



Nutritional, microbiological and sensorial characteristics of alfajor prepared with dehydrated mixture of salmon and tilapia

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ABSTRACT. Current assay deals with the preparation of alfajores with different levels (0 to 15%) of dehydrated fish mixture of salmon (10%) and tilapia (90%) to assess the sensorial characteristics and their centesimal composition and microbiological. Fish inclusion in alfajores did not affect the aroma, taste, texture, color and physical aspect, with scores ranging between 6.70 and 7.96 of a hedonic scale of 9 score. An average score of 4 in a 5-score purchasing intention scale was obtained, or rather, tasters would probably buy the product. In the case of centesimal composition, inclusion affected ($p < 0.05$) humidity (between 2.74 and 3.40%) and ash (between 0.31 and 1.01%) rates, with a quadratic effect; protein (between 5.35 and 7.12%) with positive linear effect; carbohydrates (between 72.64 and 75.79%) with negative linear effect. There was no difference ($p > 0.05$) in lipids and calorie rates in the alfajores. Results show that the inclusion of up to 15% of a dehydrated mixture of salmon (10%) and tilapia (90%) in alfajores was greatly accepted and improved their nutrition values. Further, the product was also within the microbiological standards required by Brazilian sanitary laws.

Keywords: Filleting waste, fish in sweet products, fish protein concentrate, fish carcass.

Características nutricionais, microbiológicas e sensoriais de alfajor elaborado com mix desidratado de salmão e tilápia

RESUMO. O objetivo deste estudo foi elaborar alfajores com diferentes níveis de inclusão (0 a 15%) do mix desidratado de salmão (10%) com tilápia (90%) e avaliar as características sensoriais, composição centesimal e microbiológica. A inclusão do mix de peixe no alfajor não interferiu em seus parâmetros sensoriais de aroma, sabor, textura, cor e aparência, cujas notas variaram de 6,70 a 7,96, pela escala hedônica de 9 pontos. Para intenção de compra, considerando a escala hedônica de 5 pontos, obteve-se nota média 4, cujo valor significa que os avaliadores possivelmente comprariam o produto. Em relação à composição centesimal, a inclusão do mix influenciou ($p < 0,05$) nos teores de umidade (2,74 a 3,40%) e nas cinzas (0,31 a 1,01%) proporcionando efeito quadrático, na proteína (5,35 a 7,12%) efeito linear positivo, enquanto nos carboidratos (72,64 a 75,79%) linear negativo. Não houve diferença ($p > 0,05$) para extrato etéreo e valor calórico dos alfajores. Conclui-se que a inclusão de até 15% do mix desidratado de salmão (10%) com tilápia (90%) no alfajor, teve boa aceitação e melhorou sua composição nutricional, além do produto estar dentro dos padrões microbiológicos exigidos pela legislação brasileira.

Palavras-chave: resíduos de filetagem, peixe em produtos doces, concentrado proteico de peixe, carcaça de peixe.

Introduction

It is a well-known fact that a great amount of fish industrial waste of high quality is produced in industrial plants and may be used to produce food with high nutritional value at low costs, especially for human consumption (Arvanitoyannis & Kassaveti, 2008). Moreover, may be a feasible alternative to decrease the production of organic wastes.

According to Kubitza (2006), the amount of wastes produced may vary between 8 and 16% when

the final product is eviscerated fish or between 60 and 72% when the final product is skinless fillets and frequently are discarded in inappropriate places, creating environmental problems, or intended for animal feed, which has high production costs.

The use of waste from fish filleting to human nutrition is greatly relevant since it can promote a decrease in problems with production and unit costs of prime matters. Nutritional reasons are the most important since fish wastes have a high volume of prime matter and they are a source of low cost

nutrients (Arruda & Oetterer, 2005). Fish products have high protein rates (approximately 20%) and they are rich in minerals, mainly calcium, phosphorus and iron (Ariño, Beltrán, Herrera, & Roncalés, 2013, Sartori & Amancio, 2012), vitamins A, D and B complex, especially B12, or rather, product of high nutritional value (Vila Nova, Godoy, & Aldrigue, 2005). In addition, fish of marine origin are rich in omega-3 polyunsaturated fatty acids (PUFAS) (Li et al., 2013). But, despite its high nutritional value, only 40% of fish products are used for human consumption (Dekkers, Raghavan, Kristinsson, & Marshall, 2011).

