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RESEARCH ARTICLE

Effect of Red Cabbage Extract on Minced Nile Perch Fish Patties Vacuum Packaged in High and Low Oxygen Barrier Films

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ABSTRACT

Oxidation of polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish causes loss of product quality. Oxidative rancidity causes loss of nutritional value and undesirable color changes. Therefore, powerful antioxidant extracts may provide a relatively low cost and natural means to reduce oxidation, resulting in longer, higher quality and higher value shelf life of foods.

In this study, we measured synergistic effects of red cabbage antioxidant and vacuum packaging on lipid oxidation in fresh tilapia patties using thiobarbituric acid reactive substances (TBARS) assay, peroxide value (PV), pH and color analysis.

Concentrated red cabbage extract was obtained using an efficient freeze/thawed method developed in our laboratory (citation). Fresh tilapia patties were prepared with solutions containing 68 ppm of extract concentrate for each 50 gr of fish patties. Samples were stored for 15 days at refrigeration conditions (4±1°C) and analyzed interval between two days for pH, color analysis, and lipid oxidation assessments.

Results show that treated and vacuum packaged samples had lower oxidation levels than controls. Lipid peroxide values on treated samples showed benefits through day 12. This work shows that synergistic effect of red cabbage antioxidant extracts and vacuum packaging may represent an inexpensive and natural method for retarding oxidative spoilage of fresh fish.

KEY WORDS: *fish, vacuum, packaged, oxygen barrier, modified atmosphere packaging, MAP*

1.0 INTRODUCTION

Fresh seafood is a valuable part of the human diet; however, fish is known to be highly perishable due to oxidation of lipids, microbial enzymes, and protein degradation. Numerous studies focused on application of plant antioxidants as preservatives to mitigate lipid oxidation and microbial activity in seafood [1], [2], [3], [4], [5], [6]. However, packaging films and materials also play an important role to increase the shelf life of food, often with modifications to the atmosphere in packages. Different packaging materials offer different permeabilities to oxygen and therefore, may play a role in food preservation. Several studies show synergistic benefits of different packaging systems combined with antioxidants to inhibit lipid [2], [3], [5], [9], [11].

Studies emphasized that natural antioxidants from plant extracts can increase shelf life by delaying the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ) have limited use in foods due to potential health risks and consumer preference for natural food products [7]. Therefore, development of natural antioxidants has increased recently [8].

Effects of combined antioxidants (Propyl gallate (PG) and sodium ascorbate) and packing on lipid oxidation in salted dried snakehead fish during storage at refrigerated temperature (4°C) were studied by Nitipong et al. (2014) [9].

Dallabona et al., (2013) evaluated physicochemical and microbiological stability of sausages produced from Nile tilapia filleting residues (mechanically separated fish meat, MSM) with different preservation treatments (pasteurization or smoking) and packaging systems (conventional or vacuum). They found that pH and lipid oxidation speed (TBARS) values were reduced when vacuum packaging was used. Their results show that pasteurized sausages remain stable for 10 and 15 days in conventional and vacuum packages, respectively [10].

