

Antimicrobial Properties of Grape Extract on Common Carp (*Cyprinus carpio*) Fillet During Storage in 4°C

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Abstract: Grape pomaces are low-cost natural antioxidant and antimicrobial source and surplus by product of the food processing industry. The aim of this study was to evaluate the effect of adding a red grape pomace by product extract to filleted fish and compare the shelf life of the samples with control. In this experiment, acetone 70% (v/v) was used to extract the phenolic compounds of by Colorimetric Method and concentrations of 0, 2 and 4% extraction were added to samples. Shelf life evaluation was done by determining of the Total Viable Counts (TVC) and Psychrophilic Total Count (PTC). Sensory analysis and Total Volatile Bases-N (TVB-N) were also measured to assessing quality. The sensory acceptability limit was before 6 days for fillet stored in refrigerator and 9 days for fillet prepared from 2% extract and 12 days for fillet with 4% extract. Results showed that amount of pH, TVN, TVC and PTC increased. According to bacterial analysis shelf life in treatments containing 2% extracts 3 days and in treatments with 4% extracts 6 days was larger than control. Overall, 4% concentration made available best preservation condition according to qualitative characteristics (color, odor, texture, taste and flavor). Therefore, it is suggested using preservation of fish fillet.

Key words: Grape pomace by product, extract, shelf life, antimicrobial, refrigerator

INTRODUCTION

The smell, taste, freshness, absence of specific microorganism, size and composition are the most factors for determining the marine production quality (Connell, 2002). Sea food has more spoilage than other foods with high protein (Huss, 1998). It should be noted that psychrophilic bacteria spoilage occurs in cold storage (Gram and Huss, 1996). Prior to microbial spoilage, enzymatic and chemical deteriorative changes occur in fish because of the high content of unsaturated fatty acids, free amino acids and other highly reactive compounds in fish (Huss, 1998).

Microbial spoilage leads to serious health risk to consumers. So, material with antioxidant and antibacterial activity is useful to improve quality, increase shelf life and also prevent unnecessary economic losses (Yin and Cheng, 2003). In the global food industry today, "natural" is a powerful force as there is increasing resistance at regulatory and consumer levels against chemical food preservatives (Agatemor, 2009). Numerous naturally occurring anti-microbials are present in animal and plant tissues and many studies have evaluated the antimicrobial activities of several plant extracts including *Sesamum radiatum* (Shittu *et al.*, 2007), *Allium cepa* (Agatemor, 2009), grapes and black raspberries. Grape pomace is a

waste product of grape juice (Ozkan *et al.*, 2004). These products contain high phenolic compounds because of poor extraction during this process; therefore, it makes their beneficiary valuable and support sustainable plant production (Arvanitoyannis *et al.*, 2005). Grapes are considered the world's most ordinary fruit crop. Their large amounts of phenolic compounds have made them the focus of extensive studies (Caillet *et al.*, 2006; Bozan *et al.*, 2008). Flavanols are the most abundant phenolic compounds in grape skins while grape seeds are rich in monomeric phenolic compounds such as (+)-catechins, (-)-epicatechin, (-)-epicatechin-3-ogallate, dimeric, trimeric and tetrameric procyanidins (Kammerer *et al.*, 2004). These compounds act as antimutagenic and antiviral agents (Kammerer *et al.*, 2004; Montealegre *et al.*, 2006). The main phenolic antioxidants can also be used to preserve food because of their protective effects against microorganisms (Tesaki *et al.*, 1999; Jayaprakasha *et al.*, 2003; Vattem *et al.*, 2004). Phenolic antimicrobial compounds are found in grape seeds, skins and stem extracts (Jayaprakasha *et al.*, 2003). Tesaki *et al.* (1999) confirmed that phenolics were the most important compounds active against bacteria. Also, they identified gallic acid as the most active compound for the inhibition of bacteria. The present study was carried out to evaluate the potential of grape extracts as

natural antimicrobials on common carp (*Cyprinus carpio*) fillets to increase shelf life during refrigerated storage.

MATERIALS AND METHODS

Preparation of samples: Experiments were carried out with fresh Common carp (*Cyprinus carpio*) which were caught from Caspian Sea located in north of Iran. They were transported in isothermal ice boxes to the laboratory. Fresh carp of similar weight were selected for each experiment. The fishes were cleaned and filleted. Concentrations of 0, 2 and 4% total phenolic of red grape pomace extract were added to the same weighed fillets (about 100 g). The samples were placed in moisture-impermeable plastic bags, stored in refrigerator (+4°C) and taken for analysis on 0, 3, 6, 9, 12 and 15 days. The experiences were performed with three replicates.

