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Effect of Some Plant Extracts on Quality Aspects of Frozen Tilapia (*Oreochromis niloticus* L.) Fillets

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Abstract: This study investigates the effect of some plant extracts such as rosemary (*Rosemarinus officinalis*), thyme (*Thymus vulgaris*), blackcumin (*Nigella sativa)* and their combination (0.3% concentration) on the quality aspects of tilapia (*Oreochromis niloticus* L.) fillets. Fish fillets in all treatments were glazed and storage by freezing for 4 months at -18°C. Results showed that some used plant extracts did not affect proximal analysis of frozen tilapia fillets. However, glazing was more effective in minimizing the amount of moisture, protein and ash loss. Meanwhile, plant extracts controlled the values of pH, total volatile basic nitrogen (TVB-N), Trimethylamine Nitrogen (TMA-N) and Thiobarbituric Acid Reactive Substances (TBARS) in all treatments, especially in case of thyme treatment as comparing with the control group. Also, from the microbiological point of view, these plant extract were more effective on the microbial multiplication comparing with the control sample. It could be concluded rosemary, thyme, blackcumin extracts and their combination retard the biochemical changes and inhibit the microbial load in frozen tilapia fillets.

Key words: Plant extracts · tilapia fish · biochemical analysis · glazing · frozen storage

INTRODUCTION

Freezing is an important preservation method for fish and fish products. However, [1] reported that freeze-thaw cycles directly affected the biochemical and physicochemical properties of catfish (*Silurus glanis* L.) fillets. On the other hand, plant extract have largely shown a positive effect by inhibiting the quality loss during the frozen storage of minced and filleted fish products [2]. These plant are characterized by a wide range of volatile compounds, some of which are important flavor quality factors and Generally Recognized as Safe (GRAS) as reviewed by [3-5]. Although, spices have been well know for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf-life [6, 7]. Moreover, the concentration of plant secondary compounds by extraction process leads to valuable intermediary products that are useful in food, flavor and pharmaceutical industry [8]. The present work was designed to study the quality aspects of tilapia fillets, pre-soaked in plant extracts of *Rosemarinus officinalis*, *Thymus vulgaris*, *Nigella sativa* and their combination at concentration of 0.3% before the subsequent storage by freezing (-18°C) for 4 months.

MATERIALS AND METHODS

Fish: Fresh tilapia (*Oreochromis niloticus* L.) samples (310.34±45.22g) were captured from EL-Manzala farm, EL-Dakahlyia, Egypt, during February, 2006. They transferred using ice box to the laboratory within 4 hours and were immediately eviscerated, headed and filleted.

Plant extracts: Rosemary (*Rosemarinus officinalis*), thyme (*Thymus vulgaris*) and blackcumin (*Nigella sativa*) were prepared as mentioned by [9, 10].

Preparation of plant extract-fish fillets: Clean fish fillets were frozen for 2 hours at -20° then glazed in cold various solutions (4°C); no additives, rosemary, thyme, balckcumin and their combination at a concentration of 0.3% for 1 minute at 16°C. Five batches were packaged in polyethylene bags, put in a foam dishes, wrapped in cling film and stored at -18°C for 4 months. Three fish fillets of the five batches for each intervals of storage were taken

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out randomly thawed for overnight at 4±1°C and then homogenized using Electrical mixer for about 2 minutes. All analysis was performed in triplicate.

Analytical methods: The moisture content was quantified by oven-drying at 105°C, crude protein (TN×6.25) by Kjeldahl procedure, total lipid by Soxhlet extraction and ash by incineration in a muffle furnace at 550°C [11]. pH value and TVB-N content of the samples were determined [12]. TMA-N content was calorimetrically [11]. TBARS value was calorimetrically determined [12].

Microbiological analysis: Ten g sample of fish fillets was transferred in 90 ml of sterile saline (0.85 per cent NaCl). Tenfold serial dilution was plated on the plate count agar (PCA, Oxid). The plates were incubated at 30°C for 48 days of Total Viable Count (TVC) and at 4°C for 72 days of psychrotrophic bacteria, respectively. The results were expressed as log $_{10}$ cfu per g sample.

Statistical analysis: Mean and Standard Error (SE) were calculated using SPSS 10.0 for windows [13]. An independent sample *t*-test was used to determine differences between treatments.

RESULTS

Proximal and quality aspects of raw tilapia fillets: The proximal and quality aspects of raw tilapia fillets are shown in Table 1. The proximal composition (on wet wt.) of fish fillets was 78.99% moisture, 17.40% crude protein, 2.72% lipid and 0.85% ash content. The value of pH was 6.06; the values of TVB-N and TMA-N were 11.22 and 0.61 mg per 100g flesh, respectively. In addition, lipid change as TBARS value was 0.46 mg Malonaldhyde/kg flesh. On the other hand, the initial total viable count (TVC) and psychrophilic bacteria were 4.74 and 3.12 $\log_{10}$ cfu per g, respectively.

