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"VALORIZATION OF MARINE-BASED GELATINE IN FOOD PRODUCT DEVELOPMENT"

by

Maria Teresa Dias Carvalho

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Thesis presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Biotechnology and Innovation.

by

Maria Teresa Dias Carvalho

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Recognition note

Antes de saíres estrada fora até às estrelas, ensinaste-me que sonhar é viver acordada.

Obrigada pai, é para ti.

Abstract

This research work was conducted with the purpose of studying alternative applications of fish canning by-products, to obtain alternative added-value food products. One of today's leading trends in food product development is the design of products with enhanced nutritional profile and a sustainable ethos. In this regard, marine gelatines extracted from either yellowfin tuna (*Thunnus albacares*) or from blue shark (*Prionace glauca*) skins were studied as ingredients to develop new foods that proactively integrate scientific trends in nutrition and sustainability. Hence, the role of tuna gelatine as a fat replacer in mascarpone cheese and in tuna pâté was assessed. In the latter product, its properties as a texturizing agent were also tested. The incorporation of blue shark gelatine was not very appealing for these matrices, regardless of the tested concentration level. Due to its characteristics, that type of gelatine is more suitable for consistent products, such as jelly gums. Two sets of experimental pâté samples were prepared by: i) replacing part of oil by gelatine according to the following weight proportion oil:gelatine combinations 15:25, 10:25 and 5:35 (g/100g) and gelatine replaced cornstarch; or ii) gelatine replaced 50% cornstarch (sample A 60:10:20:10) or 50% cornstarch and decrease oil percentual content (sample B 60:10:15:15; sample C 60:10:10:20 and sample D 60:10:5:25 %(w/w) for tuna:cornstarch:oil:gelatine respectively). Prepared pâtés, in duplicate, were stored in thermally treated glass bottles and stored for 28 days at room temperature. Physical (texture and colour) and chemical (protein, fat, pH, water activity and moisture) properties of developed pâtés were assayed. Tests of sensory acceptance and sensory attributes were performed on selected formulations and microbiological stability was monitored. With the inclusion of tuna gelatine in the two types of pâté, it was possible to reduce in two thirds fat content. Samples without cornstarch showed a reduction in fat content around 67% in both days. Samples with cornstach showed differences in fat content when compared with the control sample (sample A). Samples with high gelatine content reduced the fat content more than 70% at day 0 and around 65% at day 28. Concerning mascarpone cheese, six different samples of mascarpone cheese were prepared. The two fish gelatines were tested as partial fat replacers, at two different percentages: 15% and 25%; a commercial product was included for comparative purposes. For both marine species, results showed reduced fat content, when compared to the control. Fat results showed a mean variation between 22% to 25%. The samples with yellowfin tuna gelatine had similar rheological and textural properties, when compared with control and commercial product. However, mascarpone cheese with blue shark gelatine had less softness. Colour was not affected by the inclusion of gelatine. In general, the addition of 15% gelatine presented a strong similarity in protein and fat content to the commercial sample. From sensorial and nutritional perspectives, the sample which showed best results was the one with 15% tuna gelatine. Therefore, this study shows that marine gelatine, particularly tuna gelatine, has the potential to be used as a fat replacer in mascarpone cheese production and in tuna pâtés, which could offer a value-added product in terms of health and sustainability.

Keywords: marine gelatine, tuna fish, fat replacer, nutrition, mascarpone cheese, tuna pâté.

Resumo

Este trabalho de investigação foi realizado com o intuito de valorizar os subprodutos oriundos da atividade pesqueira e obterem-se produtos alimentares com valor acrescentado de mercado. Uma das principais tendências é o desenvolvimento de alimentos com melhor perfil nutricional e carácter sustentável. Assim, foram utilizadas gelatinas de origem marinha extraídas de peles de atum (*Yellowfin tuna*) e tintureira (*Prionace glauca*). Estas foram estudadas enquanto ingredientes para o desenvolvimento de novos alimentos, que respeitem as tendências em nutrição e sustentabilidade. Avaliou-se o papel da gelatina de atum enquanto substituto de gordura em queijo mascarpone e em pâté de atum, tendo neste último sido testadas as propriedades da gelatina enquanto agente texturizante. A incorporação de gelatina de tintureira não foi interessante para a incorporação nestas matrizes, independentemente da concentração testada, pois as suas características tornam-na mais adequada para produtos mais consistentes, como gomas. Dois conjuntos de amostras experimentais de pâtés foram preparadas por: i) substituição parcial do óleo por gelatina, de acordo com o seguinte peso proporcional de óleo:gelatina em combinações 15:25, 10:25, 5:35 (g/100 g) e a gelatina substituiu o amido de milho; ou ii) substituição em 50% do amido de milho (amostra A 60:10:20:10) ou substituição em 50% do amido de milho e diminuição do conteúdo de óleo (amostra B 60:10:15:15; amostra C 60:10:10:20 e amostra D 60:10:5:25 % (w/w) para atum:amido de milho:óleo:gelatina respetivamente). Os pâtés preparados, em duplicado, foram colocados em recipientes de vidro termicamente tratados e armazenados por 28 dias à temperatura ambiente. As propriedades físicas e químicas dos pâtés desenvolvidos foram testadas. Testes de aceitação sensorial e atributos sensoriais foram realizados em formulações selecionadas e a estabilidade microbiológica foi monitorizada. A inclusão da gelatina de atum nos dois tipos de pâté permitiu reduzir em cerca de dois terços o conteúdo em gordura. As amostras sem amido de milho relevaram redução no conteúdo de gordura de cerca de 67% nos dois dias. As amostras com amido de milho mostraram diferenças no teor de gordura entre si, quando comparadas com a amostra controlo (amostra A), tendo aquelas com maior conteúdo de gelatina reduzido o conteúdo de gordura acima de 70% no dia 0 e cerca de 65% no dia 28. Para o queijo mascarpone, foram preparadas seis amostras, sendo as duas gelatinas de peixe testadas como substitutos parciais de gordura, em percentagens diferentes: 15% e 25%; um produto comercial foi incluído para fins comparativos. Registou-se variação média da gordura entre 22% a 25% e as amostras com gelatina de atum apresentaram propriedades reológicas e texturais semelhantes ao controlo e ao produto comercial. Todavia, o queijo mascarpone com gelatina de tintureira apresentou menor cremosidade. A cor não foi afetada pela inclusão da gelatina. Em geral, a adição de 15% de gelatina apresentou forte similaridade no conteúdo de proteína e gordura com o produto comercial. Ao nível sensorial e nutricional, a amostra com 15% de gelatina de atum foi a que apresentou melhores resultados. Assim, este trabalho mostra que a gelatina marinha, principalmente a gelatina de atum, tem potencial para ser utilizada como substituto de gordura na produção de queijo mascarpone e pâtés de atum, podendo constituir um produto com valor acrescentado em termos de saúde e sustentabilidade.

Palavras-chave: gelatina marinha, substituto de gordura, nutrição, queijo mascarpone, pâté de atum

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1. Introduction

1.1. FISH BY-PRODUCTS

1.1.1. VALORIZATION OF FISH GELATINE

The capture and processing of fish and shellfish by fisheries and industry are processes that produce a significant amount of waste, mainly resulting from evisceration and filleting. The generated waste, which includes fish heads, skin, guts, liver, fish spawns and frame, is usually discarded, yet is still a source of important nutrients and compounds including gelatine (1). In fact, the fish processing industry produces over 60% of these by-products and, according to Lordan (2011), for example, in 2005, fishery by-products were more than 7 million tonnes (1). Since environmental laws restricting the disposal of fish discards are increasing, there is a strong demand towards the full utilization of aquatic resources. As such, the demand for alternative sustainable waste management systems to reuse these fish by-products is steadily increasing. In fact, reuse and valorisation of fish by-products is a key process for marine resources conservation. Research and innovation have explored different applications for these by-products, in particular for fish heads, guts, skin, tail, blood and shells, which offer compounds with potential functional benefits for human health, namely, gelatine, collagen, proteins, hydroxyapatite and fish oil among others (1–3).

Marine gelatine is one of the fish by-products obtained from fish skin upon thermal denaturation of collagen, and which has been gaining attention from both research and industry (4). In fact, gelatine, as an ingredient, is used for different purposes; due to its physical functionality it is widely used in the food, cosmetics and pharmaceutical industries (4). Gelatine is commonly derived from bovine, porcine or poultry sources (4–7). Fish gelatine is used lesser than the other types, but it provides technological advantages over mammalian gelatine such as a higher digestibility and greater gel strength (8); furthermore, its marine origin helps overcome religious concerns in product development. Given gelatine’s functional properties as a texturizing agent (elasticity and consistency), an emulsifier, and a stabilizer, it may be a useful ingredient for food industry applications, particularly in terms of fat replacement because gelatine may provide the creaminess that is lacking in low-fat products (3,4,7). Such functionality adds value to the product, contributing to improved nutritional profiles, a feature that many food manufacturers are continuously searching for in order to satisfy demands of current consumers who are increasingly concerned with health aspects (3,4,9,10).

This higher capacity of marine gelatines to increase the viscosity and gelling (6,8,11) in comparison to other gelatines is due to the fact that a higher temperature is required to denature marine collagen versus non-marine animal collagen. Indeed fish skins have a significant potential for the production of high-quality gelatine with different melting and gelling temperatures over a much wider range than mammalian gelatines (6,8). This property can be modulated, in the case of associations of fish collagen with other types of collagen, for example pork collagen (6,8,11).

1.2. MARINE GELATINE

1.2.1. SPECIES

Fish skins from several of fish species have been studied as sources for gelatine production. The most used species for the extraction of fish gelatine are herring, squid, sea bass, tuna, tilapia, perch, porkfish, sturgeon, hake, trout, salmon, carpe, cod, plaice, squid, sardines, deep-sea redfish, corvine, threadfin bream, walleye Pollack, unicorn leatherjacket, brown stripe red snapper and cobia (2,3,12).

1.2.2. GELATINE EXTRACTION PROCESS

Conversion of insoluble collagen into soluble gelatine can be achieved by heating the collagen in either acid or alkali pre-treatment solutions (5). Thermal solubilisation of collagen (in the presence of acid or alkali) is due to the cleavage of several intra and intermolecular covalent crosslinks that are present in collagen, resulting in a helix-to-coil transition and conversion into soluble gelatine (5). In addition, some amide bonds in the elementary chains of collagen molecules undergo hydrolysis (5,13). The extraction process can influence the length of the polypeptide chains (and consequently the molar mass distribution) and the functional properties of the gelatine. This depends on the processing parameters (extraction temperature, time, and pH), the pre-treatment, and the properties and preservation method of the starting raw material (5). Generally, the main temperature is 60-90 ºC and the choice of pH depends on the extraction rate and maintenance of physical properties. In summary, the gelatine manufacturing process involves three main steps, namely, a) pre-treatment of the raw material, b) extraction of the gelatine and finally, c) purification and drying.

Furthermore, manufactured gelatine is often blended to produce trade-quality gelatine, with specific properties for specific applications (14). Depending on the method with which the collagens are pre-treated (acid or alkaline) to disorganize the protein structure, two different types of gelatine (each with differing characteristics) can be produced. Type A gelatine (isoelectric point at pH 6–9) is produced from acid-treated collagen, and type B gelatine (isoelectric point at approximately pH 5) is produced from alkali-treated collagen (13,15,16). Acidic treatment is most suitable for the less covalently cross-linked collagens found in pig or fish skins, while alkaline treatment is suitable for the more complex collagens found in bovine hides (5). Fish gelatine has been extracted using several different methods. The direct procedures used for preparing fish gelatine typically involve a mild chemical pre-treatment of the raw material and mild temperature conditions during the extraction process (3,17). In general, a mild acid pre-treatment of the fish skin is used prior to gelatine extraction (3,17).

1.2.3. COLLAGEN AND GELATINE CHARACTERIZATION

As previously mentioned, gelatine is resultant from fibrous protein collagen (5). Collagen is the main constituent of animal skin, bone and connective tissue (5). Collagen is one of the connective tissue proteins that concentrate almost all the amino acids (3,5,18). (8). Collagen and gelatine are rich in polar amino acids: glycine, alanine, valine and proline (5). Nonetheless, Montero and Gómez-Guillén (2000) suggested that extraction conditions can disturb the hydrophobic amino acid composition and distribution (15). This has an effect on the physical properties of gelatine, even more than the imino acids content (15). Collagen consists essentially of hydroxyproline and hydroxylysine, where the total content of proline and hydroxyproline is high (3). The quantity of hydroxyproline defines the hardness of the collagen and consequently of gelatine; gelatine with high levels of imino acids tends to have a higher gel strength and melting point (5). There are three main types of collagen: collagen type I, II and III (5). Table 1 shows the several types of collagen and their specific characteristics, based on Schrieber and Gareis (2007)(19).

Table 1 – Classification types and associated characterization of collagen.

COLLAGEN TYPE	CHARACTERIZATION
TYPE I	Connective tissue such as skin, bone and tendons
TYPE II	Cartilage tissue
TYPE III	This collagen is age dependent: young skins contain more than 50 %, yet over time the presence of this collagen decreases to 5-10 %.
OTHER TYPES	Present in very low amounts and in specific organs (e.g., placenta)

In what concerns the resistance of the collagen fibres, it depends on different factors, but it is the age of the animal which will define the solubility of this protein (8). Therefore, the older the animal, the less soluble is the collagen, and the more difficult it becomes to work with collagen, which is more reluctant to pH variations, heat or denaturation (3). The composition in amino acids of gelatine is mainly dependent on the origin of the collagen (6). The usual sequence of amino acids in gelatine is glycine-proline-hydroxyproline (2,4). Normally, 33% of amino acids of gelatine correspond to glycine, 22% to proline and hydroxyproline, and 45% are other amino acids (14,20). The differences in the amino acid composition lead to the formation of unpaired gelatine and it is the presence of cross-links that will have a crucial role in the gelatine's resistance (8). However, it is the hydroxyproline, an imino acid involved in the formation of gelatine, that allows the creation of hydrogen bridges through the hydroxyl group. According to Balian and Bowes (1977), collagen is the only mammalian protein to contain large amounts of hydroxyproline and hydroxylysine, as previously mentioned (5,21). The total imino acids (proline and hydroxyproline) content is high. Normally, the composition of amino acids of collagens is very similar to the composition in amino acids of gelatine, since this is derived from collagen (5). Chains of collagen molecules are composed of three alpha-chains linked in the collagen triple-helix. This connection makes a 3D-structure in collagen, an ideal link to inter-chain hydrogen bonding (5,22,23). The triple helix is stabilized by the inter-chain hydrogen bonds (22). The direction of each chain rotates to the left and the triple-helix is approximately 300 nm in length and the molecular weight is 105 kDa, approximately (5,22,24). When denaturation of collagen occurs, the chains are total or partially separated because the hydrogen bonds are broken and the collagen chain loses the triple-helix conformation (5,19). The results of collagen denaturation are polymers in a coiled form (5,19).

