cA
	12A	0.024 ± 0aB	0.022 ± 0.015aB	0.027 ± 0bA	0.056 ± 0bC	0.169 ± 0bB	0.206 ± 0.001cA	0.027 ± 0bC	0.076 ± 0bB	0.371 ± 0.003abA
	24A	0.022 ± 0aC	0.051 ± 0.002aB	0.265 ± 0.001aA	0.178 ± 0.005aB	0.140 ± 0dC	0.394 ± 0.001aA	0.054 ± 0.006aB	0.247 ± 0.037aA	0.284 ± 0.001bcA
	12B	0.023 ± 0aA	0.007 ± 0aB	0.006 ± 0bB	0.175 ± 0.004aC	0.188 ± 0.005aB	0.315 ± 0.001bA	0.028 ± 0bC	0.205 ± 0.001aB	0.458 ± 0.005aA
	24B	0.037 ± 0.016aB	0.006 ± 0aC	0.185 ± 0.046aA	0.041 ± 0.003bA	0.180 ± 0aB	0.013 ± 0eC	0.022 ± 0bC	0.103 ± 0.004bB	0.236 ± 0.043cA
Sorbitol	Ctr	0.078 ± 0.001bA	0.072 ± 0bA	0.077 ± 0.005bA	0.112 ± 0.002bA	0.071 ± 0aB	0.063 ± 0.002bC	0.078 ± 0.001abB	0.094 ± 0aA	0.077 ± 0.006bB
	12A	0.077 ± 0bB	0.066 ± 0bC	0.081 ± 0abA	0.12 ± 0aC	0.072 ± 0.001aB	0.067 ± 0.001bA	0.079 ± 0.002aB	0.077 ± 0aB	0.112 ± 0.002aA
	24A	0.081 ± 0bC	0.084 ± 0aB	0.092 ± 0.001aA	0.058 ± 0dC	0.073 ± 0aA	0.069 ± 0bB	0.074 ± 0.001bB	0.095 ± 0.012aA	0.063 ± 0.001bC
	12B	0.099 ± 0aA	0.072 ± 0bB	0.062 ± 0cC	0.063 ± 0cC	0.069 ± 0.003aB	0.085 ± 0.002aA	0.082 ± 0.001aA	0.078 ± 0aB	0.08 ± 0.001bA
	24B	0.078 ± 0.004bB	0.081 ± 0.004aA	0.077 ± 0.002bB	0.063 ± 0cC	0.075 ± 0aA	0.069 ± 0.001bB	0.063 ± 0.001cC	0.085 ± 0.002aB	0.102 ± 0.009aA
Total	Ctr	0.256 ± 0.002abB	0.244 ± 0.018aB	0.296 ± 0.003cA	0.413 ± 0.003cC	0.529 ± 0.001cA	0.483 ± 0.003dB	0.391 ± 0.002bB	0.324 ± 0.001bC	0.555 ± 0.037cA
	12A	0.246 ± 0.001bA	0.155 ± 0.018bC	0.203 ± 0.001cB	0.424 ± 0cC	0.608 ± 0.003aA	0.719 ± 0.002cB	0.509 ± 0.001aB	0.298 ± 0.002bC	0.832 ± 0.005bA
	24A	0.234 ± 0cC	0.271 ± 0.005aB	1.078 ± 0.003aA	0.532 ± 0.007bB	0.529 ± 0.001bB	0.953 ± 0.002bA	0.345 ± 0.008cB	0.678 ± 0.035aA	0.655 ± 0.002cA
	12B	0.248 ± 0.001bA	0.172 ± 0.002bC	0.214 ± 0.002cB	0.625 ± 0.012aB	0.584 ± 0.005bC	1.253 ± 0.004aA	0.496 ± 0.004aC	0.665 ± 0.001aB	1.142 ± 0.005aA
	24B	0.266 ± 0.005aB	0.175 ± 0.003bC	0.669 ± 0.047bA	0.555 ± 0.003bB	0.576 ± 0cA	0.235 ± 0.002eC	0.349 ± 0.002cB	0.330 ± 0.017bB	0.622 ± 0.038cA

Different letters indicate significant different between treatments in each date (a, b, c) or between dates for the same treatment of biofortification (A, B, C) ($p \leq 0.05$).

Our data (**Table 3.42**) revealed that storage differently affected fructose, glucose, sucrose, and sorbitol contents in tubers of Agria, Picasso and Rossi.

3.2.6.4 Protein content

In terms of protein content in Agria tubers, significant differences were identified among treatments at harvest, after 4M and after 8M of storage, with treatment 12A displaying a significantly lower protein content when compared to the other treatments (**Table 3.43**). In the case of Picasso tubers, no significant differences in protein content were observed among treatments at harvest, while Rossi tubers exhibited significant differences among treatments at harvest and after 4M and 8M of storage. Also in Rossi tubers, a significantly higher protein content was detected in treatment 12B (at harvest and after 4M of storage) and in treatment 24B (after 8M of storage), relatively to the remaining treatments. Overall, in the three verities there were significant differences among the different analytical periods, being possible to observe a decrease of protein content from harvest to 4M of storage and an increased thereafter until the 8M of storage.

Table 3.43 Protein content at harvest (H), after four months (4M) and eight months (8M) of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error). Treatments (T).

T.	**Protein content (g/100g of fresh weight)**		
	Harvest	4M	**8M**
	Agria		
Ctr	1.52 ± 0.05aA	1.13 ± 0.07aB	1.51 ± 0.05aA
12A	1.54 ± 0.25aA	0.99 ± 0.06aB	1.23 ± 0.05bA
24A	1.63 ± 0.17aA	1.06 ± 0.1aC	1.4 ± 0.04abB
12B	1.82 ± 0.2aA	1.05 ± 0.11aC	1.54 ± 0.07aB
24B	2.1 ± 0.24aA	1.11 ± 0.13aC	1.56 ± 0.06aB
	Picasso		
Ctr	1.37 ± 0.21aA	1.04 ± 0.03aB	1.28 ± 0.11aA
12A	1.01 ± 0.01aB	0.98 ± 0.04abB	1.38 ± 0.08aA
24A	0.88 ± 0.13aB	1.15 ± 0.05aA	*Not quantified due to spectrophetometer failure
12B	1.19 ± 0.17aA	1.07 ± 0.06aA	
24B	1.04 ± 0.05aA	0.69 ± 0.14bB	
	Rossi		
Ctr	1.43 ± 0.12bA	0.63 ± 0.06bB	*Not quantified due to spectrophetometer failure
12A	1.18 ± 0.12cA	*Not quantified due to spectrophetometer failure	0.91 ± 0.04bB
24A	1.25 ± 0.09cA		0.89 ± 0.07bB
12B	1.51 ± 0.08aA	1.11 ± 0.19aB	0.85 ± 0.03bC
24B	1.41 ± 0.12bA	0.79 ± 0.06bC	1.18 ± 0.05aB

Different letters indicate significant different between treatments at harvest or after 4 or 8 months of storage for each variety (a,b) or between harvest, after 4 or 8 months of storage in the same treatment of biofortification (A,B) ($p \leq 0.05$).

3.2.6.5 Colorimetric parameters

At harvest, scanning colorimetric analysis in the visible spectral region (450 – 650 nm) were carried out in pulp of tubers from the three varieties, being possible to observe a maximum transmittance value at 550 nm (**Figure 3.29**), corresponding to the yellow color.

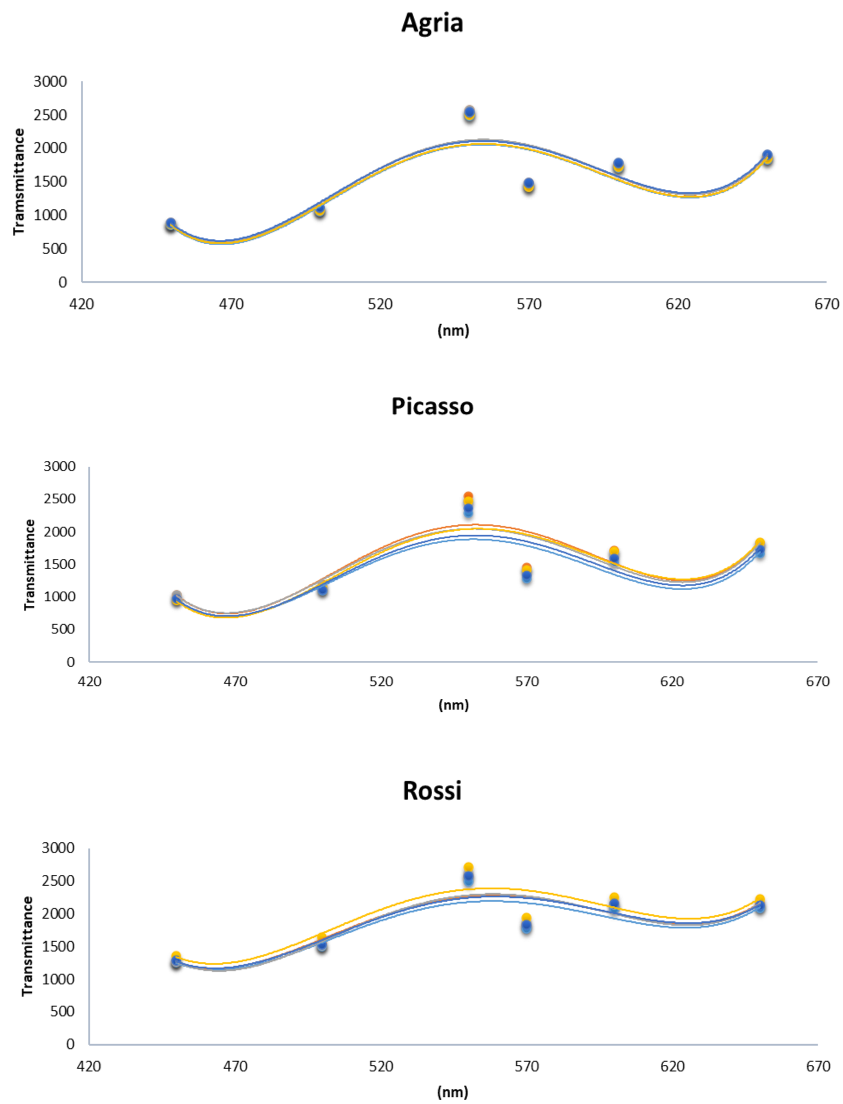


Figure 3.29 Visible spectra showing the average of transmittance (*n = 4*) in tubers of *Solanum tuberosum* L. varieties (Agria, Picasso and Rossi), at harvest, with a degree 4 polynomial (● Control, ● 12 kg ha^{-1} $CaCl_2$, ● 24 kg ha^{-1} $CaCl_2$, ● 12 kg ha^{-1} Ca-EDTA, ● 24 kg ha^{-1} Ca-EDTA).

Moreover, at harvest, as well as after 4M and 8M of storage, colorimetric parameters of the pulp of tubers were also analyzed through another colorimetric system: CieLab (**Table 3.44**). Regarding L parameter (Luminance) and considering that corresponds to a gray scale, varying between 0 (black) to 100 (white), it was found that in the three analytical periods, only Picasso (after 8M of storage) and Rossi (after 4M of storage) showed significant differences among treatments. As such, treatments with a significantly higher value of L (treatments 12B in Picasso and 24A and 12B in Rossi) presented a whiter component compared to the remain treatments. Overall, Picasso presented a lower L value in the three analytical periods relatively to Agria and Rossi. Concerning to the a* parameter, Agria and Picasso only showed significant differences among treatments after 8M of storage, and Rossi after 4M and 8M of storage. Agria and Rossi showed a significantly higher value of a* parameter in treatment 12B, but in tubers Picasso in the control. Thus, considering that a* value indicates the red (positive a*) and green (negative a*) component, those treatments, at the indicated analytical periods, revealed a more greenish color relatively to the other treatments. Considering b*parameter and Chroma (saturation), Agria in the three analytical periods revealed a higher value in all the treatments relatively to the other varieties. In this context, considering b* and Chroma, Agria showed a significantly higher values in Ca-EDTA treated tubers (after 8M of storage), whereas in Picasso this trend was detected in treatment 12B (at harvest and after 8M) and in Rossi in treatment 12B after 8M (thus, a yellowish color prevailed). Accordingly, these data suggest that Ca-EDTA treatments interferes with b* parameter and Chroma, particularly after 8M of storage, leading to an increase of yellow (b*) and brightness (Chroma) components. Hue angle, which represents the color tone, also showed significant variations in tubers of Agria after 8M, and in Picasso and Rossi at harvest and after 8M. Indeed, treatment 12B showed the lowest Hue value in Agria (after 8M), Picasso (at harvest and after 8M) and Rossi (after 8M). Additionally, in Rossi, treatments 24A and 12A showed a significantly higher Hue value at harvest and after 8M of storage, respectively. In general, for the analyzed parameters of most of treatments of the three varieties, the values were higher at harvest (relatively to 4M and 8M of storage).

Table 3.44 Color parameters considering the CieLab scale (L, a*, b*, chroma and hue) at harvest (H) and after four (4M) and eight (8M) months of storage of raw tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	L			a*			b*			Chroma			Hue		
	H	4M	8M	H	4M	8M	H	4M	8M	H	4M	8M	H	4M	8M
							Agria								
Ctr	68.23 ± 1.47a	67.98 ± 2.3a	65.23 ± 1.03a	-7.13 ± 0.11a	-4.59 ± 0.05a	-3.79 ± 0.09b	35.8 ± 0.19a	33.06 ± 0.69a	30.5 ± 0.26b	36.5 ± 0.19a	33.38 ± 0.69a	30.73 ± 0.25b	101.3 ± 0.2a	97.91 ± 0.08a	97.09 ± 0.21a
12A	67.79 ± 1.81a	68 ± 1.52a	65.17 ± 0.88a	-7.28 ± 0.35a	-4.66 ± 0.1a	-3.88 ± 0.07b	36.17 ± 0.55a	34.47 ± 1.25a	30.97 ± 0.22b	36.9 ± 0.56a	34.78 ± 1.26a	31.21 ± 0.22b	101.4 ± 0.5a	97.72 ± 0.12a	97.15 ± 0.16a
24A	68.19 ± 0.63a	69.02 ± 2.58a	66.39 ± 2.46a	-7.31 ± 0.06a	-4.58 ± 0.24a	-3.55 ± 0.26b	35.86 ± 0.47a	33.57 ± 2.8a	29.69 ± 0.86b	36.6 ± 0.47a	33.88 ± 2.81a	29.91 ± 0.82b	101.5 ± 0.1a	97.81 ± 0.24a	96.85 ± 0.68a
12B	69.44 ± 1.12a	68.73 ± 1.2a	67.74 ± 0.86a	-7.08 ± 0.12a	-4.31 ± 0.25a	-2.91 ± 0.07a	35.75 ± 1.03a	34.49 ± 0.58a	34.62 ± 0.02a	36.45 ± 0.99a	34.76 ± 0.59a	34.74 ± 0.03a	101.2 ± 0.4a	97.12 ± 0.34a	94.8 ± 0.11b
24B	70.55 ± 1.22a	69.15 ± 1.11a	65.59 ± 0.5a	-7.15 ± 0.85a	-5.04 ± 0.16a	-3.48 ± 0.06ab	36.52 ± 0.18a	37.06 ± 2.33a	33.61 ± 0.66a	37.24 ± 0.16a	37.41 ± 2.29a	33.79 ± 0.66a	101.1 ± 1.3a	97.81 ± 0.63a	95.92 ± 0.15ab
							Picasso								
Ctr	62.88 ± 1.36a	64.01 ± 2.4a	60.47 ± 1.5bc	-6.2 ± 0.09a	-3.47 ± 0.36a	-3.18 ± 0.17a	21.9 ± 0.36b	23.47 ± 0.91a	22.43 ± 1.05b	22.76 ± 0.37b	23.73 ± 0.95a	22.66 ± 1.06b	105.8 ± 0.2a	98.37 ± 0.53a	98.06 ± 0.18b
12A	62.74 ± 2.03a	60.72 ± 2.93a	60.49 ± 0.97ab	-6.21 ± 0.21a	-3.65 ± 0.36a	-3.25 ± 0.09ab	23.37 ± 0.89ab	22.94 ± 0.35a	19.41 ± 0.19c	24.18 ± 0.89ab	23.23 ± 0.4a	19.68 ± 0.2c	104.9 ± 0.4a	99.01 ± 0.75a	99.49 ± 0.2a
24A	63.51 ± 0.74a	63.21 ± 3.33a	63.27 ± 0.77c	-6.7 ± 0.1a	-4.18 ± 0.35a	-3.86 ± 0.04c	24.18 ± 0.37ab	24.52 ± 1.66a	21.64 ± 0.34bc	25.09 ± 0.38ab	24.88 ± 1.7a	21.98 ± 0.34bc	105.5 ± 0.1a	99.65 ± 0.34a	100.1 ± 0.1a
12B	62.92 ± 0.71a	61.1 ± 2.02a	65.17 ± 1.4a	-6.39 ± 0.04a	-2.87 ± 0.66a	-3.74 ± 0.15bc	29.78 ± 2.97a	29.55 ± 4.42a	30.92 ± 0.67a	30.47 ± 2.91a	29.72 ± 4.35a	31.15 ± 0.66a	102.3 ± 1.1b	96.11 ± 1.89a	96.91 ± 0.33c
24B	64.98 ± 3.12a	65.95 ± 0.39a	63.65 ± 0.41abc	-6.13 ± 0.15a	-3.86 ± 0.08a	-3.95 ± 0.06c	22.44 ± 0.82b	22.69 ± 1.18a	22.88 ± 0.28b	23.27 ± 0.82b	23.02 ± 1.18a	23.21 ± 0.28b	105.3 ± 0.2a	99.67 ± 0.27a	99.79 ± 0.17a
							Rossi								
Ctr	66.93 ± 1.39a	70.72 ± 0.44b	65.74 ± 1.09a	-6.95 ± 0.33a	-2.95 ± 0.13b	-2.98 ± 0.04ab	24.56 ± 0.88a	20.98 ± 0.87ab	21.67 ± 0.2ab	25.53 ± 0.94a	21.19 ± 0.84ab	21.87 ± 0.2bc	105.8 ± 0.2c	98.05 ± 0.65a	97.84 ± 0.15bc
12A	66.83 ± 0.29a	71.49 ± 1.55ab	65.25 ± 1.34a	-6.99 ± 0.12a	-2.56 ± 0.34ab	-2.95 ± 0.04ab	22.99 ± 0.47a	20.38 ± 0.98ab	19.42 ± 0.06c	24.03 ± 0.49a	20.55 ± 0.99ab	19.65 ± 0.06d	106.9 ± 0ab	97.15 ± 0.81a	98.64 ± 0.12a
24A	68.5 ± 0.82a	72.85 ± 0.65a	64.8 ± 0.87a	-6.95 ± 0.11a	-2.8 ± 0.1ab	-3.12 ± 0.07b	22.46 ± 0.16a	19.05 ± 0.15b	21.32 ± 0.22b	23.51 ± 0.19a	19.25 ± 0.15b	21.55 ± 0.23c	107.2 ± 0.1a	98.37 ± 0.31a	98.32 ± 0.12ab
12B	68.6 ± 0.59a	72.02 ± 1.36a	64.04 ± 0.64a	-6.55 ± 0.08a	-2.04 ± 0.12a	-2.78 ± 0.03a	22.59 ± 0.2a	18.71 ± 1.13b	23.75 ± 0.36a	23.52 ± 0.19a	18.82 ± 1.13b	23.91 ± 0.36a	106.2 ± 0.2c	96.23 ± 0.04a	96.68 ± 0.11d
24B	68.87 ± 1.52a	70.76 ± 0.6b	65.14 ± 1.04a	-6.65 ± 0.16a	-3.07 ± 0.09b	-2.92 ± 0.09ab	22.81 ± 0.45a	23.56 ± 1.16a	22.43 ± 0.13b	23.76 ± 0.47a	23.76 ± 1.14a	22.62 ± 0.12b	106.2 ± 0.1bc	97.48 ± 0.49a	97.42 ± 0.25c

Different letters (a, b, c) indicate significant different between treatments in each parameter ($p \leq 0.05$).

3.2.6.6 Heat treatment of tuber pulp

In Agria, Picasso and Rossi varieties, color and texture analyses were conducted on raw and cooked potatoes (for 20 minutes, at 100 °C) at harvest and after 4M of storage for both control and the highest calcium chloride and Ca-EDTA treatments. Additionally, sensory analysis was performed at harvest for all three varieties, encompassing both control and 24A and 24B treatments.

3.2.6.6.1 Color analysis

In both raw and cooked tubers of Agria, Picasso and Rossi varieties, significant differences in color parameters were observed at harvest (**Table 3.45**) and after four months of cold storage (**Table 3.46**). Comparing both raw and cooked tubers (**Table 3.45**), at harvest, for all three varieties, a significantly decrease was detected in all the analyzed parameters of the control and treatments 24A and 24B. Regarding Agria, in treatments 24A and 24B submitted to 4M of cold storage (**Table 3.46**), L parameter remained higher in cooked tubers (**Table 3.46**). Also, in Rossi, significant differences among treatments, and between raw and cooked tubers, were not found. For a* and b* parameters there were a significantly decreased from raw to cooked tubers. Comparing the L and a* parameters of raw tubers, at harvest (**Table 3.45**) with those after 4M (**Table 3.46**), no considerable changes were noted, but the opposite was verified for parameter b*.

Table 3.45 Colorimetric parameters (L, a* and b*) in raw and cooked tubers (20 minutes, at 100°C) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest, considering the control and the two highest treatments carried out with calcium chloride and Ca-EDTA. Mean values (*n = 3*) ± SE (Standard Error).

T.	Raw tubers			Cooked tubers		
	L	a*	b*	L	a*	b*
			Agria			
Ctr	66.94 ± 0.83aA	-4.22 ± 0.11aA	21.95 ± 0.46aA	54.6 ± 0.67aB	-7.56 ± 0.13bB	7.17 ± 0.25bB
24A	69.26 ± 0.68aA	-4.51 ± 0.04aA	22.65 ± 0.33aA	51.22 ± 0.58bB	-6.96 ± 0.07aB	7.19 ± 0.22bB
24B	67.23 ± 1.04aA	-4.43 ± 0.13aA	24.67 ± 0.82aA	54.77 ± 0.87aB	-7.27 ± 0.18bB	9.6 ± 0.47aB
			Picasso			
Ctr	70.38 ± 0.76aA	-2.78 ± 0.11aA	18.2 ± 0.58aA	70.76 ± 0.61aA	-5.3 ± 0.13aB	9.29 ± 0.32aB
24A	65.62 ± 0.8bA	-3.45 ± 0.13bA	18.25 ± 0.57aA	66.22 ± 0.68bA	-5.22 ± 0.08aB	5.83 ± 0.26cB
24B	71.88 ± 1.33aA	-2.89 ± 0.08aA	18.23 ± 0.15aA	66.26 ± 0.67bB	-5.39 ± 0.12aB	7.42 ± 0.23bB
			Rossi			
Ctr	69.89 ± 1.16aA	-4.68 ± 0.1aA	30.75 ± 0.26aA	64.47 ± 0.98aA	-9.22 ± 0.14aB	17.21 ± 0.61cB
24A	71.28 ± 0.82aA	-4.88 ± 0.15aA	29.59 ± 0.47aA	65.1 ± 0.53aB	-8.77 ± 0.87aB	20.61 ± 0.5aB
24B	73.42 ± 0.84aA	-4.73 ± 0.04aA	30.42 ± 0.71aA	65.66 ± 1.09aB	-9.52 ± 0.16aB	19.02 ± 0.67bB

Different letters indicate significant different between treatments in raw tubers or cooked tubers each variety (a, b) or between raw and cooked tubers in the same treatment (A, B) ($p \leq 0.05$).

Table 3.46 Colorimetric parameters (L, a* and b*) in raw tubers and cooked tubers (20 minutes, at 100ºC) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties after 4 months of cold storage, considering the control and the two highest treatments carried out with calcium chloride and Ca-EDTA. Mean values (*n = 3*) ± SE (Standard Error).

T.	Raw tubers			Cooked tubers		
	L	a*	b*	L	a*	b*
			Agria			
Ctr	71.32 ±0.76aA	-4.37 ±0.16aA	32.08 ±0.32aA	68.29 ±0.56bB	-8.35 ±0.04aB	8.07 ±0.33cB
24A	66.84 ±0.71bB	-3.8 ±0.15aA	25.57 ±0.2cA	70.46 ±0.24aA	-8.16 ±0.12aB	9.82 ±0.97bB
24B	68.57 ±0.51bB	-3.87 ±0.09aA	29.71 ±0.59bA	70 ±0.99aA	-8.62 ±0.28aB	3.56 ±1.25aB
			Picasso			
Ctr	64.09 ±0.68aA	-3.87 ±0.12aA	21.37 ±0.3aA	59.35 ±0.43aB	-4.85 ±0.13bB	3.17 ±0.51aB
24A	65.23 ±0.7aA	-3.86 ±0.08aA	19.97 ±0.27bA	58.92 ±0.67aB	-4.1 ±0.1aB	3.33 ±0.34bB
24B	64.18 ±0.42aA	-3.61 ±0.16aA	21.79 ±0.8aA	60.35 ±0.36aB	-5.13 ±0.12cB	3.53 ±0.38aB
			Rossi			
Ctr	69.84 ±0.65aA	-2.97 ±0.07aA	19.13 ±0.14aA	69.63 ±0.6aA	-5.74 ±0.06bB	9.54 ±0.44bB
24A	67.28 ±0.96aA	-2.39 ±0.13aA	18.16 ±0.45bA	68 ±0.51aA	-5.64 ±0.1bB	6.78 ±0.36cB
24B	69.41 ±0.76aA	-2.89 ±0.08aA	19.86 ±0.19aA	67.67 ±0.68aA	-3.15 ±0.3aB	9.08 ±1.12aB

Different letters indicate significant different between treatments in raw tubers or cooked tubers each variety (a, b) or between raw and cooked tubers in the same treatment (A, B) ($p \leq 0.05$).

3.2.6.6.2 Texture

Considering the importance of texture on the quality of food products, , the texture parameters (fracturability, hardness, number of peaks, work strength and adhesiveness) were also studied in the second-year of the experiment with Agria, Picasso and Rossi tubers, analyzing the highest treatments of Ca biofortification at harvest (**Table 3.47**) and after 4M of storage (**Table 3.48**). Concerning the raw tubers of Agria, at harvest (**Table 3.47**), there were significant differences among treatments in all the analyzed parameters (except in adhesiveness). Picasso did not show significant differences among treatments only in fracturability and number of peaks, whereas in Rossi, only in both of those parameters plus adhesiveness. Concerning to cooked tubers, the three varieties showed significant differences among treatments in the five texture parameters. In cooked tubers of Rossi, the control presented significantly lower values in all the texture parameters assessed regarding the Ca biofortification treatments (**Table 3.47**). Nevertheless, in the three varieties, for all texture parameters analyzed, comparing each treatment in raw and cooked tubers, it was found that raw tubers showed significantly higher values relatively to cooked tubers (**Table 3.47**). Considering fracturability in raw tubers, the values varied between 5.18 – 6.05, 6 – 6.37 and 6.21- 6.95 N, respectively for Agria, Picasso and Rossi. In cooked tubers, the values ranged between 0.23 – 0.31, 0.47 – 0.60 and 0.33 – 0.52 N, in Agria, Picasso and Rossi, respectively. Considering hardness, in raw and cooked tubers of Agria, Picasso and Rossi, the values ranged between 7.25 – 8.56 and 0.38 – 0.5N, 7.71 – 13.07 and 0.53 – 0.94 N, and 7.89 – 8.15 and 0.34 – 0.63 N, respectively. The number of peaks in raw tubers of Agria, Picasso and Rossi varied between 33.8

– 39.4, 44.0 – 56.0 and 41.2 – 47 and in cooked tubers between 2.0- 3.2, 1.8 – 3.25 and 1.2 – 2.5, respectively. Regarding work strength, Agria presented values varying between 79.16 – 83.43 N.mm in raw tubers and between 3.23 – 4.2 N.mm in cooked tubers. For the same parameter, Picasso, and Rossi, presented values in raw tubers that ranged between 84.03 – 116.15 and 86.76 – 94.12 N.mm, respectively, whereas in cooked tubers varied between 5.46 – 9.63 and 3.45 – 6.36 N.mm, respectively. Concerning to adhesiveness, Agria, Picasso and Rossi showed values that ranged between 2.38 – 3.24, 2.33 – 4.52 and 4.42 – 5.06 -N.mm, respectively, whereas for raw tubers and for cooked tubers varied between 0.32 – 0.52, 0.69 – 1.48 and 0.49 – 0.83, respectively.

Table 3.47 Texture parameters in raw and cooked tubers (20 minutes, at 100ºC) of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest, considering the control and the two highest treatments carried out with calcium chloride and Ca-EDTA. Mean values (*n = 3*) ± SE (Standard Error).

T.	Raw tubers					Cooked tubers				
	Fractu-rability (N)	Hardness (N)	Number of Peaks	Work stregth (N.mm)	Adhe-siveness (-N.mm)	Fractu-rability (N)	Hardnes s (N)	Num-ber of Peaks	Work stregth (N.mm)	Adhesive-ness (-N.mm)
Agria										
Ctr	6.05 ± 0.24aA	7.25 ± 0.19bA	39.4 ± 4aA	79.16 ± 3.22aA	2.38 ± 0.3bA	0.27 ± 0.02aB	0.41 ± 0.06bB	2.2 ± 0.2bB	3.86 ± 0.27aB	0.52 ± 0.05aB
24A	5.97 ± 0.12aA	8.55 ± 0.17aA	33.8 ± 1.1bA	82.58 ± 1.14aA	3.24 ± 0.25aA	0.31 ± 0.02aB	0.38 ± 0.02bB	2 ± 0.41bB	3.23 ± 0.32aB	0.32 ± 0.03bB
24B	5.18 ± 0.08bA	8.56 ± 0.8aA	38 ± 2.4aA	83.43 ± 3.35aA	3.09 ± 0.3aA	0.23 ± 0.02bB	0.5 ± 0.06aB	3.2 ± 0.2aB	4.2 ± 0.49aB	0.38 ± 0.04bB
Picasso										
Ctr	6.37 ± 0.23aA	13.07 ± 0.59aA	46 ± 1.1aA	128.4 ± 2.55aA	2.33 ± 0.61cA	0.50 ± 0.07bB	0.94 ± 0.14aB	3.25 ± 0.63aB	9.63 ± 1.23aB	1.48 ± 0.21aB
24A	6.0 ± 0.25aA	7.71 ± 0.48cA	45.6 ± 2.3aA	84.03 ± 3.59cA	4.52 ± 0.38aA	0.60 ± 0.05aB	0.67 ± 0.05bB	1.8 ± 0.37bB	8.02 ± 0.6aB	1.2 ± 0.13aB
24B	6.17 ± 0.06aA	11.41 ± 0.46bA	44 ± 1.9aA	116.15 ± 2.13bA	3.52 ± 0.34bA	0.47 ± 0.02bB	0.53 ± 0.03cB	2.75 ± 0.48aB	5.46 ± 0.58bB	0.69 ± 0.12bB
Rossi										
Ctr	6.95 ± 0.19aA	8.15 ± 0.23aA	47 ± 2.7aA	94.12 ± 3.21aA	4.42 ± 0.5aA	0.33 ± 0.03cB	0.34 ± 0.03cB	1.2 ± 0.2bB	3.45 ± 0.13bB	0.49 ± 0.06bB
24A	6.35 ± 0.17aA	7.89 ± 0.49aA	41.2 ± 0.7bA	92.07 ± 2.57aA	4.67 ± 0.56aA	0.52 ± 0.05aB	0.52 ± 0.05bB	1.6 ± 0.24bB	6.26 ± 0.26aB	0.8 ± 0.04aB
24B	6.21 ± 0.25aA	7.93 ± 0.41aA	42.6 ± 1.9bA	86.79 ± 1.65bA	5.06 ± 0.66aA	0.39 ± 0.02bB	0.63 ± 0.05aB	2.5 ± 0.29aB	6.36 ± 0.39aB	0.83 ± 0.04aB

Different letters indicate significant different between treatments in raw tubers or cooked tubers each variety considering each parameter (a, b, c) or between raw and cooked tubers in the same treatment and in the same parameter(A, B) ($p \leq 0.05$).

After 4M of storage, texture parameters of tubers from Agria, Picasso and Rossi showed (**Table 3.48**) that fracturability varied in raw tubers between 6.02 – 6.87, 5.43 – 6.77 and 7.38 – 8.59 N, respectively and in cooked tubers between 0.29 – 0.46, 0.23 – 0.34 and 0.14 – 0.41 N. In this context, hardness varied between 85.88 – 92.03, 53.85 – 80.52 and 68.17 – 77.04 N in raw tubers respectively for Agria, Picasso and Rossi. On the other hand, the values relatively to cooked tubers, varied between 5.49 – 6.46, 2.62 – 4.39 and 2.33 – 6.34 N. In raw tubers and cooked tubers of Agria, Picasso and Rossi, the number of peaks varied respectively between 33.8 – 36.0 and 2 – 3.2, 30.4 – 41.4 and 2.6 – 2.8, and 25.2 – 27.4 and 1.6 – 3.0. Agria presented values ranging between 7.89 – 8.45 N.mm in raw tubers and between 0.5 – 0.53 N.mm in cooked tubers, relatively to the work strength parameter. Also, relatively to the work strength, Picasso presented values ranging between 5.85 and 8.8 N.mm in raw tubers and between 0.31 – 0.44 N.mm in cooked tubers. In Rossi, the values varied between 7.87 – 8.6 N.mm in raw tubers and between 0.26 – 0.55 N.mm in cooked ones. Relatively to adhesiveness, in raw tubers of Agria, Picasso and Rossi, the values ranged between 2.48 – 4.85, 1.68 -3.22 and 1.33 – 1.86 -N.mm and in cooked tubers between 0.79 – 0.96, 0.28 – 0.49 and 0.30 – 0.88 -N.mm, respectively. Moreover, as verified in tubers at harvest (**Table 3.47**), also after 4M of storage (**Table 3.48**), relatively to cooked tubers, raw tubers in the three varieties showed significantly higher values considering each treatment in each parameter.

Table 3.48 Texture parameters in raw and cooked tubers (20 min at 100ºC) of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties after 4 months of storage, considering the control and the two highest treatments carried out with calcium chloride and Ca-EDTA. Mean values (*n = 3*) ± SE (Standard Error).

