



Red Cabbage Extracts as Inhibitors of Lipid Oxidation in Fresh Minced Tilapia (*Nile perch*) During Refrigerated Storage

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 06/06/2019 Accepted : 06/12/2019</p> <p>Keywords: Natural antioxidant Shelf life Fish Red cabbage Lipid oxidation</p>	<p>This study was performed to evaluate effects of red cabbage extract as a preservative against lipid oxidation for quality and shelf life of minced Tilapia (<i>Nile perch</i>) during refrigerated storage at $4 \pm 1^\circ\text{C}$. Untreated and treated samples were examined from day 0 to 10 during refrigerated storage using thiobarbituric acid reactive substances (TBARS) assay, peroxide value (PV), pH and color analysis. Samples treated with red cabbage extract showed less degradation due to lipid oxidation compared to untreated samples. Lipid peroxide values on treated samples showed benefits through day-6. This work shows that red cabbage antioxidant extracts represent an inexpensive and natural method for reducing oxidative spoilage of fresh fish.</p>

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Introduction

The importance of fish and seafood products, rich in long chain omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid), protein, vitamin D, and minerals is well documented (Faber, 2010; Sheeshka, 2002; Jaclyn, 2010; Bate 1956).

Tilapia (*Nile perch*) is one of the most cultivated species in the world. Tilapia are vegetarian so they only eat algae and plants resulting in low mercury content compared to the other seafood products. Tilapia are easy to debone and offer delicate flavour and good nutritional quality. Tilapia is a small fish compared to salmon or tuna and it grows relatively fast, reaching maturity in about 9 months compared to 2 and 4 years for salmon and tuna, respectively (Dergal, 2013; Yanar, 2006; Boari, 2008).

The major factors of spoilage in fish are enzymatic activity, bacteria and chemicals present on and in fish. Other factors also contribute to spoilage of fish including high moisture content, high fat content, high protein content, weak muscle tissue, ambient temperature and less than hygienic handling (Bate, 1956; Watanabe, 1991; Burt, 1976; Gopakumar, 2000; Karube 1984; Lima Dos Santos, 1984).

However, lipid oxidation and associated rancid off-flavours remain the most significant cause of consumer

objection of fish and foodstuff including fish oils (Pazos, 2005; Buaneow, 2008; Kanner 1994; Gandemer 1995).

To solve mitigate effects of lipid oxidation, natural and synthetic antioxidants have been used to prevent oils, and particularly fish oils, from becoming rancid prior to consumption (Pazos, 2005). Given growth of natural and organic foods, there is interest in natural antioxidants for use in foods. For instance, Pazos et al. (2005) applied grape polyphenols to fish lipids and frozen muscle and found that flavonol oligomers were the most potent inhibitors of oxidation in emulsions and frozen fish muscle. According to Sa'nchez-Alonso et al. (2007), addition of red grape fibre delayed lipid oxidation in minced horse mackerel muscle during the first 3 months of frozen storage. In another study, white grape antioxidant activity was examined on lipid oxidation in minced horse mackerel muscle during frozen storage and results showed that 2% white grape dietary fibre (WGDF) inhibited development of oxidation in minced horse mackerel muscle during storage at -20°C (Sa'nchez-Alonso, 2008).

Use of rosemary and thyme plant extracts for delaying lipid oxidation has been reported in several studies (Khalafalla, 2015; Afanso, 2008).

Maqsood et al. (2010) reported a comparative antioxidant study of different phenolic compounds (catechin, caffeic acid, ferulic acid and tannic acid) at various levels determined by different assays (Maqsood, 2010). Numerous studies have reported antioxidant properties of herbs, spices and plant extract as natural additives (Sa'ñchez-Alonso, 2007; Sa'ñchez-Alonso, 2008; Khalafalla, 2015; Afonso, 2008; Maqsood, 2010; Raghavan, 2005; Maqsood, 2013; Tang, 2001; Yarnpakdee, 2012).

