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Original Article

Effects of grape pomace extract on the quality and shelf life of silver carp (*Hypophthalmicthys molitrix*) fillets during chill storage

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Abstract: The effects of grape pomace extract (0, 2 and 4%) on quality and shelf life of silver carp (*Hypophthalmicthys molitrix*) fillets during chill storage (4°C) were investigated. The control and the treated fillets were analyzed periodically for microbiological (TVC and PTC), chemical (TVB-N), and sensory characteristics. The results showed that grape pomace-treated samples have lower TVB-N (24.2 and 21.2 mg N/100 g, respectively), TVC (7.33 and 7.09 log cfu/g, respectively) and PTC (7.26 and 7.03 log cfu/g, respectively) at the end of the storage period. The results revealed that the addition of grape pomace extract has a positive effect on the sensory quality of silver carp fillets by retaining proper quality characteristics for a longer time and extends their shelf life during chill storage.

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Introduction

The smell, taste, freshness, absence of specific microorganism, size and composition are the most important factors for determining the quality of marine products (Connell, 2002). Seafood is spoiled more than other foods containing high percentage of the protein (Huss, 1988). The psychrophilic bacteria spoilage is occurred in cold storage in seafood (Gram and Huss, 1996) and has serious health risk to consumers. Therefore, additions of the antioxidant and antibacterial substances to seafood products are useful to improve their quality, increase the shelf life and prevent economic losses (Yin and Cheng, 2003). Grape pomace is a waste product of the grape juice (Özkan et al., 2004) having high phenolic compounds (Arvanitoyannis and Van Houwelingen-Koukaliaroglou, 2005); therefore, it has drawn attention of researches to use it in seafood products (Caillet et al., 2006; Bozan et al., 2008).

As free radical, the phenolic compounds can potentially interact with biological systems and prevent the human neurodegenerative diseases, cardiovascular disorders and cancer (Poudel et al., 2008). Phenolic compounds are found in grape seeds, skins and stem extracts (Jayaprakasha et al., 2003). Flavanols are the most abundant phenolic compounds in grape skins with grape seeds being rich in monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin and (-)-epicatechin-3-Ogallate, and dimeric, trimeric and tetrameric procyanidins (Kammerer et al., 2004). These compounds act as antimutagenic and antiviral agents and can also be used to preserve food because of their protective effects against microorganisms (Vattem et al., 2004). Tesaki et al. (1999) confirmed that phenolic compounds are the important compounds acting against bacteria. Therefore, the present study aimed to determine the effect of the grape pomace extract on the quality and shelf life of silver carp (*Hypophthalmichthys molitrix*) fillets during chill storage.

Materials and Methods

Sample preparation: Experiments were carried out

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with fresh silver carp purchased from market (Zabol, southeast Iran) that had been delivered 5 hrs after slaughtering. The fresh silver carp with weight of 1.5 kg were selected and transported in isothermal iceboxes to the fish product processing laboratory at University of Zabol in 2011. The fishes were cleaned and filleted and then 0 (as control group), 2% and 4% total phenolic (TP) of red grape pomace extract (g extract/100 g flesh) were added to the same weighed fillets (about 100 g). The samples were placed in moisture-impermeable plastic bags, stored in chill room (4°C) and used for analysis on 0, 3, 6, 9, 12 and 15 days. The experiments were performed in three replicates.

Extraction: Fresh red grapes (*Vitis vinifera*) were prepared from Shahryar city (Iran) and transferred to the laboratory. They were stored at -20°C until they were made into pomace. Grape pomaces were dried at 45°C for 72 hrs and milled to a particle size less than 0.5 mm. The dried grape pomace (200 mg) was placed in a test tube, then 20 mL Diethyl ether containing 1% acetic acid was added to remove pigments and fat. The solution was thoroughly shaken at room temperature for 20 min and centrifuged at 3000 g and 4°C for 10 min, after which the supernatant was recovered. Ten milliliters of acetone 70% (V/V) was added to the residue, and shaking and centrifugation were repeated. Extractions were performed to calculate the total phenolic content (Makkar, 2000).

