



Article

# Development and Characterization of Nutritious and Sustainable Canned Fish Meal Prototype for Different Population Segments

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**Abstract:** Canning is an excellent solution to provide convenient, affordable, nutritious, and safe seafood with a long shelf life. However, many canned products use tuna, sardines, and Atlantic chub mackerel, species that raise sustainability concerns and whose overuse can put additional pressure on them. Hence, this study aimed to i) develop and assess a nutritious and sustainable canned meal prototype using the Atlantic horse mackerel (Trachurus trachurus) (whose EU allowable-catch amounts have increased, particularly, in Iberian waters) and vegetables in light brine in terms of stability, sterility, and physicochemical and sensory properties over a 4-month period at room temperature and ii) evaluate its nutritional contribution for different population groups. After preparation, the meal was stored for one month at  $\approx\!20~^{\circ}\text{C}$  and  $\approx\!40~^{\circ}\text{C}$  (to simulate the 4 months). Although the pH was not stable, the meal was considered commercially sterile according to the challenge accelerated tests. Moreover, aging did not significantly affect the meal's physicochemical and sensory properties. This innovative meal prototype can be claimed to be "low-fat", "reduced in NaCl/Na", a "source of protein, phosphorus, iron, selenium and vitamin D", and "high in vitamin B12". It proved to be both nutritious and appealing for consumption, with potential to be scaled up.

**Keywords:** Atlantic horse mackerel; eco-innovative product; accelerated aging; stability and sterility; physicochemical properties; nutritional contribution; sensory attributes



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# 1. Introduction

The regular consumption of seafood is strongly recommended by most dietary guide-lines as it is a rich source of essential nutrients such as high-quality proteins, long-chain omega-3 polyunsaturated fatty acids (*n*-3 PUFAs; healthy lipids), minerals (e.g., iodine—I, iron—Fe, zinc—Zn, and calcium—Ca), and vitamins (A, D, and B12) for all population groups (children and adults, including pregnant women and seniors) [1]. For instance, the most common recommendation in Europe is an intake of one to two portions of fish (approximately 100–150 g) (depending on the country) per week to ensure the supply of essential nutrients [2,3]. A diet rich in seafood contributes not only to the prevention of many diseases (e.g., cardiovascular, cerebrovascular, and diabetes) but also to the consumer's well-being [1,4]. Additionally, many vegetables, including grain legumes such as beans, are also nutritionally valuable, providing protein with essential amino acids, complex carbohydrates, dietary fiber, vitamins, minerals, and phytochemical compounds with several bioactive properties. They are cholesterol-free and generally low in fat, being considered excellent options to complement healthy and less monotonous meals [5,6].

On the other hand, the demand for ready-to-eat/ready-to-cook (convenient) foods is growing globally, pushing processors to integrate new approaches and culinary concepts in the production of traditional products. Among traditional technologies, canning represents a key strategy in seafood processing [7]. In this process, the seafood flesh (e.g., an edible fish portion cooked and drained) is placed in cans with other ingredients, such as vegetables and salt, and covered with a filling medium, which can be an edible oil (e.g., olive oil or sunflower oil), water, or a sauce. Then, the cans are sealed, washed, and steam-sterilized [7,8]. This process allows the market to distribute convenient products on a global scale, taking advantage of the extended shelf life for distribution, storage, and consumption (without refrigeration) under controlled food safety conditions [7]. It is expected that the canned food market in Europe will grow at a compound annual rate (CAGR) of 3.75% between 2024 and 2029, thus increasing the production volume by approximately 20% during this period [9].

The fish most commonly used in the production of canned products are small pelagics (anchovies, sardines, Atlantic mackerel, and chub mackerel) and the top predator tuna [7,8]. However, the population status and spatial distribution of small pelagic fish are highly susceptible to environmental changes and economic development [10]. Therefore, due to the sustainability of these fishery resources, it is becoming important to diversify by preparing canned products with other less-consumed and abundant species, such as the Atlantic horse mackerel (*Trachurus trachurus*). The good condition of its existing stocks, mainly due to the fact that it is a species that grows rapidly in its early life stages and has an extended breeding season, has led to increases in allowed catch amounts (e.g., proposed EU Total Allowable Catches (TAC) for horse mackerel in Iberian waters (2024): +5%) [11–13]. Since this species presents a sustainable exploitation pattern in the Iberian Peninsula and is well known to have a high nutritional value, several marketing initiatives have been implemented to encourage its consumption and valorization [11,14–16]. Moreover, it is well known that legumes are also appreciated all over the world as sustainable and economical alternatives to meat [5]. Therefore, given that many consumers consider canned fish to be tasty and versatile and often consume it with cooked or canned vegetables, the authors believe that offering a sustainable canned meal that includes both fish and vegetables could be a good option to meet their needs. The attractiveness of a new product can be strengthened if it is affordable, tastefully appealing, and nutritionally balanced (e.g., low/reduced in sodium chloride (NaCl)/sodium (Na), which is associated with high blood pressure and other health problems) [17,18]. This last fact is extremely important

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because according to [19], around 11 million deaths globally are associated with a poor diet, 3 million of which are related to high Na intake.

However, no studies were found in the literature addressing the preparation, nutritional characterization, commercial stability and sterility, physicochemical and sensory properties, and performance of canned meals based on fish (e.g., Atlantic horse mackerel) and vegetables during storage. For this reason, the focus of this work was to i) develop and assess a nutritious, sustainable, convenient, and appealing canned meal prototype using Atlantic horse mackerel, a selection of grain legumes, and other vegetables in a light brine in terms of stability, sterility, and physicochemical and sensory properties over a 4-month period of storage at room temperature and ii) evaluate its nutritional contribution for children (7–10 years), healthy adults ( $\geq$ 18 years), and pregnant women.

