International Journal of Fisheries and Aquatic Studies 2014; 1(3): 130-136

ISSN: 2347-5129
IJFAS 2014; 1(3): 130-136

www.fisheriesjournal.com
Received: 22-10-2013
Accepted: 06-11-2013

Shirin Hasani
M.Sc post Graduated of Zabol University, Faculty of natural resource, Department of Fishery, University of Zabol, Iran.
Tel: 00989113720257
E-mail: sh_hasani87@yahoo.com
E-mail: shirin.hasani88@gmail.com

Maryam Hasani
Department of Food science and Technology, Shahrood Branch, Islamic Azad University, Shahrood, Iran.
Tel: 009155094634
E-mail: mhasani81 @yahoo.com

Correspondence:
Maryam Hasani
Department of Food science and Technology, Shahrood Branch, Islamic Azad University, Shahrood, Iran.
Tel: 009155094634
E-mail: mhasani81 @yahoo.com

Antimicrobial properties of grape extract on Common carp (*Cyprinus carpio*) fillet during storage in 4 °C

Shirin Hasani and Maryam Hasani

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, Extractions were performed 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 psycrophilic total count (PTC). Sensory analysis and total volatile bases-N (TVB-N) were also measured to assessing quality. 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 and suggested using preservation of fish fillet.

Keywords: Grape pomace by-product, extract, Shelf life, Antimicrobial, Refrigerator.

1. Introduction

The smell, taste, freshness, absence of specific microorganism, size and composition are the most factors for determining the marine production quality [10]. Sea food has more spoilage than other foods with high protein [18]. It should be noted that psychrophilic bacteria spoilage occurs in cold storage [14]. 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 [18].

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 [36] 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 [1]. Numerous naturally occurring antimicrobials are present in animal and plant tissues and many studies have evaluated the antimicrobial activities of several plant extracts, including *Sesamum radiatum* [30], *Allium cepa* [1], grapes and black raspberries. Grape pomace is a waste product of grape juice [26]. These products contain high phenolic compounds because of poor extraction during this process; therefore it makes their beneficiary valuable and support sustainable plant production [4]. Grapes are considered the world’s most ordinary fruit crop. Their large amounts of phenolic compounds have made them the focus of extensive studies [8, 7].

Flavanols are the most abundant phenolic compounds in grape skins, while grape seeds are rich in monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin and (-)-epicatechin-3-Ogallate, and dimeric, trimeric and tetrameric procyanidins [21]. These compounds act as antimutagenic and antiviral agents [21, 28]. The main phenolic antioxidants can also be used to preserve food because of their protective effects against microorganisms [20, 31, 34]. Phenolic antimicrobial compounds are found in grape seeds, skins and stem extracts [20]. , Shoko et al. [31] 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.

2. Material and Methods

2.1. 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 iceboxes 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.

2.2. 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 less than 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 pigments and fat. The solution was thoroughly shaken at room temperature for 20 min and centrifuged at 3,000g at 4 °C for 10 min, and the supernatant was recovered. Ten 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 [22].

2.3. Determination of total phenolic content (TP)

Total phenolic content was determined by Folin-Ciocalteu reagent [22]. Using tannic acid as standard. A mixture of 0.1 mL of extract, 0.5 mL of distilled water, 0.25 ml of Folin–Ciocalteau 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 Model, 6305). The results were expressed as g of tannic acid equivalents per kg of grape pomace dry matter (DM).

2.4. 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 Sallam and Samejima [29]. The total volatile base-nitrogen (TVB-N) content of common carp was determined after steam distillation according to the method of AOAC [3]. 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.

2.5. 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 (Tripthic 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 psycrophilic total count. Microbial loads were expressed as $\log_{10}$ cfu/g.

2.6. Sensory evaluation

Spoilage and quality deterioration can be assessed by chemical and physical methods and sensory evaluation [9]. 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 [5]. Panel scoring of the fillets was conducted after frying fore 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.

2.7. 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 [32]. Kruskal- wallis test was used to sensory analysis. The Mann-Whitney U-test is used to Paired comparisons test.