Industrial gelatines are mixtures of different compounds: α-chains (one polymer chain), β-chains (two α-chains covalently cross-linked), and γ-chains (three covalently cross-linked a-chains) (24). Differences are found according to the distinctive sources from where collagen is derived (2). However, certain features are common to all collagens (5).

Based on the above rationale, the amino acid composition of gelatine is characterized by a replicating sequence of Gly-X-Y triplets, where X is (generally) proline and Y is, normally, hydroxyproline (5). In the case of fish gelatine, the ratio of proline and hydroxyproline is less than in mammals (1). This ratio changes according to fish species and environment temperature of fish habitat because this latter affects thermal stability of collagen. Warm water fish have major proline and hydroxyproline (e.g. tuna) (1). This content leads to the presence of a higher melting point and higher thermal stability than in colder fish environments, such as cod or pollock (1). According to Muyonga et al. (2004), the content of hydroxyproline and proline in warm water fish gelatines ranges between 22% and 25%, and around 17% in cold water fish gelatines(25,26). However, cold water fish gelatine have higher concentrations of serine and threonine in comparison with warm water fish gelatines(26,27). This explains that gelatines from cold water fish act as viscous liquids at room temperature and consequently their use in food industry is lesser than warm water fish gelatines(26). These differences between warm water and cold water fish species could be found in figure 1, from Akita et al. (2020)(27).

The summary of Tp of sample fishes and denaturation temperature, focused amino acid content, and Pro + Hyp content of ASC.

	Common name	T_p (°C)	Pro	Hyp	Ser	Pro + Hyp	T_s (°C)	T_d (°C)	Height of CD spectrum (deg·cm^2·dmol^{-1})	R_{pn} value
Warm	Double-lined fusilier	28.0	103	76	33	179	56.2	34.9	54.2	0.38
	Common dolphinfish	27.4	110	69	39	179	ND*	29.5	61.3	0.22
	Fivespot flounder	26.0	99	72	46	171	ND*	26.8	186	ND†
	Ridged-eye flounder	20.7	101	65	58	166	49.0	23.5	67.4	0.26
	Blue mackerel	18.7	105	71	44	176	53.2	27.5	25.6	0.11
	Roughear scad	16.6	112	69	41	181	56.2	30.4	39.8	0.31
	Average	22.9 ± 4.9‡	105 ± 5‡	70 ± 4‡	43 ± 8‡	175 ± 6‡	53.6 ± 3.4‡	28.8 ± 3.8‡	72.4 ± 57.7‡	0.26 ± 0.10‡
Cold	Okhotsk atka mackerel	12.8	93	58	71	151	41.3	18.1	5.2	0.10
	Deep-water arrowtooth eel	3.4	93	47	61	140	41.7	19.9	1.9	0.17
	Giant grenadier	2.6	88	49	73	137	34.5	19.2	3.9	0.08
	Pacific grenadier	2.4	84	49	73	133	42.2	18.0	9.1	0.13
	Scaly walf eelpout	0.4	87	44	77	131	43.4	20.2	5.2	0.06
	Average	4.3 ± 4.9‡	89 ± 4‡	49 ± 5‡	71 ± 6‡	138 ± 8‡	40.6 ± 3.5‡	19.1 ± 1.0‡	5.1 ± 2.6‡	0.11 ± 0.04‡

* Even when heated to more than 70 °C, no skin contraction was observed in the Common dolphinfish and Fivespot flounder.
† The negative peak necessary for calculation of R_{pn} was not observed in the Fivespot flounder.
‡ Indicate the significant difference ($p < 0.05$) between Warm and Cold.

Figure 1. Differences between amino acids and imino acids content in warm and cold water fish gelatines according to Akita et al. (2020)(27).

For industries, the melting point is very important, because of gelling and melting properties required for the different formulations. In case of fish gelatine, the melting point is lower than the human body temperature, enabling a smooth mouthfeel and melting capacity (28). Gelatines extracted at high temperatures show lower molar mass profile than gelatines extracted at a low temperature (29). The formation of gels, which tend to be less stable and have poor rheological properties when compared with mammalian gelatines, have been considered as the main problem for the use of fish gelatine (11).

Indeed, gelatine has several functional properties that can be divided into two groups (5). Firstly, gelatine exhibits properties related to the gelling functionality (e.g. gel strength, gel type, gelling time, melting temperature, viscosity, thickening ability, texturizing and binding to water molecules) (5). The second relevant functionality is related with the gelatine surface behaviour, more related with its capacity to be used as an emulsifier, stabilizer, colloidal function, foaming capacity, training films and adhesion/cohesion (5).

1.2.4. FOOD APPLICATIONS: MASCARPONE CHEESE AND TUNA PÂTÉ

Gelatine, with its unique properties, provides formulation solutions in a broad range of applications. Gelatine may be used as a beverage (beer and juices) clarifier, as a thickener in desserts (at 8-10% of dry weight) or in yogurt (at 0.3-0.5%), in ham coatings (at 2-3%), and in confectionery and dietary supplement capsules. Gelatine is also used in pastry fruit toppings; instant gravies, sauces and soups; edible films for confectionery products; as a stabilizer in ice cream, cream cheese and cottage cheese; and, also, in food foams and fruit salads. Mascarpone cheese is a type of cream cheese, originating from Lombardia in Italy, in particular from the Lodi region. Its origin is not totally well-known, although reports indicate its production and consumption dating back to the 12th century. It is a non-ripened cheese, high in fat, reaching contents up to 45-55 % fat (30,31). This noble cheese has a mild aroma, slightly acidic pH, sweet buttery flavor and soft texture with a colour that ranges from milky white to pale straw yellow (30,31). These properties promote a demand by industries for mascarpone cheese because this cheese can be applied in a multitude of products such as desserts (e.g. cheesecake, tiramisu), ice cream, cookies and bagels (30,31). Due to the high content in fat, specifically saturated fat, mascarpone has high energy density and low nutritional value. Given current consumers' higher sensitivity to environmental and health issues, the production of healthier mascarpone through the incorporation of fish gelatine, is a possible solution for overcoming the poor nutritional profile of this cheese.

From a technological perspective Mascarpone cheese is classified as an acid/heat-coagulated cheese and represents an interesting cheese processed with direct acidification (30). Its production requires heating the cream, followed by the addition of an organic acid, when the temperature reaches the desired range (30). Under high heating conditions the whey protein denatures, aggregating casein micelles and fat globule membranes (30). As a result of this protein denaturation reaction, the whey proteins remain largely in the cheese matrix upon 20 hours of draining step (30).

In what concerns fat-free mascarpone cheese, there are no studies about these specific processes or products. In his product survey Brigenthi et al. (2008) refers that Crane (1992) described a process of obtaining fat-free cream cheese, but this process is different from the traditional production of full-fat Mascarpone cheese (31,32). In Crane´s process, the author used skim milk (~25%), emulsifier salts, bulking agents and stabilizers, such as xanthan gum and carrageenan (31,32). The stabilizers have the responsibility to give a similar texture to the full-fat cheese (31,32).

Another application of fish gelatine could be in pâtés. Tuna pâté is a product with many ingredients, designed so as to increase its acceptability by the consumer. Normally, commercially available tuna pâtés have an average 11 % fat content, and vegetable oils are one of the main ingredients in this processed product. Given this reasoning, tuna pâté does not correspond to an ideal product to be incorporated in a nutritional plan because it contains salt, fat and ingredients that stimulate the appetite. Hence, the work presented herein intended to develop a new type of healthier pâté produced with more tuna, a low amount of oil, an emulsifier (cornstarch) and marine gelatine. The use of gelatine derived from fish by-products in the formulation increases the sustainability of the final product.

Apart from the latter formulation of pâté, another formulation was also tested, concerning the absence of cornstarch. This formulation allowed to verify the capacity of gelatine as an emulsifying and texturizing agent. Aside the health-concerned consumer, other potential consumers for these new types of pâtés are those people presenting digestive system diseases, as well as consumers that do not consume meat or specific types of meat (e.g., consumers of kosher and halal foods).

1.2.5. MARKET IMPORTANCE OF THE PRODUCTS UNDER ANALYSIS

The demand for gelatine increased since orthopaedic diseases started to affect an increasing number of people (33). Furthermore, people want healthier products, and gelatine can be considered within this context (33). This is reflected in expected increased margins for the global gelatine market, as it is predicted to reach a compound annual growth rate of 9.2 % from US$3.1 billion in 2019 to US$6.7 billion by 2027, while its volume is due to have a compound annual increase of 5.9 % in the same period(34).

Production and consumption of cheese has grown globally and tends to continue increasing in the next years (35). In fact, since 1980, cheese consumption has doubled (35) and, according to Euromonitor International Data, its consumption in Europe has been growing steadily. Indeed, the cheese market in North America and Europe reported 70 % of the cheese consumption in 2012 (36). In 2020, mascarpone cheese contributed for the overall cheese market growth, as it reached US$75 billion. The global cheese market is expected to grow up to US$118 billion by 2026(37). Although highly consumed, this cream cheese has a high fat content, and this aspect makes it a poor nutritional option. Hence, the development offing a more nutritionally balanced alternative will correspond to a new opportunity of the market, since there is no other similar product currently available. Besides, some brands and private label manufacturers are effectively interested in new products related to the cheese market (38).

The global pâté market was estimated at US$1.06 billion in 2018, with an expected compound annual growth of 1.3% by 2025. Among its growth drivers, one observes changes in food preferences and consumption habits, as well as technological advancements focused on shelf life and product quality(39). Regarding the tuna pâté, the new product that is being proposed is prepared mainly with marine ingredients which is more natural and simpler than other options in the market. So, the versions suggested in this work correspond to a food trend because consumers are in fact searching for healthier and more natural products. Besides, this target puts in second-place processed products with a lot of additives to make the products tastier.

2. Objectives

The main objective of this research study is the valorisation of fish skin gelatine derived from different species in food product development. This main objective will be fulfilled with the following specific objectives:

1. To study the impact of fish gelatines derived from yellowfin tuna (*Thunnus albacares*) and blue shark (*Prionace glauca*) on development of different food matrices;
2. To develop and characterize a mascarpone-like cheese with an enhanced nutritional profile, through the incorporation of fish gelatine as a fat replacer and consequent reduction of the total fat content;
3. To produce and characterize sustainable and healthy tuna pâtés, formulated with tuna gelatine as one of the main ingredients.

3. Materials and methods

3.1. GELATINE EXTRACTION

Skin fish wastes were kindly provided by Frinsa del Noroeste S.A. (yellowfin tuna, *Thunnus albacares*) and Propegal S.L. (blue shark, *Prionace glauca*), being washed and stored at -20 ºC until use. The extraction of the gelatines used in this research work was performed by Grupo de Bioquímica de Alimentos and Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), both from Instituto de Investigacións Mariñas (IIM-CSIC), Vigo, Spain. The washing and extraction of all skin portions was performed by a combination of alkaline and acid washes, as described by Sousa et al. (2017)(17). Process is presented in Annex 1.

Gelatine samples were prepared by dissolving 6.67 % (w/v) gelatine in deionized water at room temperature for 30 min, followed by 30 min in a water bath at 60 ºC (with constant agitation).

3.2. MASCARPONE CHEESE

3.2.1. SAMPLE PREPARATION

Samples were prepared utilizing Continente UHT (ultra-high temperature) cream containing 30% fat, citric acid (from lemon juice) and gelatines extracted from the skins of yellowfin tuna (*Thunnus albacares*) and blue shark (*Prionace glauca*). Formulations and their replicates (two replicates for each formulation), for both types of gelatine (tuna and blue shark), were prepared. The control formulation was prepared without fish gelatine, and the composition of each formulation is provided in Table 2. In addition, a sample from a commercially available mascarpone cheese was also tested. Ingredients of the commercial mascarpone cheese were pasteurised cream and citric acid (E330). Appendix 1 includes the process for making mascarpone cheese with marine gelatine.

Table 2 - Composition in gelatine, cream, and citric acid (% and volume), of the formulations tested for the production of mascarpone cheese.

Samples	% (v/v) Gelatine	% UHT Cream (v/v)	UHT Cream (mL)	Gelatine (mL)	Citric acid (mL)
A1	15	85	170	30	5.1
B1	25	75	150	50	4.5
Control 1	0	100	200	0	6

Preparation of marine gelatine:

Fish gelatine (6,67 g /100 g) was prepared by the method described in 3.1.

Preparation of control samples:

Control samples were prepared by placing the cream in a thermostatic water bath until the temperature range of 85-90ºC was reached; subsequently, the citric acid (lemon juice) was added in with constant stirring. Upon homogenization, the preparation was drained through a draining mesh, and refrigerated at 4 ºC overnight. The resulting mascarpone cheese, which was the drained precipitate present in the upper part of the muslin cloth was removed and further characterized.

Preparation of samples with fish gelatine:

Mascarpone-like cheese samples with fish gelatine were prepared in the same way as the control counterparts, up to the step related with the addition of citric acid. Fish gelatine was added at the same time of cream in thermostatic water bath. Afterwards, the mixture was transferred to an ice bath and mixed for 5 min until it cooled down. Then, samples were drained (through a draining mesh) and refrigerated overnight. The mascarpone-like cheese with fish gelatine, which was the drained precipitate in the upper part of the muslin cloth, was removed and further characterized.