Rajesh et al. (2002) observed that a combination of vacuum packaging and treatment with sodium acetate significantly increase storage life of seer fish in ice [11]. Chouliara et al. (2005) studied the combined effects of γ-irradiation and refrigeration on shelf-life of vacuum-packaged sea bream (Sparus aurata) fillets by monitoring microbiological, chemical and sensory changes of non-irradiated and irradiated fish samples irradiated with 1 and 3 kGy [12]. In another study, chemical, sensory and microbiological evaluation of sardines (Sardina pilchardus) in modified atmosphere packaging (MAP) and vacuum packaging (VP) was investigated [13]. They reported that the highest concentration of (spell out first) TMA was obtained from sardine stored in air, followed by sardine stored in vacuum packages (VP) and then modified atmosphere packaging (MAP). They also reported that shelf life of sardine was found to be 12 days in MAP, 9 days in VP and 3 days in air [13]. Arashisar et al. (2004) determined that microbial (psychrotrophic, mesophilic aerobic bacteria and Enterobacteriacae counts), and chemical analysis pH, total volatile bases nitrogen (TVB-N), lipid oxidation (Thiobarbituric acid reactive substance, TBARS)] of rainbow trout (Oncorynchus mykiss) fillets in air (control), vacuum and MAP with various gas mixtures conditions at 4 ±1 °C. They reported that minimum TBARS values were recorded in fillets containing packed in 100% CO2 and vacuum [14]. Another study investigated effects of MAP (60% CO2, 10% O2, 30% N2; MAP) and VP on quality of tilapia (Oreochromis niloticus) fillets stored at 4 °C [15]. They found that odor and flavor of MAP and vacuum packaged samples was more acceptable throughout storage of fifteen and twelve days, respectively.

The objective of this study was to determine

the influence of red cabbage extract on lipid oxidation, color change and pH level in raw tilapia patties using high and low oxygen barrier packaging films.

2.0 MATERIALS AND METHODS

2.1. Chemicals and instruments

Red cabbages were purchased from a local market in Gainesville, Florida. Chloroform, methanol, ammonium thiocyanate, ferrous chloride, Folin & Ciocalteu's phenol reagent (2N), cumene hydroperoxide, gallic acid, ascorbic acid, potassium chloride, sodium acetate, sodium carbonate, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH were obtained from Axxora, Switzerland and Sigma- Aldrich Co (St. Louis, MO, USA). Instruments used in this study are presented in Table 1.

Table 1. List of the used instruments

Number of the instruments	Name of instruments	Used for testing
1	Sealing machine	Sample package preparation
2	Multivac C400 Vacuum Packaging Machine (Multivac Group, Ltd. Germany)	Sample package preparation
3	Meat grinder (STX-3000-TF, USA)	Grinding fish muscle
4	Homogenizer (Biohomogenizer M 133/1281-0 2 Speed, Omni, Inc.)	Samples homogenization
5	UV-Vis Spectrophotometer	Color analysis
6	Nitrogen Evaporator (N-EVAP 112, Organomation, Berlin, MA)	Chloroform removal
7	pH meter (Accumet Model 15; Fisher Scientific, Arvada, CO)	pH measurements
8	Cenco moisture balance (CSC Scientific Co. Inc., Fairfax, Va., U.S.A.)	Moisture analysis
10	CCD color camera (Nikon D200 Digital Camera, Nikon Corp., Japan)	Color analysis

2.2. Extraction of Red Cabbage

Three red cabbages were washed with tap water and diced (about 1cm x 1cm pieces). 100g-chopped red cabbage was added to 100 mL deionized (DI) water and boiled for 1 hour. Supernatant extract was vacuum filtered and centrifuged (Beckman Coulter Ltd., Palo Alto, CA). Filtered extract was frozen in a -80 °C freezer and then thawed for 5 min to separate concentrated anthocyanin from frozen water ice (Figure 1).

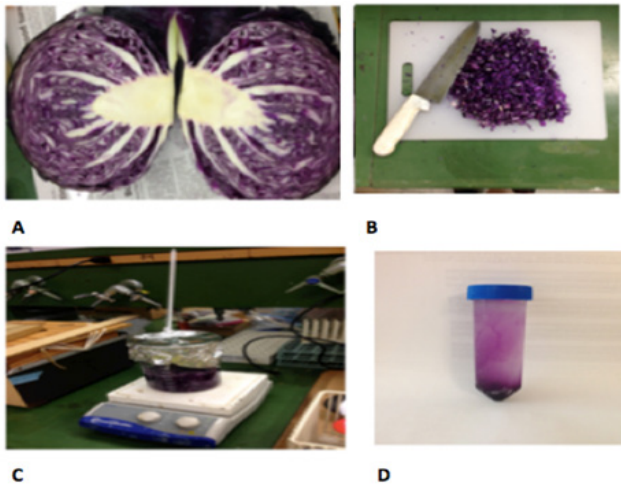

Figure 1. extraction processes of red cabbage A) Whole red cabbage, B) chopped and weighted, C) photography of boiling red cabbage D) freeze-concentrated red cabbage extract.

