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Original Research Article

Amino acid profile and protein quality related to canning and storage of swordfish packed in different filling media

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ABSTRACT

This study was conducted to determine the effect of canning, different filling media (olive oil, corn oil, sunflower oil and high oleic sunflower oil) and storage on the amino acid profile and protein quality in swordfish. Glutamic acid, aspartic acid and leucine are the predominant amino acids in raw swordfish, representing 35.62 % of the total amino acids. Thawing-salting caused significant losses of methionine, glycine and tyrosine. Frying caused moisture loss, resulting in higher protein and amino acid contents in fried swordfish than in raw or salted fish. The changes observed during the sterilization process depended on the amino acid and filling medium. The concentrations of some amino acids, such as methionine and glycine, decreased during heat treatment; however, the proline content increased. Slight changes in some amino acids were detected after storage of the canned swordfish for 12 months. The changes during storage were also influenced by the filling medium and varied depending on the amino acid considered. The highest scores in raw and canned swordfish were observed for histidine. The quality indices used showed that canned swordfish packed in olive oil was the highest quality product of those considered.

1. Introduction

The demand for high quality fish and fishery products is increasing greatly, mainly because of the abundant beneficial components that these products contain (FAO, 2020). Consumption of fish provides an excellent source of some nutrients, including polyunsaturated omega-3 fatty acids, vitamins, minerals, protein and essential amino acids (Hu and Chan, 2021). The chemical composition of fish can vary significantly between species, and it can even vary between different individuals of the same species, depending on age, sex, feeding, environment, seasonal change, sexual maturity and migration (Alaş et al., 2014; Heinsbroek et al., 2007; Tao et al., 2012). Fish quality is a complex concept relating a large number of factors, such as nutritional quality, sensory attributes, safety, availability and convenience.

The swordfish (*Xiphias gladius*) is a large pelagic, predatory and migratory fish. It is found in all tropical, subtropical and temperate oceans, including the Mediterranean Sea. There are a large number of fisheries of this species all over the world. Swordfish is an economically important species, of high commercial and social value, and demand for the species is increasing (Gobert et al., 2017). Specimens can reach up to 540 kg in weight (FAO, 2021). Swordfish is mainly marketed as a fresh or frozen product. After capture, swordfish are eviscerated, and they are usually frozen and stored at -18 °C aboard fishing vessels. This is an effective way of delaying fish spoilage and enables preservation of the freshness until the fish are sold in local markets. Due to the large size of swordfish, it is important to take advantage of damaged specimens that are not suitable for fresh sale, thus preventing large amounts of waste.

The short shelf life of fresh fish and consumer interest in healthy, easy-to-prepare foods have made canned marine products a valuable resource in the human diet. In addition, canned products can be stored for long periods at room temperature, without important loss of quality. However, canning and subsequent storage can have some negative effects on the quality parameters of fish (e.g. loss of essential nutrients, formation of undesirable compounds and browning) (Aubourg, 2001; Lucci et al., 2016). The effects on fish quality of canning, storage and the use of different filling media have been documented in some studies. Thus, for example García-Arias et al. (2004) reported that the sterilization process and storage time increased the lipid content and decreased the protein and water fractions in canned tuna, relative to raw fish. Naseri et al. (2011) pointed out that the filling medium may dilute and extract some components, thereby altering the concentrations in the final product. Mohan et al. (2015) observed changes in yellowfin tuna

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during canning which depended on the filling medium and cooking time. Gómez-Limia et al. (2020) concluded that the fatty acid profile of European eels (*Anguilla anguilla*) varied during the canning process. These researchers reported that the fatty acid components tended to be similar to those in the oil using as filling medium. They also reported that the changes in amino acids content in canned eels depended on the filling medium and the storage time. The most common oils used in canned fish are olive oil and sunflower oil, although other oils have also been used as filling media (high oleic sunflower oil, corn oil, …). These last oils have been very little studied in canned fish.

Fish is a good source of protein, which represents 65–70 % of the total dry weight of muscle. Fish protein contains large amounts of some essential amino acids. Several authors have highlighted fresh fish as an important source of essential amino acids (Usydus et al., 2008). However, little information is available on the amino acid composition of canned fish.

Swordfish is important commercial species which is appreciated for excellent quality and taste. The effect of different canning methods on amino acids composition have been studied in other species; but there is no literature available on the effect induced by canning on amino acids of swordfish. Hence, in this paper, we aimed to determine the amino acid profile and protein quality of raw swordfish, and to evaluate the changes that occur in the product during canning and storage. The effects of different filling media, i.e. olive oil, corn oil, sunflower oil and high oleic sunflower oil, were also evaluated.

2. Materials and methods

2.1. Raw materials, processing and sampling

Swordfish (*Xiphias gladius*), weighing between 50 and 70 kg, were caught in the Pacific Ocean (FAO 71) by ORPAGU (Organización de Palangreros Guardeses, Northwest of Spain). Frozen loins (total 40 kg) were transported to the laboratory in insulated boxes and stored at -20 °C until analysis. The average weight of each loin was between 13.3 and 16.6 kg.

The canning process (Fig. 1) was similar to that described by Cobas et al. (2021).

Some randomly selected samples of the frozen swordfish, hereafter referred to as "raw swordfish", were thawed in a refrigerator (4 °C) for 12 h and processed as control samples. The other samples were then cut into slices of approximately 6 cm thickness. The slices were thawed in 8 % brine at 4 °C for 12 h. They were then cleaned, to remove skin, spines and dark muscle. Prior to canning, the swordfish was cut into pieces (6 cm in length, and 2 cm in thickness) and pan-fried in olive oil (refined olive oil + virgin olive oil), at a temperature of 120 °C for 6 min. After cooling, the fried fish was placed in glass jars (130 g, diameter 8.2 cm and height 7.1 cm), and four commercially available oils were used as filling media: olive oil (the same type as used for frying), refined corn oil, refined sunflower oil and refined high oleic sunflower oil. The ratio of fish to the oil was 2:1 (p/p). A headspace of about 2.5 mm between the level of fish and the inside surface of the lid was used. Removal of air before sealing increases the stability of canning fish, due to minimizing oxidation of lipids and vitamins. The jars were vacuum-sealed with a packaging machine (VP-430 Ramón Vac Line, Barcelona, Spain) and thermally treated in an autoclave (Raypa AES-75, Barcelona, Spain) (115 °C, F_0 = 6 min) before being stored at room temperature in darkness.

The jars of swordfish (160 jars) were produced with a nominal weight of 190 g.

Three batches of canned swordfish were prepared. Each batch consisted of 16 glass jars for each type of filling oil. Each batch was sampled at different points: raw swordfish (control), and after salting, frying and sterilization, and after one year of storage in darkness at room temperature (20 ± 5 °C).

