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QUALITY OF ANIMAL PRODUCTS

USE OF NATURAL ANTIOXIDANTS IN SOUS VIDE TILAPIA FILLET

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Abstract

The objective of this study was to evaluate the use of natural antioxidants in tilapia fillets submitted to sous vide process. Four treatments were carried out using different natural antioxidants (Treatment 1 – control, without antioxidant; Treatment 2 – oregano extract; Treatment 3 - rosemary extract and Treatment 4 - basil extract) with six replicates. Analyzes of centesimal composition, microbiological composition and lipid oxidation through Thiobarbituric acid reactive substances (TBARS) were performed. There was a significant difference in moisture content, being higher for treatments with extracts, whereas the control treatment presented higher protein content. The fillets were within microbiological standards recommended by legislation. The values obtained from Malondialdehyde (MDA)/kg demonstrate the efficiency of natural antioxidants added. The use of plant extracts as natural antioxidants in sous vide has been shown to be an alternative to minimize oxidative rancidity of tilapia fillets, increasing shelf life. Thus, it is possible to use rosemary, oregano and basil extracts in the tilapia sous vide process.

Keywords

lipid oxidation, fish processing; TBARS.

USO DE ANTIOXIDANTES NATURAIS NO PROCESSO SOUS VIDE DE FILÉ DE TILÁPIA (*Oreochromis niloticus*)

Resumo

O objetivo deste estudo, foi avaliar o uso de antioxidantes naturais em filés de tilápia submetidos ao processo de Sous vide. Foram elaborados quatro tratamentos com a utilização de diferentes antioxidantes naturais no processo sous vide (Tratamento 1 – controle – sem antioxidante; Tratamento 2 - extrato de orégano; Tratamento 3 - extrato de alecrim e Tratamento 4 - extrato de manjericão), com seis repetições. Foram realizadas análise de composição centesimal, microbiológica e oxidação lipídica através das substâncias reativas ao ácido tiobarbitúrico (TBARS). Houve diferença significativa para o teor de umidade, sendo maior para os tratamentos com os extratos, enquanto no tratamento controle, os filés apresentaram maior teor de proteína. Os filés estavam dentro do padrão microbiológico recomendado pela legislação. Os valores obtidos de malonaldeído (MDA)/kg demonstram eficiência da utilização dos antioxidantes naturais. A utilização de extratos vegetais como antioxidantes naturais no processo Sous vide, mostrou ser uma alternativa para minimizar a rancidez oxidativa dos filés de tilápia, aumentando a vida de prateleira. Assim, é possível utilizar os extratos de alecrim, orégano e manjericão no processo sous vide de filé de tilápia.

Palavras chave

oxidação lipídica, processamento de pescado; TBARS.

INTRODUCTION

In recent years, the demand for tilapia fillets has grown, making them one of the species with the greatest potential for intensive farming, due to its organoleptic characteristics, making it highly accepted and demanded by consumers (LAROSA et al., 2012). However, it is interesting to diversify the products available, combining sensory and nutritional qualities, as well as speed and facility for the preparation and good customer acceptance. Associated with this, consumer habits and preferences, allied to a limited time modern lifestyle, have boosted ready-to-eat, and frozen food, therefore it is important that products have something different from the conventional options found in stores. Accordingly, the industry in general has been working on the development of equipment and products that seek to meet the needs of this market. And one of those is through *sous vide*, which is a French expression meaning under vacuum. This technique started in the 1970's in France and according to Barbosa-Cánovas et al. (2005), the technique consists of packing raw or pre-cooked food in vacuum and then subjecting it to slow cooking at low temperatures and immediately cool it. In the *sous vide* process the temperature does not reach 100 °C, while in the other processes the raw material is boiled (above 100 °C), eliminating some of the mentioned characteristics. The conditions of the heat treatment depend on the characteristics of each food, must have a suitable time, temperature and after pasteurization, they are stored at low temperatures (CREED, 1998). Therefore, this processing consists of cooking vacuum-packed foods in thermoresistant bags at low temperatures (65 to 95 ° C) (BALDWIN, 2012; CINTRA, 2016).

