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Inclusion of dehydrated mix of tilapia and salmon in pizzas

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Abstract

The objective of this study was to evaluate the nutritional, sensorial and microbiological characteristics of pizzas with the inclusion of dehydrated mix of salmon and tilapia in the dough. Tilapia and salmon carcasses were cooked, pressed, ground, dehydrated and crushed, and a mixture of salmon (20%) and tilapia (80%) was made. This mix was included in the pizza dough at different levels (0%, 5%, 10%, 15% and 20%) and afterwards the toppings were added.In the dough composition, protein, ash and phosphorus levels rose linearly, while lipids, carbohydrates and caloric value of the dough samples decreased linearly as the mix levels increased. In pizzas, the moisture, lipids and carbohydrates contents were not different. Protein and ash contents presented a linear behavior. The inclusion of the mix was effective in reducing the saturated fatty acids and raising the content of polyunsaturated fatty acids. There was no difference between the pizzas for the sensorial attributes analysed. Therefore, the inclusion of up to 20% of dehydrated mix of tilapia and salmon added protein, minerals, and polyunsaturated fatty acids and maintained the acceptance of the pizza dough samples, which makes possible the preparation of a food product with health claims and good sensorial quality.

Keywords: chemical composition; filleting waste; fish protein concentrate; sensory analysis.

Practical Application: Pizzas with inclusion of dehydrated mix of fish have high nutritional value and great sensory profile.

1 Introduction

In recent decades, lifestyle changes in many countries have led to a number of impacts on food consumption patterns, such as the frequent consumption of meals outside the home environment, or even those consumed at home are often purchased ready (Jaworowska et al., 2013). Pizza is one of the most popular ready meals, particularly among adolescents and young adults (Nielsen et al., 2002).

The consumption of take-out food and fast food is often related to higher intakes of energy, fat, saturated fatty acids, trans fatty acids, sugar, sodium, and lower intakes of fiber, macronutrients and vitamins (Paeratakul et al., 2003; Bowman et al., 2004; Jaworowska et al., 2013). Thus, several studies have been conducted with the aim of improving the nutritional profile of these foods, which can have a valuable impact on health problems (Combet et al., 2013).

Considering these facts the incorporation of fish nutrients into ready-to-eat products can be effective to increase the protein content, fatty acid profile and mineral composition of these foods (Campelo et al., 2017; Kimura et al., 2017). The health benefits of fish consumption are mainly due to the high quality protein content, vitamins and other essential nutrients (Domingo, 2014). In addition, unlike other types of meat, fish are rich in polyunsaturated fatty acids, especially the n-3 (omega-3) series in marine fish (Wang et al., 1990). Despite this, fish consumption in Brazil is still low, reaching an average of only 10.87 kg/inhab/year in 2013 (Food and Agriculture Organization Corporate Statistical Database, 2016). The development of products with fish inclusion has the potential to leverage its consumption and consequent aquaculture production.

In pizza, the flexibility in the recipe, especially when it comes to the options of toppings and modification in the dough formulation, allows the introduction of functional ingredients to obtain an improved nutritional composition (Singh & Goyal, 2011). Thus, this work aimed to evaluate the chemical composition, sensorial and microbiological profile of pizzas with inclusion of dehydrated mix of salmon and tilapia in the dough.

2 Materials and methods

2.1 Preparation of the dehydrated salmon and tilapia mix

Nile tilapia (*Oreochromis niloticus*) carcasses obtained from Smart Fish (Rolândia / PR) and Salmon carcasses (*Salmo salar*) donated by Tomita & Tomita Company (Maringá/PR) were used. The carcases or spines were made up of the vertebral column with ribs, without fins and with the meat remaining from filleting the referred fish.

The carcasses were washed and sanitized with proxitane 1512 (0.1 mg kg^{-1} of prime matter volume), weighed and

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cooked for 60 minutes in a pressure cooker with the antioxidant BHT (0.5 mg kg^{-1}). Then they were pressed (hydraulic press with capacity of 10 tons) and ground. After that, the obtained products were dehydrated in a drying oven at 60 °C for 24 hours, and milled in a knife-type mill (Willye-model TE-650). Finally, a dehydrated mix was made by mixing 20% of salmon flour and 80% of tilapia flour. This mix was used to prepare the pizza dough.