2.2. Moisture and total protein content

Moisture content was determined after drying the swordfish to constant weight in an oven at 105 ± 1 °C (AOAC, 2006). Total protein content of swordfish samples was determined according to the Kjeldalh method (AOAC, 2006).

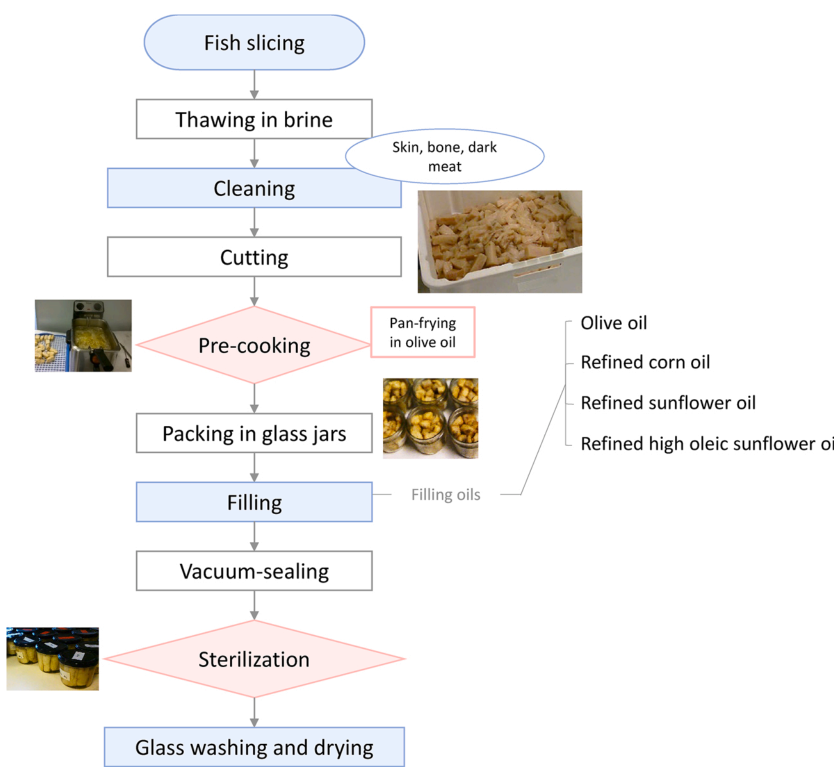

Fig. 1. Processing flowchart of canned swordfish.

2.3. Amino acid analysis

Protein hydrolysis and total amino acid extraction were performed following the procedure described by Franco et al. (2010), with some modifications.

For amino acid extraction, 100 mg of sample was transferred to a glass ampoule (Sigma-Aldrich, Sigma Chemical Co., St. Louis, USA), and 5 mL of 6 N HCl was added. The ampoules were flame sealed and held in an oven for 24 h at 110 °C to hydrolyse the samples. Aliquots of 0.625 mL of each hydrolysed sample were diluted to a final volume of 25 mL with distilled water. The pH was adjusted to a value of 7.1 ± 0.2 with 30 % potassium hydroxide (KOH) solution (w/v). The samples were then refrigerated at 2 ± 1 °C for at least 10 min before being derivatized.

For the derivatization, aliquots (200 μL) of each sample were dried in a Genevac centrifugal concentrator mod. MiVac DNA 23,050-F00 (Ipswich, UK). The derivatizing solution (mixture of ethanol -HPLC grade-, Milli-Q water, trimethylamine and phenylsothiocyanate) was then added (20 μL), and the mixture was stirred vigorously and held for 20 min at room temperature. The solution was then desiccated, and the dry residue was resuspended in 500 μL of diluent solution (disodium acid phosphate and Milli-Q water adjusted to pH 7.4 with 10 % phosphoric acid, w/v) and filtered, with 0.45 μm syringe filters, directly into glass vials.

As the hydrolysis conditions strongly affect the tryptophan content, this amino acid was not determined by this method.

2.3.1. Separation, identification and quantification of amino acids

The amino acids were separated, identified and quantified by high performance liquid chromatography (HPLC), following the procedure described by Domínguez et al. (2015) with some modifications.

The analysis was conducted using a Spectra System chromatograph system (Thermo Finnigan, San José, CA, USA) equipped with a SCM1000 degasser, a P4000 pump, an AS3000 automatic injector and a Photodiode Array UV6000LP detector. The wavelength of the UV–vis diode array detector was 254 nm. Separation was carried out on a reversed phase C18 column (250 × 4.6 mm, 5 μm particle size) from Hichrom (Reading RG7 4PE, UK). The column was held at 50 ± 1 °C throughout the determination with a thermostatically controlled column heater (Spectrasystem 3000). The detector wavelength was 254 nm. A solution of anhydrous sodium acetate in Milli-Q water, adjusted to pH 6.6 with glacial acetic acid, was mixed with acetonitrile (40:60) to produce eluent A. A solution of Milli-Q water and acetonitrile (40:60) was used as eluent B. All samples and standards were injected in the column at least in duplicate.

A commercial amino acid standard AAS18 mix from Supelco (Sigma-Aldrich Co., Missouri, USA) and containing the following compounds was used: 1.25 μmol/mL cysteine (Cys) and 2.5 μmol/mL each of aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), threonine (Thr), arginine (Arg), alanine (Ala), proline (Pro), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tyrosine (Tyr) and lysine (Lys).

Amino acids were identified by retention times and quantified by external standard technique using the amino acid standard. Data regarding amino acid composition were expressed in g/100 g of sample.

2.3.2. Calculation of protein quality indices

In order to determine the nutritional value and the importance of swordfish protein for the human diet, the scores determined for essential amino acids (EAAs) were calculated relative to the FAO/WHO/UNU reference protein (egg protein) (FAO/WHO/UNU, 2017) as follows:

$$\text{Essential amino acid (EAA) score} = \frac{\text{EAA in food protein (g/100 g)}}{\text{EAA in reference protein (g/100 g)}}$$

Oser (1959) defined the essential amino acid index (EAAI) “as the geometric mean of the "egg ratios," i.e. the ratios of the essential amino acids in a protein relative to their respective amounts in whole egg protein”. This index was also calculated in accordance to Wu et al. (2011) as follows:

$$EAAI = \sqrt[n]{\frac{100a}{ae} \times \frac{100b}{be} \times \frac{100c}{ce} \times \ldots\ldots \times \frac{100j}{je}}$$

where n = number of amino acids analysed; a-j = His, Thr, Val, Met, Ile, Leu, Phe + Tyr and Lys content of each sample (g/100 g protein); ae-je = His, Thr, Val, Met, Ile, Leu, Phe + Tyr and Lys content of the protein standard (g/100 g protein) (FAO/WHO/UNU, 2017).