3.2.2. CHARACTERIZATION OF PHYSICAL PROPERTIES

3.2.2.1. RHEOLOGY

Rheological measurements were performed on a Gemini Advanced Rheometer (Bohlin Instruments, UK), coupled with a peltier unit. Linear viscoelastic region was determined (results not shown) and a frequency of 0.1 Hz and a strain of 0.05 % were utilized. Analyses were performed at 8 ºC, in triplicate. The parameters assessed were the phase angle and the storage, or elastic, modulus (*G'*).

3.2.2.2. TEXTURE

Texture was analysed in a TA.XT*plus* Texture Analyzer (Stable Micro Systems, UK) with a 5 kg load cell. For softness (40), a 5 mm diameter cylinder probe, with a penetration speed of 1.0 mm/s and a penetration depth of 10 mm, was utilized. To measure firmness and stickiness, a 0.635 mm diameter spherical probe, with a penetration speed of 2.0 mm/s and a penetration depth of 5 mm, was utilized. Analyses were performed in triplicate.

3.2.2.3. COLOUR

Colour analysis was performed with a colorimeter (Chroma Meter CR-400, from Konica Minolta, Japan). The measured parameters were L* (lightness), a* (green to red) and b* (blue to yellow). With the obtained values, it was possible to calculate two other parameters: C (saturation) and Hue (colour) according to McGuire method (1992)(40). For each sample measurements were performed in three different sites.

3.2.3. CHARACTERIZATION OF CHEMICAL PROPERTIES

3.2.3.1. TOTAL PROTEIN

The protein content was analysed based on the Kjeldahl method for the determination of nitrogenous substances. A 200 mg sample was digested at 400 °C with 4 mL of H_2SO_4. The reaction was stopped with the addition of 20 mL deionized water. Afterwards, samples were distilled in a Kjeldahl system (1002 distilling unit from TecatorTM), in which boric acid solution was added until reaching a final volume of 150 mL. Then, titration with HCl 0.2 N was performed, until turning point of violet to orange.

3.2.3.2. TOTAL FAT

Total lipids were determined according to the Folch method (41). Briefly, 10 mL of chloroform/methanol (2:1 v/v) solvent mixture were added to 1 g of sample and vortexed for 15 sec. Then, the mixtures were sonicated for 30 min, 5 mL of solvent mixture (chloroform/methanol) and 5 mL of deionized water were added, shaken vigorously for 2 min, and centrifuged at 5000 rpm for 5 min, at 4 ºC. The resulting lower phase was transferred to a previously weighed glass balloon and the procedure was repeated with the remaining sample. The lower phases were pooled, and solvent was evaporated at 37 ºC. The glass balloon was then weighed to gravimetrically calculate the fat content.

3.2.4. APPLICATION OF MASCARPONE CHEESE: APPLE MUFFINS

The developed Mascarpone-like cheese was applied in the manufacture of cakes, namely apple muffins. These muffins were manufactured with three types of mascarpone cheese: commercial and mascarpone cheese with tuna gelatine at either 25% or 15%). A formulation for these apple muffins is presented in Appendix 2. Other ingredients in these muffins were apple, lemon, cinnamon, eggs, brown sugar, mascarpone cheese, vanilla extract, and wheat flour. Production conditions were the same for the different types of muffins (ingredient quantities, room, and oven temperatures). The resulting apple muffins were subsequently submitted to refrigeration conditions (4 ºC) for 7 days to test for stability over the storage time.

A short and informal sensorial analysis with fourteen adult consumers, that normally consume such product, was performed. No information was collected on the socioeconomic status or sensory preferences for cakes. For testing, each muffin was sliced in four (along the two perpendicular diameter axes). Each consumer evaluated three samples at a time: control apple muffin with commercial mascarpone and the two experimental apple muffins: apple muffins with mascarpone-like cheese with 25% tuna gelatine and apple muffins with mascarpone-like cheese with 15% tuna gelatine.

3.3. TUNA PÂTÉ

3.3.1. SAMPLE PREPARATION

Samples of tuna pâté were prepared with different percentages of tuna gelatine, fat and cornstarch, adapted from Lobo et al. (2015) (42). The control sample contained 60% protein, 20% cornstarch, 20% fat and 0% tuna gelatine. In this research work, the range of variation for tuna gelatine, fat and cornstarch variables were: [20, 30, 40] % for gelatine, [5, 10, 15] % for fat, and [0, 10, 20] % for cornstarch. Regarding the amount of protein, it did not vary throughout the study. Commercially available tuna (canned in water) was used, from the brand Continente. Fat used in pâtés was vegetable oil (sunflower oil) from Auchan. Concerning the spice (black pepper), it was not accounted for final yield, as the amount used (0.2 g) was insignificant. Appendix 3 includes the process of manufacturing tuna pâté.

i) Tuna pâté without starch:

Table 3 shows the experimental design for the formulations without starch. Four samples and their replicates were prepared using canned tuna in water, vegetable oil (fat) and fish gelatine, namely tuna gelatine.

Table 3 - Composition distribution (%) of the tested samples in tuna, tuna gelatine and fat.

	% tuna	% vegetable oil	% tuna gelatine
Control	60	20	20
A	60	15	25
B	60	10	30
C	60	5	35

ii) Tuna pâté with starch:

Table 4 describes the experimental design for formulations with cornstarch. The five selected formulations were prepared with canned tuna (same percentage in all samples), vegetable oil (fat), fish gelatine (tuna gelatine) and starch (20% in control sample and 10% in the remaining samples). It was used the 20 % starch in control sample based on Lobo et al. (2015) study (42). For the preparation of the pâté with cornstarch it was necessary to achieve the expansion of the starch with water before adding it to the mixture. All the formulations were prepared in duplicate.

Table 4 - Composition distribution (%) of the tested samples in tuna, tuna gelatine, fat, and cornstarch.

	% tuna	% vegetable oil	% tuna gelatine	% cornstarch
Control	60	20	0	20
A	60	20	10	10
B	60	15	15	10
C	60	10	20	10
D	60	5	25	10

3.3.2. CHARACTERIZATION OF PHYSICAL PROPERTIES

All analyses were run in duplicate and the results were expressed as mean ± standard deviation (SD).

3.3.2.1. TEXTURE

Texture was analysed in a TA.XT*plus* Texture Analyzer (Stable Micro Systems, UK) with a 5 Kg load cell. To measure firmness, a 5 mm cylinder probe, with a penetration speed of 1.5 mm/s and a penetration depth of 8 mm, was utilized. Analyses were performed in triplicate.

3.3.2.2. COLOUR

The method previously described in subsection 3.2.2.3 was applied.

3.3.3. CHARACTERIZATION OF CHEMICAL PROPERTIES

All analyses were run in duplicate and the results were expressed as the mean ± standard deviation (SD).

3.3.3.1. TOTAL PROTEIN

The method described in subsection 3.2.3.1 was applied but the titration turning point is blue to green.

3.3.3.2. TOTAL FAT

The method described in subsection 3.2.3.2 was applied.

3.3.3.3. pH

Determination of pH values was performed with a Crison pH meter (model Basic 20, Spain), with a surface electrode (Crison, reference 52 07, Spain).

3.3.3.4. WATER ACTIVITY (a_w)

Water activity was determined with a AquaLab (Series 3TE, Decagon Devices, USA) equipment.

3.3.3.5. MOISTURE

Moisture was determined by weighing 2 g of sample, which were then placed in an oven, at 100 ºC, until reaching stable weight. The difference between the initial and final weight was the amount of water that was lost, which was then utilized to calculate the moisture percentage of the sample.

3.3.4. MICROBIOLOGICAL STABILITY

Microbiological stability was evaluated at 0 and 28 days of storage, with four distinctive media, for the growth of different microorganisms:

- **PDA (Potato Dextrose Agar)** - PDA is a medium for the growth of yeasts and molds (43). PDA is appropriate for plate count of food and dairy products (43).
- **VRBGA (Violet Red Bile Glucose Agar**) - VRBGA is a selective medium for the isolation and count of enterobacteria in food (44,45). This medium has bile salts and crystal violet which is important to inhibit Gram-positive and non-enteric organisms (44,45).
- **MRS (*de* Man, Rogosa and Sharpe Agar)** - This medium was based on the formulation of *de* Man, Rogosa and Sharpe (46). It supports the growth of all lactobacilli from oral cavity, dairy products, food and other sources (46).
- **PCA (Plate Count Agar)** - Plate Count Agar is according to Buchbinder et al. (1953) and is used in food bacteriology (47). This medium enables the enumeration of aerobic bacteria in, for example, milk, meats, and meat-based products.

For the enumeration of microorganisms in the different media, a 1 g aliquot of each sample was homogenized in 9 mL of sterile peptone (1 g/L), saline (8.5 g/L) solution in a Stomacher blender. Sequential dilutions were made from the resulting homogenates and adequate aliquots were plated, in duplicate, on petri dishes with PDA, MRS and PCA agars. For VRBGA, the inoculation technique was the incorporation technique, where 1 mL of solution was poured into a Petri dish and the medium was then poured over, and homogenized. For the remaining media, the spread-plate technique, where 100 µL were placed on the agar and then spread, was utilized. Media plates were incubated aerobically, according to the following conditions:

- PDA: 30ºC; 72 hours
- VRBGA: 37ºC; 48 hours
- MRS: 37ºC; 48 hours
- PCA: 37ºC; 48 hours

3.3.5 SENSORY ANALYSIS TEST

The sensory test of three tuna pâté samples, i.e., control tuna pâté, tuna pâté with cornstarch (60% tuna, 10% cornstarch, 10% oil, 25% tuna gelatine) and tuna pâté without cornstarch (60% tuna, 15% oil, 25% tuna gelatine) was performed on the 23rd of October 2015, between 10h00 - 17h00. These samples were chosen because they had the same amount of tuna gelatine and had a softer texture and less dryness than those samples with more gelatine and less fat. The randomly coded pâté samples were assessed by a group of 76 individuals with an average age of 26 years, mainly Portuguese (93%), female (75%), male (25%) of which indicated that usually consumed pâtés several times in a month and living in Portugal who agreed to taste the pâté formulations and respond to the survey. There were no specific criteria for inclusion or exclusion of individuals from the sensorial analysis. Tuna pâtés were placed into airtight plastic containers and conditioned at room temperature for 15 min before evaluation. Between analyses, the individuals took water to eliminate the taste of the previous analysis.

Consumer acceptance was measured by overall liking ratings, provided on a 9-point hedonic scale (48–50), whereas the consumption intent was evaluated in a 4-point probability scale ranging from would buy to would not buy based on Juster (1966) (50). The questionnaire was applied through the "Qualtrics" platform and may be found in Appendix 4. Data from answers was analysed by statistical software XLSTAT Version 2015.4.01.22368.

3.3.6. STATISTICAL ANALYSIS

For the purposes of collecting and organizing databases and executing the graphic component of statistical analysis, it was used Microsoft Excel 2011 software. Descriptive and inferential statistics were performed using SPSS software version 20 (IBM). The component of descriptive statistics was based on the calculation, for numerical variables, of measures of central tendency as the average, fashion, and average and dispersion measures, such as the standard deviation, that when relevant were also presented in the form of confidence intervals for the mean. For the categorical or ordinal variables, the percentages referring to the frequencies observed, and graphs were presented. In the inferential statistics component, Pearson's Chi-square tests were used to verify the independence among categorical variables. For numerical variables and their comparison with categorical variables, normality was verified by the Shapiro Wilk and Kolmogorov-Smirnov tests, and the non-parametric alternative of ANOVA - namely the Kruskal-Wallis test - was chosen. In the case of the parameters taste, texture and overall taste, a theoretical construction of a latent variable was carried out to allow the evaluation of the ordinal response scales as if they were continuous.

3.4. OTHER APPLICATIONS: JELLY GUMS

3.4.1. SAMPLE PREPARATION

3.4.1.1. CONTROL SAMPLES

Control samples utilized 42.5 g of commercial jelly with strawberry taste and 75 mL of commercial neutral jelly. After mixing the two preparations, it was added the prepared in moulds and refrigerated these.

3.4.1.1. BLUE SHARK JELLY GUMS

Blue shark jelly gums it was prepared with 42.5 g of commercial jelly with strawberry taste and 75 mL of blue shark gelatine. After mixing the two preparations, it was added the prepared in moulds and refrigerated these.

4. Results and discussion

4.1. MASCARPONE CHEESE

4.1.1. CHARACTERIZATION OF PHYSICAL PROPERTIES

4.1.1.1. RHEOLOGY

Mascarpone control and experimental cheeses containing tuna gelatine were manufactured and duly characterized in terms of physical-chemical properties. The rheological parameters, phase angle and elastic modulus (G’) were measured and are presented in Table 5. The elastic modulus (G’) measures the elasticity of a material, the ability of the material to store energy. Elasticity corresponds to the rate at which a deformed material returns to its deformed condition after the deforming force is removed and may be determined according to the following equation:

$$G' = (stress*/strain)\cos\theta$$

On the other hand, the phase angle is a parameter sensitive to small fluctuations in rheology status. Therefore, elastic materials with solid-like behaviour have the stress and strain exactly in phase and the phase angle value is 0º, whereas in the case of viscous materials with liquid-like behaviour the stress and strain are a quarter of a cycle out of phase, and the phase angle is 90º(51).