Extraction: Fresh red grapes (*Vitis vinifera*) were prepared from Shahrood (Iran) and were transferred to laboratory. They were stored at -20°C until was made into pomace. Grape pomaces were dried at 45°C for 72 h, milled to particle size <0.5 mm. Dried grape pomace (200 mg) was placed in a test tube then 20 mL diethyl ether containing 1% acetic acid was added for removing of pigment and fat. The solution was thoroughly shaken at room temperature for 20 min and centrifuged at 3,000 g at 4°C for 10 min and the supernatant was recovered. The 10 mL of acetone 70% (v/v) were added to the residue and shaking and centrifugation were repeated. Extractions were performed to calculate the total phenolic content (Makkar, 2000).

Determination of Total Phenolic content (TP): Total phenolic content was determined by Folin-Ciocalteu reagent (Makkar, 2000) using tannic acid as standard. A mixture of 0.1 mL of extract, 0.5 mL of distilled water, 0.25 mL of Folin-Ciocalteu reagent and 1.25 mL of Na₂CO₃ were introduced in a 25 mL volumetric flask. After reacting for 40 min, the absorbance was measured at 725 nm using an ultraviolet-visible spectrophotometer (Jenway, 6305). The results were expressed as g of tannic acid equivalents/kg of grape pomace Dry Matter (DM).

Chemical analysis: pH was determined for the homogeneous mixtures of fish with distilled water (1:10, w/v) using a digital pH meter (Wagtech-Cmyber scan 510, Germany) according to Salam *et al.* (2004). The Total Volatile Base-Nitrogen (TVB-N) content of common carp was determined after steam distillation according to the method of AOAC (2002). The analysis was based on titration 0.1 N HCl, using a solution of a boric acid. The results expressed as milligram TVB-N per 100 g muscle.

Microbiological analysis: Samples were taken fillets to estimate Total Viable Counts (TVC). Common carp muscle (10 g) were mixed with 90 mL of Ringer solution and stomached for 3 min. Further decimal dilutions were made and then 0.1 mL of each dilution was pipetted onto the surface of TSA (Triphthic Soye Agar) plates in triplicate. Then, they were incubated for 2 days at 30°C for total viable count and 15 days at 4°C for psychrophilic total count. Microbial loads were expressed as log₁₀ cfu g⁻¹.

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. Sensory analysis was conducted by a panel of 30 non-trained taste panelist using 7-point hedonic scale (ASTM, 1969). Panel scoring of the fillets was conducted after frying for 3 min at 180°C. The panelists scored the sample based on the characterization and differentiation of the various sensory characters such as texture, flavor, color, odor and overall acceptability.

Statistical analyses: Data were subjected to two way ANOVA design. The Least Significant Difference (LSD) procedure was used to test for differences between means at the 5% significance level (Steel and Torrie, 1980). Kruskal-Wallis test was used to sensory analysis. The Mann-Whitney U-test is used to Paired comparisons test.

RESULTS AND DISCUSSION

Total Phenolic Component (TPH): Phenols are one of the most important groups of natural antioxidants. They occur only in material of plant origin and they are known to protect easily-oxidizable constituents of food from oxidation. The phenolic content and composition greatly differs with the type of grape and the extraction yield is greatly affected by the solvent. In this study, the total phenolic content of red grape pomace were found to be 68 g kg⁻¹ DM. Negro *et al.* (2003) found that the total phenolic content of Italian red grape pulp were 161 g kg⁻¹ DM. Alipour and Rouzbehan (2007) reported that the total phenolic content of Iranian grape pomace were 22.70 g kg⁻¹ DM. It can be concluded from these results that the phenolic content of grape pomace change according to cultivar (location, species and stage of maturity) and solvent(s) used in the extraction (Makkar, 2000; Ozkan *et al.*, 2004).

pH: Figure 1 shows the pH values for the common carp fillets subjected to grape pomace extract during refrigerated storage. The significant pH changes can be

