



Cinnamomum zeylanicum extracts reduce lipid oxidation in broadband anchovy (*Anchoviella lepidentostole*) minced fish

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Abstract

Minced fish (MF) is an interesting material for development of novel fish products; however, the mechanical deboning process interferes with MF quality, by increasing the lipid oxidation. This study reports the effect of Ceylon cinnamon (*Cinnamomum zeylanicum*) hydro-ethanolic bark extracts as natural antioxidants on both washed and unwashed MF of broadband anchovy during six months of storage at -18 °C. Spray-dried cinnamon extract (DC) and spray-dried cinnamon extract using maltodextrin 10DE (DCM) as a carrier were evaluated in relation to antioxidant activity *in vitro*. DC and sodium erythorbate were added to both washed and unwashed MF at 0.25% (w:w), whilst DCM was added at 1.055% (w:w). The DC extract presented higher antioxidant activity *in vitro* compared to DCM extract. The addition of cinnamon extracts reduced the lipid oxidation in washed and unwashed MF compared with the controls. *C. zeylanicum* extracts can be used to prevent lipid oxidation in MF during the frozen storage and are alternatives for food industries that seek to meet the demand of consumers increasingly concerned with the consumption of healthy foods.

Keywords: antioxidant activity; natural extracts; fish products.

Practical Application: Cinnamon extracts can be used as natural antioxidants to prevent lipid oxidation in minced fish.

1 Introduction

Fish and fish products are known as a good source of high quality protein (Bolat et al., 2019; Oliveira et al., 2020) and important micronutrients for health such as fatty acids (ω -3), minerals, amino acids, and vitamins (Food and Agriculture Organization of the United Nations, 2018). However, due to the high proportion of polyunsaturated fatty acids, those products are susceptible to spoil by lipid oxidation, causing the loss of nutritional value (Mathew & Abraham, 2006) and their depreciation of quality and cost (Secci et al., 2016).

The production of minced fish is known as one of the industrial process of fish that most induces lipid oxidation, mainly in marine species (Secci et al., 2016, 2017). A washing step is usually performed on the minced fish, causing leaching of sarcoplasmic proteins, pigments, enzymes, blood, lipids, flavoring components and minerals (Neiva & Gonçalves, 2011). Thus, the washed mince usually becomes lighter, less flavored and less susceptible to lipid oxidation. Mechanical separation process is an alternative for the diversification of new fish products (Freitas et al., 2012) that could be used for broadband anchovy (*Anchoviella lepidentostole*), a small anadromous fish with high nutritional value and great economic importance for the south coast of São Paulo State, Brazil (Mendonça & Sobrinho, 2013).

Following the actual demands of consumers for healthier food with less additives, several plant extracts have been tested for their ability to inhibit lipid oxidation in fish and fish products (Fernandes et al., 2017; Raeisi et al., 2016; Sampels et al., 2010; Sancho et al., 2011; Yerlikaya & Gokoglu, 2010). *Cinnamomum* species are known as natural antioxidants due to the high content of phenolic compounds, such as proanthocyanidins (Lee & Balick, 2005). Studies have proven that *Cinnamomum zeylanicum* have many beneficial effects including antioxidant (Ostroschi et al., 2018; Tulini et al., 2016), antimicrobial (Van Haute et al., 2016) and antidiabetics (Barceloux, 2009).

The antioxidant activity of Ceylon cinnamon extracts has been studied mainly *in vitro* (Abeysekera et al., 2013; Ostroschi et al., 2018). Few studies have focused on the potential antioxidant activities of cinnamon bark extracts in food (Jahangir et al., 2018; Vidanagamage et al., 2016), however, studies of the effect of the application on fish and fish products (Anal et al., 2014) are even scarcer. Regardless of, one of the great obstacles to the application of the natural antioxidants is related to the instability of the structure of antioxidants compounds such as proanthocyanidins, due to the their susceptibility to oxidative reactions (Ostroschi et al., 2018; Santiago-Adame et al., 2015). Researchers have proved that the microencapsulation technique

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(spray-drying) can protect the antioxidant compounds (Santiago-Adame et al., 2015; Tulini et al., 2016). However, the effect of microencapsulation of cinnamon bark extracts on stability of the antioxidants compounds has to be evaluated, as well as its performance in preventing the lipid oxidation in fish products with different susceptibilities to lipid oxidation. Thus, the present study aimed to evaluate the antioxidant activities of both free and microencapsulated Ceylon cinnamon bark extracts and their ability to slow up the lipid oxidation on washed and unwashed minced fish from broadband anchovy during the frozen storage (-18 °C) for six months.

2 Material and methods

This study was approved by the Ethical Committee on Animal Use (CEUA) of the Faculdade de Zootecnia e Engenharia de Alimentos, certificate number: 8355181217.