Finally, the predicted protein efficiency ratio (P-PER) was estimated using three equations proposed by Alsmeyer et al. (1974):

$$\text{P-PER1} = -0.684 + 0.456 \times \text{Leu} - 0.047 \times \text{Pro}$$

$$\text{P-PER2} = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr}$$

$$\text{P-PER3} = -1.816 + 0.435 \times \text{Met} + 0.78 \times \text{Leu} + 0.211 \times \text{His} - 0.944 \times \text{Tyr}$$

2.4. Statistical analysis

Data were analysed using the computer software STATISTICA version 8.0 (StatSoft, Inc.). One-way analysis of variance (ANOVA) was applied, where the treatments were considered as fixed effects (raw, salted, fried, sterilized and stored). The results of samples in different steps of the canning process and packed with different filling media were compared using Duncan’s test, considering a significance level of 0.05.

3. Results and discussion

3.1. Moisture and total protein contents

The moisture and total protein contents of raw and salted swordfish and of canned swordfish packed in different filling media throughout the different steps of the canning process and after 12 months of storage are shown in Table 1. The moisture content determines the nutritional composition of the fish but also the microbiological and chemical stability, physical properties and technological processes. The moisture content of fish varies greatly depending on species, size, age, sex, seasonal changes and area of capture (Doğan and Ertan, 2017). The moisture content of the raw swordfish (73.9 %) remained stable after thawing-salting (74.1 %) and exhibited no significant differences ($p > 0.05$) relative to the raw fish, possibly due to the short salting periods and to defrost under an aqueous medium. However, frying caused a reduction in moisture content (64.4 %). According to García-Arias et al. (2004), oil penetrates food during frying, after water is partially lost by evaporation. Frying also induced protein denaturation, causing

Table 1
Moisture and total protein contents (%) of raw and canned swordfish packed in different filling media throughout the different steps of the canning process and after storage for 12 months.

s	Filling media	Moisture	Total protein
Raw		73.9 ± 1.46^a	20.1 ± 0.64^a
Salting		74.1 ± 0.69^a	19.7 ± 0.70^a
Frying		64.4 ± 1.82^b	27.4 ± 0.67^b
Sterilization	*Olive oil*	60.1 ± 1.87^c	28.1 ± 0.85^b
	Corn oil	59.1 ± 0.50^c	27.1 ± 0.84^b
	Sunflower oil	59.1 ± 2.90^c	28.7 ± 2.15^b
	High oleic sunflower oil	59.7 ± 1.66^c	27.6 ± 2.23^b
Storage 12 months	*Olive oil*	60.2 ± 0.43^c	27.4 ± 0.62^b
	Corn oil	58.3 ± 0.96^c	26.7 ± 0.69^b
	Sunflower oil	60.4 ± 2.57^c	29.1 ± 1.57^b
	High oleic sunflower oil	58.9 ± 0.31^c	28.6 ± 2.29^b

${}^{a-c}$Means with different letters within the same column were significantly different ($p < 0.05$).

evaporation and loss of water. Sterilization also caused moisture loss in fish muscle (59.1–60.1 %). Water loss can be explained by exchange between water in the fish and the filling medium. There was no significant difference ($p > 0.05$) between canned swordfish in different filling media. The moisture loss is dependent on the type of food, the temperature of heating and the length of the heat treatment (Sundari et al., 2015). Paredes and Baker (1988) reported that frying and canning can cause loss of moisture in fish flesh and a corresponding increase in the quantities of other components such as proteins. There were no changes in the moisture content of canned swordfish after 12 months of room storage (58.3–60.4 %). No differences were observed when using different filling oils.

Fish is an excellent sources of high-quality protein. Fish proteins are having high biological value as they contain all the essential amino acids and have a particularly high content of the essential amino acids lysine and leucine (Dale et al., 2019). The protein contents of raw swordfish ranged from 19.2–22.1 g/100 g. Protein content can vary with age, gender, feeding environment, spawning season and nutritional density and migration season (Doğan and Ertan, 2017; Ozogul et al., 2011; Polat et al., 2009). The protein content remained stable during the thawing-salting, but increased after frying. Decreased moisture content has been described as the main change producing a significant increase in protein in processed fish (Ersoy and Özeren, 2009).

The results seem to indicate that, in our case, the time and temperature conditions used in processing were not drastic enough to produce significant variation in protein content. Mohan et al. (2015) and Gómez-Limia et al. (2021a) also reported that thermal processing had no effect on the protein content of respectively tuna and eel. The summarised total protein contents in canned swordfish did not reveal any differences in the content of this component in the different filling media, and all canned swordfish products contained substantial amounts of protein.

3.2. Total amino acid

Amino acid contents (g/100 g sample) of raw swordfish and canned swordfish packed in different filling oils sampled after thawing-salting, frying, sterilization and after room storage for 12 months are shown in Table 2. The results showed that glutamic acid (2.86 g/100 g) is the most abundant amino acid, followed by aspartic acid (1.85 g/100 g) and

Table 2
Total amino acid profiles (g/100 g) of raw and canned swordfish packed in different filling media throughout the different steps of the canning process and after storage for 12 months.