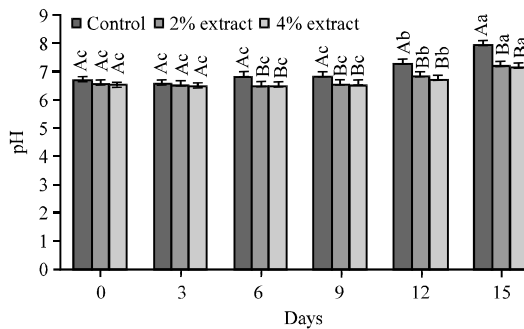


Fig. 1: Effect of grape pomace by product extracts on pH (ppm) in the treatment of common carp fish fillets in different times of storage. The values are expressed as mean±standard deviation, n = 3. Values followed by different letters (a-f) indicate significant differences during storage periods and values followed by different letters (A-C) indicate significant differences of the parameter with respect to the extract treatment

seen between treatment and storage time. There was no significant difference among the treatment after 3 days of storage showing the grape pomace extract had little effect on overall pH change in fish samples. Moreover, a significant difference in the pH was noticed among control and grape pomace extract treatment after 9 days of storage. A increase in pH was observed after storage for all treatment but this increase was more in control when compared with grape pomace extract treatment.

pH is an important and effective indicator of meat quality (Suvanich *et al.*, 2000). Lower pH in the samples treated with grape pomace extract can be attributed to the antibacterial properties (Baydar *et al.*, 2004). Phenolic compounds of grape pomace extract can be increase microbial inhibition, protection fillets against the internal protease and finally inhibit protein breakdown and amine production (Yilmaz and Toledo, 2004). The pH values of common carp fillets increase during storage. This increase may be related to the production of alkaline compounds (Manju *et al.*, 2007).

TVB-N: The TVB-N value changes in different treatments of common carp fish during storage in the refrigerator is shown in Fig. 2. The European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt as to the freshness of the fish, inspectors must use TVB-N as a chemical check (Directive, 1991). TVB-N as an indicator of freshness is measurement of the amount of basic volatile compounds recovered by distilling fish muscle or extracts of

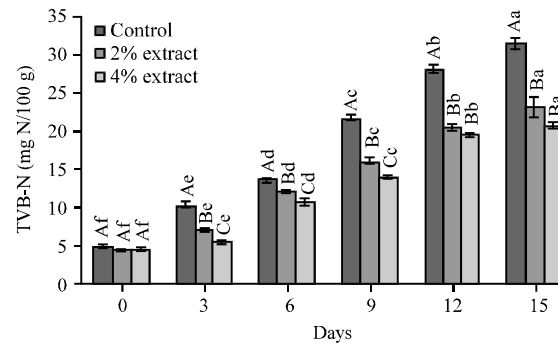


Fig. 2: Effect of grape pomace by product extracts on TVB-N (mg N/100 g) fish flesh in the treatment of common carp fillets. The values are expressed as mean±standard deviation, n = 3. Values followed by different letters (a-f) indicate significant differences during storage periods and values followed by different letters (A-C) indicate significant differences of the parameter with respect to the extract treatment

fish muscle under alkaline conditions (Howgate, 2010). In this study, TVB-N concentrations of all groups are presented in Fig. 2. TVB-N measurements were low at the beginning of the period of frozen storage, since all fish were fresh (Fig. 2).

Results showed an increase in TVB-N for fillets of both species either with or without extract by the end of the storage period. This is to be expected since enzymes are still active at low rates at cold temperatures and may increase the TVB-N values of the fish. Significant differences ($p < 0.05$) between the controls and treated samples were observed. The highest value for the controls occurred in the control, reaching 31.40 ± 0.75 mg/100 g while the lowest TVB-N value occurred in the fillets treated with 4% extract (20.7 ± 0.37 mg/100 g) (Fig. 2). Similar results for other fish species stored at freezing temperatures have been obtained previously (Ojagh *et al.*, 2010; Fan *et al.*, 2008).

The limit of acceptability of TVB-N in fish and fishery products is 30-35 mg/100 g. The value of TVB-N for fillet stored in refrigerator in control (31.40 mg/100 g) was unacceptable and other treatments were in acceptability ranges. There were significant differences for TVB-N values of samples stored in refrigerator throughout the storage period.

Antibacterial value: Figure 3 and 4 show the effects of extract addition and storage time on total viable counts and psychrotrophic counts in the common carp fish fillets. TVC and PTC increased significantly ($p < 0.05$) throughout storage, especially in control (days 6 and 9).