T.	Raw tubers					Coocked tubers				
	Fracturability (N)	Hardness (N)	Number of Peaks	Work stregth (N.mm)	Adhesiveness (-N.mm)	Fracturability (N)	Hardness (N)	Number of Peaks	Work stregth (N.mm)	Adhesiveness (-N.mm)
Agria										
Ctr	6.02 ± 1.02aA	92.03 ± 3.77aA	33.8 ± 2.4aA	8.45 ± 0.24aA	2.48 ± 0.21cA	0.29 ± 0.02bB	5.7 ± 0.51aB	2.0 ± 0.3bB	0.5 ± 0.05aB	0.79 ± 0.08bB
24A	6.87 ± 0.33aA	85.88 ± 4.93aA	36 ± 1.9aA	7.89 ± 0.26aA	4.85 ± 0.95aA	0.45 ± 0.06aB	6.46 ± 0.78aB	2.0 ± 0.3bB	0.53 ± 0.07aB	0.96 ± 0.16aB
24B	6.86 ± 0.24aA	91.03 ± 4.3aA	35.8 ± 1.7aA	8.35 ± 0.44aA	3.91 ± 0.33bA	0.46 ± 0.04aB	5.49 ± 0.55aB	3.2 ± 0.2aB	0.52 ± 0.07aB	0.71 ± 0.08bB
Picasso										
Ctr	6.68 ± 0.39aA	64.59 ± 2.64bA	32.2 ± 2.9bA	7.01 ± 0.33bA	1.68 ± 0.39cA	0.3 ± 0.07aB	4.39 ± 1.05aB	2.8 ± 0.7aB	0.44 ± 0.13aB	0.49 ± 0.09aB
24A	6.77 ± 0.32aA	80.52 ± 1.87aA	41.4 ± 1.5aA	8.8 ± 0.3aA	3.22 ± 0.52aA	0.34 ± 0.08aB	3.5 ± 0.27aB	2.6 ± 0.6aB	0.38 ± 0.07aB	0.47 ± 0.08aB
24B	5.43 ± 0.33bA	53.85 ± 3.26bA	30.4 ± 1.9bA	5.85 ± 0.26cA	2.1 ± 0.31bA	0.23 ± 0.07aB	2.62 ± 0.56aB	2.6 ± 0.7aB	0.31 ± 0.11bB	0.28 ± 0.04bB
Rossi										
Ctr	7.91 ± 0.55aA	77.04 ± 4.13aA	26.0 ± 1.7aA	8.2 ± 0.31aA	1.33 ± 0.15aA	0.41 ± 0.04aB	6.34 ± 0.55aB	1.8 ± 0.4bB	0.55 ± 0.03aB	0.88 ± 0.09aB
24A	8.59 ± 0.35aA	68.17 ± 4.6aA	25.2 ± 1.3aA	8.6 ± 0.34aA	1.42 ± 0.29aA	0.14 ± 0.02bB	2.33 ± 0.2cB	1.6 ± 0.2bB	0.26 ± 0.02cB	0.30 ± 0.04cB
24B	7.38 ± 0.44aA	69.71 ± 1.96aA	27.4 ± 2aA	7.87 ± 0.43aA	1.86 ± 0.23aA	0.35 ± 0.04aB	4.67 ± 0.31bB	3.0 ± 0.6aB	0.44 ± 0.03bB	0.58 ± 0.06bB

Different letters indicate significant different between treatments in raw tubers or cooked tubers each variety considering each parameter (a, b, c) or between raw and cooked tubers in the same treatment and in the same parameter (A, B) ($p \leq 0.05$).

At harvest, Agria exhibited the highest percentage of softening at harvest, while only after 4M, Picasso and Rossi varieties displayed the highest softening percentage (**Table 3.49**).

Table 3.49 Softening of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest (H) and after 4M (4 months) of storage, considering the control and the two highest treatments carried out with calcium chloride and Ca-EDTA.

T.	Softening (%)					
	Agria		Picasso		Rossi	
	H	4M	H	4M	H	4M
Ctr	95.5	95.2	92.2	95.5	95.3	94.8
24A	94.8	92.4	90.0	95.0	91.8	98.4
24B	95.6	93.3	92.4	95.8	93.7	95.3

3.2.6.6.3 Sensory analysis

Sensory analysis was carried out in tubers of Agria, Picasso and Rossi, submitted to the highest concentrations applied of $CaCl_2$ and Ca-EDTA (**Figure 3.30**).

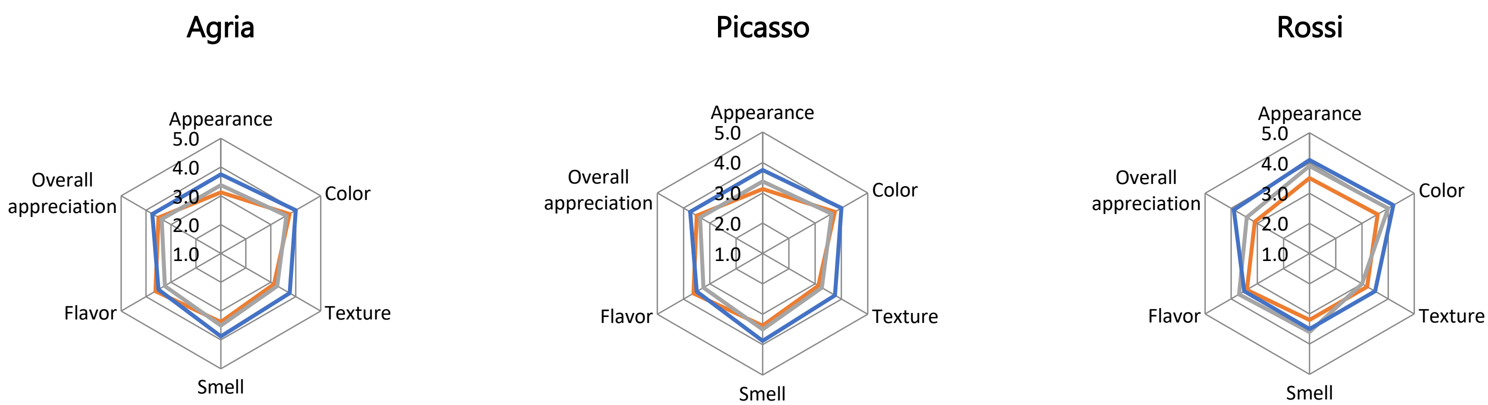


Figure 3.30 Average evaluation of the panel of tasters regarding the different sensory characteristics for *Solanum tuberosum* L., Agria, Picasso and Rossi varieties (— Control, — 24 kg ha^{-1} $CaCl_2$, — 24 kg ha^{-1} Ca-EDTA).

In Agria (**Figure 3.30**), the panel of tasters after analyzing some sensory characteristics of tubers, namely "texture" and "overall appreciation", concluded that the 24B samples revealed a higher appreciation relatively to the remain treatments. Concerning to the "appearance" and "color", the control and treatment 24A obtained the same values, and only relatively to “smell”, treatment 24A showed a higher value relatively to the control tubers and treatment 24B. In Picasso (**Figure 3.30**), treatment 24B become the favorite treatment regarding all the sensory characteristics, except for “flavor”, in which the control tubers obtained a higher value. Additionally, treatment 24A showed the second-best treatment regarding “appreciation”, “color”, “texture” and “smell”. Also, considering Rossi (**Figure 3.30**), treatment 24B showed a higher score in “appearance”, “color”, “texture” and “overall appreciation”, followed by treatment 24A. Indeed, in “smell” and “flavor”, treatment 24A displayed a higher value relatively to the remaining treatments. Overall, the control showed the lowest score regarding all the evaluated sensory characteristics. Nevertheless, variability prevailed regarding the three varieties.

3.2.7 Yield

At harvest, the assessment of potato total yield in the three varieties submitted to different Ca treatments was carried out considering 57 plants per treatment of each variety (**Table 3.50**). Regarding Agria, the treatment 12A showed the highest yield, therefore coinciding with the treatment with the highest Ca content (**section 3.5.3.1**). For the three varieties the lowest yield was obtained with treatment 12B, however in Picasso and Rossi, despite the lowest yield obtained, was the treatment that showed better results regarding Ca content. Picasso and Rossi also showed the same tendency regarding total yield, with the greater yield being obtained in control, followed by treatments 12A, 24A, 24B and 12B. Overall, Agria showed the lowest total yield relatively to the other varieties (being Picasso with the greater tubers yield).

Table 3.50 Total yield at harvest of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties.

T.	Total Yield (kgs)		
	Agria	Picasso	Rossi
Ctr	75.35	214.6	119.5
12A	81.45	210.3	101
24A	64.05	183.7	79.15
12B	28.9	80.6	50.05
24B	40.3	179.6	54.85

3.3 Third year

In the third year of the experiment, the Ca biofortification workflow was implemented with Agria, Picasso and Rossi varieties cultivated in fields C, B and A, respectively. Based on the favorable outcomes achieved with 12 kg/ha Ca-EDTA (for Picasso and Rossi varieties) and with 12 kg/ha $CaCl_2$ (12A) (for Agria variety) in the second year of experiment, the same chemical products and concentrations were employed. Seven foliar applications were maintained, after the beginning of tuberization aiming to validate the previous year's results. The concentration of 24 kg/ha was excluded due to observed adverse effects on all three varieties.

3.3.1 Irrigation Water

As carried out in the first and second year of the experiment, the physical and chemical composition of the irrigation water was analyzed to assess its effects on soil and crops (**Table 3.51**). Considering the three experimental fields, the pH ranged from 7.2 to 7.7 and EC from 691 to 1100 $\mu S.cm^{-1}$.

Table 3.51 Physical and chemical parameters of irrigation water of the experimental fields selected for Ca biofortification of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, respectively. Electrical conductivity (EC) was determined at 20 °C.

Field	pH	EC	$Ca^{2\pm}$	$K^{\pm}$	$Mg^{2\pm}$	$Na^{\pm}$	Cl^{-}	HCO_3^{-}	SO_4^{2-}
		$\mu S.cm^{-1}$	$mg.L^{-1}$ ($meq.L^{-1}$)						
A	7.2	1100	190.9 (9.5)	2.2 (0.1)	20.4 (1.7)	33.9 (1.5)	84 (2.4)	378.2 (6.2)	197.1 (24.1)
B	7.5	1001	140.9 (7.1)	2.8 (0.07)	22.2 (1.8)	46.3 (2.0)	89 (2.6)	290.4 (4.8)	172.8 (3.63)
C	7.7	691	15.4 (0.8)	0.2 (0.006)	4.1 (0.3)	6.4 (0.3)	65 (1.8)	40.8 (0.6)	52.8 (1.1)

Water classification was determined using the Piper Diagram (**Figure 3.31** and **Annex VII**). The irrigation water has an underground origin and exhibited a hydrochemical facies of: calcium bicarbonate in field A and B; and magnesium sodium chloride sulphate considering field C.

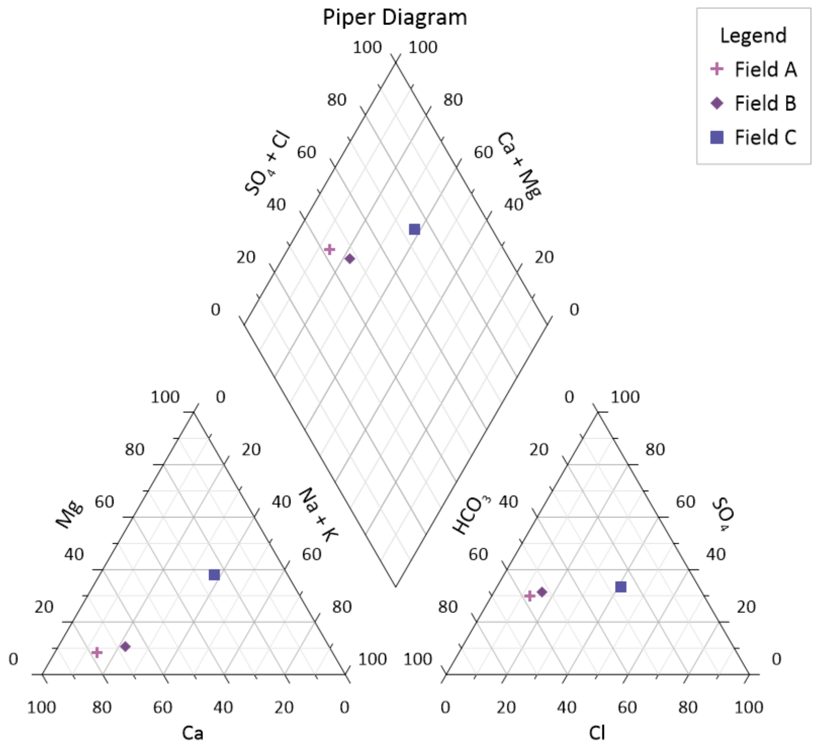


Figure 3.31 Piper Diagram of the three experimental fields (A, B and C).

Fields A and B showed high salinity in terms of EC (between 750 and 2250 µS.cm^{-1} at 20 ^{o}C) belonging to class C3S1, but field C showed medium salinity in terms of EC (between 250 and 750 µS.cm^{-1} at 20 ^{o}C) belonging to class C2S1 (**Figure 3.32** and **Annex VII**), if the SAR index is consider (**Table 3.52**).

Table 3.52 SAR index, pHe and ISL of the irrigation water of the experimental fields selected for Ca biofortification of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties, respectively. Sodium Adsorption Ration (SAR); Saturation pH (pHs); Langelier Saturation Index (LSI).

Field	SAR	pHs	LSI
A	0.63	7.1	0.05
B	0.94	7.4	0.12
C	0.40	9.1	-1.4

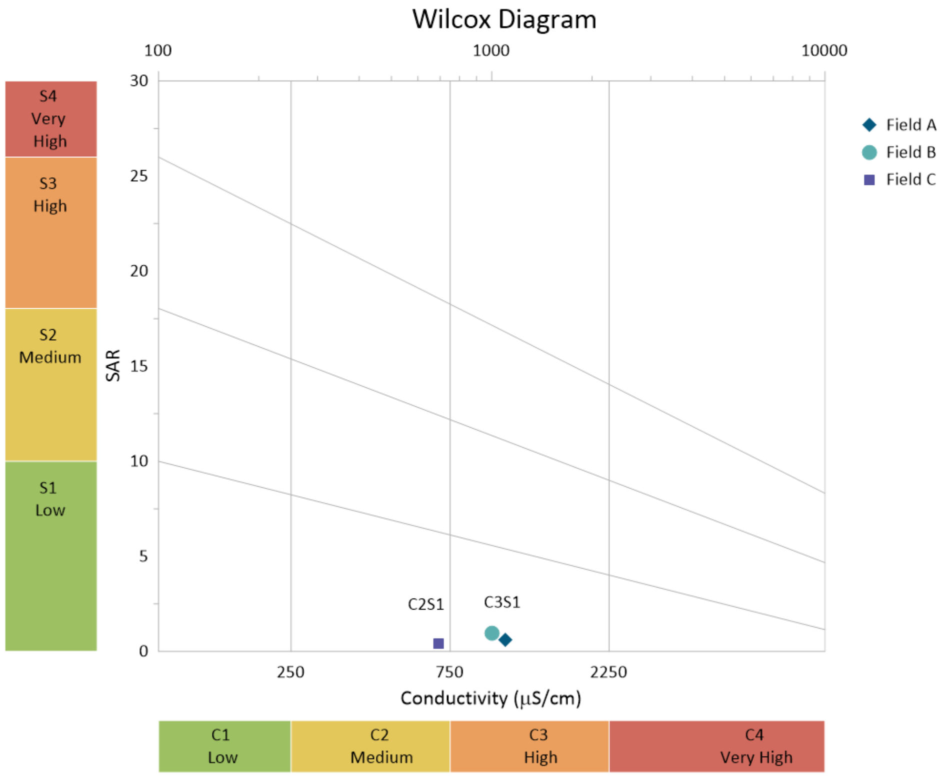


Figure 3.32 Wilcox Diagram of the three experimental fields (A, B and C).

3.3.2 Remote detection

Similarly, to the second year, the analysis of plant vigor was conducted, and the NDVI were represented (**Figure 3.33**). The color scale employed used green to illustrate dense and lush vegetation (values close to 1), the light green to yellow color for values close near zero and orange tones represents the dry vegetation and/or soil, typically associated with negatives values. In fields A and C, the higher and lower NDVI values are represented in green and red, respectively. Moreover, in field B, NDVI is represented in the opposite way (green and red for lower and higher values, respectively).

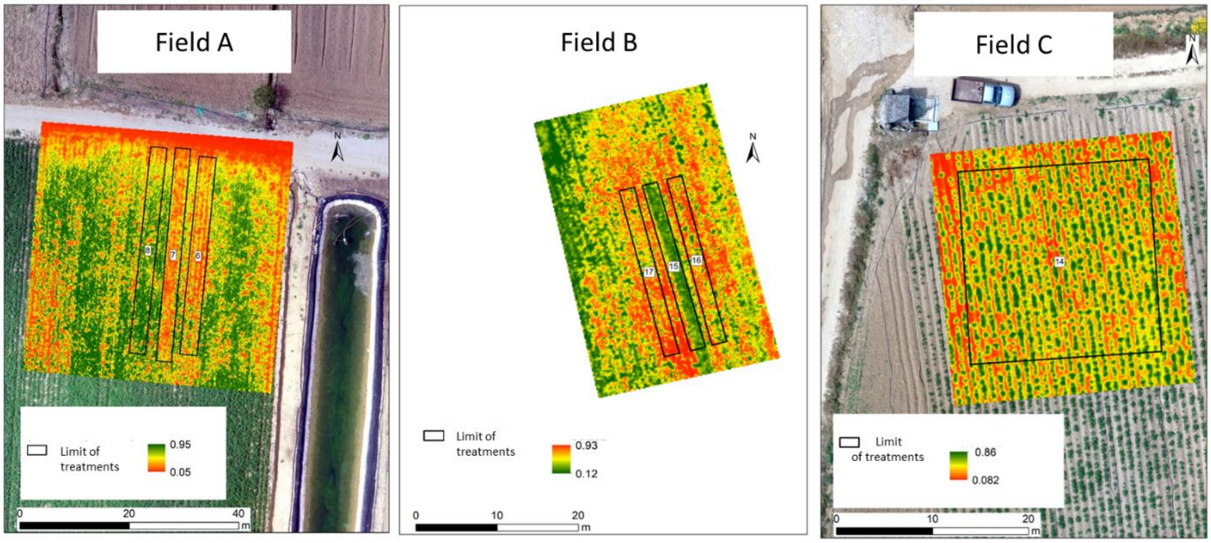


Figure 3.33 NDVI model of the three experimental fields (fields A, B and C). The NDVI was carried out in field A on June 13 of 2020 (4 days after the 4th foliar application), in field B on June 13 of 2020 (4 days after the 4th foliar application) and in field C on June 13 of 2020 (without any foliar application being carried out).

In **Table 3.53** is represented the NDVI of *Solanum tuberosum* L. plants in the three experimental fields.

Table 3.53 Minimum, maximum and the average of NDVI (± SD) of the different treatments in fields A and B and its standard deviation of the average, during the biofortification process. CaCl2 12 kg/ha (treatment 12A) and Ca-EDTA 12 kg/ha (treatment 12B).

Code	Treatment	Minimum NDVI	Max NDVI	Average NDVI
		Field A		
6	12A	0.23	0.93	0.79 ± 0.15
7	12B	0.23	0.93	0.78 ± 0.13
8	Control	0.32	0.95	0.90 ± 0.05
		Field B		
15	12B	0.28	0.92	0.76 ± 0.14
16	12A	0.34	0.91	0.85 ± 0.08
17	Control	0.36	0.92	0.87 ± 0.09

Considering **Figure 3.33** and **Table 3.53** it was found that *Solanum tuberosum* L. plants of field A and B showed a positive response after the 4th foliar spraying with Ca, as all treated plots showed a medium/high NDVI value (> 0.7), with the control showing the highest NDVI relatively to the remain treatments. Regarding field A, and relatively to the previous experimental year, after the

4th foliar application a very similar NDVI was found in the control, as well as a lower NDVI (relatively to the correspondent treatments). Moreover, as seen in the previous year, treatment 12B showed the lowest average NDVI relatively to the remaining treatments. Regarding field B, treatment 12B showed the lowest average NDVI, being the treatment with the second lowest NDVI in the previous year. There was a tendency in both field for a decreasing NDVI (average): Control > 12A > 12B (**Table 3.53**). The lower NDVI observed in the two genotypes implemented in fields A and B, was probably associated to Ca-EDTA toxicity. Following a general perspective, *Solanum tuberosum* L. plants of fields A and B also showed medium/high NDVI values (> 0.8), being plants in the same state of development (**Figure 3.34**). Relatively to field C, similarly, to the previous experimental year, the crop was not implemented, so the general lower NDVI (< 0.40) was related with the presence of soil and some sporadic weeds.

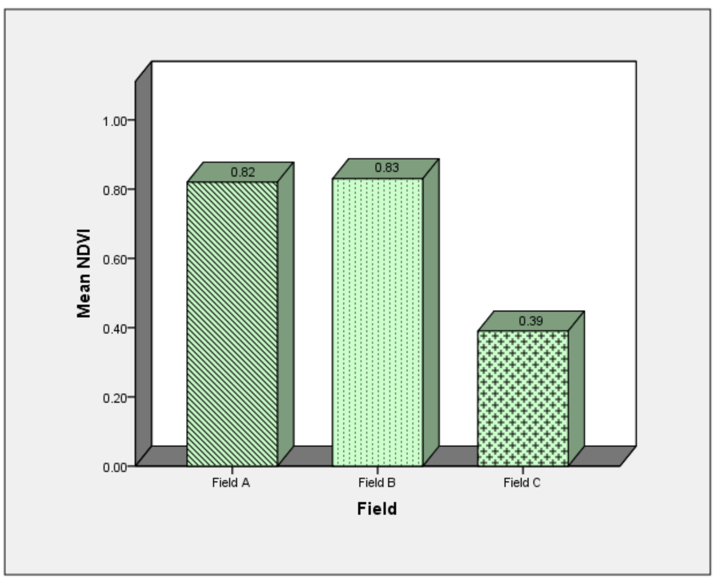


Figure 3.34 General average NDVI for field (A, B and C).

3.3.3 Monitoring of the culture in the fields

Monitoring of *Solanum tuberosum* L. plants submitted to Ca biofortification in the three experimental fields was carried out (**Figure 3.35**) to assess and evaluate the potential occurrence of stress.

Agria **Picasso** **Rossi**

Figure 3.35 Perspective of *Solanum tuberosum* L. plants in fields A, and C (Rossi, Picasso and Agria varieties, respectively) Immediately after the first foliar spraying.

As such, regarding the different treatments (control, 12 kg.ha^{-1} $CaCl_2$ and 12 kg.ha^{-1} Ca-EDTA), plants of the three varieties were monitored after the 3rd, 5th, and 7th foliar applications and at harvest (in tubers obtained in each treatment).

3.3.3.1. Agria variety

In Agria variety, seven days after the 3rd foliar application, treatment 12B exhibited symptoms of toxicity in the aerial part of *Solanum tuberosum* L. plants (**Figure 3.36** - **Figure 3.38**).

A B C

Figure 3.36 Perspective of *Solanum tuberosum* L. in field C (Agria variety), seven days after the 3rd foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

Moreover, *Solanum tuberosum* L. plants only revealed symptoms of toxicity in the aerial part with treatment 12A, six days after the 5th foliar application (**Figure 3.37 and 3.38**).

A B C

Figure 3.37 Perspective of *Solanum tuberosum* L. in field C (Agria variety), six days after the 5th foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.38 Perspective of *Solanum tuberosum* L. in field C (Agria variety), eight days after the 6th foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.39 Perspective of *Solanum tuberosum* L. in field C (Agria variety) in harvest day: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

3.3.3.2. Picasso variety

Picasso variety revealed toxicity symptoms, six days after the 3rd foliar application with treatment 12B (**Figure 3.40**), and the increase of toxicity symptoms in the aerial part of the *Solanum tuberosum* L. plants occurred after the 5th and 6th foliar applications (**Figure 3.41** and **Figure 3.42**). Regarding treatment 12A, toxicity symptoms were only observed after the 6th foliar application (**Figure 3.42**).

A B C

Figure 3.40 Perspective of *Solanum tuberosum* L. in field B (Picasso variety), six days after the 3rd foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.41 Perspective of *Solanum tuberosum* L. in field B (Picasso variety), seven days after the 5th foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.42 Perspective of *Solanum tuberosum* L. in field B (Picasso variety), seven days after the 6th foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.43 Perspective of *Solanum tuberosum* L. in field B (Picasso variety) in harvest day: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

3.3.3.3. Rossi variety

Rossi variety persisted symptom-free of toxicity until the 3rd foliar application, regardless of the $CaCl_2$ or Ca-EDTA treatments (**Figure 3.44**). However, initial toxicity symptoms emerged after the 5th foliar application with treatment 12B (**Figure 3.45**), intensifying in the aerial part of the plants after the 6th foliar applications (**Figure 3.46**).

Figure 3.44 Perspective of *Solanum tuberosum* L. in field A (Rossi variety), six days after the 3rd foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

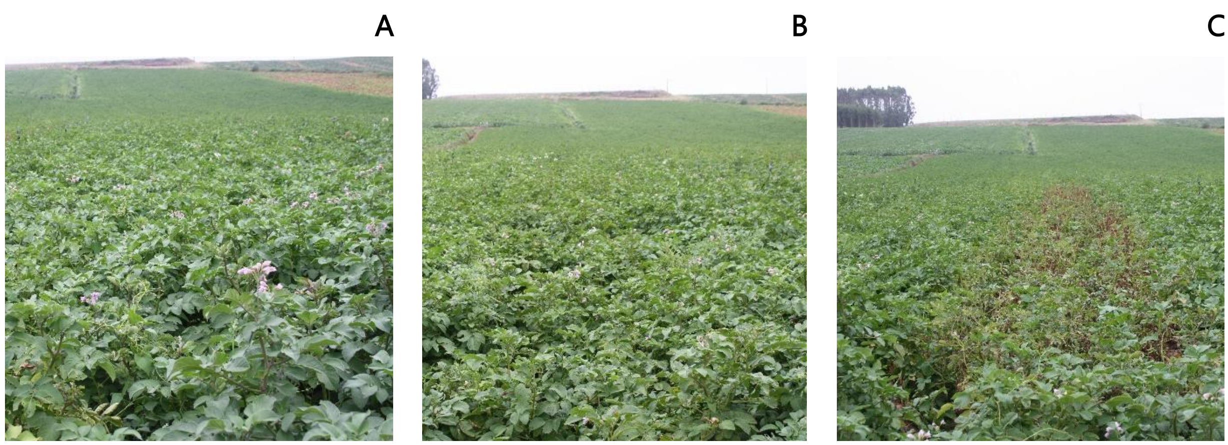


Figure 3.45 Perspective of *Solanum tuberosum* L. in field A (Rossi variety), seven days after the 5th foliar application: A – Control; B - $CaCl_2$ 12 kg·ha^{-1} (12A); C- Ca-EDTA 12 kg·ha^{-1}(12B).

A B C

Figure 3.46 Perspective of *Solanum tuberosum* L. in field A (Rossi variety), seven days after the 6th foliar application: A – Control; B - $CaCl_2$ 12 $kg \cdot ha^{-1}$ (12A); C- Ca-EDTA 12 $kg \cdot ha^{-1}$(12B).

A B C

Figure 3.47 Perspective of *Solanum tuberosum* L. in field A (Rossi variety) in harvest day: A – Control; B - $CaCl_2$ 12 $kg \cdot ha^{-1}$ (12A); C- Ca-EDTA 12 $kg \cdot ha^{-1}$(12B).

3.3.4 Photosynthetic functioning

In *Solanum tuberosum* L. plants of Agria, Picasso and Rossi varieties, foliar gas exchange and quantification of chlorophyll *a* fluorescence parameters were assessed considering the different treatments. Measurements were conducted after the 5th (22 June) foliar application in Picasso and Rossi varieties, as well as after the 7th (30 July) foliar application in Rossi. Also, in Agria, before any foliar application (22 June) and after the 5th foliar applications (30 July).

3.3.4.1. Infrared foliar gas exchange

In the third year of the experiment, the evaluation of gas exchange was focused on the later stages of the *Solanum tuberosum* L. plants cycle (**Table 3.54)**. Parameters such as the rate of net photosynthesis (P_n), the effects on stomatal conductance (g_s) and consequently on the transpiration rate (E) and instantaneous water use efficiency (iWUE) were assessed in the three varieties. Moreover, regarding iWUE in the three varieties it was possible to observe the lowest values in 12B treatment.

Table 3.54 Mean values (*n = 4 - 6*) ± SE (Standard Error) of the variation of net photosynthesis rates (Pn), stomatal conductance (gs), transpiration rate (E) and instantaneous efficiency of water use (iWUE = Pn/E), in the different treatments applied for Agria, Picasso and Rossi variety on 22 June and 30 of July of 2020.

T.	Agria		Picasso		Rossi	
	22 June	30 July	22 June	30 July	22 June	30 July
	P_n (µmol CO_2 m^{-2} s^{-1})					
Ctr	19.221 ± 1.19aA	11.756 ± 1.14bA	7.891 ± 0.54a	-	16.305 ± 0.80aA	7.903 ± 1.35bB
12A	9.458 ± 0.55bA	7.531 ± 0.88aB	8.844 ± 1.48a	-	17.718 ± 0.69aA	12.345 ± 0.71aA
12B	12.581 ± 1.8ba	-	3.519 ± 0.78b	-	17.494 ± 1.99a	-
	g_s (mmol H_2O m^{-2} s^{-1})					
Ctr	185.444 ± 16.58aA	203.947 ± 19.01aA	51.5 ± 1.95a	-	97.178 ± 10.81bA	77.133 ± 17.87aA
12A	62.811 ± 5.42bB	146.7 ± 10.88aA	60.944 ± 7.61a	-	118.367 ± 14.21bA	94.913 ± 4.39aA
12B	149.911 ± 17.04a	-	44.811 ± 2.65a	-	227.156 ± 48.56a	-
	E (mmol H_2O m^{-2} s^{-1})					
Ctr	3.507 ± 0.18aA	2.323 ± 0.15aA	1.54 ± 0.05a	-	1.918 ± 0.13bA	1.223 ± 0.23aA
12A	1.528 ± 0.11bB	2.067 ± 0.10aA	1.763 ± 0.15a	-	2.146 ± 0.20abA	1.56 ± 0.058aA
12B	2.968 ± 0.20a	-	1.408 ± 0.08a	-	2.926 ± 0.36a	-
	iWUE (mmol CO_2 mol^{-1} H_2O)					
Ctr	4.949 ± 0.08abA	5.326 ± 0.52aA	4.37 ± 0.18a	-	7.21 ± 0.135abA	6.801 ± 0.322aA
12A	6.374 ± 0.30aA	3.493 ± 0.30bB	4.706 ± 0.47a	-	7.944 ± 0.187aA	7.864 ± 0.263aA
12B	3.292 ± 0.41b	-	2.223 ± 0.15b	-	5.558 ± 0.57b	-

For each variety, in each parameter and date, the different letters express significant differences between treatments (a, b) or between treatments in each date of each variety (A, B) (*$p \leq 0.05$*). "-" means that, due to the lack of plants, measurements were not carry out.

3.3.4.2. Chlorophyll a fluorescence parameters

To complement the assessments of foliar gas exchange, measurements of chlorophyll a parameters related to the functioning of the photosynthetic machinery, was carried out in the three varieties of *Solanum tuberosum* L. plants (**Table 3.55**). Treatment 12B, showed the lowest values regarding to maximum (F_v/F_m) and current (F_v'/F_m') photochemical efficiency of PSII after the 5^{th} foliar application in Agria and Picasso. In contrast, the application of treatment 12A had no negative impact on any of the analytical dates of the three varieties. Considering the estimates of the quantum yields of non-cyclic photosynthetic electron transport ($Y_{(II)}$), regulated dissipation of energy in PSII ($Y_{(NPQ)}$), and unregulated dissipation (heat and fluorescence) in PSII ($Y_{(NO)}$), variations were found depending on each parameter, but according to the performance of the photosynthetic machinery. In general, in the three varieties, for treatment 12B, a significant decrease occurred considering $Y_{(II)}$ and an increase in $Y_{(NPQ)}$ (energy dissipation indicator). Moreover, treatment 12A did not show any negative impact on any of the varieties. Also, there were only slightly changes in the uncontrolled processes of light energy dissipation ($Y_{(NO)}$). Impacts were found regarding q_N (which reflects the proportion of energy dissipated as heat by photoprotection metabolism) and q_L (referring to the proportion of energy captured by the PSII open reaction centers and used for photochemical events). Being in accordance with Y(II), q_L values decreased in all varieties with treatment 12B. On the other hand, q_N showed a pattern of variation consistent with $Y(_{NPQ})$, increasing in the three varieties.

Table 3.55 Leaf chlorophyll a fluorescence parameters in Agria, Picasso and Rossi varieties on 22 June and 30 July of 2020. Mean values (*n = 5*) ± SE (Standard Error) of the maximum (F_v/F_m), the actual (F_v'/F_m') PSII photochemical efficiency, of the estimate of quantum yields of non-cyclic electron transport (Y(II)), of regulated energy dissipation in PSII ($Y_{(NPQ)}$), of non-regulated energy dissipation in PSII ($Y_{(NO)}$), of the photochemical quenching coefficient (q_L) and non-photochemical quenching (q_N).