#### 2. Materials and Methods

#### 2.1. Selection of Grain Legumes and Other Vegetables

The vegetables used were selected for their versatility, availability, and nutritional value. Specifically, the chickpea was mainly chosen as an ingredient since it (i) is rich in fiber and protein; (ii) contains several key vitamins and minerals [16], and (iii) has a well-accepted color. The choice of green bean and carrot was made because they are vegetables that are part of most consumers' diets and are also well accepted due to their vibrant colors. Finally, the courgette was chosen for its appearance and to smooth out the filling medium (Table 1).

Table 1.	Canned	meal	recipe	ingred	lients.

Ingredients	Quantity (%)	
Steamed horse mackerel fillet	19.7	
Sautéed onion and garlic (in olive oil) *	8.7	
Blanched chickpea	17.5	
Peeled blanched courgette	6.6	
Peeled blanched carrot	6.6	
Thawed green bean	4.4	
Xanthan gum (in powder)	0.04	
Brine (14% NaCl)	3.0	
Water (to the top)	33.6	
Total	100	

<sup>\*</sup>Cooked for around 30 min at 100–105 °C.

## 2.2. Preparation of Raw Material (Fish) and Other Ingredients

Fresh Atlantic horse mackerel (188.8  $\pm$  29.4 g total weight; 25.7  $\pm$  1.1 cm total length; mean  $\pm$  standard deviation) caught off the FAO 27 area in Portuguese waters was supplied by a local wholesaler and transported to the laboratory under controlled temperature conditions. Upon arrival, fish were beheaded, gutted, washed, drained, and steamed for 5 min at 100 °C in an oven (Combi-Master CM6, Rational Grossküchen Technik, GmbH, Landsberg am Lech, Germany). The fish were then cooled at room temperature, filleted by hand, and distributed among 32 glass jars (with a volume of approximately 290 cm³). The list of ingredients and quantities used to prepare a balanced canned meal is shown in Table 1.

The chickpeas, courgettes, and carrots were bought fresh at a local supermarket and blanched in the laboratory to avoid losses of both color and flavor as well as texture degradation that are usually caused by enzymatic action [20]. More specifically, the chickpeas were water-soaked overnight in an airy place and blanched in the next day for 10 min in boiling water (2:5 m/v; with 0.7% NaCl). After draining the blanching water, the chick-

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peas were cooled with cold water and ice to stop the blanching process, drained, and frozen at  $-20\,^{\circ}\text{C}$ . The carrots and courgettes were cleaned, peeled, and cut into slices ( $\approx$ 0.4 cm of thickness) and then into semicircles and quarters, respectively. Afterward, these pieces were blanched (1 min) and cooled with boiling and iced water, respectively. The green beans were purchased frozen also at a local supermarket (already blanched). Additionally, xanthan gum (xanthan gum clear, Sosa, Barcelona, Spain) was added as a thickener; the brine concentration (NaCl, 14%) was selected based on the NaCl contents indicated by companies aiming to supply healthy products, i.e., lower than 1 g/100 g, and the sensory results obtained in a preliminary test carried out with canned Atlantic horse mackerel fillets in olive oil, which revealed that a NaCl concentration of around 0.6 g/100 g (Na = 230  $\pm$  22 mg/100 g) is sufficient to impart a slight-to-moderate salty taste to the product. Finally, water was selected as filling medium since it was found (in a previous assay) that olive oil used for this purpose can make vegetables very oily and unappealing to consumers.

### 2.3. Canning and Accelerated Aging

The 32 glass jars were sealed and sterilized (Uniclave 88, AJ Costa, Agualva-Cacém, Portugal) for 75 min at  $115 \pm 1$  °C (well stablished and common practice of industrial units for this type of product based on previous F0 calculations [21]) at the Faculty of Veterinary Medicine of the University of Lisbon. After cooling, all glass jars were stored for 30 days (usual procedure to stabilize canned products) [21] in two different conditions: (i) 16 were kept at room temperature ( $\pm 20$  °C) in a dry place not exposed to direct sunlight and (ii) 16 were maintained at 38.9  $\pm$  0.8 °C (to simulate a 4-month aging period at 20 °C). The calculation of accelerated aging was based on the Arrhenius equation, which indicates that an increase of 10 °C at a given temperature doubles the rate of chemical reactions and/or microbiological growth [22].

# 2.4. Analytical Methods

The proximate composition and histamine, NaCl, Na, and K contents were assessed in the raw material (n = 3 homogenized fish; Grindomix GM200, Retsch, Haan, Germany; 5000 rpm). Furthermore, the canned samples stored at the two different conditions were evaluated in terms of both stability and sterility (n = 9 jars/condition) as well as physicochemical (n = 3 jars/condition) (with the exception of histamine, which was only assessed in the raw material) and sensory analyses (n = 3 jars/condition) through the methods described below (Sections 2.4.1–2.4.4). For chemical analyses and instrumental color, the filling medium of each jar was drained and the mix of fish and vegetables was homogenized (Grindomix GM200). The analytical quality control of chemical analyses is presented in Supplementary Table S1. All chemical results are expressed in wet weight.