2.3. Fish preparation

Tilapia (Nile perch) weighting 11-12 kg were purchased from a local fish market in Gainesville, Florida, USA. The fish were kept in ice during transportation to the laboratory. Fish muscle was washed with tap water, cut into small pieces using a stainless steel hygienic knife prior to grinding (STX turbo force Motor Wattage of 3000 Watts, STX-3000-TF, USA). Ground fish was stored in ice at 4°C for further analysis and treatment with red cabbage extract.

2.4. Lipid extraction and analysis

Lipids were extracted from Tilapia muscle using a modified method of Bligh and Dyer (1959) [16]. Minced tilapia muscle (1 g) was blended with 4 mL of a chloroform: methanol (1:2) mixture using a warning commercial blender (Biohomogenizer M 133/1281-0 2 Speed (115 V, 140 W) for 1 min in a disposable glass tube. 1.25 mL of chloroform was added and vortexed for 5 min, and then 2.25 mL of 0.5 % KCl solution was added and mixed for 1 min. Samples were centrifuged (Beckman Coulter Ltd., Palo Alto, CA) at 6500 rpm for 10 min and the lower phase collected through the protein disk with a pipette into a weighed glass tube. 2 mL of chloroform was then added to the remaining part of the mixture, and then vortexed, centrifuged and the lower phase of the mixture was collected into the previously weighed glass tube. Chloroform was removed under a nitrogen gas stream using an N-EVAP 112 Nitrogen Evaporator. Weights were recorded and percent lipids determined gravimetrically. Oils were flushed with nitrogen and stored in amber vials at - 80° C until analysis.

2.5. Red cabbage extract treatment and vacuum packaging (VP) of fresh minced tilapia

Concentrated red cabbage extract was stored in a -20 °C freezer until use. 50 g of minced fish samples were treated with 68 ppm concentrated red cabbage extract. Treated minced fish samples were stored under refrigeration (4 ± 1 °C) for testing and packaging. Two types of packaging film were used and cut to approximately 6in x 8in (inch) using scissors and films were sealed by sealing machine. Samples treated with and without red cabbage extract concentrate were placed into prepared film packages and sealed under vacuum (10 mbar). The samples were named as C: control (without treated), RC: treated with red cabbage extract) under vacuum packaging (F1: Film-1 (1 cc O2/m2/day) and Film-2 (3000+ cc O2/m2/day)) during storage (day 0 - day 15) at 4°C.

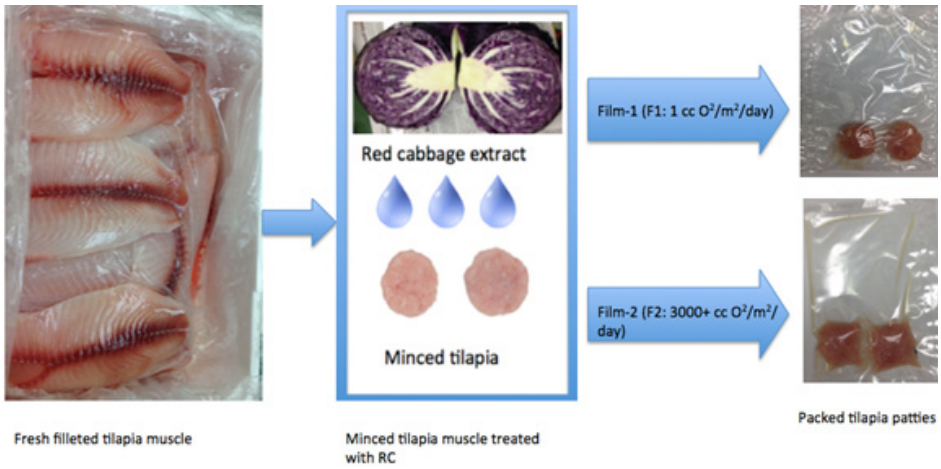

Figure 2. Illustration of prepared and packaged fish patties.