According to Godoy, Franco, Souza, Stevanato, and Visentainer (2013), aromatized tilapia fishmeal provided 1.78 mg of calcium, 2.36 mg of iron and 5.47 mg of phosphorus de in 100 g of fishmeal. The same authors reported 23 types of fatty acids, among which may be mentioned PUFAS n-3 as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

Besides the protein rates, the quality of lipids in fish, especially Omega 3, should also be assessed. Tonial et al. (2010) reported that salmon (*Salmo salar* L.) and other salmonid species provided high levels of alpha-linolenic (LNA, 18:3n-3) and linoleic acids (LA, 18:2n-6) coupled to high levels of AGPI n-3 due to their diet of unicellular algae, comprising approximately 20% of their dry weight in lipids. Further, 50% of these lipids are PUFAs, mainly Omega-3. The above facts reveal a good alternative for using salmon wastes due to the quality of lipids (fatty acids Omega 3). In fact, Omega 3 in sea fish is higher than that in fresh water fish as in the case of the tilapia in which fatty acids of Omega 6 type predominate.

In this sense, several alternatives are being analyzed to transform them into products for humans so that their nutrients could be employed and pollution problems partially solved (Chalamaiah, Hemalatha, & Jyothirmayi, 2012), encouraging the development of new products for human consumption (Feldes et al., 2010).

Several experiments have been performed to develop fishmeals for human consumption (Franco et al., 2009; Godoy et al., 2013, Petenucci et al., 2010). Fishmeal or fish protein concentrates may be defined as dehydrated ground products with variable contents of proteins, with or without the taste of fish, depending on the method for their preparation (Jesus & Almeida, 2011).

The fishmeal or protein concentration from fish carcass (spine without the head and fins) show excellent nutritional quality and can be used for inclusion in several food products, especially in low protein ones. So, protein concentrate or dehydrated mixture with the highest nutritional quality may be

manufactured and used to elaborate several products as salty tidbits, types of dough, meat (hamburgers, fries, sausages) and even candies. Dehydrated mixture of fish in candies, especially in the highly sophisticated alfajores, has been included. Alfajores are a sort of biscuits stuffed with sweetened condensed milk and covered with chocolate (Castillo, Estrada, Margalef, & Tóffoli, 2013). They are products that originated in Argentina and manufactured extensively in South America.

Current assay comprises the preparation of alfajor with inclusion of different levels of dehydrated mixture of salmon (*Salmo salar* L.) and tilapia (*Oreochromis niloticus*) and evaluation of its chemical, sensorial and microbiological composition.

Material and methods

Preparation of dehydrated mixture and alfajores

Fishmeal and alfajor were prepared at the Fish Technology Laboratory of the Iguatemi Experimental Farm of the *Universidade Estadual de Maringá*, Maringá, Paraná State, Brazil. Carcasses of the Nile tilapia (*Oreochromis niloticus*), donated by Smartfish (Rolândia, Paraná State, Brazil), and salmon carcasses, donated by Tomita & Tomita Ltda (Maringá, Paraná State, Brazil), were used. They were frozen immediately after filleting and transported in isothermal boxes to the laboratory and stored in a freezer (-18°C) until preparation of fishmeal. The carcasses (spine with ribs and meat left after filleting) without head and fins, of the two species were washed, sanitized with proxitan 1512® (0.01% of prime matter volume) and cooked for 60 minute in a pressure cooker. After cooking, the prime matter was drained, pressed (10-ton capacity), ground in a meat mincer and dehydrated in a drying buffer for 24h at 60°C. Dehydrated material was once again ground in a Willy mill and the product vacuum-packed. Fish dehydrated mixture comprised 10% salmon fishmeal and 90% tilapia fishmeal, and the mixture homogenized.

Alfajor (Figure 1) was prepared with different levels of the dehydrated mixture according to the weight of the wheat flour used. Dough was opened by a spin roll, cut in 5-cm diameter circular forms, placed in a margarine-oiled aluminum pan and cooked in a heated oven at 280°C for 15 minutes. After cooling, the alfajores were stuffed with sweetened milk, the two biscuits were placed one on top of the other and covered with hydrogenated chocolate, and decorated (Figure 1B).

Table 1 shows the formulation for the preparation of alfajores with different inclusion levels (0, 5, 10 and 15%) of dehydrated mixture of fish.



Figure 1. (A) Preparation of alfajor (ingredients and dehydrated fish mixture (arrow)) and (B) alfajor ready for consumption.

Table 1. Formulation of alfajores with different inclusion levels of dehydrated mixture of fish.

