NOVA SCHOOL OF SCIENCE & TECHNOLOGY
LISBOA UNIVERSIDADE DE LISBOA
INSTITUTO SUPERIOR DE AGRONOMIA
DEPARTMENT OF CHEMISTRY

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MSc in Chemistry

MARINATED BALTIC HERRING PRODUCED BY USING ORGANIC ACIDS OTHER THAN ACETIC ACID

MASTER IN GASTRONOMIC SCIENCES

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ABSTRACT

This thesis work was developed as part of the Blue Products Program, which aims to increase the value of underutilized domestic fish like Baltic herring, roach and sprat by increasing its use for human consumption. Marinated Baltic herring is a semi-preserved, traditional product consumed in Northern Europe; it is a "ready-to-consume" product that keeps most of the fish's health benefits and brings added value to the fish industry. These fish marinades are traditionally produced by using acetic acid, which imparts a strong, sour flavour to the products that is softened by the addition of sugar in generous amounts.

The aim of this study was to produce Baltic herring marinades using other weak organic acids, namely citric, lactic and tartaric acids - that by having a milder flavour profile would allow the development of products with less sugar content and organoleptically different.

The extensive research done on existing processing methods and adjustment of its conditions through Pilot Studies resulted in a successful two-step procedure, where fish was first pickled using a solution 6%(w/v) in acid and 14%(w/v) salt, followed by marinating in solutions with 56%(w/v) and 33%(w/v) sugar. The final products had pH levels of 3.6-4.1 after 5 weeks of storage. During the preliminary studies of the work it was concluded that only tartaric acid was not suitable to produce the fish marinades. Sensorial analysis results revealed the preference for lactic acid marinades, without a clear distinction between the higher and lower sugar concentration samples. Microbiological analysis attested that the marinades obtained using citric and lactic acids had good quality, even though citric acid had a weaker antimicrobial effect. Although it was not possible to obtain accurate results for weight change, the weight loss that occurred during pickling seemed to correlate with the pH of the solutions; during marinating there was a slight weight gain caused, most likely, by the increased density of sugar marinades .

Keywords: fish, marinades, organic acids, Baltic herring, pickling

RESUMO

Este trabalho de tese de mestrado foi desenvolvido como parte integrante do programa Blue Products, que visa aumentar o valor de peixes domésticos subutilizados (como o arenque do Báltico, pardelha-dos-alpes e espadilha), aumentando o seu uso para consumo humano. O arenque do Báltico marinado é um produto tradicional consumido no norte da Europa; é um produto "pronto a consumir" que mantém a maior parte dos benefícios para a saúde do peixe e traz valor acrescentado à indústria pesqueira. Estes produtos de peixe marinado são categorizados como semi-conservas e são tradicionalmente produzidos usando ácido acético, o que confere um sabor forte e ácido aos produtos, que é suavizado pela adição de açúcar em quantidades generosas.

O objetivo deste estudo foi produzir arenque do Báltico marinado utilizando outros ácidos orgânicos fracos, nomeadamente os ácidos cítrico, láctico e tartárico que, por terem um perfil de sabor mais suave, permitissem desenvolver produtos com menor teor de açúcar e organolepticamente diferentes.

A extensa pesquisa bibliográfica feita sobre os métodos de processamento existentes e o ajuste das condições de produção através de Estudos Piloto resultou num procedimento de produção do peixe marinado bem sucedido. O procedimento consistiu em duas etapas: primeiro o peixe foi imerso numa solução 6%(w/v) em ácido e 14% (w/w) em sal durante 35 dias e depois foi marinado em soluções com 56% e 33%(w/v) de açúcar. Após 5 semanas de armazenamento, os valores de pH medidos nos produtos de peixe marinado variaram entre 3.6 e 4.1. Durante os Estudos Piloto concluiu-se que apenas o ácido tartárico não é adequado para produzir peixe marinado. Os resultados de análise sensorial revelaram a preferência pelo peixe marinado em ácido láctico, sem uma distinção clara entre as amostras com maior e menor concentração de açúcar. A análise microbiológica atestou que os produtos obtidos com os ácidos cítrico e láctico apresentaram boa qualidade, embora o ácido cítrico tenha um efeito antimicrobiano mais fraco.

Embora não tenha sido possível obter resultados precisos para o rendimento do processo de produção, a perda de peso ocorrida durante a primeira etapa do processo apresenta alguma correlação com o pH das soluções; durante a segunda etapa verificou-se um leve ganho de peso provavelmente causado pelo aumento da densidade das soluções com a adição de açúcar.

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LIST OF ABBREVIATIONS

AA - Acetic acid
CA - Citric acid
cfu - colony forming units
DHA - docosahexaenoic acid
EMFF - European Maritime and Fisheries Fund
EPA - Eicosapentaenoic acid
FAO - Food and Agriculture Organization
FFF - Functional Foods Forum
ICES - International Council for the exploration of the Sea
IEP - Isoelectric Point
LA - Lactic acid
MSC - Marine Stewardship Council
N.d. - not detected
SK - Suomen Sillikonttori Oy
stdev - Standard Deviation
TA - Tartaric acid
UK - United Kingdom
Wk - week
WPS - Water Phase Salt
WWF - World Wildlife Fund
w/v - weight by volume
w/w - weight by weight

INTRODUCTION | 1

When Anu Hopia invited me to do my thesis work on marinated Baltic herring, I instinctively felt that it could be an engaging and joyful subject to work on. Afterall, I have always been an avid fish consumer and have enjoyed eating marinated herring, since I discovered the delicacy while living in Denmark some years back. The fact that this study also contributes to achieving a more sustainable food system also weighed heavily in my decision to accept it - despite the 150km commuting distance to the lab. Most of the work could be done remotely from my home in Espoo but I had the chance to visit the beautiful city of Turku many times during my thesis! Part of the work was done in Röölä, some 40 km further out off Turku, where the factory premises of Suomen Sillikonttori Oy, a Finnish company specializing in the production of several salted and marinated fish products, is located.

This study is a part of the Blue Products program [1], which integrates the Finnish Operational Program for the European Maritime and Fisheries Fund (EMFF). It aims to increase the value of underutilized domestic fish like Baltic herring, sprat and roach by increasing its use for human consumption. Nowadays, more than half of all Baltic herring caught in Finland ends up as animal feed for the aquaculture and fur animals industry. Consumption of Baltic herring in Finland has collapsed for the past two decades to a mere 300g per capita per year [2] and, according to health recommendations, five times this amount could actually be consumed. As reported by the World Wild Fund (WWF), the catch of Baltic herring from the Bothnian Sea is considered sustainable and is certified by the Marine Stewardship Council (MSC) [2].

Baltic herring is a good source of both high quality protein and healthy omega-3 EPA and DHA fatty acids [3]. Its use as a replacement for animal meat has not only health benefits but also contributes to lower the diet's carbon footprint and environmental impact.

This work is the follow-up of a previous study [4], also under the Blue Products program, which averiguated the effects of using different organic acids on the chemical, microbiological and sensorial properties of marinated Baltic herring. Nora Logrén, my co-supervisor, took part in the previous study and, besides guiding and helping me with the laboratory work, also conducted the sensorial analysis tests of this study as part of her PhD thesis.

The work in this thesis is based on the hypothesis that the "chemical cooking" achieved when marinating raw fish depends mainly on physico-chemical parameters like acid and salt concentrations, time and temperature and not on which acid is used. Obviously, different organic acids have different flavour profiles and properties that might lead to distinct flavour, chemical and microbiological profiles of the final products, and investigating those is part of this work.

Also, this work aims to explore how the use of less strong and less sour flavoured acids may allow to decrease the sugar content in Baltic herring marinades (it is about 20%(w/w) in the commercial products). This could be a good starting point into the development of new, organoleptically different products that can possibly attract new, younger and more health-conscious consumers in the future.

LITERATURE REVIEW | 2

2.1 Baltic Herring and its history in Finland

Baltic herring (*Clupea harengus membras*) is a subspecies of Atlantic herring (Clupea harengus) that adapted to the brackish and low-salinity waters of the Baltic Sea [5]. Compared to Atlantic herring, it is a smaller, leaner fish with less fat content. It grows to 15-20 cm in about 3-4 years but some specimens can exceed 30 cm and half a kilo in weight [6, 7].

Herring is a pelagic[1] fish that migrates during its life cycle. Large shoals of Baltic herring roam open waters in search of food and can be found everywhere from the Danish coast to the Gulf of Bothnia. Baltic herring spend the winter out in the deeper sea but form large, dense flocks that come to spawn near the coast in the Spring/Summer. Most herring off the coast of Finland are the so-called "Spring spawners" and spawn in May-June, with only a small proportion spawning in Autumn.

Baltic Herring is Finland's most commercially exploited fish species, with 92000 tonnes caught in 2020, from which only one fourth were for human consumption [8].

Despite the clear definition of Baltic herring given above, researching about the processing methods led to the discovery that within the fishing industry the distinction between Baltic and Atlantic Herring actually varies according to the country; in Finland differentiation between the fish is based on fat content (it is Baltic herring if fat content is less than 10% and Atlantic herring if over 10%) whereas for example in Sweden the distinction is based on fishing ground: Baltic herring is caught in the Baltic Sea east of an imaginary line connecting Öland's bridge (Kalmar) to Cape Rozewie on the coast of Poland (Figure 2.1); the same fish is Atlantic herring if caught west of this line [7, 9, 10, 11]. According to Matti Ruska from Suomen Sillikonttori Ltd., this discrepancy negatively affects the competitiveness of the Finnish fish industry (personal communication, October 2021).

Within the fish processing industry, herring is categorised according to its fat content and development stage of its gonads (which vary with season and fishing ground), and these are the main factors determining the types of products herring can be transformed into and their yield [9].

[1] Pelagic means that it swims neither near the surface nor near the bottom.

Figure 2.1 - Different areas of the Baltic sea and the imaginary line between Öland and Cape Rozewie, east of which caught herring is Atlantic herring and west of which is Baltic herring.
Adapted from "Map of the Baltic Sea" by Norman Einstein, used under CC BY-SA 3.0 [12]

The Finnish name for Baltic herring - silakka -derives from the Swedish words sillaka and sillake, which mean the salted water intended for the preservation of herring. The Swedish word silla means soaking, brine or salted water. Originally, any salted fish was called silakka, but over time it began to mean the name of the fish most commonly used as a raw material for salted fish in Finland - Baltic herring [7].

Being a migrating fish, herring was available at the coasts of the Baltic sea countries during the summer and then disappeared for long periods of time. It was so important for the people's livelihoods that, according to legislation dating from the 12th century, the Pope gave a special authorization to allow fishing of herring every weekday including Sunday [13]. Salt curing[2] was the main method used to preserve such vast amounts of herring, allowing it to be stored for longer periods and also transported to inland locations. Fish drying was common at the time but the local weather conditions were not optimal for this method and drying did not suit such fatty fish [14].

In Finland, salt cured Baltic herring was one of the few Finnish exports to the Hanseatic countries during the Middle Ages, being well established in the local fishing and food culture [15,16]. Salted Baltic herring also played an important role as a protein source during all the wars Finland was involved in during the last century, for being an inexpensive and widely available meat replacement.

[2] Salt cured or Barrel-salt ripened herring is a traditional process where beheaded herring is packed with salt and sugar in closed barrels and matured for 3-6 months at refrigeration temperatures. The ripening is mostly carried out by the activity of endogenous proteolytic enzymes from the internal organs of the fish.[15]

In the 15th century, the monopoly in herring trade shifted from the Hanseatic League to Germany and the Netherlands, as a consequence of the herring spawning grounds shifting westwards. The Dutch were a seafaring nation and preserved herring by curing in brine and smoking. The process was improved in the 14th - 15th century, first by adding vinegar to the brine. Then herbs and spices were also added, improving both the flavour and the longevity of the products. Vinegar pickled and marinated herring has since then become a staple food in Northern Europe, with each country developing its own methods and traditions [13, 14].

In Finland, pickling and marinating with vinegar, sugar and spices are the legacy of Swedish dominance and Scandinavian influence. Over time, Baltic herring salting was replaced by smoking, pickling and, with the emergence of the cold-chain, also became available fresh and frozen. Commercial production of fish half-preserves in Finland started in the 1960's [17, 7] and nowadays salting in brine is mainly used in the production of delicacy products of the fattier relative, Atlantic herring, caught in the North sea.

Still today, markets dedicated to selling Baltic herring products take place along the Finnish coastal cities in the Autumn. Some have a long tradition, like the Turku market (Figure 2.2) dating back to 1636 and the Helsinki Baltic herring market taking place since 1743 [7].

Figure 2.2 - Baltic herring market in the city of Turku, 1924 [18]

2.2 Definition of fish marinades

In a publication dating from 1965, Meyer defines fish marinades as "products consisting of fresh, frozen or salted fish processed by treatment with edible acids and salt with the use of adjuvants for seasoning and bleaching and put up in brines, sauces, creams, mayonnaise, remoulade or oil" [19]. Marinades are products suitable for consumption without any thermal treatment and have an extended, but still limited, shelf life. The preserving principle is the combination of acid and salt which, by lowering the pH and increasing ionic strength [20], inhibits the growth of spoilage microorganisms and most enzymatic activity. Keeping the marinated products at low temperature allows the reduction of acid and salt concentrations to more palatable levels and extends their shelf-life for several months [21, 19]. Fish marinades have characteristic flavour and texture properties that develop during both the processing and storage stages (mainly due to specific enzymatic action), resulting in products rated as delicacies in many European countries [22, 19].

Fish marinades are categorised as semi-preserves. According to the Food and Agriculture Organization of the United Nations (FAO), these are fish products with no heat-treatment applied during processing or in the preparation before consumption, with >6% water phase salt (WPS) or a pH <5.0 and requiring a storage temperature < 10°C. They may have a shelf life of 6 months or more [23].

2.3 The marinating process

2.3.1 Terminology

Before discussing the process for producing herring marinades it is important to mention that, in the literature, the terms salting, curing, brining, pickling and marinating are used differently by each author; they might refer to a particular stage of the process, more than one stage, the whole process or not be clear what is the exact meaning in terms of process. Again, this requires extra care when taking conclusions from the literature.

In the literature, while **brining** usually refers to a solution of only salt and water where the fish is immersed for a short time as a pre-treatment before further processing, **pickling** means that the raw material is stored for a longer period in the salt solution, that may or may not have an acid added to it [24]. The term **curing** always implies the use of salt. **Marinating** can refer to the whole process of producing the fish marinades, to the stage where fish is treated with acid and salt or to the final step of storing the fish in a spiced / seasoned liquid (often containing an acid) that is the final product.

Most of the marinated herring products are produced in a two-step process; in the first step the raw fish is immersed in a strong solution of salt and acetic acid for a period of 1-3 weeks and in the second step the fish is packed in a marinade where it will mature and then be ready for consumption [19, 21, 22, 25, 26].

Even if this study concerns only Baltic herring and its processing by means of treatment with an acid and salt solution, it is worth noting that the general term “marinated herring” refers to any product where herring - Baltic or Atlantic - has been treated with a non-thermal method and is marinated in its final stage. Baltic herring, due to its low fat content, is nowadays only used in the production of marinated products by processing with acetic acid and salt in the first stage and marinating in the second.. Atlantic herring marinades can be produced the same way (using fish of lower fat content) or can be made by using salted herring (which is a whole different process) in the marinating stage..

Along this thesis and in order to be clear about which stage of the process is being addressed, **pickling** will refer to a first step where the raw fish is immersed in a solution of salt and acid and **marinating** will mostly refer to the second step of the process, where the pickled fish is immersed in a solution of acid and sugar, although might also be used to refer to the whole process of producing marinated fish.

2.3.2 Research of the processes used in the production of Marinated Baltic Herring

One of the objectives of this literature review was to investigate which factors affect the quality of the final marinated fish products and the process method in itself, so that the experiments could be conducted following an established commercial procedure. One major problem encountered was the lack of published information about industrial methods and processes used in marinated herring production. Specific to Finland, most that could be found were household recipes for marinated Baltic herring, which have a very short shelf life and must be consumed within a few days of production. These recipes were not comparable to industrial ones and therefore not useful for this study.

Nevertheless, some of the processing methods and conditions used in other countries are described and/or can be inferred from published literature. Sillikontori, the company that collaborated in this study, shared some knowledge about the process and commented on the experimental conditions chosen for the study but understandably without revealing the specific conditions used in their own process.

As previously said, the production of marinated Baltic herring is generally a two stage process, consisting of pickling followed by marinating. Literature review revealed that most studies focus solely on the first part of the process - pickling - as it is the critical step of the process where the fish undergoes “chemical cooking” and its properties at the end (pH, salt content, texture, colour and flavour) define the quality of the product and possibilities for further processing.

The importance of the pickling step is reflected in the industry, where some of the big fish processing companies in Germany, Sweden and Denmark focus on producing pickled herring in addition to processing the fresh herring into fillets and other fresh and frozen products. These semi-manufactured products are sold further to smaller companies that do the final marinating of the pickled fish in their own marinades and sell them to the final customers [26, 27, 28]. In

Finland, some small companies also buy the ready pickled fish from these big companies abroad.

The following analysis of the different methods used in marinating herring will be grouped by country, as it is clear that the local traditional methods have determined how the marinated fish product is still produced nowadays.

Finland

Sillikonttori - the company that collaborated in this study - specialises in the production of high quality, traditional Atlantic herring products like salted Atlantic herring and Atlantic herring marinades as well as Baltic herring marinades. The company uses MSC certified Baltic herring caught in the Bothnian Sea and all processing and production is done in Finland [87]. Sillikonttori's Baltic herring marinades production follows a two-stage procedure where the fresh fish fillets are first pickled at the fish processing company to whom Sillikonttori provides the pickling solution. After this first stage, the barrels containing the pickled fish are transported to Sillikonttori's premises and the second stage of the process takes place: the pickled fish fillets are packed into containers and covered with a marinade; different marinades give origin to a great diversity of marinated products ready to go to the market [7]. Even if the actual acetic acid and salt concentrations used in the process were not disclosed by the company, approval was given to the values used in the experimental part of this study.

Germany

Dating from 1965, Meyer [19] wrote a comprehensive chapter on fish marinades still referred to in actual research literature. The author describes the main types of German herring marinades, the production methods and chemical and biological processes involved. It is mentioned that the marinades can be produced either from pickled or salted herring and goes to the detail of providing formulas to calculate the acetic acid amount required in the pickling solutions depending on the water and protein content of the fish, as well as the fish to solution ratio to be used. A fat content of 10% is mentioned as the minimum acceptable for the production of herring marinades. He mentions that the pickling process takes between 3-7 days at a temperature of 10-12C and that the marinades or "cover solutions" should be 1-2% in acetic acid and 2-4% in salt.

More recently in 2002, Karl and Münkner [29] investigated the processing possibilities of Baltic Herring during spawning season and concluded that the low fat content is a limitation to the use of Baltic herring caught in this season in the production of traditional herring products. They mention that 8% fat is the minimum to produce herring marinades while at least 10-12% is necessary for preserving by salting.

Another reference to the German production method is given by Laub-Ekgreen [30], who mentions that the "typical German production" method of herring marinades consists of directly submerging the fish into a solution of salt and acetic acid, whereas in Denmark the most common procedure involves a brining step (immersion in a solution of salt and water) before pickling.

Scandinavia

In a Paper dated from 1971, McLay [21], attempts at producing herring marinades with a “milder cure” than the ones locally produced in the UK He mentions that the industrial production process in the UK is secret and unknown and follows what he calls the “scandinavian process”, where marinated herring is produced in a two-stage process. First the fish is immersed for four weeks in strong acetic acid and salt solution (7% acid and 14% salt) in the proportion of 4 parts of fish to 3 parts of solution. Second, the pickled fish is drained, packed into glass jars and covered with a solution 1-2% in acetic acid and 2-4% salt and flavoured with sugar and spices; a fish to marinade ratio of 1:1 is used..

From his experiments McLay concluded that herring marinated in 3% and 4% acetic acid and 10% salt were the ones better rated by a tasting panel. He also found that, to avoid spoilage of the fish during pickling, the minimum concentration of acid and salt during pickling had to be 3% and 10%, respectively.

A Danish study by Nielsen [31] on how fishing ground and season influence the sensory properties of marinated herring describes the pickled fish samples being prepared by immersing the herring fillets in a solution of 7% acetic acid and 16% salt (1,5 parts herring to 1 part brine) and stored at 2°C for a minimum of 4 weeks. Instead of marinating, the samples were dewatered in a 1% salt solution for 2 days at 2°C (1 part herring to 1 part brine) before sensory analysis. This process is very similar to the one described by McLay [21].

Also from Denmark, Laub-Ekgreen is the author of a PhD thesis on the optimization of pickled herring production [26]. Part of the study focuses on the brining step, commonly applied in the Danish industry before pickling the fish in a salt and acid solution and how it increases the final yield of pickled herring. The author also states that a fish to solution ratio of 1:1 is standard in the industry for both brining and pickling; the fish is usually brined for 24 hours followed by pickling for a minimum of 35 days. The study compares the effects of different salt and acid concentration (among other variables) in the fish fillets during both brining and pickling, without disclosing the values used in the industry. The values presented in Table 2.1 are the ones presented in the comparative study of the effect of prior brining / no brining on weight change of the pickled fish [29] and might be indicative of the conditions practiced in the industry. Nevertheless, the salt and acid concentrations used in the experiments without brining are significantly different from the ones reported by the other danish authors and there is no explanation about the choice of those values.

From Sweden, Larsson [32] completed her degree project in collaboration with Orkla Foods (Sweden) and studied the effect of different weak organic acids in herring marinades. Fish pickled in acetic acid and salt from a supplier was used in the study and the pickling conditions were not disclosed. The author mentions the fish was marinated according to the “Abba sill på 5 minuter” recipe (not revealed); according to the company’s website [33] this product has a salt content of 3.6% and 26% of sugar. The study mentions that a final pH=4.2 was aimed for all marinades and that these were stored at a temperature of 7-8℃ up to 14 weeks.

Poland

Based in Poland, M. Szymczak is the author with most of the published research about marinated Baltic herring. His studies comprise a wide range of aspects concerning

technological and qualitative aspects affecting the production of marinated herring, from the effect of varying acid and salt concentrations to the activity of cathepsins during the process.

How marinated Baltic herring is commercially produced in Poland can be deduced from the experimental conditions used and references to the "industrial method" given in the author's work [35]. Most of his studies focus on the pickling part of the process, after which the pickled fish is put into jars with the final marinade.
In his studies, the author distinguishes between Baltic and Atlantic herring with the fat content being often mentioned. The pickling conditions used by the author as "standard" for fresh Baltic herring are 5% acetic acid 6% salt, a fish to solution ratio of 1.5:1 and a pickling period of 7 -10 days at 10-12℃ [34, 35, 36, 37]. This process is very similar to the one described by Meyer [19] for the German process, where a higher fish to solution is used and the fish is pickled at higher temperature for a shorter period of time, compared to the Scandinavian methods.

A general process for producing herring marinades is described by Boziaris [22] in a recent book on seafood processing technology. It is a two steps process where herring is first cured in a solution containing 7% acetic acid and 14% salt for 6-12 weeks at 0-4C, using a fish to solution ratio of 1:1 in closed containers. The pickled fish is then packed with a marinade consisting 1-2% acetic acid and 2-4% salt, and stored at 0-3C for a period of 6-12 months. It is also said that the acid and salt concentration in the marinade should be similar to the acid and salt concentration in the fish muscle after the first pickling step.

Table 2.1 resumes the pickling and marinating conditions used in the production of marinated herring found in the literature.

Table 2.1 - Pickling and marinating conditions used in the production of herring marinades found in the literature.

Author (reference)		Meyer [19]	McLay [21]	Szymczak [37,38,39,40]	Boziaris [22]	Nielsen [31]	Larsson [32]	Laub-Ekgreen [26,30]	
country / region		Germany	Scandinavia	Poland		Denmark	Sweden	Denmark	
PICKLING		mentions formulas for calculating the acid amount depending on the water content of the fish and fish:solution ratio; gives some examples						With previous brining in a 13,6% salt solution for 24 hours at 2C; fish: solution = 1:1	no brining
	Acetic acid concentration	5,6%	7%	5%	7%	7%	-	6,5%	5,8%
	Salt concentration	7,6%	14%	6%	14%	16%		4,3%	5,4%
	process temperature	10-12°C	3°C	10°C	0-4°C	2°C		2°C	2°C
	process time	3-7 days	35 days	10 days	6-12 wks	min 4 wks		35 days	35 days
	fish: solution ratio	3:2	4:3	1,5:1	1:1	1,5:1		1:1	1:1
MARINATING	marinade	1-2% AA + 2-4% salt	2% AA + 2-4% salt; fish:marinade = 1:1; temp 3C	-	1-2% AA + 2-4% salt 0-3C for 6-12 months	-	citric, lactic and malic acids used in concentration so that the final pH<4,2; storage at 7-8C for up to 14	-	-
fish		herring with an oil content of 7-18%	fresh BH	herring	AH	Abba pickled Atlantic herring	AH	AH	

AA, acetic acid; AH, Atlantic herring; BH, Baltic herring

2.4 The Role of salt and acid in the marinating process

2.4.1 The structure of fish muscle

Protein is the second major component (16-18%) in fish muscle after water and is classified as sarcoplasmatic, myofibrillar or connective tissue based on its solubility in salt solutions [38].

