

Effect of polyphenol extract from *Polygonum multiflorum* Thunb. root on the storage of minced red tilapia (*Oreochromis* sp.)

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This study was carried out to evaluate the effects of the polyphenol extract from *Polygonum multiflorum* Thunb. root on lipid oxidation and sensory characteristics of minced red tilapia (*Oreochromis* sp.) during frozen storage. Fresh fillets were minced in aqueous solutions of the root extract with some polyphenol concentrations of 830, 415, 277 and 208 mg GAE/L, extract solution/sample ratio of 1/20 (v/w), then stored at $-20\pm 2^{\circ}\text{C}$, for up to 100 days. The best oxidation inhibition results on minced fish were achieved at the highest polyphenol concentrations of 830 and 415 mg GAE/L. Significant differences ($p < 0.05$) in all quality parameters (pH, PoV, MDA, color parameter and sensory evaluation) of treated and control samples (blank control and water-treated or water control) were registered during storage time. The advantages of polyphenol extract were discussed. It can extend the shelf-life of products during frozen storage and was more effective to improve qualities of minced fish, especially at a polyphenol concentration of 415 mg GAE/L. Therefore, it can be concluded that the polyphenol extract from *Polygonum multiflorum* Thunb. root can potentially be used as an alternative source of natural antioxidant.

Keywords: Antioxidant, Frozen storage, Lipid oxidation, Minced fish.

INTRODUCTION

Red tilapia (*Oreochromis* sp.) is widely cultured in South Vietnam, especially Tien Giang, Vinh Long, Dong Thap province and its production is expanding every year. Red tilapia flesh is tasty and can produce various products as frozen fillet, minced fish, salted fish, etc. [1]. The current demand for aquatic products, especially frozen fish products, has increased quite quickly worldwide. These products are a valuable natural resource with high protein content and are rich in polyunsaturated fatty acids [2]. Its qualities were studied in various fish species by many storage methods. There are two main problems associated with frozen storage of fish products: protein denaturation, hydrolysis and oxidation of lipids [3]. Among them, oxidation of lipids occurs in raw material during storage, processing, heat treatment, and in the final products during subsequent storage [4]. It causes degradation of the product qualities, leading to undesirable changes in flavor, color, texture and nutrition, especially in the organoleptic characteristics.

Using antioxidant compounds of synthetic or natural origin in the process or storage is one of the methods to reduce or retard oxidation and prevent the loss of quality and sensory attributes [5]. In

recent years, consumers and food industry are concerned about food safety and health. Hence, the use of synthetic compounds in food industry was reduced, natural antioxidants as polyphenols substituting synthetics, especially polyphenols from plants, were requested. However, the activity of these compounds is quite difficult to predict, each polyphenol has different mechanisms involved in its antioxidant effect.

Polygonum multiflorum Thunb. is considered to be one of the most important natural antioxidant herbal extracts. It is valuable medicine plant and contains high levels of polyphenol compounds in its root such as tannins, anthraquinones, flavonoids, etc. In addition, these compounds were reported for hair-blackening, liver and kidney-tonifying, anti-aging effects and curing of other diseases [6]. The positive effects of polyphenols from various materials on fish products have been observed and prevented rancidity of many lipid systems, for instance, polyphenol in green tea and onion extract [7], date seed extract [8], red grape pomace (peels and seeds) [9], etc., but until now, no research has studied the combination between storage methods and polyphenols from *Polygonum multiflorum* Thunb. root to preserve minced fish.

The main objective of this study was to evaluate

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the effects of polyphenol extract from *Polygonum multiflorum* Thunb. root on the physicochemical and sensory parameters of minced red tilapia. A better understanding of the reactions that occur during the storage process enables better quality products to be obtained.