Ingredients (g)	Inclusion levels of fish dehydrated mixture			
	0%	5%	10%	15%
Margarine without salt	200	200	200	200
Eggs	250	250	250	250
Sugar	400	400	400	400
Wheat flour	560	532	504	496
Dehydrated mixture of salmon and tilapia	0	28	56	64
Maize starch	440	440	440	440
Powder chocolate	140	140	140	140
Powdered chemical yeast	9	9	9	9

Microbiological analysis of a dehydrated mixture of salmon and tilapia, alfajor

The microbiological analysis of the dehydrated mixture and prepared products was performed at the Laboratory of Food Microbiology and Microscopy of the Department of Clinical Analysis in the *Universidade Estadual de Maringá*, Maringá, Paraná State, Brazil. Samples were assessed for the Most Probable Number (MPN) of coliforms at 35 and 45°C and *Staphylococcus* positive coagulase in colony-forming unit (CFUgram⁻¹), and search for *Salmonella* spp in 25 g following APHA (2001).

Analysis of proximate composition of dehydrated mixture and alfajores

The chemical analysis of dehydrated mixture of salmon and tilapia and alfajor was performed. The proximate composition comprised moisture, ether extract and ash rates following methodology by Association of Official Analytical Chemists (AOAC, 2005). Crude protein were assessed by the semi-micro Kjeldahl method (Silva & Queiroz, 2002) and carbohydrate were estimated by difference, or rather, the sum of previous determinations (moisture, crude protein, ether extract and ash) was subtracted from 100%. Total calorie were obtained

by the sum of the multiplication of averages of protein, lipids and carbohydrates multiplied by 4, 9 and 4, respectively (Souci, Fachman, & Kraut, 2000).

Sensorial analysis of the dehydrated mixture and alfajores

Sensorial analysis was undertaken in individual white-painted cabins, under a white light, imitating daylight. The products were packed in aluminum paper analysis and coded randomly with three identification digits for sensorial analysis after 24 hours. The alfajores (0, 5, 10 and 15%) were given to 50 non-trained tasters who assessed the sensorial attributes of their aroma, color, taste, texture, coupled to a general impression of the product.

Tasters received the samples (a sub-sample of each treatment), a card for the assessment of sensorial analysis and a glass of mineral water at room temperature to evaluate the samples. The card contained two evaluation forms of the product, comprising a 1 - 9 score hedonic scale (1 = I disliked it; 9 = I liked it very much) (Dutcosky, 2007) to evaluate sensorial attributes, and a 5-score purchasing intention scale, with 5 as maximum score (I will surely buy it) and 1 as the minimum score (I won't buy it), following procedures by Damásio & Silva (1996).

University staff and students were invited to be tasters by means of advertisement fixed on the university premises. The first 50 people who declared they had no health problems with fish were enrolled.

Experimental design

Scores from the sensorial analysis and proximate composition were submitted to Univariate of SAS (Statistical Analysis System, SAS Inst. Inc. Cary, NC, USA) to verify the presuppositions of the analysis of variance. The results of variables analyzed were underwent analysis of regression compared 5% probability.

Since microbiological tests merely characterized the product, or rather, to verify whether they were fit for human consumption, statistical analysis was not performed.

Results and discussion

Microbiological analysis of dehydrated mixture and alfajor

Results of microbiological analysis of dehydrated mixture and alfajor are showed in Table 2. The alfajor of the four treatments were proper for human consumption, following established by RDC no. 12, of the Brazilian Sanitary Agency of the Health Ministry (Brasil, 2001). The above shows that dehydrated mixture and the different alfajor prepared with several inclusion levels of fish dehydrated mixture were of good quality, without contamination. They were thus prepared within the concepts of good practice in handling and manufacturing.

Table 2. Microbiological analysis of mixture and alfajor with inclusion of fish dehydrated mixture of salmon and tilapia.

	Dehydrated mixture of fish	Inclusion levels			
		0%	5%	10%	15%
Coliforms at 35°C (MPN _g ⁻¹)	<3	<3	<3	<3	<3
Coliforms at 45°C (MPN _g ⁻¹)	<3	<3	<3	<3	<3
<i>Staphylococcus</i> coagulase positive (CFU _g ⁻¹)	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Salmonella</i> sp. 25g	Absent	Absent	Absent	Absent	Absent

CFU_g⁻¹ – Colony-Forming Unitsgram⁻¹; MPN_g⁻¹ – Most Probable Numbergram⁻¹.

Analysis of proximate composition of dehydrated mixture and alfajor

Dehydrated mixture of salmon and tilapia used in the inclusion of alfajor comprised 10% salmon and 90% tilapia, had 6.51±0.35% of moisture; 49.64±0.36% of crude protein; 12.85±0.05% of ether extract; 32.10±2.60% of ash. Different levels of the above mentioned dehydrated mixture was used in alfajores, consequently, regardless of the mixture's inclusion level, there was an increase in nutrients in the final product, as may be seen in

Table 3. Dehydrated fish mixture prepared from the carcasses of salmon (10%) and tilapia (90%) had high levels of crude protein and minerals and thus corroborated results by Vidal, Rodrigues, Zapata, and Vieira (2011), reported that tilapia fishmeal had 62.39% protein. However, protein and ash may vary according to the sources of carcasses, method by which fish were fed, type of filleting and fish species.