2.1 Fish obtainment and minced fish (MF) processing

Fresh broadband anchovies (20 kg) were obtained from artisanal fishermen in the cities of Iguape and Cananéia on the coast of São Paulo State – Brazil, and carried to the laboratory (a 6h trip) in thermally insulated boxes between layers of ice (-1 °C to 1 °C) at the same day of capture. The process for obtainment of minced fish was carried out the next day. The fish were beheaded, gutted and processed in an industrial fish deboning machine (250 kg/h of capacity, HighTech, model HT 250, Chapecó, Brazil), obtaining unwashed broadband anchovy MF (UWMF). Washed MF (WMF) was obtained according to Kirschnik & Macedo-Viegas (2009) with some adaptations. The MF was washed with cooled water at 5 °C in the ratio 3:1 (water:MF), under constant agitation for 5 min. After 3 min of resting, the MF was recovered by filtration through nylon bags (mesh fabric). Antioxidants were applied in both WMF and UWMF as soon as the minces were obtained, and then the minces were packed in polyethylene bags and frozen at -18 °C.

2.2 Fish and minced fish characterization

2.2.1 Mince yield

The percent yield (EY) of unwashed mince was determined using the Equation 1:

$$EY(\%) = \frac{\text{Weight of unwashed mince (g)}}{\text{Weight of beheaded, eviscerated broadband anchovy (g)}} \times 100 \quad (1)$$

2.2.2 Proximate composition

Moisture, protein and ash contents were determined in beheaded and eviscerated broadband anchovy (BEF), WMF, UWMF, and residue of minced fish (RMF) according AOAC methods (Association of Official Analytical Chemists, 2005). The determination of total lipids was performed by cold extraction, according to the methodology described by Bligh & Dyer (1959).

2.2.3 Obtainment of cinnamon dried extracts

Ceylon cinnamon barks were imported from Chile and the extracts were obtained according to the methodology developed by Souza et al. (2018), using aqueous ethanol solution (50% by mass) in the ratio of 1:7.5 (powdered cinnamon:solution). The mixture was submitted to heating at 60 °C for 30 min and stirring at 650 rpm. Following the mixture was filtered and centrifuged (Eppendorf Centrifuge 5702, São Paulo SP, Brazil) at 2500 rpm for 5 min at 25 °C. The liquid extract was atomized in spray dryer equipment (model MSD 1.0, Labmaq do Brasil Ltda, Ribeirão Preto, Brazil) at 130 °C, 13 mL/min of feed velocity (peristaltic pump, model Labmaq OS-1, Ribeirão Preto, Brazil), 1.2 mm nozzle and 40 L/min of compressed air flow to obtain the dried extract (DC). Microencapsulation of the cinnamon extract was carried out using maltodextrin 10 DE at 5% as a carrier. The liquid extract was added to the carrier maltodextrin under constant stirring until complete dissolution, then, atomized by spray-drying following the conditions described previously for the DC, obtaining the spray-dried cinnamon microencapsulated extract (DCM). The spray-dried cinnamon extracts were stored in a freezer at -18 °C protected from light until use.

2.3 Characterization of cinnamon dried extracts

2.3.1 Determination of antioxidant compounds of cinnamon dried extracts

2.3.1.1 Total phenolic content (TPC)

The determination of total phenolic content was based to the Folin-Ciocalteu method proposed by Singleton et al. (1999). After reaction between extracts and Folin-Ciocalteu reagent, the absorbance was measured at 750 nm in 10S UV-VIS Spectrophotometer (Thermo Scientific, Genesys). TPC of cinnamon extracts were expressed as mg Gallic acid equivalents (GAE)/g of the dried extracts.

2.3.1.2 Proanthocyanidins content

The determination of total content of proanthocyanidins in dried cinnamon extracts was based on the method of 4-dimethylaminocinnamaldehyde (DMAC) (Payne et al., 2010). The results were expressed as mg procyanidin B2/g of the dried extracts.

2.3.2 Antioxidant capacity of cinnamon dried extracts

2.3.2.1 Scavenging activity of free radical (DPPH•)

The capacity of cinnamon dried extracts on sequestering DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) was evaluated according to the methodology proposed by Brand-Williams et al. (1995). The antioxidant activity (AA) of the extracts was expressed as EC50 (concentration required to achieve 50% inhibition of DPPH•).

2.3.2.2 ABTS⁺ radical scavenging activity

The capacity of sequestering the ABTS radicals was determined according to the methodology described by Melo et al. (2015).

Trolox standards or cinnamon dried extracts were added with ABTS solution. The reaction occurred at room temperature and the absorbance was read at 730 nm in a SpectraMax® M3 microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). The results were expressed as Trolox equivalents.

