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Study of the effects of freezing process on nutritional value of 17 fish species marketed in São Paulo State, Brazil

Estudo dos efeitos do processo de congelamento sobre o valor nutricional de 17 espécies de peixes comercializadas no Estado de São Paulo, Brasil

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ABSTRACT

Freezing is an important strategy to keep fish quality and make the species available the whole year. Its effects on the nutritional value of 17 fish species were studied in samples of entire fish, fillets or pieces. One portion of homogenized flesh was analyzed just after purchase (fresh sample). The other portion was packed in polyethylene bag, sealed, quick frozen (-80°C), stored properly at -18°C and analyzed after 12 months (frozen sample). Moisture, ash and protein content were tested using Brazilian Supply, Livestock and Agriculture Ministry methodologies. Lipid content was analyzed through Bligh and Dyer method. Carbohydrate content and caloric value were calculated, using NIFEXT fraction and Atwater coefficient, respectively. When fresh and frozen samples were compared, moisture and ash content showed significant difference (p<0.05) for 17.65% and 11.77% species, respectively. Lipid and protein contents were the most affected parameters, as they were altered in 29.40% of the studied species (p<0.05), and therefore, highlighted the importance of the conservation technology used on nutritional quality of fishery products. Mullet (*M. brasiliensis*) and Atlantic salmon (*S. salar*) had their nutritional composition more affected by freezing process with five and four altered parameters, respectively, from the six studied.

Keywords. Fisheries, Freezing, Nutritional Value, Food Safety.

RESUMO

O congelamento é estratégia importante para manter a qualidade do peixe e tornar inúmeras espécies disponíveis o ano todo. Seus efeitos sobre o valor nutricional de 17 espécies foram estudados em amostras de peixes inteiros, filés ou postas. A porção cárnea homogeneizada foi analisada logo após a aquisição (amostra fresca). Outra parte foi embalada em polietileno, selada, rapidamente congelada (-80°C) e analisada após 12 meses de armazenamento a -18°C (amostra congelada). O teor de umidade, cinzas e proteína foram testados com metodologias do Ministério de Agricultura, Pecuária e Abastecimento e teor de lipídios com método de Bligh e Dyer. Conteúdo de carboidrato e valor calórico foram calculados, utilizando fração NIFEXT e coeficiente de Atwater, respectivamente. Quando se comparou amostras frescas e congeladas, teor de umidade e cinzas evidenciaram diferenças significativas (p<0,05) para 17,65% e 11,77% das espécies, respectivamente. O teor de lipídios e de proteínas foram alterados em 29,40% das espécies estudadas (p<0,05), sendo os parâmetros mais afetados pelo congelamento e destacaram a importância da tecnologia de conservação utilizada sobre a qualidade nutricional do pescado. Tainha (*M. brasiliensis*) e salmão (*S. salar*) foram as mais afetadas pelo congelamento, com 5 e 4 parâmetros alterados, respectivamente, após estocagem sob congelamento.

Palavras-chave. Pescado, Congelamento, Valor Nutritivo, Segurança Alimentar.

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INTRODUCTION

Fish is the major component of the diet for more than 3 billion people around the world and is a rich source of animal protein, micronutrients, and omega-3 fatty-acids, which are vital, especially in places with low income and food deficit worldwilde[1-4]. At this moment, the world is facing a pandemic scenario caused by Covid-19 and there is neither a consensus about how it acts nor about how to control or cure it. Thus, keep ourselves healthy is an advantage, and one way to achieve it is through a rich diet.

According to the Food and Agriculture Organization of the United Nations fisheries and aquaculture activities have been affected by the global pandemic. Both artisanal and industrial fishery decreased globally about 6.5%, until the end of April 2020, when compared with previous years, due to restrictions related to Covid-19[5]. The commerce all over the world has been affected at national and international level. The majority of wholesale and retail fish markets are crowded, representing huge risk to traders and consumers, who take significant risks to buy fresh fish to eat[5]. Thus, direct sales and delivery services were alternatives adopted by some producers to compensate for the loss of the hotel and restaurant demand.

About 59.51 million people, worldwide are directly or indirectly engaged to fisheries or aquaculture activities as a source of food and/or income, and about 14% of them are women[6]. These informations can clarify the importance of these activities and also the economic impact of the pandemic situation in these sectors.

Available high-quality foodstuff is essential to guarantee a healthy diet in order to ensure that people have access to macronutrients (proteins, fats and carbohydrates including dietary fibers) and essential micronutrients (vitamins and minerals), considering also their gender, age, physical activity level and physiological state[2-4,7].

