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Physicochemical stability of bread fortified with tilapia-waste flour

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ABSTRACT

The physicochemical stability of six bread formulations with different tilapia flour (BTF) levels (0%, 2.5%, 5%, 10%, 15%, and 20%) in substitution to wheat flour was investigated regarding moisture content, water activity (a_w), pH, instrumental color parameters, texture profile, lipid and protein oxidation on days 0, 2, 4, 6 and 8 at 25°C. BTF10%, BTF15%, and BTF20% displayed lower ($P < 0.05$) moisture, a_w , lightness index, cohesiveness, springiness and resilience, and higher ($P < 0.05$) pH, redness index, yellowness index, hardness, chewiness, lipid and protein oxidation compared to BTF0%, BTF2.5%, and BTF5%. Nevertheless, pH drops, lipid and protein oxidation were less pronounced ($P < 0.05$) for BTF10%, BTF15%, and BTF20% during storage. Although quality loss has been observed in breads containing $\geq 10\%$ TF, bread at 5% TF did not display altered traditional wheat bread physicochemical characteristics and may be an attractive alternative for the health food market.

Estabilidad fisicoquímica del pan fortificado con harina de residuos de tilapia

RESUMEN

En este estudio se investigó la estabilidad de seis formulaciones de pan en cuya elaboración se sustituyó la harina de trigo con distintos niveles de harina de tilapia (BTF) (0%, 2.5%, 5%, 10%, 15% y 20%). La estabilidad de las formulaciones se midió en términos de su contenido de humedad, actividad de agua (a_w), pH, parámetros de color instrumental, perfil de textura, oxidación lipídica y proteica, en los días 0, 2, 4, 6 y 8 de su almacenamiento a una temperatura de 25°C. Se comprobó que, en comparación con los niveles BTF0%, BTF2.5% y BTF5%, los niveles BTF10%, BTF15% y BTF20% mostraron niveles de humedad, actividad de agua (a_w), índice de ligereza, cohesión, elasticidad y resiliencia más bajos ($P < 0.05$), mientras que los niveles de pH, el índice de rojez, el índice de amarillez, la dureza, la masticabilidad, así como la oxidación lipídica y proteica fueron más altos ($P < 0.05$). Sin embargo, para BTF10%, BTF15% y BTF20%, se constató que los descensos de pH y la oxidación lipídica y proteica durante su almacenamiento fueron menos pronunciados ($P < 0.05$). Si bien al compararse las características fisicoquímicas de los panes obtenidos con las del pan de trigo tradicional se observó pérdida de calidad en los panes con TF $\geq 10\%$, en los panes con un nivel de TF de 5% no se presentaron alteraciones de estas características. Ello significa que pueden ser una atractiva alternativa para el mercado de alimentos saludables.

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1. Introduction

The nutritional enhancement of food products has attracted attention due to increased interest for healthier foods in recent years (Shandilya & Sharma, 2017). Bread is the most staple cereal-based food consumed worldwide (Bagdi et al., 2016). However, bread is constituted of flour, water, and yeast, poor in protein and rich in carbohydrates, with a high glycemic index, which can lead to obesity and susceptibility to diabetes and biliary-tract cancer (Askari et al., 2013; Larsson, Giovannucci, & Wolk, 2016). On the other hand, fish and fish by-products are highly nutritional foods, due to their high protein and essential fatty acid amounts (FAO, 2016; Monteiro et al., 2016).

Among fish species, Nile tilapia (*Oreochromis niloticus*) has strongly contributed to the growth of the fish production chain (FAO, 2016). However, the fillet yield of this species is only about 30% of the live weight, resulting in high amounts

of waste (Monteiro et al., 2014). Regarding tilapia by-products, the flour obtained from meat adhered to bones and skin is an inexpensive source of essential nutrients and yields about 8% of the whole-fish weight, representing a sustainable alternative for the commercial fishery industry and an interesting way for the nutritional supplementation of bakery products (FAO, 2016; Monteiro et al., 2014, 2016). Several studies have evaluated the sensory attributes of bakery products enriched with proteins from seafood sources, with successful data (Bastos et al., 2014; Breternitz, Bolini, & Hubinger, 2017; Shaviklo, Olafsdottir, Sveinsdottir, Thorkelsson, & Rafipour, 2011). A previous study, for example, demonstrated that tilapia waste flour may be added up to 12.17% without affect the overall acceptability of breads (Monteiro et al., 2018).

Nevertheless, fish and fish by-products are highly perishable, due to their biological composition, which may affect

the physicochemical stability of bakery products during storage, leading to a faster degradation process and short shelf life (Monteiro et al., 2016), which could be a limiting factor for enriched-bread marketability. Fish present pH close to neutrality, active autolytic enzymes, high water activity and protein content, and a lipid fraction exhibiting the predominance of unsaturated fatty acids, which accelerates biochemical reactions and oxidative degradation (Lougovois & Kyranas, 2005) resulting in color and texture changes and, thus, limited shelf-life. However, it is known that biological composition and chemical stability depend on the fish species. Few studies have evaluated physicochemical changes in bakery products enriched with seafood protein powder, and no studies on the storage stability of breads fortified with tilapia-waste flour are available. Therefore, the aim of this study was to investigate the physicochemical attributes of bread fortified with different levels of tilapia-waste flour during storage at 25°C for 8 days.