EXPERIMENTAL

Materials

Extract preparation: Polygonum multiflorum Thunb. roots were harvested from Cao Bang province (Vietnam). The roots were then cleaned by tap water, sliced and dried at 60°C until the moisture level was less than 12%. The slices were then ground into fine powder (diameter less than 0.5 mm) and vacuum-packed. Polyphenols from the dried powder of *Polygonum multiflorum* Thunb. roots were extracted in a microwave system with acetone concentration of 57.35%, solid/solvent ratio of 1/39.98 (w/v), extraction time of 289 sec and microwave power of 127 W. The crude extract was filtered through Whatman paper [10]. The filtered extract was evaporated at 45°C until the solvent was completely removed and the extract was used for preparation of 830, 415, 277 and 208 mg GAE/L solution in distilled water.

Preparation of minced red tilapia: Red tilapia (*Oreochromis* sp.) fish samples (of average weight = 600-800 g) were purchased from Vinmart supermarket in Ho Chi Minh city (samples originated from Tien Giang province, Vietnam). Each sample was deheaded, cleaned, filleted and minced with various extract concentrations, using an extract solution/sample ratio of 1/20 (v/w). In addition, there were two control samples including untreated control (UC) and water control (the water/sample ratio of 1/20, v/w) (WC). Minced samples were placed in polyethylene bags and stored in the freezer at -50°C and after that, all samples were moved to a -20°C freezer and maintained at this temperature during the storage time. Samples were analyzed every 20 days until 100 days of storage at -20°C.

Chemicals and reagents: Folin-Ciocalteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) reagents were purchased from Merck (Germany). TBA (2-thiobarbituric acid) and TMP (tetramethoxy propane) were supplied by Sigma-Aldrich (USA). All other chemicals and organic solvents were of analytical reagent grade.

Determination of total phenolic content (TPC) and antioxidant capacity (AC) of extract

The TPC in the extract was determined by a slightly modified Folin-Ciocalteu colorimetric

method [11]. The results were based on a standard curve obtained with gallic acid. TPC was expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

The AC of the extract was determined by DPPH assay which was adapted and modified from studies of Soto *et al.* (2014) [12] and Chmelová *et al.* (2015) [13], with slight modification. Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) determined as μmol of Trolox per gram of dry weight ($\mu\text{mol TE/g DW}$).

Chemical analysis

pH: According to Shim *et al.* (2012) [14], the pH value of 5 g samples blended with 20 mL distilled water for 60 s in a homogenizer (Panasonic 1L MX-AC400WRA, Japan) was determined with a pH meter (Trans Instruments BP3001, Singapore).

PoV: The PoV values were determined according to Seo *et al.* (2016) [15] with slight modifications. The lipids from the minced fish samples (5 g) were homogenized with 50 mL of acetic acid-isooctane for 5 min. Then, the samples were filtered through Whatman paper, 1 mL of saturated potassium iodide solution was added to the filtered extracts, shaken gently for 1 min and then 100 mL of distilled water and 0.5 mL of 0.1% starch solution were added. The obtained solution was titrated with 0.01 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution until the violet color disappeared. The results were expressed as meq oxygen/kg sample.

Thiobarbituric acid reactive substances (TBARS): TBARS values were determined by the method of Vyncke (1970) [16] with some slight modifications. Firstly, samples (5 g) were homogenized in 20 mL of 10% trichloroacetic acid (TCA) solution and 0.5 mL of BHT. Then, the samples were filtered through Whatman paper and made up to 100 mL with 10% TCA solution. The filtrate (5 mL) was mixed with 5 mL of 0.02 M 2-thiobarbituric acid (TBA) solution, heated in a boiling water bath at 100°C for 35 min to develop the rose-pink color from the reaction between malondialdehyde and TBA; then cooled by tap water for 10 min. Absorbance was measured at 532 nm against a blank prepared with 5 mL of 0.02 M TBA solution and 5 mL of 10% TCA solution, using a spectrophotometer. TBARS values were μg of malondialdehyde (MDA)/kg of sample.