Regular fish consumption recommended by the World Health Organization[8], is of 1-2 servings per week, to be protective against coronary heart disease and ischaemic stroke. The serving should provide an equivalent of 200-500mg of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). According to FAO[7], average global per capita fish consumption raised from 9kg in 1961, to 20.5kg in 2018. Brazil has approximately 8,400km of coastal line and a high potential for fisheries and aquaculture. In 2018, average fish consumption per capita reached 10.63kg, considering products from fisheries and aquaculture[9].

Two main factors have collaborated for the global growing consumption of fish in the last century: the quick population growth and access to information about its health benefits[10]. As highlighted by Furlan et al[11], fish is rich in high quality protein and is also an important source of polyunsaturated fatty acids (PUFAs), like omega-3[2,4,12,13]. Studies have indicated that ingestion of PUFA's like EPA, can reduce heart diseases risks, as well as biochemical factors associated to arthritis, psoriasis and cancer[14]. It is also a highly digestible food, with minerals, mainly calcium, phosphorus, besides vitamins A, D and B complex[1,2,15-18].

Nowadays fish is in a process of valorization in the market, and its ingestion is highly stimulated, but information about the nutritional value of the different species traded in Brazil is very rare or difficult to be accessed by experts and consumers. Species traded at São Paulo State accounted for more than 15,452,058 tonnes in 2019. From January to December the main traded species were seabob shrimp (*Xiphopenaeus kroyeri*), followed by withemouth croaker (*Micropogonias furnieri*) and mullet (*Mugil brasiliensis*), with more than 3,047 tonnes, 1,936 tonnes and 1,044 tonnes, respectively[19]. Analysis of chemical composition indicates the main nutritional compounds found on fish are: water, lipid, protein, and ash. However, biochemical composition of fish may vary greatly between species and individuals depending on age, sex, genetic, morphology, season and also environment[1].

In order to preserve seafood quality and, consequently its nutritional value, is very important to adopt good hygienic practices along all productive chain, from capture to the ultimate moment of marketing. Time and temperature control are the main challenges in fish preservation, and low temperature is associated to degradative reactions control[20,21]. Refrigeration is a technique effectively used to preserve food because lowering temperature can avoid or slow down chemical-enzimatic reactions related to autolysis, and development of microorganisms that contribute to food deterioration[21,22].

Although refrigeration keeps the original food characteristic, shelf-life under this condition is too short[22]. But freezing, sudden inhibits microorganism development due to increased solute concentration and lowered water activity in tissues, thus, provides longer food preservation period, even to those with high water activity[21,23]. Though freezing is a relative old preservative food technology, its logistic chain is expensive, compared to other methods, but frozen food still has a wide market due to its convenience[24]. Around 67 million tonnes of fish (equivalent live weight) were traded worldwide in 2018. Over 90% of it was processed, and the highest share was frozen[7].

A study made at the Metropolitan Region of São Paulo[25], at São Paulo State/Brazil has indicated that refrigerated fresh fish is still the favorite product, which makes more important studies to show if freezing can affect fish quality, to demystify frozen products. Freezing is an important technology because some species in nature are not available in all seasons and cannot be fished all over the year. Therefore, the knowledge of the effects of freezing on nutritional quality of the different species can help to improve freezing technology or choose the best species to be frozen in order to take advantage of nutritional characteristics of each species and/or optimize freezing technology and complementary techniques to offer better products to the consumer and also to be able to regulate the market by offering different species along the year.

Considering this context, the aim of this study was to verify the effects of freezing process on nutritional value of different fish species, in order to provide economic and sustainable exploitation basis and food safety.

MATERIAL AND METHODS

A total of 17 different fish species were purchased at the Municipal Fish Market at Santos and at a fishery industry in Guarujá city, both in São Paulo State, Brazil, as described in **Table 1**. Samples size was approximately 5kg for entire fish and 2kg for samples purchased in piece or in fillet.