2. Materials and methods

2.1. Sample obtaining and bread preparation

Tilapia mechanically separated meat (MSM) (9.0 ± 0.3 kg) was purchased in a commercial processing facility (Cachoeiras de Macacu, Rio de Janeiro, Brazil). Tilapia-waste flour (TF) was obtained after MSM drying at 65°C for 12 h in a forced-air convection oven (TE-394/3, Tecnal, Piracicaba, São Paulo, Brazil) at pilot plant scale. The bread formulations were manufactured following the recommendations of Stokić et al. (2015), with some modifications. All bread formulations contained different wheat/tilapia flours ratios, as well as other ingredients, listed in Table 1. All ingredients were purchased from a local market (Torres Alimentos Ltda., Santa Genoveva, Goiás, Brazil). The TF was blended with 240 ml of water for 15 min, followed by the addition of refined white wheat flour, baker's yeast, sugar, salt, dough improver and vegetable fat. Subsequently, 400 ml of water were slowly added to every 100 ml of the previously prepared mixture, until a homogeneous dough was obtained. The dough was then transferred to a dough mixer (BP-5, Gastromaq, Rio Grande do Sul, Brazil) at a fixed speed (equivalent to position five) and time (20 min). Each dough formulation was equally divided into three portions ($n = 3$)

and was manually kneaded (25°C for 20 min), sheeted and rolled. The dough was covered with a cloth for 30 min at 25°C and then baked at 180°C during 25 min in a baking oven (FERI-90, Venancio Aires, Rio Grande do Sul, Brazil). The bread formulations were then cooled to 25°C, packed in polyethylene bags, and analyzed in triplicate regarding moisture content, water activity (a_w), pH, instrumental color indices, texture profile and lipid and protein oxidation on days 0, 2, 4, 6 and 8. The final formulations were described as bread containing tilapia flour (BTF) at 0% (BTF0%), 2.5% (BTF2.5%), 5% (BTF5%), 10% (BTF10%), 15% (BTF15%), and 20% (BTF20%).

2.2. Moisture and water activity (a_w) determinations

Moisture content was determined at 25°C on a LJ16 Moisture Analyzer (Mettler-Toledo, Greifensee, Switzerland), while a_w was directly measured at 25°C by a water activity meter (Pawkit, Decagon Devices Inc., Pullman, Washington, USA). Approximately 5 g and 2 g of ground BTF samples were used for the moisture and a_w determinations, respectively.

2.3. pH determinations

pH values were determined by a penetration electrode at a sample-water ratio of 1:9, using a digital pH meter (Hanna Instruments, Woonsocket, USA).

2.4. Instrumental color measurements

Color parameters (L^* , a^* , b^*) were determined on a CM-600D Spectrophotometer (Minolta Camera Co., Osaka, Japan) under 8 mm aperture, illuminant D65, and 10° observer at 25°C. Slices (1 cm thickness) of each bread formulation were cut into four cubes ($3 \times 3 \times 3$ cm), and two measurements for each side were recorded, totaling 8 determinations per formulation at each storage time. Chroma (C^*) and hue angle (h°) were calculated from the obtained chromaticity coordinates (a^* and b^*), while the total color difference (ΔE) between enriched-bread formulations and control bread was determined by the Equation (1) (AMSA, 2012).

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2} \quad (1)$$

Table 1. Bread formulations with different tilapia-waste flour levels.

Tabla 1. Formulaciones de pan elaborado con distintos niveles de harina de residuos de tilapia.

Ingredients	Formulations					
	BTF0%	BTF2.5%	BTF5%	BTF10%	BTF15%	BTF20%
Wheat flour (g)	840	798	756	672	588	504
Tilapia-waste flour (g)	0	42	84	168	252	336
Salt (g)	20	20	20	20	20	20
Vegetable fat (g)	33	33	33	33	33	33
Baker's yeast* (g)	40	40	40	40	40	40
Dough improver [‡] (g)	50	50	50	50	50	50
Sugar (g)	50	50	50	50	50	50
Water (ml)	640	640	640	640	640	640

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively.

*Baker's yeast composition: *Saccharomyces cerevisiae* and sorbitan monostearate. [‡]Dough-improver composition: maize starch (*Bacillus thuringiensis*, *Streptomyces viridochromogenes*, *Agrobacterium tumefaciens*), sugar, polysorbate 80, ascorbic acid, azodicarbonamide, and alpha-amylase.

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente.

*Composición de la levadura de cerveza: *Saccharomyces cerevisiae* y monoestearato de sorbitano. [‡]Composición del mejorante de masa: almidón de maíz (*Bacillus thuringiensis*, *Streptomyces viridochromogenes*, *Agrobacterium tumefaciens*), azúcar, polisorbato 80, ácido ascórbico, azodicarbonamida y alfa-amilasa.

2.5. Instrumental texture measurements

Texture-profile analyses (TPA) were performed using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Surrey, UK). Four cubes (3 × 3 × 3 cm) of each bread formulation were analyzed concerning hardness, chewiness, cohesiveness, springiness and resilience on each day of storage using a P/36R probe according to the conditions established by Coda, Varis, Verni, Rizzello, and Katina (2017).

2.6. Lipid and protein oxidation

Lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method (Yin, Faustman, Riesen, & Williams, 1993) using an UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 532 nm. The results were expressed as mg of malondialdehyde (MA)/kg of sample. Protein oxidation was evaluated by measurement of carbonyl content as described by Oliver, Levine, and Stadtman (1982), adapted by Mercier, Gatellier, Viau, Remignon, and Renner (1998) and Armenteros, Heinonen, Ollilainen, Toldrá, and Estévez (2009). The absorbance values of protein and carbonyl contents were determined at 280 and 370 nm, respectively, on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as nmol of carbonyls/mg of protein.

2.7. Statistical analysis

All experiment was carried out in triplicate (n = 3). The differences between bread formulations (BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20%) and storage days of (0, 2, 4, 6, and 8) were analyzed using a two-way ANOVA followed by a post-hoc Tukey's test (P < 0.05) using the XLSTAT software (Addinsoft, New York, NY, USA).

3. Results and discussion

3.1. Moisture and water activity (a_w)

The results of moisture content and a_w of bread formulated with different tilapia-waste flour levels are displayed in Table 2. Similar initial values for moisture and a_w in breads have been previously reported (Aguirre, Osella, Carrara, Sánchez, & Buera, 2011; Besbes, Bail, & Seetharaman, 2016). BTF0%, BTF2.5%, and BTF5% presented the highest (P < 0.05) moisture and a_w values, while BTF20% exhibited the lowest (P < 0.05). Intermediate moisture and a_w levels (P < 0.05) were observed for BTF10% and BTF15%. This may be attributed to protein denaturation and, therefore, to more interactions between proteins and polysaccharides by electrostatic forces leading to intermolecular network, water entrapment and lower free water content, which is related to decreases in moisture content and a_w in foods (Bradley, 2010; Gómez-Guillén, Borderías, & Montera, 1997; Zhang, Li, Wang, Xue, & Xue, 2016). Studies evaluating moisture and a_w levels in breads enriched with protein sources are scarce. In two studies, a decrease in moisture and a_w levels was observed in pasta fortified with fish flour (Desai, Brennan, & Brennan, 2018; Monteiro et al., 2016), in accordance to our findings.