#### 2.4.1. Stability and Sterility

The stability test was carried out in accordance with the Portuguese standard NP 4404-1 [23], which consists in the evaluation of packages stored at three different temperatures (n = 3/condition for each temperature), 20 °C (21 days), 30 °C (21 days), and 55 °C (seven days), in relation to (i) external examination of the package (glass jar and lid) during the storage time; (ii) internal examination of the glass jar content after opening, i.e., evaluation of the appearance (color and texture) and odor of the meal; and (iii) pH measurement of content (pH meter: HI99163; pH electrode: FC230B, HANNA Instruments, Póvoa de Varzim, Portugal). Thin smears of each glass jar were also prepared for fresh examination and stained using the Gram method. The microscopic observation of the microorganisms (average number per field) was focused in 20 optical fields [23].

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On the other hand, the canned samples' sterility was determined according to the Portuguese standard NP 2309-2 [24] for low-acid food. This test involved the detection of aerobic and anaerobic mesophiles and thermophiles, with the media being incubated at 30 and 55  $^{\circ}$ C (i.e., at the same temperatures that the packages were stored for 21 and 7 days, respectively).

## 2.4.2. Chemical Analyses

## Proximate Composition and Energy Value

The contents of moisture, ash, and free fat were determined according to the Association of Official Analytical Chemists methods [25]. Briefly, moisture was assessed by oven (ULE 500, Memmert, Schwabach, Germany) drying of each sample overnight at  $105 \pm 2~^{\circ}\text{C}$  while ash was obtained by incineration of previously dried sample in a muffle furnace (M104, Heraeus, Hanau, Germany) for 16 h at  $500 \pm 25~^{\circ}\text{C}$ . Free fat was quantified using a Soxhlet extraction method (in a Soxhlet apparatus, Behr Labor-Technik, Dusseldorf, Germany), with diethyl ether as a solvent (at approximately  $40~^{\circ}\text{C}$ ; 7 h), by weighing the fat residue after drying ( $105 \pm 2~^{\circ}\text{C}$ ) in an air oven. Total nitrogen was determined following the Dumas method [26] in an automatic nitrogen analyzer (FP-528, LECO Corp., St. Joseph, MI USA) calibrated with EDTA. Nitrogen was released by combustion at  $850~^{\circ}\text{C}$  and detected by thermal conductivity. Crude protein was calculated from total nitrogen through the conversion factor of 6.25 [27]. Total carbohydrates were calculated by difference and the energy value was estimated using Food and Agriculture Organization factors [27].

#### NaCl and Macroelements (Na and K)

The measurement of salt (i.e., chlorine,  $Cl^-$  as NaCl) content was performed by volumetric titration of sample with silver nitrate (AgNO<sub>3</sub>) according to Mohr's method [28]. Additionally, the Na and potassium (K) contents were quantified by flame atomic absorption spectrophotometry (Spectr AA 55 B spectrophotometer, Agilent Technologies, Santa Clara, CA, USA) with a background deuterium correction, following the method reported by [29]. The concentrations were calculated through linear calibration obtained from absorbance measurements of, at least, five different concentrations of standard solutions (NaNO<sub>3</sub> and KNO<sub>3</sub>, both dissolved in 0.5 M HNO<sub>3</sub>).

# Histamine and Total Volatile Basic Nitrogen (TVB-N)

The determination of histamine was carried out on samples previously homogenized (POLYTRON® PT 3100 D, Kinematica AG, Malters, Switzerland) with 10% (m/V) trichloroacetic acid (1:2, m/V) and filtered through a 0.2  $\mu$ m porosity filter. The amine was separated by high-performance liquid chromatography (HPLC, Agilent 1100 Series, Santa Clara, CA, USA) on a reverse-phase column, LiChrospher® 100 RP-18 (5  $\mu$ m) LiChroCART® 250-4, according to an elution gradient (mobile phase, consisting of two eluents: 0.1 M sodium acetate buffer (pH 4.5, adjusted with glacial acetic acid, containing 1-octanesulfonic acid) and 0.2 M sodium acetate buffer (pH 4.5, adjusted with glacial acetic acid, containing acetonitrile and 1-octanesulfonic acid)) at a flow rate of 1.0 mL/min and post column derivatization with ortho-phthalaldehyde reagent (OPA), following the method described by [30]. The detection of the compound was performed by fluorescence in an Agilent 1100 Series fluorimeter ( $\lambda_{\rm Excitation} = 340$  nm and  $\lambda_{\rm Emission} = 455$  nm). Histamine was identified and quantified by comparison with a calibration curve (hexanediamine was used as internal standard) in the working range of 1.25–10  $\mu$ g/mL using the Software Agilent Chem Station for LC (version A.10.02 [1757]).

TVB-N content was determined in trichloroacetic acid (5%) extracts of homogenized samples (POLYTRON $^{\circledR}$  PT 3100 D) by the microdiffusion Conway method [31] using a drying oven (INCO 2/108, Memmert, Schwabach, Germany).

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#### 2.4.3. Instrumental Color

The instrumental color was analyzed in all samples using a colorimeter (CR-400, KONICA MINOLTA, Tokyo, Japan), at standard illuminant D65 and a 2-degree observer, previously calibrated with a white standard tile ( $L^* = 93.47$ ;  $a^* = -0.16$ ;  $b^* = 3.32$ ). The  $L^*$ ,  $a^*$  and  $b^*$  coordinates from CIELAB system were recorded. In this system,  $L^*$  values describe lightness on a scale of 0 (black) to 100 (white); the  $a^*$  values denote the intensity from green (negative) to red (positive); the  $b^*$  values range from blue (negative) to yellow (positive). The chroma (saturation;  $C^*_{a,b}$ ) was calculated according to the following equation [32]:

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

# 2.4.4. Sensory Evaluation

The sensory assessment was performed after sterility confirmation of samples in the sensory laboratory of IPMA equipped with testing room according to [33]. A quantitative descriptive method was applied using seven trained panelists (both sexes) and a 5-point scale (0—absent; 1—slight; 2—moderate; 3—intense; 4—extreme) to rate the intensity of the selected attributes [34]: canned food odor, other odors, color of ingredients (fish, chickpea, green bean, courgette, and carrot) and filling medium, salty taste, other flavors, fish firmness, fish succulence, meal oiliness, filling medium consistency, ingredient cooking degree (fish, chickpea, green bean, courgette, and carrot), and mouth feeling. The jars were opened 15 min before testing and about 60 g of each sample (stored at 20 °C and 40 °C) was presented to each panelist in white coded plates. The panelists rinsed their mouths with water between portion assessments to reduce the residual flavor effects.