2.3.2.3 Oxygen radical absorbance capacity (ORAC)

The determination of antioxidant capacity by the ORAC method was based on the methodology proposed by Abeysekera et al. (2013). The results were expressed as Trolox equivalents.

2.3.2.4 Ferric reducing antioxidant power (FRAP)

The antioxidant activity by the FRAP method was determined according to the methodology proposed by Salvador et al. (2018). The results were expressed as $\mu\text{mol Fe}^{2+}/\text{g}$ of the cinnamon dried extracts.

2.4 Application of cinnamon dried extracts in the MF

Eight (8) treatments of antioxidant application in UWMF and WMF were defined (Table 1). Sodium erythorbate (positive control) and DC were added to MF at the same concentration (0.25g/100g). The amount (g) of DCM to be added in the MF (1.055g/100g) was determined accordingly to the preliminary results obtained of the total phenolic contents of the DC and DCM, in order to guarantee the equivalence of antioxidant compounds in both extracts. Around 400 g of MF was used for each treatment, which were packed in aliquots of 50 g in polyethylene bags and stored in a freezer at -18°C until the moment of analysis.

2.5 Evaluation of the MF stability

In order to evaluate the effect of cinnamon dried extracts on the reduction of the lipid oxidation in both WMF and UWMF, periodic analyzes were performed at 0, 30, 60, 90, 120, 150 and 180 days of storage at -18°C .

Table 1. Definition of treatments for the application of antioxidants in the washed and unwashed MF of broadband anchovy.

Treatment	MF	Antioxidant (%)	Amount added (g/100 g MF)
UWMF-WA	UW	Negative control - without antioxidant (WA)	0
WMF-WA	W		
UWMF-SE	UW	Positive control – sodium erythorbate (SE)	0.25
WMF-SE	W		
UWMF-DC	UW	Spray-dried cinnamon extract (DC)	0.25
WMF-DC	W		
UWMF-DCM	UW	Spray-dried cinnamon microencapsulated (DCM)	1.055 ¹
WMF-DCM	W		

¹Mass calculated as 0.25% spray-dried cinnamon extract based on the results of total phenolic compounds obtained in the characterization of the two types of extract.

2.5.1 Lipid oxidation

The 2-thiobarbituric acid reactive substances (TBARS) were determined according to the methodology developed by Vyncke (1970). Briefly, TBARS were extracted in trichloroacetic acid (TCA) (7.5%), and then added with thiobarbituric acid (TBA) 0.02 M. The reaction was performed at 98°C for 40 min. After incubation, the oxidation products were quantified in spectrophotometer (Bio Spectro, SP220, Curitiba, SP - Brazil) at 532 nm wavelength using a calibration curve of TEP (1,1,3,3-tetra-ethoxypropane) diluted in 7.5% (w/v) TCA. The results were expressed as mg malondialdehyde/kg of the sample.

2.5.2 Determination of total volatile nitrogenous bases (TVB-N)

TVB-N were determined according to the method described by Brasil (1999). Briefly, deproteinized TVB-N extract was obtained using TCA (5%). Aliquots of 20 mL of extract was alkalized with magnesium oxide (MgO) and TVB-N was distilled under vapor flow in a nitrogen distiller (Marconi, MA 036, Piracicaba, SP-Brazil). Ammonium nitrogen was received in boric acid, with methyl red and bromocresol green indicator, up to 125 mL, and then quantified by titration with 0.01N HCl.

2.5.3 Instrumental color analysis

Hunter-Lab MiniScan XE colorimeter with D65 illuminant and 10° viewing angle was utilized for colorimetric measurement of both WMF and UWMF treatments during the storage. The L^* , a^* , and b^* parameters of the CIELab system were evaluated. The equipment was adjusted to provide the mean and standard deviation of three readings. The color coordinates were used to obtain the color image in the EasyRGB color calculator (EasyRGB, 2016).

2.6 Statistical analysis

The MF was produced three times and each analysis was performed in triplicate. The statistical analysis of data was performed using the Statistical Analysis System – SAS 9.3 (SAS Institute, 2011). All the data was firstly analyzed for homoscedasticity by the Levene test. Averages from parameters of proximate composition were compared using the Tukey test ($p < 0.05$) when the variances were homogeneous and Kruskal Wallis (nonparametric Anova – $p < 0.05$) when differences in variances were significant. For the data obtained in the stability studies, the effect of treatment (T), storage time (S) and interaction T x S were evaluated. Normality of the studentized residues was verified using the Mixed procedure (PROC MIXED) and the Shapiro-Wilk test (PROC UNIVARIATE). Decomposition of the main effects term by using orthogonal polynomials was performed in order to evaluate the interactions. When interaction was not significant Tukey's pairwise comparisons were performed for treatments and storage time averages.