	Raw (control)	Salting	Frying	OO_d0	OO_d12	CO_d0	CO_d12	SO_d0	SO_d12	HSO_d0	HSO_d12
EAA											
Histidine	0.61 ± 0.08^{a}	0.55 ± 0.08^{ab}	0.51 ± 0.03^{b}	0.56 ± 0.07^{ab}	0.58 ± 0.14^{ab}	0.52 ± 0.07^{ab}	0.55 ± 0.05^{ab}	0.52 ± 0.06^{b}	0.53 ± 0.05^{ab}	0.53 ± 0.05^{ab}	0.49 ± 0.08^{b}
Threonine	0.85 ± 0.08^{a}	0.80 ± 0.08^{a}	1.05 ± 0.11^{bd}	1.06 ± 0.04^{bd}	1.00 ± 0.03^{b}	0.98 ± 0.01^{bc}	1.04 ± 0.03^{bd}	0.82 ± 0.05^{a}	0.88 ± 0.09^{ac}	1.03 ± 0.15^{bd}	1.15 ± 0.14^{d}
Valine	0.72 ± 0.07^{ab}	0.63 ± 0.09^{b}	1.00 ± 0.05^{c}	1.02 ± 0.03^{c}	0.84 ± 0.07^{a}	0.82 ± 0.11^{a}	0.77 ± 0.11^{ab}	0.80 ± 0.05^{a}	0.82 ± 0.21^{a}	0.80 ± 0.20^{a}	0.75 ± 0.18^{ab}
Methionine	0.51 ± 0.05^{a}	0.38 ± 0.05^{b}	0.60 ± 0.07^{c}	0.51 ± 0.06^{ad}	0.43 ± 0.02^{bd}	0.49 ± 0.07^{ade}	0.41 ± 0.02^{bd}	0.44 ± 0.02^{abd}	0.39 ± 0.07^{b}	0.44 ± 0.11^{abd}	0.40 ± 0.11^{be}
Isoleucine	0.75 ± 0.07^{ab}	0.65 ± 0.06^{b}	0.91 ± 0.13^{cd}	0.94 ± 0.13^{c}	0.90 ± 0.15^{cd}	0.94 ± 0.17^{c}	0.93 ± 0.06^{cd}	0.84 ± 0.08^{ac}	0.83 ± 0.14^{ac}	0.78 ± 0.16^{abd}	0.82 ± 0.23^{abc}
Leucine	1.52 ± 0.26^{ab}	1.41 ± 0.05^{b}	1.96 ± 0.17^{ce}	2.00 ± 0.07^{ce}	2.01 ± 0.09^{ce}	1.89 ± 0.13^{cde}	1.84 ± 0.17^{cd}	1.71 ± 0.13^{ad}	1.71 ± 0.28^{ad}	2.10 ± 0.28^{ef}	2.39 ± 0.39^{f}
Phenylalanine	0.67 ± 0.04^{ab}	0.57 ± 0.04^{b}	0.79 ± 0.09^{c}	0.77 ± 0.08^{cd}	0.74 ± 0.11^{cd}	0.73 ± 0.09^{ac}	0.72 ± 0.04^{ac}	0.69 ± 0.04^{ad}	0.76 ± 0.08^{cd}	0.68 ± 0.12^{ad}	0.62 ± 0.12^{ab}
Lysine	1.05 ± 0.10^{ab}	0.94 ± 0.07^{b}	1.31 ± 0.10^{c}	1.39 ± 0.02^{c}	1.29 ± 0.24^{c}	1.11 ± 0.05^{a}	0.75 ± 0.03^{d}	1.10 ± 0.05^{ab}	1.03 ± 0.11^{ab}	0.98 ± 0.26^{ab}	0.92 ± 0.18^{bd}
NEAA											
Aspartic acid	1.85 ± 0.26^{ad}	1.70 ± 0.09^{ac}	2.46 ± 0.28^{b}	2.41 ± 0.17^{be}	1.35 ± 0.24^{c}	2.38 ± 0.45^{bef}	1.60 ± 0.31^{ac}	2.08 ± 0.11^{df}	1.72 ± 0.11^{ac}	2.14 ± 0.13^{de}	1.47 ± 0.06^{c}
Glutamic acid	2.86 ± 0.42^{a}	2.67 ± 0.24^{a}	2.91 ± 0.24^{a}	2.77 ± 0.79^{a}	2.84 ± 0.53^{a}	3.08 ± 0.18^{a}	3.10 ± 0.17^{a}	3.10 ± 0.23^{a}	1.71 ± 0.17^{b}	2.78 ± 0.66^{a}	1.94 ± 0.14^{b}
Serine	0.82 ± 0.06^{ab}	0.78 ± 0.05^{be}	1.04 ± 0.06^{c}	1.01 ± 0.06^{cd}	0.90 ± 0.14^{ade}	0.94 ± 0.11^{ac}	0.73 ± 0.10^{b}	0.92 ± 0.09^{ac}	0.71 ± 0.17^{b}	1.00 ± 0.13^{cd}	0.95 ± 0.14^{acd}
Glycine	1.06 ± 0.06^{a}	0.85 ± 0.07^{b}	1.32 ± 0.08^{c}	1.10 ± 0.12^{ad}	1.22 ± 0.09^{cd}	1.11 ± 0.12^{ad}	1.03 ± 0.17^{ae}	1.07 ± 0.06^{a}	0.91 ± 0.15^{be}	1.00 ± 0.19^{ae}	1.06 ± 0.19^{ae}
Arginine	1.47 ± 0.22^{abd}	1.29 ± 0.12^{b}	1.78 ± 0.23^{c}	1.59 ± 0.34^{ac}	1.56 ± 0.25^{ac}	1.65 ± 0.24^{cd}	1.50 ± 0.26^{abc}	1.47 ± 0.14^{abd}	1.25 ± 0.17^{b}	1.42 ± 0.27^{abd}	1.34 ± 0.19^{ab}
Alanine	1.10 ± 0.09^{ac}	0.95 ± 0.09^{a}	1.46 ± 0.12^{b}	1.30 ± 0.18^{bd}	1.25 ± 0.05^{cd}	1.33 ± 0.15^{bd}	0.94 ± 0.13^{a}	1.23 ± 0.13^{cd}	1.11 ± 0.13^{ac}	1.09 ± 0.19^{ac}	0.95 ± 0.24^{a}
Proline	0.48 ± 0.02^{ab}	0.42 ± 0.11^{a}	0.48 ± 0.03^{ab}	0.56 ± 0.04^{b}	1.15 ± 0.07^{c}	0.66 ± 0.08^{df}	0.75 ± 0.04^{e}	0.71 ± 0.12^{de}	0.59 ± 0.15^{bf}	0.70 ± 0.08^{de}	0.53 ± 0.09^{b}
Tyrosine	0.69 ± 0.03^{ad}	0.54 ± 0.05^{b}	0.87 ± 0.13^{c}	0.83 ± 0.14^{ce}	0.78 ± 0.02^{cd}	0.79 ± 0.14^{cd}	0.66 ± 0.09^{abd}	0.70 ± 0.07^{ade}	0.71 ± 0.12^{ade}	0.83 ± 0.13^{ce}	0.89 ± 0.16^{c}
Total EAA	7.21 ± 0.40^{af}	6.02 ± 0.24^{b}	8.31 ± 0.52^{ce}	8.46 ± 0.09^{c}	8.15 ± 0.19^{cde}	7.76 ± 0.21^{ae}	6.78 ± 0.32^{f}	7.02 ± 0.46^{fg}	7.09 ± 0.66^{fg}	7.34 ± 0.63^{af}	7.56 ± 0.61^{adg}
Total NEAA	9.84 ± 0.94^{ad}	8.04 ± 0.67^{b}	11.16 ± 0.30^{c}	11.07 ± 0.93^{c}	10.42 ± 0.42^{ac}	11.31 ± 0.68^{c}	9.81 ± 0.83^{ad}	10.23 ± 0.77^{ac}	8.32 ± 0.74^{b}	10.52 ± 1.07^{ac}	8.77 ± 0.18^{bd}
EAA/NEAA	0.74 ± 0.07^{ab}	0.75 ± 0.06^{ab}	0.75 ± 0.04^{ab}	0.77 ± 0.06^{ac}	0.79 ± 0.03^{ac}	0.68 ± 0.02^{b}	0.68 ± 0.04^{b}	0.69 ± 0.02^{b}	0.88 ± 0.05^{d}	0.73 ± 0.04^{ab}	0.92 ± 0.03^{d}
Total Aa	17.49 ± 1.11^{ad}	14.42 ± 1.24^{b}	19.55 ± 0.72^{c}	19.53 ± 1.06^{c}	18.65 ± 0.56^{ac}	19.04 ± 0.90^{ac}	16.60 ± 1.10^{d}	17.32 ± 1.27^{ad}	16.16 ± 0.51^{d}	18.10 ± 1.58^{ac}	16.81 ± 0.59^{ad}