#### 2.5. Estimated Nutritional Composition of Other Nutrients

Other nutrient levels present in the meal recipe were estimated with @Nutrisoft—@Nutrition Information Software (version 1, Registration n°. 3208/2012) [35]. This software allowed the estimation of some macro- and micronutrients (i.e., phosphorus (P), Ca, magnesium (Mg), Fe, Zn, I, selenium (Se), and vitamins B12 and D) based on the amounts of ingredients used and their nutritional values. The calculations were carried out using the food composition table published by [16].

## 2.6. Nutritional Contribution

The meal nutritional contribution (i.e., 190 g, which corresponded approximately to the drained product) in terms of proteins, Na, K, P, Ca, Mg, Fe, Zn, I, Se, and vitamins B12 and D was calculated considering the dietary reference values (DRVs) recommended by [17,36] for different population groups (children aged 7–10 years old and adults, including pregnant women). The calculations (%) were made according to the following equation:

Nutritional Contribution(%) = 
$$\frac{C \times M}{DRV} \times 100$$

Here,

C—the mean concentration of the nutrient (mg/kg) (obtained as indicated in previous sections). In the case of protein, Na, and K, only the contents observed after 1 month at room temperature were used since the impact of aging on these parameters was nil (as can be seen in Section 3).

M—the weight of the defined meal (kg).

DRV—the PRI, Population Reference Intake (mg/day), in the case of protein, Ca, Fe, and Zn, or the AI, Adequate Intake (mg/day), in the case of the other specific nutrients.

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### 2.7. Statistical Analysis

Statistical analysis was performed using the STATISTICA© software version 12 (Stat-Soft Inc., Tulsa, OK, USA). All data were checked for normality of distribution and homogeneity of variances using Shapiro–Wilk and Levene's tests, respectively. The influence of storage at different conditions on proximate composition, energy value, NaCl and macroelement (Na and K) contents, TVB-N level, instrumental color, and sensory attributes of the canned meal was tested by Student's t-test. Statistical significance was considered at p < 0.05 for all analyses [37].

#### 3. Results and Discussion

## 3.1. Stability and Sterility

The external visual examination of the packages (glass jars and lids) analyzed did not reveal any physical damage or leakage of the filling medium. In both tested conditions, the appearance (including color and texture) and odor of the samples were considered characteristic of a canned product. However, it was observed that some vegetables (particularly the courgette) showed signs of overcooking. Furthermore, some pH differences of more than 0.5 were detected between the samples maintained at  $\approx$ 20 °C and those kept at 30 and 55 °C in both conditions (Table 2). These differences can be explained by the pH variation in the courgette based on previous experiments without this vegetable. This change could be ascribed to accelerated chemical reactions triggered by heating, such as possible Maillard reactions or other heat-induced transformations like decarboxylation [16,38]. Finally, the fresh smear and Gram coloration tests, in 20 fields, also had negative results, meaning that there was no microorganism growth.

**Table 2.** pH values found in the stability tests performed with canned meal prepared with Atlantic horse mackerel and vegetables after 1 and 4 months of storage.

Storage Time (Months)	рН
1	$6.4 \pm 0.1$ a, $5.9 \pm 0.1$ , $5.7 \pm 0.5$ (at $\approx$ 20, 30 and 55 °C, respectively)
4 *	$6.4\pm0.1$ a, $5.7\pm0.1$ , $5.5\pm0.4$ (at $\approx$ 20, 30 and 55 °C, respectively)

Results are given as mean values  $\pm$  standard deviations (n = 3). \* Simulated 4-month aging period at 20 °C (1 month at  $\approx$ 40 °C). For the glass jars maintained at  $\approx$ 20 °C, the same letter (a) indicates absence of significant differences (p < 0.05) between storage times.

Although the pH was not considered stable, the results of the sterilization assessment showed that no microbial growth (mesophilic and thermophilic aerobes and anaerobes) was observed under the specified conditions of the tests, indicating that the developed meal could be considered sterile for at least 4 months at room temperature.

#### 3.2. Proximate Composition, Energy Value, NaCl, and Macroelements (Na and K)

The proximate composition, energy value, and NaCl and macroelement (Na and K) contents of Atlantic horse mackerel and the canned meal are shown in Table 3. The values obtained for raw fish were in agreement with those reported by other studies with the same (caught in the spring season) or other pelagic species [39–44].

Additionally, there were no significant changes in the moisture content of the Atlantic horse mackerel canned meal after 1 and 4 months of storage at room temperature ( $\approx$ 79 g/100 g). Likewise, the protein ( $\approx$ 10 g/100 g) and fat ( $\approx$ 2 g/100 g) contents did not change either (p > 0.05). The values obtained also indicate that the canned meal developed in the present work can be nutritionally claimed as a "source of protein" and "low-fat" since more than 12% of the energy (i.e., approximately 45%) was supplied by proteins and it had an amount <3 g fat/100 g, respectively [45]. The mean contents of ash and carbohydrates, as

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well as the energy value, were also not significantly affected by the storage time (1 g/100 g, close to 8 g/100 g, and 93 kcal/100 g, respectively) (p > 0.05).