Table 1. Fish samples identification with common and scientific names, place of purchasing and presentation

Common English Name	Scientific Name	Place of Purchasing	Presentation
Withemouth Croaker	*Micropogonias furnieri*	Mun.Market	Entire/Fresh
Weakfish	*Cynoscion leiarchus*	Mun.Market	Entire/Fresh
Brazilian Sardinella	*Sardinella brasiliensis*	Mun.Market	Entire/Gutted
Mullet	*Mugil brasiliensis*	Mun.Market	Entire/Fresh
Acoupa Weakfish	*Cynoscion acoupa*	Mun.Market	Entire/Fresh
Brazilian Codling	*Urophycis brasiliensis*	Mun.Market	Fillet
Shark	*N.D*	Mun.Market	Pieces
Broadband Anchovy	*Anchoviella lepidentostole*	Mun.Market	Entire/Headed
Gray Triggerfish	*Balistes capriscus*	Mun.Market	Headed/skinned
Pangas Seabream	*Pterogymnus laniarius*	Mun.Market	Fillet/Fresh
Dorado	*Salminus maxillosus*	Mun.Market	Pieces
Atlantic Salmon	*Salmo salar*	Mun.Market	Fillet/skinned
Patagonian Flounder	*Paralichthys patagonicus*	Mun.Market	Entire/Fresh
Sand Drum	*Umbrina coroides*	Industry	Entire/Fresh
Southern Kingcroaker	*Menticirrhus americanus*	Industry	Entire/Fresh
Nile Tilapia	*Oreochromis niloticus*	Mun.Market	Fillet
Argentine Hake	*Merluccius hubbsi*	Mun.Market	Fillet

N.D. – Not determined because the sample did not have the required morphological features for species identification
Sample size = 5kg when Entire; 2kg When Fillet or Pieces

As shown in **Figure 1**, flesh of each sample was homogenized using a cutter (Skymsen Mod. CR-4L). Then one half of homogenized flesh sample was immediately analyzed (fresh sample), and the other half was packed in polyethylene bag, sealed, and quick frozen at -80°C (Sanyo Inc.-VIP Series ultra-freezer), to preserve its original nutritional characteristic (frozen sample). Two months later, the frozen samples were transferred to a domestic freezer (Electrolux H500) and kept for 10 more months under -18±2°C to simulate domestic storage conditions. After a total period of 12 months of frozen storage, each frozen sample was analyzed using the same methodologies as the fresh samples to evaluate the effects of freezing process on the nutritional value. 24h prior to analysis, samples were kept in domestic refrigerator (2±2°C) for adequate thawing. All analysis were performed in triplicate, using recognized or official methodologies in accordance with the Brazilian Supply, Livestock and Agriculture Ministry, as described below.