Bacteriological analysis: The total viable counts (TVC) and psychrophilic total counts (PTC) of fish fillets were estimated. Fish muscle (10 g) of each treatment was aseptically weighed and homogenized with 90 ml sterile 0.85% normal saline for 1 min at room temperature. Furthermore, the decimal dilutions were prepared and then 0.1 ml of each dilution was pipetted onto the surface of Tripthic Soye Agar (TSA). They were incubated for 48 hrs at 35°C for TVC according to Egan et al. (1997) and 10 days at 4°C for PTC based on McMeekin et al. (1993). Microbial loads were expressed as log10 cfu/g.

Chemical analysis: The TVB-N was estimated using steam distillation method (AOAC, 2002). The steam distillation was carried out by distillation after the addition of MgO to the homogenized fish samples. The distillate was collected in a flask containing a 2% aqueous solution of boric acid, and a mixed indicator produced from dissolution of 0.1 g of methyl red to 100 ml of ethanol. Then, the boric acid solution was titrated with a 0.1 N sulphuric acid solution. The TVB-N value (mg N/100 g of flesh fish) was determined based on the consumption of sulphuric acid.

Sensory evaluation: Sensory quality of fish sample was evaluated by twenty trained panelists. The panelists have scored for colour, odour, flavour, overall acceptability and texture, using a seven-point hedonic scale (0, dislike extremely to 7, like extremely) (ASTM, 1969). A sensory score of two was taken as the borderline of acceptability. For this evaluation, the fillets were fried (Sunflower oil, Bahar, Iran) for approximately 3 minutes at 180°C.

Statistical analysis: Data were analyzed using Two-Way ANOVA by SAS version 9.1 software. When differences were significant (P<0.05), the mean values were evaluated by the Least Significant Differences (LSD). Kruskal-wallis test was used to sensory evaluation. The Mann-Whitney U-test is used to Paired comparisons test.

Results

Microbiological analysis: Figure 1 and 2 show total viable counts (TVC) and psychrophilic total counts (PTC) of silver carp fillets during chill storage. The lowest and highest of TVC and PTC were found on the first and 15 days of storage between 3.96 to 7.91 and 3.87 to 7.89 log CFU/g for the 4% total phenolic and control, respectively. The TVC and PTC values were increased significantly (P<0.05) during chill storage. There were significant differences between all treatments for all days of storage.

Chemical analysis: Table 1 shows the results of the chemical analysis. The results show that the TVB-N values increase significantly (P<0.05) during chill storage. The total phenolic also had a significant effect on all the TVB-N values (P<0.005). The

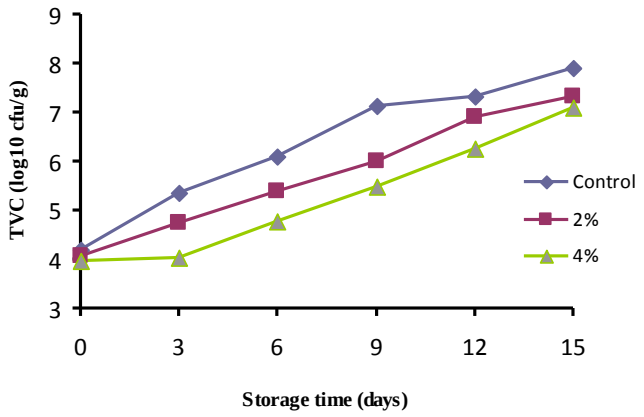

Figure 1. Changes in total viable counts of silver carp fillets during chill storage.

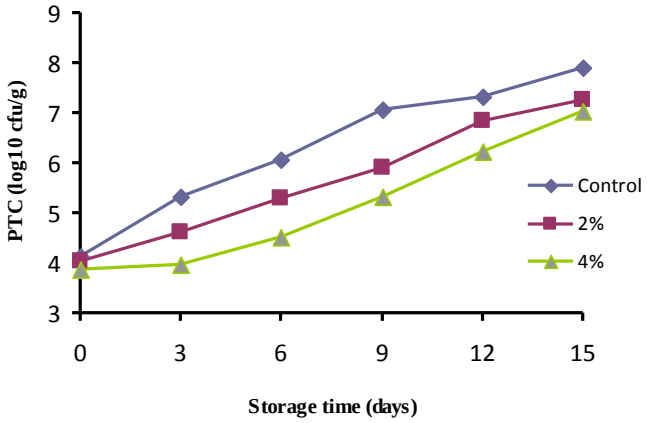

Figure 2. Changes in psychrophilic total counts of silver carp fillets during chill storage.