2.2 Processing of pizza dough

The dough was prepared according to the ingredients presented in Table 1, and the inclusion of 0%, 5%, 10%, 15% and 20% dehydrated mix was performed on the total amount of wheat flour used. For the preparation, all ingredients were placed in a vessel and mixed manually until complete homogenization. The dough was rolled out into circles, about one centimeter thick, and brought to the electric oven at 180 °C for 15 min for pre-baking.

2.3 Preparing the pizzas

For each dough with different levels of inclusion of dehydrated mix of tilapia and salmon, pizzas were prepared by adding tomato sauce (113 g), layers of ham (156 g), mozzarella (178 g), tomato (100 g), onion (56 g), oregano (0.5 g), and basil (1 g) in the same proportion for all pizzas. The pizzas were baked in oven at 200 °C for 15 min and then partitioned and directed to physicochemical, microbiological and sensorial analyses.

2.4 Centesimal composition, water activity, pH and fatty acid profile

Physicochemical analyses of the salmon and tilapia dehydration mix, of the dough samples and prepared pizzas, were performed in triplicates.

Moisture and ash contents were determined according to the methodology of the Association of Official Analytical Chemists (2005). The crude protein contents were evaluated by the Kjeldahl semi-micro method (Silva & Queiroz, 2002).

Table 1. Formulation of the pizza dough with inclusion of different levels of salmon and tilapia dehydrated mix.

Ingredients (%)	Inclusion levels of fish dehydrated mixture[1] (%)				
	0	5	10	15	20
Wheat flour	54	52	49	46	43
Salmon and tilapia dehydrated mix	0	3	5	8	11
Soybean oil	2	2	2	2	2
Margarine	2	2	2	2	2
Egg	6	6	6	6	6
Salt	1	1	1	1	1
Sugar	1	1	1	1	1
Milk	27	27	27	27	27
Active dry yeast	5	5	5	5	5
Total (%)	100	100	100	100	100

[1]Levels of the dehydrated mixture (0%, 5%, 10%, 15% and 20%) added according to the weight of the wheat flour in the dough pizza.

Lipid extraction was performed according to the methodology of Bligh & Dyer (1959).

The determination of total phosphorus was performed using ammonium phosphomolybdate by UV-VIS spectrophotometry, according to Eijsink et al. (1997)

The water activity of the dehydrated mix and the pizza dough was determined using the Aw Sprint - Novasina TH-500.

Ten grams samples were homogenized with distilled water (1:10 sample / water) for pH measurement. This homogenate was submitted to the pH meter electrodes (DM 22, Digimed, São Paulo, Brazil) for 5 minutes, when the pH was read (Instituto Adolfo Lutz, 1985).

For the prepared pizzas with inclusion of different levels of salmon and tilapia dehydrated mix in the dough, the determination of the fatty acid profile was carried out. Initially, total lipids were extracted from the samples to identify fatty acids (Bligh & Dyer, 1959). The samples were then transesterified according to ISO (International Organization for Standardization, 1978) methodology. The methyl esters were separated on a 14-A gas chromatograph (Shimadzu, Japan), equipped with flame ionization detector and fused silica capillary column (100 m x 0.25 mm di x 0.25 μm, CP-Sil 88). The peak areas (percentages of relative areas) were integrated by a CG-300 integrator-processor (scientific instruments). The fatty acid identifications were performed by the following criteria: comparison of the methyl esters retention times of the sigma (US) standards with those of the samples and comparison of the ECL (Equivalent Chain Lenght) values of the methyl esters of the samples with values of the literature of Visentainer (2003), Stránsky et al. (1997) and Silva (2000).

2.5 Sensory analyses of prepared pizzas

Sensory analysis was conducted with 50 untrained tasters, randomly selected and with no knowledge of sample composition. Methods of sensory analysis were approved by the Committee for Ethics in Research on Human Beings (COPEP) of the Universidade Estadual de Maringá, Maringá PR Brazil (CAAE: 14219213.1.0000.0104).