Some studies show that different amounts of anthocyanins in red cabbage such as 11 anthocyanins, 20 anthocyanins, 24 anthocyanins or 36 anthocyanins have been obtained from red cabbage using HPLC- MS or MS/MS (High performance liquid Chromatography & Mass spectra) (McDougall, 2007; Wlczkowski, 2013; Arapitsas, 2008; Charran, 2007). Most scientists emphasize that the major anthocyanins in red cabbage are based on a core of Cyanidin-3-O-diglucoside-5-O-glucoside, which can be non-acylated, mono-acylated or di-acylated with p-coumaric, caffeic, ferulic and sinapic acids (Wlczkowski, 2015; Pliszka, 2009; Podsedek, 2008; Piccaglia, 2002). Moreover, red cabbage represents an alternative to grape due to properties of acylation, which gives greater stability to heat and light than grape anthocyanins. Anthocyanins from red cabbage may be better suited for the food sector than grape simply due to wider availability (Henry, 1996; Shimizu, 1997). According to Simon et al., another advantage is that red cabbage is safer than grape because grape pomace may contain residues of sulphur dioxide that is used in wine-making from grape, which can cause allergic reactions in some people (Simon, 1998).

Therefore, the objective of this work was to investigate antioxidant effects of red cabbage extracts on lipid oxidation in fresh minced tilapia fish.

Materials and Methods

Chemicals

All chemicals (methanol, chloroform, 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)) used were analytical grade and were purchased from Fisher-Scientific (Fair Lawn, NJ, USA) and Sigma-Aldrich Co (St. Louis, MO, USA).

Preparation and Storage of Fish Patties

Fresh tilapia fillets were purchased from local fish market (Northwest Seafood, Inc., Gainesville, Florida) and fresh red cabbage plants were purchased from a local organic market in Gainesville, Florida. Red cabbage extracts were stored in a -20°C freezer. Fresh tilapia fillets were minced using a meat mincer (STX turbo force, 3000W, USA). 50 g ground fish samples were treated with different amounts of red cabbage extract 17, 34, 68 ppm red cabbage extract solution. 500ppm gallic acid and 500ppm ascorbic acid were separately used as positive controls on 900 g minced fish. Minced fish samples were stored under refrigeration ($4 \pm 1^\circ\text{C}$) for testing.

Extraction of Red Cabbage

Extracts of red cabbage were freeze concentrated. 100 g chopped red cabbage was added to 100 mL DI water and boiled for 1 hour. The liquid phase was vacuum filtered and centrifuged. Filtered extract was frozen in a -80°C freezer and then thawed for 5 min to collect concentrated anthocyanin from frozen water ice.

Total Polyphenols Colorimetric Assay

Total phenolic content of each extract was determined by the Folin-Ciocalteu colorimetric method of Singleton et al. (1965). A mixture of 100 μL red cabbage extract and 3900 μL distilled water, 250 μL Folin-Ciocalteu reagent (2N), and 750 μL 20% Na_2CO_3 were mixed in a tube. Samples and standards were incubated for 30 minutes in a 40°C water bath and absorbance was measured at 760 nm. Gallic acid (GA) was used as standard and expressed as Gallic acid equivalents (mg GAE/g DM, mg gallic acid/g of dry defatted matter) using an external calibration curve. The linearity range of the calibration curve was 100-800 $\mu\text{g}/\text{mL}$.

Total Antioxidant Activity by DPPH Assay

Antioxidant activity of each extract was evaluated using the DPPH radical scavenging capacity assay following procedure of Brand-Williams et al. (1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol (DPPH Solution = 0.025g/ 100mL methanol) and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm of blank (methanol) and test sample using a spectrophotometer (Beckman Instruments, CA, USA). Red cabbage extracts (100 μL) were reacted with 3900 μL of the DPPH solution for 60 minutes in the dark at room temperature. Meanwhile, 100 μL trolox solution with respective concentrations 100, 200, 400, 600, 800, 1000 μM were added to 3.9 mL DPPH working solution for generating the standard curve. Absorbance was recorded at 515 nm by an ultraviolet-visible Beckman Coulter DU-640 spectrophotometer (Beckman Instruments, CA, USA). Results were expressed in $\mu\text{M TE}/\text{g fresh mass}$.

Free radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Total Anthocyanin by pH Differential Method

Total anthocyanin content in red cabbage extracts was measured by the pH differential method of Jungmin Lee et al. (2005).