Sarcoplasmatic proteins are soluble in water and in dilute salt solutions and mostly include enzymes, myoglobin, albumins and antifreeze proteins. They are not a structural part of the muscle tissue but responsible for metabolic activities within the cells [38].

Myofibrillar proteins are the most abundant proteins in fish muscle (65-75%) and are only soluble in solutions of higher ionic strength (>0.5 M). They comprise contractile proteins (such as myosin and actin), regulatory proteins and other minor proteins and are responsible for the sensory attributes relating to the texture of fish [38].
Water solubility of myofibrillar protein varies depending on the temperature, pH, and ionic strength. Extreme pH and high temperature cause protein denaturation resulting in low solubility.

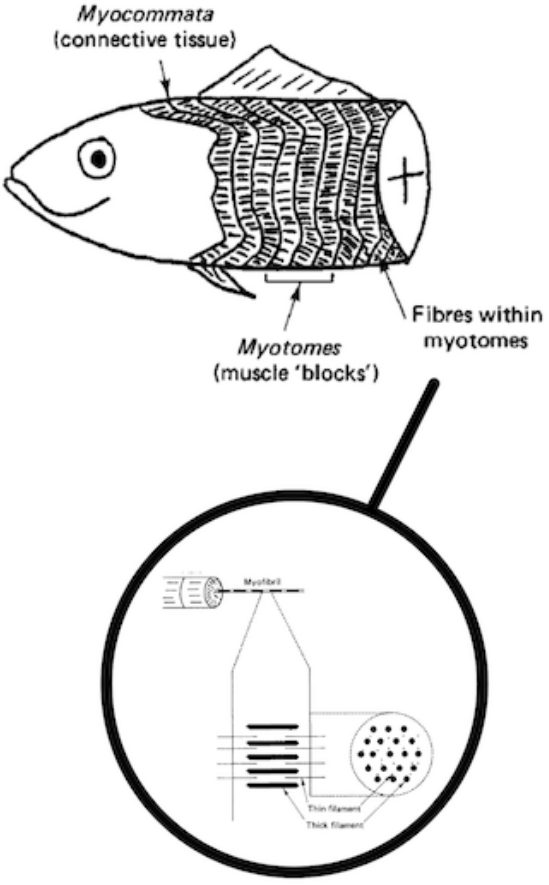

Figure 2.3 - Structure and constituents of fish muscle [39]

Connective tissue constitutes only about 2-3% of the total protein in fish and collagen is its major component. It solubilizes only in conditions of extreme temperature and/or pH, being insoluble in solutions of higher ionic strength. Connective tissue proteins contribute to the meat texture of fish and significantly affect its rheological properties. Denaturation of the connective tissue results in “gaping” of the fish fillets, the phenomenon in which slits appear between the muscle blocks and causes the fish fillets to fall into pieces. This structure can be observed as the flakes that form in cooked fish. This is because, unlike other vertebrates, fish muscles are layered instead of bundled; each layer of muscles (myomere or myotome) is separated from the next one by a sheet of connective tissue (myocommata) [39] , as illustrated in Figure 2.3.

2.4.1 Effect of salt

Marinating fish in sodium chloride and acid causes changes in both the physical-chemical and sensory properties of the muscle. Muscle is constituted mainly by water and proteins and the latter have functional groups that are affected both by pH and ionic strength [39].

When immersed in a salt solution, chlorine ions migrate by diffusion into the fish muscle where they interact with the myofibrillar proteins and affect their solubility; increased solubility results in swelling of the tissue and decreased solubility in aggregation of the proteins with shrinkage of the muscle. Swelling or shrinking depends on the concentration of salt in the muscle water phase which, in turn, is mainly the result of the salt solution concentration but also depends on factors like the fish species and fat content, fish to solution ratio and temperature, among others [40, 41, 42].

Salt brining (immersing the fish in a salt solution) is a common procedure in the fish industry where this phenomenon is explored; the fish gains weight by uptaking salt and water from the solution, resulting in increased yield and improved final texture of the marinated fish products [30].

2.4.2 Effect of acid

The effect of acid on proteins is explained by referring to the protein's isoelectric point (IEP), the pH value at which the protein has neutral charge. When inserting the fish fillets in an acidic solution, the proteins of the muscle become protonated (pH<IEP) resulting in increased electrostatic repulsion between the protein fibres. As a consequence , the structure opens allowing more water to be held in the muscle - it swells. [20, 39]
Note that, since the pH of fish flesh post rigour (6.2-6.8) is greater than its IEP (~ 5.4), initially the muscle actually shrinks as the pH lowers and approaches the IEP; then it starts to swell again as the pH continues to decrease until it reaches a pH of 4, common in fish marinades [39].

Acid also affects the fish muscle’s texture because it dissolves the connective tissue surrounding the muscle fibers, resulting in further swelling and a soft and fragile texture [19, 39].

2.4.2 Effect of acid and salt

The response of fish muscle tissue in salt and acid is different from its response in either salt or acid alone, as illustrated in Figure 2.4. When salt is present, the electrostatic repulsion between the positively charged proteins due to low pH is to some extent cancelled by the negatively charged chlorine ions and the fish muscle tightens-up, resulting in increased firmness [20, 39].

Acetic acid diffuses more rapidly into fish muscle than salt; the rates of diffusion depend on temperature but equilibration times depend mainly on curing time. Salt also prevents the complete dissolution of connective tissue that occurs in acid alone [20]

Szymczak [36] found that increasing acid concentration from 3% to 4% in the acid and salt solution had no significant effect on the content of salt in marinated herring, speculating that the moisture content of marinated products depends mainly on salt concentration in the tissue. On the other hand, increasing the acid to higher concentrations of 8% diminished the muscle water holding capacity and resulted in the release of water, proteins, lipids, free fatty acids and minerals to the solution, reducing both the quality and weight yield of the marinades.

As for salt, increasing its concentration in the pickling solution from 5% to 15% also resulted in lower weight yield of fish, with increased hardness of meat, worsened colour and stronger acidic taste. A decrease in cathepsins activity was also observed with increasing salt concentration [34].

In conclusion, while acid softens the fish muscle, salt hardens it [19]. The moisture content of fish muscle depends on the concentration of salt and acid in the tissue and therefore both are needed to get firm marinated fish fillets [20].

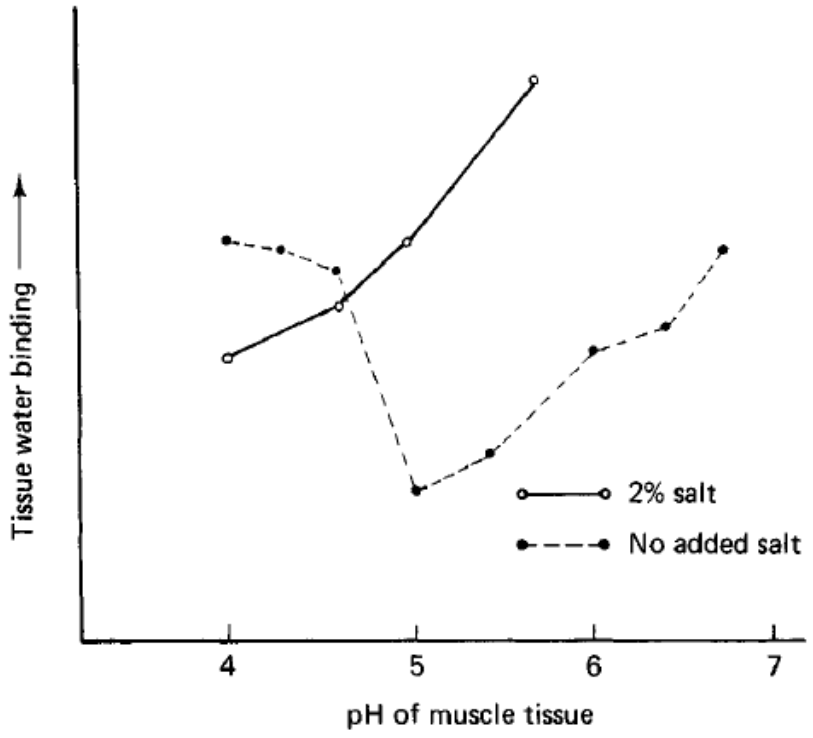

Figure 2.4 - Effect of pH on the water-holding capacity of herring muscle in the presence and absence of salt [39]

2.4.3 Proteolysis

Acid and salt are not the only factors affecting fish muscle texture. Texture of the marinated product will change during time and after salt and acid equilibrium has been established. During marinades storage, the fish muscle becomes softer due to the activity of cathepsins - proteolytic enzymes that occur naturally in fish - that are activated at low pH values and hydrolyse the muscle myofibrils [34, 39].

Besides being responsible for the ripening of fish meat during the pickling process, the activity of these enzymes also contributes to the development of many of the characteristic flavours and aromas of fish marinades, by releasing nitrogen compounds to the marinades [34, 43].

Cathepsin D is the most active protease present in fish marinades and has its highest activity in marinades with pH of 2.5-3.5 and salt concentration of 2.0-2.5% in fish muscle . It is inactivated at high salt concentrations around 12% [34].

2.4.4 Salt, acid and food safety

Fish marinades are not heat treated and instead the products are preserved mainly by the action of low pH, salt and low temperature, all the three combined preventing the growth of pathogenic microorganisms [23].

Acid is the most important component in the marinade when it comes to antimicrobial activity and pH is a decisive control parameter. The capacity of a weak acid to lower the pH of a medium or foodstuff depends on its dissociation constant (K_a) and concentration. Organic weak acids have optimal antimicrobial activity at low pH values, as it is their undissociated and uncharged form that is mostly responsible for their antimicrobial effect. These molecules are able to cross the microorganisms lipidic membrane by effect of concentration gradient and dissociate once inside, lowering the cell's internal pH and disrupting its functioning [44].

Acetic acid fish marinades usually have pH≤4.2 , as it limits the growth of dangerous pathogens that can be a risk in fish production like *Listeria monocytogenes* [23, 45] as well as quality degrading microorganisms (spoilage microorganisms) like lactic acid bacteria, yeasts and moulds [46].

Salt also plays a role in fish marinades safety, namely in guaranteeing the elimination of nematodes like Anisakis larvae, as these are not affected by acid; traditional German and Danish procedures require a pickling time of at least 5 weeks for fish not previously frozen [23, 26, 47].

2.5 Factors affecting the marinating process and quality of marinated Baltic Herring

2.5.1 Marinade ingredients and raw material

All ingredients used in the marinating process should be food grade and without impurities, as the quality of the ingredients affects the quality of the final products. For example, the presence of iron and copper ions in the pickling salt can induce oxidation during the marinating process and impart a rancid flavour to the product [48].

When it comes to sugar, it is used in marinades mainly due to its sweet taste and to counterbalance the strong flavour and sourness imparted by acetic acid; it seems to be a cultural preference as it is predominantly used in the Nordic countries [22]. There is little information in the literature on its role in fish marinades but, like for salt, the use of sugar also extends the shelf-life of products by reducing water activity in foods. In a study comparing the effect of salt (6,5% (w/v)) and salt-sugar solutions (concentration of 6.5%(w/v) and 5%(w/v), respectively) on the quality and shelf life of Atlantic bonito marinades, it was concluded that the fish preserved in the salt and sugar solution had a better overall quality in terms of texture, appearance, and odour and safe microbiological quality [49].
Besides acid, salt and other ingredients that might be used in the marinades, quality of the fish is the main and most important factor affecting the quality of the marinated products.
Quality of the fish is determined already by the catching method and the handling procedures, with low processing and transportation temperatures being key to prevent the progress of lipid oxidation [50].

Regarding the chemical composition of fish, lipid content of the fish muscle is one of the most important quality parameters in the herring industry. High lipid content gives fillets that are juicy and have a fatty mouthfeel, while low lipid content is associated with firmness and grittiness [50]. Fat content follows the cycle of feeding and spawning of Baltic fish and therefore varies according to the catching season; Baltic herring occurs in two main groups depending on the spawning period: Spring herrings and Autumn herrings [9].

In addition to affecting the lipid content of herring, spawning season also affects the activity of the hydrolytic enzymes in muscle and gastrointestinal tract. Fat content is highest before spawning; during spawning fat content decreases but there is high muscle enzymatic activity, making it ideal for the production of marinades. After spawning herring is very lean and its meat hard, diminishing its processing possibilities [9, 29, 43]. Herring with high fat content has the best taste and highest yield due to lowest loss of water [9].

The influence of freezing on fish muscle proteins and how it affects the palatability of the final products has been described in several publications [26, 43, 45]. Freezing causes damage to cellular membranes and proteins, diminishing the water holding capacity of the fish muscle, resulting in lower production yield as well as harder texture. It also affects the activity of proteolytic enzymes, which imply changes on meat texture and sensory quality of fish marinades.

2.5.2 Technological Factors

Production yield is a very important factor in the fish industry as it directly affects the final cost of the products to the consumer. Therefore, weight loss is often studied along with other quality parameters in fish marinades.

In the production of marinades, besides the effect of acid and salt concentration as discussed above, there are other technological factors of importance, like fish to solution ratio, processing times and processing temperatures. The choice of these parameters seems to be mainly cultural, as described before, and the different methods reflect how these parameters are interrelated.
These parameters are also referred to in the literature and some general conclusions can be taken as, for example, that increasing fish to solution ratio results in lower salt and acid concentrations in the fish muscle [20, 26, 41]. Temperature affects both the diffusion rates of salt and acid into the fish muscle and the activity of cathepsins (proteolytic enzymes responsible for the ripening and development of fish marinades), with the use of higher temperatures shortening the pickling time of marinades [20, 43].

2.6 Use of acids other than acetic acid for marinating

Organic acids are widely present in nature and are used to marinate meat and fish in many cultures around the world. Citric acid is present in all citrus fruits and is commonly used in meat marinades but also in the confection of ceviche, a traditional dish from Peru where raw fish is "cooked" by marinating it in lime or lemon juice. Wine - constituted mostly by malic and tartaric acids - is also widely used to marinate meat and fish prior to cooking in wine producing countries. Yoghurt, with its tangy taste given by lactic acid, is traditionally used to marinate chicken in Asia and the Middle East.
In Europe, acetic acid is the acid traditionally used in fish marinades, be it in *boquerones* (marinated fresh anchovy fillets) in Spain or in herring marinades in the north of Europe.

The use of acetic, citric and lactic acids in marinades to tenderise meat is fairly well studied, but the use of acids other than acetic in the production of fish marinades is rather scarce, even though the use of all natural organic acids in marinades has been allowed in the EU since 1996 [52].

As early as 1965, Meyer mentioned researching the antimicrobial effect of acetic, citric and tartaric acids in herring marinades and concluded that acetic acid is the most effective [19]. Later, Mclay wrote that lactic or tartaric acid could be used to replace part of the acetic acid in marinated fish products so their taste would be less astringent [21].
More recently, Babikova *et al.* [53] used cider vinegar (malic acid) to partially replace acetic acid in Irish sprat marinades, Šimat *et al.* [54] compared the use of lemon juice with acetic acid for marinating anchovies; Poligne *et al.* [52] researched the effects of using gluconic acid in marinating anchovies and there is a Master thesis work where citric, lactic and malic acids were used to marinate Atlantic herring previously pickled in acetic acid [32].
These studies [52], [53] and [54] used fish other than herring, the pickling times were very short (maximum of 7 days) and the effects of lemon juice and apple vinegar were studied instead of citric and malic acids. Therefore these studies have a very limited contribution to this work.

Regarding the use of gluconic acid, Poligne *et al.* [52] concluded that this acid alone does not have enough bactericidal effect in fish marinades, making it an unsuitable choice for the present study.

The results obtained by Logrén *et al.* [4] for this project were the starting point and the most important contribution to this thesis. In this previous study citric, lactic, malic and tartaric acids were used to produce marinated Baltic herring and the final products were studied both organoleptically and in terms of chemical composition and lipid oxidation. All acids produced Baltic herring marinades with high microbiological quality and with milder sensory profiles than the traditional acetic acid ones. Moisture content increased and protein content decreased for all acids during a 4 month storage period; a decrease in lipid content was only observed in acetic and malic acid samples. Citric acid sample had the highest level of lipid oxidation and the tartaric acid sample the lowest.

2.7 Objectives of the current study

This thesis work had the main objective to further research the effectiveness of three chosen weak organic acids - citric, lactic and tartaric - in the production of Baltic herring marinades. In order to obtain results meaningful to the local fish industry, it was crucial to research and implement conditions and processes of production as similar as possible to the ones used in the industry. Therefore securing a collaboration with a company representative of the industry that would be willing to share knowledge and facilitate the production of the marinades in a factory setting was extremely valuable.

Most published studies on fish marinades focus solely on the first part of the process, the pickling, and very little is revealed about the preparation of the marinade solutions or their properties after storage, so this study also aimed at gathering some knowledge about this part of the process. .

Texture is one of the parameters in marinated fish products which influences the most consumer acceptance and in the previous study it was found that the marinated fish fillets were extremely soft compared to the commercial products; therefore improving the texture by optimising the salt concentration and pickling time was also aimed with this work.

Last but not least, it was thought that the use of organic acids that produce milder, less sour fish marinades could be explored in order to reduce the sugar content in these products, as it is quite high (about 20% (w/w)) in store-bought products. The final objective was to produce a fish marinade using citric, lactic or tartaric acid with less sugar that could be later brought to consumer studies and evaluated against the traditional products.

3.1 Experimental Design

3.1.1 Production process of marinated Baltic herring

The marinated Baltic herring was produced using a two-step method, which comprises first pickling the fish by immersing it in a salt and acid solution for 35 days followed by draining the fish and immersing it in a solution of acid and sugar (equivalent to the commercial spiced solution) for a minimum of 4 weeks.

In the context of this study, **pickling** refers to the first step of the marinated fish production process where the fish is treated in a solution of water, acid and salt and **marinating** refers to the subsequent step of immersing the pickled fish in a solution of water, acid and sugar. Marinated fish means fish that has been through the whole process of pickling and marinating. The process is summarised in Figure 3.1.

Figure 3.1 - Production of marinated Baltic herring in the experiments

3.1.2 Study timeline

This study comprised two sets of experiments, the first one designated as Pilot Studies and the the main experiment as Factory Study. The Pilot Studies took place at University of Turku (UTU) Functional Foods Forum's (FFF) test kitchen between December 2021 and April 2022 and the final Factory Study was carried out at Suomen Sillikonttori Oy's premises in Röölä between March and May 2022. The Pilot Studies consisted of two sets of preliminary experiments and had the objective of getting acquainted with the process, adjusting the acid and salt concentrations and choosing the acids for the final experiment (Factory Study) at the factory. The study timeline is depicted in Figure 3.2.

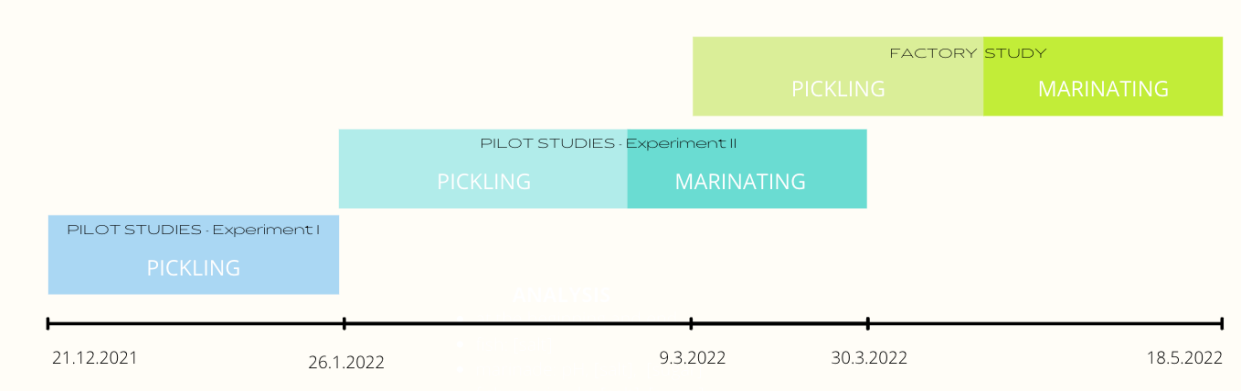

Figure 3.2 - Timeline for the Pilot and Factory Study experiments

3.2 Reagents and Materials

3.2.1 Raw material

The amount of fish used in each experiment was calculated based on the final number of marinated samples needed to be produced, taking into account the samples used for chemical, microbiological and sensorial analysis. For Factory Study the number of samples needed to take part in possible consumer studies was also accounted in.

Pilot Studies

For Pilot Studies Experiments I and II 18 kg and 30 kg, respectively, of fresh skinned Baltic Herring fillets were ordered from Kalaset Ltd. (Uusikaupunki, Finland). The fish was caught in the southern part of the Gulf of Bothnia on 21.12.2021 and 26.01.2022, respectively, and delivered to FFF's laboratory packed in styrofoam boxes with ice.

Factory Studies

For the Factory Study, 50 kgs of fresh skinned Baltic Herring fillets packed in styrofoam boxes with ice were delivered by Kalaset Ltd. (Uusikaupunki, Finland) to Sillikonttori's premises. The fish was caught on 9.3.2022 in the southern part of the Gulf of Bothnia.

3.2.2 Other reagents, materials and equipment

All reagents and materials used in this work are listed in Table 3.1 and Table 3.2, respectively.

Table 3.1 - Reagents used in the Pilot and Factory studies.

Reagent	Manufacturer	Purity	Observations
Acetic acid	Rajamäen (Berner Ltd., Helsinki, Finland)	10% (w/w)	Liquid
Citric acid monohydrate (E330)	Vinoferm (Brouwlan, Beverlo, Belgium)	91.43%	Solid. Used in Pilot Studies Experiments I and II
Citric acid monohydrate (E330)	Alfa Aesar (Thermo Fischer GmbH, Kandel, Germany)	>99%	Solid. Used in Factory Study marinades
Lactic acid	Vinoferm (Brouwlan, Beverlo, Belgium)	80%	Liquid
Tartaric acid (E334)	Vinoferm (Brouwlan, Beverlo, Belgium)	>99.8%	Solid
Sodium benzoate	Thermo Scientific Chemicals (Thermo Fisher, Germany)	99%	Solid
Salt	Meira hienosuola (Meira, Helsinki, Finland)		Solid. Used in Pilot Studies Experiments I and II
Salt	DiSal refined salt (Unión Salinera de España SA, Madrid, Spain)	100%	Solid. Used in Factory Study
Water	Activated carbon filtered water available at Functional Foods Forum's test kitchen		Used in all samples prepared in Pilot Studies; Used in preparation of samples for salt and brix analysis.
Water, MQ			Used to clean Brix reader.
Water, tap			Used in the production of all Factory study samples

Table 3.2 - Materials and equipment used in the Pilot and Factory studies.

Material / equipment	Manufacturer	Sensitivity	Local of use
Filter paper	Fisher Scientific QL100		
Precision balance / scale	PB-3002-S (Mettler Toledo)	0.01g - 3100g; e=0.01g	Functional Foods Forum's test kitchen
scale	ULTRA–30 (Ultraship; USA)	0-14 kg; e=5g	Function Foods Forum's test kitchen
Bench scale	SK-5000WP (A&D, Japan)	100g-5000g; e=2g	Suomen Silikonttori Ltd.
Bench scale	IND236 (Mettler Toledo)	100g-15kg; e=5g	Suomen Silikonttori Ltd.
Hand Blender	Bamix (Switzerland)		Functional Foods Forum's test kitchen
pH meter	IQ150 pH meter (Spectrum Technologies Inc.,USA); pHenomenal 220 electrode (VWR International, LLC, USA)		Functional Foods Forum's test kitchen
Salt meter	DTM-20, (DAEYOON SCALE INDUSTRIAL, South Korea)		Functional Foods Forum's test kitchen
Brix meter	MA871 Digital Refractometer (Milwaukee, Hungary)		Functional Foods Forum's test kitchen

3.3 Sample preparation

3.3.1. Pilot Studies

The pickling was done in bulk for each acid, that is, the fish was pickled all at once in one bucket. First, the pickling solutions were prepared by dissolving the salt in water and then the acid was added. The fish was weighed into food-grade plastic buckets, the weighed pickling solutions added and mixed well. The buckets were closed with tight fitting lids and stored in the cold room.

Marinades were made by first preparing stock solutions of acid and sodium benzoate for each acid. These were then used to prepare the several marinades with different sugar content by adding the required amounts of sugar.