3 Results and discussion

3.1 Characterization of fish and minced fish

The UWMF yield was 68.7% in relation to the beheaded and eviscerated broadband anchovy (BEA). According Leira et al. (2019) mechanical deboning process is traditionally used to obtain residual flesh from fish carcasses. Finne et al. (1980) reported the yield of mechanical deboning process from fish carcasses around 20 to 31.3%. Differently, in this study, the mechanical deboning process was used to obtain the highest yield in flesh from a small fish species, which filleting would be unfeasible. Keay (2001) reported that up to twice as much can be recovered by mechanical separation of flesh directly from headless gutted fish.

In the mechanical deboning process, minced fish with high nutritional value is obtained (Leira et al., 2019). The proximate composition values of BEA and UWMF indicated that the deboning process only affected the ash content, promoting a significant reduction of this component in UWMF (Table 2).

Comparison between UWMF and WMF revealed a reduction of protein, lipids, and ash contents and an increase of moisture, after the washing process. Similar results were reported by other authors for Nile tilapia (Kirschnik & Macedo-Viegas, 2009), African catfish (*Clarias gariepinus*) (Durães et al., 2012) and catfish (*Ictalurus punctatus*) (Hoke et al., 2000). The change in WMF composition can be justified by loss of components including sarcoplasmic proteins, pigments, enzymes, blood, lipids, flavoring compounds, and leaching of the minerals (Neiva & Gonçalves, 2011).

The residue of minced fish (RMF) was included for characterization to verify how moisture, protein, lipids and ash from the broadband anchovy would be rationed on the different materials obtained in the deboning process. RMF showed moisture and lipid contents similar to the BEA, and a lower amount of proteins. RMF presented the highest ash content, differing from the other samples and showing the positive effect of the deboning process in separating the fish flesh from the bones that contain higher amounts of minerals.

3.2 Cinnamon extract characterization

3.3 Antioxidant compounds and antioxidant capacity of cinnamon dried extracts

Cinnamon dried extract showed high values of proanthocyanidins and TPC (Table 3). Dudonné et al. (2009) found that the aqueous extract of Ceylon cinnamon was among the four extracts with the highest content of total phenolic compounds (309.23mg gallic acid/g) out of thirty different plants of industrial interest.

Significant differences were observed between the spray-dried cinnamon extracts for proanthocyanidins and total phenolic content, as well as for *in vitro* antioxidant activity measured by the methods of scavenging radicals DPPH•, EC₅₀ and ABTS⁺, ORAC and FRAP (Table 3). The results showed that the microencapsulation of the cinnamon extracts using maltodextrin as carrier diluted in 4 times the contents of TPC and, consequently, reduced the antioxidant activity of the DCM. There is a positive correlation between the amount of TPC and the antioxidant capacity (Dudonné et al., 2009). Similar results,

Table 2. Proximate composition (%) of beheaded and eviscerated anchovy (BEA), unwashed minced fish (UWMF), washed minced fish (WMF) and minced fish residue (RMF) of broadband anchovy (Wet basis).

Parameter (%) ^a	BEA	UWMF	WMF	RMF	SEM1	p value
Protein	17.9 ^A	14.9 ^A	10.7 ^B	11.29 ^B	0.72	<0.0001
Moisture	75.6 ^{BC}	76.8 ^B	84.8 ^A	74.17 ^C	0.88	<0.0001
Lipid	4.7 ^A	4.6 ^A	3.5 ^B	4.96 ^A	0.18	0.0002
Ash	1.7 ^B	1.2 ^C	0.7 ^D	3.20 ^A	0.19	<0.0001

^{A,B}Mean (n = 3) in a row with the different uppercase superscript letters are significantly different (p < 0.05); ^aWet basis; ¹Standard Error of the Mean, indicating the global variation among all treatments.

Table 3. Antioxidant compounds and antioxidant activity of cinnamon extracts.

Parameter	Cinnamon extracts	
	DC	DCM
Proanthocyanidins (mg eq procyanidin B2/g)	250.4 ^A ± 6.9	47.3 ^B ± 1.4
TPC (mg gallic acid equivalents (R ² =0.9929)/g)	424.0 ^A ± 3.9	101.9 ^B ± 1.3
ABTS ⁺ (μmol equivalents Trolox/g)	4393.5 ^A ± 29.9	1252.8 ^B ± 77.9
ORAC (μmol equivalents Trolox/g)	3782.3 ^A ± 334.1	1273.5 ^B ± 199.8
FRAP (μmol Fe ²⁺ /g)	4045.3 ^A ± 157.5	1151.7 ^B ± 28.2
DPPH•EC ₅₀ (μg of DPPH/mL)	105.7 ^B ± 4.2	483.6 ^A ± 15.7

Means ± standard deviation (n = 3) in a row with the different uppercase superscript letters are significantly different (p < 0.05). DC - spray-dried cinnamon, DCM - spray-dried cinnamon microencapsulated.

showing proportional reduction of antioxidant activity, were reported by Santiago-Adame et al. (2015) and Ostroschi et al. (2018) in spray drying-microencapsulation of *C. zeylanicum* extracts using maltodextrin 10 DE as a carrier agent.