Color parameters: Color parameters consist of of L^* (lightness), a^* (from redness to greenness), b^* (from yellowness to blueness) values were recorded and determined on a Chroma Meter CR-400

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Sensory evaluations: Samples were prepared according to Masniyom *et al.* (2005) [17] with some slight modifications. The minced frozen samples were cut into 30×20×20 mm cubes, thawed, wrapped with aluminum foil and steamed for 15 min at 70°C. 60 non-trained panelists evaluated the steamed minced fish by color, odor, taste, texture and overall acceptability; using 9-point hedonic scales from 1 (dislike extremely) to 9 (like extremely) [18].

Data analysis

The experimental data were analyzed by the one-way analysis of variance (ANOVA) method and significant differences between the means from triplicate analyses at ($p < 0.05$) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Total polyphenol content and antioxidant capacity

The TPC and AC values of the extract were 47.53±0.79 mg GAE/g DW and 334.07±3.04 µmol TE/g DW, respectively. TPC and TEAC of samples from the MAE method were higher than those of samples from China which were extracted by the decoction method with deionized water as solvent (33.91±0.62 mg GAE/g DW; 257.9±3.7 µmol TE/g DW) and maceration methods with 50% ethanol as solvent (40.42±0.63 mg GAE/g DW; 256.7±0.7 µmol TE/g DW) [19].

The results showed that factors such as extraction methods, land, gender, etc., caused the differences in TPC and AC values. The crude extract was filtered and evaporated at 45°C until the solvent was completely removed. The extract was used for preparation of 830, 415, 277 and 208 mg GAE/L solution in distilled water to mince with fish fillet.

Changes in the pH value

Table 1 showed that the pH values were significantly different ($p < 0.05$) from the storage time in the frozen storage. Indeed, they fluctuated and increased slightly after 100 days of frozen storage but all samples did not have any signs of damage such as rancid odor. However, the pH values of the initial samples were not affected by polyphenol in the extract.

The results showed that the shelf-life of the product would depend on the storage method and support of antioxidant as synthetic or natural additive, especially polyphenol from *Polygonum multiflorum* Thunb. roots. Phenolic compounds could affect the pH values and change some qualities of the product. Frozen storage can stabilize temperature of -20°C, inhibits microorganisms, minimizes the biochemical processes of the product and extends the storage time with support of antioxidant.

In general, the change of pH value depends on many factors including material, diet, harvest season, stress levels, storage methods and support agents. The pH value of fish was approximately 7 initially, but then would decrease because glycogen would be hydrolyzed into lactic acid after death. The pH value would tend to increase again during storage.

Table 1. pH values of minced fish during the storage

Storage time (Days)	Polyphenol concentration of extract (mg GAE/L)					
	UC	WC	830	415	277	208
0	6.90±0.09 ^{Aabc}	6.83±0.07 ^{Aa}	6.85±0.11 ^{Abc}	6.74±0.10 ^{Aa}	6.79±0.12 ^{Aab}	6.85±0.14 ^{Aab}
20	6.84±0.16 ^{Ca}	6.82±0.01 ^{BCa}	6.61±0.08 ^{Aa}	6.67±0.04 ^{ABa}	6.70±0.05 ^{ABCa}	6.78±0.11 ^{BCa}
40	6.88±0.03 ^{BCab}	6.90±0.01 ^{Cab}	6.80±0.05 ^{Ab}	6.83±0.04 ^{ABb}	6.86±0.06 ^{ABCbc}	6.90±0.03 ^{Cabc}
60	7.03±0.01 ^{Bc}	7.01±0.10 ^{Bc}	6.90±0.02 ^{Abc}	6.95±0.02 ^{ABc}	6.98±0.03 ^{ABd}	7.00±0.01 ^{Bc}
80	6.99±0.01 ^{BCbc}	7.01±0.02 ^{Cc}	6.94±0.02 ^{Ac}	6.94±0.01 ^{Ac}	6.98±0.02 ^{BCd}	6.96±0.02 ^{ABbc}
100	6.85±0.02 ^{Aa}	6.94±0.01 ^{Cbc}	6.91±0.02 ^{Bc}	6.92±0.01 ^{BCc}	6.93±0.01 ^{BCcd}	6.85±0.01 ^{Aab}

Different superscript lower-case letters in the same column denote significant differences ($p < 0.05$).