After 35 days of pickling, the fish was drained using kitchen sieves and weighed. The draining time was recorded for each acid using a kitchen timer. After that, predetermined amounts of fish were weighed into labelled jars and the corresponding marinade added; jars were then closed and stored in the cold room.

Two sets of consecutive experiments - Experiment I and Experiment II- were carried out in Pilot Studies. For the second set, Experiment IIA and Experiment IIB were run simultaneously.

Experiment I

Pickling solutions 14% (w/v) in salt were prepared with acetic, citric, lactic and tartaric acids with concentrations of 5%, 2%, 2% and 2.15% (w/v), respectively. The fish to pickling solution ratio was 1:1 and the fish was pickled for 35 days at 4-5°C. These samples were not marinated.

Experiment IIA

Pickling solutions with 14% (w/v) salt and 5% (w/v) acid were prepared with acetic, citric, lactic and tartaric acids. The fish to pickling solution ratio was 1:1 and the fish was pickled for 35 days at 4-5°C. These samples were further marinated.

Figures 3.3A and 3.3B - Pilot Studies: preparation of the buckets with fresh fish and pickling solutions

The marinating solutions were prepared with acetic, citric and lactic acids in the concentrations of 0.67%, 0.17% and 0.16%(w/v), respectively; these were determined experimentally in the previous study [4] and corresponded to the minimum acid concentration needed to ensure a pH<4.2 at the end of the process. Sodium benzoate was added in the concentration of 0.15% (w/v) to all marinades.

Acetic acid pickled fish was marinated in solutions of acetic, citric and lactic acid containing 18%, 10% and 10% (w/v) of sugar, respectively. Citric and lactic acid pickled fish were marinated in solutions of the same acid containing 5%, 10% and 18% (w/v) of sugar. The ratio of fish to marinade solution was 1:1 (w/w) for all samples. The jars were closed and stored in the cold room at 4-5°C. Figure 3.4 represents the different marinades prepared in Experiment IIA.

Figure 3.4 - Marinades produced in Experiment IIA.
AA, acetic acid; CA, citric acid; LA, lactic acid. All concentrations are (w/v).

Experiment IIB

Pickling solutions of acetic, citric, lactic and tartaric acids (5%, 2%, 2% and 2.15% (w/v), respectively) were prepared with 14% (w/v) salt.
The fish was weighed into food-grade plastic buckets and the pickling solution was added using a fish to solution ratio of 1:1.5 (w/w). The buckets were closed and stored in the cold room at 4-5°C for 35 days. These samples were not marinated.

Figure 3.5 resumes the experiments, experimental conditions and analysis performed for the Pilot Studies

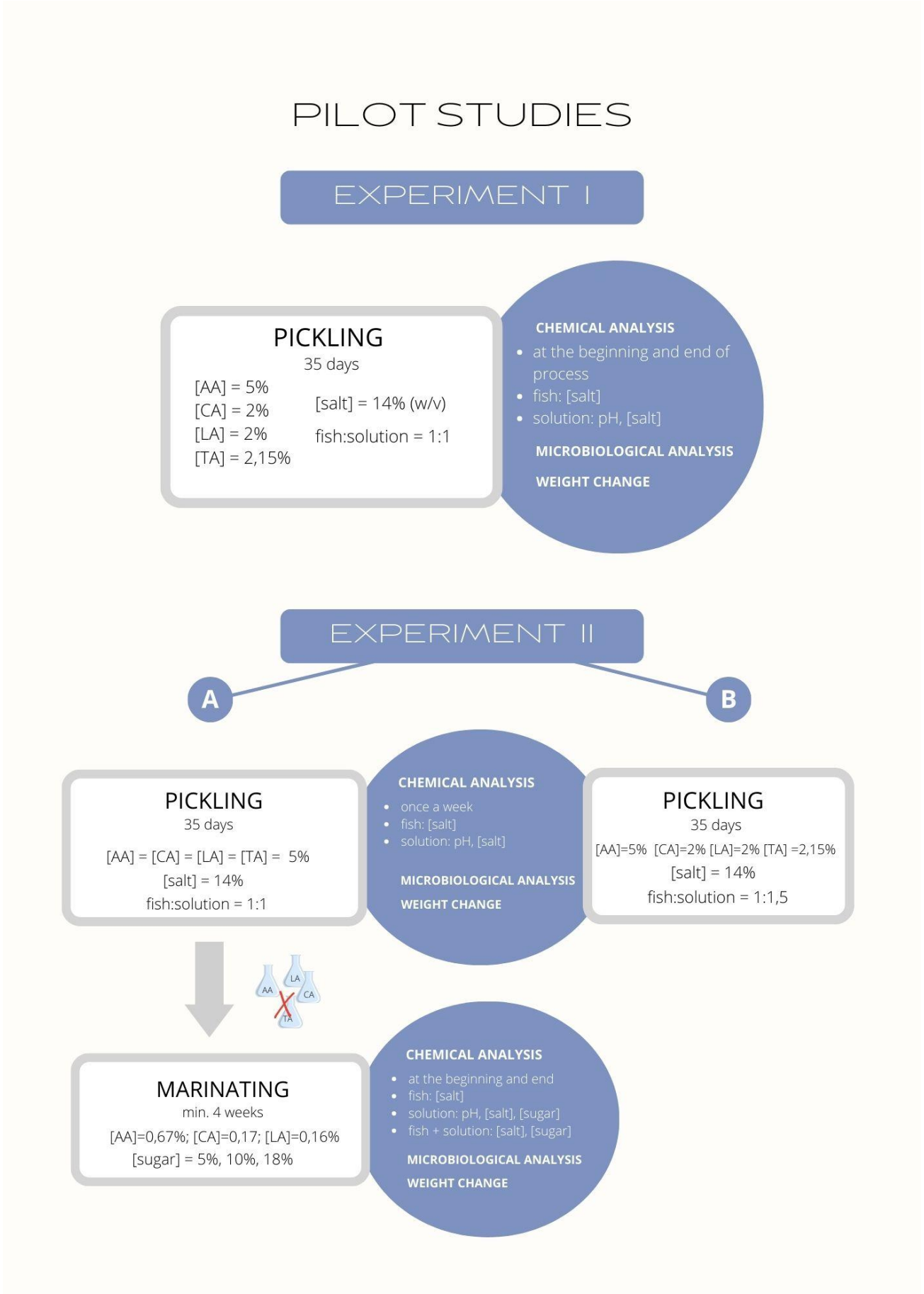

Figure 3.5 - Schematic resume of the Pilot Studies experiments, experimental conditions and analysis performed. Square brackets denote concentration; all concentrations are (w/v); ratios are (w:w). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid.

3.3.2 Factory Study

All pickling and marinating procedures were carried out at Suomen Sillikonttori Ltd.'s factory with the exception of storage of the marinated samples, which were taken to FFF's cold room after being produced.

The pickling solutions were prepared by dissolving salt in water and then adding the acid. The fish was weighed into food-grade buckets and the weighed pickling solution added. The buckets were closed with tight lids and stored at the factory's cold storage facilities.

Marinades were made by first preparing, for each acid, a solution of acid and sodium benzoate. The required amount of sugar was then added to obtain marinades of different sugar concentration.

After 35 days of pickling, the fish was drained over industrial grates and weighed. The fish and marinade were put into the jars by the professional factory workers after gauging the right weight of fish and marinade with a scale for the first samples; the marinades were produced using the vacuum production line where the jars were closed, vacuum sealed and then labelled.

The pickling solution concentration was 6% (w/v) for all acids, the salt concentration 14% (w/v) and the fish to pickling solution ratio was 1:1. The fish was stored for 35 days at -2 °C.

After pickling, the fish were marinated in a solution of the same acid as the pickling solution, except for acetic acid pickled fish that was also marinated in citric and lactic acids. The concentration of the acids used in the marinades was the same as used in the Pilot Studies Experiment IIA. Acetic acid pickled fish was marinated in three different solutions: one using acetic acid and with sugar concentration of 56% (w/v) and in citric and lactic acid solutions, both with a sugar concentration of 33 % (w/v). For citric and lactic acid pickled fish, two different marinades were produced for each, one containing 56% (w/v) sugar and the other 33% (w/v). Marinade jars were stored at 4-5°C. The marinades prepared in Factory Studies are represented in Figure 3.6 and Figure 3.7. summarizes the whole production process for Factory Studies.

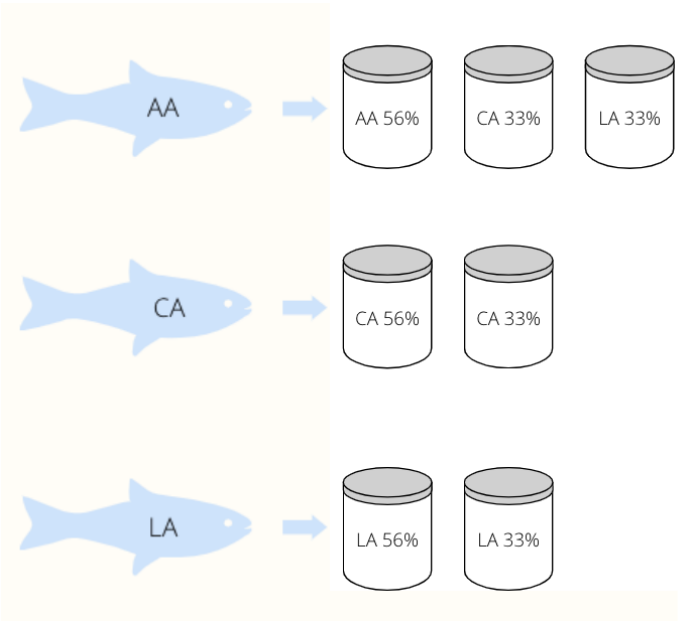

Figure 3.6 - Marinades produced for Factory Study.
AA, acetic acid; CA, citric acid; LA, lactic acid. All concentrations are (w/v).

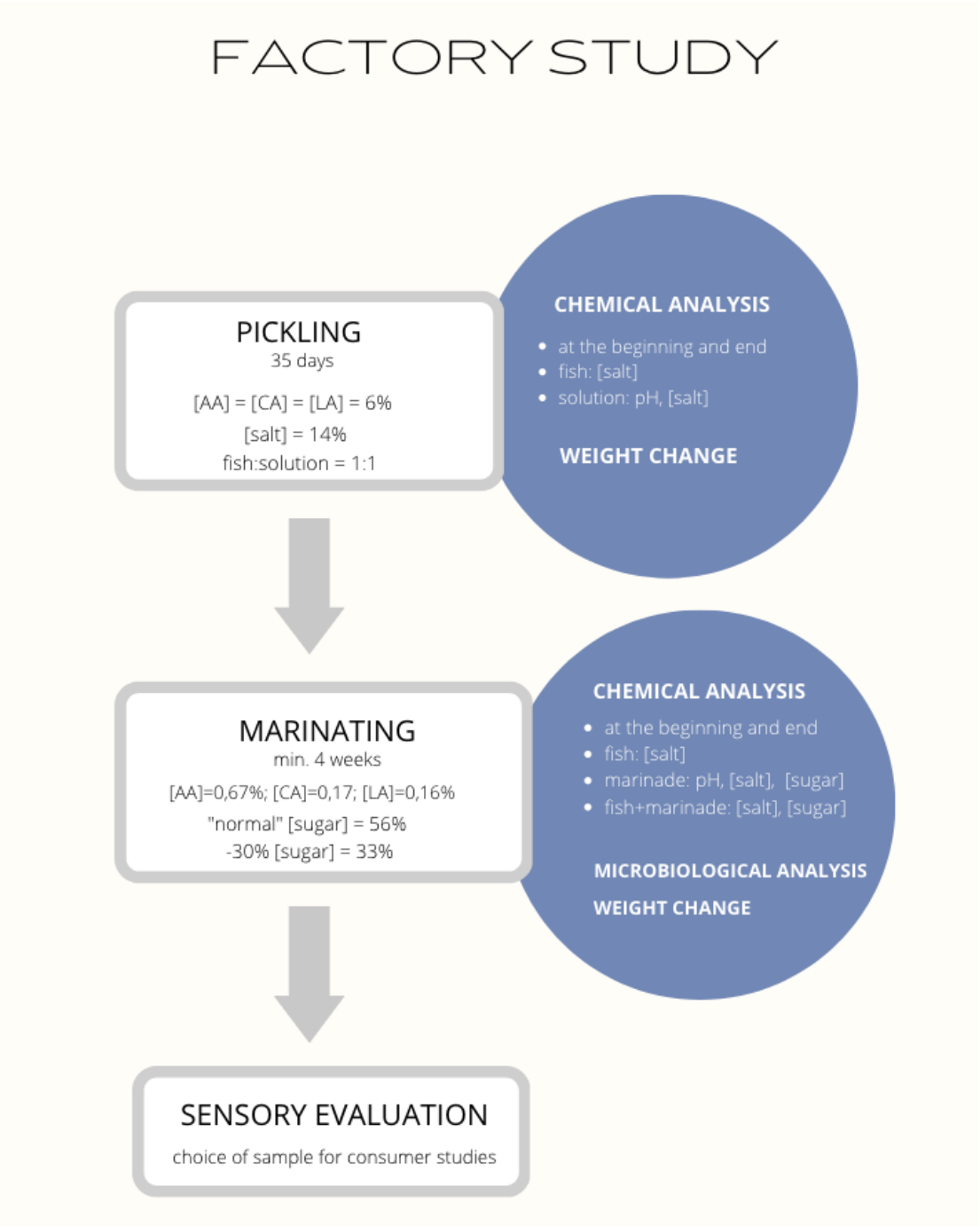

Figure 3.7 - Schematic resume of the Factory Study experiments, experimental conditions and analysis performed.
Square brackets denote concentration; all concentrations are (w/v); ratios are (w:w). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid.

3.4 Methods of Analysis

During this study the fish as well as pickling and marinating solutions were characterised by means of chemical analysis (pH, salt and sugar concentration) and evaluated sensorially. Both pickled and marinated final products were subjected to microbiological analysis to ensure their safety before tasting and sensorial analysis.

Because the nutritional values listed in commercial marinades of fish products refer to the total of fish plus marinade contained in the jars, salt and brix were analysed both in the marinade and in fish plus marinade.

The weight of the fish was taken before and after both pickling and marinating to calculate the yield of the process, an important economical factor for the industry.

Pilot Studies

In the first experiment, pH and salt concentration of the pickling solutions was measured from the pickling solution before the addition of fish and at the end of the pickling process (35 days). At the end of the process, salt concentration in the fish muscle was measured and the product was subjected to microbiological analysis. The fish was weighed before and after pickling.

In Experiment IIA and IIB the evolution of the pickling process was followed by measuring the pH and salt concentration of the pickling solution and salt concentration in fish muscle once a week. The pickled fish was weighed at the end of the process and submitted for microbiological analyses.

pH and sugar content were analysed from the marinating solutions of Experiment IIA before the addition of the fish. After 4 weeks of storage, pH, salt and sugar contents were measured from the marinades, salt and sugar content from the fish plus marinade and salt concentration was measured in fish muscle. The weight of the marinated fish was also measured. Microbiological analysis of the samples was performed after 2.5 weeks of storage, so the results would be available before the end of the four weeks storage period.

Factory Study

For the pickling process, pH and salt content were analysed from the pickling solutions before the addition of the fish. pH, salt content of the solution and in the fish muscle was measured at the end of the process (35 days). The weight of the fish was measured before and after pickling. As several buckets for each acid were used, samples of fish were taken from the different buckets and the pickling solutions were combined before sampling for analysis.

pH and sugar content were analysed from the marinating solutions before the addition of the fish and after 5 weeks of storage. Fish weight and salt concentration in the fish muscle were measured after 5 weeks of storage. Salt and sugar contents were measured from the whole contents of marinade jars - fish plus marinade - after 5 weeks of marinating. The marinades produced by Suomen Sillikonttori Ltd. (Figure 3.8) were also submitted to the same analysis as a reference from the industry.

Samples were submitted for microbiological analysis after 2 weeks of storage, in order to have the results before choosing the sample for consumer studies.

Figure 3.8 - The reference from the industry ready for analysis.
Pirkka´s Parhaat Katajanmarja silakkafileet is produced by Suomen Sillikonttori Ltd. for a private label brand.

3.4.1 Acidity (pH) analysis

All pH measurements in this study were conducted using a IQ150 pH meter (Spectrum Technologies Inc.; USA) equipped with an pHenomenal 220 electrode (VWR International, LLC; USA).

About 15 ml of the liquid sample to be analyzed was taken, covered and let come to room temperature before measuring the pH (Figure 3.9).

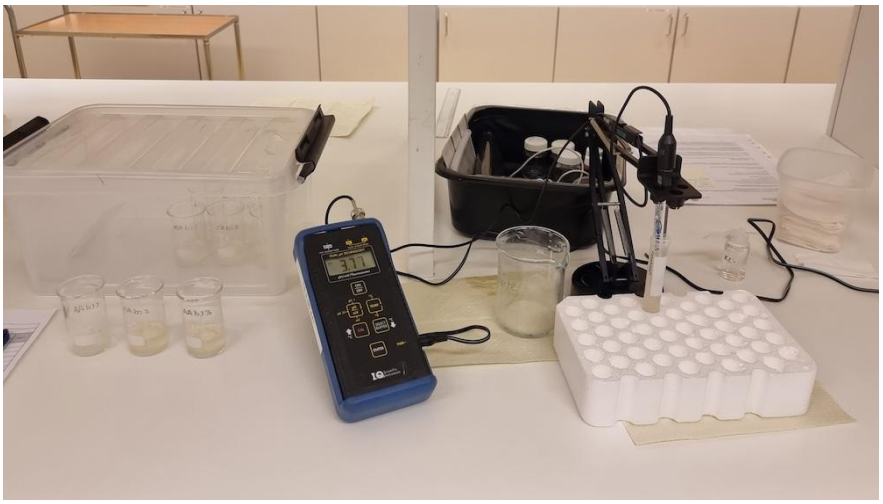

Figure 3.9 - Measuring the pH of a sample.

3.4.2 Analysis of Salt concentration

All measurements were performed using a DTM-20 saltmeter (DAEYOON SCALE INDUSTRIAL, South Korea).

Salt concentration in pickling solutions

For Pilot Studies pickling solutions, samples were diluted 1:1 and the salt concentration measured. For Factory Studies, samples were diluted 1:3.

Note: The same sample was used to measure pH and salt concentration.

Salt concentration in marinade solutions and in fish plus marinade

Salt concentration was measured directly in samples of the marinade solutions and the salt concentration of fish plus marinade was measured by blending the whole content of a jar (fish and marinade) and directly measuring the salt content.

Salt concentration in fish muscle

For pickled fish, a 10%(w/w) mixture of fish fillets and water was blended and the salt concentration was measured (Figure 3.10).

For marinated fish, this same procedure was used to measure salt concentration in fish muscle In Experiment IIA marinades. For the Factory Study, salt concentration in fish muscle was analysed using all the fish contained in one jar of marinade. The fish was drained from the marinade, weighed into a bigger jar and the same amount of water was added. The content was blended and the salt measured.

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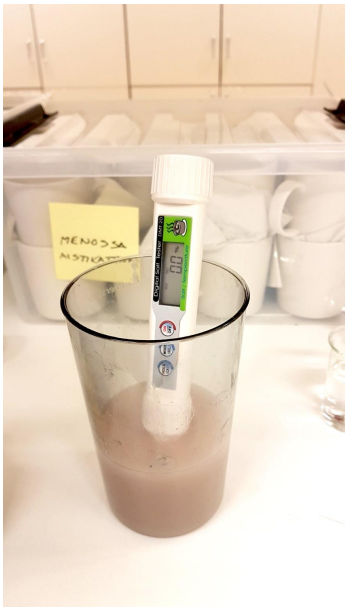

Figure 3.10 - Measuring salt in fish muscle.

3.4.3 Analysis of Sugar concentration (Brix)

Sugar concentration in the marinades was measured using a MA871 Digital Refractometer (Milwaukee, Hungary). For marinade solutions, samples were taken, filtered through a QL100 filter paper cone and the sugar content measured (Figures 3.11A and 3.11B). For fish plus marinade, the whole content of one jar was blended, a sample was taken and filtered through a QL100 filter paper cone and the sugar content measured.

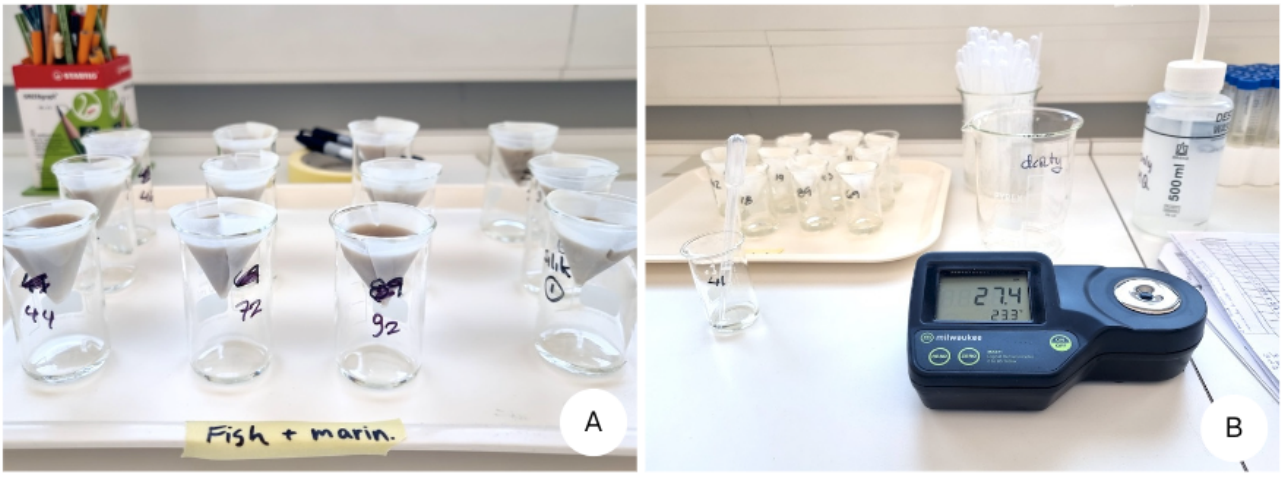

Figure 3.11A and 3.11B - Filtering samples of fish plus marinade for Brix analysis and measuring the sugar concentration of a sample.

3.4.4 Sensorial analysis and choice of sample for consumer studies

The pickling and marinating processes were evaluated qualitatively based on the fish and solution colour, smell and muscle texture (evaluated by cutting with a fork). This was done at the end of the processes and every time samples were taken for analysis along the process.

Choice of sample for consumer studies

One of the marinated Baltic herring samples produced during the Factory study was chosen to take part in consumer studies. The choice was made based on the microbiological, chemical and sensorial properties of the samples. The sensorial evaluation was performed by a panel of five staff members of FFF and Aistila Ltd. (Turku, Finland), most of whom are specialists in sensory and consumer evaluation. The samples were served at room temperature and on white porcelain plates labelled with 3-digit number codes. Water and cream-crackers were supplied as palate cleansers to be taken in between the samples (Figure 3.12). The panellists were asked to describe the odour, texture, taste, and flavour properties of the samples. They were also asked which sample they would most likely buy from a store and which they considered the most suitable for consumers.

Figure 3.12 - Samples for sensory analysis.
Water and cream-cracker were used to cleanse the palate.

3.4.5 Microbiological analysis

Samples were analysed for aerobic bacteria, enterobacteria, hydrogen-sulfide producing and sulfide reducing bacteria, yeasts, moulds and *Listeria monocytogenes*. Microbiological analyses of Pilot Studies Experiment I samples were performed by Eurofins Scientific Finland Oy (Rasio, Finland). Samples from Experiment II and Factory Study were analysed by Net-Foodlab Laboratoriot Oy (Turku, Finland).

Pilot Studies pickled fish samples were collected for analysis by randomly picking fish fillets and the same weight of pickling solution into a jar. No samples were collected for Factory Study pickled fish. For both Pilot and Factory Studies marinated samples, one jar of each marinade was sent for analysis, with the exception of Factory Study's lactic acid and citric acid marinades with higher sugar content, of which two and zero samples were analised, respectively.

3.4.6 Statistical analysis

For Factory study marinades, statistically significant differences between the different samples were determined using IBM SPSS Statistics 28 software (Armonk, NY, The United States). The limit for the statistical significance level was set at $p<0.05$. A Pairwise Comparison of Samples was done using the Kruskal-Wallis Test for all variables.

4.1 Pilot Studies

4.1.1. Choice of experimental conditions

4.1.1.1 Choice of acids

Acetic acid is the acid used in marinated herring commercial products and therefore was used as a reference throughout this study. In the previous study done for this project [4], four organic acids were tested in addition to acetic acid in the production of Baltic herring marinades: citric, malic, lactic and tartaric acids. In the present study these were narrowed down to three acids, so the number of samples would be manageable in terms of equipment, storage and within the study time frame.