The attributes aroma, flavor, color, texture and general acceptance were evaluated using a structured hedonic scale of nine points, anchored between minimum and maximum: I disliked it extremely (1) and I liked it extremely (9) (Dutcosky, 2011). In addition, the purchase intention was evaluated with the 5-point hedonic scale, 1 representing the minimum grade (would certainly not buy) and 5 representing the maximum grade (would certainly buy), according to Dutcosky (2011).

Samples were offered to the judges on disposable plastic dishes, and identified with three random numbers each. They were also offered a glass containing water with the sensory analysis card, for the removal of residual taste in the mouth.

2.6 Microbiological analyses

Microbiological analyses were performed for the most probable number (MPN) of total coliforms/gram, coagulase-positive *Staphylococcus* count in CFU/gram and *Salmonella spp*, according to APHA (Americam Public Health Association, 1992). The microbiological protocol followed the standards recommended by Resolution RDC No. 12 of January 2, 2001, of the National Health Surveillance Agency (Brasil, 2001).

2.7 Statistical analyses

For the statistical analyses of the sensorial results, the generalized linear models methodology (GENMOD procedure) was used, considering the distribution of the variables as being gamma with inverse linking function, using the Statistical Analysis System program (SAS, SAS Inst. Inc Cary, NC, USA). The treatment effect and tasters were considered, testing inclusion levels by regression analysis. For the other evaluated parameters, the regression analysis was used at 5% level of significance. For the profile of fatty acids, pH and microbiological analysis a descriptive analysis of the data was performed.

3 Results and discussion

3.1 Characterization of the pizza dough samples with inclusion of dehydrated tilapia and salmon mix

In the composition of the dough samples (Table 2), the moisture content was not different between treatments (P> 0.05). For the other parameters, linear effects (P < 0.01) were observed for protein, lipid, ashes, carbohydrate and caloric content.

It was found that with increasing levels of 0% to 20% of the dehydrated mix in the dough, crude protein and ash values rose linearly. The protein content increased from 14.43 to 18.59%, ash content went from 3.55 to 11.27%, with inclusion levels from 0 to 20% of the dehydrated mix. The mix used in the pizzas had 48.57% of crude protein and 30.89% of ashes (Table 2). Thus, the inclusion of this ingredient in the pizza dough was able to increase its nutritional value linearly.

The increase in the protein content in the pasta with inclusion of the dehydrated fish mix is important because fish and its derivatives have proteins of high biological value, easy digestibility and significant quantities of essential amino acids, particularly lysine, amino acid present in low quantity in cereals (Jabeen & Chaudhry, 2011). A previous study evaluating the amino acid composition of tilapia and salmon flours (Souza et al., 2017), demonstrated that these flours not only contain all the essential amino acids, but that the amino acids content is significantly above the standard required by the FAO Food and Agriculture) for children and adults (World Health Organization, 2007).

The increase in ash content is due to the process of manufacturing the dehydrated mix of tilapia and salmon carcasses, which aggregates the nutrients and especially the minerals naturally present in the fish. Therefore, the highest levels of ash found in the dough samples with inclusion of dehydrated fish mix are important because they are the source of several essential minerals. Fish meat is considered a valuable source of minerals, particularly calcium and phosphorus (Simões et al., 2007; Causeret, 1962). In the pizza dough the phosphorus content (Table 2) presented a positive linear effect (P < 0.05), with an increase in mean values as the inclusion levels rose (from 0.08 to 0.34 mg/kg for 0% and 20% inclusion of dehydrated mix, respectively).

The lipid, carbohydrate and caloric content in the dough samples decreased linearly as the levels of dehydrated mix of tilapia and salmon increased. Lipids decreased from 11.69% (0% inclusion) to 6.77% (dough with 20% mix). Carbohydrates ranged from 54.61% (0% inclusion) to 42.39% (mass with 20% mix), while the caloric value decreased from 381.37 kcal/100 g (0% inclusion) to 304.78 kcal/100 g (20% inclusion). The lower caloric value is a consequence of the reduction of carbohydrates and lipids and the increase in the protein content, which resulted in better nutritional value of the pizzas dough with inclusion of dehydrated mix of tilapia and salmon. The decrease in the caloric value of the food through the inclusion of fish mix was also observed for other food products, such as lasagna (Kimura et al., 2017), pasta (Goes et al., 2016), extruded snacks (Justen et al., 2017) and onion sticks (Coradini et al., 2015).

