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Carotenoids from annatto as potential antioxidants in Nile Tilapia *(Oreochromis niloticus)* burgers

Meleiro, V. C., Nascimento, K. O., Barbosa Junior, J. L., Saldanha, T. and *Barbosa, M. I. M.J.

Departament of Food Technology, Institute of Technology, Federal Rural University of Rio de Janeiro, Seropédica, Rio de Janeiro (RJ), Brazil

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Abstract

The aim of this study was to evaluate the effect of carotenoids from annatto (bixin and norbixin) on the oxidative stability and sensory acceptance of tilapia burger, and as a substitute for sodium erythorbate. Control fish burgers (0% antioxidant) and fish burgers containing antioxidant (bixin and norbixin) and sodium erythorbate (synthetic antioxidant) were prepared. The oxidative stability of the formulations was assessed by the increase in MDA (mg / kg) at 0°C on 0, 7, 14 and 21 days of storage and after heat treatment (50°C / 16 min). In general, bixin, norbixin and sodium erythorbate were able to increase the oxidative stability of tilapia burgers at 0°C for 21 days. After 21 days at 0°C, bixin was more effective in reducing lipid oxidation of the fish burgers studied. In grilled samples, bixin and sodium erythorbate were the most effective antioxidants ($p \leq 0.05$) in the control of lipid oxidation of the samples only until the 14th day of frozen storage. For the attribute appearance, the scores of the raw burgers were 7.45 and 6.98 for formulations containing bixin and norbixin, respectively, while the mean score for the formulations with sodium erythorbate was 5.39. Natural ingredients such as carotenoids from annatto can be added to fish products without harming their quality and appearance and contributing to obtain healthier burgers, free of synthetic antioxidants.

Introduction

Lipid oxidation refers to the degradation processes that occur under different oxidation mechanisms (self-oxidation, photosensitive, and enzymatic oxidation). Several factors including degree of unsaturation, temperature, light, water activity, presence of metals, photosensitizers, pro-oxidants and antioxidants significantly affect the overall quality of fish-based products during processing and storage, leading to a number of undesirable changes, such as decreased nutritional quality, health risks and economic losses (Medina-Meza *et al*., 2014; Ahmad *et al*., 2015).

In recent years, the use of natural antioxidants in fish-based products has been evaluated aimed to replace or at least minimize the use of synthetic additives, satisfying the consumers demand for products with natural characteristics (Sancho *et al*., 2011; Garcia *et al*., 2012). Among the alternatives, carotenoids stand out for being natural pigments present in many foods, and have different mechanisms of antioxidant action (Mercadante *et al*., 2010; Castro *et al*., 2011; Sancho *et al*., 2011).

Some studies have reported the potential of various carotenoids such as β-carotene (Lee and Lillard, 1997), zeaxanthin, norbixin, bixin (Mercadante *et al*., 2010; Castro *et al*., 2011; Sancho *et al*., 2011), tomato powder and extract of guava pulp, rich in lycopene (Joseph *et al*., 2012; Doménech-Asensi *et al*., 2013; Ahmad *et al*., 2015) and the combination of paprika and tomato paste (B´azan-Lugo *et al*., 2012) in the control of lipid oxidation, especially in meat products.

When compared to other meats, fish are more susceptible to lipid oxidation due to its high levels of unsaturated fatty acids, especially during processing and storage when the chemical and biochemical reactions occur, resulting in loss of quality (Sancho *et al*., 2011; Nitipong *et al*., 2014). Although the antioxidant effect of carotenoids has been reported in literature, scientific information about their use, especially in fish and fish-based products, are still incipient. Furthermore, further studies are needed on the effect of the addition of carotenoids in the fish product acceptance. The objective of this study was to evaluate the oxidative stability and sensory acceptance of tilapia burgers formulated with carotenoids from annatto, bixin and norbixin as an alternative to synthetic antioxidant sodium erythorbate.

*Corresponding author.
Email: *mivone@gmail.com*

Material and Methods

Material

Thai tilapia fillets were purchased from the Cooperativa de Aquicultores do Sul Fluminense - Peixesul Ltda (Piraí-RJ, Brazil). The powdered extract bixin and norbixin at concentrations of 27.32% and 30.15% (w/w), respectively, was supplied by Baculerê Corantes Naturais (Olímpia-SP, Brazil). Protein isolate (Solae, Barueri-SP, Brazil), monosodium glutamate (Topmart Logística e Distribuição Ltda, Barra Mansa-RJ, Brazil), spice mix for fish burgers (Kienast & Kratschmer Ltda-SP, Brazil), and the antioxidant sodium erythorbate food grade (Duas Rodas Industrial -SC, Brazil) were used. Soybean oil and refined salt were purchased from the local market (Vassouras-RJ, Brazil.)