Citric acid was chosen because it is widely used in the food industry and easily available, with a price level similar to that of acetic acid. Despite the results from the previous study [4] indicating a higher oxidation level in the citric acid marinades than in the other samples after four months of storage, this was not detected in the sensory profile obtained in the previous study.

In contrast to citric acid, tartaric acid showed the most promising results in quality terms with the least lipid oxidation and least decrease in protein content; it was therefore an interesting choice for developing a reference product in chemical terms.

Malic acid was dropped from the study because it didn't positively differentiate from any of the other acids and is considerably more expensive and harder to obtain.

Lactic acid showed promising results as it had less decrease in protein content than acetic and citric acids and the second least lipid oxidation after four months of storage; also, Larson [32] concluded that fish marinated in lactic acid was the preferred one after sensorial evaluation comparing herring marinated in acetic, citric, lactic and malic acids.

Thus, the acids chosen for this study were acetic (as reference), citric, lactic and tartaric.

The chemical formula, structure and data for the acids used in this work can be consulted on Appendix 1 on page 67.

4.1.1.2 Salt concentration

One of the objectives of this study was to improve the fish texture obtained in the previous study. The hypothesis was that the soft texture was a consequence of the salt concentration in fish muscle being too low, as fish was pickled in a 5% (w/v) salt solution and no salt was added to the marinade [4].

In this study, the salt concentration of the pickling solutions was chosen to be 14% (w/v). The aim was having a final salt concentration in fish muscle of 2% - 3%, as in most commercial marinated herring products. It was taken into consideration that the fish would first be pickled and then marinated using a 1:1 ratio and that the salt concentration in fish muscle and solution tend to reach equilibrium; salt concentration in the fish at the end of pickling should be half the initial salt concentration of the pickling solution and then halved during marinating (meaning salt will diffuse from the solution into fish muscle during pickling and from fish to solution during marinating). A salt concentration of 14% (w/v) is within the values mentioned in the literature [21, 22, 31] and also got the approval of Siilikonttori when discussing the conditions for the study.

4.1.1.3 Pickling and marinating times

In the previous study [4] the fish was pickled for just 24 hours. In this study the pickling time was increased to 35 days (approximately 5 weeks) as it is the standard time period used by the industry in Denmark and Sweden, following guidelines to ensure food safety against the eventual presence of parasites in fish meat [23, 26] . Also, at the low pickling temperatures used, a longer period of time is needed to ensure proper curing of the fish muscle [19, 43].
For marinades, samples were stored for a minimum of two weeks before analysis to ensure proper development of marinade properties (chemical, microbiological and sensorial) but respecting the study time schedule.

4.1.2 Experiment I

After five weeks of pickling, visual inspection of the fish fillets revealed that the acetic acid pickled fish fillets were white and opaque, characteristic of properly cured fish. For all other acids the fish had reddish spots and looked “uncooked”. Meyer mentioned that red spots in the pickled fish indicate defective pickling by lack of stirring and uneven contact with the solution [19]. It was also noticed that salt-like white crystals had precipitated in the tartaric acid pickling solution. These might be potassium bitartrate ($KC_4H_5O_6$), a common precipitate that appears during wine production under acidic conditions at low temperatures; the bitartrate ion is the one predominant at pH =4 [55].

The results from chemical analysis of the samples for Experiment I are resumed in Table C.1 on page 67 and the microbiological results on Table E.1 on page 73. *Listeria monocytogenes* was not found in any of the samples and all other microorganisms were below the detection limit, with exception of yeasts, that were present in all samples except acetic acid ones.

Figure 4.1 shows the pH value of each pickling solution at the beginning and end of the pickling process. The final pH of the pickling solution was 4.2 for acetic acid and above 4.2 for all other acids, which explains the presence of yeasts in all samples except acetic acid. Too low acid concentration might be also the cause for the raw appearance of fish pickled in citric, malic and tartaric acids. These high values of pH at the end of pickling can be explained by the acid concentrations used to prepare the pickling solutions being the same used in the previous study

[4] but not taking into account neither the lower fish to solution ratio (1:1,5) nor the much shorter pickling time (24 hours).

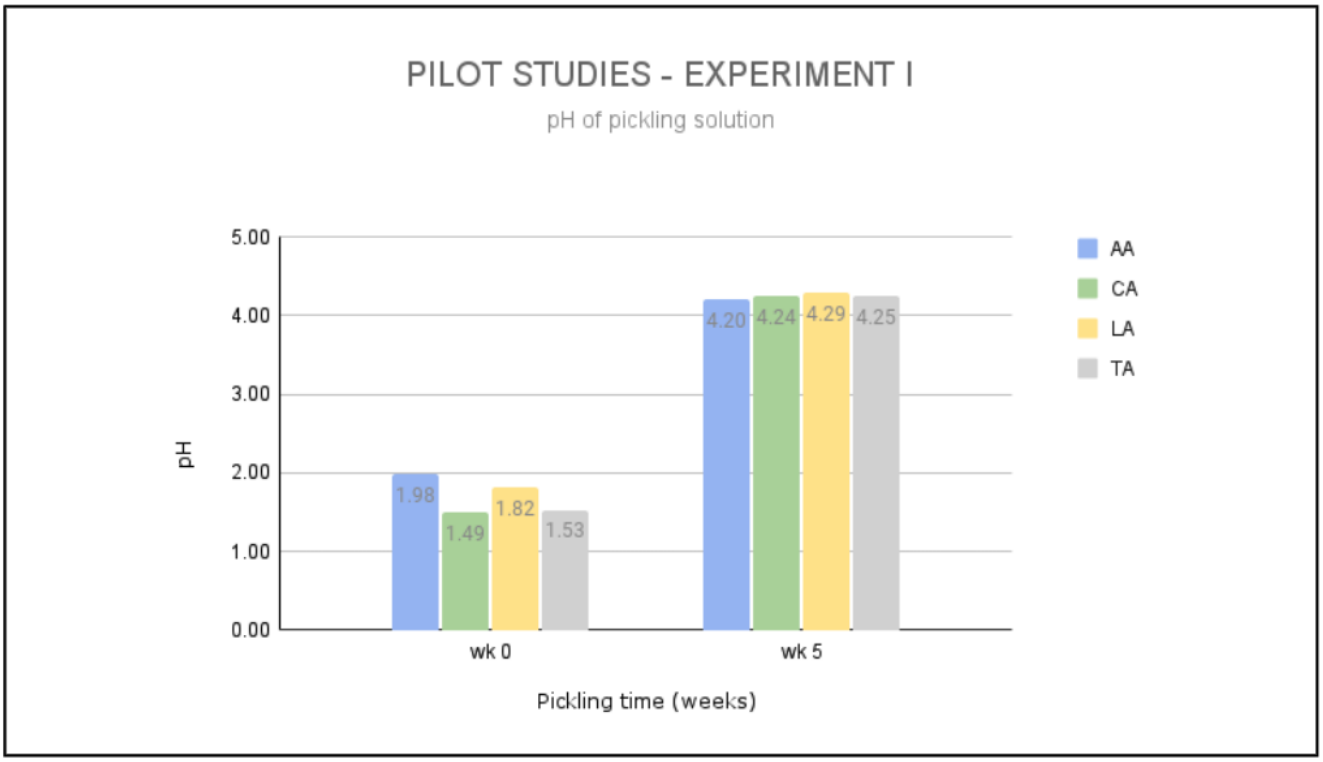

Figure 4.1 - pH of pickling solutions at the beginning and end of the process. n=1 (wk 0 is the pH of the pickling solution before inserting the fish). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid.

As for salt, its concentration in the pickling solution decreased from 10% (w/w) in the beginning to between 6.1% - 6.4% (w/w) in all acids, therefore being within the expected values (data not shown). All fish fillets had a firm texture, meaning that, relatively to the previous study for the project, increasing the salt concentration resulted in increased hardness of the fish muscle as expected.

4.1.3 Experiment II

Based on the results of Experiment I and to separately investigate the effects of increasing the acid concentration and decreasing the fish to solution ratio, two sets of experiments were run in parallel: Experiment IIA and Experiment IIB. In Experiment IIA, the acid concentration was increased to 5%(w/v) for all acids and the fish to solution ratio kept at 1:1. In Experiment IIB the fish to pickling solution ratio was lowered to 1:1.5 (as in the previous study [4]) and the acid concentrations kept the same as in both the previous study and Experiment I.

4.1.3.1 Pickling

The pickling process was monitored on a weekly basis in terms of pickling solution pH and salt concentration in solution and in fish muscle; the data collected during the process is listed in Tables C.2 and C.3 on pages 68 and 69, respectively.

4.1.3.1.1 pH and weight change

pH, concentration of salt in the solution and in the fish muscle, and weight change after 5 weeks of pickling are resumed on Table 4.1.

Table 4.1 - Resume of Experiments IIA and IIB data after 5 weeks of pickling. Except for weight change (n=1), data values are the mean ± stdev (n=9 for pH and salt in solution; n=3 for salt in fish muscle). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid. Square brackets denote concentration; all concentrations are %(w/w).

	EXPERIMENT II A				EXPERIMENT II B			
	pH	[salt] fish muscle	[salt] solution	weigh change %	pH	[salt] fish muscle	[salt] solution	weigh change %
AA	4.17±0.02	4.4±0.1	5.2±0.1	−34.6	4.05±0.05	5.1±0.1	6.6±0.3	−32.4
CA	3.79±0.05	4.6±0.0	5.7±0.2	−33.0	4.10±0.03	5.8±0.1	6.6±0.2	−27.2
LA	3.85±0.05	5.4±0.0	5.9±0.1	−32.2	4.21±0.07	6.3±0.0	6.8±0.1	−25.4
TA	3.48±0.06	4.9±0.1	5.9±0.1	−35.10	3.64±0.12	6.1±0.0	7.0±0.1	−36.3

Conclusions on the effect of increasing the acid concentration to 5% while keeping the same fish to solution ratio can be drawn by comparing the pH in Experiment IIA and Experiment I - see Figure 4.2. As expected, the final pH was lower for citric, lactic and tartaric acid solutions and kept roughly the same for acetic acid, as the same acid concentration was used. By comparing the pH values in experiments I and IIB, it can be concluded that increasing the amount of solution relative to fish also lowered the pH values, as expected. For acetic acid, its concentration was the same in both experiments IIA and IIB but the lower ratio of fish to solution in IIB explains the lower final pH - decreasing the fish to solution ratio has the same effect as increasing the acid and salt concentrations, as remarked in the literature [19, 20].

Figure 4.2 - Comparison of the average pH of pickling solutions in Experiment I, IIA and IIB after 5 weeks of pickling. AA, acetic acid; CA, citric acid; LA, lactic acid and TA, tartaric acid. For Experiments IIA and IIB data is shown as mean (n=9).

Fish in Experiment IIA looked better cured (less redness) than Experiment IIB pickled fish. In both experiments, fish pickled in acetic acid had a more pliable texture and showed less gaping than the fish pickled in the other acids. Also, as was observed in Experiment I, for citric, lactic and tartaric acids the fish had a yellow / greyish tone while acetic acid pickled fish was white. Even though the concentration used in Experiment IIA was the same for all acids, the final pH and to which extent they denature the fish muscle was different because they have different strengths and buffering capacities. As expected, the pH was lowest for the strongest acid - tartaric acid (pK_a=2.98) - and highest for the weakest one - acetic acid (pK_a=4.76).

Weight loss during the pickling process is known to increase with increasing acid concentration [20, 26] and indeed in this experiment it was observed that, with the exception of acetic acid pickled fish, the calculated weight loss for Experiment IIA (where the final values for pH are lower) was higher than for Experiment IIB. The amount of fish pickled in acetic acid was in all experiments much smaller than for the other acids (Figure 4.3), but the same amount of fish and marinade was taken for analysis from all acids, meaning this sample was subjected to a higher variation in fish to solution ratio throughout the process. Also, it was found that draining conditions (amount of fish, sieve size and draining time) greatly affect the final weight of the fish and impart a great error in the calculated values. Both factors account for the unexpected higher weight loss in acetic acid despite the higher final pH.

Figure 4.3 - Pilot Studies Experiment IIA - Draining acetic acid and citric acid pickled fish before weighing and marinating.

The pH measured along the five weeks of pickling for Experiment IIA is shown in Figure 4.4. It can be observed that the biggest change in pH occurs during the first week of pickling. Indeed, according to the literature, most changes in acid and salt concentration occur within the first 24-48 hours of pickling [26, 36, 40]. Overall, the pH is quite stable between weeks 2 and 4 and then slightly increases from week 4 to week 5. This increase can also be a consequence of the microbial contamination of the samples, later verified in the microbiological analyses.

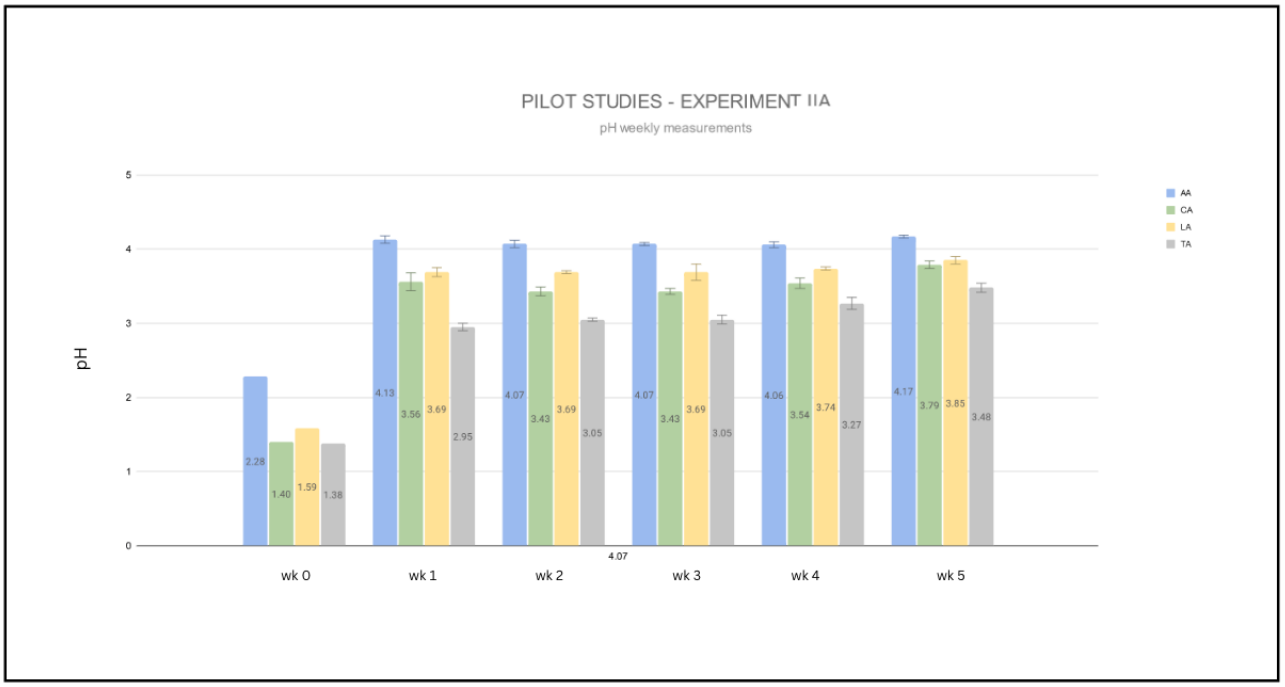

Figure 4.4 - Measured pH after 0, 1, 2, 3, 4 and 5 weeks of pickling. Wk0 is the measurement made before inserting the fish into the pickling solution. Data shown as mean± stdev (n=9 except for wk0 (n=1)). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid.

4.1.3.1.2 Salt concentration

Results for the initial and final salt concentration are listed in Table 4.1 on page 36 and the weekly analysis in Tables C.2 & C.3 on pages 68 and 69, respectively. Salt concentration decreased in the pickling solution and increased in the fish muscle as it diffused from one to the other. After 5 weeks of pickling, the salt concentration in fish muscle was between 4.4% and 5.4% in Experiment IIA and between 5.1% and 6.3% for Experiment IIB; the values in Experiment IIA are about half the initial salt concentration in the solution, as anticipated. In Experiment IIB the fish muscle ends up with a higher salt content than in Experiment IIA due to the higher solution to fish ratio.

Figure 4.5 shows how the salt concentration in fish muscle changed during the 5 weeks of pickling for Experiment IIA. The fluctuations observed in the measured values were most likely a consequence of the sampling method.

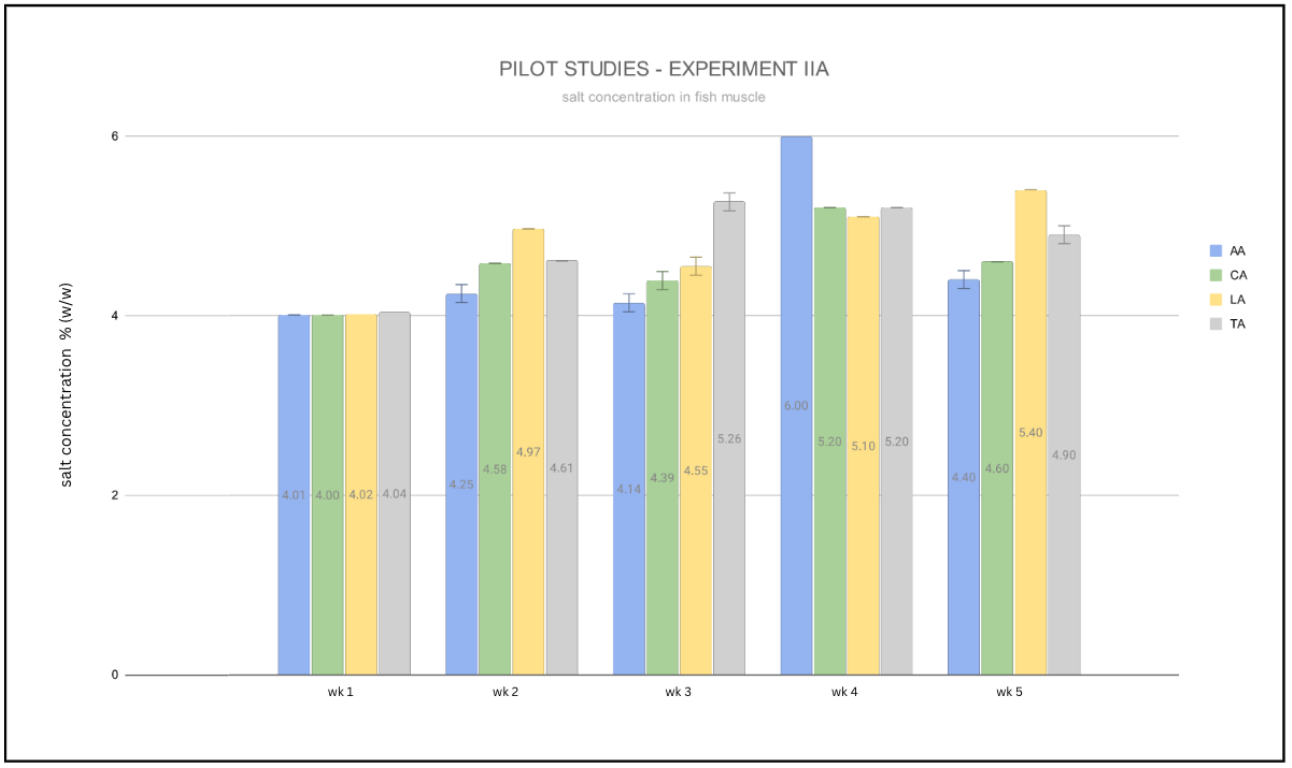

Figure 4.5 - Measured salt concentration in fish muscle after 1, 2, 3, 4 and 5 weeks of pickling.
Data shown as mean± stdev; (n=3). AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid.

4.1.3.1.3 Microbiological analysis results

The microbiological results for the pickled fish from Experiments IIA and IIB are listed in Tables E.2 and E.3 on page 73. Only acetic and tartaric acid samples had either good/ acceptable counts for all microorganisms; all other samples had too high values for at least one type of microorganism. This means that, despite having pH<4.2, weekly opening the pickling buckets to take fish and marinade for analysis and subjecting them to temperature changes constituted a source of contamination by yeasts and moulds[3].

[3] When the pickled fish was further marinated these results were not known, as the samples were collected for microbiological analysis only at the end of the pickling process.

4.1.3.2 Marinating

After pickling, the fish from Experiment IIA was further marinated and analysed after 4 weeks of storage; the results are resumed on Table C.4 on page 70. Experiment IIB pickled fish did not undergo further processing because neither the 1:1.5 fish to solution ratio is the one used in the local industry (it uses 1:1) nor it showed better pickling results than Exp. IIA in terms of fish curing.

Fish pickled in tartaric acid was also dismissed as a potential acid to produce marinades because the fish did not look properly pickled (was reddish, stiff and gritty) and, as observed in Experiment I, white crystals precipitated during the pickling process (most likely potassium bitartrate salts [55]). Furthermore, while formulating the marinades with different sugar and salt concentrations, it was noticed that tartaric acid solutions had an unpleasant sour taste.

The concentration of sugar in the marinades was chosen based on the sugar concentration used in the previous study[4] [4]. Thus, in this experiment, reference marinades were 18% (w/v) in sugar and marinades with less sugar were prepared in concentrations of 10% and 5% (w/v). For easier comparison of sugar content in the prepared marinades with the respective measured Brix readings all marinades are, from here on, labeled with their sugar concentration %(w/w).

4.1.3.2.1 pH and microbiological analysis results

The pH for the sample marinades after 4 weeks storage is shown in Figure 4.6. Acetic acid marinades were on the limit of what is considered the maximum safe value of 4.2 while the marinades of citric and lactic acid all have values below 4,0. Microbiological results (Table E.4, page 74) showed that acetic acid marinades were the only ones not contaminated by yeasts and/or moulds, despite the addition of sodium benzoate to all marinades. Citric acid samples had the highest counts of yeasts and moulds and already smelled bad when opened for analysis. All samples had high counts of aerobic microorganisms but *L. monocytogenes* was not detected in any of them. The high degree of contamination of the marinated samples can be attributed to the fact that the pickled fish was already contaminated when marinated, aggravated by the exposure of the fish to air and room temperature while draining and caning the marinades.

[4] It was later learned that the actual concentration was higher and therefore the sugar concentration was increased in the Factory Study marinades.

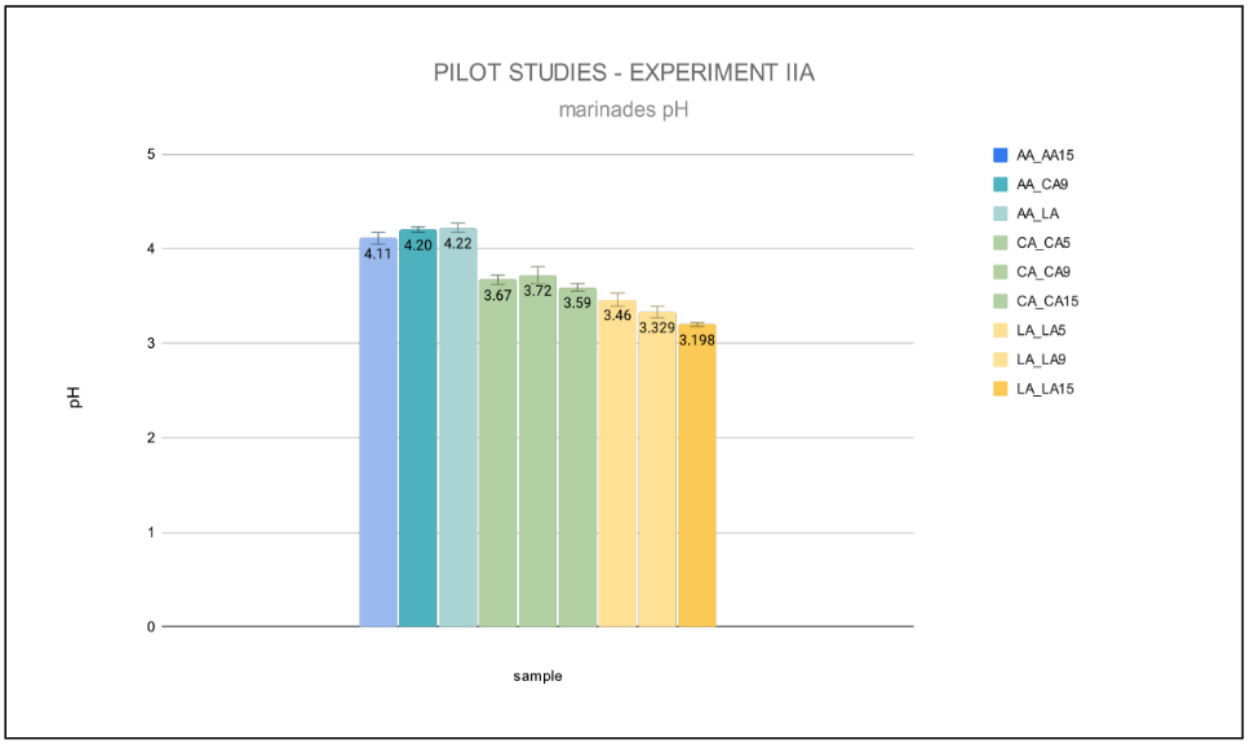

Figure 4.6 - pH for Experiment IIA marinades after 5 weeks of storage. Data shown as mean± stdev (n=3). AA, acetic acid; CA, citric acid; LA, lactic acid. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

4.1.3.2.2 Salt content

Salt concentration in the marinade solution and in fish muscle is depicted in Figure 4.7. Salt content in the marinade was about the same as in fish muscle. As contemplated when planning the process and because the marinade solutions had no salt, during marinating salt migrated from the fish muscle to the solution. The final salt content in fish muscle was around 2%(w/w) and within the expected values, meaning that the salt concentration of 14% (w/v) used to pickle the fish was adequate.