The DC presented a lower EC₅₀ (105.7 μg of DPPH/mL) compared to DCM. According to Ervina et al. (2016) the lower the value of EC₅₀, the higher the antioxidant activity of the extract. Santiago-Adame et al. (2015) reported low antioxidant activity of the Ceylon cinnamon infusion extracts with EC₅₀ values of 290 μg/mL for the free extract, and variations from 336 to 596 μg/mL for spray drying-microencapsulation of cinnamon infusions with maltodextrin. In the present study, organic solvent (ethanol 50% in water) was used to obtain the cinnamon extracts, which can explain their higher antioxidant activity, owing to more effective extraction of the antioxidant compounds.

3.4 Stability of the MF

3.4.1 Lipid oxidation

In general, it was observed that, regardless of the treatment, UWMF presented higher values of TBARS when compared to WMF (Figure 1). This result confirmed that the washing process led to higher stability of the MF related to the reduction in lipid contents (Table 2), as well as to the leaching of catalysts (blood, minerals) (Neiva & Gonçalves, 2011).

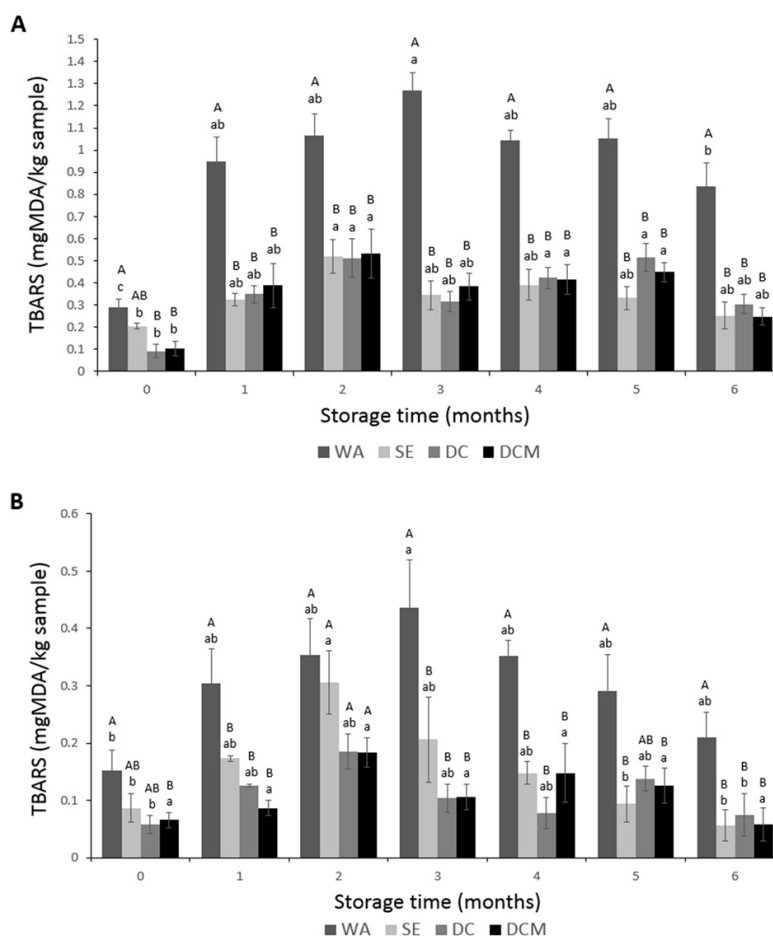


Figure 1. Lipid oxidation (TBARS, mg MDA/ kg of the sample) of unwashed *minced fish* (UWMF) (A) and washed *minced fish* (WMF) (B) stored for 6 months at -18°C . Means within the same storage time followed for the same upper-case letters are not significantly different at $p < 0.05$. Means within the same treatment followed for the same lower-case letters are not significantly different at $p < 0.05$. WA – without antioxidant (negative control); SE – sodium erythorbate (positive control); DC – spray-dried cinnamon extract; DCM – spray-dried cinnamon microencapsulated extract.

The parameters rancidity and TBARS assess the same sensory descriptor, the rancid flavor (Marques et al., 2020), which may ultimately affect the sensory acceptability and use of the MF. Ke et al. (1984) reported that fish products presenting TBARS values below 0.58 mg/kg are perceived as not rancid. According to this, only UWMF without antioxidant would not be recommended for product elaboration as it presented TBARS values above 0.58 mg/kg from the first month of storage.