2.6. Chemical analysis

2.6.1. Thiobarbituric acid reactive substances (TBARS)

TBARS was performed based on a modification of Lemon (1974) [17] according to Raghavan and Hultin (2005) [18], by measuring secondary products of oxidation in tilapia muscle. Approximately 1 g tilapia muscle and 3 mL 7.5% TCA solution was homogenized for 1 min in a disposable glass tube. Then the sample was centrifuged (Beckman Coulter Ltd., Palo Alto, CA) at 2000 rpm for 10 min. A 2 mL aliquot of supernatant was mixed with 2 mL of 0.02 M TBA solution and heated in boiling water for 40 min. Samples were cooled in ice water. Color was spectrophotometrically measured at 530 nm. A standard plot was prepared using tetraethoxypropane (TEP).

2.6.2. Peroxide Value (PV)

PV was measured according to the method of Raghavan and Hultin (2005) [18] by measuring primary products of oxidation in tilapia muscle. Approximately 1 g of tilapia muscle and 10 mL of chloroform/methanol (2:1) was homogenized for 1min and then 3 mL of 0.5 % NaCl solution was added. The mixture was vortexed for 30 sec and then centrifuged (Beckman Coulter Ltd., Palo Alto, CA) at 2000 rpm. for 10 min. Ammonium thiocyanate and ferrous chloride were prepared as in Shantha and Decker (1994). A 25 µL aliquot of each reagent was added and vortexed for 10 s. Samples were incubated for 10 min at room temperature and absorbance was measured at 500 nm. A standard curve was prepared using cumene hydroperoxide.

2.7. Color Analysis

The surface of color of treated and untreated tilapia muscle was measured during storage using a machine vision system (The machine vision system was comprised of a fluorescent light box (42.5 cm (w) x 61 cm (l) x 11.4 cm (h)) and used a digital Nikon D200 color camera (Nikon D200 Digital Camera, Nikon Corp., Japan), consisting of a light box and a CCD color camera (Nikon D200 Digital Camera, Nikon Corp., Japan) connected to a computer with a firewire connection (Figure 3). A software program was used to analyze images to provide area-averaged, region-of-interest L* (lightness), a* (redness), b* (yellowness) values [19, 20, 21].

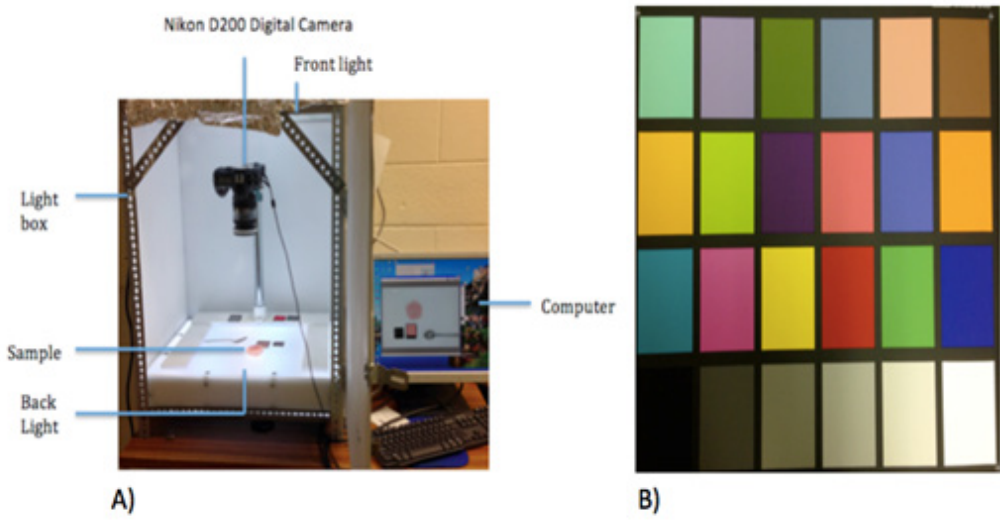

Figure 3. Machine Vision System. A) photography of the system of machine vision, B) the color chart was used in the calibration process as references.