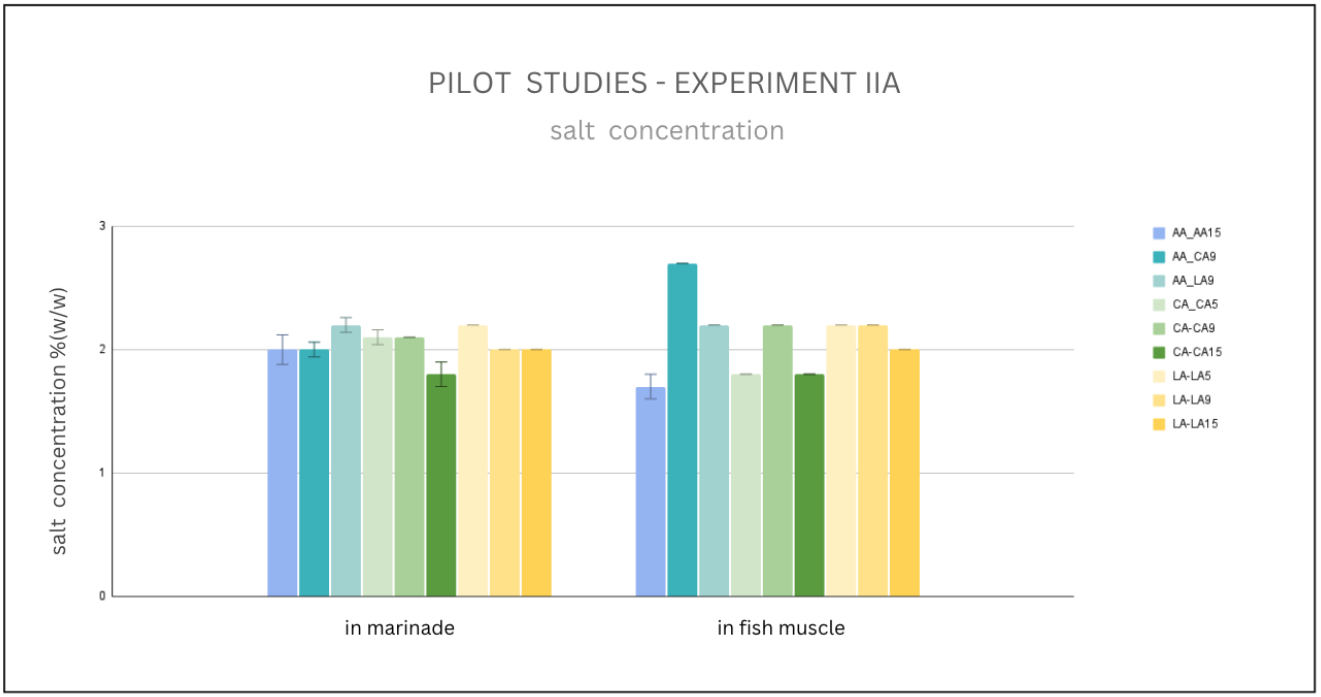

Figure 4.7 - Salt concentration in marinade solutions and fish muscle after storage of marinades for 4 weeks. Data shown as mean ± stdev (n=3 for salt in marinade; n=9 for salt in fish muscle). AA, acetic acid; CA, citric acid; LA, lactic acid. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

4.1.3.2.3 Sugar content

As for sugar concentration, the measured values in marinade solution and in fish plus marinade were very similar (Table D.2, page 72). The sugar concentration in marinade solutions is shown in Figure 4.8. For the marinades with higher sugar concentration (15%(w/w)), the readings agree with the sugar concentration used to prepare the marinades but for the lower concentration marinades the readings were higher than expected. One plausible explanation might be that in marinades with lower sugar concentration there is more free water available to dissolve other molecules from the fish into the marinade (like small peptides and amino acids) that increase the Brix readings.[5] No bibliography was found where the effects of sugar in the marinades have been studied and could support these findings.

[5] The Brix refractometer actually measures the total of dissolved solids in a solution and not solely the sugar content, as explained in Appendix G, page 79. Also, the effect of other dissolved solids on the readings will be smaller in solutions with higher sugar concentrations.

Figure 4.8 Sugar concentration in Experiment IIA marinade solutions after 4 weeks of storage.
Data shown as mean±stdev (n=3). AA, acetic acid; CA, citric acid; LA, lactic acid. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

4.2 Factory Study

4.2.1 Pickling

Because the pH of the acetic acid solution after 35 days of pickling in Experiment IIA was very close to 4.2 and the fish pickled in citric and lactic acids still showed some red patches (that might also be the result of insufficient contact between fish and solution during pickling), the concentration of all acids was increased from 5% to 6% (w/v) in this experiment.

At the end of pickling, visual inspection revealed that all fish samples looked properly cured. Again, the fish pickled in acetic acid was more white and opaque than the fish pickled in the other acids, which looked more greyish (Figure 4.9). The results from chemical analysis and calculated weight loss of the pickled samples are listed in Table 4.2.

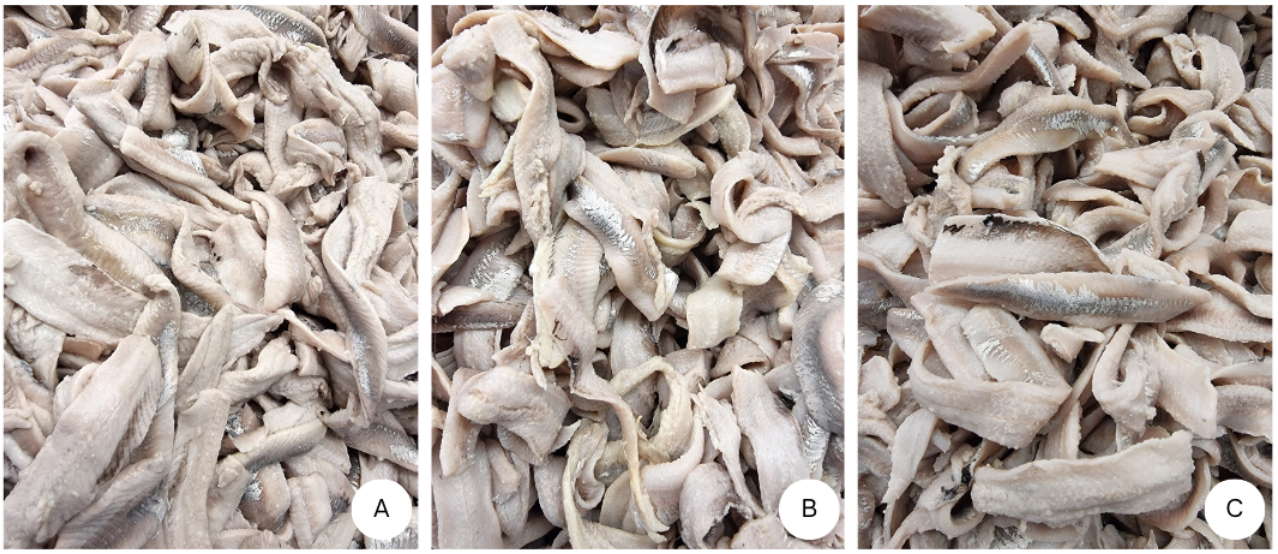

Figure 4.9A, 4.9B and 4.9C - Factory Study's pickled fish in acetic (A), citric (B) and lactic acid(C).

Table 4.2 - Chemical analysis results and calculated weight loss for the Factory Study pickled samples.
Values represent the mean±stdev; n=3. Square brackets denote concentration; all concentrations are %(w/w).

Pickling time (weeks)	AA			CA			LA		
	pH solution	[salt] solution	[salt] fish muscle)	pH solution	[salt] solution	[salt] fish muscle	pH solution	[salt] solution	[salt] fish muscle
0	1.98±0.08	11.1±0.1	-	1.18±0.01	11.2±0.0	-	1.46±0.02	11.2±0.1	-
5	4.08±0.05	5.9±0.1	4.3±0.0	3.43±0.07	5.7±0.0	4.9±0.0	3.46±0.03	6.0±0.0	4.7±0.0
weight change %		−35.0			−33.0			−27.0	

4.2.1.1 pH

All pickling solutions had a final pH<4.2, with acetic acid solution having the highest pH value (pH=4.08). Comparing the pH at the end of pickling for this study with the one in Experiment IIA (Figure 4.10) shows that the 1% increase in acid concentration decreased the final pH for all samples, as expected.

4.2.1.1 Salt content

Salt concentration in fish muscle was between 4.3-4.9% (w/w) and within the awaited values, given the initial concentration of 14% (w/v) (or 12% (w/w)) salt in the pickling solution and the fish to solution ratio of 1:1.

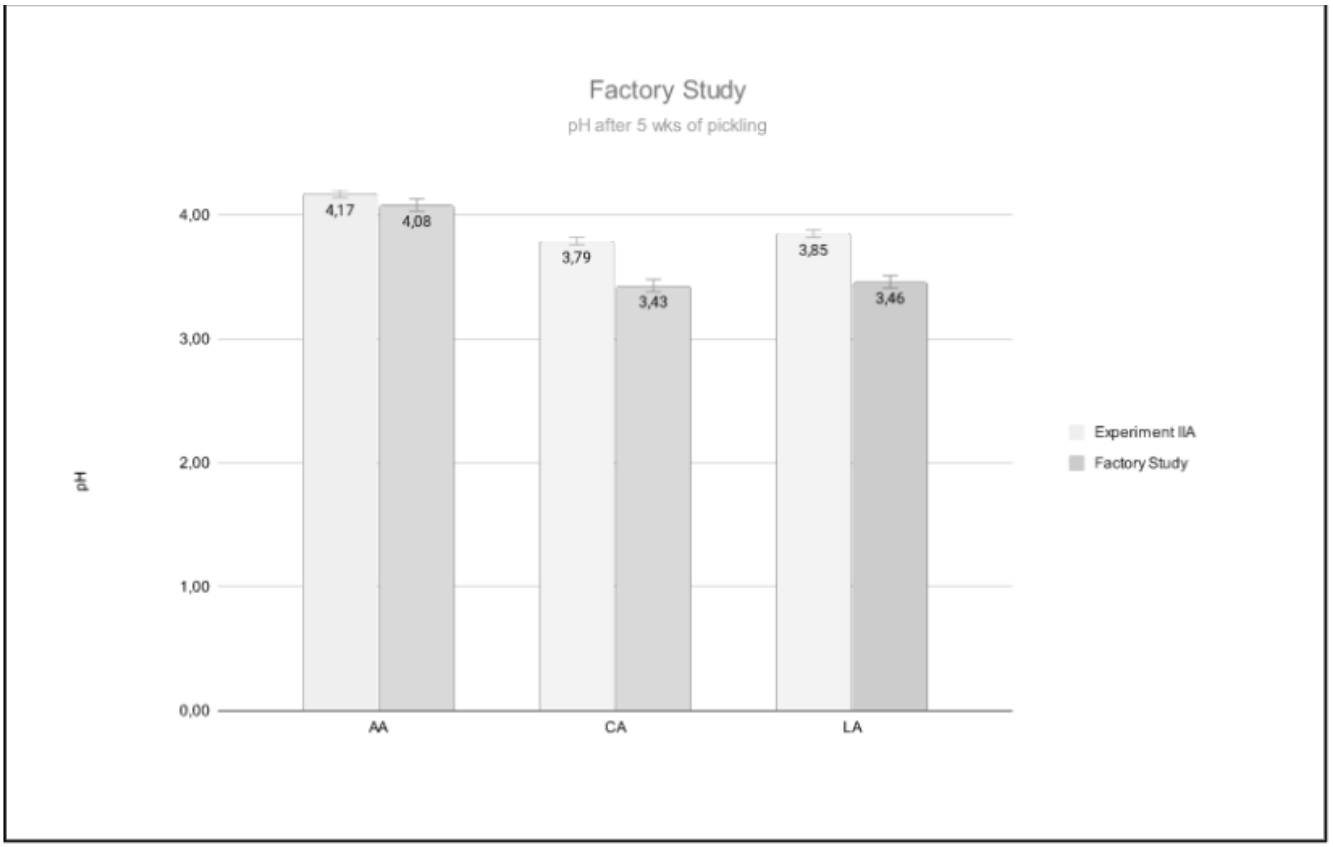

Figure 4.10 - pH after 5 weeks of pickling in Experiment IIA and Factory Study.
Data shown as mean±stdev (n=9 in Experiment IIA; n=3 in Factory Study). AA, acetic acid; CA, citric acid; LA, lactic acid.

4.2.1.3 Weight change

Also for this experiment the calculated values for weight change are just indicative, as it was not possible to set up controlled draining and weighing conditions (amount of fish, sieve size and draining time) at the factory. As for Pilot Studies, the amount of fish pickled in acetic acid in this experiment was much smaller than for the other acids and had the higher weight loss (35%) despite the higher final pH (4,08). Fish pickled in the strongest acid - citric acid - had the lowest

pH and the second highest weight loss (33%), followed by lactic acid pickled fish with a slightly lower weight loss of 27%.

It would have been interesting to see if the increase in acid concentration had any noticeable effect on the fish texture but because the two experiments (Experiment II and Pilot Study) were carried out one month apart it was not possible to sensorially evaluate and compare the pickled fish side by side.

Although acid concentrations of 5% and 6% appear in the literature, it is not possible to compare the results of this study directly with the literature because other experimental conditions like fish to solution ratio, salt concentration, temperature and processing times are different.

4.2.2 Marinating

4.2.2.1 Choice of marinating conditions

After learning that the sugar content reported in commercial products refers to the analysis of the whole content - fish plus marinade - of the jars, the sugar concentration was adjusted in Factory Study marinades. Most commercial marinated baltic herring products have 18%(w/w) sugar, so it was estimated that the concentration of the sugar in marinades should be at least double - 36% (w/w) - and this was the concentration chosen to prepare the reference marinades.

Marinades with reduced sugar were prepared using 30% less sugar because, according to Finnish regulations, it is the minimum reduction that allows for a product to be labelled as “less added sugar” if commercialised [56]. This could be an important marketing factor for this kind of product in the future.

Pirkka Parhaat Katajanmarjasilakka marinades (juniper berry marinated Baltic herring[6]) manufactured by Suomen sillikonttori Ltd. were used in this study as a reference and were also analysed chemically along with the study samples.

Results from the analyses for pH, salt and sugar concentration for the marinades prepared in this study and the reference commercial marinade (“Pirkka Parhaat Katajanmarjasilakkafileet”) are listed in Table D.2 on page 72 and their statistical analysis in Appendix F on page 75.

In this text, marinades are designated by two pairs of letters - the first denoting the acid used for pickling and the second the acid used for marinating - followed by a number indicating the sugar content expressed as %(w/w). Concentrations were converted from %(w/v) to %(w/w) for easier comparison with the concentration measurements, which are in %(w/w).

4.2.2.2 pH

The pH of the marinades after 5 weeks of storage is depicted in Figure 4.11 . All marinades had pH<4.2, the highest being measured in the commercial product (pH=4.16). No statistically significant difference was observed between the pH of acetic acid marinades and the commercial product (which also uses acetic acid). Citric acid and lactic acid samples had the lowest pH values (3.62 - 3.69), citric acid marinades being statistically significantly different from both acetic acid and reference marinades. No statistically significant difference was found

[6] Pirkka parhaat katajanmarjasilakkafileet; the product ingredients and composition are listed in Appendix B on page 65

between the lactic and acetic acid marinades, what is possibly due to the lower number of lactic acid samples analysed.

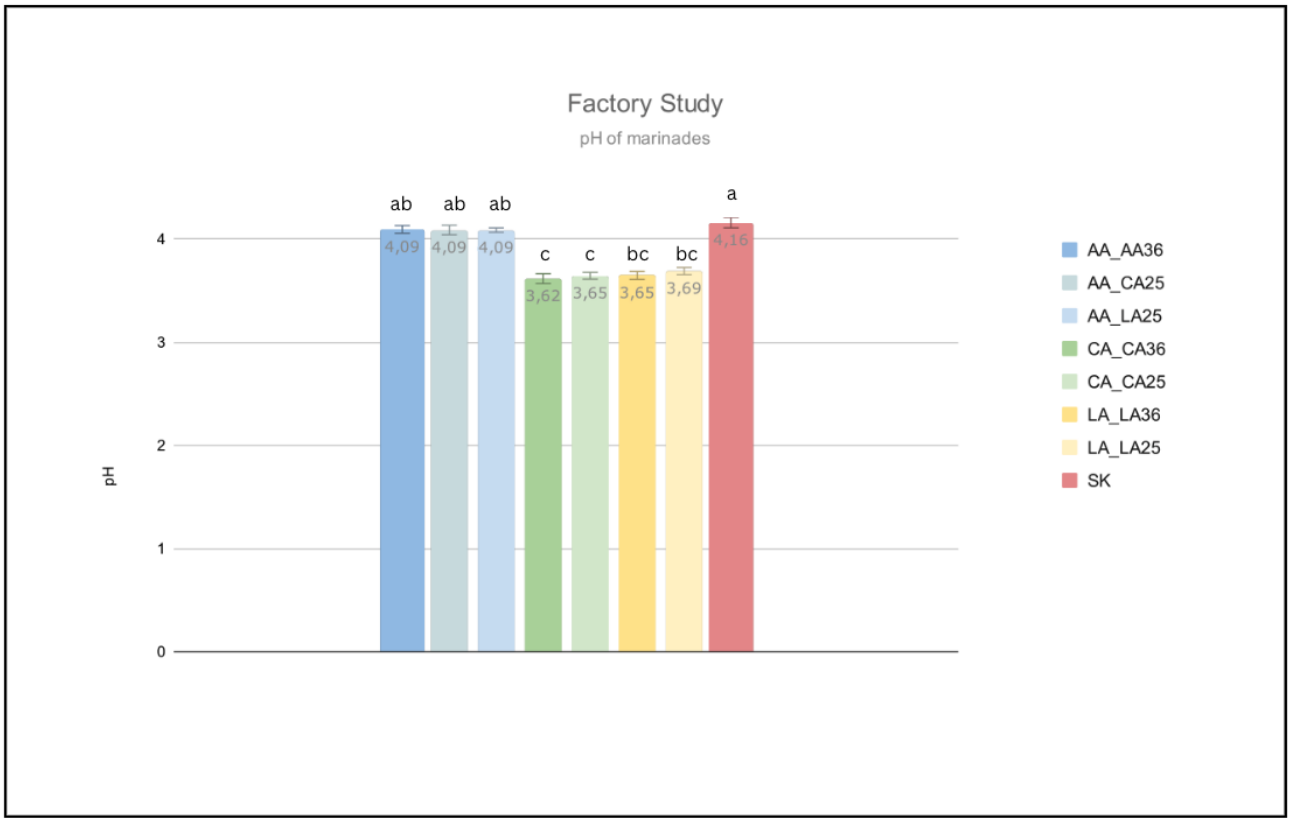

Figure 4.11 - Measured pH in Factory Study and reference marinades after 5 weeks of storage.
Data shown as mean±stdev (n=9 for all marinades except for CA_CA36 (n=12) and LA_LA36(n=6). Different small letters indicate a statistically significant difference on a 95% confidence level. AA, acetic acid; CA, citric acid; LA, lactic acid; SK, reference sample. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

4.2.2.3 Sugar concentration

The measured sugar content in fish plus marinade is shown in Figure 4.12.

There was no statistically significant difference observed between the different acids of marinades with the same sugar concentration. Despite having the highest sugar content (33% w/w), the reference product is not statistically significantly different from the study marinades with higher sugar content, except for the lactic acid marinade (LA_LA36). This might be because only two samples of this marinade were analysed, compared to 3 and 4 samples of the other marinades.
The measured concentration of sugar was higher than expected for all study samples; marinades with higher sugar had readings of 28%(w/w) and were expected to have about 18%(w/w) while marinades with lower sugar had values about 21,5%(w/w) and were expected to be about 12,5%(w/w). This is a consequence of the measuring method, as Brix measures all solids dissolved in solution and not only sugars. The reference product also had higher sugar content (33%) than the 21% of sugars listed in the label; this might be due to the measuring method but also because it contained onion and herbs, which may account for seasonal differences in the product.

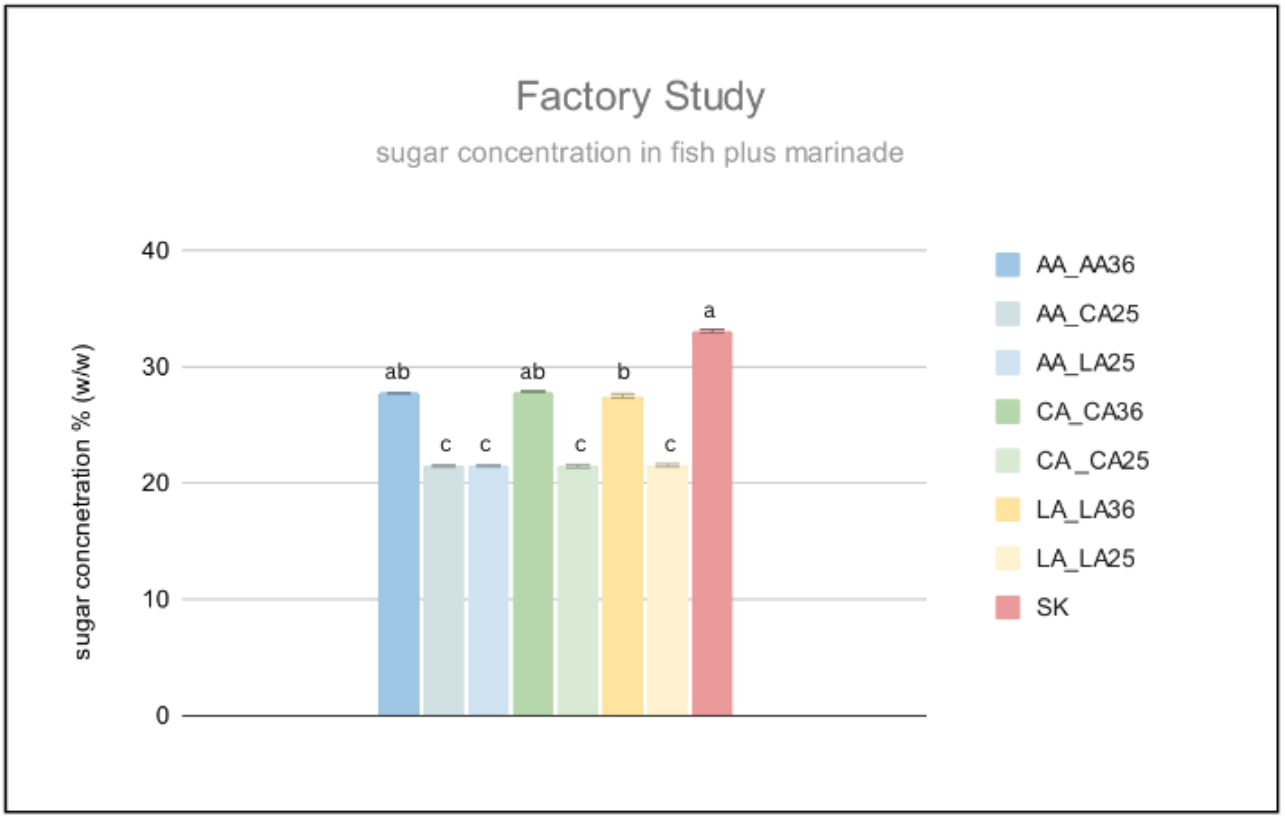

Figure 4.12 - Sugar concentration in fish plus marinade for Factory Study and reference samples after 5 weeks of storage. Data shown as mean ± stdev (For sugar content in marinades n=27 for except CA_CA36(n=36), LA_LA36(n=12) and SK(n=9). For sugar content in fish+marinade n=27 except for AA_AA36(n=18)). Different small letters indicate a statistically significant difference on a 95% confidence level. AA, acetic acid; CA, citric acid; LA, lactic acid; SK, reference sample. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

4.2.2.4 Salt concentration

Observation of the results obtained for the content of salt in fish, marinades and fish plus marinades revealed that the salt measurements are affected by the sugar concentration in the marinades - marinades with higher sugar content have lower salt readings than the ones with lower sugar concentration. The method used in this study to measure salt concentration is based on electrical conductivity and the electrical conductivity of a salt-sugar solution is known to be reduced with increased sugar concentration [57]. This explains the correlation observed between salt and sugar content for all samples and means that all salt measurements are affected by some level of uncertainty. Figure 4.13 illustrates the salt concentration in fish plus marinade, where the correlation between salt and sugar content is obvious. The measured values for salt concentration in the fish plus marinades were 1,0-1,5%(w/w), so clearly lower than the predicted 2.0-2.5% (w/w). The salt content measured in the reference product was merely 1.0%(w/w) and, according to the label, should be 2.6%(w/w).