$^{a-g}$ Means with different letters within the same row were significantly different ($p < 0.05$).
OO_d0: Sterilized swordfish in olive oil with 0 days of storage; CO_d0: Sterilized swordfish in corn oil with 0 days of storage; SO_d0: Sterilized swordfish in sunflower oil with 0 days of storage; HSO_d0: Sterilized swordfish in high oleic sunflower oil with 0 days of storage; OO_d12: Sterilized swordfish in olive oil with 12 months of storage; CO_d12: Sterilized swordfish in corn oil with 12 months of storage; SO_d12: Sterilized swordfish in sunflower oil with 12 months of storage; HSO_d12: Sterilized swordfish in high oleic sunflower oil with 12 months of storage. EAA: Essential amino acids; NEAA: Non-essential amino acids.

leucine (1.52 g/100 g), which together represent 35.62 % of the total amino acids. Glutamic acid and aspartic acid also are quantitatively the main amino acids in other species of raw fish, such as herring (Oluwaniyi et al., 2010), Atlantic bonito (Ormanci and Colakoglu, 2015) and cod (Teixeira and Mendes, 2020). Glutamic acid is an important source of nitrogen and plays an important role in amino acid metabolism. Aspartic acid and glutamic acid are also essential for enzyme solubility and protecting ionic character in enzyme activity (Özden and Erkan, 2008). Glutamic and aspartic acids have a sour taste, but produce an umami taste in the presence of sodium salts (Sarower et al., 2012).

Leucine is the major essential amino acid in swordfish muscle. This amino acid constituted approximately 8.7 % of total amino acids. This compound can stimulate muscle protein synthesis and has a therapeutic role in stress conditions (De Bandt and Cynober, 2006; Ospina-Rojas et al., 2020).

Other important amino acids in the raw swordfish were arginine (1.47 g/100 g), glycine (1.06 g/100 g), lysine (1.05 g/100 g) and alanine (1.10 g/100 g). Arginine is frequently considered a conditionally essential amino acid, as it plays an important role in hormone release, neurotransmission and maintenance of blood pressure, cell division, wound healing, etc. (Mohanty et al., 2014). Glycine plays a key role in the process of wound healing. Lysine is necessary for optimal growth and deficiency leads to immunodeficiency (Chen et al., 2003); alanine is very beneficial for supporting gluconeogenesis and leucocyte metabolism (Kudsk, 2006). On the other hand, other essential amino acids such as histidine, threonine, valine, methionine, isoleucine and phenylalanine were found in a lower range. Proline was the minor amino acid in raw samples (0.48 g/100 g).

The raw swordfish muscle contained between 38.09 and 44.00 % of total essential amino acids. The ratio between essential and non-essential amino acids (E/NE) is used as an indicator of protein quality, with higher values representing higher protein quality. The E/NE ratio was 0.74 in raw fish.

In general, the amino acids contents decreased after thawing-salting, although the changes were only significant for methionine, glycine and tyrosine. These amino acids could have transferred from the tissue to the salt solution. One of the effects due to this process is the solubilization of amino acids in brine and penetration of salt in the muscle (Teixeira and Mendes, 2020). On other hand, salting can lead to the formation of different inter and intra-molecular bonds. Consequently, protein chains unfold and free carboxylic and amino groups are exposed (Boziaris, 2013). The amounts of total acid amino acids (14.42 g/100 g), total essential amino acids (6.02 g/100 g) and total non-essential amino acids (8.04 g/100 g) decreased during thawing-salting relative to those in the raw samples. García-Arias et al. (2003) reported that the concentrations of several amino acids decreased significantly in sardine fish fillets that had been frozen and thawed.

Heat treatments such as frying tended to have a greater effect on the amino acid content and profile. In general, frying significantly increased the content of amino acids in swordfish muscle. The amino acid content is increased by the frying process due the effect of concentration. However, in this study, histidine decreased during frying as compared with raw fish, suggesting that moisture was not the only factor affecting the content of amino acids. The oxidation in some sensitive amino acids, such as histidine, can be a reason for their reduction (Jannat-Alipour et al., 2010). The highest increase rate of 27.52 % was observed for valine, followed by aspartic acid and alanine with 25.9 % increase.

The water loss that occurred during frying resulted in the fried swordfish having a higher protein content than in raw or salted fish due to a concentration effect. Longer frying times can cause a reduction in amino acid content; however, in this study, the fish were fried for a short time, and the temperature was not high enough to cause significant loss of amino acids. Previous studies have reported varying effects of frying on the protein and amino acid contents of fish samples. Ismail and Ikram (2004) found that frying did not significantly affect the amino acid content of different fish. Nurjanah et al. (2015) reported that longer frying times caused a greater reduction in amino acid content. Oluwaniyi et al. (2017) reported that frying reduced the amino acid content, with the lowest value observed in samples fried in palm oil. El Lahamy et al. (2018) reported that the concentrations of several amino acids in mullet decreased slightly after frying while others increased slightly. Erkan et al. (2010) observed a significant increase in the contents of essential, semi-essential and other amino acids relative to raw mackerel after frying and grilling. Nongmaithem et al. (2018) also reported that cooking tends to increase the amino acid content of seafood. Frying can cause the Maillard reaction, which leads to an interaction between the carbonyl group of a reducing sugar and the amino group from an amino acid or protein (García-Arias et al., 2003).

The total essential and non-essential amino acids contents of swordfish increased after frying (8.31 and 11.16 g/100 g, respectively). However, there were no significant changes in the essential/non-essential ratio (0.75).