Overall, both moisture content and a_w values decreased (P < 0.05) with increasing storage time, regardless of the formulation. Bread water loss during the storage period is due to water migration from crumb to the atmosphere and from crumb to crust (Monteau, Purlis, Besbes, Jury, & Le-Bail, 2017). According to Monteau et al. (2017), although the bread crust absorbs part of the water lost in this process, most of the water evaporates from crumb to atmosphere, resulting in water loss during storage. Similarly, Aguirre et al. (2011) and Besbes et al. (2016) also reported decreases in moisture and a_w values in breads stored at 25°C for 23 days and at 15°C for 9 days, respectively.

Table 2. Moisture content (%), water activity (a_w) and pH values of bread formulations with different tilapia-waste flour levels stored at 25°C for 8 days.

Tabla 2. Contenido de humedad (%), actividad de agua (a_w) y valores de pH de formulaciones de pan con distintos niveles de harina de residuos de tilapia, almacenadas a 25°C durante 8 días.

Parameters	Formulations	Storage days				
		0	2	4	6	8
Moisture	BTF0%	42.00 ± 0.86 ^{aA}	40.70 ± 0.56 ^{aAB}	39.07 ± 0.35 ^{aBC}	39.77 ± 0.74 ^{aB}	37.73 ± 0.68 ^{aC}
	BTF2.5%	42.17 ± 0.65 ^{aA}	40.24 ± 0.41 ^{aB}	39.63 ± 0.16 ^{aBC}	38.33 ± 1.33 ^{aCD}	37.40 ± 0.20 ^{aD}
	BTF5%	42.30 ± 0.69 ^{aA}	40.20 ± 0.61 ^{aB}	39.17 ± 0.05 ^{aBC}	38.30 ± 0.60 ^{aC}	38.49 ± 0.31 ^{aC}
	BTF10%	38.53 ± 0.49 ^{bA}	36.84 ± 0.19 ^{bA}	34.66 ± 1.41 ^{bb}	33.37 ± 0.20 ^{bBC}	32.38 ± 0.48 ^{bC}
	BTF15%	39.03 ± 1.28 ^{bA}	36.93 ± 0.50 ^{bAB}	34.87 ± 0.45 ^{bBC}	33.10 ± 0.60 ^{bC}	33.47 ± 1.10 ^{bC}
	BTF20%	36.20 ± 0.34 ^{cA}	34.16 ± 0.50 ^{cB}	32.90 ± 0.86 ^{cBC}	31.60 ± 0.50 ^{cCD}	30.97 ± 0.73 ^{cD}
a_w	BTF0%	0.96 ± 0.01 ^{aA}	0.94 ± 0.01 ^{aB}	0.94 ± 0.01 ^{aB}	0.92 ± 0.01 ^{aBC}	0.91 ± 0.01 ^{aC}
	BTF2.5%	0.96 ± 0.01 ^{aA}	0.94 ± 0.00 ^{aB}	0.94 ± 0.01 ^{aB}	0.92 ± 0.01 ^{aBC}	0.91 ± 0.01 ^{aC}
	BTF5%	0.96 ± 0.01 ^{aA}	0.94 ± 0.01 ^{aB}	0.94 ± 0.00 ^{aB}	0.92 ± 0.01 ^{aC}	0.90 ± 0.01 ^{aC}
	BTF10%	0.92 ± 0.01 ^{bA}	0.90 ± 0.01 ^{bb}	0.89 ± 0.01 ^{bb}	0.86 ± 0.01 ^{bC}	0.85 ± 0.01 ^{bC}
	BTF15%	0.93 ± 0.01 ^{bA}	0.91 ± 0.01 ^{bbAB}	0.90 ± 0.01 ^{bb}	0.86 ± 0.01 ^{bC}	0.84 ± 0.01 ^{bC}
	BTF20%	0.89 ± 0.01 ^{cA}	0.86 ± 0.00 ^{cB}	0.86 ± 0.01 ^{cB}	0.83 ± 0.01 ^{cC}	0.82 ± 0.01 ^{cC}
pH	BTF0%	5.31 ± 0.07 ^{bA}	5.16 ± 0.07 ^{bA}	4.89 ± 0.06 ^{bb}	4.86 ± 0.09 ^{bb}	4.55 ± 0.16 ^{bC}
	BTF2.5%	5.34 ± 0.02 ^{bA}	5.15 ± 0.03 ^{bA}	4.91 ± 0.04 ^{bb}	4.87 ± 0.11 ^{bb}	4.57 ± 0.07 ^{bC}
	BTF5%	5.33 ± 0.03 ^{bA}	5.19 ± 0.04 ^{bA}	4.87 ± 0.05 ^{bb}	4.90 ± 0.15 ^{bb}	4.52 ± 0.03 ^{bC}
	BTF10%	6.04 ± 0.05 ^{aA}	5.85 ± 0.08 ^{aAB}	5.72 ± 0.06 ^{aBC}	5.73 ± 0.01 ^{aBC}	5.60 ± 0.06 ^{aC}
	BTF15%	6.00 ± 0.18 ^{aA}	5.85 ± 0.10 ^{aAB}	5.71 ± 0.06 ^{aB}	5.77 ± 0.03 ^{aAB}	5.61 ± 0.03 ^{aB}
	BTF20%	6.09 ± 0.02 ^{aA}	5.93 ± 0.06 ^{aAB}	5.73 ± 0.12 ^{aBC}	5.76 ± 0.10 ^{aBC}	5.65 ± 0.06 ^{aC}

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively. Results are expressed as means ± standard deviation (n = 3). ^{a-c} Different superscripts indicate differences (P < 0.05) among formulations within same storage day. ^{A-D} Different superscripts indicate differences (P < 0.05) among storage days within same formulation.