2.8. Moisture content and pH analysis

Moisture content of samples was determined using a Cenco moisture balance (CSC Scientific Co. Inc., Fairfax, Va., U.S.A.). pH was determined according to Vareltzis et al. [48] using 1g fish sample. 1 g of minced tilapia meat and 9 mL distilled water were homogenized by a hand-held homogenizer (Biohomogenizer M 133/1281-0 2 Speed (115 V, 140 W)). The pH of the mixture was measured using a pH meter (Accumet Model 15; Fisher Scientific, Arvada, CO) for each day.

2.9. Statistical analysis

All experiments were run in triplicate. Data were analyzed by one –and two-way ANOVA. Differences between means were evaluated by Tukey`s test ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Lipid oxidation (TBARS and PV)

The impact of phenolic compounds in red cabbage and packaging with different films on TBARS formation in minced tilapia during the storage is shown in Figure 4A. TBARS values increased for all samples as storage time increased up to 15 days (Figure 4A) ($p<0.05$). TBARS values for untreated controls packaged with F1 (low barrier film) resulting in higher TBARS values than the other samples during the 15 days of cold storage ($p<0.05$). Samples treated with red cabbage extract concentrate and packaged with F2 (high barrier film), however, had the lowest TBARS values. ($p<0.05$).

PV in untreated packaged controls increased drastically from day 0 to day 12 (Figure 4B) ($p<0.05$). Thereafter, no marked change in PV was found until the end of the storage period (Figure 4B) ($p<0.05$). PV of untreated packaged controls was higher than those of samples treated with red cabbage extract concentrate and packaged with the two different package films throughout storage ($p<0.05$). Samples treated with red cabbage extract concentrate and packaged with F2 (high barrier film) provided lower PV than untreated control packed in F1 (low barrier) and F2 (high barrier) films as well as treated samples packaged with F1 (Figure 4B). Results suggest effective antioxidative