The vacuum prevents the rancidity caused by lipid oxidation, as well as food degradation by aerobic microorganisms, processes responsible for taste modification during refrigerated storage, followed by cooking of packaged foods in the presence of oxygen. Vacuum packaging allows the preservation of the constituents of food, especially meat and fresh products, thus maintaining quality and appearance for a long period (AYHAN and KAHVE, 2020).

A major concern for tilapia fillets is the issue of lipid oxidation that brings unpleasant odors and flavors, leading to decreased safety and nutritional quality, allowing the formation of potentially toxic compounds (DEL RÉ and JORGE, 2012). However, the use of antioxidants is an alternative to reduce lipid oxidation by preserving the meat for longer periods of time with better nutritional and organoleptic quality.

Structurally antioxidants are aromatic compounds that have at least one hydroxyl, and can be synthetic, widely used by the food industry, or natural, such as organosulphuris,

phenolics and terpenes, which are part of the constitution of several foods (RAMALHO and JORGE, 2006).

The main synthetic antioxidants used in human food are butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) (DAKER et al. 2008). The main natural antioxidants are tocopherols and phenolic acids obtained from plant extracts such as green-tea, sage, grape, pomegranate, etc (RAMALHO and JORGE, 2006). Both synthetic and natural are efficient in inhibiting oxidation, therefore preventing oxidation of unsaturated fatty acids. According to Brazilian legislation it is permitted the addition of 300 mg/kg tocopherols and up to 200 mg/kg for BHA and TBHG and 100 mg/g for BHT (RAMALHO and JORGE, 2006).

The main species consumed in Brazil are rosemary, basil, oregano, sage and thyme. These extracts have in their compositions phenolic diterpenes, phenolic acids and phenylpropanoids, which provide great physiological activities (PITARO et al., 2012).

Thus, the objective of this study was to evaluate the use of natural antioxidants; rosemary, basil and oregano in Nile tilapia (*Oreochromis niloticus*) fillets submitted to *sous vide*.

MATERIAL AND METHODS

The raw material, Nile tilapia fillet, was purchased fresh from a meat processing facility, located at the municipality of Toledo-PR, and the herbs (rosemary, basil and oregano) at a local supermarket.

Preparing the fillets for *sous vide*

Four treatments were elaborated, being (treatment 1 – control – no herbs added; treatment 2 - extract of oregano; treatment 3 - extract of rosemary and; treatment 4 - extract of basil). For extract preparation, the herbs were stirred with water for 3 minutes at room temperature and the treatments were immersed to marinate in a brine (1:1), according to Table 1.

Table 1. Formulation for tilapia fillet *sous vide* preparation

	Formulations (%)			
Ingredients	**T1**	**T2**	**T3**	**T4**
Water	70.6	70.3	70.0	66.9
Salt	7.06	7.03	7.0	6.69
Herbs	0	10	10	10

After marinated, tilapia fillets from all treatments were placed in a nylon-poly package, vacuum sealed and submitted to pasteurization. Temperature and time were determined according to the average thickness of fillets using Table 2 which relates

pasteurization times to reduce 6/10 of *Listeria monocytogenes* in fish. The average thickness of the fillets was 20.4 mm, subjected to pasteurization for 41 minutes at 60.5 °C, according to Table 2.

Table 2. Pasteurization time (min) and temperature (° C)

	Lean fish			Fatty fish		
Thickness (mm)/ T(°C)	55	57.5	60.5	55	57.5	60.5
5	2:18	50	16	4:30	1:27	27
10	2:22	55	21	3:59	1:32	32
15	2:31	1:04	30	4:08	1:40	41
20	2:42	1:16	41	4:20	1:52	53
25	2:58	1:31	56	4:35	2:07	1:08
30	3:16	1:50	1:12	4:53	2:26	1:25
35	3:38	2:11	1:31	5:15	2:48	1:45
40	4:03	2:35	1:52	5:40	3:13	2:08
45	4:31	3:01	2:14	6:09	3:40	2:32
50	5:02	3:29	2:39	6:40	4:10	2:58
55	5:36	4:00	3:05	7:15	4:43	3:27
60	6:12	4:32	3:33	7:52	5:18	3:57
65	6:51	5:07	4:03	8:33	5:55	4:29
70	7:33	5:44	4:35	9:16	6:34	5:03

Pasteurization Time (HH: MM) for a reduction of 6/10 of *Listeria monocytogenes*. Lean fish (such as cod) have $D_{60}^{5.59}$= 2.88 minutes, while fatty fish (such as salmon) have $D_{60}^{5.68}$= 5.13 minutes. SOURCE: Practical Guide to Cooking *sous vide*.