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente. Los resultados se reportan como medias ± desviación estándar (n = 3). ^{a-c} Los distintos superíndices indican diferencias (P < 0.05) detectadas entre las formulaciones el mismo día de su almacenamiento. ^{A-D} Los distintos superíndices indican diferencias (P < 0.05) encontradas en el pan elaborado con la misma formulación los distintos días de almacenamiento.

3.2. Bread pH

The present study demonstrated that the addition of tilapia-waste flour at 10% or higher increased ($P < 0.05$) the initial pH values of the bread (Table 2). This may be attributed to pH differences between wheat flour and fish flour; while wheat flour displays pH values about 6.05 (Tamsen, Shekarchizadeh, & Soltanzadeh, 2018), fish flour exhibits a pH close to neutrality (7.06) (Senapati, Martin Xavier, Nayak, & Balange, 2017). Moreover, high-protein flour may result in bread shrinkage, due to interactions between fish protein and the gluten network, leading to lower gas retention and, consequently, a lower oxygen supply for yeast activity, impairing pH lowering during the bread fermentation caused by the production of carbonic acid from the dissolution of carbon dioxide in water (Lin, Miskelly, & Moss, 1990; Wang et al., 2017). Similar trends in the pH values of pasta enriched with tilapia flour were also observed by Monteiro et al. (2016).

The pH values decreased ($P < 0.05$) during storage at room temperature in all formulations. However, this was less pronounced for BTF10%, BTF15%, and BTF20% (Table 2). Generally, the production of volatile compounds continues after the baking process, promoting undesirable changes in breads (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). Among volatile compounds, acids resulting from aldehyde oxidation strongly increase during bread storage (Jensen et al., 2011a), which explains the findings reported herein. However, flours display a different buffering capacity in the sourdough (Marklinder, Johansson, Haglund, Nagel-Held, &

Seibel, 1996), wherein flours with high ash content display high buffering capacity (Spicher & Stephan, 1982). This could explain the results observed in the present study, since it is known that fish flour contains higher ash content than wheat flour (USDA, 2009). Additionally, fish flour tends to alkalize during storage at room temperature, due to basic nitrogenous compound formation (Senapati et al., 2017).

3.3. Color properties

The tilapia-waste flour decreased ($P < 0.05$) the lightness index and increased ($P < 0.05$) the redness and yellowness indices, resulting in browning of the bread formulations (Table 3). The color changes in crumb are not attributed to the Maillard reaction, due to mild temperature reached by this part during the baking process (Helou, Jacolot, Niquet-Léridon, Gadonna-Widehem, & Tessier, 2016). Therefore, it may be explained by the fact that fish flour displays a typically darker color than refined white wheat flour. The same color changes in bakery products due to the addition of fish flour have been previously reported in literature (Coker et al., 2017; Monteiro et al., 2016).

Regarding total color difference, BTF10%, BTF15% and BTF20% presented ΔE higher than 5 units compared to the control bread (BTF0%), indicating that breads containing 10% or more of tilapia-waste flour underwent visually perceptible color differences to consumers (Tazart, Zaidi, Lamacchia, & Haros, 2016). Although consumers more easily accept white wheat bread, liking related to color bread

Table 3. Instrumental color parameters of bread formulations with different tilapia-waste flour levels stored at 25°C for 8 days.

Table 3. Parámetros del color instrumental del pan elaborado con distintos niveles de harina de residuos de tilapia y almacenado durante 8 días a 25°C.

Parameters	Formulations	Storage days				
		0	2	4	6	8
L^*	BTF0%	79.59 ± 2.02 ^{aA}	77.38 ± 1.86 ^{aAB}	77.90 ± 1.48 ^{aAB}	74.47 ± 3.59 ^{aB}	74.10 ± 1.32 ^{aB}
	BTF2.5%	77.93 ± 0.62 ^{abA}	76.77 ± 0.57 ^{abA}	75.20 ± 1.32 ^{ba}	71.66 ± 3.05 ^{abB}	71.23 ± 3.20 ^{abB}
	BTF5%	75.66 ± 0.94 ^{ba}	74.86 ± 0.60 ^{baB}	73.87 ± 0.74 ^{bcB}	71.74 ± 0.79 ^{abC}	71.37 ± 0.57 ^{abC}
	BTF10%	71.32 ± 0.42 ^{cAB}	71.44 ± 0.42 ^{cAB}	71.52 ± 0.27 ^{cdA}	70.44 ± 0.91 ^{dB}	67.62 ± 0.56 ^{bc}
	BTF15%	71.65 ± 0.23 ^{cA}	71.86 ± 0.25 ^{cA}	71.04 ± 0.42 ^{dA}	69.72 ± 0.54 ^{bcB}	67.82 ± 0.51 ^{bc}
	BTF20%	68.89 ± 0.91 ^{dA}	67.53 ± 1.91 ^{dA}	67.30 ± 1.07 ^{eA}	65.95 ± 1.14 ^{eB}	60.58 ± 4.94 ^{eB}
a^*	BTF0%	1.12 ± 0.06 ^{EB}	1.32 ± 0.12 ^{EB}	1.57 ± 0.09 ^{dAB}	1.84 ± 0.10 ^{eA}	1.86 ± 0.17 ^{eA}
	BTF2.5%	1.34 ± 0.10 ^{deC}	1.72 ± 0.14 ^{deB}	1.72 ± 0.12 ^{dB}	1.93 ± 0.03 ^{deA}	1.98 ± 0.03 ^{deA}
	BTF5%	1.53 ± 0.10 ^{dC}	2.05 ± 0.04 ^{cdB}	2.38 ± 0.18 ^{cAB}	2.55 ± 0.25 ^{cdA}	2.51 ± 0.03 ^{dA}
	BTF10%	2.03 ± 0.16 ^{cd}	2.33 ± 0.11 ^{bcC}	2.62 ± 0.14 ^{bcC}	3.52 ± 0.27 ^{bcB}	3.84 ± 0.12 ^{cA}
	BTF15%	2.50 ± 0.10 ^{bc}	2.52 ± 0.11 ^{bc}	2.96 ± 0.29 ^{bc}	3.82 ± 0.11 ^{bB}	4.35 ± 0.42 ^{ba}
	BTF20%	3.95 ± 0.18 ^{aC}	4.30 ± 0.29 ^{abc}	4.70 ± 0.06 ^{ab}	4.76 ± 0.08 ^{ab}	5.52 ± 0.08 ^{aA}
b^*	BTF0%	18.97 ± 0.95 ^{dB}	18.87 ± 0.70 ^{dB}	19.66 ± 0.24 ^{fAB}	20.22 ± 1.01 ^{dAB}	21.70 ± 0.19 ^{fA}
	BTF2.5%	19.93 ± 0.59 ^{dB}	21.31 ± 0.56 ^{cb}	21.69 ± 0.92 ^{abB}	21.82 ± 0.81 ^{dAB}	22.82 ± 0.69 ^{deA}
	BTF5%	21.79 ± 0.75 ^{cC}	22.57 ± 0.42 ^{cbC}	23.16 ± 0.46 ^{dBC}	23.60 ± 0.99 ^{cb}	25.82 ± 0.69 ^{dA}
	BTF10%	23.08 ± 0.84 ^{bcC}	24.49 ± 1.00 ^{bcB}	25.60 ± 0.87 ^{cAB}	26.70 ± 0.83 ^{ba}	27.27 ± 0.10 ^{cA}
	BTF15%	24.01 ± 0.21 ^{abd}	26.09 ± 0.56 ^{aC}	26.97 ± 0.59 ^{bBC}	28.03 ± 0.29 ^{abB}	29.62 ± 0.95 ^{ba}
	BTF20%	25.12 ± 0.32 ^{ad}	27.20 ± 0.18 ^{ac}	28.53 ± 0.57 ^{ab}	29.15 ± 0.47 ^{ab}	30.86 ± 0.49 ^{aA}
ΔE	BTF0%	-	-	-	-	-
	BTF2.5%	2.13	-	-	-	-
	BTF5%	4.61	-	-	-	-
	BTF10%	9.32	-	-	-	-
	BTF15%	9.48	-	-	-	-
	BTF20%	12.43	-	-	-	-