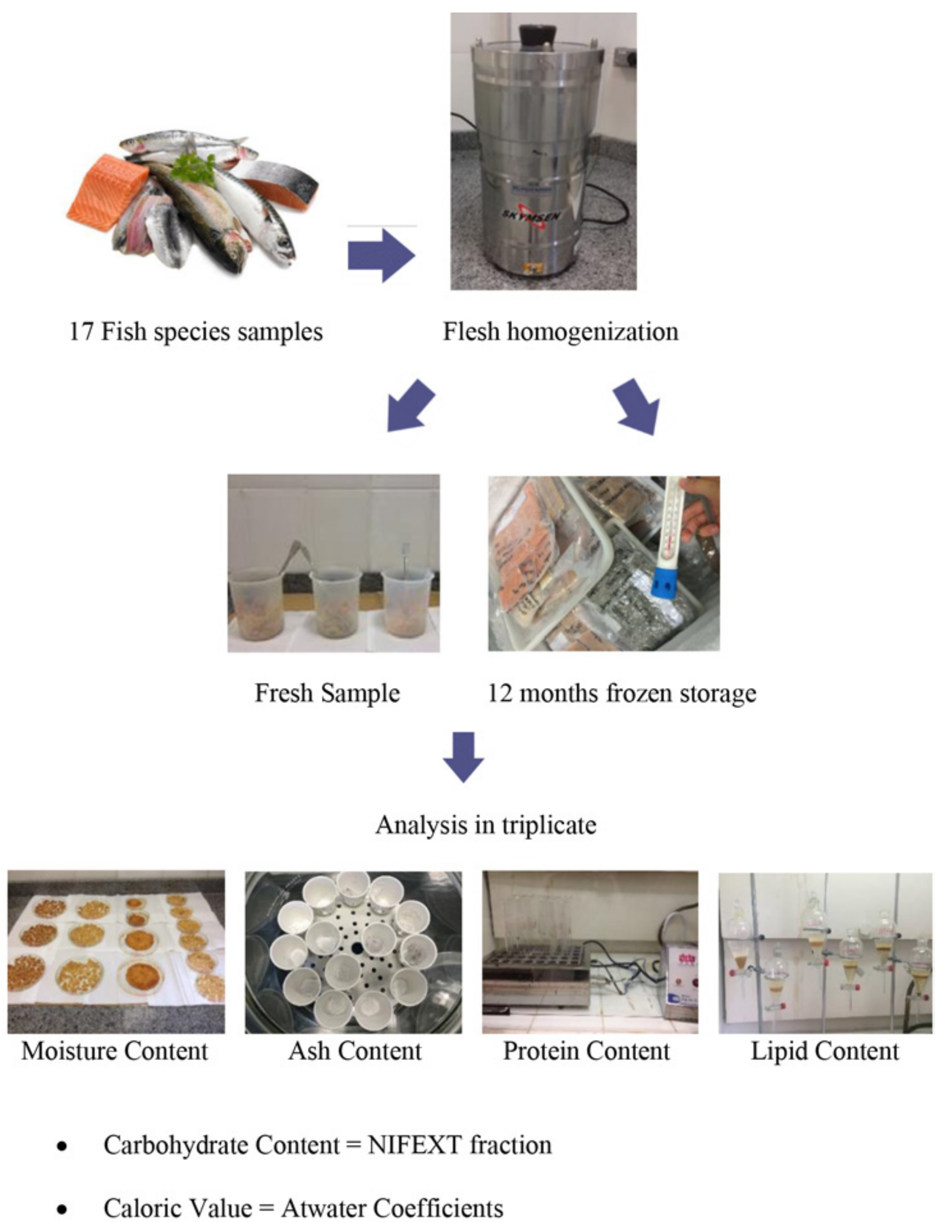

Figure 1. Schematic procedure of the study of freezing process effects on nutritional value for 17 different fish species marketed in São Paulo State, Brazil

Moisture content determination was performed according to Brasil[26], based on oven drying (105°C) to constant weight. Dried samples were carbonized and then incinerated in a mufle furnace (550°C) for the quantification of ash (total mineral residue), until white ash was obtained[26].

Lipid content was determined using dried samples and cold solvent extraction prior to quantification by weight difference, according to Bligh and Dyer[27]. For protein analyses, dry samples were digested and then steam destilled using micro Kjeldahl apparatus[26]. The carbohydrate index was determined using the NIFEXT fraction and caloric value was calculated with the Atwater coefficient[28].

Statistical analysis

Paired *t*-test was performed to determine statistically different values between fresh and frozen samples of each fish species, with 95% of confidence.

RESULTS

Mean results (% wet weight) of moisture, ash, lipid, protein, carbohydrate content and caloric value obtained from samples of fresh fish and after 12 months of freezing storage are shown in **Tables 2** and **3**.

Moisture Content

The results of moisture analysis have shown significant difference ($p<0.05$) between fresh and frozen samples for 17.65% of analyzed species (**Table 2**), broadband anchovy (*Anchoviella lepidentostole*), Atlantic salmon (*Salmo salar*) and Southern kingcroaker (*Menticirrhus americanus*). Considering only fresh samples (**Table 2**), panga seabream (*Pterogymnus laniarius*) showed the highest moisture content (82.17%), while the Atlantic salmon (*S. salar*) showed the lowest one (65.08%), and the former also showed the lowest moisture (64.47%) after 12 months of freezing storage (-18°C).

Table 2. Results (% wet weight) of moisture, ash and lipid content analysis of 17 fish species fresh samples and after 12 months under freezing storage condition (-18±2°C)

Common name	Moisture (%)		Ash (%)		Lipid (%)	
	FRESH	FROZEN	FRESH	FROZEN	FRESH	FROZEN
Withemouth Croaker	80.51^a±0.04	80.39^a±0.25	0.99^a±0.02	1.13^a±0.01	0.31^a±0.04	0.24^a± 0.05
Weakfish	79.64^a±0.36	79.80^a±1.37	0.89^a±0.02	0.88^a±0.12	0.43^a±0.02	0.50^a±0.05
Brazilian Sardinella	73.57^a±1.75	73.41^a±0.02	1.23^a±0.02	1.25^a±0.05	4.52^a±0.57	4.14^b±0.11
Mullet	77.20^a±0.18	77.88^a±0.02	1.12^a±0.01	1.01^b±0.02	0.80^a±0.02	0.40^b±0.18
Acoupa Weakfish	80.05^a±0.08	80.15^a±0.16	0.88^a±0.03	0.90^a±0.01	1.55^a±0.02	0.86^b±0.09
Brazilian Codling	81.77^a±0.05	81.79^a±0.06	1.01^a±0.01	1.05^a±0.02	0.60^a±0.04	0.35^b±0.09
Shark	77.00^a±0.01	76.72^a±0.33	1.12^a±0.02	1.10^a±0.02	0.77^a±0.08	0.36^b±0.06
Broadband Anchovy	81.46^a±0.08	80.85^b±0.10	0.94^a±0.01	1.10^a±0.01	3.49^a±0.05	3.36^a±0.07