The water activity (Aw) in the dough with inclusion of dehydrated mix of tilapia and salmon varied between 0.354 and 0.378, while the pH ranged from 5.76 to 5.91. These dough samples can be considered fresh, since, in pizza dough, the pH around 5.80 indicates the freshness of the product (Singh et al., 2012). The values found for Aw were low, and therefore, the pizza dough samples were considered as food with low Aw. This factor is important since foods with low Aw (<0.85) have clear

Table 2. Centesimal composition, calorific value, phosphorus content, water activity and pH of the dough samples with inclusion of tilapia and salmon dehydrated mix.

Parameters	Dehydrated mix	Tilapia and salmon dehydrated mix levels (%)					P. value
		0	5	10	15	20	
Moisture (%)	6.00 ± 0.10	15.73 ± 0.08	19.43 ± 0.06	18.18 ± 0.19	15.48 ± 0.18	21.00 ± 0.03	0.21
Protein (%)	48.57 ± 0.04	14.43 ± 0.06	14.74 ± 0.20	17.42 ± 0.9	17.00 ± 0.23	18.59 ± 0.35	<0.01[1]
Lipids (%)	12.95 ± 0.05	11.69 ± 0.00	6.84 ± 0.00	7.10 ± 0.00	7.13 ± 0.00	6.77 ± 0.00	<0.01[2]
Ashes (%)	30.89 ± 0.21	3.55 ± 0.24	5.44 ± 0.06	9.21 ± 0.02	6.51 ± 0.06	11.27 ± 0.08	<0.01[3]
Carbohydrates (%)	1.62 ± 3.03	54.61 ± 0.39	53.56 ± 0.20	48.11 ± 0.03	53.83 ± 0.48	42.39 ± 0.46	<0.01[4]
Caloric value (kcal/100g)	317.22 ± 0.24	381.37 ± 1.30	334.76 ± 0.00	325.95 ± 0.69	347.69 ± 1.00	304.78 ± 0.46	<0.01[5]
Phosphorus (mg/kg)	NA	0.08 ± 0.00	0.20 ± 0.01	0.37 ± 0.01	0.18 ± 0.05	0.34 ± 0.03	<0.04[6]
Water activity	0.354	0.354	0.378	0.369	0.375	0.375	-
pH	6.46	5.76	5.75	5.91	5.86	5.91	-

[1]Linear regression $y = 0.2128x+14.316$ $R^1 = 0.8603$; [2]Linear regression $-0.1911x+9.815$ $R^2 = 0.5068$; [3]Linear regression $y = 0.33x+3.895$ $R^2 = 0.7254$; [4]Linear regression $y = -04833x+55.329$ R^2 0.5334; [5]Linear regression $y = -2.8048x+366.96$ $R^2 = 0.6083$; [6]Linear regression $y = 0.0101x+0.131$ $R^2 = 0.4252$; NA = not analysed.

advantages in controlling the growth of foodborne pathogens, because the minimum Aw at which microorganisms can grow is 0.60 (Beuchat et al., 2013).

3.2 Characterization of prepared pizzas

In the analysis of the centesimal composition of prepared pizzas, containing five levels of inclusion of tilapia and salmon dehydrated mix (Table 3), caloric value, moisture, lipid, carbohydrate and phosphorus contents were not different $P > 0.05$) among the pizzas. However, both crude protein and ash presented a linear behavior ($P < 0.01$), with a gradual growth as the inclusion levels of dehydrated mix in the dough increased.

The protein content rose from 10.85% (0% inclusion) to 17.00% (20% inclusion). Considering that in commercial pizzas the protein content generally ranges from 10% to slightly more than 14% (Singh & Goyal, 2011), it can be seen that the inclusion of the dehydrated mix is effective to increase and improve the protein profile in pizzas.

The ash content ranged from 1.67% to 5.33% (0 and 20% inclusion, respectively), indicating an increase in minerals from the fish in the prepared pizza.

In the lipid profile, 19 fatty acids were identified in the prepared pizzas with different inclusion levels of dehydrated salmon and tilapia mix (Table 4). The major fatty acids in the

Table 3. Centesimal composition, caloric value and phosphorus content of prepared pizzas made with dough with different levels of tilapia and salmon dehydrated mix.