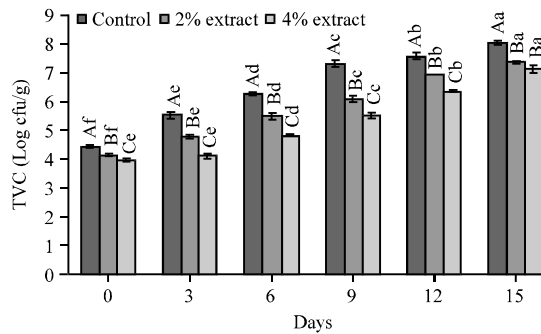


Fig. 3: Effect of grape pomace by product extracts on TVC (Log cfu/g) in the treatment of common carp fish fillets. The values are expressed as mean±standard deviation, n = 3. Values followed by different letters (a-f) indicate significant differences during storage periods and values followed by different letters (A-C) indicate significant differences of the parameter with respect to the extract treatment

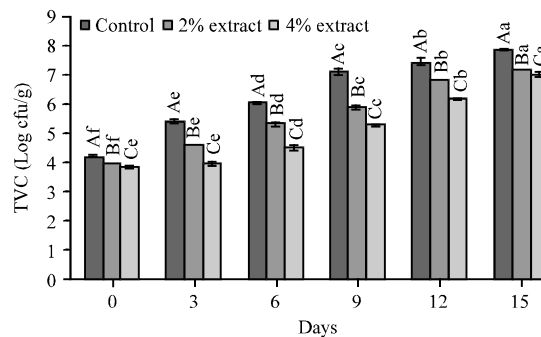


Fig. 4: Effect of grape pomace by product extracts on PTC (Log cfu/g) in the treatment of common carp fish fillets. The values are expressed as mean±standard deviation, n = 3. Values followed by different letters (a-f) indicate significant differences during storage periods and values followed by different letters (A-C) indicate significant differences of the parameter with respect to the extract treatment

Unacceptable TVC (almost 6 CFU g⁻¹) were found in control (day 6) and all groups (day 12). Significant differences ($p < 0.05$) in mean TVC and PTC between Control and groups with extract were observed in 15 days. TVC and PTC were lower in groups than in the control (all days). The shelf life of raw meat is usually limited by microbial spoilage. Bacterial count was measured to determine the shelf life of fillet in this study. Results showed that the control and group with 4% extracts had the highest and lowest value, respectively. By the time the amount of bacterial count was also increased and

Table 1: Effect of grape pomace by product extracts on sensory changes in treatment of common carp fillets

Days	Variable	Taste	Smell	Texture	Color	Total acceptance
0	Control	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}
	2%	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}
	4%	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}
3	Control	5.0 ^{Ab}	5.0 ^{Bb}	5.0 ^{Bb}	5.0 ^{Bb}	5.0 ^{Ab}
	2%	5.0 ^{Ab}	7.0 ^{Aa}	5.0 ^{Bb}	7.0 ^{Aa}	5.0 ^{Ab}
	4%	5.0 ^{Ab}	7.0 ^{Aa}	7.0 ^{Aa}	7.0 ^{Aa}	5.0 ^{Ab}
6	Control	1.5 ^{Bc}	1.5 ^{Bc}	1.5 ^{Bc}	1.5 ^{Bc}	1.5 ^{Bc}
	2%	3.0 ^{Ac}	5.0 ^{Ab}	5.0 ^{Ab}	5.0 ^{Ab}	5.0 ^{Ab}
	4%	4.0 ^{Ac}	5.0 ^{Ab}	5.0 ^{Ab}	5.0 ^{Ab}	5.0 ^{Ab}
9	Control	0.0 ^{Cd}	0.0 ^{Bd}	0.0 ^{Bc}	0.0 ^{Bd}	0.0 ^{Cd}
	2%	1.5 ^{Bd}	2.0 ^{Ac}	2.0 ^{Ac}	2.0 ^{Ac}	1.5 ^{Bc}
	4%	3.0 ^{Ad}	3.0 ^{Ac}	3.0 ^{Ac}	3.0 ^{Ac}	3.0 ^{Ac}
12	Control	0.0 ^{Bd}	0.0 ^{Bd}	0.0 ^{Bc}	0.0 ^{Bd}	0.0 ^{Bd}
	2%	1.0 ^{ABd}	1.0 ^{Ad}	1.5 ^{Ac}	1.0 ^{Bd}	1.0 ^{ABc}
	4%	1.5 ^{Ae}	1.0 ^{Ad}	1.5 ^{Bd}	1.0 ^{Bd}	1.5 ^{Ad}
15	Control	0.0 ^{Ad}	0.0 ^{Bd}	0.0 ^{Ac}	0.0 ^{Ad}	0.0 ^{Ad}
	2%	0.0 ^{Ad}	0.0 ^{Ac}	0.0 ^{Ad}	0.0 ^{Ac}	0.0 ^{Ad}
	4%	0.0 ^{Ad}	0.0 ^{Ac}	0.0 ^{Ac}	0.0 ^{Ac}	0.0 ^{Ac}