Table 1. Changes in TVB-N (mg N/100 g fish flesh) values of silver carp fillets during chill storage.

Storage time (days)	Control	2%	4%
0	4.71 ± 0.45 Af	4.2 ± 0.00Af	4.2 ± 0.00Af
3	9.8 ± 0.00Ae	7.23 ± 0.40Be	5.37 ± 0.40Ce
6	13.53 ± 0.40Ad	11.67 ± 0.40Bd	10.4 ± 0.56Cd
9	21.28 ± 0.37Ac	16.8 ± 1.40Bc	13.67 ± 0.35Cc
12	27.4 ± 0.70Ab	20.83 ± 1.10Bb	19.37 ± 0.50Bb
15	30.50 ± 0.82Aa	24.2 ± 0.30Ba	21.2 ± 0.78Ca

Values are mean ± standard deviation of three determinations.
Capital letters (A-C) in the same line indicate significant differences (P<0.05) of treatment.
Small letters (a-f) in the same column indicate significant differences (P<0.05) of storage.

lowest values were found on the first day of storage for the 2 and 4% total phenolic and the highest were found on the 15 days of storage for control group. The significant differences were observed between all treatments for all days except first day of storage.

Sensory evaluation: The results of the sensory evolution are given in Table 2. The results indicate that sensory scores showed a significant decline in all samples with increasing storage period, and the results also indicate that the sensory evolution values decreased significantly with the increasing chemical and microbial spoilage (P<0.05). The overall scores of 4% total phenolic were higher than the 2% total phenolic and control.

Discussion

Microbiological analysis: It has been estimated that about one-third of the world's food production is lost annually on account of microbial spoilage (Tingting et al., 2012). In the present study, the alternations in TVC during 15 days storage showed that microbial growth had been significantly (P<0.05) influenced by the addition of grape pomace extract. The control group attained a TVC value of 7.13 log CFU/g on day 9, which was close to the microbial acceptability limit of 7 log CFU/g for raw fish (Ojagh et al., 2010), indicating a microbiological shelf-life about 9 days for the control samples. In other works, a shelf-life of less than 10 days has been reported for Pacific salmon (*Onchorhynchus nerka*) stored at 1ºC (Sallam, 2007) and crucian carp (*Carassius auratus*) stored at 4ºC (Tingting et al., 2012).

The results showed that grape pomace extract delayed microbial growth and extended the shelf-life of the silver carp fillets. In the present study, the treated samples with grape pomace extract did not reach the microbiological acceptability limit during the 12 (2% TP) and 15 (4% TP) days storage showing significantly lower TVC values than that of the control one (P<0.05). Phenolics, with effective

Table 2. Changes in Sensory scores of silver carp fillets during chill storage.

Storage time (days)		Flavour	Odour	Texture	Colour	Overall acceptability
0	Control	7Aa	7Aa	7Aa	7Aa	7Aa
	2%	7Aa	7Aa	7Aa	7Aa	7Aa
	4%	7Aa	7Aa	7Aa	7Aa	7Aa
3	Control	5Ab	5Bb	5Bb	5Bb	5Ab
	2%	5Ab	7Aa	5Bb	7Aa	5Ab
	4%	5Ab	7Aa	7Aa	7Aa	5Ab
6	Control	1.5Bc	1.5Bc	1.5Bc	1.5Bc	1.5Bc
	2%	3Ac	5Ab	5Ab	5Ab	5Ab
	4%	4Ac	5Ab	5Ab	5Ab	5Ab
9	Control	-	-	-	-	-
	2%	1.5Bd	2Ac	2Ac	2Ac	1.5Bc
	4%	3Ad	3Ac	3Ac	3Ac	3Ac
12	Control	-	-	-	-	-
	2%	-	-	-	-	-
	4%	1.5Ae	1Ad	1.5Ad	1Ad	1.5Ad