Two buffer systems were used including potassium chloride buffer (0.025 M, pH=1.0) and sodium acetate buffer 0.4 M, pH=0.4). An aliquot of the red cabbage extract (1.0mL) was placed in a 20 mL volumetric flask, diluted to volume with pH 1.0 buffer and mixed. A second aliquot of the red cabbage extract (1.0 mL) was placed to a 20 mL volumetric flask, diluted to volume with pH 4.5 buffer and mixed. Both solutions were incubated at room temperature for 20 minutes. Then the prepared solutions were measured by using UV/VIS spectrophotometer at 510

nm and 700 nm. Absorption was calculated according to the equation 1 and expressed as mg of cyanidin-3-glucoside per gram red cabbage leaves.

$$AP = (A \times MW \times DF \times 10^3) / \epsilon \times I \quad (1)$$

Where,

AP = Anthocyanin pigment (cyanidin-3-glucoside, mg/L)

A = (A510 – A700) pH 1.0 - (A510 – A700) pH 4.5

MW = molecular weight = 611 g/mol for cyanidin-3,5-diglucoside,

DF = is dilution factor established in D,

I = Path Length in cm,

ϵ = 30,175 molar extinction coefficient in $L \times mol^{-1} \times cm^{-1}$, for cyanidin-3,5-diglucoside

10^3 = factor for conversion from gram to milligram.

Colour Analysis by Machine Vision

Colour analysis involved the method of Luzuriaga, (1997); Yoruk, (2004); Yagiz, (2009); Yagiz, (2010). Surface colour of treated and untreated (control) fresh tilapia patties was measured during storage by the machine vision (Figure 1). The machine vision system was comprised of a fluorescent light box (42.5 cm (w) \times 61 cm (l) \times 11.4 cm (h)) and used a digital Nikon D200 colour camera (Nikon D200 Digital Camera, Nikon Corp., Japan) connected to a computer with a fire wire connection. Camera settings were 36 mm focal length, ISO 100 sensitivity, 1/3 s F/11 shutter speed, \times 1.0 EV exposure compensation, and direct sunlight white balance. Average daylight illuminate D65 mode with a color temperature of 6504 K was used. Results were reported based on L^* (lightness), a^* (redness), b^* (yellowness) values for analysing total colour difference ΔE^* (Luzuriaga, 1997; Yoruk, 2004; Yagiz, 2009; Yagiz, 2010).

Fish patties were placed in the light box and the digital camera captured images of patties each day. The vision system was calibrated using a standard light skin plate ($L^* = 63.84$, $a^* = 19.8$ and $b^* = 15.79$) (Minolta) and using a professional camera calibration target as reference. Average L^* , a^* , b^* values and ΔE^* (total colour differences) were calculated against the light pink standard ($L^* 63.84$, $a^* 19.8$ and $b^* 15.79$).

Lipid and Moisture Content

Moisture content was determined using a Cenco moisture balance (CSC Scientific Co. Inc., Fairfax, Va., U.S.A.). Lipids were extracted and quantified according to the method of Bligh and Dyer (Bligh, 1959).

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was performed based on a modification of Lemon et al. (1974), according to Raghavan (2005), by measuring secondary products of oxidation in tilapia muscle. Approximately 1 g tilapia muscle and 3 mL of 7.5% TCA solution were homogenized for 1 min in a disposable glass tube. Samples were centrifuged (Beckman Instruments, CA, USA) 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 a boiling water bath for 40 min. Samples were cooled in an ice and tap water container. Colour was measured using UV-Vis Spectrophotometre at 530 nm. A standard plot was prepared using tetraethoxypropane (TEP).

Peroxide Value (PV)

PV was measured according to the method of Raghavan and Hultin (2005) by measuring by measuring primary products of oxidation in tilapia muscle. Approximately 1 g of tilapia muscle was homogenized for 1 min using a hand-held homogenizer in 10 mL of chloroform/methanol (2:1) and then 3 mL of 0.5% NaCl solution was added. The mixture was vortexed for 30 sec and then centrifuged at 2000 rpm. Two ml chloroform was used to dilute to 10 mL mix of chloroform/methanol (2:1). Ammonium thiocyanate and ferrous chloride were prepared as in Shantha (1994). A 25 μ L 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 ($C_9H_{12}O_2$).

pH Values

The pH was determined according to Varelziz et al. (1988), using 1g fish sample. 1 gr 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 (Fisher Scientific AB15 pH meter, USA) for each day.