Preparation of tilapia burgers

Tilapia burgers were prepared with natural antioxidants (F1, bixin, F2, norbixin) or synthetic (F3, sodium erythorbate) with concentration of 0.05% w / w (Mercadante *et al*., 2010). A formulation without addition of antioxidants was made as control sample (F4 control). The following ingredients and proportions (w/w) were used: Tilapia fillet (81.65%), water (10%), oil (5%), refined salt (1.50%), soy protein (1.0%), spice mix (0.50%) and monosodium glutamate (0.30%). The tilapia fillet was milled in an electric mill (Incomaf, Itaquecetuba-SP, Brazil) using a 3 mm disk until obtaining a homogeneous mass. After weighing, the other ingredients were added to the fillet mass and homogenized with the aid of electric mixer (Incomaf, Itaquecetuba-SP, Brazil) for 5 minutes. The mass of fish was shaped in a hamburger mold (Incomaf, Itaquecetuba-SP, Brazil), and the burger were individually packaged in low density polyethylene bags (thickness 0.5 mm) and stored under refrigeration (2 ± 1°C) until analysis.

Proximate composition of tilapia burgers

Moisture, ash, protein and carbohydrate contents were determined according to AOAC methodologies (AOAC, 2000). Total lipids were determined according to Bligh-Dyer method (AOAC, 2000). The calorific value (CV) was calculated using Atwater values for fat (9 kcal g^{-1}), protein (4 kcal g^{-1}) and carbohydrate (4 kcal g^{-1}) (Mansour and Khalil, 1997).

All analyses were performed in triplicate and the results expressed as mean ± standard deviation (SD), given 80 g of hamburger, which is the portion recommended by the Brazilian legislation (ANVISA, 2003a).

Lipid oxidation of tilapia burgers

The lipid oxidation of the four fish burger formulations was determined in model system, at 0 ± 1°C for 21 days. Burger samples (n = 3) were removed from the model system at 0, 7, 14 and 21 days. At the end of each storage period, all samples were grilled in a domestic grill at 150°C for 16 minutes, 8 minutes for each side. The degree of oxidation of the samples was performed by determining the thiobarbituric acid reactive substances (TBARS), according to the methodology described by Kanat *et al*. (2005). The TBARS concentration was calculated using a standard curve prepared from malonaldehyde bis (diethyl acetal; Merck), and the results were expressed in mg malonaldehyde (MDA)/kg fish burger.

Sensory evaluation of tilapia burgers

For the sensory evaluation, 97 untrained assessors, 58 female and 39 male, participated in the test. First, the overall appearance of the raw burgers prepared with bixin (F1), norbixin (F2) and sodium erythorbate (F3) was evaluated (Beserra *et al*. 2003). Then, an acceptance test was performed for the sample with better overall scores in the previous test. At this stage, 83 untrained assessors participated in the test, 48 female and 35 male. Fish burger samples were served after grilling in a domestic grill for 8 minutes per side, turning once. A hedonic 9-point scale ranging from extremely dislike to extremely like was used, according to Mello *et al*. (2012).

The acceptability index (AI) of the product was calculated using the Equation (1):

$$AI\ (\%) = \left(\frac{A}{B}\right) * 100 \qquad \text{Equation (1)}$$

where: A = mean score obtained for the product, and B = maximum score given to the product.

AII values higher than 70% are considered with good repercussion, according to Teixeira *et al.* (1987).

All sensory tests were performed at CTS Food and Beverage Laboratory - SENAI (Vassouras - RJ, Brazil) with students and staff of different ages, previously selected according to their preference for consuming fish, and availability and interest in participating in the test.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and Tukey test, using BioEstat 5.0 software and a significance level of 5%.

Results and Discussion

Proximate composition of tilapia burger

A portion of tilapia burger (80 g) presented 55.60 g moisture; 1.00 g carbohydrates; 14.30 g protein; 5.20 g lipids; 3.80 g ash, and the carbohydrate content was not significant. The caloric value (CV) was 107.60 Kcal, characterizing the fish burger as a food low in carbohydrates, lipids and calories, which is usually desired when consuming fish and fish-based products.

The present results have differed slightly from those found by Mello *et al.* (2012), who studied burger elaborated with tilapia pulp and found the following values for a serving of 80 g; 60.27 g moisture; 14.59 g protein; 3.76 lipids, and 1.70 g ash. Fogaça and Sant'ana (2007) reported 60.90 g moisture, 0.50 g lipid, 16.50 g protein, and 1.90 g ash for the portion of 80 g of tilapia burgers. The consumption of a portion of tilapia burger of this study was enough to provide approximately 5% calories, 3% carbohydrate, 19% protein, and 9% fat of the daily reference values for a diet of 2000 kcal (ANVISA, 2003b).