Table 5 – Average values of phase angle (º) and elastic modulus (G’) of the different mascarpone-like cheese samples (T: mascarpone-like cheese samples with 15 (T15) and 25% (T25) tuna gelatine; S: mascarpone-like cheese samples with 15 (S15) and 25% (S25) blue shark gelatine)

SAMPLE	PHASE ANGLE (º)	ELASTIC MODULUS (PA)
Commercial	15.8 ± 3.1^{a}	3893 ± 553^{a}
Control	25.0 ± 3.8^{b}	3415 ± 827^{ae}
T15	22.7 ± 5.1^{b}	117 ± 65^{b}
T25	15.5 ± 2.4^{a}	313 ± 70^{c}
S15	16.6 ± 2.1^{a}	465 ± 117^{d}
S25	10.4 ± 1.0^{c}	3225 ± 273^{e}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$)

The mascarpone cheese samples containing either tuna gelatine (T15 and T25) or 15% blue shark gelatine (S15) had low values of elastic modulus (G') which reflects the higher firmness of these samples. On the other hand, mascarpone cheese S25 is firmer because of its higher G' and lower phase angle. Results also showed that mascarpone-like cheese samples prepared with 25% fish gelatine had a lower phase angle than counterparts prepared with 15% fish gelatine, independent of the origin. This is a "solid-like" behaviour, which is expected because these samples have more gelatine than others and elasticity is high because the micelles of casein may aggregate in a more continuous manner (proteolysis brokers firmness in cheese). The commercial mascarpone cheese sample was similar in rheological parameters measured to mascarpone-like cheese samples T25 and S15. The control mascarpone cheese was similar in behaviour to mascarpone-like cheese sample T15 and mascarpone-like cheese sample S25 was the sample with the most differences.

4.1.1.2. TEXTURE

Texture in mascarpone cheese may be defined mainly through the softness, hardness, and stickiness parameters. Table 6 shows the results obtained for the different mascarpone cheese samples. Mascarpone-like cheese samples T15 and T25 had more similarities with the mascarpone control as far as softness was concerned. Although stickiness was more similar to control for samples with 15 % of fish gelatine. Regarding hardness, it was higher in the blue shark gelatine samples than in tuna samples. On softness, one can infer that for samples with similar percentage of gelatine, there was a statistically significant difference for both percentage levels, in which tuna gelatine sample presented more softness – no statistical differences in softness were found when comparing T25 and S15. This occurs because the gel strength of *Yellowfin tuna* skin gelatine has a low bloom value comparatively to *Prionace glauca* blue shark gelatine. This makes gelling and melting points of *Yellowfin tuna* skin gelatine lower. Furthermore, the rupture strength of *Prionace glauca* is superior to *Yellowfin tuna* and this characteristic contributes to a higher hardness of the products produced with this gelatine.

Table 6 - Texture parameters for the different mascarpone-like cheese samples (T: mascarpone-like cheese samples with 15 (T15) and 25% (T25) tuna gelatine; S: mascarpone-like cheese samples with 15 (S15) and 25% (S25) blue shark gelatine).

Sample	Softness (g)	Hardness (g)	Stickiness (g)
Commercial	66.1 ± 4.3^{a}	52.9 ± 5.1^{a}	-14.9 ± 1.3^{a}
Control	51.5 ± 8.5^{b}	36.2 ± 3.6^{b}	-21.8 ± 1.4^{b}
T15	8.2 ± 0.5^{c}	7.1 ± 0.4^{c}	-5.3 ± 0.4^{c}
T25	16.0 ± 1.5^{cd}	20.3 ± 1.4^{d}	-7.7 ± 1.1^{c}
S15	18.3 ± 1.4^{cd}	31.1 ± 1.0^{b}	-4.4 ± 1.0^{d}
S25	24.7 ± 0.9^{d}	39.5 ± 5.6^{b}	-2.6 ± 0.4^{d}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$)

4.1.1.3. COLOUR

Colour measurements were realized in three different sites of the sample. Therefore, the results in Table 7 are the average and standard deviation of these three measurements for each sample. In what concerns the measurements, some differences were found between saturation (C) and hue for S25.

Table 7 - Colour measurements for each mascarpone-like cheese sample (T: mascarpone-like cheese samples with 15 (T15) and 25% (T25) tuna gelatine; S: mascarpone-like cheese samples with 15 (S15) and 25% (S25) blue shark gelatine)

Sample	Colour		
	L*	C	Hue
Commercial	72.3 ± 1.4^{a}	8.8 ± 0.1^{a}	102.0 ± 0.1^{a}
Control	66.9 ± 10.0^{a}	11.2 ± 1.2^{ab}	99.7 ± 0.1^{b}
T15	67.9 ± 5.5^{a}	10.1 ± 0.6^{ab}	100.8 ± 0.1^{ab}
T25	69.2 ± 2.4^{a}	11.2 ± 0.3^{ab}	100.8 ± 0.1^{ab}
S15	77.0 ± 2.6^{a}	11.9 ± 1.9^{b}	100.7 ± 1.4^{ab}
S25	74.6 ± 1.0^{a}	12.3 ± 0.2^{b}	103.7 ± 0.1^{c}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$)

The high values of L may be due to the dispersibility of casein micelles, calcium phosphate and fat globules, which are together responsible for the diffusion of light, resulting in a higher reflectance, and consequently higher values of luminosity. Except for mascarpone-like cheese S25 and the control cheese, all others have similar colour (hue) to the commercial mascarpone cheese. Colour saturation is similar ($p>0.05$) between control and mascarpone-like cheese samples T15 and T25, whereas cheese samples S15 and S25 differed ($p<0.05$). This difference in colour saturation reflects the difference in colour and brightness of the tuna fish and blue shark gelatines.

4.1.2. CHARACTERIZATION OF CHEMICAL PROPERTIES

The total protein and fat contents for each mascarpone cheese sample is presented in Table 8. Protein content reported for the control and commercial mascarpone cheeses is similar to the values reported in other studies and within the 5.5-6.5% range currently available on the market. In fact, it has been reported that milk proteins in mascarpone cheese are currently found at lower concentrations than in the past, when mascarpone cheese was more consistent and sold wrapped in waxed paper rather than in the plastic cups(52). The use of fish gelatines as an ingredient enabled achieving a protein content similar to the levels found commercial and control cheese samples, when added at 15% concentration (T15 and S15); however, it resulted in a higher protein content when added at 25% concentration (Table 8), as expected. The sample with the highest protein content was mascarpone-like cheese with 25% tuna gelatine (almost two-fold higher, compared with the control and commercial mascarpone cheeses), with a significantly different value from the remaining.

Table 8 - Protein and fat content in mascarpone cheese samples (%) (T: mascarpone-like cheese samples with 15 (T15) and 25% (T25) tuna gelatine; S: mascarpone-like cheese samples with 15 (S15) and 25% (S25) blue shark gelatine).

Sample	Protein (g/100g)	Fat (mg/100g)
Commercial	5.6 ± 1.0^{a}	425 ± 43^{a}
Control	6.8 ± 1.8^{ab}	628 ± 52^{b}
T15	6.9 ± 0.3^{ab}	464 ± 78^{ac}
T25	11.4 ± 0.2^{c}	603 ± 24^{bc}
S15	6.1 ± 0.3^{ab}	491 ± 39^{abc}
S25	8.6 ± 1.0^{b}	369 ± 64^{a}

Note: Results are mean and standard deviation of duplicate assays. Values with the same superscript letter are not significantly different ($p > 0.05$)

Concerning fat content, reported values are not as consistent. Fat content reported for the commercial mascarpone cheese sample is within the range reported for literature (42%), however fat contents for the control cheese are almost 50% higher; such difference may be due to cream's original fat content used for the control cheese preparation. It is also expected that when fat content is lowered, the amount of protein required to maintain the cheese structure increases. Furthermore, it was expected that partial replacement of cream with gelatine would lower the fat content and increase the protein content accordingly. Surprisingly, adding 25 % of tuna gelatine revealed a higher fat content than adding 15% of the same gelatine, despite the increased protein content. On the other hand, the mascarpone-like cheese sample with 25 % of blue shark gelatine contained less fat, when compared with control, T25 and S15 samples. T15, S15 and S25 samples presented similar fat values to those reported for the commercial sample. Therefore, incorporating tuna or blue shark gelatine into the mascarpone cheese does not have a significant impact on the fat content, according to the samples under study.

4.1.3. APPLICATION OF MASCARPONE CHEESE: APPLE MUFFINS

Mascarpone cheese is commonly applied in the production of several desserts, cakes and pastries. The newly developed mascarpone-like cheese containing fish gelatine was tested in the production of apple muffins, a cake much sought by the general consumer. Muffins prepared with mascarpone-like cheese with fish gelatine had a better overall appearance than muffins with commercial mascarpone cheese (Figure 2). Furthermore, muffins with the new ingredient revealed a tender or lighter texture with a less compact, more aerated crumb (Figures 4 and 5). The control muffins had the typical structure of muffins, with a compact texture sprinkled with air bubbles (Figure 3), while the muffin formulations with the mascarpone-like fish gelatine cheese showed a moist crumb structure with appreciable large bubbles (Figures 4 and 5).

Muffins were also subject to an informal sensory test by untrained panellists to assess colour, taste, texture and general acceptability. All muffin batches proved to be acceptable.

Figure 2- Whole apple muffins tested (from left to right: control muffin, mascarpone-like cheese with 15% tuna-gelatine muffin - T15 and mascarpone-like cheese with 25% tuna-gelatine muffin - T25, respectively).

Figure 3 - Longitudinal section of apple muffin control, prepared with control mascarpone cheese without tuna gelatine.

Figure 4 - Apple muffin with 15 % of tuna gelatine.

Figure 5 - Longitudinal section of apple muffin, prepared with mascarpone-like cheese with 15% tuna gelatine.

Muffins prepared with mascarpone-like cheese with 25 % of tuna gelatine were the most appreciated type of muffins, followed by the muffins prepared with mascarpone-like cheese with 15 % of tuna gelatine and finally the muffin control.

4.2. TUNA PÂTÉ

PÂTÉ WITHOUT CORNSTARCH

In this food application, two strategies were studied. In the first strategy tuna fish gelatine was used to partially replace the oil used in the tuna pâté preparation whereas in the second strategy cornstarch was included as an ingredient so tuna fish gelatine was used to partially replace oil + cornstarch to test for its effectiveness as a fat replacer and texturiser.

In the former case, four different combinations of oil:gelatine were tested in the preparation of tuna pâtés containing fixed amount of tuna (Table 9). For each combination four samples with equal composition were produced in order to be assessed, in duplicate, upon production and after storage for 28 days. At day 0 samples A1-A2, A5-A6, A9-A10, A13-A14 were measured and at day 28 samples A3-A4, A7-A8, A11-A12, A15-A16 were measured (as indicated in Table 9).

Table 9 - Composition of tuna pâté samples without cornstarch.

Sample	Tuna Fish	Vegetable Oil	Tuna Fish Gelatine
A1-A4	60	20	20
A5-A8	60	15	25
A9-A12	60	10	30
A13-A16	60	5	35

4.2.1. CHARACTERIZATION OF PHYSICAL PROPERTIES OF TUNA PÂTÉ WITHOUT CORNSTARCH

4.2.1.1. TEXTURE

The results of texture evaluation, specifically on pâté samples' firmness, are presented in Table 10. Differences in firmness were detected between samples and between sampling times. In general, the higher the gelatine content used to replace the vegetable oil fraction, the higher its firmness values; this trend was observed for all pâté samples, except for those containing 30% tuna fish gelatine, which presented firmness values similar to those obtained with 20% gelatine. Regarding storage time, firmness values did not alter significantly (p>0.05) for pâté samples with 20% and 30% tuna gelatine. However, those containing 25% or 30% of tuna fish gelatine increased firmness after 28 days, reaching levels similar to those reported for the sample containing 35% tuna fish gelatine.

Table 10 - Firmness (g) in tuna pâté samples without cornstarch and containing different percentages of tuna fish gelatine.

	Day 0		Day 28	
% Gelatine	Sample	Firmness (g)	Sample	Firmness (g)
20	A1,A2	60.0 ± 2.7^{a1}	A3,A4	62.6 ± 5.3^{a1}
25	A5,A6	84.8 ± 3.7^{ab1}	A7,A8	100.2 ± 9.6^{b1}
30	A9,A10	60.6 ± 5.2^{a1}	A11,A12	102.2 ± 18.3^{b2}
35	A13,A14	108.6 ± 46.3^{b1}	A15,A16	105.3 ± 3.8^{b1}

Note: Values with the same superscript letter are not significantly different (p > 0.05), within the same sampling time. Values with the same superscript number are not significantly different (p > 0.05) between sampling times

4.2.1.2. COLOUR

The results of colour parameters are exposed in table 11. The tuna pâté samples were found to be dark in colour with a medium luminosity. The colour values showed similar profiles in all tuna pâté samples. Pâté containing 35% gelatine, however, presents a wider value range in colour, which may indicate a possible effect of higher contents of gelatine on the colour of the tuna pâtés.

Table 11 - Colour parameters of tuna pâté samples containing different percentages of tuna fish gelatine on day 0 and upon refrigerated storage for 28 days.

	Day 0				Day 28			
% Gelatine	Sample	L	C	Hue	Sample	L	C	Hue
20	A1,A2	47.9 ± 0.4^{a1}	16.1 ± 0.9^{a1}	74.5 ± 0.5^{a1}	A3,A4	45.4 ± 0.4^{a2}	17.1 ± 0.7^{a1}	77.7 ± 1.3^{ab2}
25	A5,A6	45.2 ± 1.4^{c1}	16.0 ± 0.3^{a1}	74.7 ± 1.1^{a1}	A7,A8	47.4 ± 0.6^{b2}	17.6 ± 1.2^{a2}	74.8 ± 1.6^{b1}
30	A9,A10	48.4 ± 0.2^{a1}	16.3 ± 0.3^{a1}	71.6 ± 2.1^{b1}	A11,A12	45.3 ± 0.3^{a2}	17.4 ± 0.8^{a1}	77.8 ± 0.5^{a2}
35	A13,A14	43.7 ± 0.7^{b1}	17.9 ± 2.1^{b1}	74.0 ± 0.4^{a1}	A15,A16	47.0 ± 1.3^{ab2}	16.8 ± 0.8^{a1}	76.6 ± 0.2^{ab2}

Note: Values with the same superscript letter are not significantly different (p > 0.05), within the same sampling time. Values with the same superscript number are not significantly different (p > 0.05) between sampling times

4.2.2. CHARACTERIZATION OF CHEMICAL PROPERTIES OF TUNA PÂTÉ WITHOUT CORNSTARCH

4.2.2.1. PROTEIN

The protein content on day 0 and day 28 of storage, for the tuna pâté samples with different percentages of tuna fish gelatine, is presented in table 12.