Both treatment and storage time significantly affected TBARS values in UWMF ($p < 0.001$) and WMF ($p < 0.01$), moreover, there was an interaction between treatment and storage time for UWMF ($p < 0.05$). Regardless of the washing process, the negative controls (WA) presented higher average TBARS values, 0.93 and 0.30 mg MDA/kg MF , in UWMF and WMF respectively, when compared to the other treatments under study (Figure 1). This event indicates that the lipid oxidation occurred rapidly in WA, which represents clear evidence of the positive effect of the antioxidants in the reduction of the lipid oxidation of the MF.

Cinnamon extracts were effective in inhibiting the lipid oxidation in broadband anchovy mince. The inhibition was similar to that obtained with sodium erythorbate for both UWMF and WMF at all storage times (Figure 1). Thus, both cinnamon

extracts could be used as natural antioxidants replacing sodium erythorbate for broadband anchovy mince. This is of high relevance taking into account that many natural antioxidants presenting good *in vitro* performance not always achieve the same results in food matrices owing to their complexity, as reported by Ferreira et al. (2016) who evaluated the effect of lemon verbena (*Aloysia triphylla*) infusion applied to fish pates, and Fernandes et al. (2017) who studied the effect of turmeric (*Curcuma longa*) extract applied to frozen fish fillets. This study confirm the previous findings of Anal et al. (2014) that detected high phenolic contents and good antioxidant activity in extracts of *Cinnamomum varum* barks.

3.4.2 Total volatile nitrogenous bases (TVB-N)

TVB-N content is a parameter usually measured in fresh fish as it is related to microbial quality. European Community (EC) Regulation No 1022/2008 (European Community, 2008) states limits from 25 to 35 mg TVB-N/100g for human consumption depending on the fish species. In this study, TVB-N was determined to assess the quality of the MF and to verify whether cinnamon extracts would have negative impacts on the overall quality of the MF during the frozen storage. UWMF had higher levels of

TVB-N when compared to WMF (average values of 13.22 and 5.48 mg TVB-N/100g, respectively) (Table 4), however, both values were below the limits recommended by EC. The lower value detected in WMF is related to the elimination of soluble proteins in water during the washing process. Kirschnik et al. (2013) also observed lower TVB-N values during frozen storage of unwashed and washed Nile tilapia MSM, varying from 4.25 to 6.77 mg N/100 g and 0.15 to 2.99 mg N/100g, respectively. Regardless of the washing process, the treatments had no effect on the TVB-N values, whilst storage time caused a significant ($p < 0.0001$) increase of TVB-N (Table 4).

There was no significant interaction between antioxidant treatment and storage for both UWFMF and WMF. TVB-N can be produced in fish muscle by microbial and/or endogenous enzymatic activities (Huss, 1995). In this study, as the MFs were kept at -18°C , which impairs microbial activity, the increase of TVB-N indicated that some enzymatic activity still proceeded during the storage.

3.4.3 Colorimetric analysis

The washing process significantly decreased L^* (lightness) from 56.52 to 54.49, a^* (redness) from -1.84 to -2.50 and b^* (yellowness) from 8.10 to 2.53 in MF. The decrease on color is due to the leaching of components during the washing process, mostly pigments and blood (Neiva & Gonçalves, 2011). Color changes may occur during frozen storage of fish products. Pigment oxidation usually occurs concomitantly with oxidation of lipids in meat products; thus, the addition of antioxidants may

inhibits both of these phenomena (Yu et al., 2002). Evaluating the UWMF (Table 5), it was verified that L^* was not affected by the antioxidant treatment, whilst there was a significant effect of storage. Higher L^* values were detected at 2 and 5 months of storage with neither tendency of increase nor decrease of values along the storage. The antioxidant treatment presented an effect on both a^* and b^* values, in addition there was also an effect of storage time on b^* values.

Antioxidant treatment had significant effect on L^* , a^* and b^* values in WMF (Table 5). Storage time presented significant effect on L^* values. Average L^* value was higher for SE treatment followed by DC and DCM; WA presented the lowest L^* value.

In both WMF and UWMF, a^* and b^* parameters presented the highest values in DC treatment followed by DCM. This result allows to state that the microencapsulation of the extract interferes with the color, reducing its intensity, by dilution with maltodextrin. However, the addition of dried cinnamon extracts increased both a^* and b^* parameters in comparison with samples without antioxidant or added with sodium erythorbate.