T.	Agria		Picasso		Rossi	
	22 June	30 July	22 June	30 July	22 June	30 July
			F_v/F_m			
Ctr	0.785 ± 0.011abA	0.779 ± 0.014aA	0.825 ± 0.002a	-	0.797 ± 0.008aA	0.768 ± 0.01aA
12A	0.833 ± 0.002aA	0.744 ± 0.024aA	0.832 ± 0.003a	-	0.793 ± 0.015aA	0.773 ± 0.005aA
12B	0.733 ± 0.033b	-	0.541 ± 0.041b	-	0.815 ± 0.003a	-
			F_v'/F_m'			
Ctr	0.463 ± 0.017aB	0.573 ± 0.022aA	0.458 ± 0.04a	-	0.478 ± 0.019aA	0.484 ± 0.022aA
12A	0.523 ± 0.011aA	0.451 ± 0.039bA	0.419 ± 0.055a	-	0.522 ± 0.028aA	0.471 ± 0.033aB
12B	0.168 ± 0.018b	-	0.135 ± 0.018b	-	0.425 ± 0.048a	-
			$Y_{(II)}$			
Ctr	0.291 ± 0.022aA	0.356 ± 0.025aA	0.318 ± 0.033a	-	0.325 ± 0.017aA	0.297 ± 0.036aA
12A	0.375 ± 0.019aA	0.275 ± 0.044aA	0.282 ± 0.044a	-	0.374 ± 0.037aA	0.220 ± 0.034aB
12B	0.057 ± 0.012b	-	0.044 ± 0.013b	-	0.235 ± 0.046b	-
			$Y_{(NPQ)}$			
Ctr	0.486 ± 0.024bA	0.357 ± 0.028aA	0.474 ± 0.03b	-	0.448 ± 0.018bB	0.504 ± 0.038aA
12A	0.416 ± 0.011bA	0.427 ± 0.055aA	0.537 ± 0.055b	-	0.398 ± 0.043bB	0.523 ± 0.033aA
12B	0.781 ± 0.013a	-	0.756 ± 0.008a	-	0.565 ± 0.055a	-
			$Y_{(NO)}$			
Ctr	0.223 ± 0.009aB	0.287 ± 0.01aA	0.209 ± 0.009a	-	0.227 ± 0.008aA	0.199 ± 0.003bB
12A	0.209 ± 0.012bA	0.297 ± 0.018aA	0.181 ± 0.014a	-	0.228 ± 0.014aA	0.257 ± 0.017aA
12B	0.163 ± 0.008b	-	0.200 ± 0.014a	-	0.200 ± 0.011b	-
			q_N			
Ctr	0.813 ± 0.016aB	0.669 ± 0.034bA	0.83 ± 0.018b	-	0.797 ± 0.016bA	0.818 ± 0.020aA
12A	0.79 ± 0.011aB	0.739 ± 0.053aA	0.858 ± 0.031ab	-	0.751 ± 0.043bA	0.795 ± 0.026aA
12B	0.961 ± 0.004A	-	0.95 ± 0.005a	-	0.851 ± 0.033a	-
			q_L			
Ctr	0.479 ± 0.045abA	0.41 ± 0.02aA	0.556 ± 0.051a	-	0.531 ± 0.038aA	0.450 ± 0.047aA
12A	0.553 ± 0.047aA	0.451 ± 0.041aA	0.534 ± 0.033a	-	0.548 ± 0.05aA	0.329 ± 0.059bB
12B	0.313 ± 0.057b	-	0.29 ± 0.061b	-	0.396 ± 0.042b	-

For each variety, in each parameter and date, the different letters express significant differences between treatments (a ,b) or between treatments in each date of each variety (A, B) *($p \leq 0.05$)*. "-" means that due to the lack of plants measurements were not carried out.

3.3.5 Quantification of mineral elements and Ca location

Considering that aim of enhancing the concentration of Ca through foliar sprays during the life cycle of *Solanum tuberosum* L., the quantification of Ca and other mineral elements were carried out.

3.3.5.1 Monitoring of the different organs of *Solanum tuberosum* L. during the biofortification process

Through X-ray fluorescence spectrometry, the monitoring through the production cycle was carried out in the different organs (tubers, roots, stems, and leaves) of the Agria, Picasso and Rossi varieties of *Solanum tuberosum* L. In each variety, significant differences in tubers (**Figure 3.48**), roots (**Figure 3.49**), stems (**Figure 3.50**), and leaves (**Figure 3.51**) were found between each treatment after the 3FA, 5FA and 7FA. Considering k contents of tubers (**Figure 3.48**), during each time of analysis (3FA, 5FA and 7FA) similar values were found in each variety. Relatively to the control, after 3FA, 5FA and 7FA, S contents showed increasing values linked to Ca biofortification. In general, treatment 12B showed higher values of Ca, K, S and P in the three studied varieties. Rossi showed the highest contents of Ca with treatment 12B after 3FA, 5FA and 7FA. It was further found that with increasing foliar applications, a decrease of Ca content in Agria and Picasso occurred with treatment 12B. Considering Agria and Picasso, after the 7FA, treatment 12A presented a higher Ca biofortification index relatively to the control and treatment 12B. Additionally, regarding the remaining varieties, after the 7FA, Rossi showed the highest contents of Ca, K, S and P with treatment 12B (**Figure 3.48**). Still, it was found that (**Figure 3.48**), relatively to the control, in Agria, Picasso and Rossi Ca contents increased after the 7th foliar application, (between 8.2 to 42.4 %, 40.1 % and 67.1 to 82.6 %, respectively).

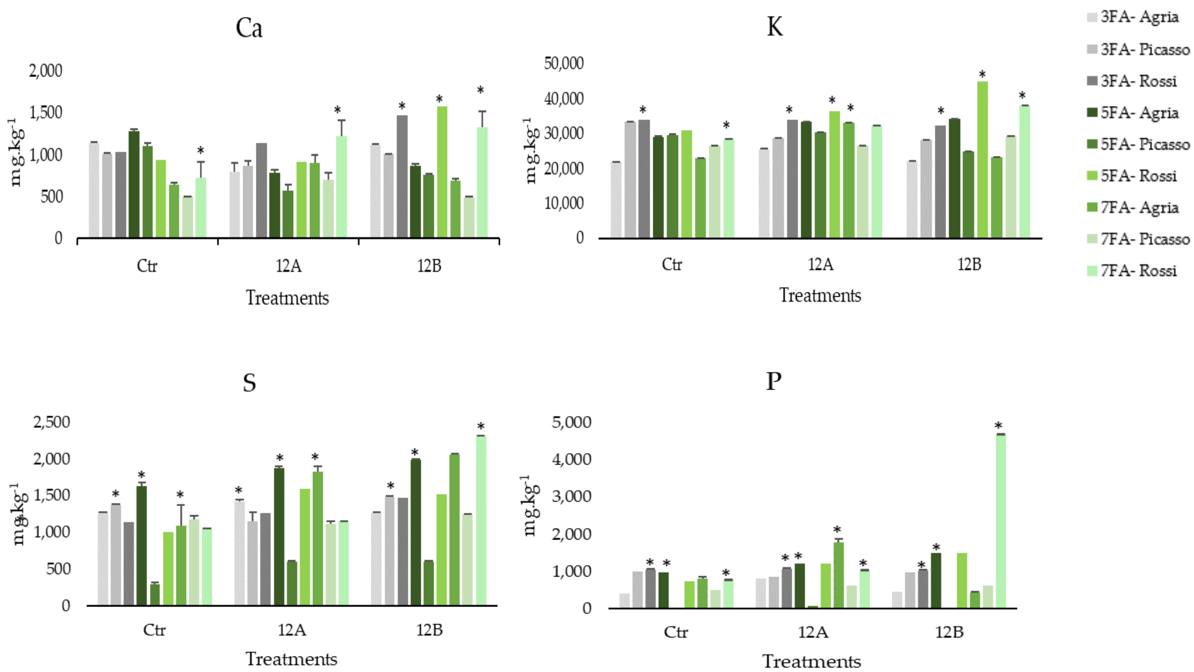


Figure 3.48 Contents of some chemical elements in tubers of *Solanum tuberosum* L. Agria, Picasso and Rossi varieties during the life cycle of plants, after thee (3FA), five (5FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) at a concentration of 12 kg ha^{-1}. Control was not sprayed. Mean values (*n = 4*) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 3FA, 5FA, and 7FA are identified (*) ($p \leq 0.05$).

Regarding roots of *Solanum tuberosum* L., Ca contents increased with each successive foliar applications in the three varieties (**Figure 3.49**). In Agria, after the 7th foliar application, the highest Ca content were obtained in treatment 12B. Moreover, for K, S and P content, oscillations between each time of analysis (after 3, 5 and 7 FA) were detected in the three varieties. After the 7FA, in Picasso, K contents was higher in treatment 12A, while Agria displayed the lowest values in all the treatments (relatively to Picasso and Rossi). Regarding to S contents, after 5 foliar applications, Agria showed the highest values in all the treatments, regarding the remaining varieties, and revealed the highest content of S in the control after 7 foliar applications. After 7FA it was possible to observe that Rossi variety did not biofortified with 12A treatment. However, Ca biofortification indexes ,relatively to Ctr, ranged between 5.7 – 26.2 %, 14.6 – 17.3 % and 42.4 %, respectively for Agria, Picasso and Rossi. Additionally, after 7FA Agria and Rossi presented a higher content of Ca with treatment 12B, whereas Picasso exhibited this trend with treatment 12A.

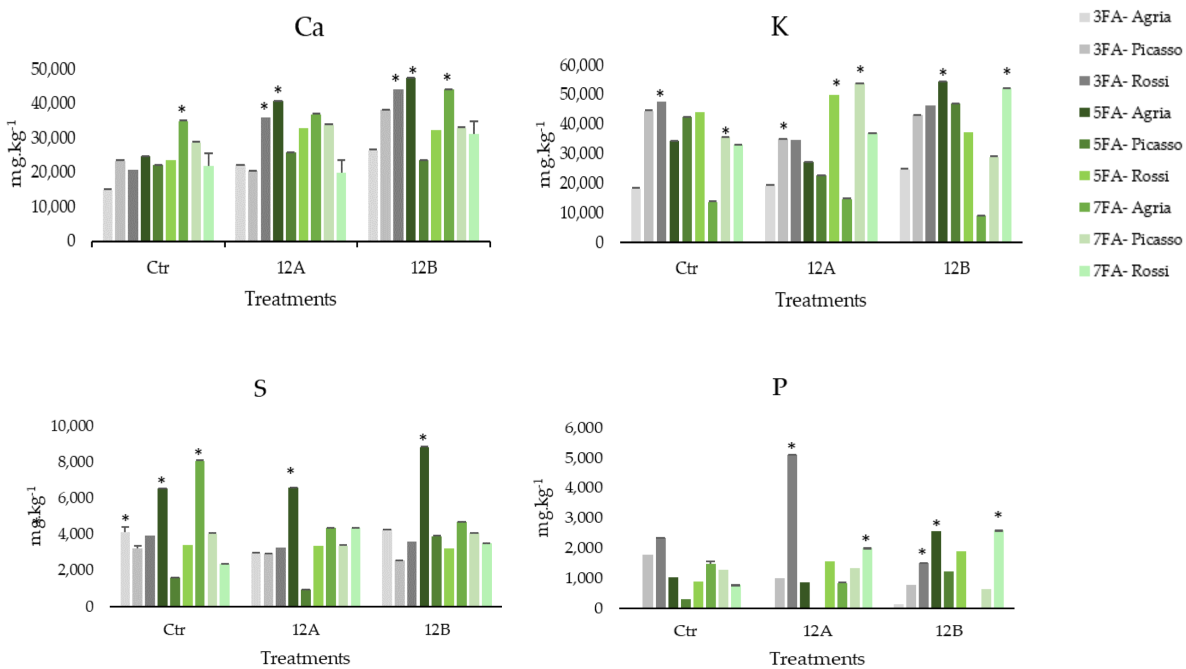


Figure 3.49 Contents of chemical elements in roots of *Solanum tuberosum* L. Agria, Picasso and Rossi varieties during the life cycle of plants, after three (3FA), five (5FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) at a concentration of 12 kg ha^{-1}. Control was not sprayed. Mean values (*n = 4*) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 3FA, 5FA, and 7FA are identified (*) *($p \leq 0.05$)*.

Considering stems of *Solanum tuberosum* L. (**Figure 3.50**), there were oscillations regarding the contents of Ca, K, S and P among the different analytical periods (thus, after 3, 5 and 7 FA), as well as among varieties. Relatively to K and P contents, there was not a clear tendency of their accumulation in stems of the three varieties. However, relatively to the remaining varieties, after 3 foliar applications, Rossi showed the highest K contents, the same being found after the 5 and 7 FA. After 7FA, with Ca biofortification, K contents increased in Agria and Picasso showing the highest content with treatment 12B. After 7FA, in Rossi, the contents of P, relatively to the other varieties, presented the highest values in the control and treatments 12A and 12B. Additionally, Agria and Picasso showed a significantly lower P content relatively to Rossi. Relatively to Picasso and Rossi, Agria showed the higher S content after 7FA, being additionally found a decrease of S content following the pattern of Ctr > 12A > 12B. After 5FA, Ca content showed significantly lower values in Agria, displaying the lowest values regarding each time of analysis and among varieties (**Figure 3.50**). Indeed, as verified in tubers (**Figure 3.48**), Rossi showed the highest Ca content after 7FA with treatment 12B. In fact, after 7FA, Agria did not biofortified and Picasso only showed Ca content higher than control with treatment 12A (28.7 % of Ca biofortification). In stems, only Rossi presented an increase of Ca content of 37.9 % (with treatment 12A) and 2-fold (with treatment 12B).

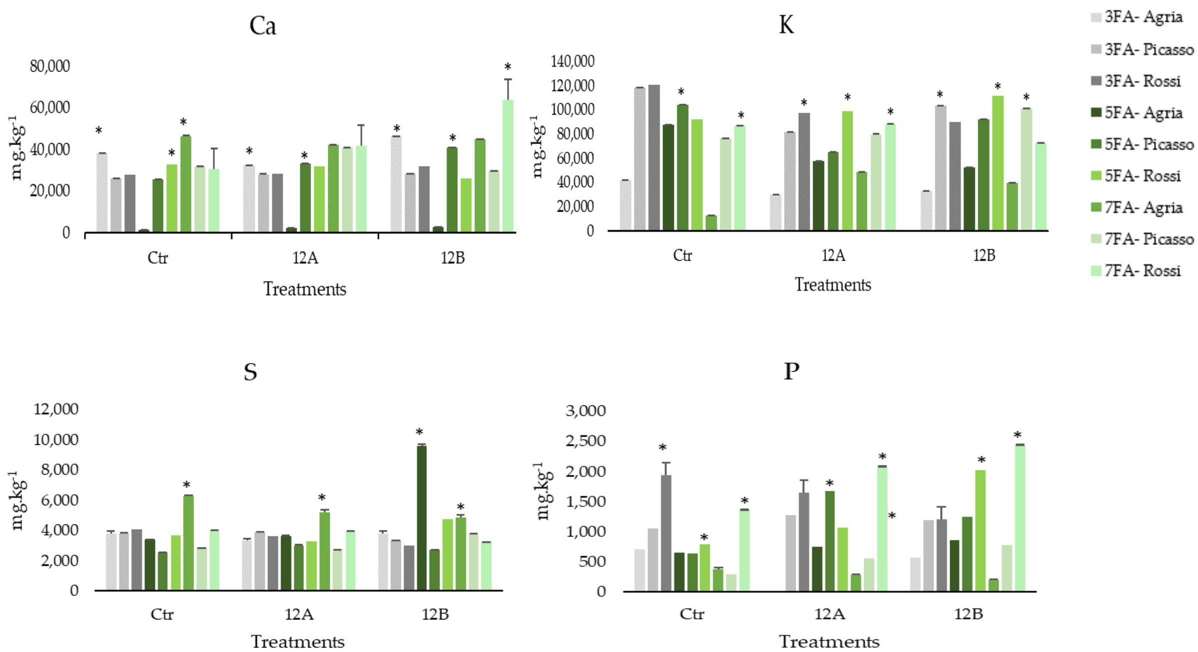


Figure 3.50 Contents of chemical elements in stems of *Solanum tuberosum* L. Agria, Picasso and Rossi varieties during the life cycle of plants, after three (3FA), five (5FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) at a concentration of 12 kg ha^{-1}. Control was not sprayed. Mean values (*n = 4*) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 3FA, 5FA, and 7FA are identified (*) ($p \leq 0.05$).

The leaves of *Solanum tuberosum* L. plants from Agria, Picasso and Rossi varieties (**Figure 3.51**), did not revealed a clear tendency regarding S and P contents. However, for Ca content, there was a similar accumulation trend after 3 and 5 FA in comparison to the control and treatments 12A and 12B. Concerning to K, a distinct pattern of accumulation was not evident in the three varieties, after 5FA. However, after 3 FA (except for treatment12B) and 7 FA, Rossi displayed higher K contents. Regarding Ca accumulation, after 7FA, Agria and Picasso exhibited the same tendency observed in stems (**Figure 3.50**), with Picasso only showing higher values with treatment 12A, relatively to control (2.9 %). Additionally, after 7FA, Rossi doubled the content of Ca with treatment 12A (relatively to the control and with treatment 12B, displaying an increase of 11.3 %).

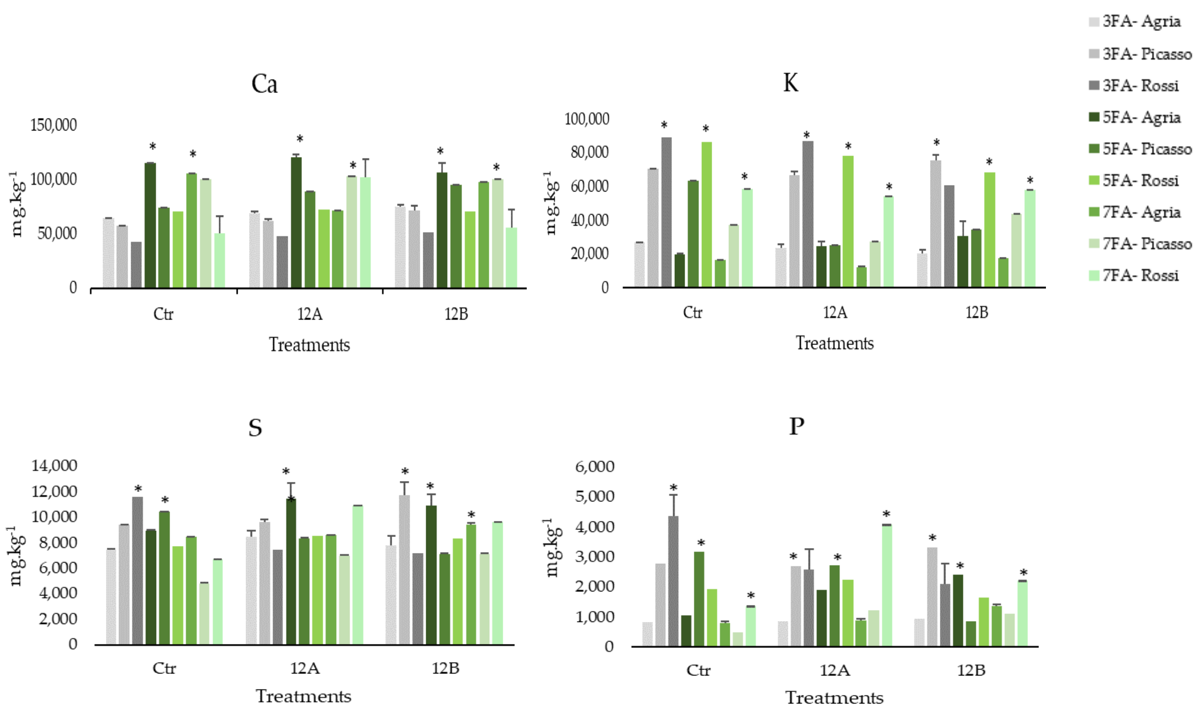


Figure 3.51 Contents of the chemical elements in leaves of Solanum tuberosum L. Agria, Picasso and Rossi varieties during the life cycle of plants, after thee (3FA), five (5FA), and seven (7FA) foliar applications with $CaCl_2$ (A) or Ca-EDTA (B) at a concentration of 12 kg ha^{-1}. Control was not sprayed. Mean values (*n = 4*) ± SE (Standard Error) and significantly higher content between varieties within each treatment after the 3FA, 5FA, and 7FA are identified (*) ($p \leq 0.05$).

3.3.5.2 Kinetic of mineral accumulation during the biofortification process

The analysis of kinetic accumulation of Ca, K, S and P during the life cycle of *Solanum tuberosum* L. plants was conducted for Agria, Picasso and Rossi varieties. Three periods of analysis were undertaken for each variety: 70, 84 and 97 days after plantation (DAP) for Agria, 93, 93 and 107 DAP for Picasso variety and 79, 93 and 107 DAP for Rossi variety.

3.3.5.2.1 Each mineral element during the development cycle

Regarding the kinetic accumulation in tubers of the three varieties (**Figure 3.52**), different accumulation patterns were observed. For Agria, after 97 DAP, treatment 12A revealed a higher Ca, K, S and P contents, relatively to the Ctr and treatment 12B. The trend of treatment 12A, expressed a higher content of Ca, K, S and P at 70 DAP, followed by a decrease at 84 DAP and a higher increase at 97 DAP. Additionally, the control in each analytical period (70, 84 and 97 DAP) presented the lowest Ca, K, S and P content. For Picasso, at the different analytical periods (83, 93 and 107 DAP), the highest content of Ca, K, S and P was obtained with treatment 12B (followed by treatment 12A only after 107 DAP with Ca, K and S). However, with the increase of DAP, a decrease of Ca, K, S and P contents was found with treatment 12B. The trend developed by Rossi with treatment 12A, relatively to the control and treatment 12B, showed the highest content of Ca, K, S and P at the different analytical periods (except at 79 DAP for P).

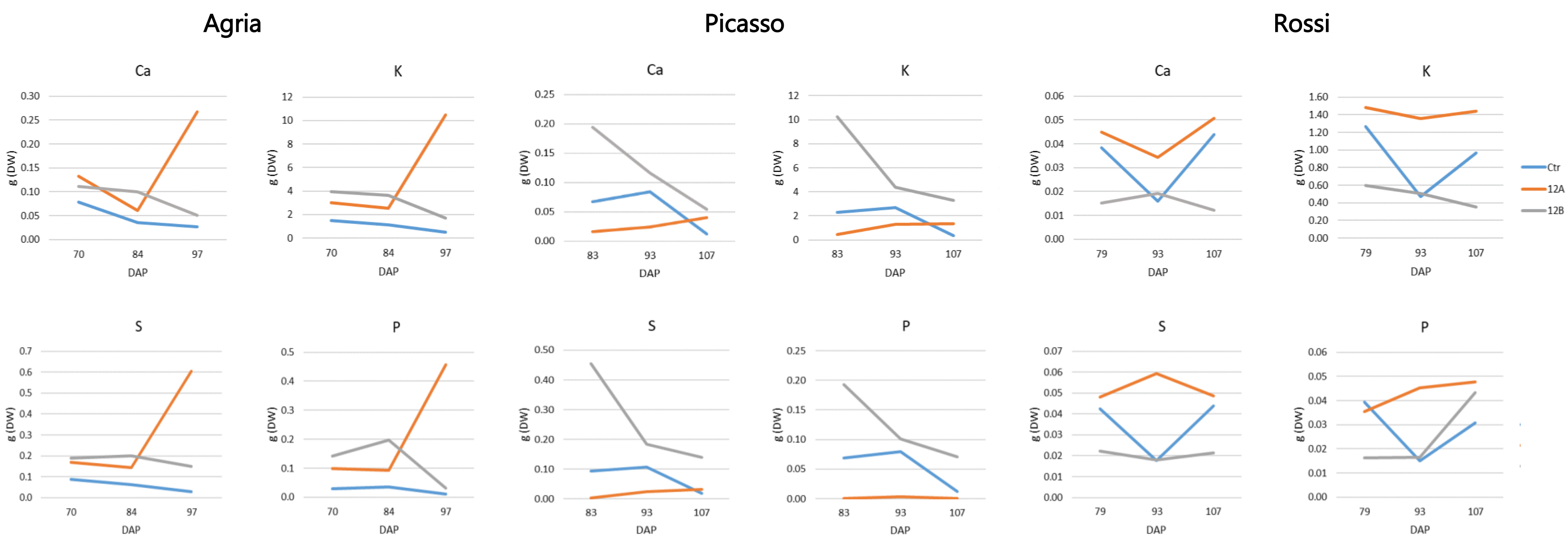


Figure 3.52 Kinetic of minerals accumulation (Ca, K, S and P) in tubers of *Solanum tuberosum* L., during the life cycle of plants, after 70, 84 and 97 days after plantation (DAP) in Agria, after 93, 93 and 107 DAP, in Picasso and after 79, 93 and 107 DAP in Rossi.

In roots of Agria, Picasso and Rossi, the kinetic accumulation of Ca, K, S and P (**Figure 3.53**) also revealed different patterns. After 97 DAP, the level of Ca in Agria presented the highest content with treatment 12B, followed by treatment 12A. However, relatively to K, S and P, the highest content was obtained with treatment 12A, as also verified for Picasso after 107 DAP. Regarding Picasso, the highest Ca content after 107 DAP, remained somewhat similar to treatment 12B as well as to the control. In Rossi, after 107 DAP, Ca and P showed the highest content in the control and with treatment 12B with K and S . Overall, there was not a clear tendency of mineral accumulation in the three varieties.

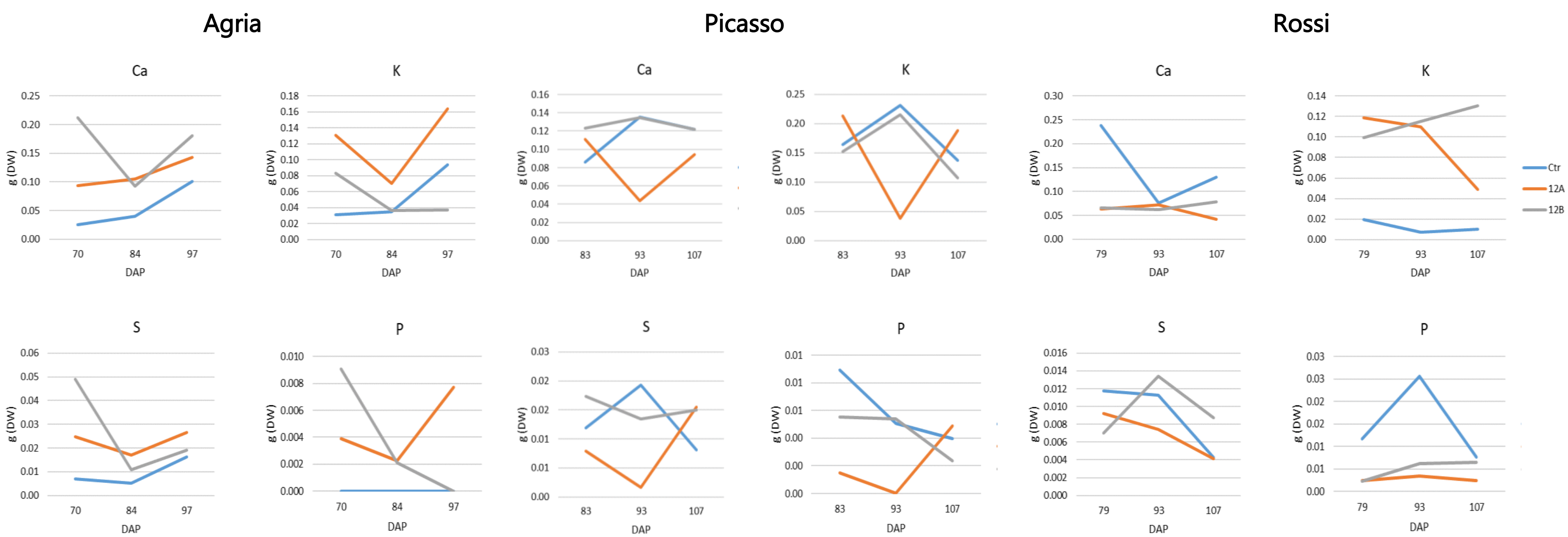


Figure 3.53 Kinetic of minerals accumulation (Ca, K, S and P) in roots of *Solanum tuberosum* L., during the life cycle of plants after 70, 84 and 97 days after plantation (DAP, in Agria, after 93, 93 and 107 DAP in Picasso, and after 79, 93 and 107 DAP in Rossi.

In the three varieties, the accumulation patterns of Ca, K, S and P In the stems from *Solanum tuberosum* L. (**Figure 3.54**), as previously seem in tubers (**Figure 3.52**) and roots (**Figure 3.53**), was found to be different. In Agria all the treatments revealed a tendency of a higher contents in 70 DAP, followed by a decrease in 84 DAP and an increase in 97 DAP. Also, after 97 DAP, Ca and S showed a higher content with treatment 12B, and K and P with treatment 12A. In Picasso the highest contents of Ca, K, S and P after 107 DAP was obtained with treatment 12A. Rossi presented a higher content of Ca and P, after 107 DAP, with treatment 12B, and with the control in K and S.

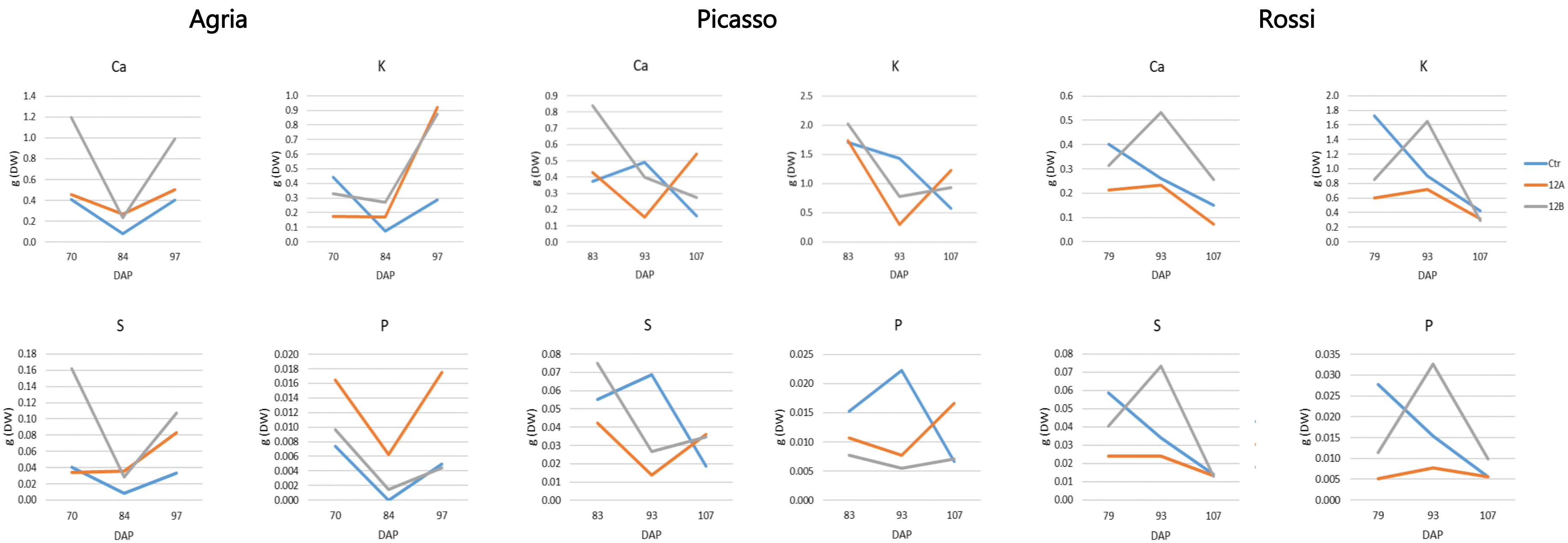


Figure 3.54 Kinetic of minerals accumulation (Ca, K, S and P) in stems of *Solanum tuberosum* L., during the life cycle of plants after 70, 84 and 97 days after plantation (DAP) in Agria, after 93, 93 and 107 DAP in Picasso, and after 79, 93 and 107 DAP in Rossi.

The kinetic accumulation of Ca, K, S and P in the leaves of *Solanum tuberosum* L., from Agria, Picasso and Rossi, further showed different patterns of accumulation regarding each variety (**Figure 3.55**). Considering Agria and Rossi, it was found that in the last analytical period, treatment 12B revealed the highest contents of Ca, K, S and P. In Agria the same tendency of accumulation was revealed for Ca, K, S and P, considering each treatment and that the lowest content of each mineral element was obtained in the control. Regarding Picasso, the same accumulation tendency observed for Agria, was found for Ca, K, S and P, considering the control as well as treatments 12A and 12B. Also, after 107 DAP, treatment 12A showed the highest content of Ca, K and S. In Rossi, after 107 DAP, the highest content of

Ca, K, S and P was found with treatment 12B. Additionally, S and P presented the same tendency of mineral accumulation regarding each treatment.

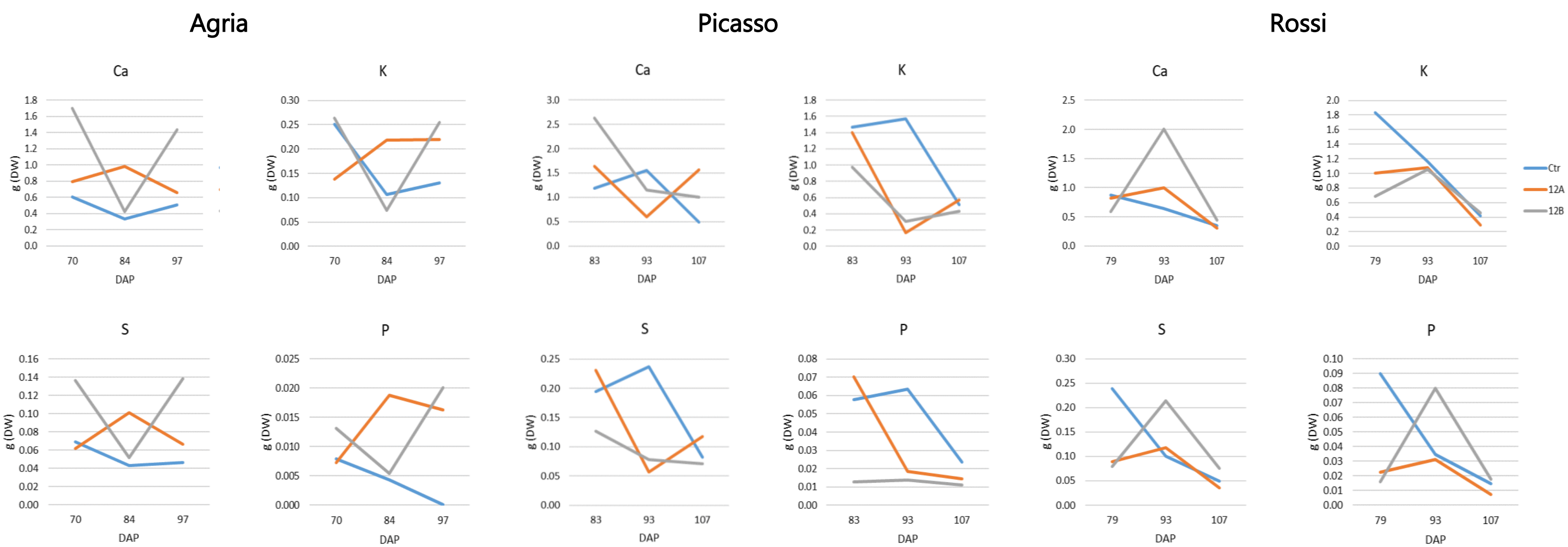


Figure 3.55 Kinetic of minerals accumulation (Ca, K, S and P) in leaves of *Solanum tuberosum* L., during the life cycle of plants after 70, 84 and 97 days after plantation (DAP) in Agria, after 93, 93 and 107 DAP in Picasso, and after 79, 93 and 107 DAP on Rossi.