Changes in amino acid content were also observed after sterilization of the product. The changes depended on the amino acid and the type of filling medium. In swordfish packed in olive oil, a significant decrease ($p < 0.05$) in methionine and glycine contents was observed. In the swordfish packed in corn oil, decreases in valine, methionine, lysine, and glycine contents was observed. Threonine, valine, methionine, leucine, phenylalanine, lysine, aspartic acid, glycine, arginine, alanine and tyrosine decreased during the sterilization process in swordfish packed in sunflower oil. In the canned swordfish packed in high oleic sunflower oil, loss of valine, methionine, phenylalanine, lysine, aspartic acid, glycine, arginine and alanine was observed. The losses during sterilization may be due to leaching of some amino acids to the filling medium. However, the proline content increased during sterilization in all canned swordfish, and other processes may therefore also be involved. The increase in proline content could indicate that collagen was progressively degraded. Some of the amino acids may have transitioned from one type of amino acid to another through different reactions, such as oxidation and deamination (Sikorski, 2001). Previous studies have shown varying effects of processing on the amino acid content of fish. Some authors reported no significant effect of processing on protein and amino acid contents (Ismail and Ikram, 2004). However, Akintola (2015) reported an increase in some amino acids, such as tyrosine, leucine and histidine, in smoked pink shrimp. Oluwaniyi et al. (2010) observed that boiling and roasting did not significantly affect the amino acid contents of four types of fish, while frying caused a significant reduction in essential, sulphur-containing and aromatic amino acids. According to these authors, the effect also depended on the processing method and type of oil used. The various findings therefore suggest that processing has different types of effects on different types of fish.

Total amino acids, non-essential amino acids and total essential amino acids did not undergo significant changes ($p > 0.05$) during the sterilization process in canned swordfish packed in olive oil and corn oil. However, in the canned swordfish packed in sunflower oil and high oleic olive oil, the total essential amino acids decreased (7.02 and 7.34 g/100 g, respectively). The value of the essential/non-essential (EAA/NEAA) ratio did not vary significantly during the sterilization process in any of the canned swordfish. This value is an index to define the quality of the protein and mirrors the optimal amino acid profile of protein.

In canned fish, storage for longer or shorter periods is frequently necessary to produce acceptable textural and optimal palatability depending on the type of fish (Aubourg, 2001). During storage, a balance is established between the fish and the filling medium. Slight changes in some amino acids were detected after 12 months of storage. The changes depended on the amino acid and the filling medium. The most stable canned swordfish was that packed in olive oil. In this case, the valine and aspartic acid contents decreased significantly ($p < 0.05$) and the proline content increased significantly ($p < 0.05$). The differences observed between filling media could be due to different interactions between amino acids and filling oil (Aubourg, 2001) and the different content of protective components in each oil (Gómez-Limia et al., 2021b).

Storage caused a significant reduction in lysine, aspartic acid, serine and alanine contents in canned swordfish packed in corn oil. In the canned swordfish packed in sunflower oil and high oleic sunflower oil, decreases in aspartic acid, glutamic acid and proline were observed. Serine and glycine also decreased during storage in canned swordfish packed in sunflower oil. Substantial changes in total essential amino acids and total amino acids content of canned swordfish packed in corn oil occurred during storage. In the canned swordfish packed in sunflower and high oleic sunflower oil, storage caused greater losses of total non-essential amino acids, increasing the EAA/NEAA ratio. The sum of essential amino acids in the canned swordfish packed in olive oil was significantly higher (8.15 g/100 g) than in the canned swordfish packed in corn oil or sunflower oil (6.78–7.09 g/100 g) after 12 months of storage.

Changes can occur during storage due to complex chemical reactions, such as protein-protein interaction, protein-fat interaction and the Maillard reaction, leading to variations in individual amino acid contents. These reactions can result in changes in the amino acid composition. In addition, different components migrate from fish to the filling medium and vice versa, depending on the characteristics of the fish muscle, the filling medium, the type of processing and type of storage. Some amino acid contents can vary due to their degradation to amines, volatile acids and other nitrogenous substances. A transition of one kind of amino acid to another through different reactions such as oxidation, deamination, etc. can also take place (Sikorski, 2001). In our previous work on canned European eels (Gómez-Limia et al., 2021a), we found that canning, filling medium and duration of storage significantly affected the amino acid composition.

International organizations that assess the nutritional needs of the population have established daily intake recommendations for essential amino acids based on gender or age (RDA). According to the WHO/FAO/UNU Commission (2007), for a healthy adult man weighing 70 kg, the daily intake of essential amino acids should be 12.9 g. Fig. 2 shows the RDA for some of the essential amino acids, in addition to the corresponding contributions of 100 g of each of the canned swordfish. Based on these data, canned swordfish can be considered a rich source of essential amino acids which could be contributing to the maintenance of human health. It can be concluded that the consumption of a portion of 150 g of canned swordfish packed in olive oil, corn oil, and high oleic sunflower oil covers more than the daily requirements of histidine, leucine, phenylalanine + tyrosine and threonine and a high percentage of isoleucine and methionine. Canned swordfish packed in corn oil (150 g) also covers more than 100 % of daily requirements of isoleucine.

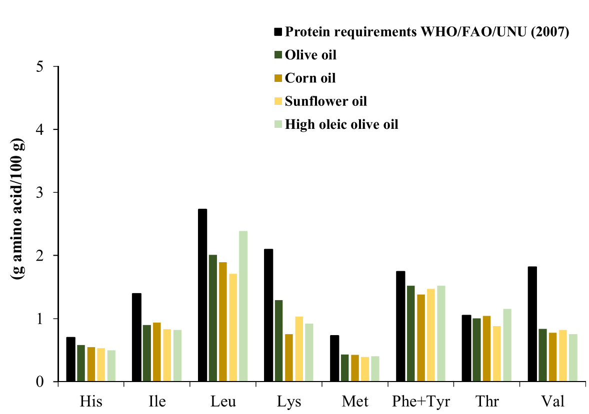

Fig. 2. Graphical representation of the recommended daily amount of some essential amino acids (RDA) for a healthy adult of 70 kg of body weight, according to the FAO/WHO/UNU (2007) recommendations, and the concentration of these amino acids, expressed in g amino acid/100 g of fish, for canned swordfish after storage for 12 months.

However, the lysine and valine contents were rather low in canned swordfish. Thus, the dairy requirements for histidine, phenylalanine + tyrosine and threonine, and a high percentage of leucine, would be met by consumption of 150 g of canned swordfish packed in sunflower oil. Pyz-Łukasik and Paszkiewicz (2018) reported a low content of sulphur amino acids and isoleucine in different fish species (grass carp, bighead carp, Siberian sturgeon, wels catfish) and the low valine content in wels catfish.