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively. Results are expressed as means ± standard deviation ($n = 3$). ^{a-f} Different superscripts indicate differences ($P < 0.05$) among formulations within same storage day. ^{A-D} Different superscripts indicate differences ($P < 0.05$) among storage days within same formulation. ΔE – Total color difference between enriched bread formulations (BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20%) and control bread (BTF0%).

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente. Los resultados se reportan como medias ± desviación estándar ($n = 3$). ^{a-f} Los distintos superíndices indican diferencias ($P < 0.05$) detectadas entre las formulaciones el mismo día de su almacenamiento. ^{A-D} Los distintos superíndices indican diferencias ($P < 0.05$) encontradas en el pan elaborado con la misma formulación los distintos días de almacenamiento. ΔE – Diferencia del color total entre distintas formulaciones de pan fortificado (BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20%) y el pan de control (BTF0%).

depends mainly on bread type preference, wherein typical whole wheat bread consumers prefer darker breads (Bakke & Vickers, 2011). The addition of 2.5% and 5% of tilapia-waste flour were not enough to change consumer visual color perception. For a complete color characterization, chroma (C^*) and hue angle (h°) were evaluated. Figure 1(a,b) show that the tilapia-waste flour affected ($P < 0.05$) both h° and C^* values of the bread formulations. Breads containing up to 5% of tilapia-waste flour tended towards a cream-yellow color, whereas breads with 10% or more of high-protein flour displayed a golden color. In addition, BTF10%, BTF15%, and BTF20% led to higher C^* values than BTF0%, BTF2.5%, and BTF5%, suggesting that the inclusion of tilapia-waste flour ($\geq 10\%$) resulted in a more vivid color for bread formulations.

Regardless of the formulation, the lightness index decreased ($P < 0.05$), while redness and yellowness indices increased ($P < 0.05$) during the storage period, resulting in dark bread color, which is usually associated to water loss and oxidation of carotenoids naturally present in wheat flour (Licciardello, Cipri, & Muratore, 2014). The water loss in all bread formulations during the evaluated storage time was confirmed by our moisture content and a_w results. Regarding carotenoids, little loss of these compounds during manufacturing stages of bread crumb compared to other bakery products is reported (Hidalgo, Brandolini, & Pompei, 2010). As carotenoids are highly instable molecules, they can oxidize during storage even when bread is stored in appropriate packaging (Licciardello et al., 2014). In agreement to the results presented herein, Naji-Tabasi and Mohebbi (2015) also reported darker breads during storage at room temperature.

3.4. Texture profile

BTF10%, BTF15% and BTF20% exhibited the highest ($P < 0.05$) hardness and chewiness, and the lowest ($P < 0.05$) cohesiveness, springiness and resilience compared to BTF0%, BTF2.5%, and BTF5% (Table 4). Previous studies confirmed the positive correlation between hardness and chewiness, springiness and resilience, as well as negative correlations between springiness and hardness, and cohesiveness and hardness (Conte, Del Caro, Balestra, Piga, & Fadda, 2018; Ergönül, 2013). Gluten is composed by

interactions between gliadin and glutenin proteins, representing one of the crucial factors for the texture of bakery products during baking process (Ortolan & Steel, 2017). Gliadin and glutenin chains form intra and intermolecular bonds through covalent (disulfide bonds) and noncovalent (hydrogen, ionic and hydrophobic bonds) interactions, resulting in a gluten network, which entraps carbon dioxide gas from yeast fermentation and generates a viscoelastic dough (Ortolan & Steel, 2017; Wang et al., 2017). Changes in texture may be due to the fact that tilapia-waste flour contains high amounts of protein that do not produce a network similar to the gluten network, leading to a firmer and more compact dough (Desai et al., 2018). It is possible that protein-polysaccharide interactions may facilitate starch entrapment in the gluten network, strengthening the dough structure and impairing bread texture (Dalbon, Grivon, & Ambrogina, 1996; Gómez-Guillén et al., 1997; Zhang et al., 2016). Similarly, Desai et al. (2018) also reported harder dough when fish flour was added to pasta.