Capital letters (A-B) in the same day indicate significant differences (P<0.05) of treatment.
Small letters (a-e) in the same column indicate significant differences (P<0.05) of storage.

antimicrobial and antioxidant activities, could significantly delay microbial growth and have been widely applied as a natural food preservative (Fan et al., 2008). Tingting et al. (2012) confirmed that shelf-life of crucian carp could be prolonged for an additional 6 days during 4ºC storage by adding natural preservative (Tea polyphenols and Rosemary extract).

Based on the results, PTC increased significantly (P<0.05) throughout chill storage in all treatments, especially in control one. The significant reduction of PTC was observed in 2% and 4% treatments showing inhibitory effect of total phenolic on spoilage bacteria (Fan et al., 2008). Based on the microbiological acceptability limit of 7 log10 cfu/g for fresh water and marine species (ICMSF, 1986), the results indicated that treatments with 2% and 4% TP have equal effect in inhibiting spoilage bacterial growth and extending the chill storage life in silver carp fillets to 12 and 15 days compared to 9 days in control group.

Chemical analysis: The results of alternation in TVB-N values in silver carp fillets during chill storage showed that the values increased gradually in all treatments during the 15-day storage. The TVB-N value is one of the most widely used indicators of seafood deterioration. It is mainly composed of ammonia and primary, secondary and tertiary amines (Beatty, 1938), resulted from degradation of the proteins and non-protein nitrogenous compounds. These compounds are chiefly produced by microbial activity (Ruiz-Capillas and Moral, 2005; Yilmaz et al., 2009) and proteolytic enzymes (Yasin and Abou-Taleb, 2007). This increase can be related to microbial activity during cold storage (Yilmaz et al., 2009; Pacheco-Aguilar et al., 2000).

TVB-N values increased from an initial value (mg N per 100 g of fish flesh) of 4.71 ± 0.45–30.50 ± 0.82 in control group, to 4.2 ± 0.00–24.20 ± 0.30 in treatment with 2% total phenolic, and to 4.2 ± 0.00–21.20 ± 0.78 in treatment with 4% total phenolic at the end of storage (day 15). The two treated groups maintained a significantly (P<0.05) lower TVB-N value than that of control one. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Fan et al., 2008; Tingting et al., 2012), which was due to the effect of the grape pomace extract on silver carp fillets. By considering a TVB-N value of 25 mg N/100 g fresh fish as the acceptable limit proposed by Kilincceker et al. (2009), the shelf-life

of the control and the treated groups were about 12 and 15 days, respectively.

Sensory evaluation: Spoilage and quality deterioration can be assessed by chemical and physical methods and sensory evaluation (Connell, 1990). Not all chemical assessments give good correlation to quality changes, hence sensory evaluation is a necessity (Hardy, 1979). The results indicated a significant decline of sensory scores in all groups with increasing storage time (Özogul et al., 2004). The sensory properties of the control group was received a lower score than that of 2% and 4% groups. Thus, the control group was acceptable up to 6 days and 2% and 4% samples were in acceptable conditions up to 9 and 12 days of chill storage. This results showed that the acceptable limits of microbial and chemical parameters is not confirmed the quality changes, thus the sensory evaluation is requirement. Fan et al. (2008) reported that sensory scores of silver carp is reduced with increasing storage period in compared to those of treated with tea polyphenols. Based on the results, 4% treatment could be retaining their good quality in terms of sensory evolution compared to others. These results are agreed with the results of microbial and chemical analyses.

As conclusion, the present study showed that treatment with 2% and 4% total phenolic of red grape pomace extract could effectively retard microbial growth, delay chemical deterioration, maintain or improve sensory attributes and extend the shelf-life of silver carp fillets for 3-6 days during chill storage. Natural preservatives such as grape pomace extract can be used as a safe method for storage of silver carp fillets, which is quite promising for food industry.

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