**Table 3.** Proximate composition, energy value, and NaCl and macroelement (Na and K) contents of the raw material (fish) and canned meal prepared with Atlantic horse mackerel and vegetables after 1 and 4 months of storage.

	Atlantic Horse	Canned Meal Storage Time (Months)		
g/100 g	Mackerel	1	4 *	
Moisture	$76.7 \pm 0.6$	$78.9 \pm 0.1$ a	$79.1\pm0.4$ a	
Protein	$20.9 \pm 0.8$	$10.2\pm0.2$ a	$10.3\pm0.6$ a	
Fat	$1.9 \pm 0.4$	$1.9\pm0.2$ a	$1.8\pm0.3~^{\mathrm{a}}$	
Ash	$1.4\pm0.1$	$1.0\pm0.0$ a	$1.0\pm0.0$ a	
Carbohydrates <sup>1</sup>		$8.0\pm0.3$ a	$7.9\pm0.7$ a	
Energy value (kcal/100 g)		$93.5\pm0.9$ a	92.4 $\pm$ 1.8 $^{\mathrm{a}}$	
NaCl	$0.2 \pm 0.0$	$0.5 \pm 0.0$ a	$0.5 \pm 0.0$ a	
Na (mg/100 g)	$54.9 \pm 0.6$	$166.9 \pm 12.0~^{\mathrm{a}}$	$162.6\pm2.8~^{\mathrm{a}}$	
K (mg/100 g)	$487.5 \pm 11.7$	$155.3\pm14.2~^{\mathrm{a}}$	$145.2\pm3.8~^{\mathrm{a}}$	

Results are given as mean values  $\pm$  standard deviations (n = 3). For each parameter, the same letter (a) indicates absence of significant differences (p < 0.05) between storage times. \* Simulated 4-month aging period at 20 °C (1 month at  $\approx 40$  °C). ¹ Calculated by difference.

The NaCl and Na contents found in the canned meal (0.5 g/100 g) and approximately 165 mg/100 g, respectively) were also not significantly influenced by the studied storage period (p > 0.05), being lower compared to those found in various canned fish products sold in supermarkets (NaCl:  $\geq 1$  g/100 g) [46]. The NaCl content was within the large variation (between 32 to 6000 mg/100 g) reported by [47] for canned fish products. Moreover, the Na value was below the global Na benchmark defined (360 mg/100 g) for canned fish (with the exception of canned anchovies) [19]. So, considering the values observed, the prepared canned meal can be considered a healthy product (with reduced NaCl/Na; [45]) whose consumption can contribute to achieve the objectives defined by the WHO (in terms of NaCl reduction) [48] and many seafood producers. Finally, the K content resulted not only from the amount naturally present in fish (e.g., Ref. [49]) but also from the quantities existing in the other ingredients used (mainly in chickpeas, around 270 mg/100 g after cooking) [16]. Storage for up to 4 months had no impact on K either (close to 150 mg/100 g) (p > 0.05). Hence, it appears that this new product can be stored at room temperature for up to 4 months without the alteration of its proximate composition and NaCl, Na, and K contents.

#### 3.3. Estimated Nutritional Composition

The most abundant macroelement was Na, followed by P, K (with identical levels), Ca, and finally Mg. Within the microelements, Fe had the highest content followed by Zn, I, and lastly Se (Tables 3 and 4). The results shown in Table 4 support the possible nutritional claim "source of phosphorus, iron and selenium" since the meal recipe had amounts > 15% of the Nutrient Reference Values, NRVs (i.e., 700 mg/100 g, 14 mg/100 g, and 55  $\mu$ g/100 g, respectively) [45,50]. Hence, the consumption of this meal based on fish and vegetables may be relevant, for instance, to avoid health constraints such as (i) muscle weakness and bone pain (due to P deficiency), (ii) anaemia (caused by P and Fe deficiencies), and (iii) organ and tissue degeneration (due to Se deficiency), which can lead to the appearance of the Keshan and Kashin–Beck diseases [36,51].

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**Table 4.** Contents of some macro- and microelements and vitamins in the meal recipe with Atlantic horse mackerel and vegetables.

Content in the Recipe (mg/100 g of Drained Product) $^{\rm 1}$
155.7
72.2
42.8
2.3
1.2
0.0142
0.0100
0.0017
0.0012

 $<sup>^1</sup>$  Nutrients contents were calculated with @Nutrisoft—@Nutrition Information Software version 1 (Registration  $n^{\circ}$ . 3208/2012).

Additionally, the value of vitamin B12 was higher than 0.75  $\mu g/100$  g (>30% of the NRV—i.e., 2.5  $\mu g/100$  g), allowing the probable nutritional claim "high in vitamin B12" (Table 4) [45,50]. Thus, the intake of the prepared meal can provide a good amount of vitamin B12, which should help, for example, keep the body's blood and nerve cells healthy in addition to reducing tiredness and fatigue [52,53]. In terms of vitamin D, the value found was higher than 0.75  $\mu g/100$  g (15% of the NRV—i.e., 5  $\mu g/100$  g), permitting the possible nutritional claim "source of vitamin D" (Table 4) [45,50]. This result shows that the consumption of this meal based on fish and vegetables may contribute to other health benefits such as the maintenance of normal bone, teeth, and muscle function, as well as the normal absorption/utilization of calcium and phosphorus and the proper functioning of the immune system [54,55]. Moreover, the vitamin D amount was well below the tolerable upper intake level/day (UL/day) set for children aged 7–10 years (50  $\mu g/day$ ) and adults/pregnant women (100  $\mu g/day$ ) [56].