Moreover, PCA analysis was carried considering Ca contents in the different analytical periods in Agria, Picasso and Rossi, regarding tubers, roots, stems, and leaves (**Figure 3.56**). Agria showed the highest dispersion of Ca values during the biofortification process, followed by Picasso and thereafter by Rossi. Treatment 12B in Rossi showed the highest Ca content. Additionally, a rise of Ca in stems and leaves occurred, followed by a fall of Ca content in tubers (in the control and in all the varieties, in treatment 12B of Agria and Picasso and with treatment 12A of Agria). There was a downward trend of Ca content in tubers, followed by a rise or maintenance, continued upward trend of Ca in the stems and leaves (in treatment 12A of Picasso and Rossi). Also, there a trend of decrease and increase of Ca in the stems and leaves, as well as a maintenance of higher values of Ca in tubers of Rossi with treatment 12B.

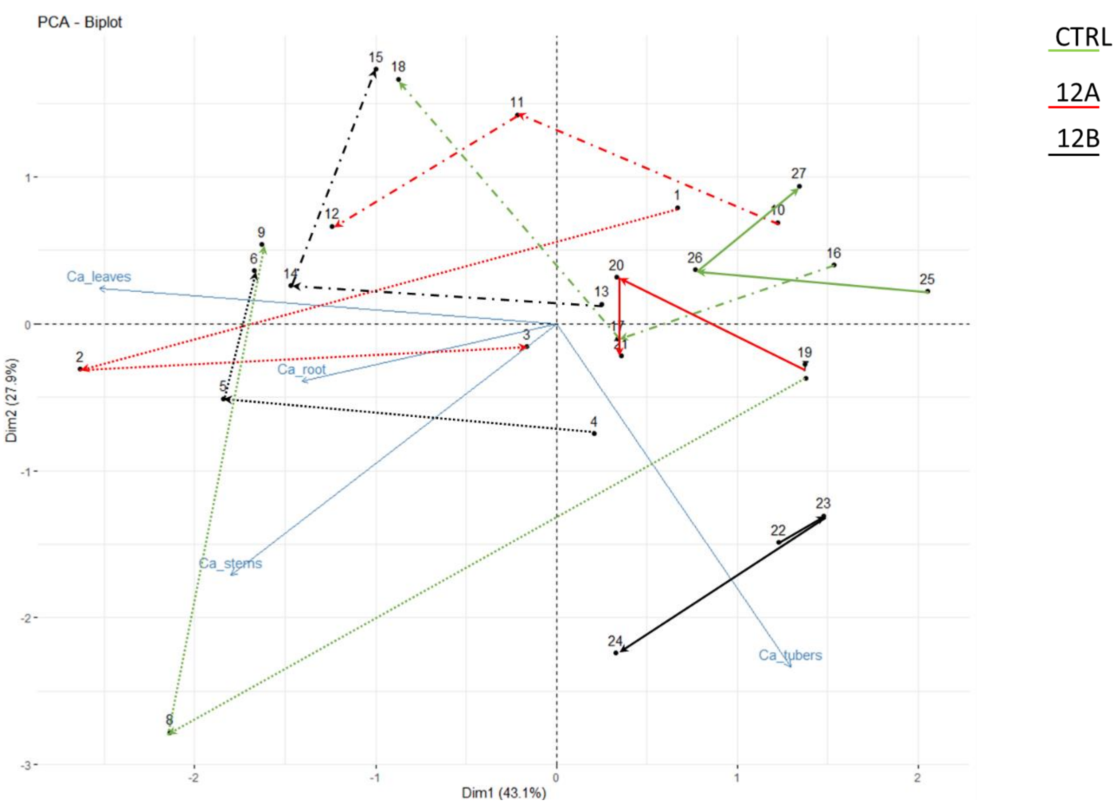


Figure 3.56 Projection of the factorial plane of individuals created with component 1 (or F1 or Dim1) (43.1 % variance) and component 2 (or F2 or Dim2) (27 % variance) axes of Ca content in tubers, roots, stems, and leaves of Agria (----), Picasso (-·-·-) and Rossi (—) varieties.

3.3.5.3 Tubers at harvest

The mineral content of potato tubers was assessed at harvest in the three varieties (Agria, Picasso and Rossi), to determine the Ca biofortification index. Additionally, further analysis of Ca localization within the tubers was conducted.

3.2.5.3.1 X-ray fluorescence spectrometry (XRF)

At harvest, the mineral contents of Ca, K, S and P were evaluated in tubers with and without skin (**Table 3.56**), revealing substantial differences. Across all mineral elements and varieties, there were notable variations among treatments in both tuber types (with and without skin). Calcium exhibited the highest content in tubers with skin, except for treatment 12A in Picasso. Furthermore, the Ca biofortification index showed variations between tubers with and without skin. In tubers without skin, relatively to the control, the increase of Ca ranged between 6.5 – 61.3 %, 17.1 – 36.6 % and 15.9 – 29.5 %, respectively in Agria, Picasso and Rossi. In tubers with skin, relatively to the control, Agria, Picasso and Rossi presented an increase of Ca content with treatment 12B (yet, relatively to the control, with treatment 12A Ca did not increase) of 8.2 %, 88 % and 78 %. Moreover, tubers without skin from Agria, submitted to treatment 12B, showed the highest contents of Ca and K (relatively to the remain treatments). Also, the lowest contents of Ca and S were found in the control, whereas for K and P were measured with treatment 12A. Tubers with skin from Agria showed the lowest content of K and S in the control, whereas the amount of Ca was the lowest with treatment 12A, being K, S and P contents, the highest. Tubers without skin from Picasso, showed the highest contents of Ca, K, S and P with treatment 12B, and the lowest for Ca and S in the control, whereas for K and P the lowest contents were obtained with treatment 12A. Additionally, in tubers with skin, the lowest contents of Ca, K, S and P were obtained with treatment 12A and the highest content of Ca, S and P with treatment 12B. For instance, in Rossi the minerals accumulation also varied, presenting, in tubers without skin, the highest contents of K, S and P in treatment 12B, and for Ca in treatment 12A. The lowest contents were obtained in the control considering Ca, K and S. Treatment 12A showed the highest Ca content and the lowest amount of P, however the same did not occur in tubers with skin. In fact, for tubers with skin, the highest content of Ca, K, S and P was obtained with treatment 12B, and the lowest content of Ca, K and P with treatment 12A. The lowest content of S was obtained in the control of Rossi, therefore keeping the tendency observed in tubers without skin.

Calcium content in tubers without skin varied between 0.031 – 0.050 %, 0.041 – 0.056 % and 0.044 – 0.057 % respectively for Agria, Picasso and Rossi. On the other hand, Ca contents in tubers with skin varied between 0.043 – 0.053 %, 0.046 – 0.094 % and 0.064 – 0.118 %, in Agria, Picasso and Rossi, respectively.

Table 3.56 Calcium, K, S and P content (on a dry weight basis) in tubers of the three varieties of *Solanum tuberosum* L. (Agria, Picasso and Rossi) at harvest. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria				Picasso				Rossi			
	Ca	K	S	P	Ca	K	S	P	Ca	K	S	P
	%				%				%			
Without skin												
Ctr	0.031 ± 0Ca	2.781 ± 0.012b	0.135 ± 0.001b	0.109 ± 0.004a	0.041 ± 0.002c	2.55 ± 0.004a	0.147 ± 0b	0.061 ± 0b	0.044 ± 0.002c	2.881 ± 0.012c	0.115 ± 0.006b	0.099 ± 0.006b
12A	0.033 ± 0.002b	2.55 ± 0.001c	0.153 ± 0.002a	0.065 ± 0.002c	0.048 ± 0.001b	2.284 ± 0.006b	0.149 ± 0.002b	0.049 ± 0.002c	0.057 ± 0.001a	2.924 ± 0.008b	0.133 ± 0.005ab	0.077 ± 0.004c
12B	0.05 ± 0.002a	2.815 ± 0.003a	0.149 ± 0.001a	0.084 ± 0.002b	0.056 ± 0.001a	2.561 ± 0.004a	0.166 ± 0a	0.082 ± 0.001a	0.051 ± 0.001b	3.521 ± 0.004a	0.153 ± 0.001a	0.137 ± 0.001a
With skin												
Ctr	0.049 ± 0.001b	1.749 ± 0.006c	0.115 ± 0.001b	<LOD	0.05 ± 0b	2.461 ± 0.005a	0.116 ± 0b	0.016 ± 0.001b	0.066 ± 0.001b	2.966 ± 0.022b	0.087 ± 0.006b	0.074 ± 0.006a
12A	0.043 ± 0.001c	2.1 ± 0.001a	0.134 ± 0a	0.044 ± 0a	0.046 ± 0c	2.222 ± 0.004c	0.106 ± 0c	0.015 ± 0.002b	0.064 ± 0.001b	2.582 ± 0.002c	0.089 ± 0.004b	0.044 ± 0.004b
12B	0.053 ± 0.001a	1.87 ± 0.002b	0.132 ± 0.003a	<LOD	0.094 ± 0.001a	2.422 ± 0.005b	0.143 ± 0.001a	0.023 ± 0.001a	0.118 ± 0.001a	3.262 ± 0.007a	0.145 ± 0.002a	0.08 ± 0.002a

Different letters (a, b, c) indicate significant differences among treatments of each mineral element *($p \leq 0.05$)*.

In Agria (**Table 3.57**), relatively to tubers with and without skin, according to the Pearson (r) and Spearman (ρ) methods, there is a positive correlation between Ca in tubers with and without skin. Moreover, Ca in tubers without skin showed a negative Pearson correlation of P content in tubers without skin and in K and P contents in tubers without skin. In tuber without skin, considering the Pearson correlation, K and P showed a strong correlation (r between 0.70 and 0.89) and, relatively to the Spearman correlation, K and Ca showed a strong correlation. Moreover, in tubers with skin, K and P showed a very strong correlation (r>0.90) with the Pearson method and, with the Spearman method, this pair also showed a strong correlation. Moreover, it is possible to observed with the Pearson index a strong correlation between Ca in tubers with skin and K in tubers without skin, and with S in tubers with and without skin. Considering the Spearman index, only Ca in tubers with skin and K in tubers without skin presented a strong correlation.

Table 3.57 Correlation matrix of Ca, K, S and P contents with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Agria variety. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	0.603*	0.245	-0.037	0.663*	-0.218	0.375	-0.514
	K	0.750	1.000	-0.535	0.742*	0.902	-0.888	-0.468	-0.989
	S	-0.117	-0.300	1.000	-0.903	-0.244	0.853	0.919	0.634*
	P	0.117	0.517	-0.817	1.000	0.488	-0.941	-0.880	-0.803
With skin	Ca	0.600*	0.900	-0.283	0.483	1.000	-0.672*	-0.228	-0.864
	K	-0.133	-0.483	0.883	-0.900	-0.417	1.000	0.785	0.940
	S	0.117	-0.083	0.650*	-0.683*	-0.217	0.617*	1.000	0.562
	P	-0.436	-.0822	0.693*	-0.822	-0.842	0.842	0.495	1.000

Correlation is significant at the 0.05 level (*).

Considering Picasso, it is possible to observe strong and negative correlations between Ca (without skin) and K (with skin) with the Pearson and Spearman methods, relatively and between S (without skin) and K (with skin) with the Spearman index (**Table 3.58**). Considering the Pearson index, it is possible to observe very strong correlations between tubers with and without skin in the pairs Ca-S, Ca-P, K-K, S-S, S-P, and between Ca and S in tubers with skin. This very strong correlation between Ca and S in tubers with skin can be observed in Picasso (**Table 3.56**), suggesting a synergistic Interaction. Moreover, with the Spearman index (**Table 3.58**), there are strong correlations between K and P in tubers without skin, as well as between S (with skin) and K (without skin), Ca (with skin) and P (without skin) and between S (with skin) and P (without skin). In this context, Ca only presented positive Pearson and Spearman correlations between K, S and P in tubers without and with skin.

Table 3.58 Correlation matrix of Ca, K, S and P contents with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Picasso. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	0.050	0.886	0.591*	0.790	-0.132	0.674*	0.706*
	K	0.383	1.000	0.406	0.806	0.592*	0.981	0.743*	0.461
	S	0.867	0.550	1.000	0.867	0.964	0.234	0.900	0.865
	P	0.400	0.967	0.633*	1.000	0.946	0.684*	0.988	0.811
With skin	Ca	0.467	0.883	0.667*	0.933	1.000	0.433	0.976	0.899
	K	-0.500	0.483	-0.133	0.500	0.417	1.000	0.606*	0.319
	S	0.433	0.967	0.583*	0.917	0.833	0.483	1.000	0.848
	P	0.617*	0.667*	0.600*	0.750	0.783	0.017	0.600*	1.000

Correlation is significant at the 0.05 level (*).

Regarding Rossi, some negative correlations were found among Ca, K, S and P with the Pearson and Spearman correlations (**Table 3.59**). According to Pearson (r) and Spearman (ρ) indexes there is a negative correlation between the pair Ca-P in tubers without skin. Considering mineral correlation in tubers without skin, there is only a very strong correlation between K and S (Spearman correlation). Thus, between tubers with skin, there is just one pair with a very strong correlation: Ca and K. Considering mineral correlation in tubers without and with skin more pairs with very strong correlations in Pearson index can be found.

Table 3.59 Correlation matrix of Ca, K, S and P contents with and without skin (on a dry weight basis) in tubers of *Solanum tuberosum* L. in Rossi variety. Pearson (blue) and Spearman (green) indexes.

		Without skin				With skin			
		Ca	K	S	P	Ca	K	S	P
Without skin	Ca	1.000	0.106	0.471	-0.276	0.011	-0.482	0.077	-0.613*
	K	0.467	1.000	0.850	0.890	0.993	0.791	0.983	0.540
	S	0.417	0.967	1.000	0.672*	0.809	0.444	0.874	0.267
	P	-0.483	0.517	0.550	1.000	0.932	0.957	0.922	0.847
With skin	Ca	-0.217	0.700*	0.667*	0.917	1.000	0.853	0.983	0.626*
	K	-0.483	0.517	0.550	1.000	0.917	1.000	0.806	0.896
	S	-0.033	0.733*	0.750	0.800	0.883	0.800	1.000	0.619*
	P	-0.567	0.200	0.183	0.783	0.750	0.783	0.767	1.000

Correlation is significant at the 0.05 level (*).

3.3.5.3.2 µ-EDXRF system – Ca location

To define the distribution pattern of Ca accumulation of the tubers, 5 regions were selected in the equatorial zone of each treatment (from epidermis/skin (1) to center (5)). This delineation, as observed previously, further revealed that Ca prevails in the epidermis region, followed by the inner regions of the tubers (**Figure 3.57**). Yet, in some treatments a higher Ca content occurred in the inner region of the tubers, namely in treatment 12B of Agria, whereas in Picasso it was found in the control treatment and in Rossi with the control and treatments 12A and 12B.

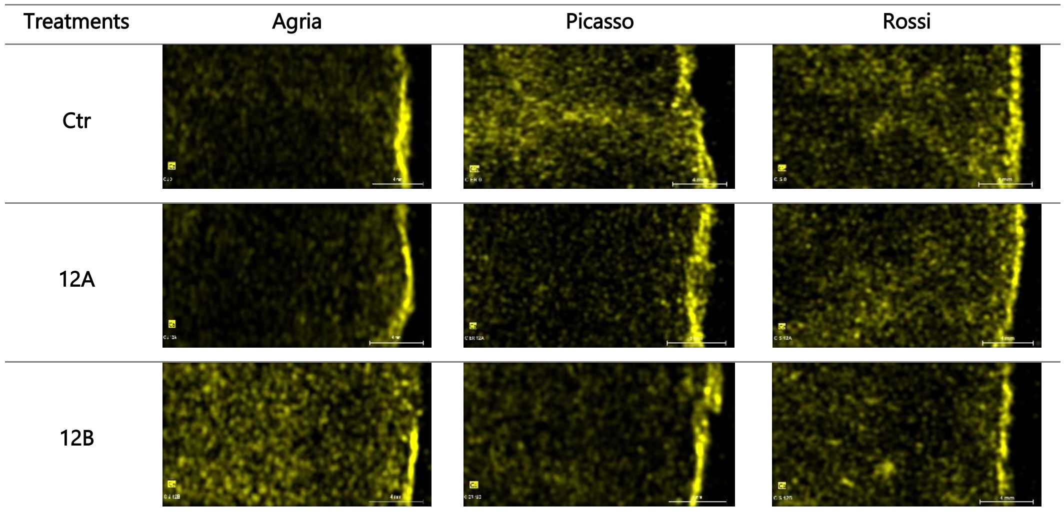


Figure 3.57 Calcium quantification in the 5 regions of the equatorial region of the tubers of *Solanum tuberosum* L. for Agria, Picasso and Rossi varieties, at harvest.

3.3.6 Morphological, physical, chemical, and organoleptic parameters

At harvest and after 7 and 8 months of storage tubers from Agria, Picasso and Rossi were analyzed regarding their morphological, physical, and organoleptic characteristics.

3.3.6.1 Height, diameter, dry weight content and total soluble solids

Height and diameter of tubers from Agria, Picasso and Rossi were assessed at harvest and after seven and eight months under cold storage conditions (**Table 3.60**). In Agria, significant differences among treatments were only verified in height and diameter after 7M of storage, and in Rossi significant differences were also found in height at harvest and after 7M of storage. Moreover, at harvest and after storage, Ca biofortification did not interfere in height and diameter of tubers from Picasso. Additionally, in the three varieties, considering the different analytical periods, there were significant differences within each treatment. At harvest, for Agria Picasso and Rossi, height and diameter varied between 9.5 - 9.83 and 6 - 6.83, 8.97 - 9.4 and 4.43 – 5.63, and 8.73 - 11.53 and 5.32 – 6.7 cm, respectively. After 7M of storage, Agria, Picasso and Rossi presented height and diameter values that ranged, respectively, between 6.77 – 11.4 and 4.27 – 5.13, 6.03 – 8.73 and 3.7 – 4.7, and 5.9 – 10.87 and 3.93 – 5.67 cm. After 8M of storage, the values of height and diameter varied between, 7.9 – 10.7 and 5.1 – 6.4, 7.87 – 9.67 and 5.8 – 7.1, and 7.9 – 11.1 and 5.03 – 6.67 cm, respectively.

Table 3.60 Height and diameter at harvest, after seven and eight (8M) months of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	Agria		Picasso		Rossi	
	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)
			Harvest			
Ctr	9.7 ± 0.67aA	6.83 ± 0.35aA	9.4 ± 0.87aA	5.37 ± 0.27aB	10.33 ± 0.88bA	6.7 ± 0.61aA
12A	9.83 ± 0.17aB	6 ± 0.32aA	9.4 ± 0.21aA	5.63 ± 0.38aB	11.53 ± 0.27aA	5.87 ± 0.41aB
12B	9.5 ± 0.7aA	6.07 ± 0.45aA	8.97 ± 0.58aA	4.43 ± 0.18aB	8.73 ± 0.06bA	5.32 ± 0.17aA
			7M			
Ctr	7.73 ± 0.67bB	5.13 ± 0.09aB	7.33 ± 0.93aB	4.1 ± 0.46aB	8.33 ± 0.2bB	5.37 ± 0.44aB
12A	11.4 ± 0.49aA	5.1 ± 0.06aB	8.73 ± 0.96aA	4.7 ± 0.35aC	10.87 ± 0.38aA	5.67 ± 0.59aB
12B	6.77 ± 0.74bB	4.27 ± 0.13bC	6.03 ± 0.77aA	3.7 ± 0.1aC	5.9 ± 0.32cC	3.93 ± 0.23bB
			8M			
Ctr	10.7 ± 0.65aA	6.4 ± 0.56aA	9.67 ± 0.88aA	7.1 ± 0.35aA	10.83 ± 0.17aA	6.67 ± 0.44aA
12A	8.57 ± 1.24aB	6.03 ± 0.43aA	9.3 ± 0.96aA	6.23 ± 0.19aA	11.1 ± 1.57aA	6.57 ± 0.48aA
12B	7.9 ± 1.36aB	5.1 ± 0.44aB	7.87 ± 0.87aA	5.8 ± 0.36aA	7.9 ± 0.49aB	5.03 ± 0.26aA

Different letters indicate significant different among treatments in each parameter (a, b) and between different times of analysis (Harvest, 7M and 8M) within each treatment (A, B, C) ($p \leq 0.05$).

Significant differences were found among treatments in Agria and Rossi at harvest and after 7M of storage, and in Picasso at harvest (**Table 3.61**). Additionally, in the three varieties, significant differences were also found among each analytical period within each treatment. At harvest, dry weight content of tubers from Agria, Picasso and Rossi ranged between 22.47 – 25.85 %, 18.66 – 26.67 % and 20.63 – 26.62 %, respectively. After 7M of storage conditions, the values varied between 18.28 – 23.57 %, 21.17 – 23.44 % and 22.44 – 26.73 %, respectively for tubers of Agria, Picasso and Rossi. On the other hand, after 8M of storage, dry weight content of tubers varied between 22.46 – 23.95 %, 19.54 – 25.68 % and 21.42 – 22.27 %, respectively for Agria, Picasso and Rossi.

Table 3.61 Dry weight content (%) at harvest (H), after seven (7M) and eight (8M) months of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n=4*) ± SE (standard error).

T.	Agria			Picasso			Rossi		
	DW (%)								
	H	7M	8M	H	7M	8M	H	7M	8M
Ctr	25.85 ± 0.28aA	23.57 ± 0.76aB	22.48 ± 5.08aC	26.11 ± 0.78aA	23.44 ± 1.41aB	19.54 ± 0.68aC	26.62 ± 0.1aA	26.93 ± 0.73aA	22.27 ± 3.35aB
12A	22.47 ± 1.07bA	20.07 ± 0.98bA	22.46 ± 1.49aA	26.67 ± 1.19aA	22.46 ± 1.91aB	22.79 ± 1.64aB	20.63 ± 0.47bA	22.44 ± 0.78bA	21.42 ± 4.23aA
12B	22.61 ± 0.05bB	18.28 ± 0.56bC	23.95 ± 0.74aA	18.66 ± 0.31bC	21.17 ± 0.98aB	25.68 ± 1.98aA	22.01 ± 0.33bA	22.91 ± 0.91bA	22.21 ± 1.01aA

Different letters indicate significant different between treatments (a, b) and between different times of analysis (Harvest, 7M and 8M) within each treatment (A, B) ($p \leq 0.05$).

Total soluble solids content in tubers was assessed in the three varieties at harvest, and after 7M and 8M of storage (**Table 3.62**). In Rossi there were only significant differences among treatments regarding TSS at harvest, as well as among each analytical period (harvest as well as 7M and 8M of storage) in the three varieties. Regarding Agria, a decrease in TSS was found with the increase of storage time: harvest > 7M > 8M. However, in Picasso and Rossi tubers that tendency did not occur, being detected a higher content of TSS at harvest, a decrease after 7M and an increase at 8M (harvest > 8M > 7M). At harvest the values of TSS, varied between 6.4 – 6.8, 6.6 – 7.33 and 5.6 – 7.8 °Brix, respectively for Agria, Picasso and Rossi. After 7M, TSS content ranged between 5.63 - 5.9, 6.2 – 6.6 and 6.33 – 6.97 °Brix, respectively for Agria, Picasso and Rossi. After 8M, Agria, Picasso and Rossi presented values of TSS ranging between 5.13 – 5.6, 5.6 – 7.8 and 6.63 – 6.97 °Brix, respectively.

Table 3.62 Total soluble solids at harvest (H), after seven (7M) and eight (8M) months of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (n = 4) ± SE (Standard Error).

T.	Agria			Picasso			Rossi		
	TSS (°Brix)								
	H	7M	8M	H	7M	8M	H	7M	8M
Ctr	6.67 ± 0.18aA	5.9 ± 0.06aB	5.2 ± 0.12aC	7.33 ± 0.35aA	6.6 ± 0.12aB	5.93 ± 0.09aC	7.8 ± 0.31aA	6.9 ± 0.21aB	6.97 ± 0.18aB
12A	6.8 ± 0.12aA	5.83 ± 0.12aB	5.13 ± 0.18aC	6.6 ± 0.23aA	6.2 ± 0.12aB	6.37 ± 0.28aAB	5.6 ± 0.7bB	6.33 ± 0.24aA	6.9 ± 0.67aA
12B	6.4 ± 0.12aA	5.63 ± 0.09aB	5.6 ± 0.12aB	7 ± 0.12aA	6.53 ± 0.18aB	6.67 ± 0.09aB	6.73 ± 0.37abA	6.97 ± 0.2aA	6.63 ± 0.3aA

Different letters indicate significant different among treatments (a , b) and between different times of analysis (Harvest, 7M and 8M) within each treatment (A, B, C) ($p \leq 0.05$).

3.3.6.2 Total fatty acid content

In the third year of the experiment, for the three varieties, total fatty acid content was carried out at harvest (**Table 3.63**). In tubers of Agria variety significant differences were found among treatments for <C16:0, C18:0 and C18:1, with the control presenting a significantly higher content relatively to the Ca biofortification treatments. In Picasso only <C16:0 presented significant differences among treatments, being the highest content obtained with treatment 12A. In Rossi, there were only significant differences among the biofortification treatments in C18:3, with the control presenting the highest content. Furthermore, as previously observed in the three varieties, the fatty acids profile remained similar, being characterized by the highest abundance of linoleic acid (C18:2), followed by linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0) (**Table 3.63**). Nevertheless, considering the three varieties TFA, <C16:0, C16:0, C18:0, C18:1, C18:2, C18:3 and DBI contents varied between 0.17 – 0.30 g/100 g FW, 0.28 – 1.25 mol %, 5.56 – 12.68 mol %, 1.31 – 3.33 mol %, 0.34 – 0.77 mol %, 61.33 – 67.51 mol %, 17.77 – 29.87 mol %, 13.62 – 29.35, respectively.

Table 3.63 Total fatty acid content (TFA), fatty acids profiles and double bound index (DBI) of lipids at harvest, in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	TFA	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3	DBI
	g/100g FW	mol %						
				Agria				
Ctr	0.18 ± 0.01a	0.83 ± 0.11a	12.68 ± 1.76a	1.91 ± 0.1a	0.49 ± 0.04a	61.33 ± 0.97a	22.75 ± 0.96a	13.64 ± 2.44a
12A	0.20 ± 0.01a	0.37 ± 0.04b	10.31 ± 0.49a	1.74 ± 0.13ab	0.36 ± 0.02ab	64.19 ± 0.94a	23.02 ± 0.67a	16.33 ± 0.51a
12B	0.17 ± 0.02a	0.28 ± 0.04b	11.97 ± 1.08a	1.31 ± 0.11b	0.34 ± 0.04b	62.25 ± 0.75a	23.86 ± 0.45a	15.15 ± 1.45a
				Picasso				
Ctr	0.23 ± 0.02a	0.54 ± 0.03b	6.67 ± 0.19a	1.78 ± 0.11a	0.41 ± 0.07a	63.89 ± 0.34a	26.72 ± 0.32a	24.03 ± 0.67a
12A	0.27 ± 0a	0.78 ± 0.07a	5.56 ± 0.63a	1.56 ± 0.13a	0.38 ± 0.03a	61.86 ± 2.21a	29.87 ± 2.13a	29.35 ± 2.77a
12B	0.27 ± 0.03a	0.40 ± 0.03b	5.74 ± 0.36a	1.97 ± 0.11a	0.40 ± 0.05a	64.24 ± 1.25a	27.25 ± 1.44a	26.69 ± 1.43a
				Rossi				
Ctr	0.17 ± 0.01b	1.16 ± 0.08a	8.18 ± 0.39a	3.33 ± 0.36a	0.61 ± 0.08a	64.10 ± 0.85a	22.62 ± 0.98a	16.82 ± 1a
12A	0.23 ± 0.02b	1.25 ± 0.19a	10.27 ± 0.91a	3.12 ± 0.24a	0.77 ± 0.13a	66.57 ± 0.67a	18.03 ± 0.61b	13.62 ± 0.84a
12B	0.30 ± 0.03a	1.29 ± 0.13a	9.71 ± 1.33a	3.14 ± 0.31a	0.57 ± 0.09a	67.51 ± 1.13a	17.77 ± 0.77b	14.95 ± 2.03a

Different letters indicate significant different among treatments (a, b) *($p \leq 0.05$).*

3.3.6.3 Soluble sugar content

In tubers of Agria, Picasso and Rossi, sucrose, glucose, fructose, and sorbitol were quantified at harvest and after seven and eight months of storage (**Table 3.64**). There were significant differences among treatments in each variety, regarding sucrose, glucose, fructose, sorbitol, and total soluble sugar content, except in: sorbitol content at 7M and 8M in Agria; Picasso at 7M; Rossi at 7M of storage. Significant differences were also found in each treatment considering the different analytical periods (harvest, 7M and 8M of storage) in each variety. Considering Agria, in treatment 12B (at harvest), and after 7M and 8M of storage in treatment 12A, sucrose content presented a significantly higher content. Also, treatment 12A revealed a glucose content significantly higher in the different analytical periods, as well as sorbitol at harvest. In tubers of Picasso, relatively to the control, depending on the soluble sugar, Ca biofortification treatments showed a significantly higher content. Rossi showed a significantly higher content of sucrose in the control after 7M, but for remaining soluble sugars, the highest content was obtained in Ca biofortification treatments (at harvest, 7M and 8M of storage). Considering all the treatments of the three varieties at harvest, sucrose, glucose, fructose, and sorbitol content varied between 0.081 – 0.475, 0.97 – 0.918, 0.024 – 0.801, 0.072 – 0.097 mg/g (on a fresh weight basis), respectively. In this context, after 7M of storage, sucrose, glucose, fructose, and sorbitol content ranged between 0.027 – 0.258, 0.071 – 1.023, 0.017 – 0.856 and 0.070- 0.084 mg/g (on a fresh weight basis), respectively. Additionally, after 8M, content of sucrose, glucose, fructose, and sorbitol varied between 0.002 – 0.206, 0.069 – 0.830, 0.014 – 0.429, 0.070 – 0.084 mg/g (on a fresh weight basis). Considering sucrose, glucose, fructose, and sorbitol, in Agria an increase was found with treatment 12A after 7M to 8M of storage and, in general, a higher content at harvest, followed by tubers stored for 7M and 8M. In Picasso and Rossi, comparing data from harvest to 7M of storage, an increase was found (except in sucrose and sorbitol).

Table 3.64 Soluble sugar content in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest, after seven (7M) and eight months (8M) of storage. Mean values (*n = 4*) ± SE (Standard Error).

Soluble Sugar	**T.**	**Agria**			**Picasso**			**Rossi**		
		Harvest	7M	8M	Harvest	7M	8M	Harvest	7M	8M
Sucrose	Ctr	0.081 ± 0.001cA	0.059 ± 0.001bB	0.013 ± 0.003bC	0.254 ± 0.001cA	0.14 ± 0.005bC	0.079 ± 0.003cB	0.213 ± 0.001cB	0.258 ± 0.001aA	0.035 ± 0.002bC
	12A	0.158 ± 0bA	0.027 ± 0.001cC	0.084 ± 0aB	0.475 ± 0.001aA	0.149 ± 0.001bC	0.206 ± 0.001aB	0.235 ± 0bA	0.028 ± 0.003cC	0.037 ± 0.001bB
	12B	0.216 ± 0.001aA	0.067 ± 0.003aB	0.002 ± 0.001cC	0.278 ± 0.006bA	0.208 ± 0aB	0.139 ± 0.001bC	0.247 ± 0.003aA	0.042 ± 0.001bC	0.047 ± 0.001aB
Glucose	Ctr	0.114 ± 0bA	0.077 ± 0bB	0.069 ± 0.002bC	0.421 ± 0.01cC	0.674 ± 0.001cA	0.519 ± 0cB	0.145 ± 0.003bC	0.338 ± 0.003cB	0.347 ± 0.001bA
	12A	0.121 ± 0aA	0.1 ± 0.001aC	0.109 ± 0aB	0.918 ± 0.001aB	1.023 ± 0.003aA	0.556 ± 0.002bC	0.208 ± 0.011aC	0.424 ± 0.002bA	0.305 ± 0cB
	12B	0.097 ± 0cA	0.071 ± 0cB	0.069 ± 0.005bB	0.671 ± 0.02bC	0.874 ± 0.003bA	0.83 ± 0.005aB	0.202 ± 0.001aC	0.671 ± 0.001aA	0.378 ± 0aB
Fructose	Ctr	0.024 ± 0cA	0.017 ± 0aB	0.015 ± 0.004bB	0.39 ± 0.003cB	0.567 ± 0.004cA	0.216 ± 0.001cC	0.134 ± 0.001bC	0.33 ± 0.004cA	0.197 ± 0.001bB
	12A	0.044 ± 0aB	0.022 ± 0.004aC	0.075 ± 0aA	0.801 ± 0aB	0.856 ± 0.002aA	0.251 ± 0bC	0.224 ± 0.009aB	0.445 ± 0.003bA	0.179 ± 0cC
	12B	0.031 ± 0bA	0.019 ± 0aB	0.014 ± 0.006bB	0.522 ± 0.017bB	0.752 ± 0.003bA	0.429 ± 0.006aC	0.228 ± 0.001aB	0.726 ± 0aA	0.216 ± 0aC
Sorbitol	Ctr	0.076 ± 0.002bB	0.082 ± 0aA	0.077 ± 0.01aB	0.083 ± 0aA	0.068 ± 0.009aB	0.07 ± 0.001bB	0.083 ± 0.001bA	0.081 ± 0.003aA	0.078 ± 0abB
	12A	0.085 ± 0.001aA	0.077 ± 0.008aB	0.083 ± 0aA	0.073 ± 0bA	0.064 ± 0aB	0.075 ± 0aA	0.084 ± 0bA	0.08 ± 0.001aB	0.078 ± 0bC
	12B	0.078 ± 0.001bC	0.094 ± 0aA	0.084 ± 0aB	0.072 ± 0.003bA	0.07 ± 0aA	0.075 ± 0aA	0.097 ± 0aA	0.076 ± 0.001aC	0.079 ± 0aB
Total	Ctr	0.295 ± 0.001cA	0.235 ± 0.001abB	0.175 ± 0.012bC	1.147 ± 0.011cB	1.45 ± 0.009cA	0.885 ± 0.003cC	0.575 ± 0.003cC	1.006 ± 0.008bA	0.657 ± 0.002bB
	12A	0.409 ± 0.001bA	0.226 ± 0.008bC	0.351 ± 0aB	2.267 ± 0.001aA	2.092 ± 0.006aB	1.088 ± 0.002bC	0.752 ± 0.003bB	0.978 ± 0.008bA	0.599 ± 0.001cC
	12B	0.421 ± 0.001aA	0.251 ± 0.002aB	0.154 ± 0.011bC	1.543 ± 0.019bB	1.904 ± 0.007bA	1.473 ± 0.01aC	0.774 ± 0.004aB	1.515 ± 0.002aA	0.721 ± 0.001aC

Different letters indicate significant different among treatments in each date (a, b) or between dates for the same treatment of bio-fortification (A, B, C) ($p \leq 0.05$).