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Continuation

Common name	Moisture (%)		Ash (%)		Lipid (%)	
	FRESH	FROZEN	FRESH	FROZEN	FRESH	FROZEN
Gray Triggerfish	79.74^a±0.11	79.44^a±0.18	1.05^a±0.01	1.03^a±0.01	0.26^a±0.02	0.34^a±0.15
Pangas Seabream	82.17^a±0.21	81.62^a±1.10	1.56^a±0.03	1.43^a±0.03	4.56^a±0.05	4.56^a±0.10
Dorado	76.46^a±0.17	76.26^a±0.39	1.10^a±0.09	1.23^a±0.02	0.94^a±0.02	1.49^a±0.07
Atlantic Salmon	65.08^a±0.13	64.47^b±0.32	1.08^a±0.01	1.05^b±0.01	14.37^a±0.53	13.62^a±0.21
Patagonian Flounder	79.30^a±0.23	78.99^a±0.49	0.97^a±0.02	1.05^a±0.02	0.45^a±0.03	0.41^a±0.17
Sand Drum	77.46^a±1.39	76.90^a±0.04	1.68^a±0.43	1.75^a±0.13	0.79^a±0.20	0.86^a±0.17
Southern Kingcroaker	79.56^a±0.02	79.22^b±0.09	1.28^a±0.01	1.30^a±0.01	0.93^a±0.10	0.78^a±0.09
Nile Tilapia	79.82^a±0.12	79.65^a±0.13	0.86^a±0.08	0.79^a±0.08	1.51^a±0.01	2.28^a±0.06
Argentine Hake	81.69^a±0.66	81.59^a±0.29	0.71^a±0.19	0.75^a±0.04	1.13^a±0.09	1.06^a±0.14

Mean values ±standard deviation (n=3)
Different letters in the same line mean significant difference between fresh and frozen samples ($p<0.05$)

Ash Content

Ash content varied from 0.71% in fresh Argentine hake (*Merluccius hubbsi*) to 1.68% in fresh sand drum (*Umbrina coroides*), and after 12 months of freezing storage it varied from 0.75% to 1.75%, in the same species, respectively (**Table 2**). Results also indicated that mullet (*Mugil brasiliensis*) and the Atlantic salmon (*S. salar*) showed significant difference in ash content ($p<0.05$) in fresh and frozen samples (**Table 2**).

Lipid Content

Lipid content varied in fresh samples from 0.31% in Withemouth croaker (*Micropogonias furnieri*) to 14.37% in the Atlantic salmon (*S. salar*), and in frozen samples, it varied from 0.24% to 13.62% in these same species, respectively (**Table 2**). When lipid content in fresh and frozen samples were compared, among all studied species, 29.40% showed significant difference ($p<0.05$), and included: Brazilian sardinella (*Sardinella brasiliensis*), mullet (*M. brasiliensis*), acoupa weakfish (*Cynoscion acoupa*), Brazilian codling (*Urophycis brasiliensis*) and shark, as can be seen in **Table 2**.

Protein Content

Table 3 shows the protein content for the 17 species analyzed as fresh and after 12 months of freezing storage. Shark showed the highest protein content in both samples, 22.35% and 22.91%, for fresh and frozen samples, respectively, and panga seabream (*P. laniarius*) was the unique species with low protein content (~9.45%) as can be seen in **Table 3**. The freezing process has affected the protein content in 29.40% of analyzed species ($p<0.05$): mullet (*M. brasiliensis*), gray triggerfish (*Balistes capriscus*), dorado (*Salminus maxillosus*), Atlantic salmon (*S. salar*) and Patagonian flounder (*Paralichthys patagonicus*). The last one was the most affected species by the freezing process, since protein content in fresh sample was 19.05% and after 12 months under frozen storage it was 17.59% (**Table 3**).

Carbohydrate Content

Table 3 shows that 88.24% of studied species had carbohydrate content varying from 0.15 to 2.57% (wet weight). Statistical analysis showed difference ($p<0.05$) between fresh and frozen samples in 29.40% of studied species: Brazilian sardinella (*S. brasiliensis*), mullet (*M. brasiliensis*), gray triggerfish (*B. capriscus*), Patagonian flounder (*P. patagonicus*) and Nile tilapia (*O. niloticus*).