Table 12 - Protein content (g/100g) on day 0 and day 28 in tuna pâté samples without cornstarch.

	Day 0		Day 28	
% Gelatine	Sample	Protein (g/100g)	Sample	Protein (mg/g)
20	A1,A2	18.6 ± 1.6^{a1}	A3,A4	18.8 ± 2.6^{a1}
25	A5,A6	19.1 ± 1.9^{a1}	A7,A8	19.2 ± 1.5^{a1}
30	A9,A10	17.0 ± 1.0^{a1}	A11,A12	19.0 ± 1.8^{a1}
35	A13,A14	20.6 ± 3.7^{a1}	A15,A16	18.5 ± 1.4^{a1}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

The protein content of the different samples was very similar; no statistical differences were observed between formulations or over storage time. Even though it is not statistically significant, the only pâté sample with a slightly different behaviour was the sample containing 30% of gelatine which reported the lowest protein content – despite its higher gelatine content when compared with samples with 20% and 25% of gelatine. On the other hand, and as expected, the pâté containing the highest content of tuna fish gelatine (35%) reported the highest protein content. Protein content remained stable over the 28 days storage period.

4.2.2.2. TOTAL FAT CONTENT

Total Fat content on day 0 and day 28 is presented in table 13.

Table 13 - Fat content (mg/g (wet-weight)) in tuna pâté samples without cornstarch on day 0 and day 28 of storage.

	Day 0		Day 28	
% Tuna Gelatine	Sample	Total Fat (mg/g)	Sample	Total Fat (mg/g)
20	A1,A2	234 ± 27^{a1}	A3,A4	240 ± 12^{a1}
25	A5,A6	196 ± 49^{a1}	A7,A8	166 ± 27^{c1}
30	A9,A10	114 ± 13^{b1}	A11,A12	107 ± 5^{b1}
35	A13,A14	76 ± 27^{b1}	A15,A16	79 ± 21^{b1}

Note: Values with the same superscript letter are not significantly different (p > 0.05), within the same sampling time. Values with the same superscript number are not significantly different (p > 0.05) between sampling times

A good relationship between fat content and percentage gelatine was denoted. The higher the percent addition of tuna gelatine, the lower the fat content; on day 0, the samples with more fat were A1,A2, which had the lowest gelatine content (20%), whereas samples with less fat were A13,A14, which incorporated with the highest gelatine content (35%). No changes in fat content were reported over the 28 day-storage period. These results demonstrate the positive impact of tuna gelatine addition in the reduction of the pâtés fat content. These values were very similar to the ones expected.

4.2.2.3. WATER ACTIVITY (a_w), MOISTURE and pH

Chemical analysis was done on all experimental tuna pâtés on day 0 upon manufacture and after 28 days of storage. Values are presented in Table 14. Water activity was high (>0.99) in all experimental tuna pâtés upon manufacture – among the four different formulations, only tuna pâté with 30% added gelatine reported slightly lower values of statistical significance ($p<0.05$) (Table 14). Over storage, a_w values dropped slightly but such drop was not of statistical significance.

Concerning moisture, replacing oil with tuna gelatine showed an increasing effect on moisture content of tuna pâté formulations. This is due to the increase of water in formulations with higher content of gelatine. This higher content of water in tuna samples is in accordance with previously discussed a_w values. On day 28 of storage, no significant statistical differences were found between samples with 25% and 30% of gelatine. However, samples with 20% of added gelatine had lower a_W values on day 28 than on day 0, as well as when compared with samples with higher percentage of added gelatine.

pH values of tuna pâté formulations were found between 5.46 and 5.56, where an increase in the gelatine concentration above 20% resulted in a statistically significant ($p<0.05$) decrease in pH. Over the 28 days of storage pH became slightly more acidic when compared with day 0.

Table 14 - Water activity, moisture, and pH values in tuna pâté samples without cornstarch on day 0 and upon 28 days of storage.

	Day 0				Day 28			
% Gelatine	Sample	a_w	Moisture (%)	pH	Sample	a_w	Moisture (%)	pH
20	A1,A2	0.997 ± 0.001^{a1}	64.6 ± 0.7^{a1}	5.56 ± 0.01^{a1}	A3,A4	0.988 ± 0.007^{a1}	60.6 ± 4.3^{a1}	5.46 ± 0.06^{a2}
25	A5,A6	0.996 ± 0.002^{a1}	71.0 ± 0.8^{d1}	5.50 ± 0.02^{c1}	A7,A8	0.997 ± 0.002^{a1}	66.8 ± 2.8^{ac2}	5.47 ± 0.03^{a2}
30	A9,A10	0.994 ± 0.002^{b1}	72.5 ± 1.2^{b1}	5.46 ± 0.01^{b1}	A11,A12	0.995 ± 0.001^{a1}	70.8 ± 3.2^{bc1}	5.42 ± 0.03^{a2}
35	A13,A14	0.998 ± 0.001^{a1}	76.0 ± 0.5^{c1}	5.46 ± 0.03^{b1}	A15,A16	0.988 ± 0.008^{a1}	74.8 ± 2.8^{b1}	5.41 ± 0.03^{a2}

Note: For each physical-chemical parameter, values with the same superscript letter are not significantly different (p > 0.05), within the same sampling time. Values with the same superscript number are not significantly different (p > 0.05) between sampling times dropped slightly, yet with statistical significance (p<0.05)

PÂTÉ WITH CORNSTARCH

The identification and ingredient composition/distribution of tuna pâté samples with cornstarch are listed in Table 15.

Table 15 - Ingredient composition (tuna, vegetable oil, cornstarch and tuna gelatine) and distribution in the different formulations of tuna pâté with cornstarch.

Samples	A1-A4	A5-A8 (A)	A9-A12 (B)	A13-A16 (C)	A17-A20(D)
Tuna:oil:starch:gelatine	60:20:20:0	60:20:10:10	60:15:10:15	60:10:10:20	60:5:10:25

4.2.3. CHARACTERIZATION OF PHYSICAL PROPERTIES OF TUNA PÂTÉ WITH CORNSTARCH

4.2.3.1. TEXTURE

Tuna pâté formulations containing 50% replacement of cornstarch and partial substitution of oil (25, 50 and 75% replacement) with tuna gelatine were prepared. A control sample containing full amount of cornstarch and vegetable oil was also manufactured for comparative purposes. In terms of texture, the results obtained could be identified in table 16. On day 0 of storage, only the sample with 20% of added gelatine had significantly higher firmness than the control sample. However, after 28 days of storage all samples with added gelatine had more firmness than the control sample. This means that replacement of starch with gelatine could be effectively achieved with different percentages of gelatine. Cornstarch is used by a fat replacer Starches, like cornstarch, are used by food industry because of several functions, including gelling agent, bulking agent and water retention agent(53). Also, cornstarch has a good potential to be a fat replacer, due to its property of binding extra water and its capacity to give similar amount of lubricity and smoothness as its full fat counterparts(54). In this case, maybe it was necessary to increase starch amount due to the humidity in samples. Alternatively, it would require cooking the products, so that a major gelatinization of starch might be achieved, and consequently a gel increase and more firmness is observed in samples.

Table 16 - Firmness (g) in tuna pâté samples with cornstarch, on day 0 and 28 of storage.

		Day 0		Day 28	
% Gelatine	% Starch	Sample	Firmness (g)	Sample	Firmness (g)
0	20	A1,A2	26.1 ± 6.6^{ac1}	A3,A4	17.1 ± 1.5^{a2}
10	10	A5,A6	19.8 ± 7.4^{a1}	A7,A8	30.3 ± 4.2^{b2}
15	10	A9,A10	23.3 ± 4.4^{ac1}	A11,A12	38.0 ± 8.9^{bc2}
20	10	A13,A14	38.6 ± 3.5^{b1}	A15,A16	49.0 ± 4.8^{cd2}
25	10	A17,A18	29.9 ± 2.0^{bc1}	A19,A20	42.8 ± 2.8^{d2}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

4.2.3.2. COLOUR

Regarding colour parameters, the results obtained could be identified in table 17. On day 0 of storage, only the samples with 20% gelatine presented significantly different values when compared with the remaining samples. Concerning C and Hue, no relevant differences were found on day 0.

After 28 days of storage, the samples with 20% gelatine remain with lower level of L, when compared with samples with less gelatine – no statistical differences can be inferred, however, between 20% gelatine samples and 25% ones. Regarding C, samples without gelatine have lower values than samples with 10% and 25% gelatine. On Hue, due to volatility found in values for samples with 15% and 20% gelatine, no significant statistical differences can be inferred.

Table 17 - Colour parameters of tuna pâté samples with cornstarch on day 0 and 28 of storage.

		Day 0				Day 28			
% Gelatine	% Starch	Sample	L	C	Hue	Sample	L	C	Hue
0	20	A1,A2	52.6 ± 1.0^{a1}	14.7 ± 0.4^{a1}	69.9 ± 5.2^{a1}	A3,A4	54.4 ± 1.4^{a2}	14.7 ± 0.3^{a1}	71.1 ± 0.8^{a1}
10	10	A5,A6	52.8 ± 0.6^{a1}	15.1 ± 0.1^{b1}	70.3 ± 0.9^{a1}	A7,A8	53.8 ± 1.2^{a1}	16.6 ± 0.9^{b2}	73.1 ± 0.9^{a2}
15	10	A9,A10	52.7 ± 0.3^{a1}	14.8 ± 0.1^{ab1}	71.0 ± 0.9^{a1}	A11,A12	51.6 ± 0.3^{b2}	15.5 ± 0.5^{a2}	70.5 ± 2.2^{a1}
20	10	A13,A14	47.3 ± 1.4^{b1}	14.9 ± 0.3^{ab1}	67.1 ± 1.4^{a1}	A15,A16	50.0 ± 0.5^{c2}	15.2 ± 0.6^{a1}	73.1 ± 3.7^{a2}
25	10	A17,A18	51.0 ± 0.7^{c1}	14.6 ± 0.2^{a1}	68.4 ± 2.9^{a1}	A19,A20	50.9 ± 0.7^{bc1}	17.2 ± 0.1^{b2}	69.7 ± 0.8^{a1}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

4.2.4. CHARACTERIZATION OF TUNA PÂTÉ CHEMICAL PROPERTIES WITH CORNSTARCH

4.2.4.1. PROTEIN

After analysing the samples, the protein values identified in table 18 were considered. The most significant statistical conclusion which can be made is that all samples had an increase in protein content from day 0 to day 28 of storage. No differences were found between samples with different gelatine content after identical numbers of days in storage. The same conclusion is reached when compared with control version.

Table 18 - Protein (g/100g(wet-weight)) in tuna pâté samples with cornstarch.

		Day 0		Day 28	
% Gelatine	% Starch	Sample	Protein (g/100g)	Sample	Protein (g/100g)
0	20	A1,A2	18.8 ± 1.7^{a1}	A3,A4	36.8 ± 6.0^{a2}
10	10	A5,A6	16.6 ± 4.9^{a1}	A7,A8	30.9 ± 2.8^{a2}
15	10	A9,A10	17.3 ± 2.7^{a1}	A11,A12	33.0 ± 0.8^{a2}
20	10	A13,A14	17.1 ± 3.2^{a1}	A15,A16	32.8 ± 4.2^{a2}
25	10	A17,A18	17.6 ± 2.2^{a1}	A19,A20	34.1 ± 3.1^{a2}

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

4.2.4.2. TOTAL FAT

Fat content values are listed in table 19 for day 0, upon manufacture, and after 28 days of storage. At day 0, the samples with more total fat content had 0% and 10% of gelatine and the samples with less total fat content were the ones with the highest gelatine content (25%). These results were expected because samples with more protein tend to have less fat content. On day 28 of storage, identical conclusion can be inferred – the higher the protein content, the lower the total fat content. Between day 0 and day 28, fat content decreases in samples with 0 %, 15 % and 20 % of gelatine. On days 0 and 28, no significant difference was found between samples with 0% and 10% of gelatine. Also, no significant differences were found on day 28 between 0% and 15% gelatine samples, as well as between 10% and 15% gelatine samples. Between the two days, a fat reduction around 22% was observed in samples with 20% of gelatine, while a reduction around 16% was registered in samples with 15% of gelatine. It was observed that samples with less fat had more firmness after 28 days than samples with 0 % and 10 % gelatine, which is expected due to the cornstarch fat replacer function in tuna pâtés and the gelatine properties.

Table 19- Total fat (mg/g) in tuna pâté samples with cornstarch.

		Day 0		Day 28	
%Gelatine	% Starch	Sample	Total Fat (mg/g)	Sample	Total Fat (mg/g)
0	20	A1,A2	260 ± 2[a1]	A3,A4	209 ± 16[ab2]
10	10	A5,A6	283 ± 54[a1]	A7,A8	218 ± 31[a1]
15	10	A9,A10	204 ± 5[b1]	A11,A12	172 ± 20[b2]
20	10	A13,A14	149 ± 6[c1]	A15,A16	117 ± 7[c2]
25	10	A17,A18	71 ± 3[d1]	A19,A20	73 ± 13[d1]

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

4.2.4.3. WATER ACTIVITY (a_w), MOISTURE and pH

Water activity, moisture and pH values are presented in table 20 after 0 and 28 days of storage. a_w parameter was high (>0.99) in all experimental tuna pâtés upon manufacture. After 28 days, the a_w values in samples with gelatine present statistically significant lower amounts than samples without gelatine.

In terms of moisture, reducing starch to half and increasing gelatine cause significant changes between the sample without gelatine and samples with more than 20% gelatine, on day 0. As the samples show high a_w values, it was expected that moisture percentage would increase with increasing gelatine. From day 0 to day 28, statistical differences were identified in the samples with 20% of gelatine and 10% of starch.

Regarding pH values at day 0 and 28 of storage, pH was acid and did not reveal differences between the samples. Still, pH became slightly more acidic after 28 days of storage, when compared with day 0 levels.