3. Results and discussion

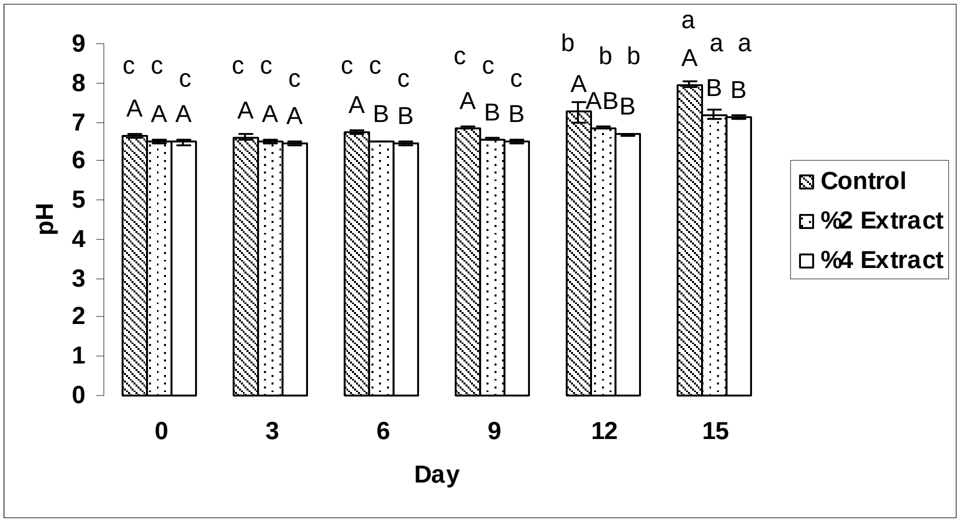
3.1. Total phenolic component (TPH)

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 constitutes 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 DM. Negro et al [24] found that the total phenolic content of Italian red grape pulp were 161 g/ kg DM. Alipour and Rouzbehan [2] reported that the total phenolic content of Iranian grape pomace were 22.70 g/kg 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 [22, 26].

3.2. *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 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 [33]. Lower pH in the samples treated with grape pomace extract can be attributed to the antibacterial properties [6]. 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 [35]. The pH values of Common carp fillets increase during storage. This increase may be related to the production of alkaline compounds [23].



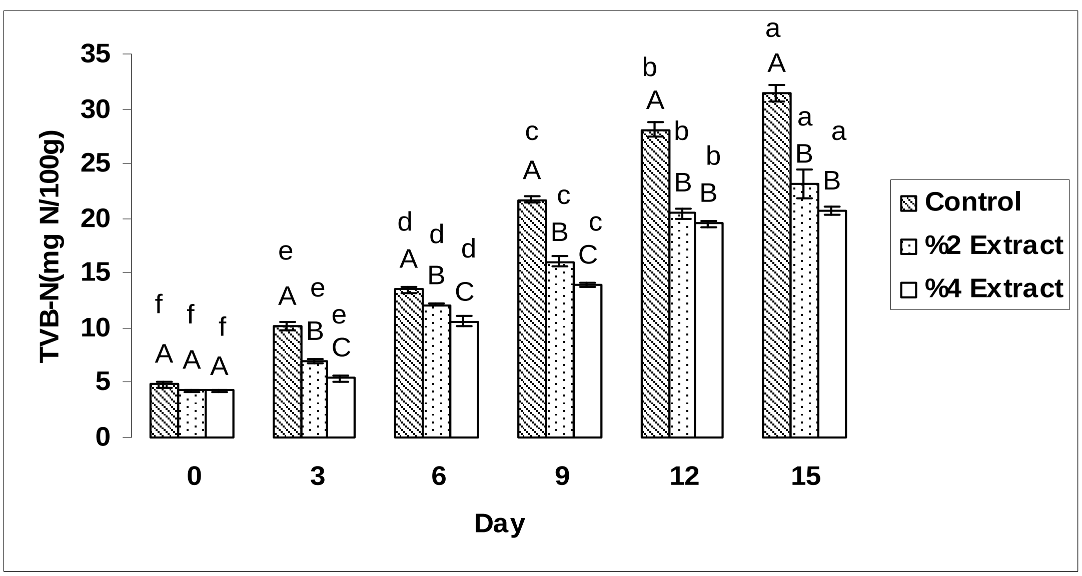
The values are expressed as mean±standard deviation, n=3. Values followed by different letters (a–f) indicate significant differences during storage periods, and Values followed by different letters (A–C) indicate significant differences of the parameter with respect to the extract treatment.

Fig 1: Effect of grape pomace by-product extracts on pH (ppm) in the treatment of Common carp fish fillets in different times of storage.

3.3. TVB-N

The TVB-N value Changes in different treatments of common carp fish during storage in the refrigerator is shown in Figure 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 [11]. TVB-N as an indicator of freshness is measurement of the amount of basic volatile compounds recovered by distilling fish muscle, or extracts of fish muscle, under alkaline conditions [16]. In this study, TVB-N concentrations of all groups are presented in Figure 2. TVB-N measurements were low at the beginning of the period of frozen storage, since all fish were fresh (Figure 2).