Table 3. Results (% wet weight) of protein content, carbohydrate and caloric value calculation of 17 fish species fresh samples and after 12 months under freezing storage condition (-18±2°C)

Common name	Protein (%)		Carbohydrate (%)		Caloric Value (Kcal/100g)	
	FRESH	FROZEN	FRESH	FROZEN	FRESH	FROZEN
Withemouth Croaker	$16.71^{a}\pm1.63$	$17.58^{a}\pm0.02$	$2.19^{a}\pm1.50$	$0.66^{a}\pm0.21$	75.42^{a}	74.48^{a}
Weakfish	$16.55^{a}\pm1.50$	$16.40^{a}\pm0.06$	$2.49^{a}\pm0.78$	$2.42^{a}\pm1.16$	80.35^{a}	79.78^{a}
Brazilian Sardinella	$19.87^{a}\pm0.06$	$21.10^{a}\pm0.19$	$0.81^{a}\pm0.06$	$0.10^{b}\pm0.09$	123.40^{a}	122.06^{b}
Mullet	$20.02^{a}\pm0.27$	$19.16^{b}\pm0.13$	$0.87^{a}\pm0.20$	$1.05^{b}\pm0.31$	90.98^{a}	88.70^{b}
Acoupa Weakfish	$17.00^{a}\pm0.07$	$17.54^{a}\pm0.20$	$0.53^{a}\pm0.04$	$0.55^{a}\pm0.22$	84.01^{a}	80.14^{b}
Brazilian Codling	$16.61^{a}\pm0.08$	$16.50^{a}\pm0.07$	$0.02^{a}\pm0.10$	$0.31^{a}\pm0.21$	71.87^{a}	70.41^{b}
Shark	$22.35^{a}\pm0.36$	$22.91^{a}\pm0.01$	-	-	96.33^{a}	94.88^{b}
Broadband Anchovy	$14.18^{a}\pm0.28$	$14.90^{a}\pm0.16$	-	-	88.13^{a}	89.84^{a}
Gray Triggerfish	$18.54^{a}\pm0.44$	$17.25^{b}\pm0.04$	$0.41^{a}\pm0.03$	$1.94^{b}\pm0.20$	78.12^{a}	80.62^{a}
Pangas Seabream	$9.44^{a}\pm0.67$	$9.45^{a}\pm0.18$	$2.27^{a}\pm0.46$	$2.94^{a}\pm0.17$	87.32^{a}	89.10^{a}
Dorado	$20.84^{a}\pm0.22$	$19.24^{b}\pm0.88$	$0.66^{a}\pm0.34$	$1.77^{a}\pm0.66$	94.47^{a}	97.50^{a}
Atlantic Salmon	$18.47^{a}\pm0.82$	$17.04^{b}\pm1.48$	$1.01^{a}\pm0.09$	$3.83^{b}\pm0.45$	207.24^{a}	206.63^{a}
Patagonian Flounder	$19.05^{a}\pm0.19$	$17.59^{b}\pm0.05$	$0.23^{a}\pm0.06$	$1.96^{b}\pm0.38$	81.15^{a}	81.04^{a}
Sand Drum	$19.84^{a}\pm3.12$	$19.82^{a}\pm1.05$	$0.23^{a}\pm0.12$	$0.67^{a}\pm0,08$	87.36^{a}	89.25^{a}
Southern Kingcroaker	$18.38^{a}\pm0.00$	$18.17^{a}\pm0.89$	$-^{a}$	$0.53^{a}\pm0.06$	81.89^{a}	81.82^{a}
Nile Tilapia	$17.07^{a}\pm0.14$	$17.84^{a}\pm0.11$	$0.74^{a}\pm0.28$	$-^{b}$	84.55^{a}	89.68^{a}
Argentine Hake	$16.22^{a}\pm0.63$	$16.25^{a}\pm0.27$	$0.26^{a}\pm0.14$	$0.35^{a}\pm0.04$	76.04^{a}	76.74^{a}

Mean values ± standard deviation (n=3)
Different letters in the same line mean significant difference between fresh and frozen samples ($p<0.05$)

Caloric Value

Results of caloric value ranged in fresh samples from 71.87 to 207.24 kcal/100g in Brazilian codling (*U. brasiliensis*) and Atlantic salmon (*S. salar*), respectively, and in the samples kept 12 months under freezing storage, from 70.41 to 206.63 kcal/100g in these same species, respectively (**Table 3**). Statistical analysis showed difference ($p<0.05$) in caloric value results between fresh and frozen samples for 29.40% of studied species: Brazilian sardinella (*S. brasiliensis*), mullet (*M. brasiliensis*), acoupa weakfish (*C. acoupa*), Brazilian codling (*U. brasiliensis*) and shark, as can be seen in **Table 3**.