Table 3 shows results of the analysis of the proximate composition of alfajores, with significant difference ($p < 0.05$) for humidity, crude protein, ashes and carbohydrates. There was no difference ($p > 0.05$) for the product's lipid and calorie rates (Table 3).

Linear effects ($p < 0.01$) were observed for crude protein ($y = 5.52 + 0.06x$, $R^2 = 0.72$) and carbohydrates ($Y = 75.64 - 0.11x$, $R^2 = 0.79$) contents. This helped to verify that with an increase in the levels of dehydrated mixture, the crude protein increased linearly (5.35 to 7.12%), whereas the carbohydrate content decreased (75.79 to 72.79%). Moisture ranged between 2.74 and 3.40%, regardless of treatment; and ashes between 0.31 and 1.01%, with an increase proportional to the rise in inclusion levels of dehydrated mixture of fish in alfajor. Consequently, equations were linearly positive for crude protein ($R^2 = 0.72$) and quadratic for humidity ($R^2 = 0.67$) and ashes ($R^2 = 0.56$).

Table 3. Analysis of proximate composition and calorie rates of alfajores with inclusion of fish dehydrated mixture of salmon and tilapia.

	Inclusion levels				P value
	0%	5%	10%	15%	
Moisture	2.74±0.21	3.11±0.11	3.40±0.15	3.22±0.31	0.005 ¹
Crude protein	5.35±0.26	6.25±0.24	6.96±0.30	7.12±0.74	<0.0001 ²
Ether extract	15.82±0.46	15.02±0.23	16.28±0.28	15.87±0.37	0.28
Ash	0.31±0.08	0.93±0.05	0.71±0.10	1.01±0.08	0.001 ³
Carbohydrates	75.79±0.48	74.71±0.35	72.64±0.23	72.79±0.76	<0.0001 ⁴
Calorie(Kcal100g ⁻¹)	466.89±1.89	458.94±1.47	464.94±1.62	462.40±1.81	0.35

Data given in means ± standard deviation. ¹($Y = 2.72 + 0.058x - 0.0014x^2$, $R^2 = 0.67$), ²($Y = 5.52 + 0.06x$, $R^2 = 0.72$), ³($Y = 0.37 + 0.04x - 0.0008x^2$, $R^2 = 0.56$), ⁴($Y = 75.64 - 0.11x$, $R^2 = 0.79$).

The inclusion of fish dehydrated mixture increased crude protein and mineral rates in the alfajores. Dehydrated mixture of salmon and tilapia contained 49.64% crude protein, and 32.10% ash. Thus, the inclusion of this ingredient in the alfajores was responsible for the linear increase in protein and ash, and negative linear equation in carbohydrates.

Proteins from tilapia meat have high nutritional value with a balance of essential amino acids, especially rich in lysine (Pizato, Kraieski, Sarmiento, & Prentice, 2012). Increase in mineral rates may be possibly due to the spine of the carcasses of the tilapia and salmon used as prime

matter in the preparation of the protein concentrates. Further, mineral enrichment in alfajores is also due to the amount of minerals in sea water fish (calcium, phosphorus, iron, and others) (Ariño et al., 2013).

Franco et al. (2013) assessed chocolate cookies with the inclusion of tilapia fishmeal at different levels (0, 10, 15, 20, 25 and 30%) and obtained a product with 6.25- 7.88% humidity; 9.41-15.30% crude protein; 8.21-10.37% ether extract; 1.44-6.43% ashes and a decrease in carbohydrate rates from 74.13 to 61.59%, caused by the respective inclusions of fishmeal in chocolate cookies. Results by Franco et al. (2013) showed that the inclusion of fishmeal also increased the rates of crude protein and minerals and reduced carbohydrate rates, similar to what occurred in the alfajor experiment. Also, Justen et al. (2011) developed snacks with flavored tilapia carcass meal, observing that the addition of up to 12% increases the contents of proteins, lipids, minerals, without affecting sensory characteristics of the product.

Sensorial analysis of alfajores

Ingredients, such as chocolate and sweetened milk as stuffing matter, associated with characteristics proper to the mixture (less intense fish odor due to a higher percentage of tilapia fishmeal and technological method in the preparation of the fishmeal), provided satisfactory results for the alfajor, which may be seen from tasters' scores on the product (Table 4).