3.3.6.4 Protein content

Protein content was evaluated in the three varieties submitted to Ca biofortification at harvest and after 7M and 8M of storage (**Table 3.65**). Considering Picasso, protein accumulation in tubers followed the pattern: 7M of storage > harvest > 8M of storage, in most of treatments. In tubers of Agria, protein accumulation followed a pattern of 8M of storage > harvest > 7M of storage, and in tubers of Rossi the pattern was 8M of storage > 7M of storage > harvest, in most of the treatments.

Table 3.65 Protein content at harvest (H), after seven months (7M) and eight months (8M) of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (n = 4) ± SE (Standard Error).

T.	Protein content (g/100g $_{FW}$)		
	Harvest	7M	8M
	Agria		
Ctr	1.32 ± 0.06aA	1.1 ± 0.05aB	1.65 ± 0.28aA
12A	1.38 ± 0.09aA	0.98 ± 0.1aC	1.13 ± 0.06bB
12B	1.25 ± 0.19aA	0.86 ± 0.11aB	1.29 ± 0.1abA
	Picasso		
Ctr	0.98 ± 0.04abB	1.4 ± 0.1aA	0.67 ± 0.06bC
12A	1.09 ± 0.08aB	1.23 ± 0.03aA	0.87 ± 0.03bC
12B	0.73 ± 0.09bC	1.21 ± 0.07aA	1.13 ± 0.03aB
	Rossi		
Ctr	0.87 ± 0.12aC	0.93 ± 0.05abB	1.21 ± 0.05aA
12A	0.89 ± 0.04aC	0.99 ± 0.04aB	1.16 ± 0.08aA
12B	0.81 ± 0.02aA	0.77 ± 0.06bB	0.73 ± 0.01bB

Different letters indicate significant different among treatments at harvest or after 7 or 8 months of storage for each variety (a, b) or between harvest, after 7 or 8 months of storage in the same treatment of biofortification (A, B, C) *($p \leq 0.05$).*

Significant differences among treatments were found in tubers of Agria after 8M of storage, as well as in tubers of Picasso at harvest and after 8M of storage. In tubers of Rossi significant differences were also found after 7M and 8M of storage (**Table 3.65**). At harvest, protein content of Agria, Picasso and Rossi varieties varied between 1.25 – 1.38, 0.73 – 1.09 and 0.81-0.89 g/100 g (on a fresh weight basis), respectively. After 7M of storage, protein content oscillated between 0.86 – 1.1, 1.21 – 1.40 and 0.77 – 0.99 g/100 g (on a fresh weight basis), respectively for Agria, Picasso and Rossi. Regarding 8M data, the values of protein obtained for Agria, Picasso and Rossi, ranged between 1.13 – 1.65, 0.67 – 1.13 and 0.73 – 1.21 g/100g (on a fresh weight basis), respectively.

3.3.6.5 Starch content

At harvest and after 7M and 8M of storage, the starch contents of tubers from Agria, Picasso and Rossi, did not exhibit significant variations among treatments at the different analytical periods (**Table 3.66**). Independently of the treatment, Agria presented a higher starch content at harvest and after 7M of storage. After 8M of storage, Rossi presented the highest starch content. Moreover, there were significant differences in each treatment from each variety comparing to the different experimental periods (at harvest, and 7M and 8M of storage). Besides, the trend of starch accumulation in the three varieties was: harvest > 8M of storage > 7M of storage. Considering Agria and Rossi, there weren't significant differences between treatments in each experimental period (harvest, 7M and 8M after storage). However, Picasso showed a significant difference among treatments after 7M and 8M of storage, with treatment 12B always presenting a significantly higher content of starch relatively to the remain treatments.

Table 3.66 Starch content at harvest (H), after seven months (7M) and eight months (8M) of storage of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values (*n = 4*) ± SE (Standard Error).

T.	**Starch content** (mg/g FW)		
	Harvest	7M	8M
		Agria	
Ctr	218.63 ± 37.37aA	60.83 ± 5.27aC	84.81 ± 0.61aB
12A	210.89 ± 12.04aA	66.27 ± 1.25aB	63.27 ± 1.36aB
12B	193.99 ± 36.81aA	105.95 ± 38.71aB	109.2 ± 37.07aB
		Picasso	
Ctr	159.22 ± 30.64aA	65.27 ± 6.26aB	75.98 ± 4.52bB
12A	115.47 ± 20.04aA	39.68 ± 6.17bC	93.3 ± 2.3aB
12B	199.55 ± 5.73aA	75.37 ± 1.41aC	92.85 ± 3.28aB
		Rossi	
Ctr	131.3 ± 12.02aA	37.27 ± 10.93aC	103.81 ± 2.37aB
12A	125.53 ± 5.95aA	35.85 ± 4.37aC	95.54 ± 0.53aB
12B	106.67 ± 8.2aA	67.12 ± 5.99aC	76.32 ± 11.37aB

Different letters indicate significant different among treatments at harvest or after 7 or 8 months of storage for each variety (a, b) or between harvest, after 7 or 8 months of storage in the same treatment of biofortification (A, B, C) ($p \leq 0.05$).

3.3.6.6 Colorimetric parameters

At harvest, in the three varieties, through scanning colorimetric analysis was carried out considering the visible spectral region (450 – 650 nm), being found a maximum transmittance value at 550 nm (**Figure 3.58**), corresponding to the yellow color.

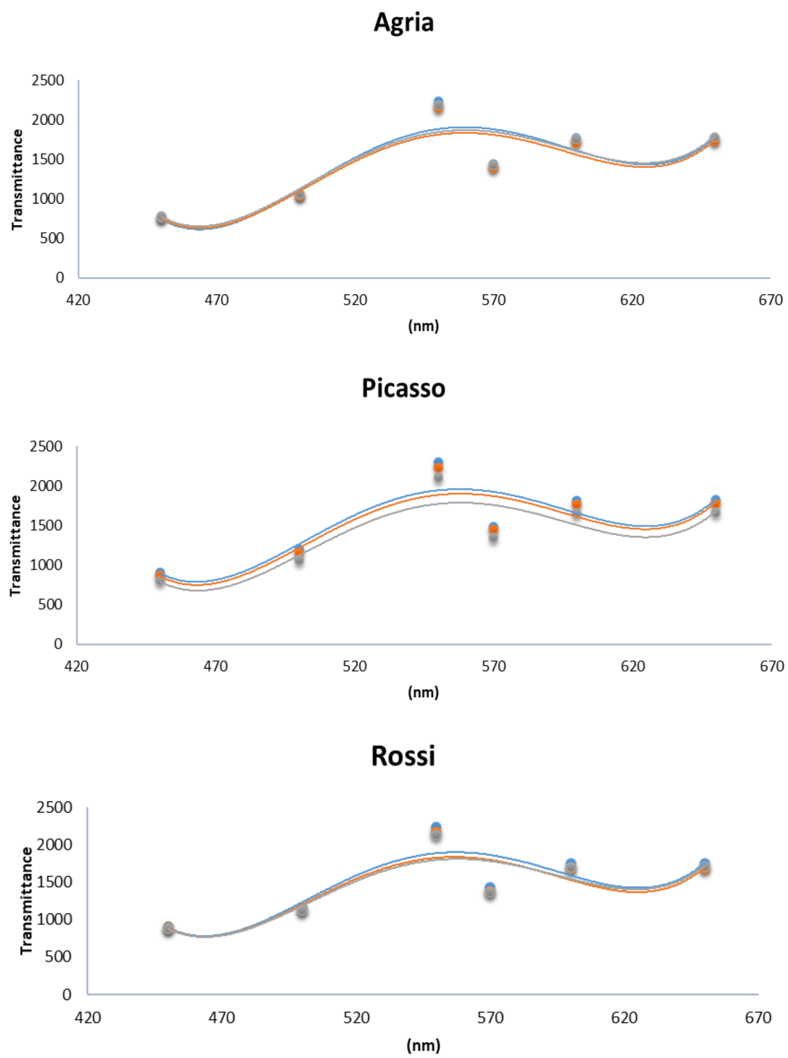


Figure 3.58 Visible spectra showing the average of transmittance (*n = 4*) in fresh tubers of *Solanum tuberosum* L. varieties (Agria, Picasso and Rossi), at harvest, with a degree 4 polynomial. (● Control, ● 12 kg ha^{-1} $CaCl_2$ and ● 12 kg ha^{-1} Ca-EDTA).

Considering the pulp of Agria, Picasso and Rossi tubers at harvest, after 7 and 8 months of storage, colorimetric parameters were also analyzed through the CieLab system (**Table 3.67**). In Agria, after

7M and 8M of storage, only significant differences were found among treatments in L, b*, Chroma and Hue. In fact, for parameter L of the control significantly lower values were found relatively to the Ca biofortification treatments, indicating a darker color (considering a gray scale). Relatively to parameter b* and Chroma, treatment 12B presented significantly higher values, indicating a more yellow and brightness color. Considering the Hue parameter, treatment 12B revealed significantly lower values after 7M and 8M of storage. Concerning Picasso, only significant differences were found among treatments in L, b*, Chroma and Hue after 7M of storage, presenting the similar tendencies relatively to Agria (control with lower values of L; treatment 12B with higher values of b*; lower values of Hue). Nevertheless, in tubers of Rossi only significant differences were found among treatments: after 7M of storage in L, b*, Chroma and Hue; at harvest in a*, b*, Chroma and Hue; after 8M of storage in a* and Hue. After 8M of storage, treatment 12B presented significantly higher values: at harvest and in parameter a*; after 7M of storage in b* and Chroma. However, treatment 12B showed a significantly lower values in Hue at harvest and after 7M. In the three varieties, Ca biofortification treatments, especially treatment 12B, showed slight changes in a*, b*, chroma and Hue, mainly after 7M of storage.

Table 3.67 Color parameters considering the CieLab scale (L, a*, b*, chroma and hue) at harvest (H) and after seven (7M) and eight (8M) months of storage of fresh tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties. Mean values ($n = 4$) ± SE (standard Error).

T.	L			a*			b*			Chroma			Hue		
	H	7M	8M	H	7M	8M	H	7M	8M	H	7M	8M	H	7M	8M
Agria															
Ctr	67.96 ± 0.09a	60.77 ± 0.4b	60.60 ± 0.2b	-4.18 ± 0.11a	-2.69 ± 0.11a	-2.60 ± 0.13a	37.23 ± 1.14a	10.46 ± 0.08b	11.46 ± 0.04b	37.46 ± 1.12a	30.81 ± 0.09b	30.00 ± 0.03b	96.43 ± 0.36a	104.44 ± 0.53ab	102.20 ± 0.53ab
12A	67.98 ± 0.4a	62.24 ± 0.87a	62.40 ± 0.80a	-3.64 ± 0.23a	-2.89 ± 0.07a	-2.82 ± 0.02a	35.13 ± 1.78a	10.62 ± 0.16b	11.62 ± 0.10b	35.32 ± 1.79a	31.01 ± 0.14b	30.05 ± 0.15b	95.9 ± 0.1a	105.23 ± 0.55a	106.2 ± 0.4a
12B	68.08 ± 1.49a	62.96 ± 0.44a	62.90 ± 0.42a	-3.38 ± 0.47a	-2.85 ± 0.06a	-2.70 ± 0.04a	35.63 ± 0.31a	12.04 ± 0.16a	12.00 ± 0.05a	35.8 ± 0.35a	32.37 ± 0.17a	12.40 ± 0.15a	95.41 ± 0.71a	103.3 ± 0.13b	103.1 ± 0.11b
Picasso															
Ctr	70.57 ± 0.98a	60.77 ± 0.4b	61.94 ± 2.72a	-3.42 ± 0.46a	-2.69 ± 0.11a	-3.09 ± 0.69a	23.91 ± 2.87a	10.46 ± 0.08b	23.91 ± 1.41a	24.15 ± 2.9a	30.81 ± 0.09b	24.13 ± 1.44a	98.11 ± 0.15a	104.44 ± 0.53ab	97.31 ± 1.54a
12A	66.67 ± 2.06a	62.24 ± 0.87a	65.38 ± 0.87a	-3.99 ± 0.29a	-2.89 ± 0.07a	-3.68 ± 0.19a	27.34 ± 0.77a	10.62 ± 0.16b	25.75 ± 0.89a	27.64 ± 0.81a	31.01 ± 0.14b	26.02 ± 0.9a	98.28 ± 0.37a	105.23 ± 0.55a	98.12 ± 0.25a
12B	65.55 ± 0.75a	62.96 ± 0.44a	65.45 ± 2.15a	-3.7 ± 0.06a	-2.85 ± 0.06a	-3.63 ± 0.13a	27.25 ± 0.48a	12.04 ± 0.16a	25.27 ± 0.61a	27.5 ± 0.47a	12.37 ± 0.17a	25.53 ± 0.59a	97.74 ± 0.23a	103.3 ± 0.13b	98.2 ± 0.47a
Rossi															
Ctr	71.63 ± 0.64a	60.77 ± 0.4b	65.71 ± 1.38a	-3.29 ± 0.04b	-2.69 ± 0.11a	-2.96 ± 0.07b	22.42 ± 0.61a	10.46 ± 0.08b	20.74 ± 0.44a	22.66 ± 0.61a	30.81 ± 0.09b	20.95 ± 0.44a	98.36 ± 0.12a	104.44 ± 0.53ab	98.12 ± 0.18a
12A	65.04 ± 1.36a	62.24 ± 0.87a	66.67 ± 2.26a	-2.88 ± 0.15ab	-2.89 ± 0.07a	-2.87 ± 0.11b	20.11 ± 0.32b	10.62 ± 0.16b	19.84 ± 0.73a	20.31 ± 0.3b	31.01 ± 0.14b	20.05 ± 0.73a	98.17 ± 0.53a	105.23 ± 0.55a	98.23 ± 0.04b
12B	66.97 ± 2.33a	62.96 ± 0.44a	66.99 ± 0.82a	-2.21 ± 0.25a	-2.85 ± 0.06a	-2.24 ± 0.06a	21.39 ± 0.44ab	12.04 ± 0.16a	21.19 ± 0.7a	21.5 ± 0.46ab	32.37 ± 0.17a	21.31 ± 0.7a	95.87 ± 0.52b	103.3 ± 0.13b	96.04 ± 0.2a

Different letters (a, b) indicate significant different between treatments in each parameter ($p \leq 0.05$).

3.3.6.7 Heat treatment of tuber pulp

Color (**Table 3.68**) and texture (**Table 3.69**) of both raw and cooked tubers were analyzed at harvest in Agria, Picasso and Rossi varieties. Sensory analysis was also performed (**Figure 3.59**).

3.3.6.7.1 Color analysis

Significant differences among treatments in tubers color (L, a* and b* parameters) were found in Agria, Picasso and Rossi, except for parameter L of raw tubers in Agria and Picasso and in cooked tubers of Rossi (**Table 3.68**). Indeed, regarding raw and cooked tubers in the same treatment and variety, significant differences were also found. Considering parameter L, in the three varieties a decrease was found from raw to cooked tubers, evolving a darker color. In fact, there was also a decrease of parameters a* and b* from raw to cooked tubers in the three varieties, indicating a greenish and less yellowish tone, respectively. Considering cooked tubers, colorimetric parameters in Agria and Rossi (**Table 3.68**) presented a significantly higher value with treatment 12B. Only Picasso showed with treatment 12B a significantly higher value of b* parameter.

Table 3.68 Colorimetric parameters (L, a* and b*) in raw and cooked tubers (20 minutes, at 100 ºC) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest. Mean values (*n = 3*) ± SE (Standard Error).

T.	Raw tubers			Cooked tubers		
	L	a*	b*	L	a*	b*
			Agria			
Ctr	70.48 ± 0.46aA	-5.51 ± 0.11cA	36.55 ± 0.51aA	67.18 ± 0.85aB	-9.33 ± 0.28bB	22.04 ± 0.7bB
12A	66.67 ± 0.72aA	-4.46 ± 0.17bA	31.29 ± 0.36bA	65.02 ± 1.05bA	-9.14 ± 0.26bB	22.6 ± 0.83bB
12B	65.47 ± 0.52aB	-3.02 ± 0.09aA	29.43 ± 0.43cA	67.39 ± 0.9aA	-9.88 ± 0.15aB	25.29 ± 0.32aB
			Picasso			
Ctr	69.53 ± 0.5aA	-5.02 ± 0.07cA	26.14 ± 0.33aA	58.07 ± 1.17aB	-6.14 ± 0.33aB	4.01 ± 0.86cB
12A	68.48 ± 0.51aA	-4.72 ± 0.09bA	22.63 ± 0.4cA	59.89 ± 0.78aB	-7.17 ± 0.22bB	8.39 ± 0.67bB
12B	67.67 ± 0.43aA	-4.44 ± 0.07aA	25.28 ± 0.39bA	61.71 ± 1.13aB	-8.2 ± 0.22cB	12.77 ± 0.92aB
			Rossi			
Ctr	69.51 ± 0.39aA	-3.24 ± 0.07bA	19.35 ± 0.3aA	52.61 ± 1.37bB	-6.11 ± 0.36cB	4.88 ± 0.34bB
12A	66.65 ± 0.25cA	-2.37 ± 0.08aA	17.10 ± 0.19cA	61.52 ± 1.58aB	-5.87 ± 0.23bB	5.56 ± 0.52bB
12B	67.05 ± 0.29bA	-2.47 ± 0.04aA	18.48 ± 0.2bA	61.89 ± 1.53aB	-5.25 ± 0.22aB	7.82 ± 0.37aB

Different letters indicate significant different among treatments in raw tubers or cooked tubers each variety (a, b, c) or between raw and cooked tubers in the same treatment (A, B) ($p \leq 0.05$).

3.3.6.7.2 Texture

Texture parameters (fracturability, hardness, number of peaks, work strength and adhesiveness) were studied in Agria, Picasso and Rossi in the control and in Ca biofortified samples (**Table 3.69**). A remarkable difference was found in the behavior of the texture of raw samples, with a more indented texture profile (greater number of peaks) and with higher hardness values when compared to cooked samples, with values of low hardness, associated with a low number of peaks (**Table 3.69)**. Considering fracturability in raw tubers, Picasso presented a lower value, followed by Agria and Rossi. However, this tendency did not occur in cooked tubers. In the three varieties, the hardness parameters, in generally varied between 83.54 and 154.36 N for raw tubers, whereas for cooked tubers ranged between 2.32 and 9.35N in. The number of peaks remained similar in the three varieties of raw tubers but varied considerable between each treatment and variety. For instance, in Agria the number of peaks were found to be significantly lower with treatment 12B and significantly higher in Picasso and Rossi tubers. In general, in raw tubers from the three varieties, work strength presented a lower value in Picasso, followed by Agria and Rossi, but in cooked tubers that tendency did not match. In cooked tubers of the three varieties, relatively to Ca biofortification treatments, the control presented significantly lower values, with Agria and Rossi showing the highest values in treatment 12A and Picasso in treatment 12B. In fact, in all the texture parameters, cooked tubers from Picasso presented a significantly higher value in treatment 12B. For instance, considering adhesiveness of cooked tubers, Agria and Picasso showed a significantly higher value in treatment 12B and Rossi in treatment 12A.

Table 3.69 Texture parameters in raw tubers and cooked tubers (20 minutes at 100ºC) in tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest. Mean values ($n = 3$) ± SE (Standard Error). Treatments (T).

T.	Raw tubers					Cooked tubers				
	Fracturability (N)	Hardness (N)	Number of Peaks	Work stregth (N.mm)	Adhesiveness (-N.mm)	Fracturability (N)	Hardness (N)	Number of Peaks	Work stregh (N.mm)	Adhesiveness (-N.mm)
Agria										
Ctr	6.46 ± 0.19aA	127.86 ± 0.4bA	52.3 ± 0.6bA	12.61 ± 0.48aA	4.21 ± 0.55aA	0.42 ± 0.07aB	5.35 ± 0.78aB	2.6 ± 0.2bB	0.46 ± 0.06cB	0.67 ± 0.12bB
12A	6.67 ± 0.29aA	136.21 ± 5.47aA	56.5 ± 2.4bA	13.94 ± 0.85aA	2.34 ± 0.6bA	0.46 ± 0.08aB	6.42 ± 0.56aB	4.8 ± 0.7aB	0.60 ± 0.04aB	0.68 ± 0.06bB
12B	6.65 ± 0.31aA	120.24 ± 1.35cA	60.6 ± 2.1aA	11.28 ± 0.38bA	4.52 ± 0.64aA	0.46 ± 0.05aB	6.61 ± 0.56aB	1.6 ± 0.2cB	0.55 ± 0.04bB	0.89 ± 0.09aB
Picasso										
Ctr	5.44 ± 0.07bA	94.41 ± 3.4aA	44.6 ± 1.4bA	8.65 ± 0.44aA	4.14 ± 0.27bA	0.33 ± 0.08cB	3.54 ± 0.59cB	2.6 ± 0.5cB	0.37 ± 0.06cB	0.47 ± 0.09bB
12A	5.15 ± 0.09cA	83.54 ± 3.55bA	52.4 ± 1aA	8.15 ± 0.49aA	3.34 ± 0.46cA	0.52 ± 0.11bB	5.84 ± 0.84bB	4.1 ± 0.8bB	0.60 ± 0.09bB	0.56 ± 0.04bB
12B	6.48 ± 0.36aA	98.91 ± 2.52aA	52.6 ± 4aA	8.51 ± 0.31aA	6.45 ± 0.5aA	0.85 ± 0.15aB	9.35 ± 1.67aB	6.8 ± 0.5aB	0.95 ± 0.12aB	0.71 ± 0.09aB
Rossi										
Ctr	8.13 ± 0.51aA	154.36 ± 10.9aA	52.2 ± 1.7aA	15.84 ± 1.11aA	2.34 ± 0.26bA	0.21 ± 0.01cB	2.32 ± 0.06cB	2.0 ± 0.4bB	0.21 ± 0cB	0.25 ± 0.01cB
12A	8.1 ± 0.22aA	117.41 ± 4.5bA	40.2 ± 1.2bA	10.89 ± 0.57bA	4.06 ± 0.29aA	0.40 ± 0.09aB	7.26 ± 1.08aB	3.2 ± 0.8aB	0.84 ± 0.15aB	0.83 ± 0.11aB
12B	7.23 ± 0.15bA	111.74 ± 3.55bA	49.4 ± 2aA	10.59 ± 0.47bA	4.08 ± 1.14aA	0.29 ± 0.03bB	4.88 ± 0.57bB	3.4 ± 0.2aB	0.58 ± 0.04bB	0.51 ± 0.07bB

Different letters indicate significant different among treatments in raw tubers or cooked tubers each variety considering each parameter (a, b, c) or between raw and cooked tubers in the same treatment and in the same parameter (A, B) ($p \leq 0.05$).

Considering softening at harvest (**Table 3.70**), it was found that Rossi presented the highest percentage of softening, followed by Agria and Picasso.

Table 3.70 Softening of tubers *of Solanum tuberosum* L., Agria, Picasso and Rossi varieties at harvest.

	Softening (%)		
T.	Agria	Picasso	Rossi
Ctr	93.5	93.9	97.4
12A	93.1	89.9	95.1
12B	93.1	86.9	96.0

3.3.6.7.3 Sensory analysis

Sensory analysis was carried out in tubers of the three varieties, submitted to Ca biofortification (**Figure 3.59**). In this context, the panel of tasters analyzed the "texture", "flavor", "appearance", "color" and "overall appreciation" in cooked tubers from the control and treatments 12A and 12B treatments from Agria, Picasso and Rossi varieties.

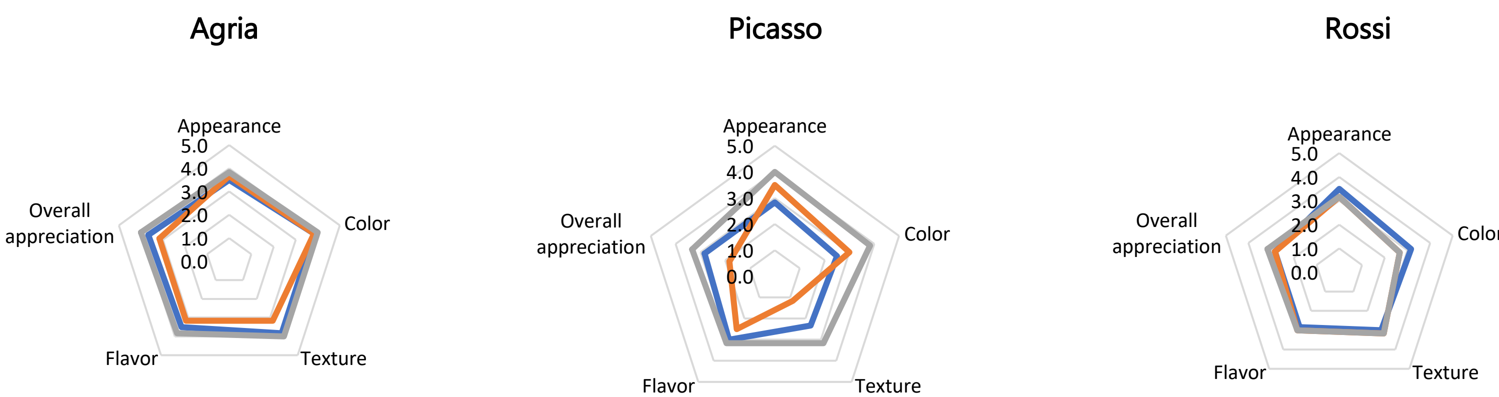


Figure 3.59 Average evaluation of the panel of tasters regarding the different sensory characteristics for *Solanum tuberosum* L. Agria, Picasso and Rossi varieties (— Control, — 12 kg ha^{-1} $CaCl_2$, — 12 kg ha^{-1} Ca-EDTA).

Regarding each sensory characteristics (**Figure 3.59**), it was found that tubers from Agria and Rossi presented values between 3 and 4, which corresponds to "I didn't like" and "I liked it", respectively. The Ca biofortification treatments did not show significant differences from the control samples. However, treatment 12A from Picasso in "texture" and "overall appreciation" presented averages below 1, corresponding to "I disliked it a lot", showing also significant differences from control samples. Furthermore, treatment 12B samples of Agria and Picasso were highly appreciated by the panel of tasters in all the attributes analyzed. In Rossi, samples from treatment 12B were highly appreciated by the panel of tasters in "texture", "flavor" and "overall appreciation". As such, in the three varieties, samples from treatment 12B presented a higher "score "in the different sensory characteristics analyzed.

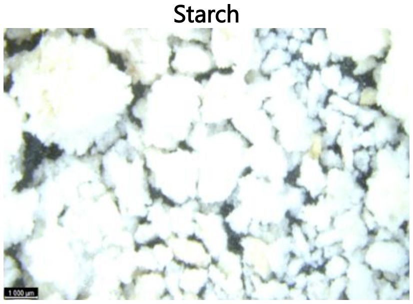
3.3.7 Starch and dehydrated mashed potatoes

Starch and dehydrated mashed potatoes were produced with the three varieties, to assess potential differences among treatments within each variety. Additionally, food additives were incorporated (E223 and E320) in both starch and dehydrated mashed potatoes. After 41 and 82 days after production of starch and dehydrated mashed potato, in samples with or without addition of food additives (**Table 3.71**), development of molds were not found (**Figure 3.60**). In fact, considering that both products presented a very low water activity, the molds development is hardly expected.

Table 3.71 Verification through magnifying glass to assess the development of microorganisms on samples without any food additive and on samples with the incorporation of E223 and, alternatively E320 (✖ - not detected).

Processed potato product	Days after additives incorporation	
	41	82
Starch	✖	✖
Dehydrated mashed potatoes	✖	✖

Starch



Dehydrated mashed potatoes

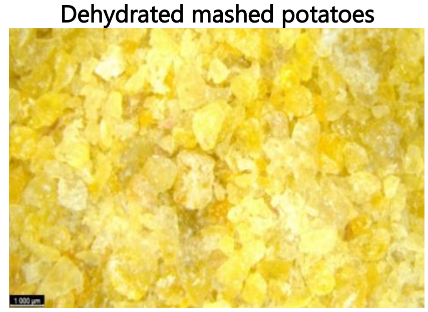


Figure 3.60 Example of starch and dehydrated mashed potatoes through magnifying glass after 41 days of production and incorporation of food additives.

3.3.8 Yield

Potato yield in the three varieties submitted to Ca biofortification was carried out at harvest (**Table 3.72**), being found the highest value in the control and the lowest with treatment 12B. Relatively to the control and treatment 12A, treatment 12B revealed the lowest yield.

Table 3.72 Total yield at harvest of tubers of *Solanum tuberosum* L., Agria, Picasso and Rossi varieties.

T.	**Total Yield (kgs)**		
	Agria	Picasso	Rossi
Ctr	100.65	104.7	168.8
12A	81.1	93.75	159.85
12B	56.5	59.3	46.9

4

DISCUSSION

4.1 Soil and irrigation water

In plants, soil is the primary source of their production, and its type can affect crop production (Benjamin et al., 2003). Accordingly, soil characterization was carried out in the three experimental fields (**Table 3.1**) before the implementation of the biofortification workflow for the culture, considering some of the most important characteristics regarding mineral availability (White & Broadley, 2009). Furthermore, the pH of the three experimental fields was found to be within the ideal range for agriculture (pH 6.5 – 7.5) (IPNI, 2010). However, potato plants are tolerant regarding pH (Muthoni, 2006), being grown in a wide range of pH levels (5.7 to 8.4), despite the maximum nutrient availability being between 6.5 and 7.5 (Thornton, 2020). In fact, the availability of nutrients is strongly affected by soil pH (**Figure 1.15**) (Power & Prasad, 1997), yet in the three experimental fields the pH could be considered optimal for nutrient availability. Moreover, CEC (cations exchange capacity, which is proportional to clay content), was not analyzed because it is usually not a limitation to potato production (Stark & Thornton, 2020). Electrical conductivity is influenced by a combination of both physical and chemical factors (Corwin & Lesch, 2005), that can be used as a precision farming diagnostic tool and is further considered an integrated measure of different soil properties (namely, clay content, salinity, and water content) related to crop productivity (Clay et al., 2001). Hence, EC measure the amount/content of salts present in soil solution. This is an important parameter since the excess of salts in the root zone, harms the germination, development, and productivity of plants, leading to a greater energy expenditure for water absorption by the root system (*i.e.*, osmotic effect) and, therefore, impairs the essential metabolic processes of the plant (leading to a loss of productivity) (Brandão & Lima, 2002).

Considering this background, it was found that the three fields showed an EC lower than 1.7 $dS.m^{-1}$, which avoids yield losses of the culture (Thornton, 2020). Following Smith & Doran (1996), It was therefore concluded that the three experimental fields belong to the class of non-saline soils (having an EC between 0 and 2 $dS.m^{-1}$ regardless of the soil texture). Additionally, sandy fields have low EC, silts have intermediate EC, and clays have high EC (Cambouris et al., 2006).

In Portugal, soils usually have a low content of OM, as well as a tendency towards its progressive decrease. This tendency is mainly due to the prevailing climacteric conditions, which are favorable to decomposition, as well as cultural practices carried out (namely, lack of adequate replacement of OM content) (Gonçalves, 2005). Hence, OM is significantly related to crop nutrient composition. As such, more OM can lead to a higher nutrient content in crops (Wood et al., 2018). In this context, further considering this parameter in the three experimental fields (**Table 3.1**), it was found that field C had a significantly higher OM content relatively to field B, and A. Field A showed significantly lower content compared to the remaining field. Additionally, as Indicated in **Annex VIII**, the clay content of field A is less than 10 % and field B and C higher than 30 %. Therefore, the OM of field A was less than the expected and field B and C are according with was expected (**Annex IX)**. Nevertheless, as stated by Gonçalves (2005), soil can be categorized into five classes of OM richness according to soil texture (**Annex X**). The soil of the three fields can be integrated on the "medium and fine texture", with, field A belonging to "Low" class, being in accordance with data from Gonçalves (2005) that showed that, in Ribatejo and Oeste region of Portugal, the major soil from agricultural regions belongs to class "Low". Field B and C belongs to "Medium" Class, which is the third most common class in the region according to Gonçalves (2005).