It is well known that cooking techniques that require heat can affect the nutritional composition of food [57]. However, and according to the literature, the retention factors of the food constituents reported in Table 4 by cooking (boiling) fish and fish-based dishes (low fat fish; <5%) are high (i.e., between 80 and 100%) [16,57–59]. Moreover, vitamins D and B12 are heat stable and fat-soluble vitamins, meaning that both are not easily leached into cooking water [60,61]. So, it is expected that most of the estimated values presented in Table 4 are close to those that can be obtained after heat treatment (sterilization).

#### 3.4. Nutritional Contribution

# 3.4.1. Proteins and Macro- and Microelements

The nutritional contribution of the meal based on fish and vegetables in terms of proteins and macro- and microelements is presented in Table 5. The consumption of 190 g corresponds to a greater protein contribution for children (74% of the PRI) and a lower one for pregnant women in the last trimester (22% of the PRI). In all cases, such contributions are valuable for tissue maintenance [36]. The nutritional contribution is at least 50% of the daily AI of P and Se for children. The same can be also observed in the case of P for adults. The 190 g meal also contributes with at least a quarter ( $\geq$ 25%) of the DRVs (AIs or PRIs) in the following cases: Mg for children and women (adults, including pregnant women); I for children; Se for adults (including pregnant women); Fe for all population groups; and Zn for children and women (adults, including pregnant women). Assuming that the population

in Portugal consumes up to five meals a day [62], this percentage ( $\geq$ 25%) can be a good contribution to reach the recommended amounts if the consumer eats other nutritious foods throughout the day. The contributions of Mg for male adults; I for adults, including pregnant women; Zn for male adults; and Na, K, and Ca for all population segments were less pronounced, ranging from 8% to 24% of the daily recommended amounts. Specifically, the nutritional contribution of Na corresponded to 16–19% for both groups, values far below the recommended limit proposed by [17].

**Table 5.** Nutritional contribution of the meal prepared with Atlantic horse mackerel and vegetables in terms of proteins and macro- and microelements for different population segments, considering a dose of 190 g.

Target Nutrient	Target Population	DRV: AI or PRI (mg/day) <sup>1</sup>	Nutritional Contribution (%)
Protein	Children (7–10 y)	26,261 <sup>3</sup>	$73.6 \pm 1.1$
	Adults (≥18 y)	<b>52,539</b> <sup>3</sup>	$36.8 \pm 0.6$
	Pregnant women (3rd trimester)	86,515 <sup>3,4</sup>	$22.3 \pm 0.3$
Macroelements			
NT.	Children (7-10 y)	1700 <sup>3,5</sup>	$18.7 \pm 1.3$
Na	Adults $(\geq 18 \text{ y})^2$	2000 <sup>3,5</sup>	$15.9 \pm 1.1$
K	Children (7–10 y)	1800 <sup>3</sup>	$16.4\pm1.5$
K	Adults $(\ge 18 \text{ y})^2$	3500 <sup>3</sup>	$8.4\pm0.4$
D	Children (7–10 y)	440 <sup>3</sup>	67.2
P	Adults $(\geq 18 \text{ y})^2$	550 <sup>3</sup>	53.8
C	Children (7–10 y)	800 <sup>3</sup>	17.1
Ca	Adults $(\geq 18 \text{ y})^2$	950/1000 <sup>3</sup>	13.7/14.4
	Children (7–10 y)	230–250 <sup>3</sup> (W) <sup>6</sup> /230–300 <sup>3</sup> (M) <sup>6</sup>	27.1–35.4%
Mg	• • • • • • • • • • • • • • • • • • • •		$(M)^{6}/32.5-35.4\% (W)^{6}$
	Adults ( $\geq$ 18 y) <sup>2</sup>	300 <sup>3</sup> (W) <sup>6</sup> /350 <sup>3</sup> (M) <sup>6</sup>	23.2 (M) <sup>6</sup> /27.1 (W) <sup>6</sup>
Microelements		_	
	Children (7–10 y)	0.09 3	30.0
I	Adults (≥18 y)	0.15 3	18.0
	Pregnant women	0.2 <sup>3</sup>	13.5
0	Children (7–10 y)	0.035 <sup>3</sup>	53.7
Se	Adults $(\geq 18 \text{ y})^2$	0.07 <sup>3</sup>	26.9
	Children (7–10 y)	11 <sup>3</sup>	39.7
Fe	Adults (≥18 y)	$11^{3}$ (M, postmenopausal W) $^{6}/16^{3}$ (premenopausal W) $^{6}$	27.3 (premenopausal W) <sup>6</sup> /39.7 (M, postmenopausal W) <sup>6</sup>
	Pregnant women	16 <sup>7</sup>	27.3
	Children (7–10 y)	7.4 <sup>3,8</sup>	30.8
Zn	Adults (≥18 y)	$7.5^{3,9}$ (W) <sup>6</sup> /9.4 <sup>3,9</sup> (M) <sup>6</sup>	$24.3 (M) ^{6} / 30.4 (W) ^{6}$
	Pregnant women	9.1 <sup>3,10</sup>	25.1