Different superscript capital letters in the same row denote significant differences ($p < 0.05$).

These results are similar to those of Khalafalla *et al.* (2015) [20] who preserved Nile tilapia fillet (*Oreochromis niloticus*) using natural herbs such as

thyme (*Thymus vulgaris*) extract and rosemary (*Rosmarinus officinalis*) extract, or Patagonian hake (*Merluccius hubbsi*) minces and fillets which were

P. T.Q. Le and V. M. Nguyen: Effect of polyphenol extract from *Polygonum multiflorum* Thunb. root on the storage ... preserved by the frozen method [21]. The increase of pH value might be due to endogenous enzymes in the material or microbial enzymes, which produced volatile bases such as ammonia or trimethylamine, which would increase the pH value [22]. Conversely, in some cases the pH did not change significantly, as shown in the study of Aubourg *et al.* (2004) [23] that preserved horse mackerel (*Trachurus trachurus*) by the freezing method with citric and ascorbic acid; or slightly decreased when Rostamzad *et al.* (2011) [24] preserved Persian sturgeon (*Acipenser persicus*) fillets by the freezing method with ascorbic acid. Although the change of pH values is negligible it is quite important in preservation, because it is related to structural changes and deterioration in the quality of materials during storage time.

Changes in the PoV value

The PoV values of all samples of this storage method was significantly different during storage time ($p < 0.05$). The PoV values increased steadily after 100 days of storage, especially in control samples including UC and WC (6.53 and 6.84 meq/kg, respectively). The others kept a lower PoV value during storage time. The concentration of polyphenol extract increases with the decrease of PoV value (Table 2). Results show that polyphenol extract from *Polygonum multiflorum* Thunb. roots strongly affects the PoV value.

During storage time, the PoV value of the fish product steadily increases in spite of the storage methods used. The level of increase of PoV value could depend on many factors such as composition of raw material, storage method and antioxidants. There are some different mechanisms which affect lipid oxidation such as lipoxygenase enzyme, photo-oxidation, especially a free radical mechanism. Among them, autoxidation is a spontaneous reaction of molecular oxygen with

lipids, leading to oxidative deterioration and proceeds by a free radical chain mechanism. The lipid oxidation is stopped by antioxidants that interrupt the free radical chain reaction. In addition, photo-oxidation also leads to the formation of hydroperoxides because of the presence of sensitizer and light which excite the reaction between oxygen and unsaturated fatty acids [25].

The presence of polyphenol extract may retard lipid oxidation by preventing the formation of free radicals or by interrupting the propagation of them through several mechanisms including scavenging species that initiate peroxidation, quenching *O_2 - preventing formation of peroxides, breaking the autoxidative chain reaction, reducing localized O_2 concentrations and chelating metal ions so that they would be unable to generate reactive species or decompose lipid peroxides [26]. This result is in agreement with Ozen *et al.* (2011) [27] who stored minced chub mackerel (*Scomber japonicus*) muscle by the freezing method by adding red grape seed extracts and pomegranate seed extracts; or the study of Ozogul *et al.* (2010) [28] who refrigerated sardine (*Sardinella pilchardus*) fillets by immersing them in a solution of rosemary extract.

Changes in the TBARS value

Based on table 3, TBARS values of all samples are significantly different ($p < 0.05$) and would tend to increase during storage time. Polyphenol concentrations increase with the decrease of the TBARS values. The combination of frozen storage and polyphenol extract produced positive effect on the inhibition of lipid oxidation. Extracts with concentrations of 830 and 415 mg GAE/L had the lowest TBARS values of 440.57 and 517.78 $\mu\text{g}/\text{kg}$ while those of control samples were 781.35 and 1016.18 $\mu\text{g}/\text{kg}$ after 100 days of storage. Those showed that TBARS values were dependent on storage method, raw material and antioxidant.