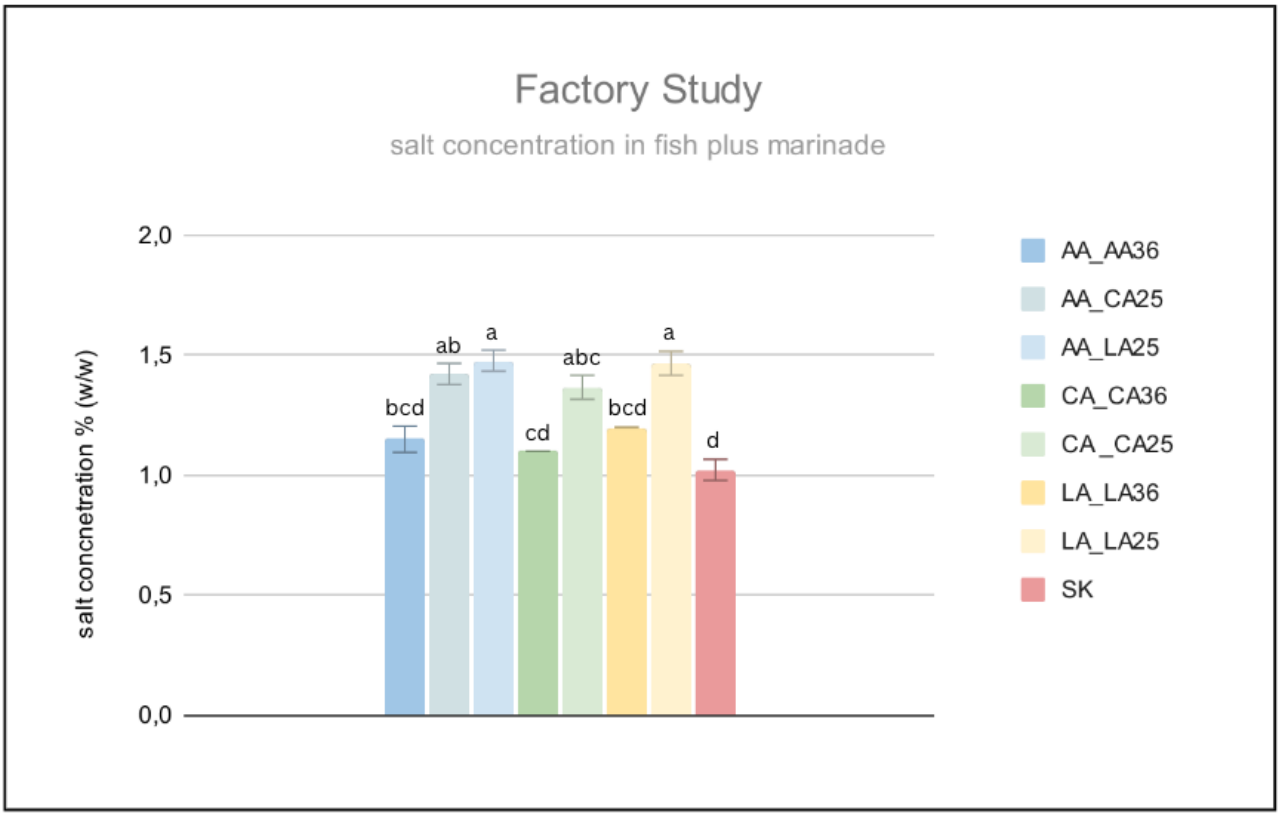

Figure 4.13 - Salt concentration in fish plus marinade for Factory Study and reference samples after 5 weeks of storage. Data shown as mean ± stdev (n=9 except AA_AA36(n=6). Different small letters indicate a statistically significant difference on a 95% confidence level. AA, acetic acid; CA, citric acid; LA, lactic acid; SK, reference sample. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

Despite this, useful information could be drawn from comparing the salt concentration in marinades with the same sugar content: there was no statistically significant difference in salt content in fish muscle among marinades with the same sugar content. This means that using acetic, citric or lactic acid in the marinade has no effect on how the salt diffuses into and out of the fish muscle during pickling and marinating, respectively, despite the slight difference in final pH of marinades. This observation confirms the findings of Szymczak et al. [36] that increased acid concentration in the brine does not have a significant effect on the content of salt in marinated herring .

4.2.2.5 Fish weight after marinating

The weight of fish after marinating for 5 weeks is plotted in Figure 4.14. Marinated fish weight was highest for acetic acid pickled and acetic acid marinated samples (156g/jar) and lowest for the reference marinade (132g/jar). Both marinades were prepared using acetic acid and had similar pH - so the acid can't explain the difference - but it was not controlled how the commercial product was manufactured.

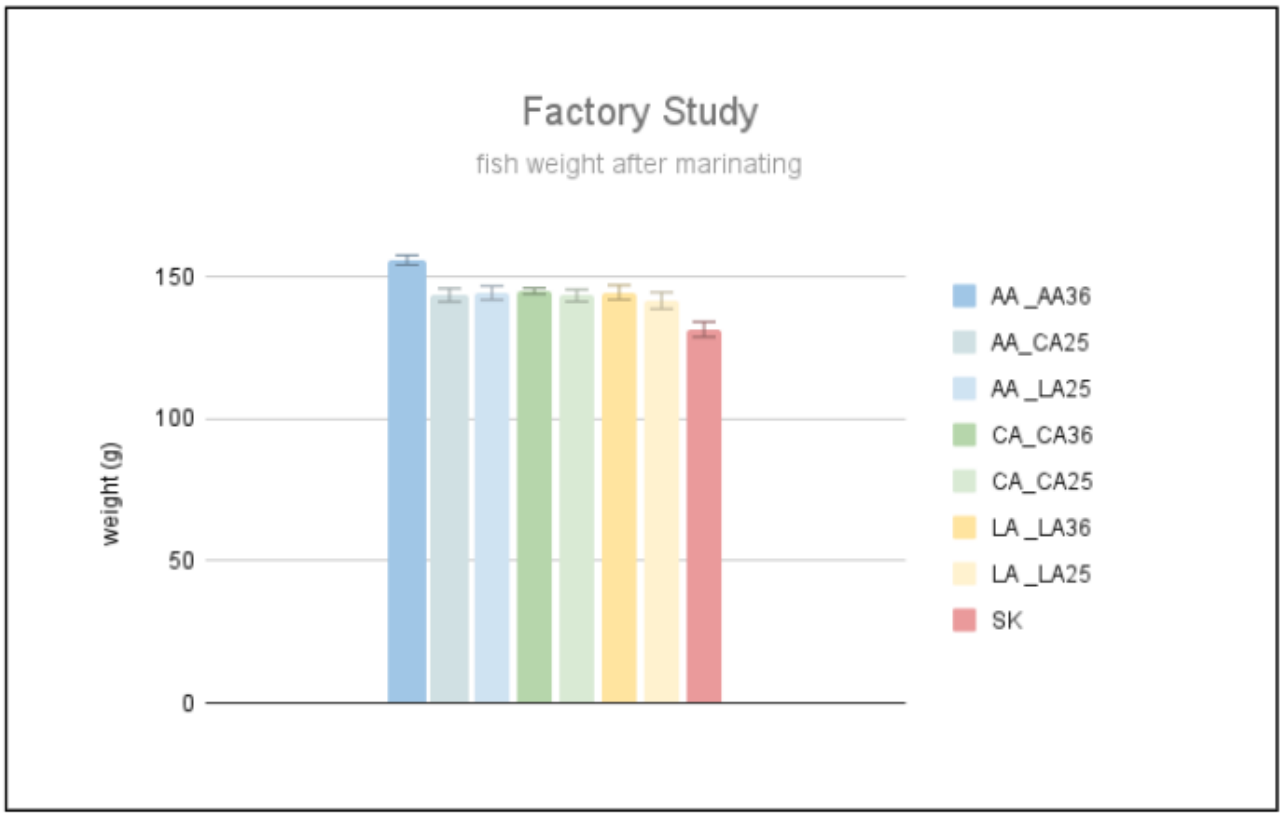

Figure 4.14 - Weight of fish for Factory Study and reference samples after marinating for 5 weeks.
Data shown as mean ± stdev (n=3 except CA_CA36(n=4) and LA_LA36(n=2)). AA, acetic acid; CA, citric acid; LA, lactic acid; SK, reference sample. The first pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

The average weight of fish after marinating was, for all study samples, above the approximately 140g put into the jars. This is opposite to what was observed with Experiment IIA marinades, where weight loss was registered. Besides the lack of a standard procedure for measuring weight change, another possible explanation might be the fact that Pickling IIA marinades had a considerably lower sugar content than Factory Study marinades (15%, 12% and 9%(w/w) vs. 36% and 25%(w/w)). It can also be observed that for Factory Study marinades, the samples marinated in solutions with higher sugar concentration had slightly higher weight (CA_CA36 - 145.17g; LA_LA36 - 144.73g) than the lower sugar versions (CA_CA25 - 143.57g; LA_LA25 - 141.83g) . So the positive weight change observed can merely be the effect of increased density of the marinades with higher sugar concentration, as the marinade penetrates into the fish and also remains on its surface, total separation being impossible. Statistical analysis for the fish weight was not performed because the fish put into each jar was not accurately measured by weighing when producing the marinades.

4.2.2.6 Microbiological quality

The results of microbiological analyses of Factory Study marinades are resumed in Table E.5 on page 74. All marinades were evaluated as of good microbiological quality. The number of enterobacteria, sulphide reducing Clostridia, hydrogen-sulphide producing bacteria and moulds was below the detection limit of <10 cfu/g for all samples. *L. monocytogenes* bacteria was not found in any of the samples tested (qualitative test per 25g of sample). The number of aerobic microorganisms was below the detection limit of <10 cfu/g for all samples except acetic acid

pickled and both citric acid and lactic acid marinated samples with 25% of sugar, which had an estimate of 40 cfu/g and 10 cfu/g, respectively. As for yeasts, its presence was estimated to be of 45 cfu/g (detection limit of 10 cfu/g) for the citric acid pickled and citric acid marinated samples with sugar concentration of 25%. The presence of microorganisms in marinades with lower sugar content might not be a coincidence; it is known that sugar acts as a preservative by binding with water and lowering water activity, so it might improve the microbiological quality of marinades with higher sugar content.
Even if samples containing citric acid had a lower pH than acetic acid ones , the presence of yeasts was still detected in citric acid marinades, as it had been earlier in Pilot Studies samples and in the previous study [4]. It is generally agreed that the ability of weak acids to inhibit microbial growth is related to their pKa, as it is their uncharged form that passes across the lipidic membrane of microorganisms cells, the lipophilicity also varying from weak acid to weak acid [58]; this might explain why citric acid has weaker antimicrobial effect than the other acids.

4.3 Choice of a sample for consumer studies

Choice of a sample to take part in consumer studies was made based on the microbiological, chemical and sensorial properties of the samples. The descriptives for the samples obtained from the sensorial session are resumed in Table 4.3.

Table 4.3 - Sensory evaluation results for Factory Study marinated samples.
Data collected, compiled and used with permission of Nora Logrén. AA, acetic acid; CA, citric acid; LA, lactic acid. First pair of letters denotes the acid used for pickling and the second the acid used for marinating; the number indicates the sugar content (% w/w).

Sample	Descriptives	
	Odour	Taste
AA_AA36	sour / vinegary	vinegary, gentle, sweet, fruity, not very good aftertaste
AA_CA25	sour, fishy, vinegary, not very strong	sour, vinegary, neutral, dark, coffee like, salty, astringent
AA_LA25	fishy, mushy, vinegary	sour, sweet, stingy, strong vinegary, spicy, musty, less salty
CA_CA36	mild, not very sour, berry-like, sweet, herring-like, fresh	fishy, sea-like, sour, sweet, rancid, vinegary, sweetness covers acidity and saltiness
CA_CA25	mild, fresh, herring-like	soft, vinegary, sweet, fishy, sea-like, astringent
LA_LA36	fishy, mild	sour, sweet, neutral, fishy, easy, mild, fresh, sweetness covers acidity
LA_LA25	fishy, fresh	sour, sweet, Atlantic herring-like, spicy, strong, different, vinegary

Evaluation of the sensorial analysis results showed that the panel had a preference for lactic acid pickled and marinated samples, without a clear distinction between the higher and lower sugar concentration samples. The sample chosen for consumer studies was Baltic herring pickled and marinated in lactic acid and with 25%(w/w) of sugar, because one of the objectives of this study was to develop a product with lower sugar content. This result is in accordance with Larson's work, where lactic acid marinated sample was the preferred one in sensorial tests due to its milder, not so sour flavour [32].

Citric acid pickled and marinated samples also performed quite well but aerobic microbes and yeasts were still detected in microbiological analysis (Table E.5 on page 74), indicating that citric acid might not have such an efficient antimicrobial action as acetic or lactic acid. Also, in the previous study for this project citric acid samples showed the highest oxidation of all acids, which means an undesirable deterioration of the product's quality. Larsson [32] found that citric acid also had a lower impact than lactic and acetic acids against the lactic acid bacteria *L. plantarum*, which has been found in spoiled marinated herring products .

CONCLUSIONS | 5

The two-step process devised for producing the marinades and the conditions chosen and adjusted through the Pilot Studies resulted in the successful production of Baltic herring marinades with good microbiological quality and sensorial properties. It was possible to produce Baltic herring marinades using both citric and lactic acids. The experiments carried out during the Pilot studies revealed that tartaric acid is not suitable for producing the marinades, due to the precipitation of crystals during the pickling stage. Comparison of Experiments I and IIB during Pilot Studies showed that the fish to solution ratio is an important parameter to take into account in the pickling process.

The increase of acid concentration from 5% to 6% (w/v) in the pickling process guaranteed proper pickling of the fish using citric and lactic acids in the Factory Study. It also had a positive effect on the microbiological quality of the pickled fish and marinades. Using a salt concentration of 14%(w/v) for the pickling process resulted in fish fillets with texture similar to the reference product, successfully improvIng the result obtained in the previous study done for the project.

All marinades produced in the Factory Study had good microbiological quality and that can be attributed mainly to their low pH values (<4.2), as it is considered a determining factor in the safety of this kind of semi-preserved products. Production of the marinades in factory conditions, where strict hygienic practices are in place, working premises are kept at low temperature and marinade jars could be vacuum sealed, all ensured a lower risk of contamination and inhibition of microbial growth. As learned from Experiment IIA marinades, the use of sodium benzoate as a preservative can't guarantee good microbiological quality of the products without the adequate hygiene and processing practices in place.

The presence of yeasts, even if very in very low counts, was still detected in Factory Study's citric acid marinades, as it had been earlier in Pilot Studies samples and in the marinades from the previous study, leading to the conclusion that citric acid has a weaker antimicrobilogical effect than acetic and lactic acids. It is also worth noting that all Factory Study marinades where micro organisms were detected are all marinades with lower sugar content, so this might possibly affect the products microbiological quality and shelf life. Acetic acid, the weakest acid used in the study, had the best antimicrobial results throughout the study.

The choice of the lactic acid, low sugar marinated sample for consumer studies testifies that it is possible to produce a Baltic herring marinade with 30% less sugar than the average commercial products that is organoleptically highly scored. This opens the possibility for further research and development of new products.

STUDY LIMITATIONS AND FUTURE WORK | 6

Only a limited number of samples could be produced within the time frame and facilities of this study, which limited the scope of the results obtained after statistical treatment of data.

Results from this study were difficult to compare with the literature because not only is there little literature published on the subject but the experimental conditions were mostly different from the ones used in this study.

The limitations of the method used for measuring the salt content, with little sensitivity and affected by sugar concentration, did not allow any more detailed conclusions to be drawn about how using different acids influences the salt concentration in fish muscle. Also, for sugar concentration, use of a Brix refractometer resulted in readings with poor accuracy because the method is sensitive to all solids dissolved in solution and not only saccharose, what is particularly problematic in marinades with high concentration of sugar. In future work, more sensitive and analyte-specific methods of analysis should be considered for salt and sugar measurements.

A replicable procedure to calculate the yield of both the pickling and marinating processes was not in place during this study, and so it was not possible to reach reliable results about the influence of using different acids in the processes yield. This is an important factor for the industry and could be further improved.

In the future, it would be useful to evaluate the texture of fish quantitatively, using a texturometer. This data might contribute to a better perception of the effects of the different conditions used in the process on the quality of the product and could be correlated with sensorial evaluation. Also, further studies could be done in order to clarify how the texture of the fish marinated with different acids develops with a longer storage period, similar to that of commercial products.

The role of sugar did not become clear in these studies and it would be of interest to research its effect on fish weight change during the marinating process. Also, if lower sugar versions of marinades are to be developed, it is recommended that the possible impact of lower sugar concentration in the shelf life of marinades is followed up.

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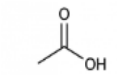
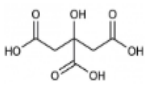
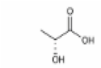
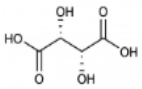
APPENDIX | A
ACIDS CHEMICAL DATA

Table A.1 - Chemical data for acetic, citric, lactic and tartaric acids [59].

Organic acid	Molecular formula	Molecular weight (g/mol)	Structural formula	pK_a	K_a	Physical appearance	Solubility in water (g/l)
Acetic acid	$C_2H_4O_2$	60.05		4,76	$1{,}73 \times 10^{-5}$	liquid	miscible
Citric acid	$C_6H_8O_7$	192,12		3,13 4,76 6,40	$7{,}4 \times 10^{-4}$ $1{,}7 \times 10^{-5}$ $3{,}9 \times 10^{-7}$	solid	750
Lactic acid	$C_3H_6O_3$	90,07		3,9	$1{,}3 \times 10^{-4}$	liquid	miscible
Tartaric acid	$C_4H_6O_6$	150,08		2,89 4,40	$6{,}8 \times 10^{-4}$ $1{,}2 \times 10^{-5}$	solid	1330

APPENDIX | B

COMMERCIAL MARINADE INGREDIENTS AND COMPOSITION

“Pirkka parhaat katajanmarjasilakkafileet”

Ingredients:
Baltic herring (Clupea harengus membras, water, sugar, juniper berries (3.5 %), onion, salt, dill, sipuli, suola, tilli, acidity regulator (E260), preservatives (E202, E211)

Table B.1 - Composition of “Pirkka parhaat katajanmarjasilakkafileet” per 100g of product [60].

Energy	662 kJ / 157 kcal
Fat	4.8g
From which saturated	1.3g
Carbohydrates	21g
From which sugars	19g
Protein	7g
salt	2.6g

APPENDIX | C
PILOT STUDIES RESULTS

C.1 Pilot Studies - Experiment I

Table C.1 - Results for the analyses of Pilot Studies Experiment I samples at the beginning and end of pickling. AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid. Square brackets denote concentration. n=1 for all attributes except salt concentration in solution after 5 weeks (n=3).

PILOT STUDIES - EXPERIMENT I						
acid	**week**	**pH solution**	**[salt] sol. % (w/w)**	**stdev**	**[salt] fish % (w/w)**	**weight change (%)**
AA	0	1,98	10,5	-	0,0	-22,4
	5	4,20	6,3	0,1	4,9	
CA	0	1,49	10,8	-	0,0	-25.7
	5	4,24	6,4	0,0	6,1	
LA	0	1,82	10,5	-	0,0	-46,5
	5	4,29	6,4	0,0	5,8	
TA	0	1,53	10,5	-	0,0	-27,4
	5	4,25	6,1	0,1	5,1	

C.2 Pilot Studies - Experiment II

Table C.2 - Results for the weekly analyses of Pilot Studies Experiment IIA samples during pickling. AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid. Square brackets denote concentration. n=9 for pH and salt concentration in solution except for week 0, n=1. n=3 for salt concentration in fish muscle.

PILOT STUDIES - EXPERIMENT IIA PICKLING								
acid	week	SOLUTION				FISH		Weight change %
		pH	stdev	[salt] % (w/w)	stdev	[salt] % (w/w)	stdev	
AA	0	2,28	-	9,6	-	-	-	-34,6
	1	4,13	0,05	5,2	0,3	4,0	0,0	
	2	4,07	0,05	5,7	0,1	4,2	0,1	
	3	4,09	0,02	5,8	0,2	4,1	0,1	
	4	4,06	0,04	4,7	0,8	6,0	0,0	
	5	4,17	0,02	5,2	0,1	4,4	0,1	
CA	0	1,40	-	11,1	-	-	-	-33,0
	1	3,56	0,12	5,3	0,3	4,0	0,0	
	2	3,43	0,06	5,8	0,2	4,6	0,0	
	3	3,54	0,04	6,0	0,0	4,4	0,1	
	4	3,54	0,07	5,8	0,2	5,2	0,0	
	5	3,79	0,05	5,7	0,2	4,6	0,0	
LA	0	1,59	-	11,4	-	-	-	-32,2
	1	3,69	0,06	4,7	0,8	4,0	0,0	
	2	3,69	0,02	5,9	0,1	5,0	0,0	
	3	3,64	0,11	6,0	0,1	4,6	0,1	
	4	3,74	0,02	5,8	0,2	5,1	0,0	
	5	3,85	0,05	5,9	0,1	5,4	0,0	
TA	0	1,38	-	11,1	-	-	-	-35,1
	1	2,95	0,05	5,7	0,1	4,0	0,0	
	2	3,05	0,03	5,9	0,1	4,6	0,0	
	3	3,22	0,06	5,9	0,1	5,3	0,1	
	4	3,27	0,08	5,8	0,3	5,2	0,0	
	5	3,48	0,06	5,9	0,1	4,9	0,1	

Table C.3 - Results for the weekly analyses of Pilot Studies Experiment IIB samples during pickling. AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid. Square brackets denote concentration. n=9 for pH and salt concentration in solution except for week 0, n=1. n=3 for salt concentration in fish muscle.

PILOT STUDIES - EXPERIMENT IIB PICKLING								
acid	week	MARINADE				FISH		Weight change %
		pH	stdev	[salt] % (w/w)	stdev	[salt] % (w/w)	stdev	
AA	0	2,09	-	10,8	-	-	-	-32,4
	1	3,78	0,04	6,8	0,1	5,0	0,0	
	2	3,83	0,06	6,9	0,1	5,7	0,0	
	3	3,86	0,02	7,0	0,1	4,9	0,1	
	4	3,95	0,05	6,7	0,6	6,6	0,0	
	5	4,05	0,05	6,6	0,3	5,1	0,1	
CA	0	1,65	-	11,1	-	0,0	-	-27,2
	1	3,84	0,04	7,0	0,0	4,1	0,0	
	2	4,07	0,04	7,0	0,1	5,2	0,0	
	3	4,08	0,05	7,0	0,0	5,9	0,0	
	4	4,10	0,02	6,8	0,2	6,6	0,0	
	5	4,10	0,03	6,6	0,2	5,8	0,1	
LA	0	1,80	-	9,6	-	0,0	-	-25,4
	1	3,84	0,06	7,0	0,0	5,1	0,0	
	2	4,05	0,03	7,0	0,1	6,4	0,0	
	3	4,12	0,04	7,1	0,1	6,4	0,0	
	4	4,14	0,02	6,8	0,4	6,4	0,0	
	5	4,21	0,07	6,8	0,1	6,3	0,0	
TA	0	1,57	-	10,5	-	0,0	-	-36,3
	1	3,33	0,03	6,8	0,1	5,0	0,0	
	2	3,57	0,02	6,7	0,1	5,4	0,0	
	3	3,56	0,03	7,2	0,1	6,1	0,0	
	4	3,60	0,09	7,4	1,4	6,9	0,1	
	5	3,64	0,12	7,0	0,1	6,1	0,0	

Table C.4 - Results for the analyses of Pilot Studies Experiment IIA marinades.
AA, acetic acid; CA, citric acid; LA, lactic acid; TA, tartaric acid. Samples are identified by the acid used for pickling followed by the acid used in marinating and the sugar concentration used to prepare the marinade % (w/w). Square brackets denote concentration. n=3 for pH, salt and sugar concentrations in marinade. n=9 for salt concentration in fish muscle.