Table 20 - Water activity, moisture, and pH values in tuna pâté without cornstarch on day 0 and upon 28 days of storage.

			Day 0			Day 28			
% Gelatine	% Starch	Sample	a_w	Moisture (%)	pH	Sample	a_w	Moisture (%)	pH
0	20	A1, A2	0.997 ± 0.002[a1]	60.1 ± 1.7[a1]	5.75 ± 0.20[a1]	A3,A4	0.997 ± 0.001[a1]	62.3 ± 0.8[a1]	5.46 ± 0.04[a2]
10	10	A5,A6	0.992 ± 0.005[ab1]	59.7 ± 1.7[ab1]	5.75 ± 0.04[a1]	A7,A8	0.991 ± 0.001[b1]	60.6 ± 1.2[a1]	5.48 ± 0.09[a2]
15	10	A9,A10	0.992 ± 0.004[ab1]	63.8 ± 2.2[bc1]	5.69 ± 0.05[ab1]	A11,A12	0.990 ± 0.001[b1]	65.8 ± 1.4[b1]	5.39 ± 0.07[a2]
20	10	A13,A14	0.990 ± 0.003[b1]	67.1 ± 1.9[c1]	5.60 ± 0.06[ab1]	A15,A16	0.994 ± 0.002[c2]	71.7 ± 0.6[c2]	5.44 ± 0.04[a2]
25	10	A17,A18	0.994 ± 0.003[ab1]	76.4 ± 1.2[d1]	5.57 ± 0.02[b1]	A19,A20	0.992 ± 0.001[bc1]	75.4 ± 0.5[d1]	5.40 ± 0.03[a2]

Note: Values with the same superscript letter are not significantly different ($p > 0.05$), within the same sampling time. Values with the same superscript number are not significantly different ($p > 0.05$) between sampling times

4.3. MICROBIOLOGICAL STABILITY OF TUNA PÂTÉ WITH OR WITHOUT CORNSTARCH

Microbiological quality of all experimental tuna pâtés was maintained below detectable limits for microbial groups under study throughout the 28 days of storage. No spoilage organisms were present at detectable viable cell numbers upon manufacture, and they did not proliferate throughout storage. All samples reported absence of colony forming units at the lowest dilution tested (<1x10^1 to <3x10^2 CFU/g) for both pour plate and spread plate techniques respectively. This is a very promising result and supports the food innovation trend of safety with minimum additives used.

4.4 SENSORY ANALYSIS OF TUNA PÂTÉ

4.4.1. OVERVIEW OF CONSUMER PANEL

The study consumer panel was composed of 76 individuals, 75% (n=57) females and 25% (n=19) males. There was a total of 5 foreigners (6.6 %) from several origins, but the sample was mostly Portuguese (n=71). The average age of the respondents was 26.53 years (95% confidence interval [CI 95%] [24,61-28,44]) with a 31-year range, where the youngest was 19 years old and the oldest was 50. The distribution of the sample ages is given in the box plot diagram that follows (Figure 6).

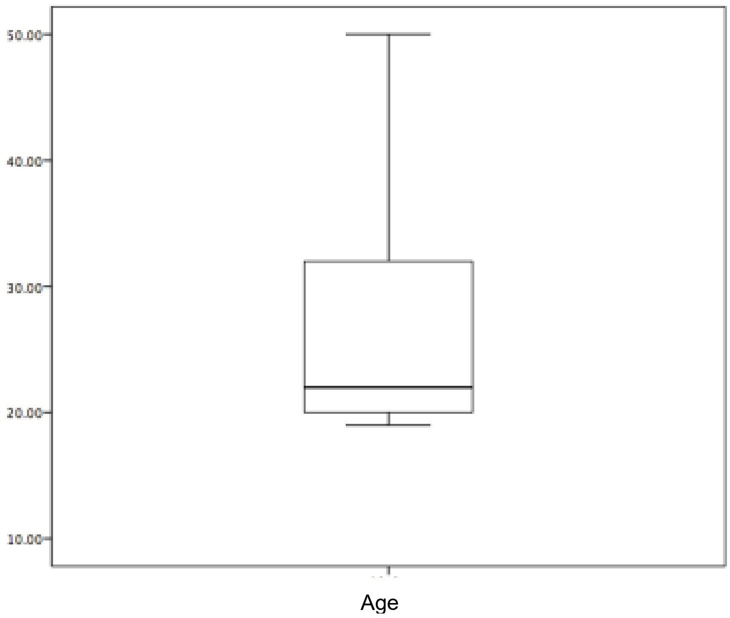


Figure 6 - Distribution of among consumer panel members.

Regarding the academic degree, 52.6% (n=40) held a bachelor's or master's degree, 42.1% (n=32) had the 11th or 12th grade and only 5.3% (n=4) held a PhD.

Only 10.5% (n=8) of respondents consumed pâtés once or more times per week, but this cumulative percentage rises to 42.1% if we add consumers with a monthly habit that represent 31.6% (n=24). Figure 7 illustrates the frequency of distribution of consumption.

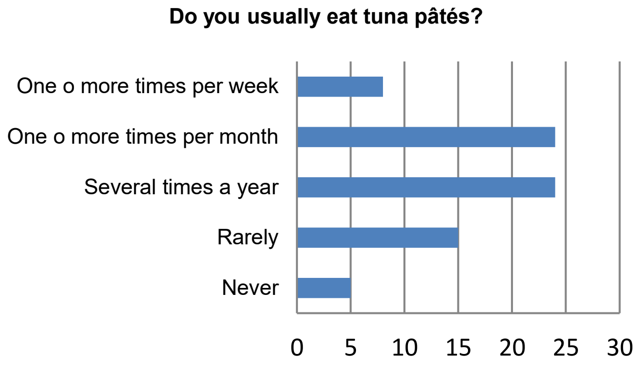


Figure 7 - Frequency of pâtés consumption among consumer panel.

The remaining consumption frequencies and respective percentages are shown in the table below.

Table 21 - Consumption frequencies of pâtés and respective percentages.

Do you usually eat tuna pâtés?	n	%
Never	5	6.6
Rarely	15	19.7
Several times a year	24	31.6
One or more times per month	24	31.6
One or more times per week	8	10.5
Total	76	100.0

4.4.2. EVALUATION OF TASTE, TEXTURE AND TASTE

4.4.2.1. Preference of the consumer panel members

The tasters were asked to indicate the order of preference of the three samples. In the following evaluation, 4 responses were excluded due to an error of introduction or non-completion.

The samples were randomly coded with 3 digits:

807 – Control sample

243 – Pâté with cornstarch (60% tuna:10% cornstarch:5% oil:25% tuna gelatine)

408 – Pâté without cornstarch (60% tuna:15% oil:25% tuna gelatine)

Table 22 - Order of samples preference evaluation.

Sample		Preference order 1st option	2nd option	3rd option
Pâté with cornstarch (60:10:5:25 tuna gelatine) (243)	n	14	30	31
	% of this sample	18.7%	40.0%	41.3%
	% in the respective position	18.7%	39.5%	42.5%
Pâté without cornstarch (60:15:25 tuna gelatine) (408)	n	23	33	18
	% of this sample	30.7%	44.0%	24.0%
	% in the respective position	30.7%	43.4%	24.7%
Control tuna pâté (807)	n	38	13	24
	% of this sample	50.7%	17.3%	32.0%
	% in the respective position	50.7%	17.1%	32.9%
Total	n	75	76	73

The control tuna pâté was the preferred sample for more than 50% (n=38) respondents (Table 22), followed closely by the tuna pâté without cornstarch with 30.7% (n=23) and finally by the tuna pâté with cornstarch with only 18.7% (n=14) of the respondents pointing the tuna pâté sample as their first choice. Interestingly, the control sample was classified as the third option by 32% (n=24) of the respondents and only 17.3 % (n = 13) as the second option. In the case of the second most chosen sample, i.e., tuna pâté without cornstarch, most of the respondents,44% (n=33), indicated it as their second option, followed by the already mentioned 30.7 % (n = 23) as their first option and 18% (n=18) as their third option. Indeed, the least popular sample was the tuna pâté with cornstarch (similar content in gelatine to previous sample tuna pâté without cornstarch), where most of the answers, 41.3 % (n=31), classified it as the third option, followed by the second option with 40% (n = 30) and only 18,7 % choosing it as the first option (n=14).

The Chi-square test suggests that there is a statistically significant relationship between the order of choice and the sample (p value <0.001). Such association occurs because the control sample was selected as the first option more times than expected whereas, the tuna pâté with cornstarch sample was selected fewer times than expected.

4.4.2.2. Evaluation of flavour, texture, and taste

The evaluation of the flavour, texture and taste attributes of the tuna pâté samples, was made in a range varying from appreciating it extremely or not appreciating it at all (dislike extremely). Table 23 summarizes the response frequencies according to such scale.

Table 23 - Hedonic evaluation of flavour, texture, and taste of tuna pâté samples.

Scale		**Overall taste**			**Texture**			**Flavour**		
		243	408	807	243	408	807	243	408	807
Dislike extremely	n	2	3	3	0	1	2	3	1	2
	%	2.6%	3.9%	3.9%	0.0%	1.3%	2.6%	3.9%	1.3%	2.6%
Dislike very much	n	3	3	2	0	2	1	4	3	4
	%	3.9%	3.9%	2.6%	0.0%	2.6%	1.3%	5.3%	3.9%	5.3%
Dislike moderately	n	6	3	3	6	2	2	8	5	3
	%	7.9%	3.9%	3.9%	7.9%	2.6%	2.6%	10.5%	6.6%	3.9%
Dislike slightly	n	13	7	9	12	7	7	8	6	9
	%	17.1%	9.2%	11.8%	15.8%	9.2%	9.2%	10.5%	7.9%	11.8%
Neither like nor dislike	n	8	9	5	10	16	6	7	7	5
	%	10.5%	11.8%	6.6%	13.2%	21.1%	7.9%	9.2%	9.2%	6.6%
Like slightly	n	15	14	10	17	13	13	19	13	12
	%	19.7%	18.4%	13.2%	22.4%	17.1%	17.1%	25.0%	17.1%	15.8%
Like moderately	n	20	20	23	17	17	19	13	20	19
	%	26.3%	26.3%	30.3%	22.4%	22.4%	25.0%	17.1%	26.3%	25.0%
Like very much	n	6	17	18	13	18	22	12	21	17
	%	7.9%	22.4%	23.7%	17.1%	23.7%	28.9%	15.8%	27.6%	22.4%
Like extremely	n	3	0	3	1	0	4	2	0	5
	%	3.9%	0.0%	3.9%	1.3%	0.0%	5.3%	2.6%	0.0%	6.6%

Regarding the overall taste, sample 807 presented a percentage of positive responses distributed as follows: 3.9% (n=3) liked extremely, 23.7% (n=18) liked very much and 30.3% (n=23) liked moderately. Similarly, sample 408 presented responses that were compatible with 22.4% (n=17) liking very much, followed by 26.3% (n=20) of responses that expressed moderation. In relation to this parameter, sample 243 has the highest expression in the moderately liking category, with 26.3% (n=20) of the total responses, and the remaining frequencies are expressed in the less favourable categories as shown in the table 23. Figure 8 (a) illustrates the distribution of number of responses on the overall taste.

Regarding texture, results followed a similar trend to that observed for overall taste. Sample 807 was the sample with the best results. It is observed that 5.3 % (n = 4) likes extremely, 28.9% (n=22) likes very much and 25 % (n=19) likes moderately. The sample with the most expressive positive results, after sample 807, is sample 408. In this case the responses with greater expression are: like very much with 23.7% (n=18) and like moderately with 22.4% (n=17). In the sample with less favourable responses, sample 243, like moderately had an expressive similarity to that of sample 408, with 22.4%, but there is, in this case, another category with a positive tendency, to like slightly, which is equally significant reaching 22.4%

(n=17). Figure 8(b) illustrates the distribution of the number of responses on the texture of the tuna pâté

As expected, flavour scores followed the same trends as overall taste and texture. Sample 807 was the sample with the highest number of positive responses (5 respondents liked extremely, 17 liked very much and 19 liked moderately) followed closely by sample 408 (21 respondents liked very much and 20 liked moderately). Similarly, sample 243 showed the highest occurrence frequency in the like moderately category with 25% (n=19) of the total responses (Figure 8(c)).

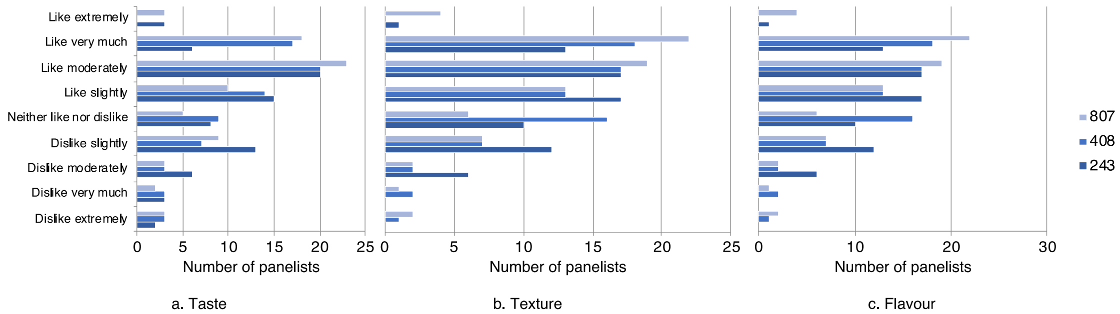


Figure 8. Preferences of panellists in terms of taste (a), texture (b) and flavour (c).

For the purpose of the evaluation and inferential comparison, the ordinal scale of the global taste, texture and flavour parameters, shown above, was converted into a latent variable with a numerical score of 1 to 9, where 9 is the best appreciation of the parameter, equivalent to like extremely, by the tasters and 1, equivalent to dislike extremely, the less acceptable. Thus, higher scores reflect a more positive response to the parameter by the tasters.