Table 2. PoV values (meq/kg) of minced fish during the storage

Storage time (Days)	Polyphenol concentration of extract (mg GAE/L)					
	UC	WC	830	415	277	208
0	1.12±0.30 ^{Aa}	1.27±0.11 ^{Aa}	1.27±0.12 ^{Aa}	1.26±0.21 ^{Aa}	1.06±0.12 ^{Aa}	1.18±0.21 ^{Aa}
20	1.85±0.22 ^{ABb}	1.99±0.35 ^{Bb}	1.53±0.23 ^{Aa}	1.79±0.21 ^{ABb}	1.86±0.31 ^{ABb}	1.79±0.19 ^{ABb}
40	3.05±0.11 ^{Cc}	3.29±0.28 ^{Cc}	2.20±0.21 ^{Ab}	2.52±0.12 ^{ABc}	2.58±0.22 ^{Bc}	2.62±0.17 ^{Bc}
60	4.31±0.10 ^{CDd}	4.62±0.27 ^{Dd}	2.66±0.09 ^{Ac}	3.29±0.29 ^{Bd}	3.62±0.27 ^{Bd}	4.09±0.15 ^{Cd}
80	5.12±0.08 ^{De}	5.24±0.13 ^{De}	3.38±0.19 ^{Ad}	4.11±0.15 ^{Be}	4.31±0.29 ^{Bce}	4.50±0.33 ^{Ce}
100	6.53±0.13 ^{Df}	6.84±0.33 ^{Df}	4.91±0.23 ^{Ae}	5.45±0.14 ^{Bf}	5.57±0.20 ^{Bf}	6.09±0.21 ^{Cf}

Different superscript lower-case letters in the same column denote significant differences ($p < 0.05$).

Different superscript capital letters in the same row denote significant differences ($p < 0.05$).

Table 3. TBARS values ($\mu\text{g MDA/kg}$) of minced fish during the storage

Storage time (Days)	Polyphenol concentration of extract (mg GAE/L)					
	UC	WC	830	415	277	208
0	238.39 \pm 16.11 ^{Aa}	246.17 \pm 9.19 ^{Aa}	243.27 \pm 9.28 ^{Aa}	233.49 \pm 24.65 ^{Aa}	233.02 \pm 24.6 ^{Aa}	241.34 \pm 9.21 ^{Aa}
20	355.09 \pm 9.26 ^{Db}	388.65 \pm 9.17 ^{Eb}	244.72 \pm 9.14 ^{Aa}	272.16 \pm 18.34 ^{Bbc}	277.64 \pm 9.36 ^{Bb}	319.82 \pm 24.26 ^{Cb}
40	408.57 \pm 9.26 ^{Cc}	485.88 \pm 9.21 ^{Dc}	276.37 \pm 9.14 ^{ABb}	271.63 \pm 18.31 ^{Ab}	276.53 \pm 9.32 ^{ABb}	298.64 \pm 18.67 ^{Bb}
60	480.56 \pm 18.42 ^{Dd}	547.61 \pm 9.26 ^{Ed}	330.99 \pm 9.34 ^{Bc}	302.74 \pm 9.14 ^{Ac}	361.88 \pm 9.30 ^{Cc}	373.36 \pm 9.32 ^{Cc}
80	786.69 \pm 18.49 ^{De}	872.08 \pm 18.49 ^{Ee}	544.36 \pm 24.36 ^{Ae}	606.94 \pm 18.38 ^{Be}	706.63 \pm 24.46 ^{Ce}	780.67 \pm 9.30 ^{De}
100	781.35 \pm 16.01 ^{De}	1016.18 \pm 9.24 ^{Ef}	440.57 \pm 18.75 ^{Ad}	517.78 \pm 9.21 ^{Bd}	653.26 \pm 27.73 ^{Cd}	750.82 \pm 16.04 ^{Dd}

Different superscript lower-case letters in the same column denote significant differences ($p < 0.05$).