After reaching the indicated temperature and pasteurization time, the fillets were cooled for 18 minutes in iced water, reaching 5 °C, according to Table 3. Soon after all treatments were stored in a freezer at -18 ° C.

Centesimal Composition

Centesimal composition analysis of the tilapia fillets prepared by *sous vide* process was performed according to the methodology proposed by AOAC (2005). For moisture analysis a pre-drying at 55 °C for 72 hours, followed by drying at 105 °C for 8 hours, proteins by the Kjeldhal method, lipids by Soxhlet extraction with ether as solvent, mineral matter by calcination of samples at 550 °C for 6 hours. Caloric value (CV) was obtained by summing the multiplication of factors 4, 9 and 4, respectively, for protein, fat and carbohydrate contents, respectively, obtaining the result in Kcal/ 100g (SOUCI, 2000). Carbohydrates were obtained by the sum of the contents of moisture, protein, lipids and ashes subtracted of 100%.

Table 3. Cooling time in iced bath

Thickness (mm)/ T(°C)	55	60.5	80
5	1	1	1
10	4	4	5
15	10	10	11
20	17	18	20
25	27	28	30
30	38	40	43
35	52	54	59
40	1:07	1:10	1:17
45	1:25	1:28	1:37
50	1:45	1:49	1:59
55	2:07	2:11	2:24
60	2:30	2:36	2:51
65	2:56	3:03	3:21
70	3:24	3:31	3:53

*Approximate cooling time (HH: MM) in iced water bath (at least half ice) so that the center of the meat reaches 5 ° C. SOURCE: Practical Guide to Cooking Sous vide.

Microbiological analyzes

Microbiological evaluations of the fillets *in natura* and processed by *sous vide* were performed for *Coliforms* at 35 ° C and 45 ° C, *Salmonella sp.* and *Staphylococcus aureus* for food safety of the tasters during sensory analysis. The analyzes were performed according to Resolution RDC No. 12 of January 2, 2001 of the Brazilian National Health Agency (ANVISA).

Lipid oxidation analysis

To verify the fillets lipid stability on storage days 0, 7, 14 and 21, lipid oxidation analysis was performed by the thiobarbituric acid reactive substances (TBARS) method (VYNCKE, 1970). The results were expressed as mg of malonaldehyde (MDA) per kg of sample.

Experimental Design and Statistical Analysis

A completely randomized design with 4 treatments and 6 replicates was performed for analysis of centesimal composition and lipid oxidation. The fillets were the experimental unit. The data was submitted to tests of normality and homoscedasticity assumptions, ANOVA analysis at 5% of probability and if significant ($p < 0.05$) Tukey test, using Statistic software 7.1.

RESULTS AND DISCUSSION

Microbiological analyzes

Table 4 presents the microbiological analysis results for *sous vide* tilapia and *in natura* fillets.

Table 4. Microbiological analysis results for *sous vide* tilapia.

Treatments	*S. aureus* UFC/g	Coliforms 35°C a 45°C NMP		*Salmonella sp.* In 25g
Treat. 1	<1.0 x 10^1 UFC/g	<3	<3	Absent
Treat. 2	<1.0 x 10^1 UFC/g	<3	<3	Absent
Treat. 3	<1.0 x 10^1 UFC/g	<3	<3	Absent
Treat. 4	<1.0 x 10^1 UFC/g	<3	<3	Absent
Fillets *In natura*	<1.0 x 10^1 UFC/g	<3	<3	Absent

NMP: Most likely number. UFC: Colony Forming Unit

The results obtained show that the fillets were within the parameters of normality required by Brazilian legislation, obeying all hygienic-sanitary procedures. In particular coliforms that are indicative of probable fecal water contamination in fish capture environments (FARIAS, 2007). Therefore, the low microorganism count in this experiment is related to good sanitary conditions of the environment in which the fish were captured, associated with adoption of good handling practices during filleting and *sous vide* process.