Hardness and chewiness increased ($P < 0.05$) in all bread formulations, while no difference ($P > 0.05$) was observed in cohesiveness, springiness and resilience during the entire storage period (Table 4). Increasing hardness and chewiness can be attributed to decreasing moisture content during the storage period (Monteau et al., 2017), reinforcing the findings reported herein. This phenomenon has been confirmed by recent studies on traditional breads and breads with high-in-protein flours stored at room temperature (Bize, Smith, Aramouni, & Bean, 2017; Conte et al., 2018).

3.5. Lipid and protein oxidation

Regardless of the storage day, BTF10%, BTF15%, and BTF20% displayed higher ($P < 0.05$) malondialdehyde (MA) values and carbonyl content than BTF0%, BTF2.5%, and BTF5% (Table 5). The heat during the baking process induces oxidation through the generation of radicals (Jensen, Oestdal, Clausen, Andersen, & Skibsted, 2011b). The incorporation of tilapia-waste flour in wheat-based foodstuffs results in increased amounts of amino acids (essential and non-essential) and polyunsaturated fatty acids, which are susceptible to protein and lipid oxidation, respectively (Monteiro et al., 2016). In agreement with these findings, the inclusion of ingredients with high protein and/or lipid content was

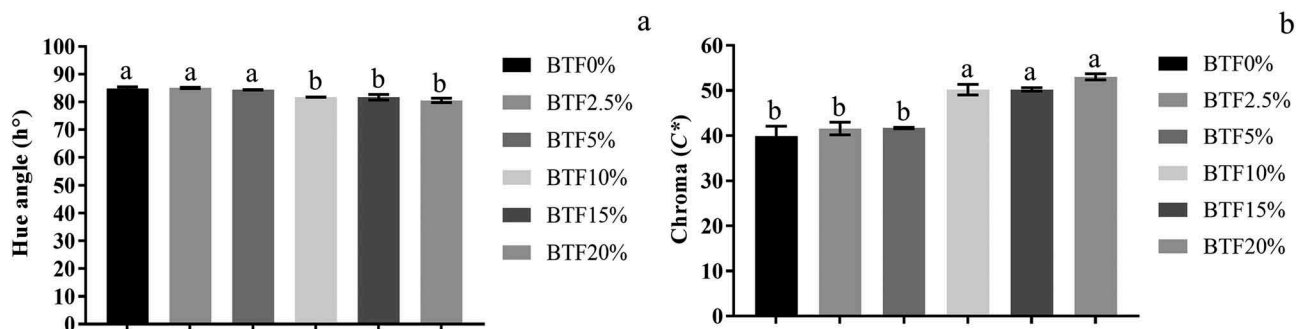


Figure 1. Hue angle (h°) (a) and Chroma (C^*) (b) values of bread formulations with different tilapia-waste flour levels. BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively. Bars are means \pm standard deviation ($n = 3$). ^{a-b} Different superscripts indicate differences ($P < 0.05$) among formulations.

Figura 1. Valores del ángulo del tono (h°) (a) y croma (C^*) (b) de las formulaciones de pan elaboradas con distintos niveles de harina de residuos de tilapia. BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente. Las barras son medias \pm desviación estándar ($n = 3$). ^{a-b} Los distintos superíndices indican la existencia de diferencias ($P < 0.05$) entre las distintas formulaciones.

Table 4. Instrumental texture parameters of bread formulations with different tilapia-waste flour levels stored at 25°C for 8 days.**Tabla 4.** Parámetros de textura instrumental de las formulaciones de pan elaborado con distintos niveles de harina de residuos de tilapia y almacenado durante 8 días a 25°C.