Effect of plant extracts during frozen storage on Proximal analysis: Moisture was ranged from 80.45 to 81.28% of all treatments at the first time of storage (zero time). A slight loss in water content of treatments was found with the advance of time. From the same Table, crude protein (on dry wt.) ranged from 83.15 to 83.95% for all treatments at the first day of storage. Following storage at -18°C, it decreased slightly up to the end of fourth month in all fish samples. Lipid content in all sample were similar at the first time of storage. Data showed that fat

Table 1: Proximal analysis, quality criteria and bacteriological aspects (Mean±SE) of raw tilapia fillets

Constituent	(%)	Quality criteria	Value	Bacteriological aspects	Log_{10} CFU per g
Moisture	78.99±0.37	$_{p}$H	6.06±0.03	Total Viable Count (TVC)	4.74±0.13
Crude protein	17.42±0.15	[1]TVB-N (mg per 100g sample)	11.22±1.40		
Lipid	2.72±0.23	[2]TMA-N (mg per 100g sample)	0.61±0.18	Psychrophilic count	3.12±0.11
Ash	0.85±0.09	[3]TBARS (mgMalonaldhyde per kg sample)	0.46±0.04		

[1]TVB-N: Total Volatile Basic Nitrogen; [2]TMA-N: Trimethylamine Nitrogen; [3]TBARS: Thiobarbituric acid reactive substances

Table 2: Effect of some plant extracts on the proximal analysis (Mean±SE) of frozen tilapia fillets

	Some plant extracts				
Storage period (month)	Control	Rosemary	Thyme	Black cumin	Combined
Moisture					
0	80.94±0.05	81.28±0.47	80.45±0.20	81.27±0.21	80.63±0.18
2	80.69±0.09	80.13±0.18	79.37±0.23	80.59±0.18	80.14±0.19
4	79.70±0.30	80.00±0.30	79.17±0.93	80.05±0.11	79.36±0.23
Crude protein (on dry wt. basis)					
0	83.15±0.82	83.66±0.46	83.75±0.48	83.71±0.63	83.95±0.88
2	82.92±0.66	82.52±0.38	83.33±0.22	82.20±0.58	83.15±0.68
4	81.32±0.57	81.85±0.82	82.42±0.13	82.11±0.91	82.26±0.66
Lipid (on dry wt. basis)					
0	12.98±0.02	12.91±0.16	12.92±0.06	12.56±0.14	12.87±0.08
2	12.35±0.19	12.40±0.27	12.65±0.15	12.31±0.07	12.58±0.14
4	11.95±0.12	12.29±0.11	12.35±0.13	11.86±0.10	12.30±0.07
Ash (on dry wt. basis)					
0	3.57±0.12	3.32±0.06	3.23±0.11	3.43±0.06	3.10±0.05
2	3.44±0.16	3.25±0.08	3.12±0.13	3.34±0.15	3.08±0.19
4	3.38±0.16	3.16±0.11	3.06±0.08	3.22±0.06	3.00 ±0.13

Table 3: Effect of some plant extracts on the quality criteria (Mean±SE) of frozen tilapia fillets

	Some plant extracts				
Storage period (month)	Control	Rosemary	Thyme	Black cumin	Combined
pH					
0	6.01±0.07	5.92±0.04	6.07±0.10	5.89±0.01	5.72±0.04
2	6.18±0.04	6.06±0.02	6.09±0.03	6.12±0.05	6.00±0.11
4	6.50±0.06	6.32±0.05	6.17±0.07	6.13±0.03	6.21±0.08
Total volatile basic nitrogen-TVBN (mg per 100g sample)					
0	11.90±1.40	11.60±1.82	11.90±1.54	11.20±1.82	11.90±1.54
2	14.60±1.82	12.60±2.52	12.80±1.40	12.80±1.40	12.60±1.82
4	15.40±1.54	13.30±1.40	13.60±1.82	14.20±2.80	14.80±1.40
Trimethylamin nitrogen-TMAN (mg per 100g sample)					
0	0.63±0.31	0.67±0.09	0.48±0.24	0.84±0.06	0.46±0.22
2	0.83±0.02	1.33±0.11	0.61±0.17	1.01±0.03	0.54±0.04
4	2.75±0.12	1.48±0.20	0.77±0.06	1.06±0.0.7	0.67±0.31
Thiobarbituric acid-TBARS (mg Malonaldhyde per kg sample)					
0	2.44±0.07	1.51±0.03	1.25±0.05	1.22±0.08	1.21±0.05
2	3.35±0.06	1.96±0.04	2.15±0.10	2.80±0.02	2.96±0.02
4	1.41±0.01	1.04±0.08	0.72±0.07	0.94±0.01	1.01±0.05

Table 4: Effect of some plant extracts on bacteriological aspects (Mean±SE) as log_{10} cfu/g of frozen tilapia fillets