3.3. Protein and amino acid quality indices

In view of the fact that amino acid composition provides important measures of the nutritional quality of protein, essential amino acid scores are commonly used for the evaluation of protein quality (Iqbal et al., 2006).

The mean values of the essential amino acid (EAA) score, expressed as g/100 g of protein, as proposed by FAO/WHO/UNU (2017) for humans (adult indispensable amino acid requirements) are shown in Table 3. This index reflects the amino acid content of proteins compared to the ideal protein and provides a means of predicting how efficiently a protein will meet the amino acid requirements of an individual. The present results demonstrate the high nutritive value of swordfish protein, as indicated by the high values of amino acid scores for the different essential amino acids relative to the standard protein. The highest scores in raw swordfish were observed for histidine (2.03) and threonine (1.82); and the lowest scores in raw swordfish were obtained for valine, with a value of 0.92. Adeyeye (2014) reported that valine was also limiting in *Acanthurus monroviae* and *Lutjanus goreensis.*

Furthermore, changes in amino acid composition due to canning process led to changes in the concentration of the different essential amino acids. From the results presented in Table 3, salting clearly caused a decrease in the EEA score for methionine, leucine and phenylalanine + tyrosine. Frying significantly affected ($p < 0.05$) the EAA score for histidine. The sterilization process decreased the EAA score for valine and lysine in canned swordfish packed in corn oil, sunflower oil and high oleic sunflower oil. Sterilization caused a decrease in the EAA score for methionine in all canned swordfish. During this thermal treatment, EAA scores for leucine and phenylalanine + tyrosine also decreased in canned swordfish packed in sunflower oil; and the EAA score for isoleucine decreased in canned swordfish packed in high oleic sunflower oil. Storage significantly affected the EAA score for valine in canned swordfish packed in olive oil and for lysine in canned swordfish packed in corn oil.

The results indicated that limiting amino acids in canned swordfish stored for 12 months at room temperature were valine in canned swordfish in olive oil, and valine, methionine and lysine in canned swordfish packed in corn oil, sunflower oil or high oleic sunflower oil. These were not limited in aromatic amino acids (Phe + Tyr) relative to the reference protein, the percentage of which varied from 1.29 to 1.48 in canned swordfish after storage for 12 months. Nonetheless, the values for most of the amino acids in canned swordfish were greater than 1 or close to 1, implying that this fish has high quality protein. Canned swordfish is rich in histidine. Liao et al. (2015) reported the requirement of histidine in the growth and repair of tissues, maintenance of myelin sheaths and removal of heavy metals from the body.

Table 4 shows the essential amino acid index (EAAI) and predicted protein efficiency ratio (P-PER). The EAAI was 146.11 in raw swordfish, indicating that the sum of essential amino acids in the protein of this raw fish was higher than in the reference standard protein (FAO/WHO/UNU, 2017). The EAAI value did not confirm the lower valine content in the protein of swordfish. However, the EAAI is more closely associated with the biological quality of proteins than the EAA score (Dawczynski et al., 2007). The EAAI value decreased during salting (131.61), but not during frying (126.61). The sterilization process caused losses in canned swordfish packed in corn oil (107.77), sunflower oil (107.39) and high oleic sunflower oil (105.46). Storage did not result in significant changes

Table 3
Essential amino acid (EAA) scores for raw and canned swordfish packed in different filling media throughout the different steps of the canning process, and after storage for 12 months.

	Histidine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phe + Tyr	Lysine
Raw	2.03 ± 0.28[a]	1.82 ± 0.12[a]	0.92 ± 0.09[ac]	1.59 ± 0.15[a]	1.23 ± 0.11[a]	1.38 ± 0.07[a]	1.73 ± 0.05[a]	1.16 ± 0.10[a]
Salting	1.84 ± 0.24[a]	1.75 ± 0.23[ab]	0.87 ± 0.11[abc]	1.30 ± 0.12[b]	1.11 ± 0.14[abd]	1.22 ± 0.05[bd]	1.54 ± 0.12[bd]	1.06 ± 0.09[a]
Frying	1.24 ± 0.09[b]	1.68 ± 0.20[abc]	0.94 ± 0.08[c]	1.43 ± 0.17[ab]	1.16 ± 0.17[ab]	1.21 ± 0.13[bd]	1.55 ± 0.20[ab]	1.07 ± 0.11[a]
OO_d0	1.32 ± 0.24[b]	1.65 ± 0.08[abc]	0.93 ± 0.02[c]	1.09 ± 0.14[c]	1.12 ± 0.16[abd]	1.20 ± 0.07[bd]	1.54 ± 0.21[bd]	1.10 ± 0.04[a]
OO_d12	1.27 ± 0.28[b]	1.59 ± 0.06[bce]	0.79 ± 0.07[abd]	1.00 ± 0.06[cd]	1.11 ± 0.19[abd]	1.26 ± 0.06[ab]	1.48 ± 0.14[bcd]	1.06 ± 0.21[a]
CO_d0	1.23 ± 0.25[b]	1.49 ± 0.18[cd]	0.74 ± 0.16[bd]	1.06 ± 0.12[c]	1.10 ± 0.18[abd]	1.13 ± 0.11[bcd]	1.39 ± 0.19[bcd]	0.87 ± 0.10[bd]
CO_d12	1.29 ± 0.07[b]	1.33 ± 0.07[d]	0.77 ± 0.07[abd]	0.89 ± 0.05[cd]	1.10 ± 0.06[abcd]	1.10 ± 0.07[cd]	1.29 ± 0.16[c]	0.58 ± 0.02[c]
SO_d0	1.24 ± 0.19[b]	1.47 ± 0.18[cd]	0.74 ± 0.15[bd]	1.00 ± 0.06[cd]	1.02 ± 0.12[bcd]	1.06 ± 0.07[c]	1.33 ± 0.11[c]	0.89 ± 0.06[b]
SO_d12	1.31 ± 0.09[b]	1.43 ± 0.14[de]	0.78 ± 0.19[bd]	0.94 ± 0.12[cd]	1.04 ± 0.17[bcd]	1.09 ± 0.10[cd]	1.45 ± 0.16[bcd]	0.84 ± 0.09[bd]
HSO_d0	1.23 ± 0.20[b]	1.55 ± 0.29[bcd]	0.70 ± 0.17[d]	0.93 ± 0.21[cd]	0.90 ± 0.19[c]	1.18 ± 0.18[bcd]	1.37 ± 0.16[cd]	0.74 ± 0.18[d]
HSO_d12	1.11 ± 0.12[b]	1.69 ± 0.17[abc]	0.65 ± 0.11[d]	0.84 ± 0.19[d]	0.92 ± 0.21[cd]	1.27 ± 0.21[ab]	1.34 ± 0.12[c]	0.77 ± 0.06[bd]
Protein standard*	2.4	3.6	4.6	3.1	3.4	7	6.3	6.5