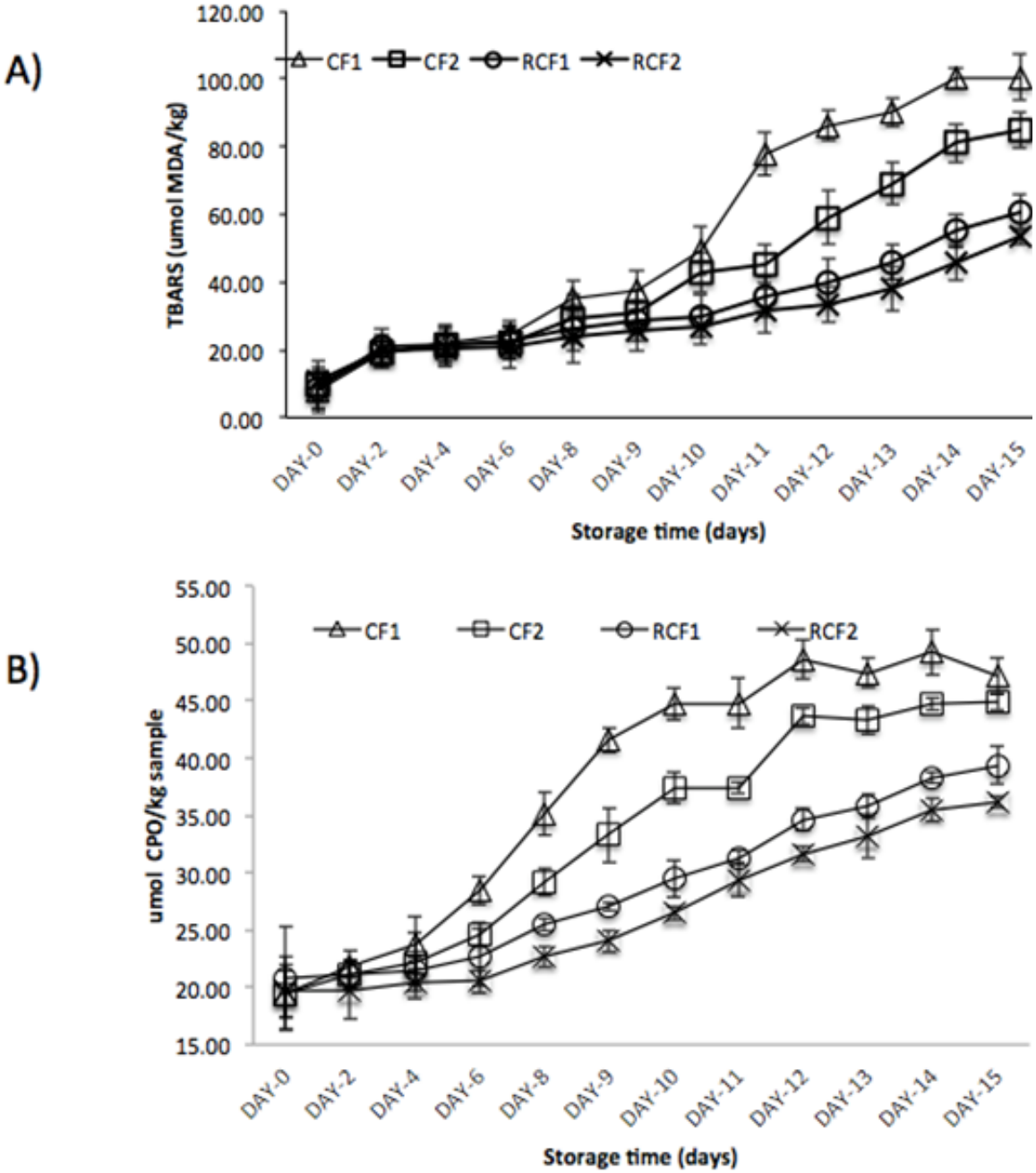

Figure 4. Changes in lipid oxidation products of minced treated without and with red cabbage extract. A) Peroxide value, B) TBARS values. Bars represent the standard deviation (n=6).