EXPERIMENT IIA - MARINADES													
sample	MARINADE						FISH		FISH + MARINADE				weight change %
	pH	stdev	[salt] %(w/w)	stdev	[sugar] %(w/w)	stdev	salt %(w/w)	stdev	[salt] %(w/w)	stdev	[sugar] %(w/w)	stdev	
AA_AA15	4.11	0,06	2.0	0,12	15.9	0,0	1.7	0,1	a)	-	a)	-	a)
AA_CA9	4.20	0,03	2.0	0,06	12.0	0,1	2.7	0,0	a)	-	a)	-	a)
AA_LA9	4.22	0,05	2.2	0,06	13.5	0,1	2.2	0,0	a)	-	a)	-	a)
CA_CA5	3.67	0,05	2.1	0,06	10.1	0,1	1.8	0,0	1.5	0.0	10.7	0,1	-6.4
CA_CA9	3.72	0,09	2.1	0,00	12.1	0,4	2.2	0,0	1.5	0.0	13.1	0,1	-4.1
CA_CA15	3.71	0,04	1.8	0,10	15.7	0,0	1.8	0,0	1.4	0.0	16.5	0,1	-5.5
LA_LA5	3.84	0,07	2.2	0,00	10.3	0,0	2.2	0,0	1.7	0.0	10.8	0,1	-9.0
LA_LA9	3.80	0,06	2.0	0,00	13.9	0,0	2.2	0,0	1.6	0.0	14.6	0,1	1.6
LA_LA15	3.85	0,02	2.0	0,00	14.3	0,2	2.0	0,0	1.7	0,1	14.8	0,1	-4.9

a) Not measured

APPENDIX | D
FACTORY STUDY RESULTS

Table D.1 - Results for the analysis of Factory Study samples at week 0 and week 5 of pickling. AA, acetic acid; CA, citric acid; LA, lactic acid. Square brackets denote concentration. n=3 for pH, salt concentration in solution and in fish muscle.

FACTORY STUDY - PICKLING								
acid	wk	solution				fish		weight change %
		pH	stdev	[salt] %(w/w)	stdev	[salt] %(w/w)	stdev	
AA	0	1.98	0.08	11.10	0.1	-	-	-35.0
	5	4.08	0.05	5.9	0.1	4.3	0.1	
CA	0	1.18	0.01	11.20	0.0	-	-	-33.0
	5	3.43	0.07	5.7	0.0	4.9	0.0	
LA	0	1.46	0.02	11.20	0.1	-	-	-27.0
	5	3.46	0.03	6	0.0	4.7	0.0	

Table D.2 - Results for the analysis of Factory Study marinades.
AA, acetic acid; CA, citric acid; LA, lactic acid; SK, reference sample. Samples are identified by the acid used for pickling followed by the acid used in marinating and the sugar concentration used to prepare the marinade % (w/w). Square brackets denote concentration. n=9 for pH, salt concentrations in marinade and in fish muscle except for CA_CA36 (n=12)and LA_LA36 (n=6). n=27 for sugar concentration in marinades except for CA_CA36 (n=36), LA_LA36 (n=12) and SK (n=9). n=9 for salt concentration in fish plus marinade for all samples except AA_AA36 (n=9). n=27 for sugar concentration in fish plus marinade for all samples except AA_AA36 (n=18). n=3 for weight of fish for all samples except CA_CA36 (n=4) and LA_LA36 (n=2).

FACTORY STUDY - MARINADES								
sample	marinade						fish	
	pH	stdev	[salt] %(w/w)	stdev	[sugar] %(w/w)	stdev	[salt] %(w/w)	stdev
AA_AA36	4.09	0.04	1.4	0.1	28	0.1	1.5	0.1
AA_CA25	4.09	0.05	1.8	0.1	22	0.2	1.6	0.0
AA_LA25	4.09	0.02	1.7	0.1	22	0.1	1.7	0.1
CA_CA36	3.62	0.05	1.4	0.1	28	0.3	1.5	0.1
CA_CA25	3.65	0.03	1.8	0.1	22	0.3	1.6	0.0
LA_LA36	3.65	0.04	1.5	0.1	28	0.3	1.6	0.0
LA_LA25	3.69	0.03	1.8	0.0	22	0.1	1.8	0.1
SK	4.16	0.05	1.3	0.0	33	0.1	1.2	0.0

sample	fish + marinade				fish weight	
	[salt] %(w/w)	stdev	[sugar] %(w/w)	stdev	g	stdev
AA_AA36	1.2	0.1	27.7	0.0	151.94	1.7
AA_CA25	1.4	0.0	21.5	0.1	143.75	2.3
AA_LA25	1.5	0.0	21.5	0.1	144.52	2.5
CA_CA36	1.1	0.0	27.9	0.0	145.17	1.1
CA_CA25	1.4	0.0	21.5	0.1	143.57	2.1
LA_LA36	1.2	0.0	27.5	0.2	144.73	2.5
LA_LA25	1.5	0.1	21.5	0.1	141.83	2.9
SK	1.0	0.0	33.1	0.2	131.68	4.4

APPENDIX | E
MICROBIOLOGICAL RESULTS

Table E.1 - Microbiological analyses results of Experiment I samples.

PILOT STUDIES - EXPERIMENT I

Acid	Aerobic microorganisms (cfu/g)	Sulfide reducing Clostridia (cfu/g)	Hydrogen-sullfide producing bacteria (cfu/g)	Yeasts (cfu/g)	Moulds (cfu/g)	L. monocytogenes (/25g)
AA	<10000	<10	<10000	<10	<10	n.d.[1]
CA	<10000	<10	<10000	>4500	<10	n.d.[1]
LA	<10000	<10	<10000	>4500	<10	n.d.[1]
TA	<10000	<10	<10000	>4500	<10	n.d.[1]

[1] not detected

Table E.2 - Microbiological analyses results of Experiment IIA pickled samples.

PILOT STUDIES - EXPERIMENT IIA PICKLED SAMPLES

Acid	Aerobic microorganisms (cfu/g)	Enterobacteria (cfu/g)	Sulfide reducing Clostridia (cfu/g)	Hydrogen-sullfid e producing bacteria (cfu/g)	Yeasts (cfu/g)	Moulds (cfu/g)	L. monocytogenes (/25g)
AA	50 *	<10	<10	<10	<10	<10	n.d.[1]
CA	1900	<10	<10	<10	30000	2400*	n.d.[1]
LA	1500	<10	<10	<10	580	1200	n.d.[1]
TA	580000	<10	<10	<10	<10	<10	n.d.[1]

* estmated value; [1] not detected

Table E.3 - Microbiological analyses results of Experiment IIB pickled samples.

PILOT STUDIES - EXPERIMENT IIB PICKLED SAMPLES

Acid	Aerobic microorganisms (cfu/g)	Enterobacteria (cfu/g)	Sulfide reducing Clostridia (cfu/g)	Hydrogen-sullfid e producing bacteria (cfu/g)	Yeasts (cfu/g)	Moulds (cfu/g)	L. monocytogenes (/25g)
AA	50	<10	<10	<10	<10	<10	n.d.[1]
CA	65000	<10	<10	<10	230000	60*	n.d.[1]
LA	18000	<10	<10	<10	110000	<10	n.d.[1]
TA	690000	<10	<10	<10	15*	<10	n.d.[1]

* estmated value; [1] not detected

Table E.4 - Microbiological analysis results of Experiment IIA marinated samples.

PILOT STUDIES - EXPERIMENT IIA MARINADES

Acid	Aerobic microorganisms (cfu/g)	Enterobacteria (cfu/g)	Sulfide reducing Clostridia (cfu/g)	Hydrogen-sullfide producing bacteria (cfu/g)	Yeasts (cfu/g)	Moulds (cfu/g)	L. monocytogenes (/25g)
AA_AA15%	630000	<10	<10	<10	<10	<10	n.d.[1]
AA_CA9%	1100000	<10	<10	<10	<10	<10	n.d.[1]
AA_LA9%	1500000	<10	<10	<10	<10	<10	n.d.[1]
CA_CA5%	1400000	<10	<10	<10	20000	3600*	n.d.[1]
CA_CA9%	160000000	<10	<10	<10	150000	2300	n.d.[1]
CA_CA15%	58000	<10	<10	<10	88000	4400	n.d.[1]
LA_LA5%	63000	<10	<10	<10	<10	300	n.d.[1]
LA_LA9%	720000	<10	<10	<10	30*	340	n.d.[1]
LA_LA15%	160000	<10	<10	<10	30*	490	n.d.[1]

* estmated value; [1] not detected

Table E.5 - Microbiological analysis results of Factory Study marinated samples[7] .

FACTORY STUDY - MARINADES

Acid	Aerobic microorganisms (cfu/g)	Enterobacteria (cfu/g)	Sulfide reducing Clostridia (cfu/g)	Hydrogen-sullfide producing bacteria (cfu/g)	Yeasts (cfu/g)	Moulds (cfu/g)	L. monocytogenes (/25g)
AA_AA36%	<10	<10	<10	<10	<10	<10	n.d.[1]
AA_CA25%	40*	<10	<10	<10	<10	<10	n.d.[1]
AA_LA25%	10*	<10	<10	<10	<10	<10	n.d.[1]
CA_CA25%	<10	<10	<10	<10	45*	<10	n.d.[1]
LA_LA36%	<10	<10	<10	<10	<10	<10	n.d.[1]
LA_LA25%	<10	<10	<10	<10	<10	<10	n.d.[1]

* estmated value; [1] not detected

[7] CA_CA36 marinade was not analised because of a labelling mistake

APPENDIX | F

STATISTICAL ANALYSIS OF FACTORY STUDY RESULTS

Descriptives

Salt concentration in fish muscle

Sample_name			Statistic	Std. Error
AA_AA36	Mean		1.5333	0.03333
	95% Confidence Interval for	Lower Bound	1.4565	
		Upper Bound	1.6102	
	5% Trimmed Mean		1.5370	
	Median		1.6000	
	Variance		0.010	
	Std. Deviation		0.10000	
	Minimum		1.40	
	Maximum		1.60	
	Range		0.20	
	Interquartile Range		0.20	
	Skewness		-0.857	0.717
	Kurtosis		-1.714	1.400
AA_CA25	Mean		1.6333	0.01667
	95% Confidence Interval for	Lower Bound	1.5949	
		Upper Bound	1.6718	
	5% Trimmed Mean		1.6315	
	Median		1.6000	
	Variance		0.002	
	Std. Deviation		0.05000	
	Minimum		1.60	
	Maximum		1.70	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		0.857	0.717
	Kurtosis		-1.714	1.400
AA_LA25	Mean		1.7000	0.02887
	95% Confidence Interval for	Lower Bound	1.6334	
		Upper Bound	1.7666	
	5% Trimmed Mean		1.7000	
	Median		1.7000	
	Variance		0.008	
	Std. Deviation		0.08660	
	Minimum		1.60	
	Maximum		1.80	
	Range		0.20	
	Interquartile Range		0.20	
	Skewness		0.000	0.717
	Kurtosis		-1.714	1.400
CA_CA36	Mean		1.5250	0.03917
	95% Confidence Interval for	Lower Bound	1.4388	
		Upper Bound	1.6112	
	5% Trimmed Mean		1.5222	
	Median		1.5000	
	Variance		0.018	
	Std. Deviation		0.13568	
	Minimum		1.40	
	Maximum		1.70	
	Range		0.30	
	Interquartile Range		0.28	
	Skewness		0.246	0.637
	Kurtosis		-2.000	1.232

Sample_name			Statistic	Std. Error
CA_CA25	Mean		1.6000	0.00000
	95% Confidence Interval for	Lower Bound	1.6000	
		Upper Bound	1.6000	
	5% Trimmed Mean		1.6000	
	Median		1.6000	
	Variance		0.000	
	Std. Deviation		0.00000	
	Minimum		1.60	
	Maximum		1.60	
	Range		0.00	
	Interquartile Range		0.00	
	Skewness			
	Kurtosis			
LA_LA36	Mean		1.6000	0.00000
	95% Confidence Interval for	Lower Bound	1.6000	
		Upper Bound	1.6000	
	5% Trimmed Mean		1.6000	
	Median		1.6000	
	Variance		0.000	
	Std. Deviation		0.00000	
	Minimum		1.60	
	Maximum		1.60	
	Range		0.00	
	Interquartile Range		0.00	
	Skewness			
	Kurtosis			
LA_LA25	Mean		1.7667	0.04410
	95% Confidence Interval for	Lower Bound	1.6650	
		Upper Bound	1.8684	
	5% Trimmed Mean		1.7685	
	Median		1.8000	
	Variance		0.018	
	Std. Deviation		0.13229	
	Minimum		1.60	
	Maximum		1.90	
	Range		0.30	
	Interquartile Range		0.30	
	Skewness		-0.463	0.717
	Kurtosis		-1.714	1.400
SK	Mean		1.2000	0.00000
	95% Confidence Interval for	Lower Bound	1.2000	
		Upper Bound	1.2000	
	5% Trimmed Mean		1.2000	
	Median		1.2000	
	Variance		0.000	
	Std. Deviation		0.00000	
	Minimum		1.20	
	Maximum		1.20	
	Range		0.00	
	Interquartile Range		0.00	
	Skewness			
	Kurtosis			

pH in marinade

Sample_name			Statistic	Std. Error
AA_AA36	Mean		4.0944	0.01248
	95% Confidence Interval for	Lower Bound	4.0657	
		Upper Bound	4.1232	
	5% Trimmed Mean		4.0916	
	Median		4.0800	
	Variance		0.001	
	Std. Deviation		0.03745	
	Minimum		4.06	
	Maximum		4.18	
	Range		0.12	
	Interquartile Range		0.04	
	Skewness		1.736	0.717
	Kurtosis		3.094	1.400
AA_CA25	Mean		4.0889	0.01532
	95% Confidence Interval for	Lower Bound	4.0536	
		Upper Bound	4.1242	
	5% Trimmed Mean		4.0915	
	Median		4.0900	
	Variance		0.002	
	Std. Deviation		0.04595	
	Minimum		3.99	
	Maximum		4.14	
	Range		0.15	
	Interquartile Range		0.06	
	Skewness		-1.131	0.717
	Kurtosis		2.069	1.400
AA_LA25	Mean		4.0878	0.00722
	95% Confidence Interval for	Lower Bound	4.0711	
		Upper Bound	4.1044	
	5% Trimmed Mean		4.0870	
	Median		4.0900	
	Variance		0.000	
	Std. Deviation		0.02167	
	Minimum		4.06	
	Maximum		4.13	
	Range		0.07	
	Interquartile Range		0.03	
	Skewness		0.840	0.717
	Kurtosis		0.559	1.400
CA_CA36	Mean		3.6175	0.01382
	95% Confidence Interval for	Lower Bound	3.5871	
		Upper Bound	3.6479	
	5% Trimmed Mean		3.6161	
	Median		3.6250	
	Variance		0.002	
	Std. Deviation		0.04789	
	Minimum		3.55	
	Maximum		3.71	
	Range		0.16	
	Interquartile Range		0.08	
	Skewness		0.233	0.637
	Kurtosis		-0.270	1.232

Sample_name			Statistic	Std. Error
CA_CA25	Mean		3.6456	0.01094
	95% Confidence Interval for	Lower Bound	3.6203	
		Upper Bound	3.6708	
	5% Trimmed Mean		3.6462	
	Median		3.6500	
	Variance		0.001	
	Std. Deviation		0.03283	
	Minimum		3.59	
	Maximum		3.69	
	Range		0.10	
	Interquartile Range		0.05	
	Skewness		-0.283	0.717
	Kurtosis		-0.848	1.400
LA_LA36	Mean		3.6483	0.01558
	95% Confidence Interval for	Lower Bound	3.6083	
		Upper Bound	3.6884	
	5% Trimmed Mean		3.6493	
	Median		3.6650	
	Variance		0.001	
	Std. Deviation		0.03817	
	Minimum		3.60	
	Maximum		3.68	
	Range		0.08	
	Interquartile Range		0.08	
	Skewness		-0.812	0.845
	Kurtosis		-1.907	1.741
LA_LA25	Mean		3.6911	0.01160
	95% Confidence Interval for	Lower Bound	3.6644	
		Upper Bound	3.7179	
	5% Trimmed Mean		3.6907	
	Median		3.6800	
	Variance		0.001	
	Std. Deviation		0.03480	
	Minimum		3.64	
	Maximum		3.75	
	Range		0.11	
	Interquartile Range		0.05	
	Skewness		0.361	0.717
	Kurtosis		-0.474	1.400
SK	Mean		4.1600	0.01691
	95% Confidence Interval for	Lower Bound	4.1210	
		Upper Bound	4.1990	
	5% Trimmed Mean		4.1583	
	Median		4.1400	
	Variance		0.003	
	Std. Deviation		0.05074	
	Minimum		4.10	
	Maximum		4.25	
	Range		0.15	
	Interquartile Range		0.09	
	Skewness		0.635	0.717
	Kurtosis		-0.717	1.400

Salt concentration in marinade

Sample_name			Statistic	Std. Error
AA_AA36	Mean		1.4000	0.01667
	95% Confidence Interval for	Lower Bound	1.3616	
		Upper Bound	1.4384	
	5% Trimmed Mean		1.4000	
	Median		1.4000	
	Variance		0.003	
	Std. Deviation		0.05000	
	Minimum		1.30	
	Maximum		1.50	
	Range		0.20	
	Interquartile Range		0.00	
	Skewness		0.000	0.717
	Kurtosis		4.000	1.400
AA_CA25	Mean		1.7889	0.02003
	95% Confidence Interval for	Lower Bound	1.7427	
		Upper Bound	1.8351	
	5% Trimmed Mean		1.7877	
	Median		1.8000	
	Variance		0.004	
	Std. Deviation		0.06009	
	Minimum		1.70	
	Maximum		1.90	
	Range		0.20	
	Interquartile Range		0.05	
	Skewness		-0.018	0.717
	Kurtosis		1.126	1.400
AA_LA25	Mean		1.7444	0.01757
	95% Confidence Interval for	Lower Bound	1.7039	
		Upper Bound	1.7850	
	5% Trimmed Mean		1.7438	
	Median		1.7000	
	Variance		0.003	
	Std. Deviation		0.05270	
	Minimum		1.70	
	Maximum		1.80	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		0.271	0.717
	Kurtosis		-2.571	1.400
CA_CA36	Mean		1.3500	0.01946
	95% Confidence Interval for	Lower Bound	1.3072	
		Upper Bound	1.3928	
	5% Trimmed Mean		1.3556	
	Median		1.4000	
	Variance		0.005	
	Std. Deviation		0.06742	
	Minimum		1.20	
	Maximum		1.40	
	Range		0.20	
	Interquartile Range		0.10	
	Skewness		-1.068	0.637
	Kurtosis		0.352	1.232

Sample_name			Statistic	Std. Error
CA_CA25	Mean		1.8000	0.02887
	95% Confidence Interval for	Lower Bound	1.7334	
		Upper Bound	1.8666	
	5% Trimmed Mean		1.8000	
	Median		1.8000	
	Variance		0.008	
	Std. Deviation		0.08660	
	Minimum		1.70	
	Maximum		1.90	
	Range		0.20	
	Interquartile Range		0.20	
	Skewness		0.000	0.717
	Kurtosis		-1.714	1.400
LA_LA36	Mean		1.5000	0.02582
	95% Confidence Interval for	Lower Bound	1.4336	
		Upper Bound	1.5664	
	5% Trimmed Mean		1.5000	
	Median		1.5000	
	Variance		0.004	
	Std. Deviation		0.06325	
	Minimum		1.40	
	Maximum		1.60	
	Range		0.20	
	Interquartile Range		0.05	
	Skewness		0.000	0.845
	Kurtosis		2.500	1.741
LA_LA25	Mean		1.7889	0.01111
	95% Confidence Interval for	Lower Bound	1.7633	
		Upper Bound	1.8145	
	5% Trimmed Mean		1.7932	
	Median		1.8000	
	Variance		0.001	
	Std. Deviation		0.03333	
	Minimum		1.70	
	Maximum		1.80	
	Range		0.10	
	Interquartile Range		0.00	
	Skewness		-3.000	0.717
	Kurtosis		9.000	1.400
SK	Mean		1.2778	0.01470
	95% Confidence Interval for	Lower Bound	1.2439	
		Upper Bound	1.3117	
	5% Trimmed Mean		1.2809	
	Median		1.3000	
	Variance		0.002	
	Std. Deviation		0.04410	
	Minimum		1.20	
	Maximum		1.30	
	Range		0.10	
	Interquartile Range		0.05	
	Skewness		-1.620	0.717
	Kurtosis		0.735	1.400

Salt concentration in fish + marinade

Sample_name			Statistic	Std. Error
AA_AA36	Mean		1.1500	0.02236
	95% Confidence Interval for	Lower Bound	1.0925	
		Upper Bound	1.2075	
	5% Trimmed Mean		1.1500	
	Median		1.1500	
	Variance		0.003	
	Std. Deviation		0.05477	
	Minimum		1.10	
	Maximum		1.20	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		0.000	0.845
	Kurtosis		-3.333	1.741
AA_CA25	Mean		1.4222	0.01470
	95% Confidence Interval for	Lower Bound	1.3883	
		Upper Bound	1.4561	
	5% Trimmed Mean		1.4191	
	Median		1.4000	
	Variance		0.002	
	Std. Deviation		0.04410	
	Minimum		1.40	
	Maximum		1.50	
	Range		0.10	
	Interquartile Range		0.05	
	Skewness		1.620	0.717
	Kurtosis		0.735	1.400
AA_LA25	Mean		1.4778	0.01470
	95% Confidence Interval for	Lower Bound	1.4439	
		Upper Bound	1.5117	
	5% Trimmed Mean		1.4809	
	Median		1.5000	
	Variance		0.002	
	Std. Deviation		0.04410	
	Minimum		1.40	
	Maximum		1.50	
	Range		0.10	
	Interquartile Range		0.05	
	Skewness		-1.620	0.717
	Kurtosis		0.735	1.400
CA_CA36	Mean		1.1000	0.00000
	95% Confidence Interval for	Lower Bound	1.1000	
		Upper Bound	1.1000	
	5% Trimmed Mean		1.1000	
	Median		1.1000	
	Variance		0.000	
	Std. Deviation		0.00000	
	Minimum		1.10	
	Maximum		1.10	
	Range		0.00	
	Interquartile Range		0.00	
	Skewness			
	Kurtosis			

Sample_name			Statistic	Std. Error
CA_CA25	Mean		1.3667	0.01667
	95% Confidence Interval for	Lower Bound	1.3282	
		Upper Bound	1.4051	
	5% Trimmed Mean		1.3685	
	Median		1.4000	
	Variance		0.002	
	Std. Deviation		0.05000	
	Minimum		1.30	
	Maximum		1.40	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		-0.857	0.717
	Kurtosis		-1.714	1.400
LA_LA36	Mean		1.2000	0.00000
	95% Confidence Interval for	Lower Bound	1.2000	
		Upper Bound	1.2000	
	5% Trimmed Mean		1.2000	
	Median		1.2000	
	Variance		0.000	
	Std. Deviation		0.00000	
	Minimum		1.20	
	Maximum		1.20	
	Range		0.00	
	Interquartile Range		0.00	
	Skewness			
	Kurtosis			
LA_LA25	Mean		1.4667	0.01667
	95% Confidence Interval for	Lower Bound	1.4282	
		Upper Bound	1.5051	
	5% Trimmed Mean		1.4685	
	Median		1.5000	
	Variance		0.003	
	Std. Deviation		0.05000	
	Minimum		1.40	
	Maximum		1.50	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		-0.857	0.717
	Kurtosis		-1.714	1.400
SK	Mean		1.0222	0.01470
	95% Confidence Interval for	Lower Bound	0.9883	
		Upper Bound	1.0561	
	5% Trimmed Mean		1.0191	
	Median		1.0000	
	Variance		0.002	
	Std. Deviation		0.04410	
	Minimum		1.00	
	Maximum		1.10	
	Range		0.10	
	Interquartile Range		0.05	
	Skewness		1.620	0.717
	Kurtosis		0.735	1.400