[a–e] Means with different letters within the same row were significantly different ($p < 0.05$).
Phe + Tyr: phenylalanine + tyrosine; pattern proteins are expressed in (g/100 g protein).
EAAS: one essential amino acid content in sample/one essential amino acid content in FAO reference protein (FAO/WHO/UNU, 2017).
OO_d0: Sterilized swordfish in olive oil with 0 days of storage; CO_d0: Sterilized swordfish in corn oil with 0 days of storage; SO_d0: Sterilized swordfish in sunflower oil with 0 days of storage; HSO_d0: Sterilized swordfish in high oleic sunflower oil with 0 days of storage; OO_d12: Sterilized swordfish in olive oil with 12 months of storage; CO_d12: Sterilized swordfish in corn oil with 12 months of storage; SO_d12: Sterilized swordfish in sunflower oil with 12 months of storage; HSO_d12: Sterilized swordfish in high oleic sunflower oil with 12 months of storage.
* FAO/WHO/UNU (2017) (g/100 g protein).

Table 4
Essential amino acid index (EAAI) and predicted protein efficiency ratio (P-PER) for raw and canned swordfish packed in different filling media throughout the different steps of the canning process and after storage for 12 months.

	P-PER 1	P-PER 2	P-PER 3	EAAI
Raw	2.80 ± 0.20[a]	2.86 ± 0.19[a]	3.03 ± 0.34[a]	146.11 ± 4.64[a]
Salting	2.51 ± 0.15[ab]	2.51 ± 0.14[b]	2.75 ± 0.28[ab]	131.61 ± 5.03[b]
Frying	2.49 ± 0.34[ab]	2.45 ± 0.26[b]	2.35 ± 0.29[bc]	126.61 ± 9.39[b]
OO_d0	2.46 ± 0.18[ab]	2.45 ± 0.14[b]	2.26 ± 0.18[c]	123.32 ± 9.71[b]
OO_d12	2.51 ± 0.17[ab]	2.56 ± 0.11[ab]	2.37 ± 0.13[bc]	122.48 ± 0.96[b]
CO_d0	2.15 ± 0.21[b]	2.25 ± 0.30[bc]	2.20 ± 0.38[c]	107.77 ± 6.82[c]
CO_d12	2.15 ± 0.18[b]	2.23 ± 0.16[bc]	2.18 ± 0.35[c]	102.46 ± 4.81[c]
SO_d0	2.12 ± 0.23[b]	2.02 ± 0.17[c]	2.13 ± 0.25[c]	103.78 ± 4.77[c]
SO_d12	2.20 ± 0.36[b]	2.06 ± 0.32[c]	2.16 ± 0.21[c]	103.64 ± 6.15[c]
HSO_d0	2.23 ± 0.38[b]	2.24 ± 0.32[bc]	1.98 ± 0.38[c]	105.46 ± 14.33[c]
HSO_d12	2.28 ± 0.23[b]	2.32 ± 0.20[bc]	1.98 ± 0.02[c]	104.99 ± 9.36[c]

[a–c]Means with different letters within the same row were significantly different ($p < 0.05$).
P-PER: predicted protein efficiency ratio. P-PER1 = - 0.684 + 0.456 × Leu - 0.047 × Pro; P-PER2 = - 0.468 + 0.454 × Leu - 0.105 × Tyr; P-PER3 = - 1.816 + 0.435 × Met + 0.78 × Leu + 0.211 × His − 0.944 × Tyr.
OO_d0: Sterilized swordfish in olive oil with 0 days of storage; CO_d0: Sterilized swordfish in corn oil with 0 days of storage; SO_d0: Sterilized swordfish in sunflower oil with 0 days of storage; HSO_d0: Sterilized swordfish in high oleic sunflower oil with 0 days of storage; OO_d12: Sterilized swordfish in olive oil with 12 months of storage; CO_d12: Sterilized swordfish in corn oil with 12 months of storage; SO_d12: Sterilized swordfish in sunflower oil with 12 months of storage; HSO_d12: Sterilized swordfish in high oleic sunflower oil with 12 months of storage.

in this parameter. The EAAI value was highest in canned swordfish packed in olive oil. In general, proteins with high quality and efficiency were characterized by high EAAI values. EAAI values higher than 100 indicate that the amino acid is not limited and will meet the requirements of children or adults.

The protein efficiency ratio (PER) is one of the most important parameters used to estimate the overall nutritional quality of proteins. The values of this index depend on the concentrations of leucine and proline (P-PER1), leucine and tyrosine (P-PER2) or of methionine, leucine, histidine and tyrosine (P-PER3). Foods with PER values below 1.5 have low protein quality, foods with PER values higher than 2 have high protein quality and with PER values greater than 2.5 (standard value of casein protein) are considered excellent sources of protein (Oluwaniyi et al., 2010; Zengin et al., 2012). The protein in all swordfish samples can be considered of high or excellent quality. The highest PER values corresponded to raw fish. The canned swordfish with the highest PER values were those packed in olive oil. The results indicated that PER was influenced by the type of filling medium used.

The different heat penetration characteristics of the different oils (Mohan et al., 2015) together with their different antioxidant capacity (Medina et al., 1998) could also explain the different results obtained in the oils under study.

4. Conclusion

In conclusion, swordfish are a good source of amino acids and high quality protein, according to the FAO/WHO/UNU standards. Canning steps, filling medium and storage significantly affected the amino acid contents and quality parameters of the fish. The changes varied depending on the amino acid considered. The observed differences in amino acid content can be attributed to leaching into the water during salting or into the frying medium or filling medium, to degradation due to heat treatment and to decomposition or conversion to other compounds by chemical reactions. The quality indices used indicated that canned swordfish packed in olive oil was the highest quality product of those considered.

Author statement

N. Cobas: Investigation, Writing- Original draft preparation, Writing- Reviewing; **L. Gómez-Limia**: Investigation, **I. Franco**: Conceptualization, Methodology, Validation; **S Martínez**: Conceptualization, Methodology, Supervision, Validation, Writing- Reviewing and Editing.

Declaration of Competing Interest

The work contained in the paper is an original research carried out by the authors. All the four authors agree with the contents of the present manuscript and its submission to the JOURNAL OF FOOD COMPOSITION AND ANALYSIS

All authors listed have contributed significantly to the work and agree to be in the author list. No part of the research has been published in any form elsewhere.

There are not conflicts of interest.

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