When the sensorial analysis of alfajores is taken into account, the scores of tasters that evaluated the product with the inclusion of fish dehydrated mixture showed that there was no influence on the sensorial attributes under analysis (aroma, taste, texture, color, acceptance and purchase intention). The product was well received by the tasters at all inclusion levels. Scores varied between 6.70 and 7.96, which correspond to "I liked the product slightly" and "I like the product a lot".

There was no significant difference in the purchase intention of alfajores, with scores by the non-trained taster reaching between 3.72 and 4.00. In the 5-score scale, this means "I may probably buy the product" (Table 4).

Purchase intention revealed that approximately 40% of tasters "would certainly buy" the alfajor with a 15% inclusion of dehydrated mixture and only less than 5% of the population "would not purchase" the product. There was a good acceptance of the product with the inclusion of fish dehydrated mixture.

Table 4. Sensorial analysis of alfajor with the inclusion of fish dehydrated mixture of salmon and tilapia.

Attributes	Inclusion levels				P value
	0%	5%	10%	15%	
Aroma ¹	7.76±1.35	7.46±1.14	7.30±1.55	7.54±1.37	0.359
Taste ¹	7.51±1.82	7.10±1.68	6.96±1.77	7.32±1.80	0.534
Texture ¹	7.22±1.69	7.08±1.3	6.70±1.66	7.30±1.38	0.912
Color ¹	7.89±1.37	7.56±1.37	7.46±1.54	7.96±1.19	0.882
General acceptance ¹	7.53±1.62	7.18±1.35	6.98±1.36	7.34±1.75	0.436
Purchasing intention ²	4.00±1.29	3.88±1.09	3.72±1.19	3.96±1.10	0.647

Data given in means ± standard deviation. ¹Hedonic scale between 1 (I didn't like it very much) and 9 (I liked it very much). ²Hedonic scale between 1 (I will surely not buy it) and 5 (I will surely buy it).

Since the tasters failed to detect the taste of the fish, the aroma associated with the fish mixture, and changes in texture and color, the results were high scores in the 5-score scale by Damásio and Silva (1996).

Results of sensorial analysis revealed no significant effect of the inclusion level of dehydrated mixture on the product (Table 4). Consequently, up to 15% inclusion of dehydrated mixture may be used in the alfajor. The dehydrated mixture may be added to the alfajor, a candy product, to raise the latter's nutritional rate, without affecting its sensorial characteristics.

Few assays with the inclusion of fish or fish protein concentrates have been performed in candy products. Veit et al. (2012), who developed and characterized chocolate and carrot cakes with tilapia fillets, reported the possibility of including fish in candies such as cakes, cookies, sweet bread and which may be given in children's school meals. Veit et al. (2012) evidenced that chocolate and carrot cake with tilapia filets was greatly accepted. In fact, averages were close to maximum score of the five-score scale by Dutcosky (2007) mentioned above. In the alfajor assay with chocolate icing and stuffing with sweetened milk, the typical fish's taste and aroma failed to emerge in the prepared candy and good results in organoleptic characteristics could be displayed in the evaluated product.

Inclusion of more fishmeal depends on the type of candy used. According to Franco et al. (2013), who assessed chocolate cookies with fishmeal from tilapia carcass, up to 30% may be included without interfering in the acceptance of the prepared product. On the other hand, the same authors reported that a maximum of 12% inclusion may be added to homemade biscuits.

Food enriched with a dehydrated mixture of fresh (tilapia) and sea water fish (salmon) may endorse high expectations for adequate feeding due to its sources of crude protein, minerals and essential amino acids and polyunsaturated fatty acids, mainly Omega 3. This is especially true when one observes the results of the centesimal

composition in the dehydrated mixture of salmon and tilapia, as referred to above.

Results show that the inclusion of dehydrated fish mixture in candies for nutritional enrichment is feasible and acceptable. Further research may be undertaken which includes different aspects, such as people in different age brackets, other inclusion levels and the preparation of a mixture with more salmon fishmeal. Further, although current assay included 10% of salmon fishmeal, in other experiments 20% for the product may be included.

Conclusion

The dehydrated mixture of fish and alfajores complied with Anvisa standards and thus proper for human consumption.

The alfajor prepared with a dehydrated mixture of fish was well-accepted by tasters at all levels of inclusion, besides the inclusion of fish mixture with increasing protein and mineral enrichment by an increase in the product's inclusion levels. Results show that up to 15% of dehydrated mixture of salmon (10%) and tilapia (90%) may be included.

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