Lipid oxidation of tilapia burgers

The lipid oxidation of fish burgers determined by the formation of malondialdehyde (MDA) mg/kg at 0, 7, 14 and 21 days of frozen storage is presented in Table 1. The oxidative rancidity is the chemical oxidation of polyunsaturated fatty acids present in fish, and the content of malondialdehyde (MDA), considered as a secondary oxidation product determined by TBARS, is an important parameter for the study of shelf life of fish burgers (Angelini *et al*., 2013; Dallabona *et al*., 2013; Medina-Meza *et al*., 2014; Nitipong *et al*., 2014).

In general, there was a gradual increase in the formation of MDA mg / kg of raw burger within 21 days of frozen storage ($p \leq 0.05$), indicating that the storage time had significant influence on the development of lipid oxidation (Table 1). MDA values ranged from 0.522 mg/kg to 1.029 mg/kg in control formulations, from 0.393 mg/kg to 0.987 mg/kg for formulations containing bixin, from 0.412 mg/kg to 1.031 mg/kg for formulations containing norbixin. For the samples with addition of sodium erythorbate, these values ranged from 0.406 mg/kg to 1.055 mg/kg (Table 1).

At time 0, 7, 14 and 21 days, the control samples (F4) presented higher MDA values (mg/kg), which were statistically different ($p \leq 0.05$) from the other samples containing bixin and norbixin (Table 1), suggesting that carotenoids acted as antioxidant agents, reducing the formation of secondary products during lipid oxidation of burgers. Similar results were found for hake meatballs prepared with annatto dye and stored at -18°C (Sancho *et al.*, 2011).

After 7 days of storage, the burgers formulated with synthetic antioxidant showed slightly lower MDA contents (mg/kg), which were statistically different ($p \leq 0.05$) from the samples formulated with bixin, norbixin and control. The natural antioxidants showed similar performance of sodium erythorbate in controlling lipid oxidation of tilapia burgers, at 14 and 21 days under frozen storage (Table 2). However, at the end of 21 days, the burger made with bixin had MDA contents (mg/kg burger) slightly lower ($P \leq 0.05$) than those burgers formulated with norbixin and erythorbate (Table 1). This fact can be justified by the difference in polarity of antioxidants. According to Mercadante *et al*. (2010) and Dimakou and Oreopoulou (2012), the antioxidant mechanism of carotenoids in food matrix can be justified by its polarity and location in the emulsion interface, which favor greater contact with free radicals with subsequent inactivation. This mechanism may explain the behavior of natural pigments added to tilapia burgurs of the present study, since during the sample preparation, a good distribution of bixin in the mass may have occurred, probably favored by the mixing and homogenization steps, increasing their contact with free radicals in the environment, promoting its inactivation.

The effect of heat treatment on lipid oxidation is presented in Table 2. Overall, there was a gradual increase in the formation of MDA (mg/kg) in burgers stored for 21 days and grilled, indicating that the storage time followed by heat treatment had a significant effect ($p = 0.05$) on the development of lipid oxidation (Table 2) in all samples. A similar result was reported for chicken burgers grilled and stored at -18°C (Castro *et al.*, 2011), containing colorifico, a brazilian condiment rich in carotenoids from annatto.

Table 2 shows that the antioxidants bixin and sodium erythorbate were more effective ($p \leq 0.05$) against lipid oxidation until the 14th day of storage prior to heat treatment. At time 0, no significant difference ($p \leq 0.05$) was found for MDA values (mg/kg) of the samples containing norbixin, grilled, and control. Similar results were observed for the grilled burgers containing bixin and sodium erythorbate, whose values also showed no statistically significant difference ($p \leq 0.05$). Similar behavior was found for the control and grilled burgers formulated with norbixin after 7 days of frozen storage. However, for the grilled samples containing bixin and erythorbate, the synthetic antioxidant was slightly more effective

Table 1. Malonaldehyde contents (MDA) of raw tilapia burgers during 21 days of storage at 0°C

Time (Day)	MDA contents (mg /kg burger)*			
	Control	Antioxidant		
		Bixin	Norbixin	Erythorbate
0	0.522 a C± 0.028	0.393 b D± 0.031	0.412 b D± 0.017	0.406 b D± 0.024
7	0.647 a B± 0.024	0.590 b C± 0.010	0.599 b C± 0.009	0.555 c C± 0.008
14	1.052 a A ± 0.026	0.766 bc B± 0.004	0.785 b B± 0.006	0.756 c B± 0.005
21	1.029 a A ± 0.005	0.987 b A± 0.003	1.031 a A± 0.003	1.005 ab A± 0.004

*All values present the average of three independent replicates plus standard deviation; [2] Different capital letters (A-D) in different columns mean different MDA content among burger samples ($p \leq 0.05$); [3] Different small letters (a-c) in different rows mean different MDA content during storage ($p \leq 0.05$).