DISCUSSION

Freezing is considered a successful and one of the most used technology to preserve natural quality of foodstuffs for long periods of time[21,29]. In the case of fish, if correctly applied, it lowers the rate of spoilage, extends shelf-life to several months, while not excessively affecting the physical properties and quality[21,30]. Although, considerable drip loss, colour, texture and sensory changes can occur in meat and vegetable products after thawing[21,24,30], leading to alterations in nutritional value. It is highlighted by these authors that this technology is still being studied and there are great advances specially related to anti-freezing proteins, that in near future will be an important tool for controlling the formation of ice cristals and may represent an ally for preservation of original quality of many foodstuffs. The best way for thawing fish and fish products for processing without loss of quality[21,23,30] has also been studied in order to manufacture higher quality products.

In Brazil, the "*Regulamento de Inspeção Industrial de Produtos de Origem Animal* (RIISPOA)" stablishes the official regulation for industrial and sanitary inspection of animal products since 1952. In its 2017 revised edition[31], the article 335 defines "frozen fish" as "that one submitted to process of quick freezing, in a way that the product quickly exceeds the temperature of maximum crystallization". According to paragraph 1° of this article, the "process of quick frozen can be considered finished only when the product reaches -18°C"[31].

Fish is important from both the economic and health points of view, and its importance for a healthy diet is undeniable in many countries[2,3,30]. Freezing is considered an important fish preservation technology, and alteration in protein content with time of frozen storage has already been reported[29,30], but data must be generated in order to demonstrate the extension of alteration and what must be done to guarantee high quality products, especially with Brazilian species, due to lack of nutritional data of raw material and effects of freezing processes on it. Nowadays, when the world must face the Covid-19 pandemic, it is particularly important to debate freezing technology, because it is an important tool to prevent contamination.

Moisture content is one of the most important determinations used in food analysis and is based on the physicochemical property of the water to volatilize at high temperature (>100°C). All studied species showed moisture varying from 65 to 90%, in agreement to Hart and Fisher[32], in both samples, fresh or after 12 months of frozen storage, respectively, as shown in **Table 2**. Fish moisture content is related to its stability, quality and nutritional composition[33], and it is strongly related to protein and carbohydrate[32]. Results showed that moisture content was slightly altered by the freezing process in 17.65% of studied species, which could reflect in texture loss and general quality alteration of product after thawing and/or processing.

Ash content reflects the total mineral content of a fish, and it varies depending on the amount and quality of the fish nutrition and the amount of movement it makes[1], generally total mineral residue in fish ranges from 1 to 2%[32] and also indicates the richness of species in mineral elements like calcium, zinc, iron, and phosphorus, among others[2,3,34]. It is obtained after heating sample until complete combustion of organic matter is reached[35]. In the present study, 11.77% of the studied species have shown difference ($p<0.05$) between fresh and frozen samples, that could be reflected in lack of nutritional value for the final consumer.

Statistically significant difference ($p<0.05$) was found for results of moisture content in broadband anchovy (*Anchoviella lepidentostole*), Atlantic salmon (*Salmo salar*) and Southern kingcroaker (*Menticirrhus* *americanus*). For ash content, results of fresh and frozen samples were different (*p*<0.05) in mullet (*Mugil brasiliensis*) and the Atlantic salmon (*S. salar*).

Lipid from fish is important due to the presence of polyunsaturated fatty acids (PUFA's), especially those of long chain omega-3 series, known for their health benefits[36]. Besides PUFA's, there are others nutrients, like vitamin D, selenium, zinc, magnesium and potassium, associated with protective effects for cardiovascular diseases that can also be present[36]. Results shown in **Table 2** have indicated that all studied species presented lipid content <5%, and were considered lean, according to Stansby[37]. Only the Atlantic salmon was classified as moderately fat[37] and showed lipid content of 14.37% and 13.62%, in fresh and frozen samples, respectively. Significative difference (*p*<0.05) in lipid content was observed in 29.40% of the studied species: Brazilian sardinella, mullet, acoupa weakfish, Brazilian codling, and shark, when fresh and frozen samples were compared. A considerable loss of nutritional value to the consumer that seeks for healthy lipid in any fish species was observed, especially considering that for acoupa weakfish it was observed a loss of about 55.48% in lipid content, from 1.55% in fresh sample to 0.86% for sample kept storage 12 months under freezing condition (**Table 2**). In mullet, Brazilian codling and shark about 50% of loss in lipid content was observed, when results of fresh and frozen sample were compared (**Table 2**). Brazilian sardinella also showed loss (*p*<0.05) in lipid content, from 4.52% in fresh sample to 4.14% in sample kept under freezing condition, but it was slight, considering the previous results (**Table 2**).