Parameters	Formulations	Storage days				
		0	2	4	6	8
Hardness (g)	BTF0%	2175.10 ± 104.06 ^{dC}	2244.67 ± 49.80 ^{dC}	3353.29 ± 142.72 ^{dB}	4332.51 ± 470.42 ^{CA}	4642.48 ± 81.06 ^{CA}
	BTF2.5%	2379.27 ± 418.78 ^{dC}	2390.95 ± 138.85 ^{dC}	3577.03 ± 210.33 ^{dB}	4070.17 ± 367.73 ^{CB}	4720.45 ± 120.02 ^{CA}
	BTF5%	2284.45 ± 43.75 ^{dD}	2385.84 ± 163.71 ^{dD}	3539.75 ± 201.35 ^{dC}	4287.68 ± 53.18 ^{CB}	4669.23 ± 174.78 ^{CA}
	BTF10%	3338.26 ± 366.24 ^{CB}	3583.31 ± 126.99 ^{CB}	4588.02 ± 264.83 ^{CA}	4653.02 ± 140.36 ^{CA}	4702.86 ± 74.07 ^{CA}
	BTF15%	5212.12 ± 224.53 ^{BC}	5487.14 ± 252.31 ^{BC}	6132.08 ± 493.69 ^{BB}	6775.58 ± 629.90 ^{BAB}	7747.66 ± 295.82 ^{BA}
	BTF20%	6792.58 ± 249.76 ^{AC}	6919.88 ± 850.61 ^{AC}	7683.00 ± 188.01 ^{ABC}	8812.97 ± 97.04 ^{AB}	9898.73 ± 690.86 ^{AA}
	BTF0%	1159.41 ± 24.73 ^{CC}	1175.92 ± 65.61 ^{CC}	1369.83 ± 128.25 ^{CB}	1641.91 ± 61.72 ^{CAB}	1804.83 ± 105.67 ^{dA}
Chewiness (g × mm)	BTF2.5%	1197.17 ± 96.64 ^{CD}	1151.45 ± 59.31 ^{CD}	1373.78 ± 89.40 ^{CB}	1610.85 ± 15.65 ^{CAB}	1862.26 ± 62.13 ^{cdA}
	BTF5%	1157.09 ± 88.01 ^{CB}	1128.96 ± 78.71 ^{CB}	1327.77 ± 105.60 ^{CB}	1671.52 ± 83.26 ^{CA}	1857.72 ± 38.19 ^{cdA}
	BTF10%	1581.69 ± 141.24 ^{BB}	1517.15 ± 27.73 ^{BB}	1738.75 ± 81.68 ^{BAB}	1877.50 ± 141.75 ^{BAB}	2088.51 ± 137.04 ^{BA}
	BTF15%	1830.44 ± 7.54 ^{ABC}	1833.95 ± 2.57 ^{ABC}	2016.44 ± 77.74 ^{AB}	2206.27 ± 20.50 ^{BA}	2329.90 ± 61.44 ^{BA}
	BTF20%	2061.99 ± 158.11 ^{AC}	2058.94 ± 98.45 ^{AC}	2368.04 ± 52.25 ^{ABC}	2600.73 ± 101.59 ^{AB}	2869.10 ± 38.53 ^{AA}
	BTF0%	0.522 ± 0.030 ^{AA}	0.499 ± 0.046 ^{AA}	0.501 ± 0.047 ^{AA}	0.524 ± 0.028 ^{AA}	0.520 ± 0.006 ^{AA}
	BTF2.5%	0.520 ± 0.045 ^{AA}	0.528 ± 0.044 ^{AA}	0.493 ± 0.037 ^{AA}	0.514 ± 0.008 ^{AA}	0.527 ± 0.038 ^{AA}
Cohesiveness	BTF5%	0.530 ± 0.017 ^{AA}	0.526 ± 0.016 ^{AA}	0.520 ± 0.018 ^{AA}	0.532 ± 0.025 ^{AA}	0.527 ± 0.011 ^{AA}
	BTF10%	0.394 ± 0.031 ^{BA}	0.391 ± 0.039 ^{BA}	0.387 ± 0.010 ^{BA}	0.373 ± 0.019 ^{BA}	0.393 ± 0.027 ^{BA}
	BTF15%	0.390 ± 0.028 ^{BA}	0.376 ± 0.025 ^{BA}	0.372 ± 0.032 ^{BA}	0.381 ± 0.016 ^{BA}	0.378 ± 0.027 ^{BA}
	BTF20%	0.393 ± 0.027 ^{BA}	0.381 ± 0.031 ^{BA}	0.383 ± 0.022 ^{BA}	0.386 ± 0.028 ^{BA}	0.392 ± 0.027 ^{BA}
	BTF0%	0.899 ± 0.026 ^{AA}	0.895 ± 0.022 ^{AA}	0.870 ± 0.028 ^{AA}	0.900 ± 0.052 ^{AA}	0.879 ± 0.053 ^{AA}
	BTF2.5%	0.862 ± 0.036 ^{abA}	0.876 ± 0.054 ^{abA}	0.847 ± 0.037 ^{abA}	0.893 ± 0.051 ^{AA}	0.911 ± 0.039 ^{AA}
	BTF5%	0.882 ± 0.046 ^{abA}	0.852 ± 0.013 ^{abA}	0.863 ± 0.027 ^{abA}	0.829 ± 0.040 ^{AA}	0.893 ± 0.021 ^{AA}
Springiness (mm)	BTF10%	0.757 ± 0.015 ^{CA}	0.766 ± 0.058 ^{CA}	0.781 ± 0.015 ^{CA}	0.747 ± 0.037 ^{BA}	0.740 ± 0.056 ^{BA}
	BTF15%	0.784 ± 0.071 ^{CA}	0.790 ± 0.024 ^{CA}	0.759 ± 0.034 ^{CA}	0.771 ± 0.049 ^{BA}	0.756 ± 0.044 ^{BA}
	BTF20%	0.748 ± 0.055 ^{CA}	0.757 ± 0.018 ^{CA}	0.755 ± 0.007 ^{CA}	0.751 ± 0.066 ^{BA}	0.742 ± 0.027 ^{BA}
	BTF0%	0.207 ± 0.016 ^{AA}	0.203 ± 0.020 ^{AA}	0.223 ± 0.007 ^{AA}	0.225 ± 0.011 ^{AA}	0.227 ± 0.018 ^{AA}
	BTF2.5%	0.220 ± 0.020 ^{AA}	0.218 ± 0.004 ^{AA}	0.244 ± 0.031 ^{AA}	0.227 ± 0.009 ^{AA}	0.223 ± 0.017 ^{AA}
	BTF5%	0.217 ± 0.012 ^{AA}	0.218 ± 0.012 ^{AA}	0.227 ± 0.016 ^{AA}	0.221 ± 0.022 ^{AA}	0.225 ± 0.014 ^{AA}
	BTF10%	0.129 ± 0.010 ^{BA}	0.136 ± 0.008 ^{BA}	0.130 ± 0.010 ^{BA}	0.140 ± 0.007 ^{BA}	0.138 ± 0.013 ^{BA}
Resilience	BTF15%	0.133 ± 0.011 ^{BA}	0.137 ± 0.005 ^{BA}	0.132 ± 0.007 ^{BA}	0.139 ± 0.003 ^{BA}	0.137 ± 0.012 ^{BA}
	BTF20%	0.136 ± 0.006 ^{BA}	0.138 ± 0.008 ^{BA}	0.134 ± 0.009 ^{BA}	0.139 ± 0.010 ^{BA}	0.135 ± 0.011 ^{BA}

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively. Results are expressed as means ± standard deviation (n = 3). ^{a-d} Different superscripts indicate differences (P < 0.05) among formulations within same storage day. ^{A-D} Different superscripts indicate differences (P < 0.05) among storage days within same formulation.

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente. Los resultados se reportan como medias ± desviación estándar (n = 3). ^{a-d} Los distintos superíndices indican diferencias (P < 0.05) detectadas entre las formulaciones el mismo día de su almacenamiento. ^{A-D} Los distintos superíndices indican diferencias (P < 0.05) encontradas en el pan elaborado con la misma formulación los distintos días de almacenamiento.

Table 5. Lipid and protein oxidation of bread formulations with different tilapia-waste flour levels stored at 25°C for 8 days.**Tabla 5.** Oxidación lipídica y proteica de las formulaciones de pan elaborado con distintos niveles de harina de desperdicio de tilapia y almacenado durante 8 días a 25°C.