Uppercase and lowercase letters between treatments indicate significance at different times

15th day had the highest value (Fig. 3). Bacterial spoilage in fish under aerobic conditions takes place by negative-Gram psychrophilic micro-organisms such as *Pseudomonas alteromonas*, *Shewanella* and the *Flavobacterium* species during the storage in 4°C (Hubbs, 1991). The literature reported that total bacteria count of different species of fresh water is 2-6 log₁₀ g⁻¹ cfu (Gelman *et al.*, 2001). This result is similar to Salam *et al.* (2004) on the effects of plant extracts on the meat. Harpaz *et al.* (2003) reported that the shelf life of sea bass treated with oregano essential oil comparison with the control sample increased to 33 days in 0-2°C. It can be attributed to the antioxidant and anti-microbial essential oil of pennyroyal (Harpaz *et al.*, 2003). Tesaki *et al.* (1999) have reported antimicrobial activity of methanol extract from grape seeds. The active compound for the inhibition of *E. coli* and *Salmonella enteritidis* was identified as gallic acid. It has been reported that Gram-negative bacteria have low susceptibility to plant extracts when compared to Gram-positive bacteria. The resistance of Gram-negative bacteria to antibacterial substances is related to lipopolysaccharides in their outer membrane. Generally, the extent of the inhibitory effects of the extracts could be attributed to their phenolic composition. Results of this study showed that GP extraction may be exploited as antibacterial agents to prevent the deterioration of stored fish fillet by bacteria.

Sensory analysis: The sensory analysis scores changes in different treatments of fish fillets during storage in the refrigerator are shown in Table 1. Ismail (2000) reported that the necessity of conducting sensory evaluation. Sensory testing is important to quality evaluation since the ultimate test of food quality is consumer response.

Instrumental methods to determine some physical, chemical or biological properties of food have been developed and are being used to assess flavor, color, odor and texture. However, sensory evaluation panels must be used to ensure that instrumental methods are properly correlated with sensory data. Figure 5 showed the changes in sensory evaluation (color, odor, flavor, texture and overall acceptability) from days 0-15 for fried fillets. The sensory scores of fillets in all groups decreased with the storage time. In all indices decreased scores with time and were not significantly different at 9, 12 and 15th days from each other. There were significant differences between the flavor score and was the lowest score in the 15th day. There was no significant difference in the smell of the 1st 2 days of measurement. Recently, the fillets odor control samples remained up to the 9th day and rancidity was more emotional after the 10th day. Ozogul *et al.* (2004) reported a significant decrease in sensory scores with increasing during storage. Color, odor, flavor, texture and overall acceptability scores of the control group were significantly lower than the groups with 2 and 4% extract at 15 days, it can be indicated that extract could be used as easily accessible natural source since they have better consumer acceptance. Also, Fan *et al.* (2008) reported that sensory scores in both common carp treated with increasing duration has been reduced and sensory properties of samples that were treated with tea polyphenols had a higher score than the treatments were immersed in distilled water. Sensory panelist emphasized slight grape aroma for both groups.

CONCLUSION

In this study, experiment on indices of spoilage factors in fillets of common carp showed that the amount of volatile nitrogen bases and pH increased in fillet production during refrigeration. Microbial counts showed increased the total number of bacteria and psychrophilic bacteria control treatments and treatments with extracts. Refrigeration containing GP extract improved the sensory and some chemical quality of fish which resulted in a significant extension of the shelf life of common carp fillets. Refrigeration with grape extract had a positive effect causing low bases nitrogen, pH and bacterial count. Consequently, the application of grape extract improved the quality of fillets which is quite promising for the food industry.

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