Viable treatment		DAY - 0	DAY - 2	DAY - 4	DAY - 6	DAY - 8	DAY - 9	DAY - 10	DAY - 11	DAY - 12	DAY - 14	DAY - 15
L*												
	CF1	67.79 (0.12)	70.14 (0.47)	70.31 (1.99)	69.40 (1.46)	70.16 (0.35)	70.16 (0.35)	70.18 (0.68)	69.03 (0.16)	69.41 (0.51)	68.61 (0.44)	68.35 (0.74)
	CF2	67.62 (0.03)	69.08 (0.01)	69.43 (0.09)	71.01 (2.07)	69.94 (0.02)	69.94 (0.02)	70.99 (1.02)	69.64 (1.07)	71.61 (0.06)	70.01 (1.61)	69.73 (1.70)
	RCF1	67.11 (0.02)	67.91 (0.15)	67.14 (0.57)	68.87 (0.31)	67.04 (0.13)	67.04 (0.13)	68.44 (0.35)	68.88 (0.36)	68.24 (0.42)	68.16 (2.04)	68.34 (0.37)
	RCF2	66.20 (0.28)	66.75 (0.23)	67.63 (0.62)	69.09 (0.21)	68.64 (0.16)	68.64 (0.16)	68.56 (0.06)	69.15 (0.31)	69.25 (0.04)	68.95 (0.06)	70.25 (0.42)
a*												
	CF1	16.26 (0.20)	12.74 (0.08)	13.36 (1.21)	13.47 (0.50)	12.2 (0.23)	12.2 (0.23)	12.12 (0.04)	12.97 (0.67)	12.72 (0.16)	12.25 (0.18)	12.82 (0.55)
	CF2	16.40 (0.01)	21.22 (1.43)	14.12 (0.03)	13.64 (1.77)	13.26 (0.18)	13.26 (0.18)	12.43 (1.41)	13.39 (0.48)	12.21 (0.53)	13.4 (1.00)	12.97 (1.04)
	RCF1	12.68 (0.01)	10.40 (0.32)	13.16 (0.30)	13.42 (0.16)	13.26 (0.33)	13.26 (0.33)	12.83 (0.01)	12.75 (0.28)	12.49 (0.25)	12.5 (0.55)	12.09 (0.21)
	RCF2	12.50 (0.09)	12.60 (0.23)	14.05 (0.07)	13.5 (0.41)	12.75 (0.13)	12.75 (0.13)	13.47 (0.40)	13.55 (0.12)	12.97 (0.35)	13.03 (0.36)	12.79 (0.30)
b*												
	CF1	14.10 (0.18)	12.28 (0.21)	11.47 (0.41)	11.6 (0.03)	11.39 (0.39)	11.39 (0.42)	11.18 (0.46)	11.63 (0.38)	11.97 (0.04)	11.75 (0.51)	11.43 (0.69)
	CF2	14.21 (0.01)	18.54 (0.26)	11.89 (0.21)	11.73 (0.36)	11.52 (0.45)	11.52 (0.83)	11.49 (0.07)	11.91 (0.16)	11.54 (0.01)	11.33 (0.01)	11.29 (0.40)
	RCF1	10.16 (0.01)	9.365 (0.25)	9.65 (0.13)	10.63 (0.01)	10.81 (0.19)	10.81 (0.10)	11.3 (0.15)	11.08 (0.58)	10.89 (0.14)	11.7 (0.10)	11.48 (0.35)
	RCF2	10.07 (0.08)	10.30 (0.08)	10.39 (0.28)	10.98 (0.23)	10.88 (0.12)	10.88 (0.01)	11.34 (0.05)	11.48 (0.86)	11.18 (0.26)	11.42 (0.31)	11.4 (0.07)
ΔE												
	CF1	0.00	4.61 (0.03)	1.75 (0.22)	2.75 (1.23)	1.90 (0.86)	1.90 (0.18)	0.96 (1.00)	1.51 (0.04)	0.71 (0.44)	1.17 (0.47)	1.20 (0.58)
	CF2	0.00	9.54 (1.05)	9.82 (0.98)	2.15 (1.98)	2.34 (0.55)	2.34 (1.27)	1.88 (0.63)	1.88 (0.84)	2.36 (2.25)	2.05 (0.01)	0.56 (0.26)
	RCF1	0.00	2.54 (0.41)	2.90 (0.79)	2.00 (0.27)	1.85 (0.44)	1.85 (0.43)	1.57 (0.46)	0.66 (0.22)	0.85 (0.29)	1.94 (0.17)	1.78 (0.40)
	RCF2	0.00	0.62 (0.46)	1.73 (0.04)	1.68 (0.89)	0.96 (0.08)	0.96 (0.34)	0.90 (0.27)	0.63 (0.65)	0.85 (0.26)	0.56 (0.19)	1.46 (0.76)

Figure 5. During the 15 days of storage, changes on the color values (L, a*, b* and ΔE*) of fish sample. Parenthesis represents the standard deviation (n=6).*

activity of red cabbage extract concentrate in packaged minced fish (Figure 4B). Medina et al., emphasized that the reducing capacity of phenolic antioxidants was realized as a key function for preventing and controlling lipid oxidation in fish tissues [22, 23].