Values of protein, Na, and K are means  $\pm$  standard deviations (n = 3); other values were calculated from the contents obtained using the @Nutrisoft program; <sup>1</sup> DRVs (Dietary Reference Values): AIs (Adequate Intakes) are presented in ordinary type and PRIs (Population Reference Intakes) in **bold type**; <sup>2</sup> including pregnant women; <sup>3</sup> Ref. [36]; <sup>4</sup> +28,000 mg/day in addition to the PRI for protein of non-pregnant, non-lactating women. <sup>5</sup> Ref. [17]; <sup>6</sup> W—Women, M—Men. <sup>7</sup> The PRI covers the requirement of approximately 95% of premenopausal women. <sup>8</sup> The fractional absorption of Zn considered in setting PRIs for children was based on data from mixed diets expected to contain variable quantities of phytate; therefore, no adjustment for phytate intake was made; <sup>9</sup> considering a phytate intake level of 300 mg/day; <sup>10</sup> +1.6 mg/day—in addition to the PRIs for non-pregnant, non-lactating women.

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#### 3.4.2. Vitamins

The nutritional contributions of vitamins are shown in Table 6. The amounts of vitamin B12 provided by the meal were high for all segments. In other words, the meal (190 g) almost fulfils or fulfils the daily AIs of vitamin B12 for children (>100%) and adults including pregnant women (72–81%). Hence, this product can be a good option for vitamin B12 intake, especially for individuals that have a special need. The nutritional contribution of vitamin D for daily AIs was 15% for all population segments analyzed.

**Table 6.** Nutritional contribution of the meal prepared with Atlantic horse mackerel and vegetables in terms of vitamins for different segments of the population, considering a dose of 190 g.

Target Nutrient	<b>Target Population</b>	DRV: AI (mg/day) <sup>1</sup>	Nutritional Contribution (%)
Vitamin			
	Children (7-10 y)	$0.0025^{3}$	129.2
B12	Adults (≥18 y)	$0.004^{3}$	80.8
	Pregnant women	$0.0045^{3}$	71.8
D	Children (7–10 y) Adults ( $\geq$ 18 y) <sup>2</sup>	0.015 3,4	15.2

Values were calculated from the contents obtained with the @Nutrisoft program. <sup>1</sup> DRVs (Dietary Reference Values): AIs (Adequate Intakes); <sup>2</sup> including pregnant women; <sup>3</sup> Ref. [36]; <sup>4</sup> under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may be even zero.

#### 3.5. Histamine and TVB-N

Since some publications observed histamine poisoning from other fish species not considered in the EU legislation [63], the control of this parameter was performed in raw Atlantic horse mackerel because its ingestion may be responsible for cases of food intolerance, which may include allergies-like symptoms [64]. Additionally, histamine was only determined in the fish because this type of food has been identified as the predominant causative agent (over 90% of cases) of intoxication by this amine [63,64]. However, the histamine level found was below the detection limit (0.6 mg/kg), which means that the analyzed samples were in compliance with the current EU regulations [65] and were kept following the recommendations, i.e., adequate chilling conditions and hygienic measures [66]. Therefore, these levels (<10 mg/kg,) also indicated the utilization of good-quality fish [67,68]. For this reason, and due to the very low levels of histamine found in canned fish produced with species associated with histamine risk (probably related to the improving quality of the canning process over the years due to the widespread application of HACCP principles, i.e., from the fish caught in the vessel to the processed product) [63], this analysis was not carried out for the canned meal.

On the other hand, the TVB-N levels obtained in the canned meal prepared with Atlantic horse mackerel and vegetables were around 20 mg/100 g after 1 and 4 months of storage. Such contents were not significantly affected by the aging period (p > 0.05), being lower than (i) the satisfactory and acceptable range reported for this type of product (40–45 mg/100 g muscle; [39]) prepared from raw material of good quality (i.e., with contents ranging between 25–30 mg/100 g) and also (ii) the limits (25–35 mg N/100 g) set for the fish species or families mentioned in Commission Regulation (EC) No. 1022/2008 [69]. Thus, fish degradation could not be considered in all samples evaluated. Hence, it seems that the canned meal can be stored for up to 4 months while keeping its good quality.

## 3.6. Instrumental Color and pH

It is well known that chlorophylls and carotenoids, i.e., the pigments responsible for the characteristic colors (green and red/orange/yellow, respectively) of vegetables (e.g., green bean and carrot) are highly susceptible to degradation during storage, resulting

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in color changes, which can be perceived by the consumer as a loss of quality [38,70]. However, in the present work, the color of the canned meal remained constant with storage (p > 0.05; Table 7). The L\* mean value ranged from 57 to 58 between the storage periods analyzed, being in a medium range of luminosity. The a\*, b\*, and C\*<sub>a,b</sub> mean values were close to 6.0, 27.0, and 27.0, respectively, at both times. The geometry of the CIELAB color model and a\*and b\* results showed that the color of the meal was in the orange-to-yellow range [71]. The absence of significant differences suggests that the time tested does not seem to cause any color degradation (e.g., associated to degradation of the pigments present in vegetables). So, it is not expected that there will be changes in the sensory perception and consumer acceptance (in terms of color) of the developed canned meal over a 4-month storage period at room temperature.

**Table 7.** Color parameters of the canned meal prepared with Atlantic horse mackerel and vegetables after 1 and 4 months of storage.

Color Parameters		d Meal ne (Months)
	1	4 *
L*	$57.2 \pm 1.3$ a	$58.0 \pm 0.5$ a
a*	$6.0\pm0.6$ a	$5.6\pm0.1$ a
b*	$26.7\pm0.8$ a	$26.5\pm1.3~^{\mathrm{a}}$
Chroma (C* <sub>a.b</sub> )	$27.4\pm0.8$ a	$27.1\pm1.2$ a

Results are given as mean values  $\pm$  standard deviations (n = 3). For each parameter, the same letter (a) indicates absence of significant differences (p < 0.05) between storage times; \* simulated 4-month aging period at 20 °C (1 month at  $\approx$ 40 °C).