Statistical Analysis

The experimental data were performed by one –and two-way ANOVA. The differences between means were evaluated by Tukey's test ($P < 0.05$). We used Microsoft Excel Version 2016 (MS Excel 2016) for data analysis.

Results and Discussion

Figure 1 shows total phenolic content of red cabbage extract as a function of thaw time (5 min, 1 hour, 5 hours, 24 hours, and ice part). Highest observed values were obtained after 5 min of thawing (16.99 μ g/g (FW) ($P < 0.05$). Figure 2 shows antioxidant activity of freeze-concentrated red cabbage extract after different thaw times. The first 5 min thawed time showed highest value antioxidant values of 2069.81 μ mol/g with little remaining in the water ice (123.69 μ mol/g). Anthocyanin content were significantly observed in 5 min thawed time as highest value (25.47 μ g/g) compared to other thawed time (1, 5, 24 hours, and ice part of extraction) ($P < 0.05$). The lowest value was 3.96 μ g/g in ice part of extraction in Figure 3.

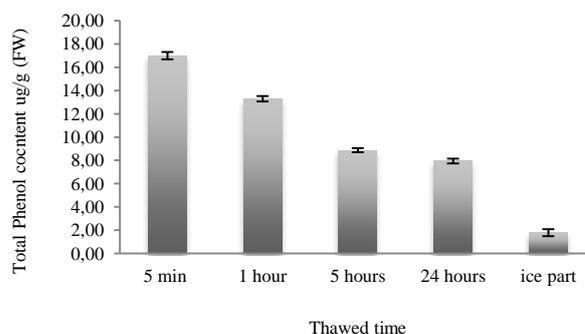


Figure 1. Total phenolic content of red cabbage extracts at different thawed times for freeze/thawed method.

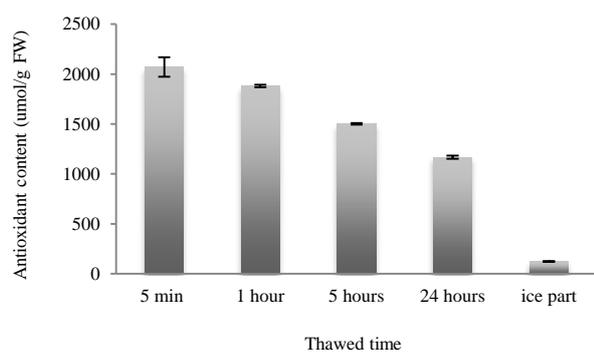


Figure 2. DPPH values of red cabbage extracts at different thaw times for freeze/thawed method.

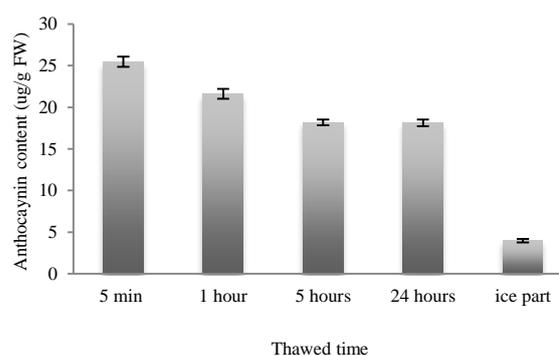


Figure 3. Anthocyanin values of freeze-concentrated red cabbage extracts at different thawed time for freeze/thawed method.