L^* , a^* and b^* values can be combined to generate Chroma (C^*) and Hue parameters, however, these color parameters did not raise different results in this study (data not shown). In contrast, the observation of Figure 2 that shows the colors obtained using the RGB calculator, may better translate the color sensation produced by each UWMF and WMF treatment. UWMF samples presented brownish tones that were enhanced by cinnamon extracts, mainly DC. Washing interfered with WMF color that became more grayish for treatments WA and

Table 4. Total volatile nitrogenous bases (mg N/100 g sample) of unwashed (UWMF) and washed minced fish (WMF) stored for 6 months at -18°C .

Treatment ³ (T)	Storage (S) (months)							Treatment mean
	0	1	2	3	4	5	6	
UWMF-WA	13.2	11.4	11.6	13.5	13.3	13.6	16.6	13.3
UWMF-SE	13.5	11.1	11.9	14.3	14.1	14.4	13.3	13.2
UWMF-DC	13.0	10.7	11.9	13.8	13.4	14.3	15.3	13.2
UWMF-DCM	12.9	10.9	11.1	13.4	13.4	15.0	15.5	13.2
Storage time mean ²	13.1 ^B	11.0 ^C	11.6 ^C	13.8 ^{AB}	13.5 ^B	14.3 ^{AB}	15.2 ^A	13.2
UWFM mean	13.22							
SEM ¹	0.18							
Effect of treatment (T)	NS							
Effect of storage (S)	<0.0001							
Interaction T x S	NS							
WMF-WA	5.1	5.0	4.4	5.1	5.5	5.9	6.7	5.4
WMF-SE	5.4	4.1	5.0	5.6	5.7	5.8	6.7	5.5
WMF-DC	5.8	4.4	4.9	5.2	5.6	6.1	6.9	5.6
WMF-DCM	5.6	4.2	4.8	5.1	6.1	6.0	6.6	5.5
Storage time mean ²	5.5 ^{BC}	4.4 ^E	4.8 ^{DE}	5.3 ^{CD}	5.7 ^B	6.0 ^B	6.7 ^A	5.5
WMF mean	5.48							
SEM ¹	0.07							
Effect of treatment (T)	NS							
Effect of storage (S)	<0.0001							
Interaction T x S	NS							

¹Standard Error of the Mean indicating the global variation among all treatments; ²Storage means ($n = 4$) in a row with the different uppercase superscript letters are significantly different ($p < 0.05$). NS: not significant ($p > 0.05$); ³Data were obtained from three replicates. WA – without antioxidant (negative control); ES – sodium erythorbate (positive control); DC – spray-dried cinnamon extract; DCM – spray-dried cinnamon microencapsulated extract.

Table 5. Color (L^* , a^* and b^*) parameter of unwashed and washed minced fish stored for 6 months at $-18\text{ }^{\circ}\text{C}$