3.2. Lipid and moisture content

The result shows that lipid content of raw fish tissue was 0.85 ± 0.07 (% Wet weight basis), and moisture content was 78.0 ± 0.12.

3.3. Colour analysis

ΔE* values of packaged samples treated with red cabbage extract concentrate were reduced relative to packaged controls at all tested storage days (Figure 5). There was no marked change on L* values for all samples throughout storage. Control samples packed in F1 (low barrier) and F2 (high barrier) films, had decreased a* values compared to other samples from day 0 to day 15 (Figure 5). For controls, b* value decreased from day 0 to day 15, but samples treated with red cabbage extract concentrate and packaged in F1 and F2 films were changeable during the 15 days of storage (Figure 5).

3.3. pH level

pH values increased throughout storage (Figure 6) ($p<0.05$). pH rose faster in packaged untreated controls throughout storage. Samples treated with red cabbage extract concentrate and packed in F2 (high barrier) film showed rise in pH (Figure 6) ($p<0.05$).

4. CONCLUSION

Water-soluble red cabbage extract inhibits lipid oxidation and associated color and pH changes in packaged fish even when using high oxygen transmitting films (3000+ cc O2/m2/day). This Natural antioxidant offer greater consumer acceptance and offer a valid alternative to synthetic antioxidants currently used in commercial meat and seafood products. Red cabbage extracts may prove to be commercially viable antioxidant food additives.

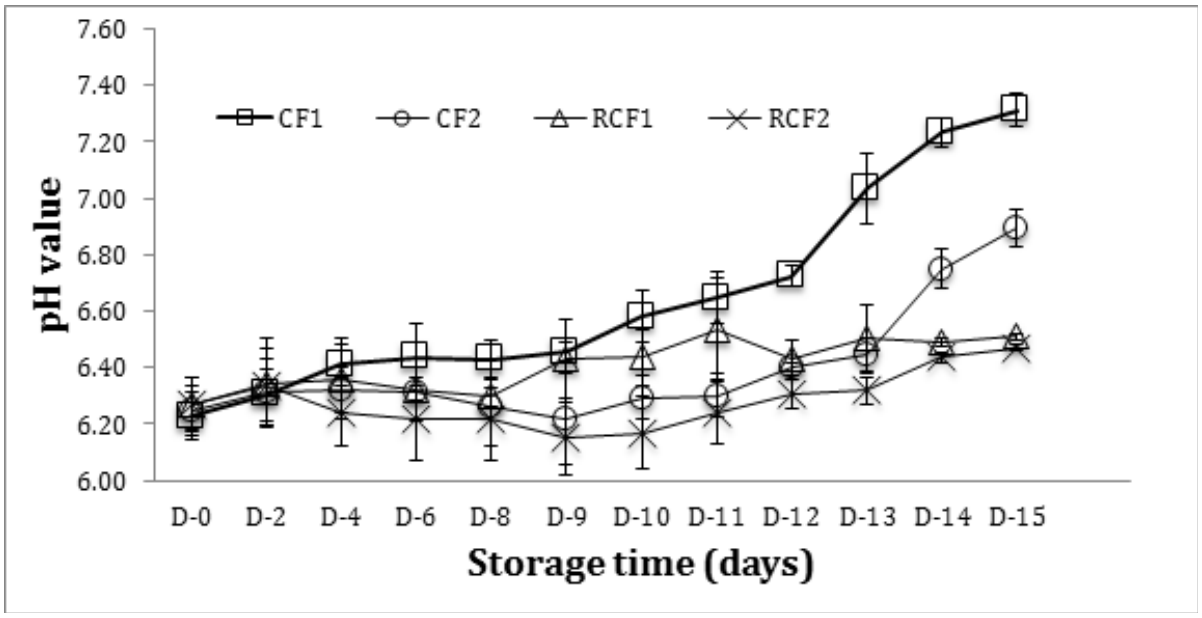

Figure 6. pH values of fish samples at cold storage for 15 days.

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