Protein is the main constituent of edible part of fish and has high biological value, which means that human organism takes high profit when consumes it[33]. Fish accounted for 17% of the animal protein and 7% of all protein consumption in the world[6] and is an important source of high-quality animal protein[3,29], with digestibility coefficient of 90-98%, higher than bovine and other meats, due to the amount of myofibrillar fraction[34]. According to Stansby[37], fish may be classified as high protein content when varies from 15 to 20% of lipid, moderate protein content from 10 to 15% and low protein content when is below 10%.

The results (**Table 3**) indicated that majority of species (88.24%) were considered with high protein content, independent if fresh or frozen, and shark was the one with the highest protein content with 22.35%. Broadband anchovy (*A. lepidentostole*) was classified with moderate protein content with 14.18 and 14.90% of protein in the fresh and frozen sample, respectively, Panga seabream (*P. laniarius*) was the unique species with low protein content (~9.45%), as can be seen in **Table 3**. Protein content showed difference (*p*<0.05) for 29.40% of studied species when fresh and frozen samples were compared (mullet, gray triggerfish, dorado, Atlantic salmon and Patagonian flounder). Our results indicated loss of approximately 7.7% in protein content between fresh and frozen samples of dorado (18.47% to 17.04%), Atlantic salmon (18.47% to 17.04%) and Patagonian flounder (19.05% to 17.59%). Gray triggerfish showed a difference of 6.96% between fresh (18.54%) and frozen samples (17.25%), and mullet showed 4.3%, the smallest loss in protein content between fresh (20.02%) and frozen samples (19.16%). Similar results were observed by Abemourand[29], when studied the effect of freezing on four fish species after 30 and 60 days of storage.

The same amount of samples (29.40%) showed difference (*p*<0.05) in lipid and protein content between fresh and frozen samples, but for different species. Results showed that although freezing is an important fish conservation technology, because lowering temperature reduces the speed of degradative processes, its utilization demands high quality fish to obtain satisfactory results, and deficient use can lead to protein denaturation and lipid oxidation[29], especially in fat species, because will cause rancification odour and flavour[3,29,38]. Our results can corroborate these affirmations, since it has shown that these two were the most affected parameters after freezing storage under experimental condition.

Complementary techniques as glazing, is also of great importance in order to preserve the quality of products[3,29,38]. Glazing and the use of cryoprotective substances can grant greater stability and avoid protein denaturation, lipid oxidation and dehydration, main causes of nutritional value loss[29,38,39]. As observed by Oetterer et al[38], biochemical interactions can occur between proteins and fatty acids in fish muscles, during prolonged frozen storage, thus proteins can react with products of lipid oxidation and the amino acids with formaldehydes.

The results have shown significant difference ($p<0.05$) in all analyzed parameters, but with different incidences depending on species. It must be emphasized that fish may present important contents of micronutrients and it is variable depending on the species[40], which were not investigated here, but must be studied because storage condition can affect them either[36].

Carbohydrate content and caloric value were different ($p<0.05$) in 29.40% of species for fresh and frozen analyzed samples. The species with difference in caloric value were the same of those in lipid content because calculation is highly influenced by it.

Our study detected nutritional alterations in all studied species and in all studied parameters after frozen storage, thus it must be considered when the technology is applied in order to guarantee that frozen fish or processed product do not loose nutritional value, especially because lipid and protein content are the most important nutritional features of interest to fish consumers.

CONCLUSION

Mullet (*M. brasiliensis*) and Atlantic salmon (*S. salar*) were the most affected species by the frozen storage, with 5 and 4 affected parameters, respectively, out of a total of 6. Results showed that lipid and protein contents were the most impaired parameters after 12 months of frozen storage and highlighted the importance to study nutritional value of raw material and the type of storage and conservation technology used in order to avoid of nutritional value loss in frozen products.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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