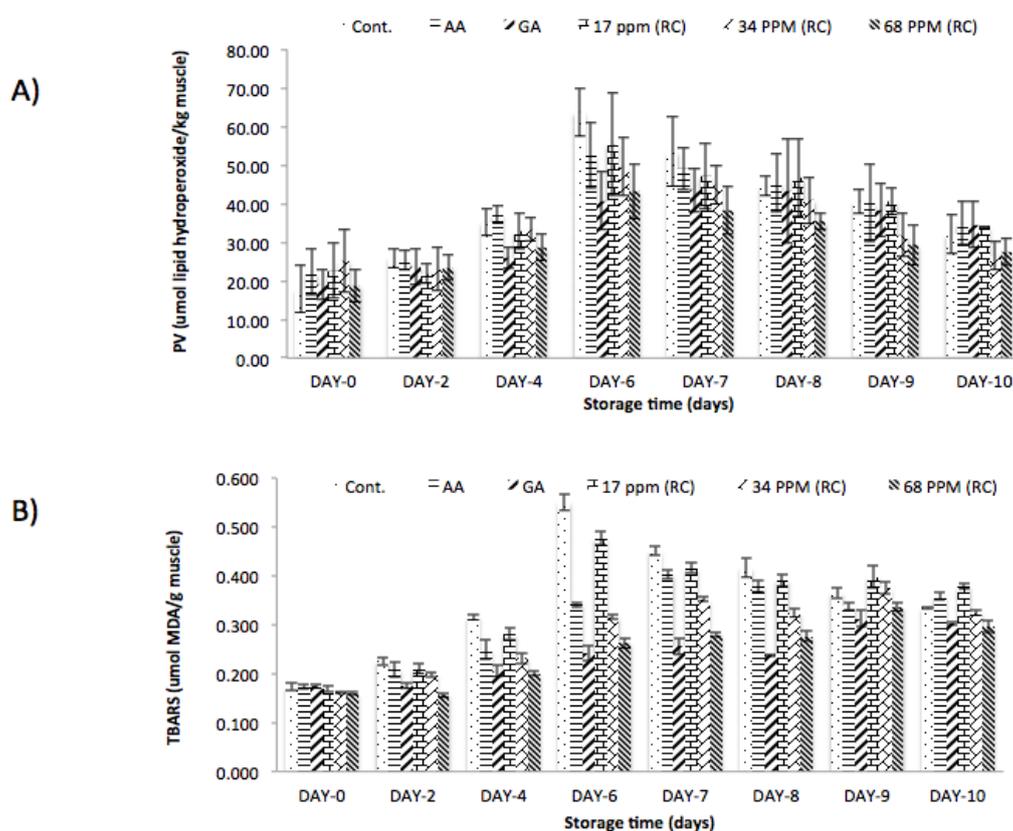


Figure 4. Changes in TBARS and PV value of minced tilapia muscle during storage. Error bars represent the standard deviations.

Results show that after freezing at -80°C , 5 min of thawed time resulted in highest antioxidant activity, anthocyanin amount and total phenols content ($P < 0.05$) in concentrated extracts.

TBARS values of tilapia patties during refrigerated storage (Figure 4A) show continuous increase in TBARS value up to 6 days ($P < 0.05$). Thereafter, TBARS values decreased notably ($P < 0.05$). The TBARS value was expressed as umol malonaldehyde/g sample since Malondialdehyde (MDA) is the most abundant aldehydes generated during secondary lipid oxidation, and MDA is commonly used as oxidation marker (Shahidi, 2005).

TBARS values of controls (untreated) were 0.17 umol MDA/g at Day 0, which reflected presence of lipid oxidation products in minced muscle after capture and

during handling. This value reached 0.55 umol MDA/g in controls by day. During extended storage, lipid oxidation proceeded as evidenced by the increases in TBARS values until Day 6 ($P < 0.05$). Samples treated with 68 ppm concentrated of red cabbage extract showed a low inhibition on the formation of TBARS, compared with the control for all storage days (Figure 4A) ($P < 0.05$).

The first compounds formed during the oxidation process are peroxides, specifically hydroperoxides; thus, they are called primary oxidation products (Shahidi, 2005; Barriuso, 2013; Dobarganes, 2002; Melton, 1983).

PV of tilapia muscle during refrigeration storage of 10 days is shown in Figure 4B. Peroxide value (PV) increased up to Day 6 and subsequently decreased to day 10 (Figure 4B) ($P < 0.05$).



Figure 5. Pictures of tilapia patties (untreated and treated with red cabbage antioxidants at different concentration, gallic and ascorbic acid) during refrigeration storage time (day-0)

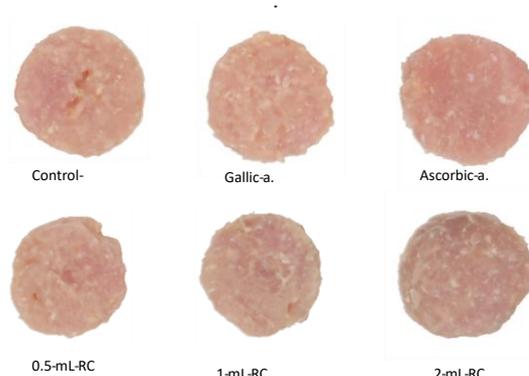


Figure 6. Pictures of tilapia patties (untreated and treated with red cabbage antioxidants at different concentration, gallic and ascorbic acid) during refrigeration storage time (day-10)