Sugar concentration in marinade

Sample_name			Statistic	Std. Error
AA_AA36	Mean		27.7630	0.01427
	95% Confidence Interval for	Lower Bound	27.7336	
		Upper Bound	27.7923	
	5% Trimmed Mean		27.7588	
	Median		27.7000	
	Variance		0.005	
	Std. Deviation		0.07415	
	Minimum		27.70	
	Maximum		27.90	
	Range		0.20	
	Interquartile Range		0.10	
	Skewness		0.739	0.448
	Kurtosis		-0.739	0.872
AA_CA25	Mean		21.6630	0.02979
	95% Confidence Interval for	Lower Bound	21.6017	
		Upper Bound	21.7242	
	5% Trimmed Mean		21.6630	
	Median		21.7000	
	Variance		0.024	
	Std. Deviation		0.15479	
	Minimum		21.40	
	Maximum		21.90	
	Range		0.50	
	Interquartile Range		0.30	
	Skewness		0.141	0.448
	Kurtosis		-1.279	0.872
AA_LA25	Mean		21.6593	0.00964
	95% Confidence Interval for	Lower Bound	21.6395	
		Upper Bound	21.6791	
	5% Trimmed Mean		21.6603	
	Median		21.7000	
	Variance		0.003	
	Std. Deviation		0.05007	
	Minimum		21.60	
	Maximum		21.70	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		-0.399	0.448
	Kurtosis		-1.994	0.872
CA_CA36	Mean		27.7194	0.05743
	95% Confidence Interval for	Lower Bound	27.6028	
		Upper Bound	27.8360	
	5% Trimmed Mean		27.7074	
	Median		27.7000	
	Variance		0.119	
	Std. Deviation		0.34461	
	Minimum		27.30	
	Maximum		28.40	
	Range		1.10	
	Interquartile Range		0.65	
	Skewness		0.495	0.393
	Kurtosis		-0.780	0.768

Sample_name			Statistic	Std. Error
CA_CA25	Mean		21.5074	0.04919
	95% Confidence Interval for	Lower Bound	21.4063	
		Upper Bound	21.6085	
	5% Trimmed Mean		21.4971	
	Median		21.4000	
	Variance		0.065	
	Std. Deviation		0.25559	
	Minimum		21.30	
	Maximum		21.90	
	Range		0.60	
	Interquartile Range		0.50	
	Skewness		0.717	0.448
	Kurtosis		-1.437	0.872
LA_LA36	Mean		27.6889	0.06098
	95% Confidence Interval for	Lower Bound	27.5602	
		Upper Bound	27.8175	
	5% Trimmed Mean		27.6877	
	Median		27.7500	
	Variance		0.067	
	Std. Deviation		0.25870	
	Minimum		27.40	
	Maximum		28.00	
	Range		0.60	
	Interquartile Range		0.50	
	Skewness		-0.046	0.536
	Kurtosis		-2.033	1.038
LA_LA25	Mean		21.6815	0.02617
	95% Confidence Interval for	Lower Bound	21.6277	
		Upper Bound	21.7353	
	5% Trimmed Mean		21.6794	
	Median		21.7000	
	Variance		0.018	
	Std. Deviation		0.13598	
	Minimum		21.50	
	Maximum		21.90	
	Range		0.40	
	Interquartile Range		0.30	
	Skewness		-0.035	0.448
	Kurtosis		-1.147	0.872
SK	Mean		33.1222	0.03643
	95% Confidence Interval for	Lower Bound	33.0382	
		Upper Bound	33.2062	
	5% Trimmed Mean		33.1191	
	Median		33.1000	
	Variance		0.012	
	Std. Deviation		0.10929	
	Minimum		33.00	
	Maximum		33.30	
	Range		0.30	
	Interquartile Range		0.20	
	Skewness		0.188	0.717
	Kurtosis		-1.232	1.400

Sugar concentration in fish + marinade

Sample_name			Statistic	Std. Error
AA_AA36	Mean		27.7222	0.01008
	95% Confidence Interval for	Lower Bound	27.7009	
		Upper Bound	27.7435	
	5% Trimmed Mean		27.7191	
	Median		27.7000	
	Variance		0.002	
	Std. Deviation		0.04278	
	Minimum		27.70	
	Maximum		27.80	
	Range		0.10	
	Interquartile Range		0.03	
	Skewness		1.461	0.536
	Kurtosis		0.137	1.038
AA_CA25	Mean		21.4667	0.01925
	95% Confidence Interval for	Lower Bound	21.4271	
		Upper Bound	21.5062	
	5% Trimmed Mean		21.4685	
	Median		21.5000	
	Variance		0.010	
	Std. Deviation		0.10000	
	Minimum		21.30	
	Maximum		21.60	
	Range		0.30	
	Interquartile Range		0.10	
	Skewness		-0.498	0.448
	Kurtosis		-0.698	0.872
AA_LA25	Mean		21.4704	0.01835
	95% Confidence Interval for	Lower Bound	21.4327	
		Upper Bound	21.5081	
	5% Trimmed Mean		21.4671	
	Median		21.4000	
	Variance		0.009	
	Std. Deviation		0.09533	
	Minimum		21.40	
	Maximum		21.60	
	Range		0.20	
	Interquartile Range		0.20	
	Skewness		0.657	0.448
	Kurtosis		-1.644	0.872
CA_CA36	Mean		27.8704	0.00896
	95% Confidence Interval for	Lower Bound	27.8520	
		Upper Bound	27.8888	
	5% Trimmed Mean		27.8726	
	Median		27.9000	
	Variance		0.002	
	Std. Deviation		0.04653	
	Minimum		27.80	
	Maximum		27.90	
	Range		0.10	
	Interquartile Range		0.10	
	Skewness		-0.946	0.448
	Kurtosis		-1.201	0.872

Sample_name			Statistic	Std. Error
CA_CA25	Mean		21.4556	0.02465
	95% Confidence Interval for	Lower Bound	21.4049	
		Upper Bound	21.5062	
	5% Trimmed Mean		21.4506	
	Median		21.4000	
	Variance		0.016	
	Std. Deviation		0.12810	
	Minimum		21.30	
	Maximum		21.70	
	Range		0.40	
	Interquartile Range		0.20	
	Skewness		0.578	0.448
	Kurtosis		-0.997	0.872
LA_LA36	Mean		27.4889	0.03261
	95% Confidence Interval for	Lower Bound	27.4219	
		Upper Bound	27.5559	
	5% Trimmed Mean		27.4877	
	Median		27.5000	
	Variance		0.029	
	Std. Deviation		0.16946	
	Minimum		27.30	
	Maximum		27.70	
	Range		0.40	
	Interquartile Range		0.40	
	Skewness		0.187	0.448
	Kurtosis		-1.645	0.872
LA_LA25	Mean		21.5111	0.02522
	95% Confidence Interval for	Lower Bound	21.4593	
		Upper Bound	21.5630	
	5% Trimmed Mean		21.5179	
	Median		21.6000	
	Variance		0.017	
	Std. Deviation		0.13107	
	Minimum		21.30	
	Maximum		21.60	
	Range		0.30	
	Interquartile Range		0.20	
	Skewness		-0.884	0.448
	Kurtosis		-1.163	0.872
SK	Mean		33.0889	0.02988
	95% Confidence Interval for	Lower Bound	33.0275	
		Upper Bound	33.1503	
	5% Trimmed Mean		33.0877	
	Median		33.0000	
	Variance		0.024	
	Std. Deviation		0.15525	
	Minimum		32.90	
	Maximum		33.30	
	Range		0.40	
	Interquartile Range		0.30	
	Skewness		0.599	0.448
	Kurtosis		-1.492	0.872

Pairwise Comparisons of Samples

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
SK-LA_LA25	6.333	5.774	1.097	0.273	1.000
SK-CA_CA25	9.000	5.774	1.559	0.119	1.000
SK-AA_CA25	9.333	5.774	1.617	0.106	1.000
SK-AA_LA25	11.667	5.774	2.021	0.043	1.000
SK-LA_LA36	12.500	6.455	1.936	0.053	1.000
SK-CA_CA36	13.750	5.401	2.546	0.011	0.305
SK-AA_AA36	21.000	5.774	3.637	0.000	0.008
LA_LA25-CA_CA25	2.667	5.774	0.462	0.644	1.000
LA_LA25-AA_CA25	3.000	5.774	0.520	0.603	1.000
LA_LA25-AA_LA25	5.333	5.774	0.924	0.356	1.000
LA_LA25-LA_LA36	6.167	6.455	0.955	0.339	1.000
LA_LA25-CA_CA36	7.417	5.401	1.373	0.170	1.000
LA_LA25-AA_AA36	14.667	5.774	2.540	0.011	0.310
CA_CA25-AA_CA25	0.333	5.774	0.058	0.954	1.000
CA_CA25-AA_LA25	2.667	5.774	0.462	0.644	1.000
CA_CA25-LA_LA36	-3.500	6.455	-0.542	0.588	1.000
CA_CA25-CA_CA36	4.750	5.401	0.880	0.379	1.000
CA_CA25-AA_AA36	12.000	5.774	2.078	0.038	1.000
AA_CA25-AA_LA25	-2.333	5.774	-0.404	0.686	1.000
AA_CA25-LA_LA36	-3.167	6.455	-0.491	0.624	1.000
AA_CA25-CA_CA36	-4.417	5.401	-0.818	0.413	1.000
AA_CA25-AA_AA36	11.667	5.774	2.021	0.043	1.000
AA_LA25-LA_LA36	-0.833	6.455	-0.129	0.897	1.000
AA_LA25-CA_CA36	-2.083	5.401	-0.386	0.700	1.000
AA_LA25-AA_AA36	9.333	5.774	1.617	0.106	1.000
LA_LA36-CA_CA36	1.250	6.124	0.204	0.838	1.000
LA_LA36-AA_AA36	8.500	6.455	1.317	0.188	1.000
CA_CA36-AA_AA36	7.250	5.401	1.342	0.179	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

pH of marinade

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
CA_CA36-CA_CA25	-5.722	9.218	-0.621	0.535	1.000
CA_CA36-LA_LA36	-7.167	10.452	-0.686	0.493	1.000
CA_CA36-LA_LA25	-16.167	9.218	-1.754	0.079	1.000
CA_CA36-AA_LA25	38.167	9.218	4.140	0.000	0.001
CA_CA36-AA_AA36	38.722	9.218	4.201	0.000	0.001
CA_CA36-AA_CA25	40.444	9.218	4.387	0.000	0.000
CA_CA36-SK	-53.333	9.218	-5.786	0.000	0.000
CA_CA25-LA_LA36	-1.444	11.018	-0.131	0.896	1.000
CA_CA25-LA_LA25	-10.444	9.855	-1.060	0.289	1.000
CA_CA25-AA_LA25	32.444	9.855	3.292	0.001	0.028
CA_CA25-AA_AA36	33.000	9.855	3.349	0.001	0.023
CA_CA25-AA_CA25	34.722	9.855	3.523	0.000	0.012
CA_CA25-SK	-47.611	9.855	-4.831	0.000	0.000
LA_LA36-LA_LA25	-9.000	11.018	-0.817	0.414	1.000
LA_LA36-AA_LA25	31.000	11.018	2.814	0.005	0.137
LA_LA36-AA_AA36	31.556	11.018	2.864	0.004	0.117
LA_LA36-AA_CA25	33.278	11.018	3.020	0.003	0.071
LA_LA36-SK	-46.167	11.018	-4.190	0.000	0.001
LA_LA25-AA_LA25	22.000	9.855	2.232	0.026	0.716
LA_LA25-AA_AA36	22.556	9.855	2.289	0.022	0.619
LA_LA25-AA_CA25	24.278	9.855	2.464	0.014	0.385
LA_LA25-SK	-37.167	9.855	-3.771	0.000	0.005
AA_LA25-AA_AA36	0.556	9.855	0.056	0.955	1.000
AA_LA25-AA_CA25	2.278	9.855	0.231	0.817	1.000
AA_LA25-SK	-15.167	9.855	-1.539	0.124	1.000
AA_AA36-AA_CA25	-1.722	9.855	-0.175	0.861	1.000
AA_AA36-SK	-14.611	9.855	-1.483	0.138	1.000
AA_CA25-SK	-12.889	9.855	-1.308	0.191	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Salt concentration in marinade

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
SK-CA_CA36	8.917	9.031	0.987	0.323	1.000
SK-AA_AA36	14.778	9.654	1.531	0.126	1.000
SK-LA_LA36	24.000	10.794	2.224	0.026	0.733
SK-AA_LA25	41.278	9.654	4.276	0.000	0.001
SK-AA_CA25	48.000	9.654	4.972	0.000	0.000
SK-LA_LA25	48.389	9.654	5.012	0.000	0.000
SK-CA_CA25	49.000	9.654	5.076	0.000	0.000
CA_CA36-AA_AA36	5.861	9.031	0.649	0.516	1.000
CA_CA36-LA_LA36	-15.083	10.240	-1.473	0.141	1.000
CA_CA36-AA_LA25	32.361	9.031	3.583	0.000	0.009
CA_CA36-AA_CA25	39.083	9.031	4.328	0.000	0.000
CA_CA36-LA_LA25	-39.472	9.031	-4.371	0.000	0.000
CA_CA36-CA_CA25	-40.083	9.031	-4.439	0.000	0.000
AA_AA36-LA_LA36	-9.222	10.794	-0.854	0.393	1.000
AA_AA36-AA_LA25	-26.500	9.654	-2.745	0.006	0.169
AA_AA36-AA_CA25	-33.222	9.654	-3.441	0.001	0.016
AA_AA36-LA_LA25	-33.611	9.654	-3.482	0.000	0.014
AA_AA36-CA_CA25	-34.222	9.654	-3.545	0.000	0.011
LA_LA36-AA_LA25	17.278	10.794	1.601	0.109	1.000
LA_LA36-AA_CA25	24.000	10.794	2.224	0.026	0.733
LA_LA36-LA_LA25	-24.389	10.794	-2.260	0.024	0.668
LA_LA36-CA_CA25	25.000	10.794	2.316	0.021	0.575
AA_LA25-AA_CA25	6.722	9.654	0.696	0.486	1.000
AA_LA25-LA_LA25	-7.111	9.654	-0.737	0.461	1.000
AA_LA25-CA_CA25	-7.722	9.654	-0.800	0.424	1.000
AA_CA25-LA_LA25	-0.389	9.654	-0.040	0.968	1.000
AA_CA25-CA_CA25	-1.000	9.654	-0.104	0.918	1.000
LA_LA25-CA_CA25	0.611	9.654	0.063	0.950	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Salt concentration in fish muscle

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
SK-AA_AA36	24.000	9.195	2.610	0.009	0.253
SK-CA_CA36	25.875	8.601	3.008	0.003	0.074
SK-CA_CA25	31.500	9.195	3.426	0.001	0.017
SK-LA_LA36	31.500	10.280	3.064	0.002	0.061
SK-AA_CA25	39.000	9.195	4.241	0.000	0.001
SK-AA_LA25	49.000	9.195	5.329	0.000	0.000
SK-LA_LA25	53.000	9.195	5.764	0.000	0.000
AA_AA36-CA_CA36	-1.875	8.601	-0.218	0.827	1.000
AA_AA36-CA_CA25	-7.500	9.195	-0.816	0.415	1.000
AA_AA36-LA_LA36	-7.500	10.280	-0.730	0.466	1.000
AA_AA36-AA_CA25	-15.000	9.195	-1.631	0.103	1.000
AA_AA36-AA_LA25	-25.000	9.195	-2.719	0.007	0.183
AA_AA36-LA_LA25	-29.000	9.195	-3.154	0.002	0.045
CA_CA36-CA_CA25	-5.625	8.601	-0.654	0.513	1.000
CA_CA36-LA_LA36	-5.625	9.753	-0.577	0.564	1.000
CA_CA36-AA_CA25	13.125	8.601	1.526	0.127	1.000
CA_CA36-AA_LA25	23.125	8.601	2.689	0.007	0.201
CA_CA36-LA_LA25	-27.125	8.601	-3.154	0.002	0.045
CA_CA25-AA_CA25	7.500	9.195	0.816	0.415	1.000
LA_LA36-AA_CA25	7.500	10.280	0.730	0.466	1.000
CA_CA25-AA_LA25	17.500	9.195	1.903	0.057	1.000
LA_LA36-AA_LA25	17.500	10.280	1.702	0.089	1.000
CA_CA25-LA_LA36	0.000	10.280	0.000	1.000	1.000
CA_CA25-LA_LA25	-21.500	9.195	-2.338	0.019	0.543
LA_LA36-LA_LA25	-21.500	10.280	-2.091	0.036	1.000
AA_CA25-AA_LA25	-10.000	9.195	-1.088	0.277	1.000
AA_CA25-LA_LA25	-14.000	9.195	-1.523	0.128	1.000
AA_LA25-LA_LA25	-4.000	9.195	-0.435	0.664	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Salt concentration in fish + marinade

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
SK-CA_CA36	8.167	9.254	0.882	0.378	1.000
SK-AA_AA36	14.667	10.346	1.418	0.156	1.000
SK-LA_LA36	21.167	9.254	2.287	0.022	0.621
SK-CA_CA25	35.667	9.254	3.854	0.000	0.003
SK-AA_CA25	42.833	9.254	4.629	0.000	0.000
SK-LA_LA25	50.167	9.254	5.421	0.000	0.000
SK-AA_LA25	52.000	9.254	5.619	0.000	0.000
CA_CA36-AA_AA36	6.500	10.346	0.628	0.530	1.000
CA_CA36-LA_LA36	-13.000	9.254	-1.405	0.160	1.000
CA_CA36-CA_CA25	-27.500	9.254	-2.972	0.003	0.083
CA_CA36-AA_CA25	34.667	9.254	3.746	0.000	0.005
CA_CA36-LA_LA25	-42.000	9.254	-4.539	0.000	0.000
CA_CA36-AA_LA25	43.833	9.254	4.737	0.000	0.000
AA_AA36-LA_LA36	-6.500	10.346	-0.628	0.530	1.000
AA_AA36-CA_CA25	-21.000	10.346	-2.030	0.042	1.000
AA_AA36-AA_CA25	-28.167	10.346	-2.722	0.006	0.181
AA_AA36-LA_LA25	-35.500	10.346	-3.431	0.001	0.017
AA_AA36-AA_LA25	-37.333	10.346	-3.608	0.000	0.009
LA_LA36-CA_CA25	14.500	9.254	1.567	0.117	1.000
LA_LA36-AA_CA25	21.667	9.254	2.341	0.019	0.538
LA_LA36-LA_LA25	-29.000	9.254	-3.134	0.002	0.048
LA_LA36-AA_LA25	30.833	9.254	3.332	0.001	0.024
CA_CA25-AA_CA25	7.167	9.254	0.774	0.439	1.000
CA_CA25-LA_LA25	-14.500	9.254	-1.567	0.117	1.000
CA_CA25-AA_LA25	16.333	9.254	1.765	0.078	1.000
AA_CA25-LA_LA25	-7.333	9.254	-0.792	0.428	1.000
AA_CA25-AA_LA25	-9.167	9.254	-0.991	0.322	1.000
LA_LA25-AA_LA25	1.833	9.254	0.198	0.843	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Sugar concentration in marinade

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
CA_CA25-AA_LA25	18.815	15.539	1.211	0.226	1.000
CA_CA25-AA_CA25	20.500	15.539	1.319	0.187	1.000
CA_CA25-LA_LA25	-24.241	15.539	-1.560	0.119	1.000
CA_CA25-CA_CA36	106.319	14.535	7.315	0.000	0.000
CA_CA25-LA_LA36	-109.917	17.373	-6.327	0.000	0.000
CA_CA25-AA_AA36	116.130	15.539	7.474	0.000	0.000
CA_CA25-SK	-155.389	21.975	-7.071	0.000	0.000
AA_LA25-AA_CA25	1.685	15.539	0.108	0.914	1.000
AA_LA25-LA_LA25	-5.426	15.539	-0.349	0.727	1.000
AA_LA25-CA_CA36	-87.505	14.535	-6.020	0.000	0.000
AA_LA25-LA_LA36	-91.102	17.373	-5.244	0.000	0.000
AA_LA25-AA_AA36	97.315	15.539	6.263	0.000	0.000
AA_LA25-SK	-136.574	21.975	-6.215	0.000	0.000
AA_CA25-LA_LA25	-3.741	15.539	-0.241	0.810	1.000
AA_CA25-CA_CA36	-85.819	14.535	-5.904	0.000	0.000
AA_CA25-LA_LA36	-89.417	17.373	-5.147	0.000	0.000
AA_CA25-AA_AA36	95.630	15.539	6.154	0.000	0.000
AA_CA25-SK	-134.889	21.975	-6.138	0.000	0.000
LA_LA25-CA_CA36	82.079	14.535	5.647	0.000	0.000
LA_LA25-LA_LA36	85.676	17.373	4.932	0.000	0.000
LA_LA25-AA_AA36	91.889	15.539	5.914	0.000	0.000
LA_LA25-SK	-131.148	21.975	-5.968	0.000	0.000
CA_CA36-LA_LA36	-3.597	16.481	-0.218	0.827	1.000
CA_CA36-AA_AA36	9.810	14.535	0.675	0.500	1.000
CA_CA36-SK	-49.069	21.277	-2.306	0.021	0.591
LA_LA36-AA_AA36	6.213	17.373	0.358	0.721	1.000
LA_LA36-SK	-45.472	23.308	-1.951	0.051	1.000
AA_AA36-SK	-39.259	21.975	-1.787	0.074	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Sugar concentration in fish + marinade

Pairwise Comparisons of Sample_name

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.a
CA_CA25-AA_CA25	3.056	16.171	0.189	0.850	1.000
CA_CA25-AA_LA25	4.333	16.171	0.268	0.789	1.000
CA_CA25-LA_LA25	-14.833	16.171	-0.917	0.359	1.000
CA_CA25-LA_LA36	-75.389	16.171	-4.662	0.000	0.000
CA_CA25-AA_AA36	92.944	18.080	5.141	0.000	0.000
CA_CA25-CA_CA36	117.463	16.171	7.264	0.000	0.000
CA_CA25-SK	-145.056	16.171	-8.970	0.000	0.000
AA_CA25-AA_LA25	-1.278	16.171	-0.079	0.937	1.000
AA_CA25-LA_LA25	-11.778	16.171	-0.728	0.466	1.000
AA_CA25-LA_LA36	-72.333	16.171	-4.473	0.000	0.000
AA_CA25-AA_AA36	89.889	18.080	4.972	0.000	0.000
AA_CA25-CA_CA36	-114.407	16.171	-7.075	0.000	0.000
AA_CA25-SK	-142.000	16.171	-8.781	0.000	0.000
AA_LA25-LA_LA25	-10.500	16.171	-0.649	0.516	1.000
AA_LA25-LA_LA36	-71.056	16.171	-4.394	0.000	0.000
AA_LA25-AA_AA36	88.611	18.080	4.901	0.000	0.000
AA_LA25-CA_CA36	-113.130	16.171	-6.996	0.000	0.000
AA_LA25-SK	-140.722	16.171	-8.702	0.000	0.000
LA_LA25-LA_LA36	60.556	16.171	3.745	0.000	0.005
LA_LA25-AA_AA36	78.111	18.080	4.320	0.000	0.000
LA_LA25-CA_CA36	102.630	16.171	6.346	0.000	0.000
LA_LA25-SK	-130.222	16.171	-8.053	0.000	0.000
LA_LA36-AA_AA36	17.556	18.080	0.971	0.332	1.000
LA_LA36-CA_CA36	42.074	16.171	2.602	0.009	0.260
LA_LA36-SK	-69.667	16.171	-4.308	0.000	0.000
AA_AA36-CA_CA36	-24.519	18.080	-1.356	0.175	1.000
AA_AA36-SK	-52.111	18.080	-2.882	0.004	0.111
CA_CA36-SK	-27.593	16.171	-1.706	0.088	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

APPENDIX | G

SALT AND BRIX METERS OPERATING PRINCIPLES

Salt meter

Salt was measured using a DTM-20 saltmeter (DAEYOON SCALE INDUSTRIAL, South Korea), which uses electrical conductivity to measure the salt content of the sample. . Electrical conductivity is the measure of the concentration of ions present within the sample.

The amount of ions present within a sample can be calculated by the substance's ability to transmit an electrical current over a defined area. The salt meter operates by transferring a current between two electrodes that are immersed in the sample being tested. The higher concentration of ions present, the more conductive the sample is and higher the electrical conductivity reading will be. Usually, the saltmeter's software will automatically apply a conversion factor to the electrical conductivity reading and display an estimated salinity measurement. While the salt meter measures in units of Milli Siemens per centimetre mS/cm or Micro Siemens per cm µS/cm, concentration is displayed in parts per thousand(ppt), parts per million (ppm), grams per litre (g/L) or other concentration units.

When using electrical conductivity salt meters, caution must be taken measuring liquid solutions that contain other electrolytes, as they increase the electrical conductivity of the sample. Sample temperature also affects the salinity readings but many devices have automatic temperature compensation (ATC) [61, 62].

Brix meter

Sugar was measured using a Brix Refractometer MA871 (Milwaukee, Hungary). A refractometer measures the sugar content of a sample by passing light through a sample and measuring its refractive index (nD) - the amount that the light bends - and converting it into degree Brix (^{0}Bx). Brix refractometers are calibrated using the brix scale, where one degree Brix (^{0}Bx) is equivalent to 1 gram of sucrose in 100 grams of aqueous solution and is expressed as a percentage.

As well as sucrose, any other dissolved solids can also affect the refractive index of a mixture or solution. Therefore, the brix scale only provides an approximate measurement of the sugar content for real food substances. Refractive index depends on temperature and if not using an instrument with automatic temperature compensation (ATC), readings should be made with samples at 20^{0}C [63].