Parameters	Tilapia and salmon dehydrated mix levels (%)					P value
	0	5	10	15	20	
Moisture (%)	53.48 ± 3.93	45.65 ± 1.47	46.81 ± 3.43	48.81 ± 4.82	40.10 ± 0.47	0.07
Protein (%)	10.85 ± 0.47	12.85 ± 0.61	13.89 ± 0.72	15.01 ± 0.97	17.00 ± 0.51	<0.01[1]
Lipids (%)	5.48 ± 0.54	5.11 ± 0.2	4.87 ± 0.46	5.28 ± 0.48	6.64 ± 0.94	0.15
Ashes (%)	1.67 ± 0.08	2.97 ± 0.18	4.17 ± 0.25	3.34 ± 0.45	5.33 ± 0.47	<0.01[2]
Carbohydrates(%)	28.52 ± 3.01	33.43 ± 0.98	30.15 ± 2.33	27.93 ± 3.38	30.94 ± 1.62	0.82
Caloric value(kcal/100 g)	206.8 ± 3.52	231.12 ± 2.39	219.94 ± 4.63	219.25 ± 3.89	251.46 ± 2.50	0.16
Phosphorus	0.2 ± 0.04	0.4 ± 0.05	0.39 ± 0.14	0.31 ± 0.08	0.21 ± 0.06	0.70

[1]Linear regression $y = 0.1883x + 11.48$ $R^2 = 0.59$; [2]Linear regression $y = 0.094x + 2.2554$ $R^2 = 0.52$.

Table 4. Fatty acid profile of prepared pizzas made with dough containing different levels of tilapia and salmon dehydrated mix.

Fatty acids	Tilapia and salmon dehydrated mix levels (%)				
	0	5	10	15	20
10:0	1.64	1.26	1.02	1.2	1.67
12:0	2.37	2.1	1.81	2	2.5
14:0	8.2	7	4.94	6.27	7.24
16:0	25.82	24.76	19.58	22.71	23.85
16:1n-7	1.3	1.18	1	1.17	1.21
17:00	0.54	0.49	0.34	0.46	0.37
17:1 n-9	0.22	0.19	0.16	0.18	0.18
18:0	12.21	11.88	9.11	10.4	9.62
18:1n-9 c	22.25	22.74	20.57	21.62	20.4
18:1 n-7	3.11	2.97	2.25	2.68	2.46
18:2n-6	11.09	14.33	18.88	16.47	14.61
18:3n-3	1.21	1.44	2.07	1.72	1.52
CLA	0.74	0.67	0.54	0.58	0.52
20:1 n-9	0.25	0.32	0.41	0.32	0.5
20:2 n-6	0.1	0.1	0.25	0.38	0.39
20:3 n-9	0.07	0.08	0.15	0.21	0.11
20:4	0.15	0.2	0.41	0.45	0.4
20:5 n-3	0.06	0.14	0.29	0.19	0.25
22:00	0.13	0.18	0.27	0.23	0.17
SFA	50.91	47.67	37.07	43.27	45.42
MUFA	27.13	27.40	24.39	25.97	24.75
PUFA	13.42	16.96	22.59	20.00	17.80
Sum n-3	1.27	1.58	2.36	1.91	1.77
Sum n-6	11.19	14,43	19.13	16.85	15.00
PUFA/SFA	0.26	0.36	0.61	0.46	0.39
n6/n3	8.81	9.13	8.11	8.82	8.47

CLA = conjugated linoleic acid; SFA = sum of saturated fatty acids; MUFA = sum of monounsaturated fatty acids; PUFA = sum of polyunsaturated fatty acids; n-6 and n-3 = sum of n-6 and n-3 fatty acids series, respectively; PUFA/SFA = ratio between the sum of polyunsaturated and saturated acids; and n-6/n-3 = ratio between the sum of n-6 and n-3 series acids.

Table 5. Sensory analysis of prepared pizzas with the inclusion of dehydrated salmon and tilapia mix in the dough.