	Treatment (T)	Storage (S) (months)								SEM ¹	Effect of T	Effect of S	Interaction T X S
		0	1	2	3	4	5	6	Mean ²				
L^*	UWMF-WA	56.52	56.41	58.47	55.34	55.52	60.52	52.12	56.41	0.28	NS	<0.0001	NS
	UWMF-SE	55.45	57.34	59.33	56.16	56.59	61.38	56.16	57.49				
	UWMF-DC	55.65	56.55	59.68	56.18	57.15	59.71	55.68	57.23				
	UWMF-DCM	56.49	55.62	59.86	56.29	55.70	57.87	55.92	56.82				
	Mean ³	56.03 ^C	56.48 ^{BC}	59.34 ^{AB}	55.99 ^C	56.26 ^C	59.87 ^A	55.23 ^C	56.99				
a^*	UWMF-WA	-1.84	-1.85	-2.05	-1.74	-1.82	-1.92	-1.90	-1.87 ^D	0.06	<0.0001	NS	NS
	UWMF-SE	-1.76	-1.49	-1.62	-1.33	-1.48	-1.53	-1.53	-1.54 ^C				
	UWMF-DC	0.15	-0.12	0.01	-0.01	0.13	-0.18	0.03	0.00 ^A				
	UWMF-DCM	-0.67	-0.75	-0.81	-0.64	-0.80	-0.78	-0.72	-0.74 ^B				
	Mean	-1.03	-1.00	-1.12	-0.93	-0.99	-1.10	-1.03	-1.04				
b^*	UWMF-WA	8.10	6.81	7.31	7.45	7.61	6.79	6.28	7.19 ^D	0.11	<0.0001	<0.0001	NS
	UWMF-SE	9.86	8.24	8.38	8.56	7.86	7.22	8.21	8.33 ^C				
	UWMF-DC	12.39	9.33	10.32	9.96	10.11	9.27	10.01	10.20 ^A				
	UWMF-DCM	11.00	9.00	9.01	8.71	8.44	8.15	9.26	9.08 ^B				
	Mean ³	10.34 ^A	8.34 ^B	8.76 ^B	8.67 ^B	8.50 ^B	7.86 ^B	8.44 ^B	8.70				
L^*	WMF-WA	54.49	54.64	57.74	54.01	53.28	57.46	54.53	55.16 ^B	0.24	0.033	<0.0001	NS
	WMF-SE	55.03	55.10	57.72	56.52	55.38	59.91	55.90	56.51 ^A				
	WMF-DC	54.82	55.62	57.84	55.67	55.52	57.35	53.82	55.81 ^{AB}				
	WMF-DCM	54.92	54.19	58.15	54.75	55.01	56.04	54.52	55.37 ^{AB}				
	Mean ³	54.81 ^C	54.89 ^C	57.86 ^A	55.24 ^{BC}	54.80 ^C	57.69 ^{AB}	54.69 ^C	55.71				
a^*	WMF-WA	-2.50	-2.12	-2.22	-2.15	-2.19	-2.23	-2.11	-2.22 ^C	0.06	<0.0001	NS	NS
	WMF-SE	-2.33	-1.94	-2.09	-1.88	-1.91	-2.25	-2.00	-2.06 ^C				
	WMF-DC	-0.11	0.13	0.06	0.14	0.08	-0.03	0.00	0.04 ^A				
	WMF-	-0.91	-0.95	-1.14	-0.97	-0.95	-0.81	-0.94	-0.95 ^B				
	Mean	-1.46	-1.22	-1.35	-1.22	-1.24	-1.33	-1.26	-1.30				
b^*	WMF-WA	2.53	1.83	2.10	2.20	1.72	1.81	1.81	2.00 ^D	0.18	<0.0001	NS	NS
	WMF-SE	3.63	3.20	3.12	3.11	3.23	3.24	3.10	3.23 ^C				
	WMF-DC	9.84	8.58	8.54	8.01	8.34	7.90	8.33	8.51 ^A				
	WMF-DCM	6.62	6.05	5.76	5.64	5.30	5.83	5.77	5.85 ^B				
	Mean	5.66	4.92	4.88	4.74	4.65	4.69	4.76	4.90				

¹Standard Error of the Mean indicating the global variation among all treatments; NS: not significant; ²Storage means (n = 4) with different uppercase superscript letters are significantly different (p < 0.05); ³Treatment means (n = 7) with different uppercase superscript letters are significantly different (p < 0.05). WA – without antioxidant (negative control); ES – sodium erythorbate (positive control); DC – spray-dried cinnamon extract; DCM – spray-dried cinnamon microencapsulated extract.

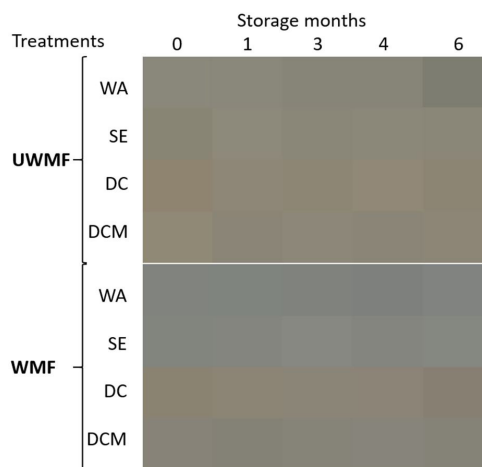


Figure 2. Color of each treatment of minced fish (MF) throughout the frozen storage obtained from the color coordinates using the EASYRGB color calculator (EasyRGB, 2016). WA – without antioxidant (negative control); ES – sodium erythorbate (positive control); DC – spray-dried cinnamon extract; DCM – spray-dried cinnamon microencapsulated extract.

SE, whilst the brownish tones remained mainly in DC treatment. UWMF samples presented more visible and unpredictable color changes during frozen storage, regardless of the treatment, while color of WMF samples were more stable.

4 Conclusions

The cinnamon dried extracts present high antioxidant capacity *in vitro*. The microencapsulation of cinnamon extracts with maltodextrin dilutes the contents of total phenolic compounds, but do not interfere with their antioxidant capacity in broadband anchovy MF. Cinnamon dried extracts inhibit the formation of TBARS in washed and unwashed broadband anchovy minced fish stored at -18 °C for 6 months, thus they are effective in slow down an important phase of lipid oxidation. The effect of cinnamon extracts is similar to the sodium erythorbate for both UWMF and WMF, suggesting that cinnamon extracts could be used as natural antioxidants for fish products and offering an alternative for food industries that seek to meet the demand of consumers increasingly concerned with the consumption of healthy foods. Future studies should address the use of fish mince added with cinnamon extracts in fish products such as fish burgers and nuggets evaluating their sensory acceptability.

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