The values are in accordance with what is established in RDC n. 12, where fresh or frozen fish should be free of *Salmonella sp* in 25 g, and the value of *Staphylococcus* coagulase positive/ g should be limited to 10^3 (ANVISA, 2001).

The absence of *Salmonella sp.*, the reduced count of Coliforms at 35 ° C and 45 °C and *Staphylococcus aureus* shows that the fillets were fit to be consumed without any risk of toxic infections. Although Listeria is the quality indicator, in this work we do not use it since Brazilian legislation excels in other microorganisms.

Centesimal Composition

Table 5 presents the results obtained through the analysis of centesimal composition of *sous vide* tilapia.

The *in natura* fillets presented 78.93% humidity, 18.26% protein, 1.78% lipids and 0.80% ash. These values are close to those described by some authors for tilapia: 19.9% protein, 1.37% lipids and 75.01% ash (ALCÂNTARA et al., 2019; MORAIS et al., 2020). However, there are lower values for protein (16.1 to 17.88%) reported by Talab et al. (20016) and higher levels (24.80%) according to Colpini et al. (2017).

Table 5. Mean values for centesimal composition of *sous vide* tilapia fillets.

Treatments	Humidity (%)	Protein (%)	Lipids (%)	Ashes (%)	CH (%)	Caloric Value (Kcal/100g)
Treat. 1	77.24	18.35	1.28	1.35	1.75	91.98
Treat. 2	83.48	13.94	1.21	1.07	0.28	67.78
Treat. 3	84.83	13.47	0.61	0.96	0.10	59.87
Treat. 4	83.94	13.66	1.74	0.92	-0.27	69.25

Values obtained through duplicates mean. CH: Carbohydrates Treat. 1: Control; Treat. 2: Oregano; Treat. 3: Rosemary; T4: Basil.

Morais et al. (2020) reported moisture content ranging from 74.3% to 75.5%, protein from 19.9% to 20.2%, lipids from 2.38% to 2.71%, and ashes from 2.2% to 2.4%. Mantovani et al., (2012) also studied tilapia fillets and found humidity 75.0%, protein 19.8%, lipids 1.19% and ashes 0.99%.

Bouzgarrou et al. (2020) obtained 77.1% moisture, 21.4% protein, 0.89% lipids and 1.09% ashes for tilapia fillets. It has already been observed that besides protein content, there is a great variation in lipid content, this being due to feeding and raising environment. Neves et al. (2015) showed that Nile tilapia fillets obtained from extractive fishing and intensive farming varied in composition according to the type of environment they were raised in, for humidity 81.68% for fishing and 78.40% for farming and lipids 1.0 and 3.83%, respectively. However, lipid content in commercial fish feed is one of the factors that most influence their final composition (NEVES et al., 2015).

As for the centesimal composition of *sous vide* fillets it was observed that there was a change in crude protein and moisture contents with the addition of natural extracts compared to the control. This may be related to higher fillet water absorption because of antioxidant extracts used, resulting in dilution of the protein concentration of these fillets. Therefore, the protein values were higher for the control treatment, as were the values of ash, carbohydrates and caloric value. According to Contreras-Guzman (2012), ash values in freshwater fish can range from 0.90 to 3.39%.

Alves et al. (2010) evaluated the chemical composition of Nile tilapia fillets submitted to salting and natural drying, where values for lipids (5.69%) and ashes (2.42%) were found for fillets *in natura*, this may be related to the processing method, where there is addition of salt and dehydration occurs, thus concentrating these two nutrients.

Oxidative rancidity results in loss of meat juiciness, changes in texture and destruction of essential constituents, causing a decrease in nutritional value during processing (PIEDADE, 2007). Thus, it can be inferred that with the use of natural antioxidant extracts the fillets lose less water during *sous vide* process in comparison to control treatment without antioxidants.