Regarding the overall taste, the sample with the highest score, that is the most appreciated by the respondents, is the sample 807 with a mean score of 6.17 95% CI [5,71-6,62], followed by sample 408 with an average score of 5.93 CI 95% [5,49-6,37] and with a slightly lower mean score, the sample 243, with a mean of 5.57 CI 95% [5,13-5,99]. Figure 9(a) shows a box plot diagram with the distribution of scores on the overall taste.

On texture, it is verified that sample 807 presents an average score of 6.46 95% CI [6.04-6.87], followed by the 408 sample with a mean score of 6.05 95% CI [5,67-6,43] and with a slightly lower mean score, the sample 243, with an average of 5.93 CI 95% [5,55-6,28]. The Kruskal-Wallis test, applied for failure to verify the normality of the scores, suggests that there are statistically significant differences between the samples (p value=0.044) with respect to the texture. Figure 9(b) shows a box plot diagram with the distribution of scores on the texture.

Concerning flavour, the 807 sample shows the highest score with a mean of 6.13 95% CI [5,66-6,59], followed by the 408 sample with a mean score of 6.14 95% CI [5,72-6,56] and, with a considerably lower mean score, sample 243, with an average of 5.54 95 % CI [5.07-6.01]. The following figure shows a box plot diagram with the distribution of the scores on the taste:

The Kruskal-Wallis test, applied for failure to verify the normality of the scores, suggests that there are no statistically significant differences between the samples (p value=0.07).

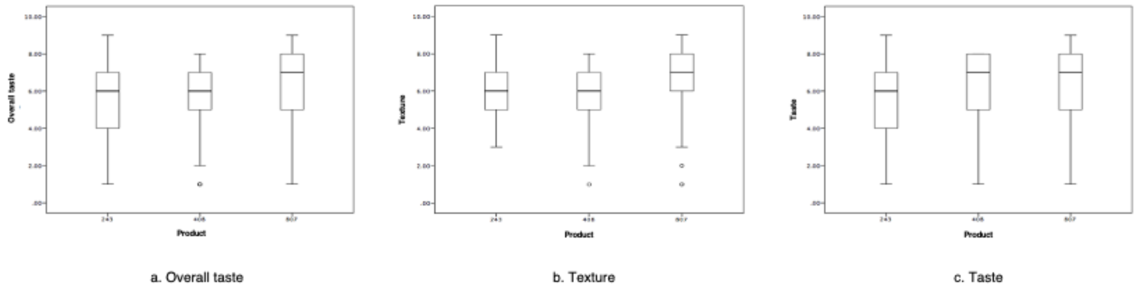


Figure 9. Diagram of the distribution of the scores on overall taste (a), texture (b) and taste (c).

4.4.2.3. Relationship between preferred pâté and gender

Despite the different distributions in preference between men and women among the samples, gender did not have a statistically significant impact on the overall preference for the different pâtés (Table 24). For sample 807, the percentage of men and women did not vary significantly over the options. The Chi-square test suggests that there is an independence between sex and preference for this particular pâté (p value=0.787). The same applies to sample 408 (p=0.309) although in this case the percentage of men who chose the first option was considerably lower (13.04%) than in the other options. Sample 243 was where the largest fluctuations of percentages within the same gender could be found in the various options, yet neither are statistically significant (p value=0.177).

Table 24 - Relationship between preferred pâté and gender.

		Gender				
		Female		Male		**p value**
Sample 243		n	%	n	%	
	1st option	8	57,14	6	42,86	
	2nd option	25	83,33	5	16,67	0,177
	3rd option	23	74,19	8	25,81	
Sample 408						
	1st option	20	86,96	3	13,04	
	2nd option	23	69,70	10	30,30	0,309
	3rd option	13	72,22	5	27,78	
Sample 807						
	1st option	28	73,68	10	26,32	
	2nd option	9	69,23	4	30,77	0,787
	3rd option	19	79,17	5	20,83	

In terms of descriptive statistics, it is suggested that, for men the most expressive option in sample 243 is the first option with 42.86 % (n=6) of the total of the two genders who chose it as the first option. In sample 408, this reference is the second option with 30.3 % (n = 10) and finally, in sample 807, also in the second option, with 30.77 % (n=4). Compared with men, in sample 243, the second option is the one with the highest percentage, in which women represent 83.33% of the total of this option. In sample 408, women represented the highest percentage of the first option reaching 86.96 % (n=20) while in sample 807, the highest percentage of women compared to men relapsed in the third option.

4.4.2.4. Intention to buy

For the evaluation of the purchase intention and the samples under study, the following table was calculated.

Table 25 – Tasters' intention to buy.

		Sample			
Would you buy the product?		243	408	807	Total
I would not buy	n	18	10	10	38
	%	23.7%	13.3%	13.2%	16.7%
I probably would not buy	n	25	24	17	66
	%	32.9%	32.0%	22.4%	29.1%
I would probably buy	n	25	28	33	86
	%	32.9%	37.3%	43.4%	37.9%
I would buy	n	8	13	16	37
	%	10.5%	17.3%	21.1%	16.3%
Total	n	76	75	76	227

The highest purchase rejection expression is 23.7% (n=18) for sample 243, while the highest purchase acceptance is that of sample 807 with 43.4 % (n=33). Regarding the final purchase, if the price was adequate, it is verified that the sample that would have greater acceptance would be 807 with a little more than a fifth of the respondents, 21.1% (n = 16) confirming the purchase, following sample 408 with 17.3% (n=13) and finally with 10.5% (n = 8) sample 243. Although the descriptive statistics enable such suggestion, the statistical findings on the absence of a statistically significant relationship between the overall taste and either product or flavour (only the texture was statistically significant) - led to the performance of the evaluation of the relationship of the two variables in the population by the Chi-square test. This test suggests that, in the population, there is no statistically significant association between the intention to purchase the product and the sample type (p value = 0.168), which constitutes an interesting aspect for the commercialization of these products.

4.4.2.5. Other issues

Which pâté would be the purchase option among tasters who are regular consumers of pâté?

For this purpose, 32 tasters who consumed up to at least once a month (as usual) were selected. Repeating the contingency table calculated at 4.4.2.4 (relation of the sample with intention to buy) but in this case for the regular consumers, almost the same trends are verified, however there are some important differences:

Table 26 – Regular pâté consumers' intention to buy.

Would you buy the product?		Sample 243	408	807	Total
I would not buy	n	11	5	6	22
	%	34.4%	15.6%	18.8%	22.9%
I probably would not buy	n	10	12	5	27
	%	31.3%	37.5%	15.6%	28.1%
I would probably buy	n	6	7	16	29
	%	18.8%	21.9%	50.0%	30.2%
I would buy	n	5	8	5	18
	%	15.6%	25.0%	15.6%	18.8%
Total	n	32	32	32	96

There was a worsening in the purchase rejection of sample 243 to 34.4 % (n = 11), and an increase in certainty of purchasing product 408 to 25 % (n = 8). For this specification, the Chi-square test suggests that there is a statistically significant relationship between the purchase intention and the sample (p=0.043). Standardized residue analysis of the contingency table suggests that there are more individuals who would likely buy the product 807 than expected. It is important to reflect in the proximity that this value of p has at the level of significance (5%) - being important that future studies contain a larger sample to clarify this trend.

As for the relation between preference order and the sample, the same trend is observed in the usual consumers as the one in 4.4.2.1 (all types of consumers), that is, a preference for the product 807.

Table 27 - Relationship between order of preference and the samples.

Preference order		Sample 243	408	807	Total
1st option	n	6	8	18	32
	%	18.8%	25.0%	56.3%	33.3%
2nd option	n	12	16	5	33
	%	37.5%	50.0%	15.6%	34.4%
3rd option	n	14	8	9	31
	%	43.8%	25.0%	28.1%	32.3%
Total	n	32	32	32	96

The Chi-square test suggests that there is a statistically significant relationship between the order of preference and the sample (p=0.004). This relationship is explained by the greater number than expected of first options to revert to the sample 807.

4.5. OTHER APPLICATIONS: JELLY GUMS

The results revealed that the blue shark gelatine is the best choice for this type of products because this gelatine has more hardness, which is important for the final product. Next, the first proposal for the formulation of this product and the first results are presented. From the data obtained it was shown that for the product to become closer to jelly gums, some conditions required improvement including a possibility of inserting a technological auxiliary to make a texture of jelly gums and mask the opacity and some particles of the gelatine. First tests applied in this product were texture and colour.

4.5.1. COLOUR

Table 28 – Jelly gums colour: control and blue shark jelly gums.

	L*	A*	B*
Jelly gums control	41.90	34.56	20.63
	41.89	32.78	18.50
	41.68	35.95	22.49
Blue shark jelly gums control (type of tie 1)	32.60	28.40	23.43
	34.68	29.23	24.19
	36.09	28.24	22.59
Blue shark gums control (type of tie 2)	43.03	26.17	18.90
	44.60	25.03	18.23
	43.58	26.34	19.00

4.5.2. TEXTURE

It was not possible to test the samples because the thickness of the jelly gums was different and had a lot of variabilities into gums (jelly gums with different moulds). Another limiting factor was the temperature of the laboratory. Although the air conditioning was on, the jelly gums melted after some time, upon removal from the freezer and it was not possible to obtain a texture hard enough. Figure 10 show the jelly gums tested.

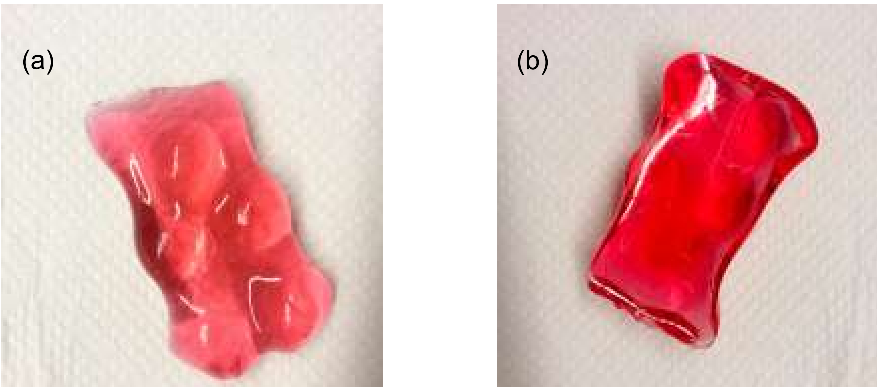


Figure **10** - Jelly gums without fish gelatine (a) front jelly gum mould – control and (b) back jelly gum mould - control.

5. General conclusions

Nowadays, populations are more sensitive to health issues, and they know that food is one of the key tools for a proper health status. Besides, given the consumers' growing interest for healthy and sustainable food products, it would be ideal to produce i) a mascarpone cheese with lower fat content and identical flavour, physical and chemical characteristics, as well as ii) a tuna pâté with lower fat content and a more balanced nutritional profile. Indeed, using fat replacers and efficient incorporation systems to achieve nutritionally improved food products is one of the current approaches in the food industry. Therefore, tuna and blue shark fish gelatines were tested as fat replacers, more specifically as cream or vegetable and/or cornstarch replacers in mascarpone cheese and tuna pâté formulations, respectively. Gelatine works as an emulsifier, thickener, gelling agent and has a stabilizing capacity, which may enable the production of mascarpone cheese or tuna pâté with lower fat contents. Besides testing gelatine's technological multifunctionality, the present study also intended to give economic added value to a fish industry by-product. Indeed, the fish gelatine used in this work was extracted at 45 ºC from fish waste, namely, tuna (*Yellowfin tuna*) and blue shark skins.

Mascarpone cheese is a type of soft cheese with high-fat content and mild flavour. The mascarpone cheese's fat content is normally within the 40 to 60 % range. These features make this cheese an ingredient commonly used in a wide range of products, namely, desserts (e.g., cheesecake, dips, bagels, cakes). However, these products have too much fat and sugar – a less favourable nutritional profile which may contribute to increase health impairment, as fat and sugar could cause many diseases such as obesity, metabolic syndrome, cardiovascular diseases, among others. In our study, marine gelatines were used as partial fat replacers in mascarpone model cheese systems. The results of the study showed that partial replacement of cream with marine gelatines could be used for mascarpone-like cheese production. It could improve texture and nutritional profile, namely, fat and protein profile of the products. No significant effects on protein content and stability were found when compared with control samples, for replacement levels up to 25% of marine gelatine.

Concerning protein, the experimental mascarpone cheeses had higher – though not statistically significant – protein content than commercial mascarpone. Rheological, colour and textural parameters of mascarpone-like cheeses containing tuna fish gelatine were very similar to those of the traditional product. State of the art knowledge did not demonstrate evidence that fish gelatine is unsafe for consumers and previous studies had already shown the safety of these gelatines for human consumption (20). Nevertheless, it is suggested as a preventive measure that individuals who suffer from food allergy, particularly allergy to fish proteins, should not consume products with fish gelatine.

Furthermore, such newly developed product - mascarpone-like cheese with less fat - contributes to innovation in the dairy sector since the market does not yet offer mascarpone cheese with low-fat content. If this becomes an option, both consumers and industry will prefer this new product version over the traditional mascarpone cheese, due to its more balanced nutritional profile. Also, it was better accepted by panellists and led to better matrix outputs when applied in bakery (preparation of apple muffins) – important effects were found on crumb structure and texture. Regarding industries, they can produce food applications with less fat, one of the trends of the food market. Indeed, several foods formulated with fat replacers do not compare favourably with the flavour of full-fat counterparts and food manufacturers strive to find fat replacers that taste and function like conventional fat without the potential adverse health impact. Our study shows the innovative potential of marine gelatines as fat replacers in cream cheese given its overall acceptance. Even though the final developed product did not have a differential fat content in comparison to the commercial version, it adds value to the food industry since this is a completely new opportunity. Further studies are needed to optimise the cheese production and effectively reduce fat in comparison to commercial conventional counterparts.