For the normal growth and development of plants, at least 17 essential elements are requiredc (Uchida, 2000; Thorton, 2020; Kumar et al., 2021), being N, P, K, Ca, S, Fe, Zn, Mg, Mn, Cu, B, Mo, Cl, and Ni (Thornton, 2020) obtained by soil minerals and OM or by fertilizers (Uchida, 2000). Accordingly, Ca, K, Mg, P, Fe, S, Zn, Mn, Pb and, As were quantified (**Table 3.1**). Nitrogen, P, and K are considered primary nutrients (Thornton, 2020) and previous data using the Kjeldahl method showed that N content was 850, 830 and 1320 mg/kg in fields A, B and C, respectively. According to a study carried out by Wekesa et al. (2014) in Kenya for potato production, it was found that a higher content of N relatively to the ones found In fields A and B, but similar relatively to field C. Additionally, the study of Wekesa et al. (2014) also showed that different spots have different N contents, despite the close location of the studied locations. Phosphorus on the surface of soils usually varies between 0.02 - 0.1 % and the concentration present in the soils do not reflect the availability index to plants (Mia, 2015). Our data revealed that P contents varied between 0.19 - 0.29 % in the three experimental periods (**Table 3.1**), being higher than the average P in soil surface, probably due to the fertilization that occurred throughout the years in the fields. The contents of P found in our study can be consider in the range of soils concentration presented by Strawn et al. (2020), that stated that P in soil can varied from 35 to 53000 $mg.kg^{-1}$, being the median 800 $mg.kg^{-1}$. Regarding K, the three experimental field showed values ranging between 2.20 - 2.64 % (Table 3.1), being in accordance with the range of soils concentration presented by Strawn et al. (2020). Iron, although being a micronutrient in plants (Thornton, 2020; Kumar et al., 2021), was the second most abundant element in the soils of fields A and C. Iron showed a significantly higher content in field C (2.59 %), followed by field A (1.19 %) and with a significantly lower content in field B (0.50 %) (**Table 3.1**). However, generally Fe contents in soils range between 2000 and 550 000 mg/kg (Strawn et al., 2020), being our data within that range. Calcium, Mg and S are considered secondary nutrients (Thornton, 2020). Moreover, Ca is the third most abundant element in the soils (**Table 3.1**) and high content of this nutrient indicates a near-neutral pH, which it is desirable for most plants (Bohn et al., 2001). In fact, the positive correlation between exchangeable Ca and soil organic carbon should be considered, as an increase in soil organic carbon concentration generally leads to an increase of the cation exchange capacity (CEC) (Rowley et al., 2018). According to Strawn et al. (2020), the average of Ca in the soils is 15 000 mg/kg, being the minimum around 700 $mg.kg^{-1}$ (0.07 %). Considering the data of Ca obtained in the three experimental fields (0.39 – 0.71 %), it was found that this nutrient content remained higher than the minimum and lower than the average. Magnesium showed a significantly higher content in field B, relatively to the remain fields, varying between 0.15 - 0.24 % (**Table 3.1**), and being in accordance with the concentration range for soils (400 – 9000 $mg.kg^{-1}$) (Strawn et al., 2020). Sulfur can range between small concentrations (30 $mg.kg^{-1}$) to higher concentrations (1600 $mg.kg^{-1}$) (Strawn et al., 2020). According to our data, S contents was in the normal range for soil, showing an amount close to the lowest concentration (between 55.9 and 77.6 $mg.kg^{-1}$) (Table 3.1). Thus, field C showed a significantly higher content of S relatively to the other two fields (A and B). It must also be pointed out that the content of mineral elements of soils can vary a lot, namely for nitrogen, sulfur, or phosphorus (Strawn et al., 2020). Zinc and Mn contents were significantly different among the experimental fields (**Table 3.1**), varying between 19.6 – 62.7 $mg.kg^{-1}$ and 270 – 703 $mg.kg^{-1}$, respectively. Nevertheless, these values remained in accordance with the considered normal range in soils (*i.e.,* between 1 – 900 $mg.kg^{-1}$ for Zn and 20 – 10000 $mg.kg^{-1}$ for Mn) (Strawn et al., 2020). Hence, regarding the micronutrients, Mn was the third most abundant mineral element in the soils (after Fe and Mg). Despite the presence of relatively high levels of contaminating mineral elements (**Table 3.1**), Pb contents in the soils were below the concentration limit value indicated by the Portuguese legislation, considering that for a pH greater than 7.0, the limit value is 450 $mg.kg^{-1}$ of dry matter (DL nº 118/2006). In an uncontaminated soil, As concentration can also vary between 0.2 and 40 $mg.kg^{-1}$ (Cunha & Duarte, 2008). Nevertheless, field C showed significantly higher content of Pb and As, followed by field B and A. As seen in **Table 3.5**, our data pointed out to an interference of organic matter in the colorimetric parameters.

In the first year, the irrigation water (**Table 3.6**) exhibited higher salinity based on EC, however, this elevated EC does not pose a risk of soil alkalinization, due to its low Na concentration relatively to Ca and Mg. The irrigation water of the fields is subsaturated in calcium carbonate, having a corrosive tendency when circulating in pipes, considering that pHs > pH, which leads to a negative LSI (MECC, 2022). Nonetheless, the water has a relatively low corrosion impact on pipes or metallic components in the distribution system considering that the LSI is between -1 and +1 (MECC, 2022). Moreover, field C has almost a perfect balanced irrigation water, considering that the LSI is close to zero.

In the second year (**Table 3.25**), fields B and C shared the same hydrochemical facies, while relatively to the first year field A presented a different facies (**Table 3.6**). Although there were slight changes in pH and EC in the second year, the water quality remained generally stable, with an increase in certain ion concentrations. For instance, field B exhibited a slight increase in pH, and showed a shift in its behavior in relation to precipitation and dissolution of calcite, making it slightly encrusting. Yet, this aspect is merely indicative since the pH and the bicarbonate concentration are very unstable. Additionally, the irrigation water has a relatively low corrosion impact on pipes or metallic components in the distribution system, considering that the LSI is between -1 and +1, being zero the perfect LSI, indicating a perfectly balanced water and saturated with the perfect content of calcium carbonate and a stable pH (MECC, 2022). Regarding field C (pHs = pH), the water is balanced, meaning that calcium carbonate will not be dissolved or even precipitated. Concerning field B (pHs < pH), it was found that the water is non-aggressive, tending to deposit calcium carbonate. However, in field A (pHs > pH) the water was considered corrosive (negative LSI) (MECC,2022).

Relatively to the second (**Table 3.25**) and third years (**Table 3.51**), field A exhibited a slight increase in pH (7.1 to 7.2, thus close to the pH of the first year) and fields B and C revealed a considerable increase (7.3 to 7.5 and 7.2 to 7.7, respectively), with the electrical conductivity decreasing considerably in the three experimental fields. Additionally, Mg^{2+} (except in field C), Cl^{-} (except in field C), HCO_3^{-} (except in field B) and So_4^{2-} (except in field A) showed an increased content, whereas Ca^{2+} (except in field A), K^+, Na^+ revealed a decrease. In the third year, the hydrochemical facies were: calcium bicarbonate, considering field A (thus, remained the same in the three years); calcium bicarbonate in field B (therefore, the same of the first year); and magnesium sodium chloride sulphate, considering field C. Overall, the irrigation water in all three years, and in the three fields, was found to be of underground origin, with specific hydrochemical facies for each field. Nevertheless, irrigation is essential for potato production, mostly in regions of inadequate precipitation or with soils with low capacity of water holding. Also, without irrigation would be difficult to meet the standards for fresh, process or seed potatoes (Ojala et al., 1990). Thus, irrigation affects positively tubers yield, as well as the quality in terms of shape or size (Begum et al., 2018). The more suitable methods are sprinkler and drip irrigation, which can increase tubers yield (Begum et al., 2018). Regarding the three experimental fields, the irrigation was carried out through sprinklers in the different stages of phenological development of *Solanum tuberosum* L. and did not represent danger of soil alkalinization.

4.2 Remote detection and monitoring of the culture in the fields

In recent years, agriculture has evolved due to advances mostly in the areas of chemistry and robotics (Radoglou-Grammatikis et al., 2020). Regarding advances in robotics, some RGB and multispectral cameras coupled to UAVs (Unmanned Aerial Vehicles) are being used to provide data for decision making, for instance: vegetation indices - which provide crop status, growth, and vigor to be monitored (Xue & Su, 2017). Through data obtained by UAVs, it is possible to create NDVI maps, which ranged from -1 to 1 (USGS, 2022). The NDVI index is one of the most used indexes (Karnieli et al., 2010), providing information to detect stress (Xue & Su, 2017), diseases, infestations, or even nutrient deficiencies in crops (Radoglou-Grammatikis et al., 2020). In fact, NDVI maps are interpreted by considering the differences between the green color of the plant's leaves (Xue & Su, 2017), in which low values indicating to stress symptoms in the vegetation (Karnieli et al., 2010). Moreover, NDVI serves as a valuable tool for estimating the primary productivity of crops (Zaeen et al., 2020). In this context, and considering the previously studies that has been carried out to monitor potato crops using remote sensing technology (Hunt & Rondon, 2017; Zaeen et al., 2020; Fernández et al., 2020), in the second (**Figure 3.13**) and third year (**Figure 3.33**) was possible to obtain some valuable data relative to crop health and growth dynamics. Nevertheless, in the first year (**Figure 3.7**) - without any assessment during the Ca biofortification process - there were distinct drainage characteristics among the three experimental fields. In fact, field A exhibited a higher propensity for water accumulation and infiltration, while field B could lead to runoff and limited drainage capabilities. Considering the second year (both **Figure 3.13** and **Table 3.27**), only treatment 12B showed a lower NDVI when compared to the control. These lower NDVI values can be due to Ca-EDTA, since induced toxicity (as seen by the appearance of chlorosis and necrosis on the edges of plant leaves) (Souza et al., 2020). Hence, the NDVI map already indicated the onset of toxicity symptoms in plants. Notably, these Ca-EDTA toxicity symptoms may be due to higher loads of Na or Cl of EDTA (Souza et al., 2020), because potato plants are known as Na-sensitive plants and Cl-nonsentive plants (Hutsch et al., 2018). Additionally, the toxicity symptoms (specially with Ca-EDTA) were observed during the field monitoring (**Figure 3.15** and **Figure 3.16**). Nevertheless, also in the third year, the lower NDVI observed in the two genotypes implemented in fields A and B (**Figure 3.33** and **Table 3.53**), was probably associated to Ca-EDTA toxicity, which can promote chlorosis and necrosis on the edges of plant leaves (Souza et al., 2020). The expand of Ca-EDTA toxicity symptoms in the aerial part of plants was also observed during the field monitoring (**section 3.3.3**).

4.3 Photosynthetic functioning

Photosynthesis plays an essential role in crops, whose efficiency stands as a crucial factor that can determine the final yield (Simkin et al., 2019; Mu & Chen, 2021). Thus, photosynthetic metabolism is virtually responsible for the entire biomass production of plants and is therefore fundamental for the productivity of crops. Moreover, it is considered a good indicator of the ability of plants to acclimatize to prevailing environmental conditions, which include periods of less suitable conditions for plant development, namely extreme temperatures, or water availability (drought or flooding) or even mineral (deficiency or toxicity). In fact, changes in the photosynthetic rates, changes in water uptake, and evapotranspiration occurred frequently under environmental stress conditions (Morales et al., 2020). Whitin this framework, during the three years both infrared gas analysis (IRGA) at leaf leveland chlorophyll *a* fluorescence parameters were evaluated (**sections 3.1.5**; **3.2.4** and **3.3.4**). In this context, it is important to considered that Ca is involved in a wide range of physiological processes, including growth and development, as well as the ability to tolerate environmental stresses. These effects are interconnected with the photosynthetic functioning, yet depending on Ca content, negative impacts can develop (Ramalho et al., 1995; Hochmal et al., 2015). Moreover, Ca^{2+} plays a regulatory role in several photosynthetic pathways such as, on stomatal closure, to photosystems functioning, non-photochemical quenching, and xanthophyll cycle functioning (Hochmal et al., 2015; Wang et al., 2019). Considering that when the toxicity threshold is exceeded, the synthesis of photoassimilates is hindered, during the first year we use the variety Agria as a test system (**Figure 3.8**), being found that, regardless of dosage or product used (calcium chloride or calcium nitrate), Ca-biofortification promoted a low to moderate decrease of P_n, and an even a stronger decline of g_s with a consequent iWUE increase. The reduction of P_n could be associated with decreases in the maximum and current efficiency of photosystem II, as well as to the quantum yield of the photosynthetic electron transport. However, the strong stomatal closure led to substantial declines in the internal CO_2 concentration (Ci), pointing a stomatal limitation on P_n. Additionally, stomata guard cells integrate a response to environmental and endogenous signals to regulate stomatal opening and closure, with cytoplasm Ca^{2+} oscillations playing a critical role in this process (Allen et al., 2001). Moreover, it was observed that an increase in external Ca content promotes stomatal closure, likely through Interference on Ca^{2+}-sensing receptor (CAS) protein (Allen et al., 2001; Weinl et al., 2008). This seemed to be the case, as regards g_s reduction in Agria plants (**Figure 3.8**). Still, regarding the first year of the experiment, by comparing the two dates of monitoring (**Table 3.9- Table** 3.11), overall, a global decline in the use of energy through photochemistry was verified (q_L and $Y_{(II)}$), being probably associated with the close date to harvest and the respective end of *Solanum tuberosum* L. plants life cycle. Furthermore, the increased foliar applications with Ca can alter the photosynthetic performance by reducing stomatal opening, a process that is mediated by the Ca^{2+}-sensing receptor (CAS) protein (Weinl et al., 2008). This is in line with the significant g_s decline previously observed in **Figure 3.8**, although that usually promoted only a (consistent) tendency to reduce the net C assimilation rate after the 3rd application. Furthermore, it was possible to verify (**Table 3.9- Table** 3.11) that after the 3rd application, no impacts were observed regarding the photochemical performance of the photosynthetic machinery, or in the photoprotective dissipation process. This suggests that until this stage, the potential functioning of the photosynthetic apparatus remained intact, regardless the doses or chemical forms applied. However, a marginal impact on C assimilation was observed, primarily due to a decrease in gs (**Figure 3.8**), that restricted the CO_2 supply (as reflected in the lowered C_i) to the carboxylation sites. After the 4th application with $CaCl_2$, the photosynthetic performance was either maintained or slightly improved, in contrast to the effects of $Ca(NO_3)_2$ doses. These results suggested a negative impact on the photosynthetic apparatus performance, although without increases in deregulated energy dissipation usually associated with photoinhibition ($Y_{(NO)}$) (Rodrigues et al., 2016). Regarding the second year (**section 3.2.4**), it was possible to observe different effects regarding the concentration/dose or product (calcium chloride or Ca-EDTA) applied. Overall, Ca-EDTA presented negative impacts in the three varieties regarding the different photosynthetic parameters analyzed. Therefore, in the third year of the experiment (**section 3.3.4**), the evaluation of gas exchange was carried out more directed towards the final phase of plants life cycle (**Table 3.54**). Regarding the rate of net photosynthesis (Pn), treatment 12A showed a slightly negative effect (non-significant), contrary to the effect of treatment 12B, which clearly showed negative effects on the three varieties. In fact, our data revealed an aggressive effect with the application of 12 $kg.ha^{-1}$ concentration of both products in Agria. In Picasso, only Ca-EDTA promoted negative effects after five foliar applications, while in Rossi there was no negative effects of $CaCl_2$ or Ca-EDTA after 5 foliar applications. However, as seen in Agria, close to harvest date, Ca-EDTA treatment had burned all plants. The effects on stomatal conductance (g_s), and consequently on the transpiration rate (E), were variable. In Agria tending to decrease, but increased in Rossi and remaining quite similar in Picasso after 5FA of both products. Instantaneous water use efficiency (iWUE) varied, despite presenting a decreased in all varieties (not significantly in Rossi) with Ca-EDTA treatment. In this context, it was found that application of treatment 12B had important negative implications in terms of the photoassimilates production (as it shorts the life cycle of *Solanum tuberosum* L. plants) and in the iWUE. Considering **Table 3.55**, treatment 12B, stand out negatively regarding to maximum (F_v/F_m) and current ($F_{v'}/F_{m'}$) photochemical efficiency of PSII after the 5th foliar application in Agria and Picasso. Also, this treatment promoted necrosis of leaves in the last evaluation of Agria, therefore confirming the extend of toxicity previously verified in the second year of experiment. Considering the estimates of the quantum yields of non-cyclic photosynthetic electron transport ($Y_{(II)}$), regulated dissipation of energy in PSII ($Y_{(NPQ)}$), and unregulated dissipation (heat and fluorescence) in PSII ($Y_{(NO)}$), variations were found depending on each parameter, but according to the performance of the photosynthetic machinery. In general, in the three varieties, and treatment 12B, a significant decrease occurred considering $Y_{(II)}$ and an increase of $Y_{(NPQ)}$ (energy dissipation indicator). Moreover, treatment 12A did not show any negative impact on any of the varieties. Also, there were only slightly changes in the uncontrolled processes of light energy dissipation ($Y_{(NO)}$). Furthermore, impacts were found regarding q_N (which reflects the proportion of energy dissipated as heat by photoprotection metabolism) and q_L (referring to the proportion of energy captured by the PSII open reaction centers and used for photochemical events). Being in accordance with $Y_{(II)}$, q_L values decreased in all varieties with treatment 12B. On the other hand, q_N showed a pattern of variation consistent with $Y_{(NPQ)}$, increasing in the three varieties. Considering treatment 12A, relatively to the control, any impact on both dates of analysis were not found. In this context, our data showed (**Table 3.54** and **3.55**) that the application of treatment 12B had negative implications in terms of the photosynthesis metabolism.

4.4 Quantification of mineral elements and Ca location

4.4.1 Mineral monitoring and kinetic of mineral accumulation during the biofortification process

Interactions occur among different mineral elements (Reboredo, 1991, 1994; Fageria, 2001; Romera et al., 2021), as seen in the organs of *Solanum tuberosum* L. and at the different stages of plant development. Besides, interactions among minerals can trigger different effects, namely if they are essential at physiological levels (Romera et al., 2021). In fact, understanding the mineral interactions can facilitate the enhancement of certain nutritional disorders, or even in the biofortification of selected mineral elements (Zhou et al., 2020; Romera et al., 2021), like Ca. Overall, it was possible to observe (**Figure 3.17 - 3.20**) a decrease in Ca content in the different organs of *Solanum tuberosum* L. plants (leaves > stems > roots > tubers) in most of the biofortification treatments, and after the different times of analysis (*i.e.*, after 4FA, 6FA and 7FA) in both varieties (Agria and Picasso). It was further possible to verify (**Figure 3.17 - 3.20**) that, despite some toxicity symptoms triggered by $CaCl_2$ and Ca-EDTA (**section 3.2.3**), overall Ca content increased in the *Solanum tuberosum* L. organs of Picasso and Agria. It was also possible to observe that Ca, K, S and P showed different accumulation trends during Ca biofortification in both varieties. Nevertheless, considering that mineral accumulation occurs mainly from the soil solution by roots uptake (White & Boradley, 2009), and that potato plants are usually classified as inefficient regarding nutrients acquisition, due to the poor root system - shallow and less extended (Subramanian et al., 2011), foliar applications with Ca can lead to an increase of the mineral element in the plant organs. Additionally, although K and P are considered very mobile minerals in the phloem tissue, Ca like S are less mobile (White & Boradley, 2009). Besides, even similar edaphoclimatic conditions, depending on the genotypes, differences can occur in shoot absorption of minerals (Almekinders & Struik, 1996). As such, this can justify the different trends of Ca accumulation in the different organs of Picasso and Agria plants (**Figure 3.17 -3.20**). Considering that after the last two foliar applications, the highest Ca content in roots (**Figure 3.18**) was obtained with Ca-EDTA treatments, and in tubers after the 7FA (**Figure 3.17**), and that the highest Ca content were obtained in treatments 24A (in Agria) and in 24B (in Picasso), these data seemed to indicate a remobilization of that nutrient. In fact, as seen in roots and tubers of both varieties, the increase in Ca accumulation in both organs of *Solanum tuberosum* L. plants seemed to point that Ca is redistributed through the phloem (Coelho et al., 2021), despite being identified as a relatively immobile mineral element (Koch et al., 2020a; White & Broadley, 2009). Regarding tubers, Ca content showed the lowest values regarding the other studied minerals, probably because Ca transport is mainly accumulated through the xylem, and because tubers hardly transpire (Koch et al., 2020b). However, despite the negative effects observed in *Solanum tuberosum* L. plants with both foliar spraying products (**section 3.2.3**) used in the biofortification workflow, the highest Ca content was obtained in tubers almost ready for harvest (**Figure 3.17**). In general, Ca-EDTA treatments showed a higher content in Picasso organs, while in Agria was not possible to obtain a clear tendency regarding Ca-EDTA and $CaCl_2$ applications. For instance, in the leaves of both varieties (**Figure 3.20**), Ca biofortification treatments showed a higher Ca content relative to control, being in accordance with a previous study carried out on *Solanum tuberosum* L. plants (El-Hadidi et al., 2017). In potato tubers, the highest content was obtained with K during the Ca biofortification process (**Figure 3.17**), probably because of its central role in carbohydrates translocation from leaves to tubers (Abdel-Salam et al., 2012), as well as in establish tubers and starch (Koch, 2020b), and the fact that tuber composition and tuber bulking are dependent on the availability of K content (Ewais et al., 2020). Moreover, the organs of potato plants, which contain more K than tubers, are in the vegetative part (stems and leaves) (Zhang et al., 2022b), as verified in our data (**Figure 3.19 and 3.20**). Thus, this was probably due to the fact that K is readily redistributed within the plant, after being acquired by the roots and provided to the shoots through the xylem pathway (Karley & White, 2009). In the leaves (**Figure 3.20**) Ca biofortification, had an irrelevant effect on K content, probably due to the antagonistic effect of Ca on the absorption of K by roots at higher levels (El-Hadidi et al., 2017), despite the essential role of K an in leaf area development, and in the photosynthetic functioning (Koch, 2020b). In fact, with the development of *Solanum tuberosum* L. plants, K showed a decrease (more notorious in Agria) (**Figure 3.20**), which is in accordance with Sharma & Arora (1989). In the different organs both genotypes of *Solanum tuberosum* L., P did not vary considerably during the biofortification process (**Figure 3.17 - 3.20**), eventually because this nutrient (in the form of Pɨ) can be translocated in the xylem and phloem and has a constant loading and unloading into the different plant organs (Raghothama, 2005). Additionally, the main source of P is accumulated in the aerial parts of the plants, being a crucial mineral for the rate of tuber expansion (Potarzycki & Grzebisz, 2019). The content of P content in tubers was also lower than in leaves (**Figure 3.20**), which was in accordance with a study carried out in other potato varieties (White et al., 2018), and previously reported by Potarzycki & Grzebisz (2019). Moreover, P accumulation during the potato production cycle was different in both varieties, probably due to the variation among the different genotypes (White et al., 2018). Yet, considering that other organs of *Solanum tuberoum* L. plants, such as roots, can contribute to P supply to the growing tubers

(Potarzycki & Grzebisz, 2019), this can explain the P decrease in roots of Agria during the biofortification process and, in parallel, to the production cycle (**Figure 3.18**). In higher plant, S is mostly absorbed by roots and, additionally by the atmospheric source (as SO_2) (Koch, 2020b), which might explain the highest S content in roots (**Figure 3.18**) and leaves (**Figure 3.20**) during the biofortification workflow. Thus, in most of treatments, S contents showed the following tendency: leaves > roots > stems > tubers, being different with the observed in other studies (Grzebisz et al., 2022). This different pattern of S accumulation plants organs is probably due to differences in methodologies and the experimental workflow applied. Furthermore, different patterns of mineral accumulation in both varieties during the workflow of Ca biofortification and plant development seemed to be due to the remobilization of mineral elements during the different stages of plant development (Marschner, 1995), and the difference mineral content between potato genotypes (Navarre et al, 2009), justifying the different contents obtained. Accordingly, it Is important to consider that plant genotypes might differ in minerals uptake, translocation, and accumulation rates (Clárk, 1983). Moreover, considering the absorption rate of each mineral element, the differences observed are probably due to the fact that plants require a higher nutrient content during the development season. Another factor triggered to this Item might be the nutrient uptake during the rapid vegetative growth and in the early stages of tuber developments, which improves and tends to stabilize during tuber maturation and in the end of plant development (Thornton, 2020). As such, in both varieties it was possible to observe the phase of stabilization of nutrient uptake with small variations between the three periods of analysis (**Figure 3.25** and **Figure 3.26**). As observed in the second year of experiment, also in the third year (**Figure 3.48 - 3.51**), a decrease of Ca content was found from the areal part of *Solanum tuberosum* L. plants to the tubers of the three varieties (thus, with the following pattern: leaves > stems > roots > tubers) in most of the biofortification treatments and in the different analytical periods (3, 5 and 7 foliar applications). It must be also pointed that nutrition interactions occurs among different minerals and among the different stages of plant development (Reboredo, 1991, 1994; Fageria, 2001; Romera et al., 2021), as observed in our data (**Figure 3.48 - 3.51**). In this context It was found that Ca, K, S and P showed different accumulation patterns during the Ca biofortification process (**Figure 3.48 - 3.51**). Furthermore, differences of the translocation rates in the shoots, linked to genotypic variations and microclimate variations could have influenced (Almekinders & Struik, 1996) mineral accumulation. In fact, the differences observed in Ca, K, S and P contents for the different varieties seemed to be coupled to these factors, being K and P considered very mobile in the phloem tissues, and Ca as well as S the less mobile minerals (White & Broadley, 2009). Besides, previous studies indicated that Ca is redistributed though the phloem (Coelho et al., 2021), which supports the increase of Ca content in biofortified tubers of *Solanum tuberosum* L. mediated by $CaCl_2$ and Ca-EDTA. Under this background, despite other studies suggesting that Ca is a relatively immobile mineral element (Koch et al., 2020a; White & Broadley, 2009), our data suggest that there is mobilization of Ca from leaves to the tubers. Similarly, there were different patterns of Ca, K, S and P accumulation in Agria, Picasso and Rossi during the Ca biofortification process. Indeed, considering plants development, remobilization of different mineral elements seems to occur during the different stages of plant development (Marschener, 1995) and due to the different potato genotypes (Navarre et al., 2009).

4.4.2 Tubers at harvest

Considering data from **Table 3.12**, it was possible to verify the different accumulations of nutrients analyzed in each variety that implicates different mobility of nutrients through phloem and xylem pathways. In fact, the accumulation of nutrients being dependent on genotype is in accordance with Goméz et al. (2017), which mention that the accumulation of nutrients is related to the tuberization dynamics per cultivar, although also related to soil fertility. Regarding Ca, its accumulation in tubers of *Solanum tuberosum* L. relies upon the interaction of various factors, such as the development of tubers, phloem and xylem delivery, and other chemical interactions that occur within the tubers (Subramanian et al., 2011). Despite plant species variability, Ca might interfere by stimulating the uptake of P under defined concentration ranges (Ishizuka & Tanaka, 1960; Fageria, 2001), however, Ca accumulation promoted by the two fertilizers in the tubers did not significantly affected P content only in Rossi (**Table 3.12**). Moreover, considering that plants require P for growth, despite being poorly mobile in plants (Hinsinger et al., 2011), Ca content in Agria and Picasso significantly affected its availability/content. Potassium is one of the main nutrients present in tubers (Navarre et al., 2009), being considered highly mobile within plants (White & Karley, 2010), determining its high content in the potato tubers of the three varieties. Overall, Ca accumulation in tubers showed an increase of K content, providing evidence of a synergistic interaction, probably mostly due to the similar chemical properties, namely size, charge, or electronic configuration (Robson & Pitman, 1983). On the other hand, although more evidence must be provided, as suggested by Aulakh & Dev (1978), and found in our data, a higher content of S also could have improved the absorption of K and P. As noted in the first year (**Table 3.12**), also in the second year (**Table 3.32**), the patterns revealed for Ca, K, S and P indicated the occurrence of different mobilities of minerals though the phloem and xylem pathways, which is well known to be dependent of the genotypes characteristics (Goméz et al., 2017).

Regarding Ca accumulation, all the varieties showed a significantly increased of Ca, relatively to the control tubers, with different biofortification indexes. Considering data obtained in the first year (**Table 3.12**), for the three varieties, with a different Ca biofortification workflow, it was possible to observe that data obtained in tubers with skin, despite some variations, remained similar. Furthermore, in the three varieties, Ca accumulation in tubers, triggered by $CaCl_2$ and Ca-EDTA, affected positively the levels of P, as previously reported in other studies (Ishizuka & Tanaka, 1960; Fageria, 2001). Also, in the second year, K showed, relatively to Ca, P and S, the highest content, since is the main mineral element in tubers (Navarre et al., 2009), as it is high mobile within plants (White & Karley, 2010). Nevertheless, S can improve the absorption kinetics of K and P, as reported by Aulakh & Dev (1978). Overall, considering the third year (**Table 3.56**), K, S and P content obtained in tubers without skin were higher than the ones obtained in tubers with skin. Furthermore, as previously found (Navarre et al., 2009), relatively to Ca, S and P, potassium showed the highest content, as it has the highly mobile within plants (White & Karley, 2010). Considering treatment 12B (**Table 3.56**), as verified in the second year of experiment (**Table 3.32**), Ca showed the highest content both in tubers with and without skin. Also, the mineral content obtained in the second and third years were similar. Moreover, the different accumulation in Ca, K, S and P observe in the three varieties is probably due to the different mineral mobility through phloem and xylem pathways, being dependent on the genotype's characteristics (Goméz et al., 2017). In both first (**Figure 3.9**) and third year of the experiment (**Figure 3.57**), the distribution of Ca location was assessed in five distinct zoned within the equatorial region of the tubers. Our findings suggest that the translocation of Ca from the shoot did not reveal any effect on the pattern of mineral accumulation in tissues of tubers within all the varieties. Additionally, data revealed a consistent trend where Ca content was higher in the skin region compared to the inner region of the tubers, which was consistent with previous studies (McGuire & Kelman, 1984; Wszelaki et al., 2005; Subramanian et al., 2011). Glosek-Sobjeraj et al. (2019) also supported this observation, highlighting elevated Ca levels in the skin relatively to the flesh of tubers. However, it Is essential to acknowledge potential variations within different regions of tubers, specifically between the stem end and the bud end of the tubers, as documented by Johnston et al. (1968) and Subramanian et al. (2011). This variability aligns with our third-year data, where certain treatments exhibited higher Ca content in the inner region of the tubers. For instance, treatment 12B of Agria showed higher Ca content in the inner regions, while in Picasso it was found in the control Ctr treatment and in Rossi in the control and treatments 12A and 12B (**Figure 3.57**).

4.5 Morphological, physical, and organoleptic parameters

4.5.1 Height, diameter, dry weight content and total soluble solids

Analyzing data from the first year in terms of height and diameter (**Table 3.14**), at harvest Ca biofortification process did not interfere with the caliber of the tubers. Moreover, according to the Portuguese Law (Portaria nº 587/87), in the case of potatoes for consumption, the size/tubers caliber (diameter) cannot be less than 35 mm, or 30 g (in oblong tubers varieties) and 40 mm, or 30 g (in rounded tubers varieties), and therefore all the three varieties remained in accordance with the Portuguese Law, having more than 40 mm of size. Besides, according to Amaral & Militão (2015), the tubers are considered suitable for industrial production when the caliber of tubers is higher than 40 mm. Thus, the three varieties can be used for consumption or for industrial production. The dry weight content, as presented in **Table 3.15**, meets the processing industry standards outlined by Braun et al. (2010) in the majority of treatments for Agria, Picasso (excluding 1A, 3A and 0.5N treatments) and Rossi (excluding 0.5N treatment). Specifically, the dry weight content surpassed the threshold of 20 %, demonstrating suitability for processing industries. Additionally, at harvest Agria exhibited a higher content of dry weight than those reported for the same variety by Romano et al. (2018) (18.6 ± 0.42 %), but similar to the report of AGRICO (2022) (21.8 %) and slightly lower relatively to the mean values obtained by Bordoloi et al. (2012) (23.8 %), Arvanitoyannis et al (2012) (23.27 %) and Abbasi et al. (2011) (22.95 %). According to AGRICO (2023), the average dry weight in Picasso is 20.8 %, but most of our treatments have higher dry weight contents. Also, according to HZPC (2022), the average dry weight for Rossi is also 20.8 %, which was in accordance with most of our treatments. The variation of tubers weight loss is dependent of tuber genotype and storage conditions (Haider et al., 2023). Indeed, lower storage temperatures (4 and 8 ºC) are more successful in keeping dry weight of tubers than higher temperatures (12 – 25 ºC) (Freitas et al., 2012), but there are still losses as verified in our data (**Table 3.15**), and in a study carried out by Freitas et al. (2012) with other varieties. In fact, a study carried out with different varieties showed a weight loss in tubers stored at 10 ºC (Yosuke et al., 2000). The decrease dry weight is probably linked to the respiration rates after harvest (Ozturk & Polat, 2016), which determines that during storage tubers may considerably lose weight and quality (namely, at a nutritional basis) (Gottschalk & Ezhekiel, 2006). Besides, coupled to respiration, dry weight losses in tubers during storage are also mainly due to transpiration and sprout development (although after 3 months storage sprout development still does not occur - Burton et al., 1992). Thus, a loss of tubers weight loss of tubers of *Solanum tuberosum* L. before and after storage is expected (Hamideldin et al., 2013). Across all three varieties (**Table 3.15**), the TSS data at harvest aligns with findings from Feltran et al. (2004) for different varieties, such as Agata (5.46 °Brix) Mondial (4.88 °Brix), Asterix (5.94 °Brix), Iac Aracy Ruiva (6.23 °Brix) or Remarka (6.72 °Brix), cultivated under different edaphoclimatic conditions. Furthermore, these varieties exhibit higher TSS values compared to other varieties, such as Agata (4.00), Atlantic (4.8), BRS Clara (3.2.) (Braun et al., 2010), Asterix (4.25) or Mondial (3.95) (Virmond et al., 2014). For instance, Agria's TSS values at harvest align with studies by Abbasi et al. (2011) (5.44 °Brix) and Escuredo et al. (2018), which demonstrated variability according to the year of production (2014 – 5.1 °Brix; 2015 – 4.7 °Brix and 2016 – 5.6 °Brix). Overall, there was a significant decrease in TSS after 3M, which pointed to some changes in the ratio of glucose/fructose probably occurring during storage (Javanmardi & Kubota, 2006). As verified in the first year, as well in the second year (**Table 3.36**), there were no significant differences among treatments for both heigh and diameter in the three varieties, suggesting that Ca biofortification did not interfere in tuber's caliber. Moreover, regarding dry weight (**Table 3.37**) and TSS content (**Table 3.38**), the differences observed were align with the ones observed in the first year. In terms of the third year, heigh and diameter (**Table 3.60**) the three varieties were found suitable for industrial processing at harvest and after 7M and 8M of storage (Portaria n.o 587/87) (*i.e.* tubers calibers need to be higher than 3.5 cm). Nevertheless, in Rossi, treatment 12A, independently of the analytical period, always presented a higher height, relatively to the control and treatment 12B. Relatively to the second year of the experiment (**Table 3.36**), height and diameter of tubers from the three varieties at harvest, were similar in each treatment (control and treatments 12A and 12B). Considering dry weight content in each time of analysis (harvest, 7M and 8M of storage) in the three varieties (**Table 3.61**), as previously observed in the second year of experiment (**Table 3.37**), in most treatments the trend was H > 8M > 7M. As previously reported, probably due to evaporation and decay of tubers, there were a slightly increase of dry weight after 8M of storage, relatively to 4M of storage (considering that the loss of dry matter after 4M of storage is mainly due to sprout development, as well as to respiration and transpiration of tubers - Burton et al., 1989). Relatively to the second year, in the three varieties (**Table 3.37**), the values obtained for the control and treatments 12A and 12B were similar, despite some minor differences, mainly with 12B treatment. Since the requirement in processing industries requires a dry weight of tubers greater than 20 % (Braun et al., 2010), only treatment 12B of Agria after 7M of storage, as well as Picasso at harvest and the control of Picasso after 8M of storage, were not suitable for processing industries. In terms of TSS the data obtained in the third year (**Table 3.62**) in most of the treatments were similar to data from the second year of experiment (**Table 3.38**).