Different superscript capital letters in the same row denote significant differences ($p < 0.05$).

TBARS value of the fish product always appears during storage time and was determined by the MDA content. During the secondary oxidation process, aldehydes, especially MDA, are formed [29], which are used to test for rancid food products. Changes of TBARS values also depend on lipoxygenase, peroxidase enzyme, autoxidation and photo-oxidation [25]. In addition, several proteins are bound to iron such as myoglobin, hemoglobin, ferritin and transferrin. During storage time, iron is released and then activates oxygen, which results in lipid oxidation [30]. The increase in TBARS value agreed with Maqsood *et al.* (2015) [8] who preserved minced mackerel (*Rastrelliger kanagurta*) by combination of iced storage date seed extract or with Aubourg *et al.* (2004) [23] who preserved horse mackerel (*Trachurus trachurus*) by the freezing method with citric and ascorbic acid.

Results proved that the presence of polyphenol extract from *Polygonum multiflorum* Thunb. roots inhibited the increase of TBARS value. The antioxidants had different abilities in preventing primary and secondary oxidation products formation [25]. However, the composition and content of fatty acid in the raw material also affected TBARS values during storage time; for instance, Tang *et al.* (2001) [31] refrigerated raw minced red meat, poultry and fish muscle during 10 days, and observed that the increase in TBARS values of all samples was completely different. Hence, to evaluate the quality of product, many quality factors are required to be assessed besides TBARS values.

Changes in the color parameters

The results of color analysis are presented in table 4. Color parameters (L^* , a^* , b^*) of the initial samples were slightly affected by the color of extract and they were significantly different ($p < 0.05$) during storage time. The trend of color change was quite complex, L^* , a^* and b^* fluctuated

slightly after 100 days of storage, but in general, they changed negligibly (except L^* values). The results obtained were similar with those of Paola and Isabel (2015) [32] with frozen mackerel fillet (*Scomber japonicus*); the L^* value slightly decreased, whereas a^* and b^* values changed insignificantly after 12 months.

Changes of fish product color were caused by the amount of soluble proteins which could strongly affect color parameters, especially L^* value. Protein structural changes resulted in the reflection of incident light and the light becomes scattered, causing interference, thus affecting the appearance of the flesh [33]. In addition, the color parameters were influenced by the retention of connective tissue and lipid content in the initial materials [34]. Besides, lipid oxidation could lead to an increase in the oxidation of oxymyoglobin to myoglobin, which would also causes color change [35]. Therefore, the color changes of the product could depend on several different factors including storage methods, antioxidants, raw material and process methods. Therefore, antioxidant usage would only restrict parts of the color changes in the products.

Changes in sensory attributes

Frozen storage proved most effective in extending shelf-life of products and in retaining quality index. In addition, the presence of polyphenol, especially in high polyphenol concentrations of 830 and 415 mg GAE/L, could inhibit the rise in PoV and TBARS values and would lessen the change of product color. However, the dark color of the botanical extracts could alter the visual perception of some food products. Sample of 415 mg GAE/L was chosen for sensory attributes evaluation and compared with control samples after 100 days of storage because this lower concentration did not affect natural odor and color of product.