Chemical composition and quantitative values of fish fillets are of great value for formulating diets and adjusting technical procedures for fish processing industries (SALES and SALES, 1990).

Lipid oxidation

Lipid oxidation results for *sous vide* tilapia fillets are shown in Figure 1.

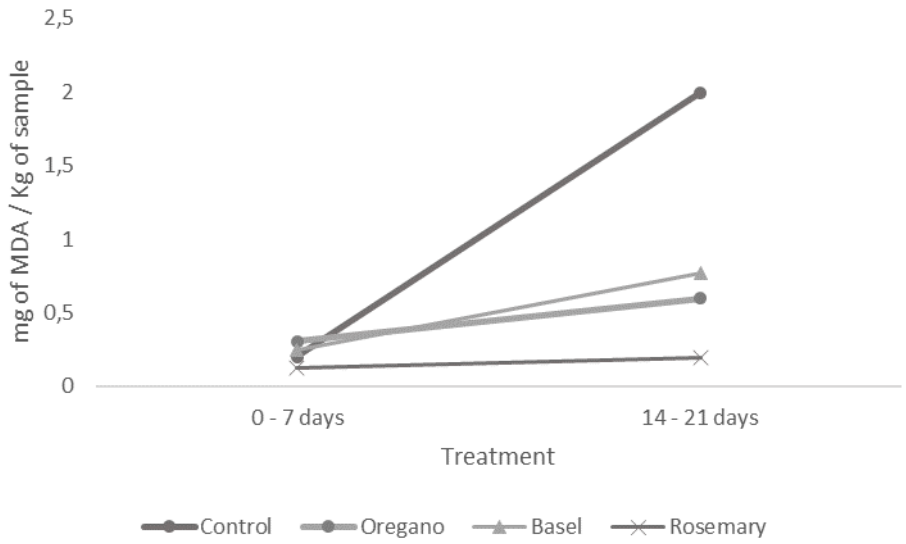

Figure 1. Analysis of lipid oxidation by formation of reactive substances to thiobarbituric acid (mg malonaldehyde/ kg of sample) of *sous vide* tilapia

The results showed a low malonaldehyde/ kg (MDA/ kg) index during the evaluation period for treatments with extracts, differing only from the control treatment that presented a high MDA/ kg value, demonstrating oxidative rancidity characteristics. According to Ogawa (1999), values above 2mg of MDA/ kg increase oxidation presenting characteristics of oxidative rancidity.

The values of MDA/ kg found in the present study differ from those found by Larosa et al. (2012) who used natural antioxidants in the development of a tilapia meat product. The authors reported that the lowest oxidation occurred in treatment with oregano (0.158 mg of MDA/ kg). However, treatment with rosemary was less effective when compared to other treatments. This may be related to the higher phenolic concentration found in oregano.

Piedade (2007) used different aromatic herbs to verify the oxidative stability of processed sardine fillets (*Sardinella brasiliensis*). In this study positive values were observed for parsley, oregano and rosemary presenting greater efficiency in prevention of oxidation. However, in the present study, basil was not as effective when compared to other natural extracts used.

Also there are alternatives to minimize oxidation, would be the use of vacuum

packaging (PIEDADE, 2007). Soccol et al. (2005) worked on a new product based on tilapia, being submitted to processing where, part of it was packed in polyethylene trays, covered with ethylene vinyl alcohol (EVOH) film, another part packed in Modified Atmosphere Packaging (EAM) (modified atmosphere of 60% CO_2 + 40% O_2) and another part was only in vacuum. The products packed through EAM presented high values of MDA, presenting characteristic rancid odor. The vacuum packed product did not change in relation to MDA, maintaining normal organoleptic characteristics until the end of the experiment.

Therefore, the reduction of synthetic products added to industrialized foods, in a certain way, strengthens the appeal that these foods can provide benefits and do not pose health risks, corroborating the results obtained in the *sous vide* experiment.

CONCLUSION

The use of vegetable extracts as natural antioxidants in *sous vide* proved to be an alternative to minimize the oxidative rancidity of tilapia fillet, increasing shelf life. It can be used as a natural alternative to reduce product rancidity.

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