4.5.2 Total fatty acid content

Despite potato tubers being rich in carbohydrates, they also contain essential lipids, being the total lipid content ranging between 0.15 – 0.5 % of the fresh weight (Lal et al., 2020). Also, according to Kärenlampi & White (2009), the lipid content varied from 0.2 to 2 g, being 1.2 g the average per kg considering the fresh weight. Nevertheless, seventeen fatty acids have been quantified in raw potato tubers, but it must be noticed that lipids are primarily synthesized from sucrose, and in tuber cells after phloem unloading, sucrose is converted in fructose and UDP-glucose. Also, for fatty acid synthesis which occurs in the amyloplast, a small amount of carbohydrate is converted to acetyl-CoA and malonyl-CoA (Kärenlampi & White, 2009). Thus, lipid accumulation affects the tuber metabolism and increases the content of sugars (Lal et al., 2020). Additionally, the predominant fatty acid in potato tubers is C18:2 (which represents about 50 % of total fatty acids) followed by C18:3 and C16:0 (which both represents about 20 % of total fatty acids) (Kärenlampi & White, 2009). In the first year (**Table 3.16 - Table** 3.18) the values obtained for C18:2, C18:3, and C16:0, in the three varieties, and in the three periods of analysis is in accordance with Kärenlampi & White (2009). Nevertheless, foliar application with Ca, using both chemical forms at the different concentrations applied, did reveal impacts in lipid content in the three varieties and at the different times of analysis (harvest, 3M and 6M) (**Table 3.16 - 3.18**), however did not alter the shares of C18:2, C18:3, and C16:0. Furthermore, according to different studies (Liljenberg et al. 1978; Palta et al. 1993), changes of lipid composition in potato tubers were found during long periods of storage under low temperatures. Nevertheless, considering the quality lipids in potatoes, which are mostly unsaturated fatty acid (like linolenic and linoleic acid), makes this crop relevant for producing healthy food products. However, it is important to consider that the interaction of starch, protein, and fat in potato can affect the processing of potatoes (Lal et al., 2020). Furthermore, it has been reported that the presence of more unsaturation of fatty acids can provide tolerance to cold (Upchurch, 2008). In this context, considering that potato in our study were stored for 3 and 6 months under cold conditions (**Table 3.16 - 3.18**), it was unlikely to occur the autoxidation of unsaturated fatty acids present in tubers (Lal et al., 2020). A such, considering that lipids are a major constituent of tubers membrane, potatoes storage between 7 to 26 months showed the decline of membrane integrity under cold storage due to increase of peroxidation, which damaged the membrane lipids (Kumar & Knowles, 1993; Lal et al., 2020). Besides, it is also important to consider that the effects of storage on lipids are dependent on potato varieties (Mondy et al., 1963). As such, the differences in lipids content observed between the three varieties, during the two different storage times (**Table 3.16 - 3.18**), was probably due to the fact that lipids during storage are variety dependent (Mondy et al., 1963). Nevertheless, the values obtained (**Table 3.16 - 3.18**) further revealed higher values of TFA relatively to other *Solanum tuberosum* L. varieties (Galliard, 1973). Considering the research of Cotrufo & Lunsetter (1964) with potato tubers, there were also significant differences in fatty acids during storage (2 months), being in that time that occurs the break in the rest period in tubers, which was in accordance with our data (**Table 3.16 - 3.18**). Another study carried out by Dobson et al. (2004) also showed that TFA contents increased in the initial storage period and then revealed a decrease (after more than 5 months), as also seen in few treatments regarding the three varieties of our study (**Table 3.16 - 3.18**). Besides, with ageing of potato tubers during storage, DBI increases, therefore affecting membrane permeability, as it changes membrane bilayers, leading to a more resistance to leakage of metabolites (Knowles & Knowles, 1989). Overall, after 6M of storage, only a significant decrease in the DBI content occurred in all the treatments of Agria tubers (but not in Picasso and Rossi), suggesting that the decrease is variety dependent regarding the storage time.

Furthermore, considering the second (**Table 3.39 - 3.41**) and third year (**Table 3.63**), in the three varieties studied, the fatty acids profile remained similar, being characterized by the highest abundance of linoleic acid (C18:2), followed by linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0). Additionally, in the third year (**Table 3.63**) and considering the three varieties, the amounts of C18:2 was found similar to values previously reported Kärenlampi & White (2009), representing about 50 % of total fatty acids. Additionally, our data was also similar to the relative fatty acid's abundance in potato tubers: C18:2 > C18:3 > C16:0 (Kärenlampi & White, 2009). In this context, our data further point that Ca biofortification did not interfere with fatty acids synthesis in Agria, Picasso and Rossi varieties.

4.5.3 Soluble sugar content

Burton (1989) identified fructose, glucose, and sucrose as the major sugars in potato tubers. Yet, our analysis revealed that in several treatments, sorbitol presented a higher content than glucose and sucrose in the three varieties in the first year (**Table 3.19**). Storage conditions had a discernible impact on fructose, glucose, sucrose, and sorbitol content in tubers, as seen in our data for the three varieties (**Table 3.19**). Cold storage, as noted by Haider et al. (2023), affects sugar levels in potato tubers, and Javanmardi & Kubota (2006) reported that the ratio of glucose/fructose occurs during storage, decreasing TSS. Indeed, according to Haider et al. (2023), sucrose, fructose, and glucose content in potato tubers were significantly affected by storage periods and varied between genotypes. Besides, there is an extent of biochemical changes that can occur during tuber storage (Mazza et al., 1983) and the reducing sugars, glucose, and fructose in tubers can interfere with tubers color when frying (Mazza et al., 1983). For instance, a study carried out with the Snowden variety by Matsuura-Endo et al. (2006) showed that sucrose, fructose, and glucose content after 18 weeks at 8 to 10ºC of storage showed similar values among the three types of sugars, however this tendency was not verified in our data, which is a further evidence of the chemical variability among varieties. Also, in the same study (Matsuura-Endo et al., 2006) was found that storage of potato tubers at lower temperatures than 8 ºC can lead to the increase of reduced sugar content, which can result in an increase in acrylamide (a carcinogen compound) when potatoes are fried. Considering a study carried out with Agria Cabezas-Serrano et al., 2009), the contents of sucrose, glucose, and fructose were 0.2, 0.6 and 0.6 g/100 g_{FW}. Thus, data obtained in our study with Agria showed lower values at 3M, similar values at 6M and higher values at harvest regarding sucrose. Besides, according to Haider et al. (2023), reducing (glucose and fructose) and non-reducing (sucrose) sugars were lower at the beginning of tubers dormancy and increased over time, being dormancy induced in tubers from the inhibition of the flow of reduced sugars. Additionally, dormancy is considered a stage in which sprouting will not occur for a specific period of time (Hairder et al., 2023). As such, it is important to highlight that, after 3M under storage conservation, tubers did not show sprout development, contrary to what was observed after 6M, justifying the lower values of the soluble sugars at 3M in most of the treatments and the increase at 6M in the three varieties. In the second year (**Table 3.42**), sucrose content found in tubers of the different varieties agrees with the report of Mazza et al. (1983) about the Norchip variety. In fact, according to Mazza et al. (1983), sucrose content in tubers (as mentioned before) is closely dependent on the variety, being 2.8 mg/g_{FW} the maximum acceptable intended for long term storage (Mazza et al., 1983). Indeed, high sucrose content is found in immature potatoes and serves as a substrate for reducing sugar production through the storage activated enzyme invertase (Sowokinos, 1978). Accordingly, it has been suggested that, at harvest, both sucrose content and availability can affect the initial rate of reduced sugar synthesis, leading to a decrease in processing quality in tubers submitted to storage (Sowokinos, 1978). Besides, the endogenous sucrose pool can increase above the harvest contents due to starch degradation triggered by low temperatures (Sowokinos, 1978). Interesting was to note that although Burton (1989) reported that glucose is the second major sugar in potato tubers, in some treatments and analytical periods, some soluble sugars (**Table 3.42**) revealed higher content (namely, sucrose or sorbitol), as previously observed in the first year (**Table 3.19**). Additionally, according to Burton (1898), fructose is the major sugar in potato tubers, yet in most of our treatments this pattern was not found (**Table 3.42**). Furthermore, in the three varieties, sorbitol contents showed higher content than glucose and sucrose in most treatments (**Table 3.42**). Our data (**Table 3.42**) also revealed that storage differently affected fructose, glucose, sucrose, and sorbitol contents in tubers of Agria, Picasso and Rossi. In fact, sugars content in potato tubers is affected by different factors, such as genotype and post-harvest factors including storage (Kumar et al., 2004). In this context, Cabezas-Serrano et al. (2009) also reported that after harvest, fructose, and glucose Agria, Agata, Almera, Marabel and Vivaldi varieties presented similar contents to sucrose. In this context, our data did not show that tendency (**Table 3.42**). Additionally, post-harvest handling (such as storage) is associated with an increase in sugar contents of potato tubers (Kumar et al., 2004). In fact, during storage, tubers are submitted to an extent of biochemical changes (Mazza et al., 1983) leading to changes in sugar composition. Also, as reported by Sowokinos et al. (1987), potatoes tubers showed an increase in sucrose content as storage time increased, being the increase of glucose lower compared to sucrose content during storage. Following Kumar et al. (2004) there are several factors/processes that lead to the accumulation of reduced sugars during storage: lower temperature than 10 ºC (which was the case in this study); dormancy break and sprouting (which occurs after 8M in the three varieties studied); tuber senescence after long-term in storage and tuber's atmosphere during storage. There was not a clear tendency of sucrose, glucose, fructose, and sorbitol accumulation in tubers during the different analytical periods (harvest, 4M and 8M after storage) (**Table 3.42**), although in most treatments of the three varieties, after 8M of storage, the content of the four soluble sugars analysis increased. In fact, at 8M of storage eventually this can be linked with the sprouting development, which can lead to an increase of reducing and non-reducing sugars (Haider et al., 2023), or of the low temperatures, that might lead to sugars accumulation, namely glucose, fructose, and sucrose (Kumar et al., 2004), despite temperatures between 8-12 ºC minimize this process (which is commonly called 'cold sweetening'). However, storage of tubers with higher temperatures are also associated with the increase in sugar levels (Kumar et al., 2004). Moreover, despite the variety, Ca biofortification seemed to interfere with soluble sugar accumulation in tubers of *Solanum tuberosum* L. namely with treatments 24A and 12B. In the third year (**Table 3.64**), Ca treated tubers presented much lower sucrose content regarding what is the maximum acceptable content indicated by Mazza et al. (1983). Remembering what was previously mentioned: during storage with low temperatures, sucrose content increases above harvest mostly due to starch degradation (Sowokinos, 1978); however, an opposite trend was found with our experiment (**Table 3.64**). It was found a reduction on sucrose contents from harvest to 7M and 8M of storage (except in Agria and Picasso in treatment

12A after 8M of storage and in Rossi in treatments 12A and 12B after 8M). Accordingly, our data suggested that sucrose content in stored tubers of Agria, Picasso and Rossi pointed that the rate of starch degradation is minimum. Besides, as verified in the previous years, our data for most Ca treatments (**Table 3.64**) are not in agreement with Burton (1898). The author reported that fructose is the major sugar in potato tubers, followed by glucose. Furthermore, as previously mentioned by Kumar et al. (2004) and Haider et al. (2023), storage affected fructose, glucose, sucrose, and sorbitol content differently in the three varieties studies (**Table 3.64**), which suggest an interaction by genotype and storage. As verified in the previous years, Ca biofortification did not affect significantly soluble sugar accumulation in this third year of the experiment.

4.5.4 Protein content

Protein content in tubers was assessed throughout the three years (**Table 3.20**; **Table 3.43** and **Table 3.65**), considering its importance for the definition of potato quality (Mitrus et al., 2003; Lachman et al., 2005). Regarding the first year (**Table 3.20**), the protein content obtained for the three varieties at harvest, 3M or 6M, showed lower values comparing to the reference values for raw potatoes (2.5 g/ 100 g_{FW}) presented elsewhere (INSA, 2023). In addition, another study has shown that protein content in potatoes can vary between 1.6 to 2.5 g/100 g_{FW} (Harris, 1992). Based on the indicated value of 1.6 to 1.6 g/100 g_{FW} (Harris, 1992), only Agria in 4N treatment after 6M, and Picasso in 2N treatment ant harvest and after 6M, agree with that value.

In the second year (**Table 3.43**) the values of protein contents obtained at harvest remained similar to ones obtained in the first year of experiment (**Table 3.20**), despite the implementation of a different biofortification workflow. However, considering that protein contents can vary between 1.6 to 2.5. g /100 g of fresh weigh (Harris, 1992), some treatments still presented lower contents of protein than 1.6 g / 100 g_{FW}. In this framework, at harvest only a few treatments (24B, 12B and 24B) of Agria showed a higher protein content than 1.6 g / 100 g_{FW}. In the third year (**Table 3.65**), protein content differ from contents obtained both in the first (**Table 3.20**) and second years (**Table 3.43**). In fact, excepting the control tubers of Agria, after 8M of storage, none of the varieties showed similar values to the range reported by Harris (1992), for protein in tubers.

Although potatoes are not typically regarded as a protein source, they can make a significant nutritional contribution to a person's nutritional intake, due to their high consumption rate (Lachman et al., 2005). The different protein content among the three varieties could be attributed to their genetic predisposition to protein accumulation in tubers, which is further influenced by various factors, such as soil quality, tillage, or environment (Mitrus et al., 2003). Moreover, more than 40 % of the total soluble protein in potato tubers (*Solanum tuberosum* L.) is patatin (a common name given to a family of glycoproteins), which serves as a storage protein (Liu et al., 2003). Furthermore, the differences observed during the three years, eventually are due to patatin content. Nevertheless, Ca biofortification does not appear to interfere with the protein content in tubers.

4.5.5 Starch content

In *Solanum tuberosum* L. tubers, starch stands out as the predominating carbohydrate (FAOb, 2009; Mu et al., 2017; Burgos et al., 2023), being synthesized by polymerization of glucose (Gould, 1999), or hydrolyzation due to respiration or stress conditions (Gould, 1999). As such, the assessment of starch content was carried out in the third year at harvest, after 7 and 8 months under storage conditions (**Table 3.66**). The obtained data revealed a consistent trend in starch accumulation in the three varieties: harvest > 8M of storage > 7M of storage, which suggested that probably due to respiration (**Figure 1.11**) the process of starch was reversed or hydrolyzed. Additionally, the lower starch content in tubers can probably be coupled to the beginning of sprouting in tubers from the three varieties. In terms of total starch content, according to INSA (2022), the reference value Is 180 mg/ g_{FW} (**Table 1.1**), but at harvest, in Agria, higher values were found, whereas lower values were detected in tubers of Picasso (except for treatment 12B) and Rossi (**Table 3.66**). Additionally, in our study we found lower starch contents relatively to these obtained for unwounded tubers in Ma et al. (2023) (320 - 330 mg/g_{FW}), but higher values relatively to Islam et al. (2022) (21.15 - 26.06 mg/g_{FW}). When compared to the control, most Ca biofortification treatments showed lower starch content, although not statistically significant. In fact, as previously mentioned in Ginkel & Cherfas (2023), different food matrix submitted to biofortification may lead to a decrease in starch yield. Considering starch contents in tubers of the three varieties, Ca biofortification treatments did not seems to interfere with starch accumulation.

4.5.6 Colorimetric parameters

Color is considered an important parameter in potato tubers, impacting consumer acceptability (Xiao et al., 2020). In this context, it was found that there was not any relevant change in the pulp color of the tuber regarding the Ca applications through the visible spectra of transmittance, which further

indicated the absence of depreciative effects in the three years of experiment (**Figure 3.10, 3.29, 3.58**). In the first year, considering the CieLab system (**Table 3.21**), most colorimetric parameters in the three varieties showed a higher value at harvest, relatively to those obtained after 3 months of storage, which indicated a slightly interference of color after the cold storage. Similar changes in color were reported by Copp et al. (2000), who observed a correlation between respiration and chip color change (*i.e.*, with the beginning of respiration occurring the decline in chip color quality).
Regarding Agria, all the parameters analyzed were lower than those obtained by Pardo et al. (1999) for the same variety (except for the Hue parameter, which showed higher values). Regarding our data about Agria, relative to other studies carried out on the same variety, L (brightness) presented a higher value compared to those obtained by Bordoloi et al. (2012) and a lower compared to Cabezas-Seerano et al. (2009), Mesías et al. (2017) and Picouet et al. (2019). Besides, a* parameter showed higher values relatively to those found by Cabezas-Seerano et al. (2009) and Picouet et al. (2019) and lower when compared to those published by Bordoloi et al., 2012 and Mesías et al. (2017). Regarding b* parameter, Cabezas-Seerano et al. (2009) showed similar values to those obtained in this study. Thus, Bordoloi et al., 2012 and Mesías et al. (2017) presented higher values, and Picouet et al. (2019), lower values when compared to our data. Hue angles showed similar values and a higher chroma relatively to data obtained by Cabezas-Seerano et al. (2009). Considering Yang et al. (2016), chroma showed higher values and hue similar values relative to our data. All three varieties showed similar values to those obtained by Pardo et al. (1999) in different varieties produced worldwide (Bartina, Caesar, Desiree, Agria, Edzina, Monalisa, and Victoria). Indeed, also in a study carried out by Yang et al. (2016) with other varieties (Agata, Agria, Caesar, Cherie, Kennebec, Monalisa, Red Pontiac, and Spirit), the values obtained for Chroma and Hue angle were similar to those obtained in our data. Nevertheless, prior to processing, a large number of biochemical changes occurs during tuber storage, which can affect quality triggering finished product discoloration (Mazza et al., 1983). In this context, the color of the three studied varieties changed along the different periods of analysis (harvest and after 3M). Furthermore, despite the various advantages of low-temperature storage, there is an association in most cultivars to hexoses accumulation, resulting in tubers unsuitable for processing (Blenkinsop et al., 2002). Additionally, regarding chip color, the reduced sugar concentration can explain the variation in color (Blenkinsop et al., 2002). Thus, as previously reported by Parkin & Schwobe (1990), cold storage led to accumulation of sucrose and hexoses and a decline in color quality in chips from two *Solanum tuberosum* L. varieties (Norgold and Russet Burbank). Accordingly, considering that sucrose values (**Table 3.19**) on most of the treatments did not show increases from

harvest to 3M of storage, the phenomenon of accumulation of hexoses can probably indicate why occurred a slightly change in the color of our fresh tubers after 3 months under cold storage conditions. Overall, in the first year, and considering the three varieties, most parameters showed a higher value at harvest compared to those obtained after 3 months of storage, indicating a slightly interference of color after the cold storage. Considering the second year of the experiment (**Table 3.44**) as previously mentioned, the different values obtained in the analytical periods (harvest, after 4M and 8M of storage) can be due to the biochemical changes that occurs during tubers storage. In Agria, at harvest, L and a* parameter revealed lower values, b* and Chroma showed higher values and Hue similar values (**Table 3.44**), relatively to the average data obtained for the same variety by Pardo et al. (1999). Moreover, after 8M of storage, a*, b* and Chroma presented similar values to these reported by Pardo et al. (1999), despite the data were obtained at harvest. Following the study with Agria by Cabezas-Serrano et al. (2009), the average of L, a* and b* parameters were 71.3, -4.3 and 28, respectively. In this context, our data from Agria (**Table 3.44**), showed lower L and a* parameters and higher values of b* parameter relatively to the report of Cabezas-Serrano et al. (2009). Nevertheless, it must be pointed that cultivation and environmental conditions were different from these applied in our study. Furthermore, in Messias et al. (2017), the average of values obtained for L, a* and b* parameters in Agria were 66.8, 0.4 and 24.1, which relatively to our data (**Table 3.44**) was lower in L and b* parameters and higher in a* parameter. Moreover, considering the inexistent or inaccessible research carried out with Picasso and Rossi, only was possible to compare our data (**Table 3.44**) with other varieties. In this context, Picasso showed similar L and b* values with these obtained for Monalisa and Milva varieties in Messias et al. (2017). Additionally, Rossi showed similar values for b* parameter to Milva variety and a considerably lower value of parameter a* compared to Caesar, Monalisa and Milva varieties (Messias et al., 2017). As reported by Cabezas-Serrano et al. (2009), and in our data (**Table 3.44**), during storage some changes occurred in L, a*, b*, Chroma and Hue parameters. Furthermore, data from the third year of the experiment (**Table 3.67**) didn't vary considerable from the one obtained in the second year. Overall, during the three years of the experiment, in the three varieties, despite the Ca biofortification workflow implemented and some minor changes due storage, color did not suffer any substantial depreciative effects.

4.5.7 Heat treatment of tuber pulp: color, texture, and sensory analysis

Texture and color are considered two important parameters regarding the quality of cooked potatoes (Chiavaro et al., 2006). Indeed, when potatoes are cooked (*i.e*, submitted to a heat treatment), food safety and sensory qualities improve, namely the texture of the tubers (Jayanty et al., 2019). As such, in the first year of the experiment, color and texture analysis were carried out considering different cooking times at 100ºC (**Table 3.22** and **Table 3.23**). In fact, boiling in water at 100ºC is one of the primary cooking methods used by consumers for potatoes (Jayanty et al., 2019). Nevertheless, it is important to mention that temperatures above 120ºC can lead to the formation of Maillard reaction products, which have been reported as being potentially carcinogenic (Jayanty et al., 2019). Regarding color analysis (**Table 3.22**), over the different cooking time, there were no significant differences between the two highest treatments with $CaCl_2$ and $Ca(NO_3)_2$, suggesting that biofortification treatments with Ca, did not interfere in color parameters (except in Agria at 12.5 min in L and Chroma, in Picasso at 0 and 7.5 min in L and at 15 min in Chroma and in Rossi at 5 min in L, 10 min in L and Chroma and at 12.5 min in Hue).

Nevertheless, there was an increase in interest in the development of different potato cultivars that can be processed (namely, into potato chips) with an acceptable color despite being stored at low temperatures (Blenkinsop et al., 2002). Additionally, it might be important to mention that food materials are complex biological matrices, which can show variability due to different unit operations carried out through food processing, and therefore can influence the compositional and sensorial properties of the final product (Picouet et al., 2019). As such, color analysis in the biofortified tubers had the purpose of assessing the potential differences that can occur during cooking, considering that the three varieties are suitable for being eaten after cooking. After 17.5 minutes of cooking time, there were no significant differences between treatments in the three varieties, showing that Ca biofortification did not interfere with colorimetric parameters. In terms of texture (**Table 3.23**), it is important to consider that is an essential factor to the consumer perception of the quality in products, namely in potatoes (Garcia-Segovia et al., 2008). In fact, the texture of cooked and processed potatoes is considered an important quality attribute for consumer acceptance (Burton, 1989), as the texture of potatoes has been associated with the content of starch and pectic substances, dry matter content, amylase, sugars, proteins (Linehan & Hughes, 1969; Reeve, 1972; Garcia-Segovia et al., 2008), or even calcium and magnesium content (Linehan & Hughes, 1969). Additionally, textural variations have been associated with differences among varieties and/or among the chemistry of tubers (Linehan & Hughes, 1969). On the other hand, one of the major constituents of potato tubers is starch, which together with pectic substances such as hemicellulose, cellulose, and lignin, contributes to the texture, consistency, and to the organoleptic characteristics of cooked tubers (Jayanty et al., 2019). In fact, the formation of cross-links between pectin molecules through Ca^{2+} in potato tuber cell wall is vital for the processing properties of the potatoes and the quality of the transformation products (Murayama et al., 2017). The cross-linking of pectin via Ca^{2+} is a factor that determines the processing properties of potato tubers and its products, considering that it leads to an increase of thermal stability of Ca^{2+} cross-linked adhesion, as the adhesion in plant tissue is regulated by the middle lamella (being pectin the major constituent) (Murayama et al., 2017). Therefore, probably the increased availability of Ca^{2+} during the growth of potato tubers to the middle lamella, can contribute to an improvement in the processing properties as in the quality of its products (Murayama et al., 2017). Additionally, in a study carried out by Linehan & Hughes (1969) it was found a relationship between intercellular adhesion and calcium and magnesium content in tubers, which further indicated that in tubers with low amylose and high calcium content, the intercellular adhesion is controlled by Ca content. Thus, despite the increase of Ca in biofortified tubers, no significant differences were observed after 20 minutes of cooking relative to the control tubers, which suggested that in Agria, Picasso, and Rossi, Ca does not seem to interfere with adhesiveness (**Table 3.23**). Nevertheless, the texture of cooked tubers varied according to the cultivar and the cooking method (Jayanty et al., 2019), being found in our study that there were small differences in texture parameters regarding the three different varieties (**Table 3.23**). Indeed, according to Garcia-Segovia et al. (2008), texture also depends on the dry matter content of potato tubers, but despite the different dry matter content (**section 3.1.7.1**), at 20 minutes in our study, the texture of the three varieties remained similar (**Table 3.23**). In a study carried out by Alvarez et al. (2002), using tubers of the Kennebec variety, it was found that hardness (after 6 minutes at 100 °C) was 3.97 N and, comparing with the data obtained for Agria, Picasso, and Rossi, similar values of hardness were obtained only at 10 minutes of cooking time, which suggested that according to the time of cooking, the varieties in our study have a higher hardness than the Kennebec variety. Moreover, according to Bordoloi et al. (2012), the cell wall of parenchyma cells decreased after cooking, in thickness, most likely due to the loss of the primary cell wall to a bigger extent. In the same study (Bordoloi et al., 2012), carried out with raw and cooked potatoes (at 99 ± 1 °C, until the core of the tuber was cooked) from different varieties (Agria, Moonlight, Nadine, and Red Rascal) the values obtained for fracturability (N), hardness (N) and adhesiveness (N.s) were different from the ones obtained in our study, showing lower values in fracturability and hardness and higher values in adhesiveness. Yet, it must be pointed that the conditions of the study (Bordoloi et al., 2012) were different from the ones in our study. Moreover, it also must be reported that heat treatments affect both mealiness and firmness, and that cooked potatoes have lower chewiness due to the decrease of the intracellular adhesion, leading to the break of the potato structure

(Jayanty et al., 2019). For Agria, Picasso, and Rossi, the values obtained after 20 minutes of cooking for softening in control samples, were calculated and were 95.2 %, 94 %, and 94.3 %, respectively. Softening remained higher in Agria, followed by Rossi and Picasso. On the 12A treatment, the softening percentage was 93.8 %, 93.8 % and 94.7 % for Agria, Picasso, and Rossi, respectively. On the other hand, in the 4N treatment, the softening values were 92.8 %, 93.1 % and 93.6 %. Therefore, with the Ca biofortification treatments, there was a slightly decrease in the softening of potato tubers (except in Rossi submitted to treatment 12A). Moreover, relatively to data about softening found by Bordoloi et al. (2012), which showed values between 85.4 and 88.4 %, our experiment revealed higher values. Additionally, it is important to consider that dry matter content might influence softening values in potato tubers (Bordoloi et al., 2012). In terms of the second year (**Tables 3.45, 3.46**), the comparison of raw tubers from Agria and Picasso, collected at harvest after 4M of storage, showed an increase of parameter b*, suggesting an increase of the yellow color, whereas in Rossi a decrease of parameter b* was measured. On the other hand, comparing cooked tubers at harvest (**Table 3.45**) with tubers after 4M of storage (**Table 3.46**), was possible to verify an increase of parameter L after 4M of storage, as well as different values for a* and b* parameters regarding the different varieties. Considering tubers of Agria, parameter b* presented lower values after cooking, prevailing a dark yellow color (HZPC, 2023). Picasso revealed a light-yellow color (AGRICO, 2023), being in accordance with the values of parameter b*. The tubers pulp of Rossi after cooking showed a light-yellow color, being in accordance with the decrease of parameter b* (HZPC, 2022). Furthermore, it was found that cold storage affects colorimetric parameters, as previously mentioned before. Besides, despite some observed differences, Ca biofortification did not seem to interfere in colorimetric parameters of raw and cooked tubers. Considering texture (**Table 3.47, 3.48**), relatively to cooked samples, significantly differences in texture behavior of raw samples were measured, showing a more indented texture profile (greater number of peaks), and with higher hardness values, suggesting that the cooking process leads to the decrease of intracellular adhesion, and therefore to the breakdown of potato structure (Jayanty et al., 2019). Overall, Agria and Rossi have higher resistance to penetration (hardness and work of penetration/strength). Additionally, Ca biofortification seems to interfere with adhesiveness, namely in cooked tubers from Rossi at harvest, which suggests that Ca controls the intercellular adhesion (Linehan & Hughes, 1969). Still, as previously mentioned, it must be pointed that the texture of cooked tubers is dependent of the variety (Jayanty et al., 2019). At harvest Agria also showed the highest percentage of softening (**Table 3.49**), as verified in the first year of the experiment. After 4M, Picasso and Rossi showed the highest softening percentage. Our data also suggest that Ca biofortification affect Rossi softening after 4M of storage, in a process that surpasses the softening reported by Bordoloi et al. (2012). Overall, Ca biofortification did not seem to affect the physical parameters of raw and cooked samples, except in cooked tubers from Rossi variety at harvest, which presented higher values in all the texture parameters. In evaluating sensory analysis data (**Figure 3.30**), it is crucial to acknowledge the inherent variability that occurs during food processing, determining not only the compositional properties, but also the sensorial attributes (Picouet et al., 2019). In this context, the impact of Ca biofortification on the sensory preferences varied among the different varieties. Interestingly, it appeared that the panel of tasters did not favor Ca biofortified potatoes in the case of Agria, whereas the opposite trend was observed with Picasso and Rossi. In fact, Ca biofortification affected positively some sensory characteristics of tubers from Picasso and Rossi varieties, leading to higher scores of the different assessed parameters. Considering the third year, the analysis of color (**Table 3.68**) revealed distinctive characteristics for each variety. Agria exhibited a lower value of parameter b* considering that, after cooking, tubers flesh remained dark-yellow (HZPC, 2023) and lower values were obtained for both Picasso and Rossi that become light-yellow as found elsewhere (AGRICO, 2023; HZPC, 2022). Moreover, considering texture (**Table 3.69**), as previously noted, Ca biofortification interferes with texture parameters, especially regarding cooked tubers. Notably, Picasso variety seemed to have a positive effect in fracturability, hardness, number of peaks, work strength and adhesiveness with treatment 12B. In terms of softening (**Table 3.70**), the recorded values exceeded those reported by Bordoloi et al. (2012) (86.1 % for Agria and varied between 85.4 to 85.7 % for Moonlight and Red Racal, respectively). Besides, across all the three varieties, the softening triggered by Ca biofortification treatments exhibited lower values compared to the control samples. In this context, considering the sensory analysis (**Figure 3.59**) was possible to observed that Ca-EDTA seemed to influence positively most of sensory characteristics in the three varieties.

4.6 Starch and dehydrated mashed potatoes

Relatively to the three varieties, in the third year of the experiment, starch and dehydrated mashed potatoes were produced to assess potential differences among treatments within each variety. Food additives were incorporated (E223 and E320) in both starch and dehydrated mashed potatoes. After 41- and 82-days post-production, no mold development was observed in both samples with or without addition of food additives (**Figure 3.60** and **Table 3.71**). Given the low water activity in both potato products, further studies were deemed necessary to assess mod development, although constraints related to time prevented immediate analysis.