Parameters	Formulations	Storage days				
		0	2	4	6	8
Lipid oxidation [§]	BTF0%	0.46 ± 0.03 ^{BC}	0.48 ± 0.02 ^{BC}	0.55 ± 0.05 ^{BB}	0.64 ± 0.02 ^{BB}	0.76 ± 0.03 ^{BA}
	BTF2.5%	0.47 ± 0.03 ^{BD}	0.47 ± 0.04 ^{BD}	0.59 ± 0.02 ^{BC}	0.66 ± 0.03 ^{BB}	0.79 ± 0.01 ^{abA}
	BTF5%	0.45 ± 0.03 ^{BC}	0.46 ± 0.04 ^{BC}	0.58 ± 0.03 ^{BB}	0.63 ± 0.03 ^{BB}	0.79 ± 0.07 ^{abA}
	BTF10%	0.76 ± 0.03 ^{AB}	0.75 ± 0.03 ^{AB}	0.76 ± 0.05 ^{AB}	0.87 ± 0.05 ^{AA}	0.88 ± 0.06 ^{AA}
	BTF15%	0.76 ± 0.04 ^{AB}	0.78 ± 0.04 ^{AB}	0.77 ± 0.08 ^{AB}	0.90 ± 0.01 ^{AA}	0.89 ± 0.02 ^{AA}
	BTF20%	0.75 ± 0.05 ^{AB}	0.75 ± 0.05 ^{AB}	0.78 ± 0.04 ^{AB}	0.89 ± 0.03 ^{AA}	0.88 ± 0.05 ^{AA}
	BTF0%	3.49 ± 0.21 ^{BD}	5.19 ± 0.55 ^{BC}	6.51 ± 0.49 ^{BB}	6.40 ± 0.36 ^{BB}	9.96 ± 0.73 ^{AA}
Protein oxidation [¶]	BTF2.5%	3.63 ± 0.19 ^{BD}	5.12 ± 0.34 ^{BC}	6.34 ± 0.57 ^{BB}	6.22 ± 0.43 ^{BB}	9.56 ± 0.52 ^{AA}
	BTF5%	3.56 ± 0.22 ^{BD}	5.24 ± 0.25 ^{BC}	6.51 ± 0.23 ^{BB}	6.18 ± 0.40 ^{BB}	9.37 ± 0.31 ^{AA}
	BTF10%	5.87 ± 0.40 ^{AC}	7.44 ± 0.55 ^{ABC}	7.63 ± 0.75 ^{ABC}	8.08 ± 0.07 ^{AB}	9.75 ± 0.95 ^{AA}
	BTF15%	5.82 ± 0.48 ^{AC}	7.40 ± 0.41 ^{AB}	7.41 ± 0.44 ^{AB}	8.29 ± 0.51 ^{AB}	10.15 ± 0.54 ^{AA}
	BTF20%	5.80 ± 0.30 ^{AC}	7.34 ± 0.38 ^{AB}	7.37 ± 0.45 ^{AB}	8.19 ± 0.65 ^{AB}	10.50 ± 0.77 ^{AA}

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15%, and BTF20% means bread with tilapia flour at 0%, 2.5%, 5%, 10%, 15%, and 20%, respectively. Results are expressed as means ± standard deviation (n = 3). ^{a-b} Different superscripts indicate differences (P < 0.05) among formulations within same storage day. ^{A-D} Different superscripts indicate differences (P < 0.05) among storage days within same formulation. [§]Lipid oxidation is expressed as mg of malondialdehyde (MA)/kg of sample. [¶]Protein oxidation is expressed as nmol of carbonyls/mg of protein.

BTF0%, BTF2.5%, BTF5%, BTF10%, BTF15% y BTF20% significan pan elaborado con harina de tilapia a niveles de 0%, 2.5%, 5%, 10%, 15% y 20%, respectivamente. Los resultados se reportan como medias ± desviación estándar (n = 3). ^{a-b} Los distintos superíndices indican diferencias (P < 0.05) detectadas entre las formulaciones el mismo día de su almacenamiento. ^{A-D} Los distintos superíndices indican diferencias (P < 0.05) encontradas en el pan elaborado con la misma formulación los distintos días de almacenamiento. [§]La oxidación lipídica se reporta como mg de malondialdehído (MA)/kg de la muestra. [¶]La oxidación proteica se reporta como nmol de carbonilos/mg de proteína.

shown to increase the lipid oxidation of bread and pasta immediately after the baking step (Monteiro et al., 2016; Takeungwongtrakul, Benjakul, & H-Kittikun, 2015).

Moreover, Monteiro et al. (2016) reported an increase in the initial protein oxidation of pasta fortified with tilapia-waste flour equal to or above 12%.

MA values and carbonyl content increased ($P < 0.05$) during the entire storage period in all formulations, although this increase was less pronounced in BTF10%, BTF15%, and BTF20% (Table 5). Although lipase and lipoxygenase are inactivated during the baking process, their action on the lipid fraction before inactivation leads to increased amounts of free fatty acids, which are more readily oxidized than polyunsaturated fatty acids during storage (Jensen et al., 2011b). Other key factors concerning oxidative damage during storage of breads are the oxygen permeability of the packaging material and the amount of gas in the dough (Jensen et al., 2011b; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013). Increases in lipid oxidation during storage of traditional breads has been previously reported (Jensen et al., 2011b, 2011a). Nevertheless, a lack of studies on the oxidative stability of breads enriched with fish flour stored at room temperature exists. Additionally, water content and mobility is crucial for the occurrence of oxidative reactions, although this is very variable among bakery products (Besbes et al., 2016; Monteiro et al., 2016) making it difficult to compare the results of the present study with published reports in the literature. Herein, all formulations were packed in oxygen permeable polyethylene bags, but breads containing high level of tilapia-waste flour (BTF10%, BTF15%, and BTF20%) resulted in a firmer and more compact product, decreasing the amount of carbon dioxide retained by the gluten network, which contributes to the oxidation process (Pa, Chin, Yusof, & Aziz, 2014).

4. Conclusions

The addition of up to 5% TF maintained the original physico-chemical attributes of wheat breads, representing a promising health food market product. Nevertheless, although pH lowering and the evolution of oxidative degradation during storage were minimized, higher levels of tilapia-waste flour ($\geq 10\%$) led to initial negative changes in the color, texture, lipid and protein oxidation of the bread formulations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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