Table 4. Color parameters of minced fish during the storage

Storage time (Days)	Polyphenol concentration of extract (mg GAE/L)					
	UC	WC	830	415	277	208
<i>L</i> *						
0	71.34±0.05 ^{Ac}	70.78±0.53 ^{Ac}	73.51±1.43 ^{BCbc}	74.50±0.63 ^{Ce}	74.07±0.16 ^{BCf}	73.06±0.53 ^{Bf}
20	71.37±0.06 ^{Ec}	69.49±0.09 ^{Ab}	69.92±0.07 ^{Ba}	70.25±0.04 ^{Cc}	73.08±0.01 ^{Fe}	70.62±0.04 ^{Dc}
40	74.55±0.02 ^{Fd}	70.83±0.07 ^{Cc}	72.66±0.04 ^{Db}	73.42±0.04 ^{Ed}	68.74±0.06 ^{Bc}	68.18±0.02 ^{Ab}
60	76.47±0.04 ^{Fe}	75.48±0.03 ^{Ed}	74.22±0.06 ^{Cc}	74.68±0.06 ^{De}	72.56±0.03 ^{Bd}	72.25±0.04 ^{Ae}
80	69.31±0.03 ^{Db}	62.66±0.03 ^{Aa}	73.38±0.01 ^{Fbc}	67.11±0.07 ^{Cb}	65.43±0.06 ^{Bb}	71.14±0.02 ^{Ed}
100	66.93±0.01 ^{Da}	75.46±0.06 ^{Fd}	70.41±0.01 ^{Ea}	65.26±0.03 ^{Ba}	64.69±0.01 ^{Aa}	66.42±0.04 ^{Ca}
<i>a</i> *						
0	2.23±0.02 ^{Aa}	2.42±0.04 ^{Bb}	4.79±0.05 ^{Fd}	3.80±0.07 ^{Df}	4.64±0.03 ^{Ef}	3.60±0.09 ^{Cd}
20	2.44±0.02 ^{Bb}	1.02±0.02 ^{Aa}	4.46±0.07 ^{Ec}	3.62±0.03 ^{De}	3.13±0.02 ^{Cb}	4.51±0.01 ^{Ef}
40	2.75±0.01 ^{Ec}	5.83±0.05 ^{Ff}	2.67±0.02 ^{Da}	2.12±0.01 ^{Ca}	1.39±0.06 ^{Aa}	1.67±0.02 ^{Bb}
60	2.47±0.02 ^{Bb}	5.29±0.07 ^{Ee}	3.76±0.03 ^{Db}	3.53±0.03 ^{Cd}	3.74±0.03 ^{De}	1.29±0.03 ^{Aa}
80	2.49±0.05 ^{Ab}	3.04±0.04 ^{Bc}	4.79±0.07 ^{Dd}	3.36±0.03 ^{Cc}	3.42±0.08 ^{Cd}	2.45±0.09 ^{Ac}
100	2.42±0.07 ^{Ab}	3.25±0.04 ^{Cd}	3.69±0.06 ^{Db}	2.62±0.06 ^{Bb}	3.26±0.05 ^{Cc}	3.74±0.06 ^{De}
<i>b</i> *						
0	9.79±0.32 ^{Aa}	10.27±0.02 ^{Bc}	12.3±0.24 ^{Dd}	11.35±0.30 ^{Cc}	11.73±0.29 ^{Cd}	10.39±0.06 ^{Cd}
20	10.32±0.01 ^{Bb}	9.51±0.07 ^{Aa}	12.00±0.06 ^{Ec}	10.41±0.05 ^{Bb}	10.61±0.08 ^{Cb}	10.97±0.04 ^{De}
40	10.52±0.02 ^{Eb}	11.36±0.05 ^{Fd}	9.44±0.04 ^{Ca}	9.56±0.02 ^{Da}	7.25±0.03 ^{Aa}	7.84±0.03 ^{Ba}
60	12.03±0.06 ^{Bc}	12.42±0.07 ^{Cf}	12.67±0.01 ^{De}	12.9±0.06 ^{Ee}	14.87±0.06 ^{Fe}	9.09±0.05 ^{Ab}
80	10.37±0.08 ^{Bb}	9.83±0.05 ^{Ab}	14.63±0.06 ^{Ef}	11.87±0.10 ^{Dd}	10.92±0.04 ^{Cc}	9.73±0.09 ^{Ac}
100	9.80±0.08 ^{Aa}	11.54±0.05 ^{Ee}	10.60±0.08 ^{Bb}	11.18±0.07 ^{Dc}	10.87±0.06 ^{Cc}	11.61±0.03 ^{Ef}

Different superscript lower-case letters in the same column denote significant differences (p<0.05).