4.7 Yield

Regarding potatoes yield, Mohammed et al. (2020), showed that foliar spraying with $CaCl_2$ combined with chitosan increased the marketable tuber yield by more than 40 % in two potato varieties (Arosa and Neveske). In another study, also carried out in potatoes (El-Hadidi et al., 2017), tuber yield augmented with the increase of foliar application of Ca. Also, according to Hamdi et al. (2015) working with *Solanum tuberosum* L., it was found that calcium nitrate enhanced tuber yield. Additionally, Helal & AbdElhady (2015) showed that Ca and K fertilization enhances potato tubers yield and Seifu & Deneke (2017) additionally mentioned that both calcium chloride and calcium nitrate, through foliar application, increased significantly tubers yield in two varieties of *Solanum tuberosum* L. (Shenkola and Gera). In this context, in the first year (**Table 3.24**), Agria showed a higher tuber yield with $CaCl_2$ application, and Picasso and Rossi with $Ca(NO_3)_2$ leaves spraying, which was a similar trend previously found by Seifu & Deneke (2017) relative to the increase of tubers yield after the application of both products. Besides, it was also found in another food matrix, namely oregano in which, together with Mg^{2+}, Ca^{2+}, foliar application as $CaCl_2$ increased the yield by 22 % (Dordas, 2009). Also, in sweet pepper, the yield increased through Ca foliar applications (Buczkowska et al., 2016), whereas in wine grapes, the yield increased with foliar application of chelated calcium sugar alcohol (Ma et al., 2023). In peanuts, through foliar spraying with sorbitol calcium chelated, the yield also improved (Liu et al., 2021). Additionally, other studies carried out, namely in pomegranate (Korkmaz & Askin, 2015), apples (Asgharzade et al., 2012) or pears (Raese & Drake, 1995), also showed an increase in yield though Ca foliar spraying. In a study carried out by Tchapga et al. (2023) with the Desirée variety, a yield of 23438 kg/ha was found, considering 31250 plants planted per hectare, whereas in another study carried out with the Granola variety, by Alveno et al. (2022), in a plot with 30 plants the total yield varied between 10 - 28 kgs. Through extrapolation of the data obtained by Tchapga et al. (2023) and Alveno et al. (2022), for 57 plants a higher yield was obtained with Agria, Picasso, and Rossi varieties. However, these correlations must be viewed with caution since the edaphoclimatic conditions as well as the varieties, are different from both studies previously mentioned in this paragraph. Considering the second year of the experiment (**Table 3.50**) and Agria variety, treatment 12A showed the highest yield, therefore coinciding with the treatment with the highest Ca content (**section 3.2.5.3.1**). On the other hand, relatively to the first-year of the experiment (**Table 3.24**) the following second-year (**Table 3.50**), showed that Agria had a lower yield, whereas Picasso displayed greater total yield. Furthermore, Picasso after 4FA showed a maximum NDVI of 0.91 in treatment 12A, and Agria after 6FA showed a maximum NDVI of 0.65 in control plants (**section 3.2.2.**). In this context, the NDVI closely predicted the highest total yield, despite after 4FA and 6FA, considering that the second highest total yield were in treatment 12A and control in Picasso and Agria, respectively. Considering a study carried out by El-Hadidi et al. (2017), which mention that tuber yield increased with the increase of Ca foliar spraying, only Agria showed a higher total yield in a Ca biofortification treatment. As such, those tendency might probably be dependent on variety and edaphoclimatic conditions. Moreover, considering other studies carried out in different potato varieties (Alveno et al., 2022; Tchapga et al., 2023), our data also showed highest yields. In the third year of the experiment, in comparison to the control and treatment 12A, treatment 12B exhibited a higher Ca biofortification index in tubers with skin (**Table 3.56**) but recorded the lowest yield (**Table 3.72**). As verified in the second (**Table 3.50**) and third years of the experiment (**Table 3.72**), Agria showed the lowest total yield relatively to Picasso and Rossi. However, contrary to the second year of experiment, Picasso was the variety with the greater yield. Moreover, the yield of Rossi presented a higher impact triggered by Ca-EDTA, leading to the lowest yield of the three varieties. Still, relatively to the second year of the experiment (**Table 3.50**), the control, of Agria and Rossi showed higher total yield in this third year (**Table 3.72**). However, Picasso presented about half of the yield compared to the second year. Moreover, after 4 foliar applications was already visible the impact of Ca biofortification treatments in Agria and Picasso (in the NDVI analysis) and in the monitoring of the culture in the fields of the three varieties, after the 3rd foliar application of Ca treatments, especially with Ca-EDTA. In this context, it was verified that tubers yield of Agria, Picasso and Rossi, submitted to calcium chloride or Ca-EDTA applications, did not increase with the augmentation of Ca foliar applications, as previously reported by El-Hadidi et al. (2017).

5

FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

5.1 Final considerations

5.1.1 First year

The results of the first year of study indicated that the soils of the three different fields are suitable for potato cultivation, allowing potato production. The statistical analysis conducted for these fields revealed different correlations between mineral elements, due to variations in soil content and type. Irrigation plays an important role in tuber yield and quality, being the sprinkler method the one most effective and the one used during the first year of Ca biofortification workflow. With remote sensing data was possible to verify that field A showed a greater aptitude for water accumulation and infiltration, while field B had poor drainage conditions, promoting runoff, and field C had a lower percentage of area with low drainage capabilities. As such, the three fields presented different drainage conditions. The study also assessed the impact of calcium chloride and calcium nitrate foliar applications on the photosynthetic apparatus of the Agria variety (used as a test system in the first year of experiment). Results indicated that the photosynthetic performance remained intact until the 3rd foliar application, but a decrease in g_s restricted CO_2 supply, affecting carbon assimilation. Also, after the second assessment, was possible to verified that calcium chloride had a negative impact on photosynthetic performance, while calcium nitrate had less detrimental effects. The analysis of mineral elements in tubers of *Solanum tuberosum* L. using X-ray fluorescence spectrometry revealed variations in their accumulation and content among the different potato varieties. Calcium showed a higher content in certain treatments, indicating the potential for Ca biofortification in potatoes (*Solanum tuberosum* L.). In fact, Ca presented a biofortification index which varied between 1.9 - 27.4 %, 34.7 - 57 % and 3.3 % to 2.5-fold for Agria, Picasso and Rossi, respectively. Calcium was predominantly located in the epidermis/skin region of the tubers in the three varieties. Calcium biofortification process did not significantly affect the morphological parameters of tubers, such as height and diameter. At harvest, only tubers from all the treatments in Agria showed a dry weight content suitable for the processing industry. Total soluble solids content varied among treatments and decreased with tuber storage. Overall, there is only a significant decrease in the DBI content in all the treatments of Agria tubers after 6M of storage conditions and not in Picasso and Rossi varieties, suggesting that the decrease is variety dependent, and that Ca sprays did not affect the permeability of the lipid's membrane in *Solanum tuberosum* L. tubers regarding the storage time.

5.1.2 Second year

Throughout the second year of experiment, the obtained results provided valuable insights into the effects of Ca biofortification through foliar applications on the growth, physiology, and nutrients contents of *Solanum tuberosum* L. plants from Agria, Picasso and Rossi varieties and in their potato tubers. Moreover, the comparative analysis of data obtained in the second year of the experiment, with the results from the first year, revealed some changes regarding water analysis in the experimental fields. In fact, field A exhibited a slight decrease in pH, while fields B and C showed an increase. The electrical conductivity increased in all the three fields and fields A and B maintained the same water classification as in the previous year, while field C had a different one. Treatments carried out with calcium chloride and treatment 24B demonstrated higher average NDVI values, relatively to the control plants, except for treatment 12B, which exhibited lower NDVI values, due to potential Ca-EDTA toxicity. In contrast, field B (with cultivation of the Agria variety), faced a decrease in foliage density after the 6th foliar application. All treatments in field B resulted in lower NDVI values relatively to the control plants. Furthermore, when comparing fields A and B, it was found that field A displayed higher overall NDVI values, although it is essential to consider that they were in different stages of plant development. An *in-situ* evaluation, revealed that Picasso demonstrated signs of negative effects with treatment 12B, while treatment 24B and the control plants exhibited similar external aspects. In field B, Agria exhibited toxicity symptoms with calcium chloride treatments (especially with treatment 24A) and with treatment 12B. Regarding the net photosynthetic rate (P_n), treatment 12A had no significant impact on Agria, but showed moderately positive or negative effects on Picasso and Rossi, especially in the last assessments. Stomatal conductance (g_s) and the transpiration rate (E) varied in response to Ca treatments, affecting the overall photosynthetic parameters. Instantaneous water use efficiency (iWUE) showed similar values between treatment 12A and the control plants, at the end of the life cycle of Agria and Picasso. However, Ca-EDTA had negative impacts on the photosynthetic parameters of all three varieties. The cumulative or toxic effect of treatment 12B caused plant death in Picasso and even in Rossi before the final assessment. In contrast, a partial recovery was observed in plants treated with treatment 24B, especially in Agria. The actual photochemical efficiency of PSII (F_v'/F_m') exhibited a similar pattern to F_v/F_m, with treatment 12B, causing significant losses in the PSII functioning efficiency, particularly in Picasso and Rossi. Overall, treatment 12B had a negative impact on the photosynthetic functioning of all three varieties, while other treatments and applications showed varying effects. As such, became essential to monitor Ca foliar treatments in potato crop, considering their potential implications on plant health and productivity. During the Ca biofortification process along the production cycle of Agria and Picasso, it was possible to observe significant differences in Ca, K, S and P contents of tubers, roots, stems, and leaves among the different treatments, after the 4th, 6th, and 7th foliar applications in each variety. After the 7th foliar application, the 24 kg/ha treatments (24B in Picasso and 24A in Agria) triggered a higher Ca content. The highest K and P contents were obtained with treatments 24A and 12B for Picasso and Agria, respectively. Additionally, S content was higher in treatment 12B relatively to the other treatments (in both varieties) and P content in Picasso was generally higher relatively to Agria in the different analytical periods. Furthermore, after the 7th foliar application, relatively to the control, Ca content in tubers ranged from 5.7 to 95.6 % for Picasso, and 20.7 to 33 % for Agria. In roots, after the 6th and 7th foliar applications, Ca content was higher with Ca-EDTA treatments in both varieties. The increase in Ca content in roots, relatively to the control, ranged from 9.7 to 41.1 % for Picasso and 16.5 to 55.1 % for Agria after the 7th foliar application. In stems, relatively to the control, Ca increased between 28.2 % to 3-fold for Picasso and 2-fold to 3.1-fold for Agria after the 7th foliar application. Also, in leaves, the control had the lowest contents of Ca, S, and P, but the highest content of K, in Agria. Moreover, in Picasso, treatment 12B showed the highest Ca and S contents, but the lowest in K and P, after the 7th foliar application. After the 7th foliar application, relatively to the control, both varieties showed an increase of Ca contents, ranging from 32.8 to 73.1 % for Picasso and 16.8 to 84.3 % for Agria. Overall, despite some toxicity symptoms found with $CaCl_2$ and Ca-EDTA treatments, globally Ca content increased in the different organs of both. Also, different patterns of mineral accumulation were observed during the Ca biofortification process and plant development, which could be attributed to the remobilization of mineral elements during different stages of plant development and the inherent differences in mineral content between potato genotypes. Regarding the kinetic accumulation in tubers of both varieties (Agria and Picasso), different accumulation patterns were observed. The absorption rate of Ca, K, S, and P in tubers appeared to depend on the Ca biofortification treatments. In the last analytical period, treatments 12A, 24A, and 12B showed higher absorption rates, indicating a positive effect on mineral uptake in tubers. Moreover, it was also possible to verified, due to the differences observed in Picasso and Agria absorption rates, that the absorption rate of minerals in tubers is genotype dependent. At harvest, it was possible to verify that treatments 12A and 24A resulted in a significantly higher Ca content in tubers without and with skin of Agria, respectively. In tubers with skin, Picasso and Rossi showed a significantly higher Ca content in treatment 12B. Overall, treatment 12B positively affected the content of K, S and P in Agria, Picasso and Rossi. Tubers with skin in general had higher Ca, K, S, and P contents relatively to tubers without skin. Besides, Ca accumulation significantly increased in all the varieties relatively to the control tubers, with different biofortification indices being found. As such, tubers without skin showed a Ca biofortification index between 10.5 to 63.2 %, 3.9 %, 17.2 % to 2.3-fold, respectively for Agria, Picasso and Rossi. Considering tubers with skin, Ca biofortification index varied between 1.3 to 52.6 %, 7 % to 2.2-fold and 1.7 % to 3.9-fold. Significant differences among treatments and varieties were found regarding morphological and physical characteristics of tubers, height, diameter, and dry weight. However, the Ca biofortification process did not significantly affected the height and diameter of tubers but, regarding dry weight content, there were variations among the varieties, with harvest tubers generally having higher dry weight relatively to the cold stored tubers. Also, total soluble solids increased after 8 months of storage, eventually due to sucrose accumulation, and Ca biofortification did not interfere in fatty acids synthesis in the three varieties. However, soluble sugar accumulation in tubers was influenced by Ca biofortification, particularly with treatments 24A and 12B. Protein content, presented variations among the different varieties, but can be affected by different factors, including genetic predisposition and environmental conditions. Colorimetric parameters of tubers did not seem to be affected negatively by the Ca biofortification process, but storage affected the colorimetric parameters, probably due to tuber senescence. Indeed, Ca biofortification treatments did not significantly interfered with colorimetric parameters of raw and cooked tubers. Considering texture, Ca biofortification influenced softening after 4 months in cooked tubers from Rossi. Thus, in general, Ca biofortification did not significantly affected the physical parameters of raw and cooked tubers (except for cooked tubers from the Rossi variety). Regarding the sensory characteristics of tubers, Ca biofortification positively affected sensory characteristics in Picasso and Rossi, resulting in higher scores of the analyzed parameters. Nevertheless, Agria showed the highest yield with treatments 12A, coinciding with the highest Ca content in tubers. Also, the lowest yield was obtained in the three varieties with treatment 12B, despite showing the best results regarding Ca content in tubers (specially in Picasso and Rossi varieties). These findings suggest that Ca biofortification can affect minerals contents, tuber characteristics, sensory attributes, and yields, in Agria, Picasso and Rossi varieties.

5.1.3 Third year

To conclude, the study aimed to investigate the effects of Ca biofortification on three potato field crops (Agria, Picasso and Rossi), specifically focusing in the third and last year of the experiment on the use of $CaCl_2$ and Ca-EDTA with a concentration of 12 $kg.ha^{-1}$. Considering the water used in culture irrigation, field A showed a slight increase in pH, while fields B and C experienced considerable increases. In terms of electrical conductivity, all three experimental fields showed a significant decrease, and the hydrochemical facies of the irrigation water differed among the fields. Fields A and B revealed high salinity, belonging to class C3S1, while field C had medium salinity, belonging to class C2S1, considering the SAR index. The response of potato plants to Ca biofortification varied across the fields, still fields A and B demonstrated positive responses after the 4th foliar application with Ca (as indicated by medium/high NDVI values in all plots of Ca treatments). However, in both fields, the control plots exhibited the highest NDVI, while treatment 12B showed the lowest average of that index. Moreover, as seen in the second year of study, treatment 12B showed a decreasing trend in NDVI across the fields, pointing that Ca-EDTA, may induce toxicity symptoms, namely chlorosis and necrosis. Monitoring of crops during the Ca biofortification process confirmed the obtained values of NDVI, being possible to observe toxicity symptoms in the aerial parts of the plants, particularly with treatment 12B. In Agria and Picasso varieties, those symptoms were observed after the 3rd foliar application with treatment 12B and started to appear also with treatment 12A after the 5th foliar applications in Agria, and after the 6th foliar spraying in Picasso. In Rossi, no toxicity symptoms were observed with treatment 12B until the 5th foliar applications. The analysis of net photosynthesis (P_n) revealed that treatment 12A had a slightly negative effect (non-significant), while treatment 12B clearly showed negative effects on all three potato varieties. In fact, Agria, showed aggressive negative effects with treatment 12B and, before the last assessment, the plants were "burned". Additionally, Picasso exhibited negative effects, after 5 foliar applications, only with the Ca-EDTA treatment, while Rossi showed no negative effects, either with $CaCl_2$ or Ca-EDTA. The effects on stomatal conductance (g_s) and transpiration rate (E) varied among the varieties, with a decreasing trend in Agria, an increasing trend in Rossi, and no significant changes in Picasso, after 5 foliar applications of both products. Instantaneous water use efficiency (iWUE) showed variations, decreasing in all varieties (not significant in Rossi) with treatment 12B. Also, treatment 12B had important negative implications for the photosynthetic rate and iWUE, and it also negatively affected the maximum (F_v/F_m) and current (F_v'/F_m') photochemical efficiency of PSII in Agria and Picasso. In contrast, in the three varieties, treatment 12A had no negative impact on any of the analyzed parameters. Mineral monitoring of the different organs was carried out, showing significant differences in Ca, K, S and P content in tubers, roots, stems, and leaves among the treatments and varieties. In this context, tubers from Rossi accumulated the highest Ca content in the different analytical periods with treatment 12B, while Agria and Picasso showed higher Ca biofortification with treatment 12A after seven foliar applications. Indeed, after seven foliar applications, Rossi exhibited higher Ca, K, S, and P contents in tubers with Ca-EDTA treatment. Moreover, in the last assessment, relatively to the control, Agria, Picasso and Rossi showed an increase of Ca content, which varied between 8.2 to 42.4 %, 40.1 % and 67.1 to 82.6 %, respectively. Overall, Ca biofortification showed varying effects on the different organs of Agria, Picasso and Rossi, with a decrease in Ca content from the areal part to tubers (leaves > stems > roots > tubers). On the other hand, it was possible to verify that Ca is redistributed through the phloem, supporting the increase of Ca contents in tubers with foliar applications (calcium chloride or Ca-EDTA). Also, our study points that Ca is mobilized from leaves to the tubers and that there were different patterns of Ca, as well as K, S, and P accumulations during the Ca biofortification process, indicating that occurs remobilization of the different mineral elements during the different stages of plant development, being that remobilization genotypic dependent. The kinetic accumulation of Ca, K, S and P in tubers, roots, stems, and leaves of the three potato varieties exhibited different patterns. Though the PCA analysis, and considering the Ca content in the different organs and at the different times of analysis, it was found that Agria had the highest dispersion of Ca values during the biofortification process, followed by Picasso, while Rossi had the lowest dispersion, with treatment 12B in Rossi consistently resulting in the highest Ca content. Moreover, regarding the variations observed in the absorption rate of Ca, K, S, and P in tubers, different nutrients uptake rates were detected during different stages of development. At harvest, tubers with and without skin showed significant differences in Ca, K, S, and P contents between the two types of tuber preparation, and among the different treatments in Agria, Picasso and Rossi. In general, tubers without skin had higher mineral contents than tubers with skin, especially K, S and P. Relative to the control tubers, the highest Ca biofortification index was obtained with treatment 12B in tubers without skin, with increases ranging from 6.5 to 61.3 % in Agria, 17.1 to 36.6 % in Picasso, and 15.9 to 29.6 % in Rossi. In tubers with skin, the increase in Ca content with treatment 12B was, relatively to the control, 8.2 % in Agria, 88 % in

Picasso, and 78 % in Rossi. Treatment 12B consistently resulted in the highest Ca content in tubers, both with and without skin, as observed in the previous year of the experiment. As such, considering the mineral analysis of tubers at harvest, data suggested that foliar applications of Ca redistribute the mineral through the phloem and complement xylem mass flow, resulting in increased Ca content in tubers. Also, considering Ca distribution in tubers, it was observed that Ca content was higher in the epidermis region, followed by the inner regions of the tubers, being in accordance with the verified in the first year of experiment. Additionally, some treatments revealed higher Ca contents in the inner regions, indicating a variation depending on the treatment and variety. Rossi consistently showed a uniform distribution of Ca in the inner regions, regardless the treatment. Also, comparing with data from the previous year, it was found that the values of tubers height, diameter and dry weight were similar across treatments considering each variety (*i.e*, indicating minimal variations). Moreover, in Rossi, at harvest significant differences were verified in the total soluble solids among treatments. There were also significant differences in total soluble contents among the different analytical periods (harvest, 7 and 8 months of storage), within each treatment, for all three varieties. At harvest, regarding fatty acid composition of tubers, the obtained data remained consistent with previous studies and, additionally, it was found that Ca biofortification did not substantially interfered with fatty acid composition in the three varieties. Relatively to the soluble sugar contents, at harvest and after storage, there were significant differences among treatments in each variety, except for sorbitol contents. However, overall, Ca biofortification did not substantially affected soluble sugar accumulation in the third year of the experiment. Regarding protein content in tubers, at harvest and after storage, varied among the three varieties and was dependent on multiple factors, including genotype. Moreover, in the three varieties, Ca biofortification did not appear to have any significant effect on protein content, starch content and in colorimetric parameters. Considering texture, Ca biofortification had an impact on some parameters, particularly in cooked tubers. Besides, Picasso showed improvements in fracturability, hardness, number of peaks, work strength, and adhesiveness with treatment 12B. In all the three varieties that samples from treatment 12B received higher scores in the different analyzed sensory characteristics, indicating a positive influence of Ca-EDTA treatment on these attributes. In this third year of the experiment, incorporation of the food additives E223 and E320 was carried out in both starch and dehydrated mashed potatoes, with no development of molds after 41 and 82 days of post-production. In this framework, through Ca biofortification, it was possible to obtain a functional food product based on potato tubers, adding product value that can be commercialized worldwide. In terms of yield, it was observed that in the three varieties, the highest yield was obtained in the control, while the lowest yield was obtained in treatment 12B (despite being the treatment which presented the highest Ca bioforticiation index). Likewise, the yield of Rossi was the most affected variety by Ca-EDTA, leading to the lowest yield among the three varieties. In this context, it was observed that Agria, Picasso, and Rossi, when subjected to calcium chloride or Ca-EDTA applications, did not show an increase in tubers yield and is likely dependent on the genotype. Overall, the findings highlight the variability in responses to Ca biofortification and the potential risks of toxicity in *Solanum tuberosum* L. plants, emphasizing the importance of a careful monitoring and adjusted dosage to ensure optimal results in potato cultivation. Based on all the findings throughout these three years of research, further research and optimization of Ca foliar application strategies are necessary to ensure optimal results and minimize any adverse effects on potato crops, especially regarding the quality of potatoes and yield. Hence, it is important to integrate different techniques to monitor the culture during the biofortification process, including analyses of photosynthetic metabolism, the use of technologies such as NDVI obtained by drone, soil and water analyses and the determination of calcium content throughout the biofortification process.

5.2 Future perspectives

Calcium biofortification workflow of *Solanum tuberosum* L. plants hold great potential for enhancing Ca content in potatoes and, consequently, in potato-based food products (namely, starch or dehydrated mashed potatoes). Considering the goal of increasing Ca content in tubers of Agria, Picasso, and Rossi varieties, in the third year of the study, which aimed to optimize the Ca biofortification itinerary, the 12 $kg.ha^{-1}$ Ca-EDTA treatment presented the best results (higher Ca content in tubers). However, it is important to highlight that each variety can responds differently at a morphological, physiological, and organoleptic levels.

Further analysis and assessment are required to fully understand the bioavailability of Ca in the human body when consuming these Ca biofortified potatoes or process potato products. Moreover, bioavailability studies (namely, through *Caco-2-cells*) need to me done, which involves assessing different factors such as the absorption, utilization, and retention of calcium from these products. Also, a comparative study of the different bioavailability of Ca from biofortified potato or processed potato products with other commonly consumed calcium-rich foods (such as dairy products) is required to establish the effectiveness of these products. On the other hand, it is essential to realize further research and optimization of the formulation and processing techniques of biofortified potato, or process potato products, which can not only increase Ca content as well as contributing to enhancing

Ca bioavailability. This type of optimization involves exploring Ca biofortification workflows (specially, the number of foliar applications and products applied), as well as methods involved in the industrial process of potatoes, such as cooking, blanching, or enzymatic treatments to improve Ca solubility and accessibility in the final food products. Additionally, studying the impact of the different food matrices and additives on Ca absorption can further optimize the formulation of Ca biofortified potato or potato products. Additional studies to assess mold development are necessary in potato products. Also, trials involving the consumption of Ca biofortified potato products aiming to evaluate the effects of sustained consumption of biofortified potato on bone health, prevention of calcium deficiency-related diseases, and overall nutritional status must be carried out. Furthermore, consumer acceptance and market potential are two essential factors to the success of Ca biofortified potatoes or potato products. As such, consumers acceptance and market potential are crucial. On the other hand, exploring ways to incorporate circular economy principles to minimize resource consumption as well as reduce waste and optime nutrient utilization during potato cultivation. Additionally, evaluate the potential benefits of integrating organic and regenerative farming practices alongside Ca biofortification in order to enhance, for instance soil health and biodiversity. In this framework, the information obtained for instance, by sensory evaluations or consumer preferences can guide marketing strategies and facilitate wider adoption of Ca biofortified potato or its derived products worldwide. Overall, by pursuing these future perspectives, the knowledge obtained in this study will facilitate the development of effective strategies for utilizing biofortified potatoes, as a valuable source of dietary calcium, contributing to improved nutrition and public health.

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Annexes

Annex I - Equations for the determination of the Langelier saturation index (LSI) and the sodium adsorption index (SAR)

$$\text{SAR} = \frac{Na^{+}}{\sqrt{\frac{1}{2} \times (Ca^{2+} + Mg^{2+})}} \text{ (meq/L)}$$

$$\text{LSI} = \text{pH} - \text{pHs}$$

pH_s = (9.3 + A + B) - (C + D) where:

A = (Log10[TDS] - 1)/10

B = -13.12 x Log10(^{o}C + 273) + 34.55

C = Log10[Ca^{2+} as $CaCO_3$] - 0.4

D = Log10[alkalinity as $CaCO_3$]

TDS- total dissolved solids

Annex II- equation for the determination of the NDVI index

$$\text{NDVI} = \frac{NIR - Red}{NIR + Red}$$

Annex III- equations for the determination of Chroma (C) and Hue-Angle (H)

$$C^* = \sqrt{a*^2 + b*^2}$$

$$H^* = \text{arctg}\ \frac{b*}{a*}$$

Annex IV- equation for the determination of dry matter content (%), suggested by Agle & Woodbury (1968)

$$\%\ Dry\ matter = \frac{\text{Weight of sample after drying (g)}}{\text{Initial weigh of sample (fresh) (g)}} \times 100\%$$

Annex V- equations for the determination of kinetics accumulation

Absorption rate in terms of time (by the different organs of the plant)

$$Rate = \frac{amount\ of\ analyte\ absorbed\ (in\ mg.kg^{-1})}{time\ (days\ after\ planting)}$$

Mass of analyte present in the sample (mg)

$$mass\ of\ analyte\ = \frac{m_{analyte}.kg^{-1}\ \times m_{sample\ total}}{1000000}$$

$m_{analyte}$- mass of the analyte present in each part of the plant (in mg)

$m_{analyte}.kg^{-1}$- mass of analyte/kg of sample (in mg)

m_{sample} – mass of the sample (in mg)

Absorption rate by mineral element
(mg.dia^{-1})

$$AbsRate = \frac{\sum m_{analyte}}{\sum time\ (days\ after\ planting)}$$

$\sum m_{analyte}$- corresponds to the sum of the masses of analyte present in each part of the plant (tubers, roots, stems and leaves) (in mg)

Translocation rate
(%.dia^{-1})

$$TransRate = \left(\frac{\left(\frac{\sum m_{analyte}\ Stems\ and\ Leaves}{\sum m_{analyte} Tubers, Roots, Stems\ and\ Leave}\right)}{\sum time\ (days\ after\ planting)}\right) \times 100$$

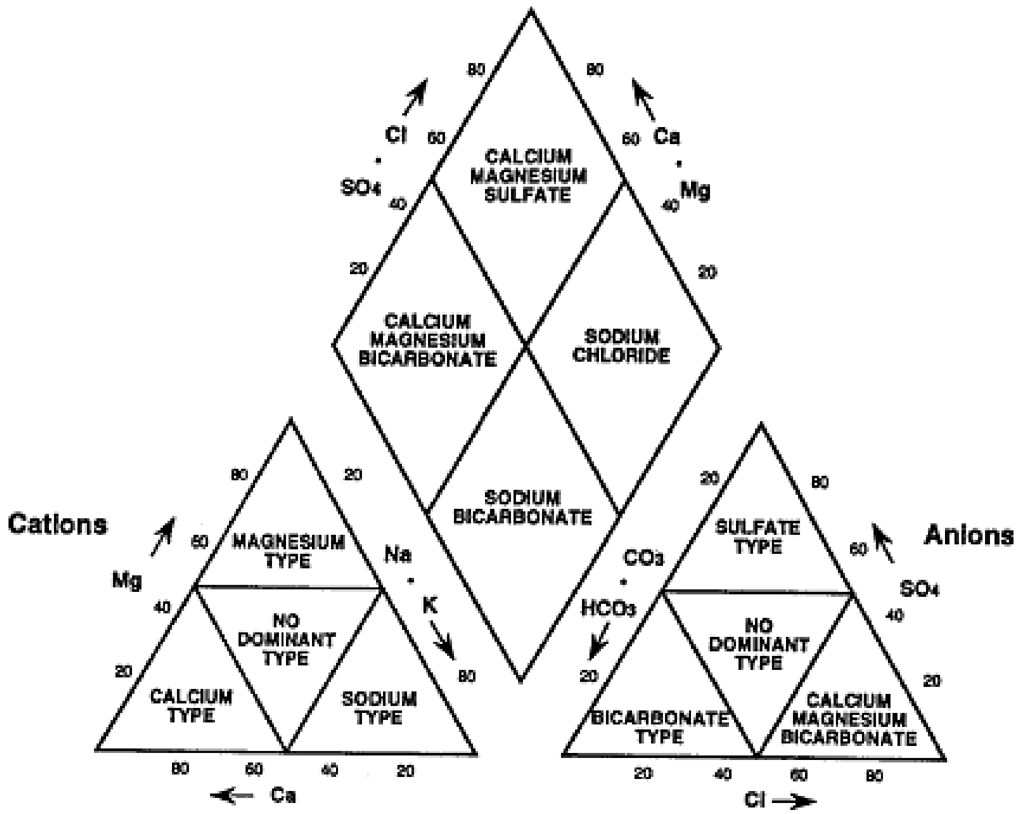
Represent the daily average of translocation from the underground organs (roots and tubers) to the aerial part (stems and leaves)

Annex VI- Food Additives allowed by European regulations, in processed potato products (Adapted from: European Commission Regulation N°. 1129/2011, of 11 November 2011. Amends Annex II of Regulation (EC) N°. 133/2008 of the European Parliament and Council, establishing a union on the list of food additives).

Group	Additives	Quantity	Notes	Utilization
E100	Curcumin	*Quantum satis*		Only in granulated or flaked dry potatoes
E220-228	Sulfur Dioxide - Sulphites	100 mg.kg-1	(1)	
E310-320	Gallates, TBHQ and BHA	25 mg.kg-1		Only in dehydrated potatoes products
E392	Rosemary extracts	200 mg.kg-1	(2)	Only in dehydrated potatoes products

Notes: (1)- No more than 10 mg.kg-1 SO_2; (2)- considering the sum of carnasol and carnosic acid.

Annex VII- Piper diagram (Source: Lonerganand & Change, 1994) and Water quality for irrigation purposes – Wilcox Diagram (Source: Cordeiro, 2001).



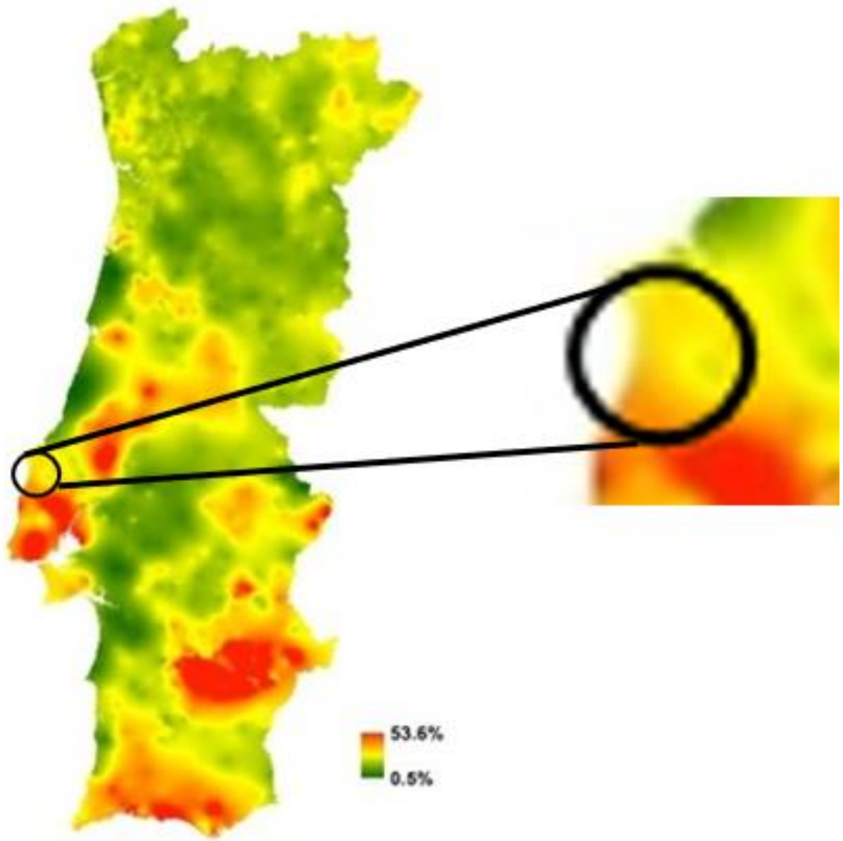
C1	Low salinity water (CE < 250 µS/cm) -can be used for irrigation on most crops in almost all types of soil, with little likelihood of developing salinity problems.
C2	Medium salinity water (CE between 250 and 750 µS/cm) – can be used whenever there is a moderate degree of leaching. Plants with moderate salt tolerance can be grown, in many cases, without the need for special control practices.
C3	High salinity water (CE between 750 and 2250 µS/cm) – cannot be used in soils with poor drainage and even with adequate drainage, special practices for salinity control may be necessary and should only be applied for irrigation of salt-tolerant plants.
C4	Very high salinity water (CE > 2250 µS/cm) – cannot be used under normal conditions, only occasionally, in very special circumstances, such as in very permeable soils and highly tolerant plants to salts.

S1	Water with low sodium content - can be used for irrigation in almost all soils, with little danger of developing sodification problems.
S2	Water with medium sodium content – should only be used in soils with a sandy texture or in organic soils with good permeability, since in fine textured soils (clayey) the sodium represents a danger.
S3	Water with a high sodium content – it can produce toxic levels of exchangeable sodium in most soils, thus requiring special management practices such as: drainage, easy washing, application of organic matter.
S4	Water with a very high sodium content – it is generally unsuitable for irrigation except when the salinity is low or medium or the use of gypsum or other amendment makes it possible to use this water.

Annex VIII- Relationship between the desired content of organic matter and clay content (Source: Gonçalves, 2005).

Clay content (%)	OM (%)
< 10	1.5 - 2
10 - 30	2 – 2.5
> 30	2.5 - 3

Annex IX- Clay content in Portugal, with the region of the three experimental fields highlighted in black (Source: INFOSOLO, 2020).



Annex X- Classes of soil richness according to organic matter content (Adapted from: Gonçalves, 2005).

Class	Medium and fine texture	Coarse texture
Very Low	OM < 1 %	MO < 0.5 %
Low	1.1 % < OM < 2 %	0.6 % < MO < 1.5 %
Medium	2.1 % < OM < 7 %	1.6 % < MO < 5 %
High	7.1 % < OM < 10 %	5.1 % < MO < 7 %
Very High	MO > 10.1 %	MO > 7.1%

2024

ANA RITA FONSECA COELHO

DESIGN AND IMPLEMENTATION OF A TECHNICAL ITINERARY FOR BIOFORTIFICATION OF CALCIUM IN *SOLANUM TUBEROSUM* L. TUBERS OF AGRIA, PICASSO AND ROSSI VARIETIES

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