Table 2. Malonaldehyde contents (MDA) of grilled tilapia burgers during 21 days of storage at 0°C

Time (Day)	MDA contents (mg /kg of burger)*			
	Control	Antioxidant		
		Bixin	Norbixin	Erythorbate
0	0.935 a B± 0.057	0.515 b D± 0.022	0.948 a B± 0.087	0.514 b D± 0.025
7	1.011 a AB± 0.044	0.816 b C± 0.006	0.981 a B± 0.017	0.668 c C± 0.005
14	1.062 a A± 0.026	0.864 cd B± 0.004	0.985 b B± 0.006	0.854 d B± 0.005
21	1.093 a A± 0.020	1.094 a A± 0.022	1.081 a A± 0.021	1.090 a A± 0.017

*All values present the average of three independent replicates plus standard deviation; [2] Different capital letters (A-D) in different columns mean different MDA content among burger samples ($p \leq 0.05$); [3] Different small letters (a-c) in different rows mean different MDA content during storage ($p \leq 0.05$).

($p \leq 0.05$) against oxidation than bixin. In contrast, after 14 days of frozen storage, the control samples had higher MDA content (mg / kg burger), followed by the samples with norbixin, bixin, and erythorbate (0.756), which did not differ ($p \leq 0.05$). Mercadante *et al.* (2010) reported that natural antioxidants (the carotenoids zeaxanthin and norbixin) were more effective in controlling lipid oxidation in sausages than the synthetic antioxidant (sodium erythorbate), within 45 days of storage at 4°C.

For the grilled burger, no significant difference in the MDA content (mg/kg) was observed after 21 days of storage for all samples. A possible interaction between time of frozen storage and heat treatment may have contributed to the loss of effectiveness of the synthetic and natural antioxidants, making the MDA contents were similar within 21 days of frozen storage prior to heat treatment (Table 2). Although Brazilian legislation has not established limits for TBARS in fish-based products, the mean values for the frozen and grilled samples (Tables 1 and 2) in this study were lower than 2 mg MDA / kg, which is considered the threshold value for a rusty meat product (Campo *et al.*, 2006).

Sensory evaluation of tilapia burgers

Besides the antioxidant activity, the addition of annatto carotenoids also contributed to improve the sensory properties of tilapia burgers. With respect to overall appearance, the mean scores were 7.45 and 6.98 for the raw burgers with bixin and norbixin, respectively, which did not differ statistically ($p \leq 0.05$). The sample containing the synthetic antioxidant presented lower acceptance score (5.39), and was significantly different from the others ($p \leq 0.05$). This result can be explained by the report of the assessors who claimed that the sample containing sodium erythorbate was pallor, suggesting little spiciness. Similar results were observed by Mendiratta *et al.* (2013), who reported that mutton (kind of Indian meat) nuggets formulated with carrot juice presented higher appearance scores (6.93) than the control (6.50). The authors explained that the carotenoids from carrot juice contributed to improve the appearance of nuggets.

Since the sample containing bixin received the highest appearance scores, it was selected for the acceptance tests. Most assessors liked the burgers made with bixin, and 2% did not like or disliked the burgers, followed by 7% who liked very much and 20%, who liked little. The percentage of 70% of the assessors liked (35%) or liked very much (35%) the fish burgers containing bixin, whereas no assessor disliked the product.

The acceptability index of the burgers with bixin was 80.44%, suggesting good acceptance for this sample. According to Teixeira *et al*. (1987), an acceptability index of at least 70% is necessary for a product to be considered accepted by consumers. The high rate obtained for this formulation may be due to the attractive appearance caused by the addition of bixin. These results suggest that functional natural ingredients such as bixin can be incorporated into fish-based products, contributing to improve their quality and appearance and to obtain healthier fish burgers.

Conclusion

Natural antioxidants (bixin and norbixin) have increased the oxidative stability of tilapia burgers during frozen storage when compared to control samples. In general, the interaction between time of frozen storage and heat treatment contributed to the loss of effectiveness of the natural and synthetic antioxidants during storage. The addition of carotenoids to the raw and grilled burgers provided more attractive characteristic under the sensory point of view. Natural ingredients such as bixin, can be incorporated into fish-based products without harming their quality and appearance, contributing to improve stability, as well as obtaining healthier burgers and free of synthetic antioxidants.

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