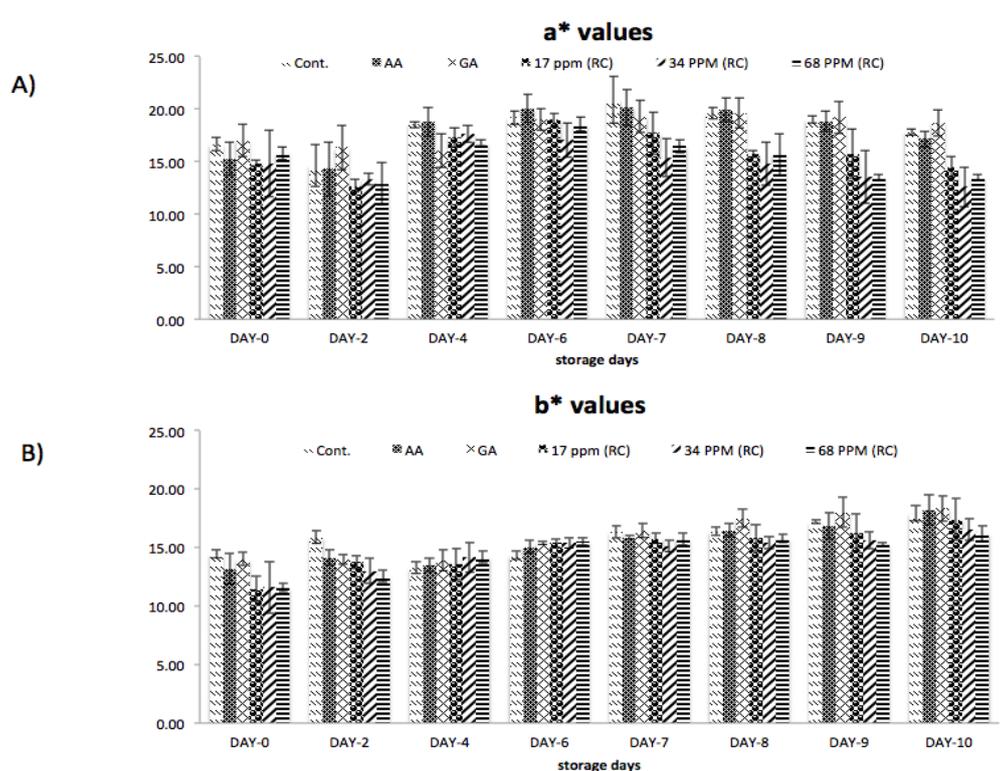


Figure 7. Changes in a^* and b^* values of tilapia patties (untreated and treated with red cabbage antioxidants at different concentration, gallic and ascorbic acid) during refrigeration storage time.

At Day 0, PV of controls was of 17.86 μmol hydroperoxide/kg. PV increased through 6 days of iced storage ($P < 0.05$). Subsequently, PV decreased through day 10 ($P < 0.05$). The increase in PV was likely due to the formation of hydroperoxide during early stages of lipid oxidation. These results are in accordance with the lower PV values of gallic acid treatment as positive controls. Moreover, samples with 2 ml concentrated of red cabbage extracted resulted in lowest PV values observed during storage ($P < 0.05$). The result shows that lipid oxidation occurred during refrigerated storage, probably because of the high content of unsaturated fatty acids (Figure 4.)

Chemical composition analysis of tilapia fish was presented in Table 1. Including moisture and lipid content at day 0 (Table 1.). The literature reports higher moisture and lipid values than those the found in the present study (Yarnpakdee, 2014; Angelini, 2013). The differences

between literature and present study may be attributed to environmental factors, seasons, and feeding quality of fish.