Sensory attributes	Tilapia and salmon dehydrated mix levels (%)					P value
	0	5	10	15	20	
Aroma[1]	8.03 ± 1.21	7.88 ± 1.38	7.78 ± 1.26	7.82 ± 1.32	8.01 ± 1.14	0.65
Taste[1]	8.14 ± 1.42	7.99 ± 1.60	7.93 ± 1.43	8.05 ± 1.34	8.11 ± 1.39	0.89
Color[1]	7.99 ± 1.19	8.11 ± 0.99	7.89 ± 1.04	7.99 ± 1.03	8.07 ± 1.02	0.77
Texture[1]	8.08 ± 1.31	7.96 ± 1.27	7.78 ± 1.39	7.83 ± 1.37	8.04 ± 1.19	0.55
General Impression[1]	8.05 ± 1.11	7.91 ± 1.38	7.95 ± 1.19	7.97 ± 1.21	8.13 ± 1.15	0.79
Purchase Intention[2]	4.52 ± 0.81	4.43 ± 0.84	4.29 ± 0.94	4.36 ± 0.99	4.42 ± 0.94	0.56

[1]Hedonic scale from 1 (I disliked it extremely) to 9 (I liked it extremely); [2]Hedonic scale from 1 (I would certainly not buy) to 5 (I would certainly buy). Values expressed in Average ± Standard Deviation.

pizzas were palmitic, oleic and linoleic acid. This must have occurred because of the higher content of these in the dehydrated mix. In addition, soybean oil was used in the formulation of the dough, which, like other vegetable oils (sunflower, safflower, corn, soybean, cotton), has a high content of n-6 fatty acids, mainly linoleic acid (18: 2n-6) (Novello et al., 2008).

The pizzas with inclusion of the dehydrated mix in the dough presented an increase in the polyunsaturated fatty acids contents and a decrease in the levels of saturated fatty acids when compared to the control pizzas. In particular, the sum of fatty acids of the ω-3 series grew in the pizzas with the dehydrated mix. It is emphasized that polyunsaturated fatty acids are important for maintaining the integrity of the membranes of all living cells; for the production of prostaglandins that regulate many body processes, such as inflammation and blood clotting (Jabeen & Chaudhry, 2011). Therefore, the referred pizza dough samples can contribute to a balanced diet by providing a strategy to increase the ingestion of ω-3 series fatty acids.

Pizza dough prepared with tuna dehydrated mix also presented improvements in the lipid profile, ω-3 series fatty acids went up and saturated fatty acids decreased (Campelo et al., 2017), which shows the potential of including fish mix in the development of high nutritional value products.

In the sensory analysis of the prepared pizzas made from the dough with different levels of tilapia and salmon dehydrated mix no significant differences were observed (P > 0.05) for the sensorial attributes analysed (Table 5). The purchase intention was also not affected by the different treatments (P > 0.05). These results suggest that up to 20% of the dehydrated mix can be included in the pizza dough without sensory impairment.

These results are important, since in a previous study with levels of inclusion of tuna flour in pizza dough (0, 5, 10, 15 and 20%), it was observed that, despite the improvement in nutritional profile, the inclusion of tuna flour decreased the sensorial acceptance, indicating a maximum of 5% inclusion of tuna mix (Campelo et al., 2017). On the other hand, the sensorial analysis in the tuna mix study was performed considering the consumption of the dough itself, while in this experiment the sensory analysis was performed considering the prepared pizza (with toppings).

The results obtained in the microbiological analysis of the pizzas showed <3 NMP/g of coliforms at 35 °C and coliforms at 45 °C for all the pizzas with different levels of inclusion of dehydrated mix of tilapia and salmon. The pizzas also presented less than 1x10 2 CFU/g of *Staphylococcus* coagulase positive, absence of *Salmonella sp*. in 25 g of sample and counting of less than 1x10 2 CFU/g of *Bacillus cereus*, showing that all pizzas were fit for human consumption, indicating that the samples were produced under the necessary conditions of hygiene, within the microbiological standards required by the legislation (Brasil, 2001).

4 Conclusions

The inclusion of up to 20% of dehydrated mix of tilapia (80%) and salmon (20%) added protein, mineral matter, polyunsaturated fatty acids and maintained the sensorial acceptance of the pizza dough samples, which makes possible the preparation of a food with health claims and sensory quality. The pizzas presented microbiological standards within those established by the legislation, being suitable for consumption.

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