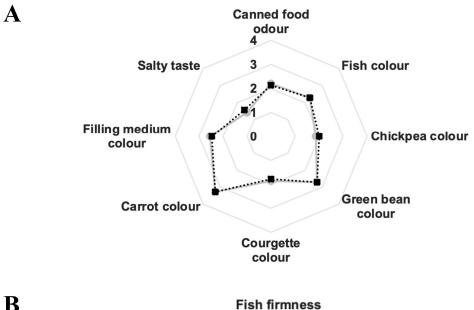
On the other hand, accelerated aging (simulated 4 months at 20 °C) also did not promote significant changes in the pH of the meal (close to 6 at both times; p > 0.05), revealing a good maintenance of the initial quality (Table 2).

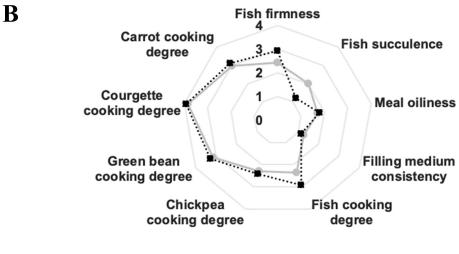
### 3.7. Sensory Evaluation

The results of the sensory properties (i.e., odor, color, taste, and texture) of the drained canned meal obtained after 1 and 4 months of storage can be found in Figure 1A,B. The meal was always scored by the panel as having a moderate canned food odor. The panelists also detected other odors (mainly sautéed or garlic), but with a slight intensity. Regarding color, the fish, chickpea, and courgette were scored with a moderate intensity while the green bean and carrot were described as having an intense color after both times. The filling medium was scored with a moderate-to-intense color (reported as yellow by most of the panelists) and a slightly thick consistency in the two periods. Furthermore, the prepared product was rated as having a slight-to-moderate salty taste regardless of the storage time evaluated. This result indicates that the NaCl content used was adequate to prepare a tasty and healthy meal with reduced NaCl/Na (Table 3). Other flavors (e.g., bitter and sweet) were considered imperceptible by the panel.

The fish fillet was classified as moderately firm and succulent after 1 month and intensely firm and slightly succulent after 4 months, which indicated a slight tendency of the product to become firmer and less succulent with storage. In both times, the oiliness of the meal was rated as moderate. The fish cooking degree was scored from moderate (1 month) to intense (4 months). The cooking degree of the chickpea was identified as moderate, that of the green bean and carrot as intense (i.e., slightly overcooked), and that of the courgette as extreme (i.e., overcooked) in both periods. Finally, the panel did not detect negative sensations such as astringency (at both times). Therefore, and according to Figure 1A,B, the sensory properties analyzed were not significantly influenced by storage

(p > 0.05). These results indicate that the developed canned meal can be stored for up to 4 months at room temperature without sensory changes, although they also point out that the pre-cooking time of the fish (before canning) can be slightly reduced to obtain a product with a more appreciable texture. Additionally, it can also be noted that the recipe could be improved by replacing the courgette with another nutritionally rich ingredient since it was classified as overcooked and disintegrated.





**Figure 1.** Sensory profile of the canned meal prepared with Atlantic horse mackerel and vegetables after 1 and 4 \* months of storage (\* corresponds to a simulated 4-month aging period at 20 °C; 1 month at  $\approx$ 40 °C) in terms of (**A**) odor, color, and taste and (**B**) textural properties. Results correspond to mean values ( $0.4 \le SD \le 1.5$ ) (n = 3). Intensity scale: 0 (absent); 1 (slight); 2 (moderate); 3 (intense); 4 (extreme). For each parameter, no significant differences (p < 0.05) were found between storage times.

-- 1 month -- 4 months

# 4. Conclusions

The findings allow us to conclude that the chosen ingredients (horse mackerel, chick-pea, carrot, and green bean) are very suitable for preparing fish-based canned meals. As for the courgette, it ended up not being a good option because it did not provide a smooth consistency to the filling medium and contributed to a slight change in the meal's stability

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(based on pH values), although it did not compromise sterility. Hence, further development is needed, especially in exploring vegetable alternatives, to ensure long-term stability.

Based on chemical (proximate composition, energy value, and NaCl, Na, and K contents and TVB-N values), physical (instrumental color and pH), and sensory properties (e.g., canned food odor, salty taste, fish firmness, and fish succulence), this prototype is stable for at least 4 months under normal storage conditions. Furthermore, it could possibly be labelled as "low-fat", "reduced in NaCl/Na", "source of protein, phosphorus, iron, selenium and vitamin D", and "high in vitamin B12". Moreover, its consumption (190 g; drained product) will contribute to meeting the daily needs of several nutrients (8- > 100%) essential for consumers' health at all ages.

The present results provide valuable information for scaling up an innovative, nutritious, sustainable, and convenient prototype based on the studied formulation (without the courgette) but with a more appealing filling medium, for instance spiced tomato sauce, so that the consumer considers its consumption (as happens with canned fish in tomato sauces) and not its disposal. Therefore, the market application prospects are promising, considering the growing demand for this type of product. Potential consumers include conscious individuals concerned about health and sustainability issues and busy professionals and their children.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su17115050/su17115050/s1, Table S1: Analytical quality control of chemical analyses (proximate composition, histamine, TVB-N, and macroelement contents).

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