Myoglobin and hemoglobin are generally responsible for colour changes in fish flesh and oxidation of these proteins tends to cause yellow-brownish colours (Yarnpakdee, 2012; Venugopol, 1996). Decreases in a^* were observed during storage after day 2 (Figure 7A). Surface redness decreased with increasing yellowness during storage for all samples ($P < 0.05$). At the end of storage, yellowish discolorations were observed when compared to day 0 ($P < 0.05$). Increases in b^* are shown in Figure 7B. Yellowness on sample surfaces increased with decreasing redness during storage for all samples ($P < 0.05$) (Figure 5 and 6). There is a significant colour change from pink to yellowness in figure 5 and 6. Lipid oxidation causes to yellow discoloration of fish muscle (Khantaphant, 2011).

Table 1. Mean of Proximate composition of raw minced tilapia (Nile perch)

Sample component	Minced tilapia
Lipid	0.039 (0.002)
Moisture	77.3 (0.70)

(a) Values in parenthesis shown standard deviation.

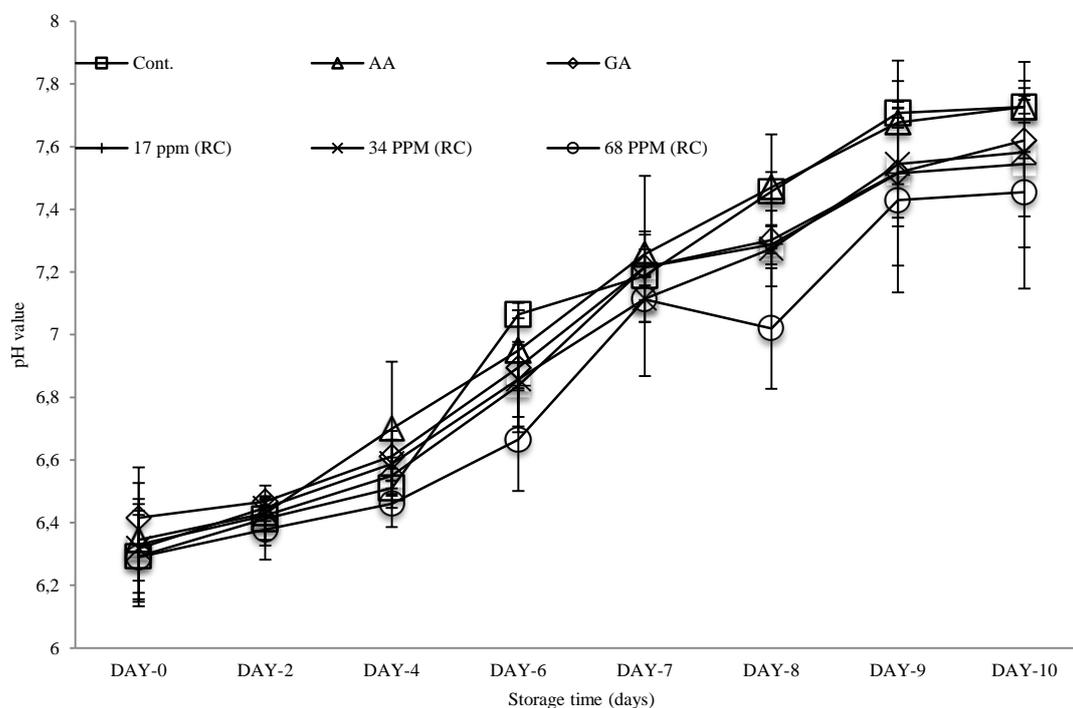


Figure 8. Changes in pH values of tilapia patties (untreated and treated with red cabbage antioxidants at different concentration, gallic and ascorbic acid) during refrigeration storage time.

A continuous increase in pH values was noticeable with increasing storage time ($P < 0.05$) (Figure 8). pH values increased ($P < 0.05$) during refrigerated storage time (Figure 8) in the control samples and in those treated with antioxidants (red cabbage extract, gallic and ascorbic acid).

pH value of fresh fish meat generally varies from 5.7-6.6. Fresh fish muscle is close to neutral pH (7.0), but after death, lactic acid is produced causing pH to fall initially. As spoilage occurs, pH tends to rise (Sa'nchez-Alonso, 2007; Bilgin, 2007; Oguzhan, 2013).

Conclusion

Results from this study showed that decreasing thawed time yielded higher polyphenols content during extraction of red cabbage. Increasing application of red cabbage extract in minced tilapia decreased lipid oxidation during refrigerated storage. This work shows that red cabbage antioxidant extracts may represent a natural antioxidant alternative for reducing oxidative spoilage of fresh fish.

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