	Some plant extracts;				
Storage Period (month)	Control	Rosemary	Thyme	Black cumin	Combined
Total viable count (TVC)					
0	6.65±0.12	6.26±0.10	5.97±0.09	6.31±0.08	5.71±0.12
2	5.67±0.13	4.65±0.11	5.13±0.12	5.46±0.16	5.26±0.15
4	6.05±0.10	5.92±0.12	5.91±0.11	5.83±0.23	5.32±0.12
Psychrophilic bacteria					
0	4.52±0.12	4.20±0.20	4.45±0.25	4.26±0.13	4.19±0.10
2	4.88±0.18	4.53±0.13	4.49±0.19	4.83±0.13	4.45±0.15
4	5.42±0.12	4.76±0.16	4.70±0.20	4.93±0.03	4.83±0.13

was slightly decreased at the end of frozen storage. In addition, ash of investigated treatments decreased during different times of freezing (Table 2).

Quality attributes:

pH value: pH values ranged from 5.72 to 6.07 at the beginning of frozen storage. A slight increase in the values of pH was obtained following increased storage periods. However, plant extracts retard these values as comparing with the control value (Table 3).

TVB-N content: The values of TVB-N of fish fillets at the first time of freezing ranged from 11.20 to 11.90 mg per 100g flesh. Values increased with advancing of the storage periods in all treatments. However the increasing rate was the highest in control samples as compared plant extract treated samples (Table 3).

TMA-N content: TMA-N values at the first day of freezing were low (ranged 0.48 to 0.84 mg per 100g flesh). During freezing periods, values increased in all treatments (Table 3).

TBARS value: At the beginning of storage, TBARS value (Table 3) in control fillets was the highest (2.44 mg MA per kg fillets) as compared with plant extracts treated samples. A continuous increase was observed in all treatments after 2 months storage. However, values decreased at the end of fourth month in all treatments (Table 3).

Bacteriological aspects: TVC: At the beginning of storing, TVC in control sample (6.65 log_{10} cfu per g) showed the highest count. After 2 months of storage, TVC reduced in all trials (Table 4). On the other hand, from the same Table, initial psychrophilic load in control fillets was 4.52 log_{10} cfu per g and then reduced in fillets which treated with plant extracts, especially combined trial (3.30 log_{10} cfu per g).

DISCUSSION

The current investigation reveals that plant extracts aid in inhibiting the quality loss and biochemical changes of raw fish fillets. Similar findings were reported [2, 14]. A slight loss in water content of treatments was found as the advance of time. However, the glazing process and addition of plant extracts caused a slight loss in moisture and this loss is due to drip separated throughout thawing time [16, 17]. Decreased protein content may be due to the partial hydrolysis of protein [18]. It well known that there is an inverse relationship between the moisture and lipid content of fish fillets [18, 19]. The loss in fat may be due to separated drip through thawing, oxidation and hydrolysis of lipid [20]. In addition, loss in ash content of frozen fish samples may be due to some minerals were lost in separated drip throughout thawing time.

Concerning plant extracts induced retardation in pH values of tilapia fillets, this may be due to the containing phenolic compounds. In addition, increase of pH values is referring to formation and accumulation of TVB-N content. Besides, the increment in TVB-N content during frozen storage may be due to bacterial activity. However, the breakdown occurred in fillets proteins in presence plant extracts was of low rate and might be due to antimicrobial agent of plant extracts. Concerning TMA its content slightly increased may be due the conversion of TMAO oxide to TMA [21]. An increase in TBARS value may be due to the ice crystals formed could injure the cell and cause the release of pro-oxidants for lipid oxidation, especially free iron [1]. These results are in agreement with those reported by [20, 22]. Furthermore, plant extracts, especially thyme retarded lipid the occurred charges, so plant extracts played an important role as antioxidants indicated by decrease TBARS values after 4 months storage. Also, some aldehydes and ketons components reacted or combined with protein derivatives [23]. The increase of TVC may be due to the multiplication of microbial counts that can able to growing under freezing conditions [20]. While the reduction in TVC may be due to the damage of bacteria cell caused by grown crystals [14]. Psychrophilic load counts developed markedly with prolongation of frozen storage may be due to multiplication of microflora at low temperature [20]. However, it was observed that plant extracts were more effective on bacterial multiplication comparing with control sample. The inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi and microbial toxins synthesis is well documented [1]. Moreover, significant differences ($P<0.05$) were found among different periods of storage and between different treatments.

In conclusion, this study recommends using of some plant extracts, especially thyme followed by rosemary in glazing solutions of fish fillets that intended for storage at low temperature, since these plant extract have a role in inhibiting the quality loss and biochemical changes, especially TBARS value and microbial load. Therefore, spices extracts are available and can be used as an antimicrobial and antioxidant agents to improve fish fillets quality during long-term freezing.

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