Concerning tuna pâté development, in the formulation without cornstarch, protein did not vary significantly ($p>0.05$) between samples with different added gelatine concentrations. Lower fat contents were registered among tuna pâtés containing ≥15% gelatine. Regarding pH, a_w, and moisture, statistical differences ($p<0.05$) were found mainly among samples A13-A16 and A17-A20, in which pH and moisture values were lower. Microbiological quality was maintained at required legal levels throughout 28 days of storage. In tuna pâté with cornstarch, the protein content did not vary significantly ($p>0.05$) between samples with different added gelatine concentrations. Lower fat content ($p>0.05$) was noted among tuna pâtés A9-A10 and A13-A14 containing more than 15% of gelatine. Concerning pH and moisture, statistical differences were found in samples A13,A14 and A15,A16 (20% gelatine). Microbiological quality was maintained at required legal levels throughout 28 days of storage at room temperature. Firmness was increased among samples with higher gelatine contents. Sensory analysis indicated good acceptability between samples. Tuna pâtés produced with tuna gelatine, a by-product of fishery industries, revealed a promising impact as a partial fat and cornstarch replacer for manufacturing of reduced fat tuna pâtés.

Preliminary studies also revealed that marine gelatines may be used in other food applications such as the manufacture of jelly gums where its acceptable protein content, low fat content and good flavour are essential ingredients for the development of nutritionally balanced gums targeting different age groups.

6. Future work

For future works, authors could refine the development of gums with fish gelatine. For this, it is necessary to study the quantities required for the development of gums with consistent texture and how to control the environment temperature. Strategies to overcome the presence of residues in gums of blue shark gelatine, including the addition of a food colouring or an increase in stirring time, are also required.

It is essential to carry out further laboratory tests to improve and analyse how marine gelatines can contribute to the reduction of fat and increase protein in mascarpone cheese so that its content is better in comparison to the commercial versions.

It is also necessary to study the lipid profile of marine gelatines, particularly gelatines resulting from fatty fish, and to understand if it contains omega-3 fatty acids, which are of interest in the health-products market.

In addition to product optimization, it would also be important to promote studies on the possibility of extending the use of marine gelatines to other food products, in order to make them more nutritionally interesting and natural. Examples of products which could improve their nutritional composition (mainly in fat and protein composition) from incorporating marine gelatine include desserts, soups, confectionary products, sauces, butter, cocoa cream and gums or other candies.

Another interesting market segment in which marine gelatine could be adopted is elderly food products. It could be done by adding marine gelatine in products like cereal breakfast, purees, porridge (instant porridge), soup and other type of food applications. This addition could be a way of increasing the consumption of protein among elderly people and reducing the increment of salt in these products.

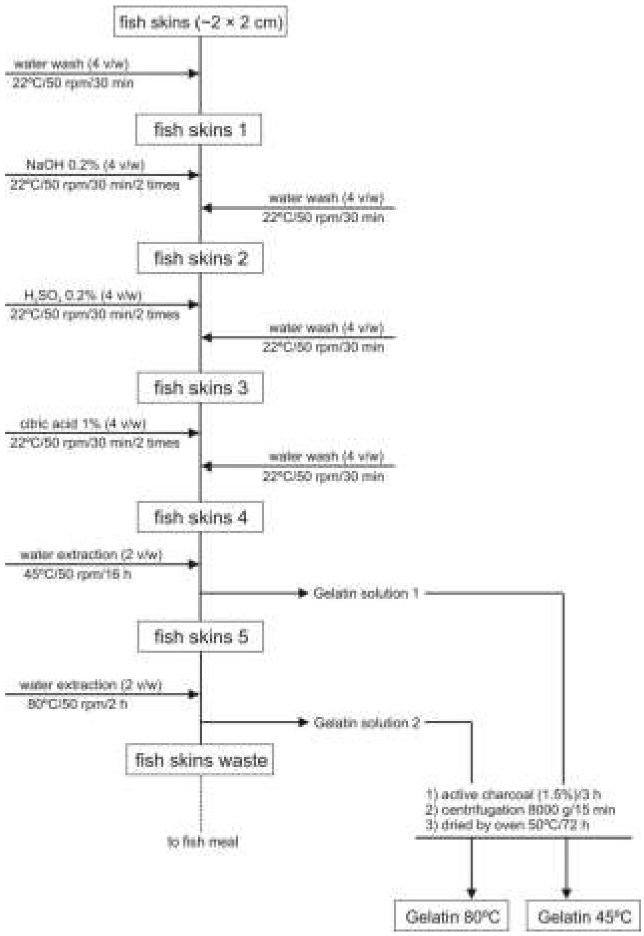
7. Scientific dissemination

The scientific material produced in this thesis was presented at some conferences (supplement 1). It took part in national and international marketing events and innovation awards (supplement 2), and scientific articles are being prepared.

8. Appendices and supplements

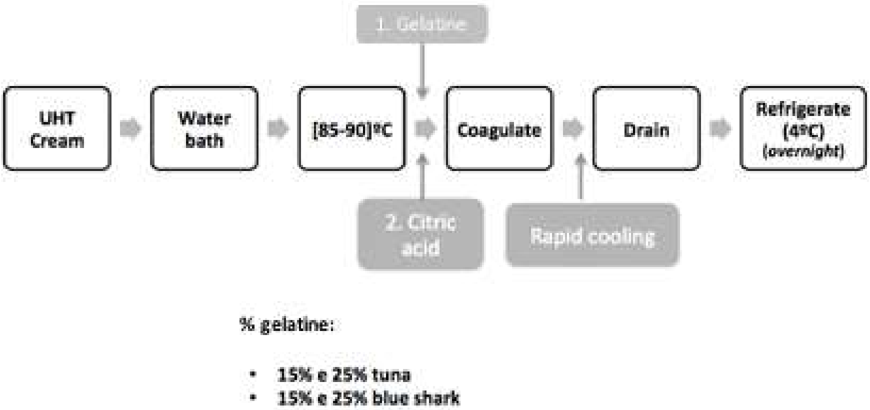
ANNEX 1

<u>Gelatine extraction process according to Sousa et al. (2017)(17)</u>



APPENDIX 1

Process of mascarpone cheese with marine gelatine.



APPENDIX 2

Formulation of apple muffins

CONTROL

Ingredients:

- 1 apple
- 1 egg
- 58 g brown sugar
- 80 g mascarpone cheese
- 2 g cinnamon in power
- 5 mL lemon
- 80g flour
- 2 g of vanilla

WITH 25% OF TUNA MASCARPONE CHEESE

Ingredients:

- 1 apple
- 1 egg
- 58 g brown sugar
- 80 g mascarpone cheese with 25% of tuna gelatine
- 2 g cinnamon in power
- 5 mL lemon
- 80 g flour
- 2 g of vanilla

WITH 15% OF TUNA MASCARPONE CHEESE

Ingredients:

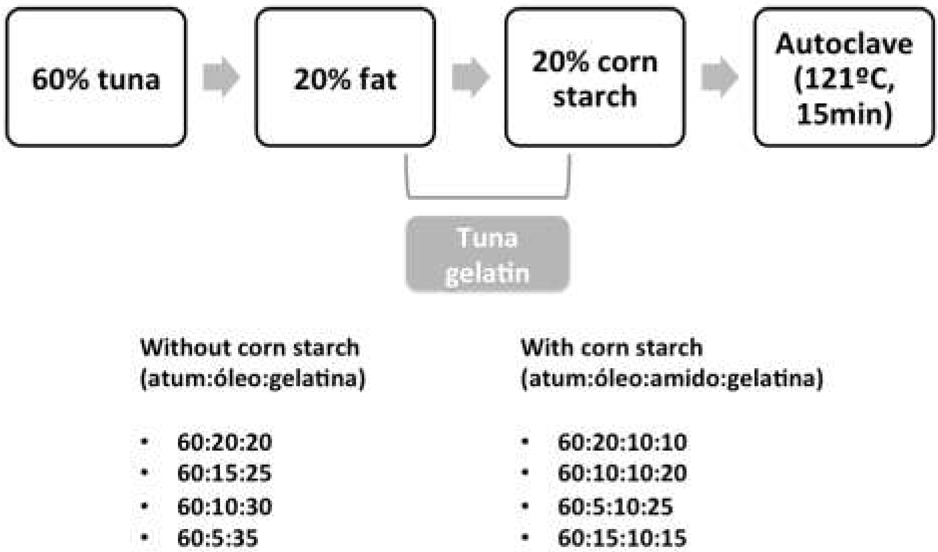
- 1 apple
- 1 egg
- 58 g brown sugar
- 80 g mascarpone cheese with 15% of tuna gelatine
- 2 g cinnamon in power
- 5 mL lemon
- 80 g flour
- 2 g of vanilla.

PROCEDURE:

1. Peel the apples and cut the apples into small cubes. Next, add lemon juice and cinnamon.
2. Pre-heat the oven at 180ºC.
3. Beat eggs with sugar.
4. Add mascarpone cheese and vanilla. Beat to 2 minutes.
5. Drain the lemon juice of apples and add to the prepared, mixing well.
6. Add flour and mix.
7. Join the diced apples and involve.
8. Put the prepared into the ant-adherent muffins tins.
9. Bake to 40 minutes.
10. Take it from the cake pan.
11. Allow it to cold.
12. Refrigerate each sample at 4 ºC for 8 days.

APPENDIX 3

Process of tuna pâtés



APPENDIX 4

<u>Survey of sensory analysis</u>

Novomar Project – Tuna Pâté

Thank you for collaborating in this study carried out under the NOVOMAR research project. The products have been prepared in compliance with all applicable hygiene and safety rules. If you have food allergies, we appreciate your availability, but please DO NOT participate in this sensory test. The individual information will be treated confidentially and the results of this study will be reported without your personal data being disclosed. Your participation is voluntary and we are at your disposal to provide any additional information that you may consider important. You can leave the study if you want at any time.

❍ I have read and understood the information above and I want to participate in the study.
❍ I do not want to participate in the study.

Your participation will only take a few minutes.

Read the instructions carefully when they appear on your computer screen. Please do not exchange impressions with your colleagues. There are no right or wrong answers, the important is your opinion.

After each question is answered, the software will not allow you to return to the previous screen. Do not hesitate to ask for help if you have any question or need some assistance.

Thank you.

Gender:

❍ Female

❍ Male

Birth year:________

Nationality:

❍ Portuguese

❍ Other: ______________________

Country of residence

❍ Portugal

❍ Other: ______________________

Level of education: ______

Do you usually eat tuna pâtés?

- ❍ Yes, one or more times a week
- ❍ Yes, one or more times a month
- ❍ Yes, several times a year
- ❍ Rarely
- ❍ Never

Please, taste product 243.

Globally, how much do you like 243?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

How much do you like of 243 texture?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

How much do you like of 243 taste?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

Would you buy the product if it was available in the market at an appropriate price?

- I would buy
- I would probably buy
- I probably would not buy
- I would not buy

Comments:_______

Please, taste the product 408.

Globally, how much do you like 408?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike much
- Dislike extremely

How much do you like of 408 texture?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

How much do you like of 408 taste?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

Would you buy the product if it were available in the market at an appropriate price?

- I would buy
- I would probably buy
- I probably would not buy
- I would not buy

Comments:_______

Please, taste the product 807.

Globally, how much do you like 807?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike much
- Dislike extremely

How much do you like of 807 texture?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

How much do you like of 807 taste?

- ❍ Like extremely
- ❍ Like very much
- ❍ Like moderately
- ❍ Like slightly
- ❍ Neither like nor dislike
- ❍ Dislike slightly
- ❍ Dislike moderately
- ❍ Dislike much
- ❍ Dislike extremely

Would you buy the product if it were available in the market at an appropriate price?

- ❍ I would buy
- ❍ I would probably buy
- ❍ I probably would not buy
- ❍ I would not buy

Comments:_______

Please, organize the samples according to your preference, in which "1" corresponds to your favourite sample, "2" to the second most liked sample, and "3" to the least liked one (insert the number in the box on the left).

______ sample 243

______ sample 408

______ sample 807

Thank you very much for taking part in this study.

SUPPLEMENT 1

Conferences and Congresses

3rd International Conference
viana do castelo
2015
14>16 september

WASTES
solutions
treatments
opportunities

We hereby confirm that **Maria Teresa Dias Carvalho** attended the 3rd *International Conference - WASTES: solutions, treatments and opportunities* in Viana do Castelo, Portugal on September 14th to 16th, 2015, with the oral presentation «PHYSICAL AND CHEMICAL CHARACTERIZATION OF MASCARPONE CHEESE WITH FISH GELATIN AS A FAT SUBSTITUTE».

Cândida Vilarinho
Chair of 3rd International Conference
Wastes: solutions, treatments and opportunities

DIPLOMA

XVI
Congresso de
Nutrição e Alimentação

sustentabilidade alimentar
desafios

Certifica-se que no XVI Congresso de Nutrição e Alimentação, realizado nos dias 4 e 5 de maio de 2017, no Centro de Congressos de Lisboa, foi apresentada e discutida a seguinte comunicação oral:

Development and characterization of tuna pâté using tuna fish gelatine

Teresa Carvalho[1]; Sérgio C. Sousa[1]; Ricardo I. Pérez-Martín[2], Xosé A. Vásquez[2], Ana P. Carvalho[4] and Ana M. Gomes[1]
[1] CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia da Universidade Católica Portuguesa
[2] IIM-CSIC -Grupo de Bioquímica de Alimentos, Instituto de Investigacións Mariñas
[3] REVAL - IIM-CSIC – Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), Instituto de Investigacións Mariñas
[4] REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto

Lisboa, 5 de maio de 2017

Célia Craveiro (Presidente)
Comissão Organizadora

DIPLOMA

Certifica-se que no XVI Congresso de Nutrição e Alimentação, realizado nos dias 4 e 5 de maio de 2017, no Centro de Congressos de Lisboa, foi apresentada e discutida a seguinte comunicação oral:

Marine gelatine as a substitute for fat and cornstarch in tuna pâté

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Lisboa, 5 de maio de 2017

Célia Craveiro (Presidente)
Comissão Organizadora

SUPPLEMENT 2

Awards Participation

- Nutraingredients Awards, 2016
- Ecotrophelia Award, 2017

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