Different superscript capital letters in the same row denote significant differences (p<0.05).

Table 5. Sensory evaluation of steamed minced fish

Samples	Texture	Color	Taste	Odor	Overall acceptability
UC	5.90±0.99 ^a	6.22±0.85 ^b	5.33±1.11 ^a	5.03±0.92 ^a	5.77±0.85 ^b
WC	5.83±0.69 ^a	5.87±0.77 ^a	5.58±0.72 ^a	5.18±0.7 ^a	5.28±0.74 ^a
415 mg GAE/L	6.27±0.86 ^b	6.77±0.93 ^c	6.75±0.97 ^b	7.00±0.76 ^b	7.03±0.76 ^c

Different superscript lower-case letters in the same column denote significant differences (p<0.05).

The minced fish was evaluated in terms of consumer acceptability including odor, color, taste, texture and overall acceptability. Table 5 showed the results of the sensory evaluation of minced fish. There were no significant differences in texture, taste and odor (p>0.05) between control samples. However, all sensory scores of the sample of 415 mg GAE/L were higher than those of control samples and there were significant differences in all sensory attributes (p<0.05). Addition of polyphenol extract in minced fish did not adversely affect the odor, color, taste, texture and overall acceptability of steamed minced fish. It tended to increase the scores of sensory attributes. The overall acceptability scores of samples ranged from 5.28 to

7.03, with maximum acceptability obtained with polyphenol concentration of 415 mg GAE/L.

CONCLUSION

According to the present results, the use of polyphenol extract from *Polygonum multiflorum* Thunb. roots rendered minced fish less prone to oxidation than untreated samples. Polyphenol which acts as an antioxidant during minced fish storage offers many advantages such as extending the shelf-life of products and improving important qualities indexes. The antioxidant activities of polyphenol extract were recorded without any adverse effect on the sensory acceptability of the treated minced fish.

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ВЛИЯНИЕ НА ПОЛИФЕНОЛОВ ЕКСТРАКТ ОТ КОРЕНИ НА *Polygonum multiflorum* THUNB. ВЪРХУ СЪХРАНЕНИЕТО НА НАРЯЗАНА ЧЕРВЕНА ТИЛАПИЯ (*Oreochromis* SP.)

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(Резюме)

Целта на това изследване е да се оцени влиянието на полифенолов екстракт от корените на *Polygonum multiflorum* Thunb. върху окислението на липидите и сензорните характеристики на нарязана червена тилапия (*Oreochromis* sp.) по време на съхранението ѝ в замразено състояние. Пресни рибни филета, потопени в 5% (v/w) водни разтвори на екстракта с концентрация на полифенола 830, 415, 277 и 208 mg GAE/L, са съхранявани при $-20\pm 2^\circ\text{C}$ за срок до 100 дни. Най-добри резултати за инхибицията на окислението на нарязаната риба са получени при най-високите концентрации на полифенол - 830 и 415 mg GAE/L. По време на съхранението са установени значителни разлики ($p < 0.05$) при всички качествени параметри (pH, PoV, MDA, цвят и сензорна оценка) на обработените и контролните проби (празна проба и проба, обработена с вода). Обсъдени са предимствата на полифенолния екстракт – може да удължи търговския живот на продуктите при съхранението им в замразено състояние, като е най-ефективен при концентрация на полифенол 415 mg GAE/L. Следователно, може да се заключи, че полифенолният екстракт от корените на *Polygonum multiflorum* Thunb. би могъл да се използва като алтернативен източник на природни антиоксиданти.