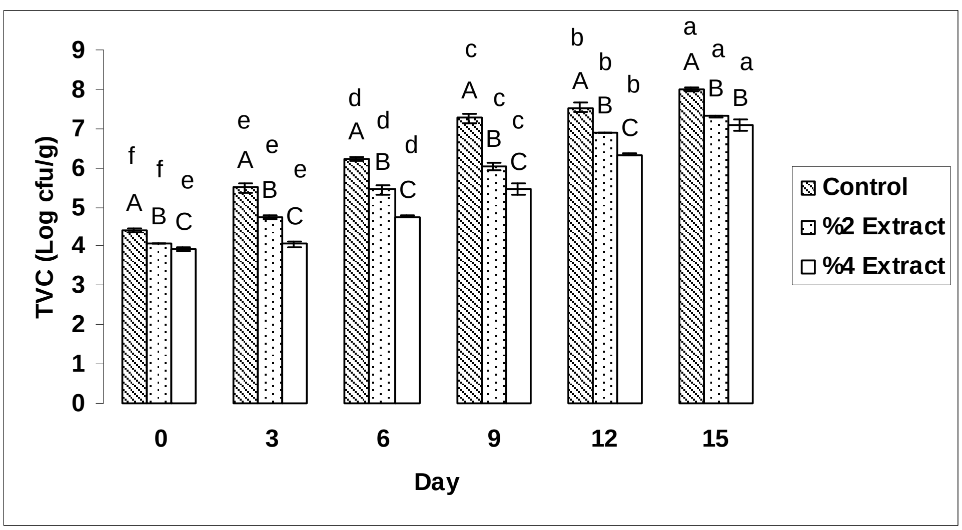
The values are expressed as mean±standard deviation, n=3. Values followed by different letters (a–f) indicate significant differences during storage periods, and Values followed by different letters (A–C) indicate significant differences of the parameter with respect to the extract treatment.

Fig 2: Effect of grape pomace by-product extracts on TVB-N (mg N/100 g fish flesh in the treatment of Common carp fillets

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) (Figure2). Similar results for other fish species stored at freezing temperatures have been obtained previously [12, 25]. 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.

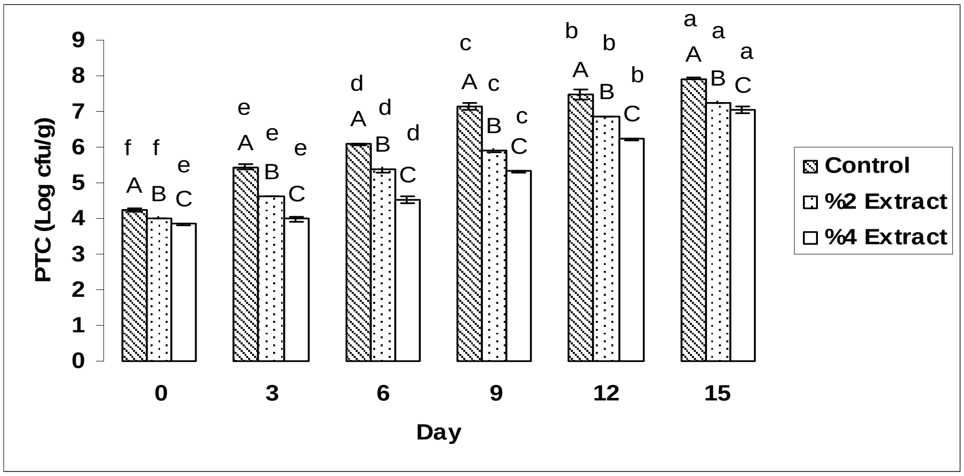
3.4. Antibacterial value

Figure 3 and 4 shows the effects of extract addition and storage time on total viable counts and Psychrothrophic counts in the Common carp fish fillets. TVC and PTC increased significantly ($P < 0.05$) throughout storage, especially in Control (days 6 and 9). Unacceptable TVC (almost 6 CFU/g) 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 15th day had the highest value (Figure 3). Bacterial spoilage in fish under aerobic conditions takes place by negative- gram psychrophilic micro-organisms, such as *Pseudomonas alteromonas*, Shuwanella and the Flavobacteriom species, during the storage in 4 °C [17]. The literature reported that total bacteria count of different species of fresh water, is 2-6 log_{10} /g cfu [13]. This result is similar to Sallam et al [29] on the effects of plant extracts on the meat. Harpaz et al [15] 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 [15]. Shoko et al [31] 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 lipo poly saccharides 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 exploit Figure as antibacterial agents to prevent the deterioration of stored fish fillet by bacteria.



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 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/gr) in the treatment of Common carp fish fillets

3.5. Sensory analysis

The sensory analysis scores Changes in different treatments of fish fillets during storage in the refrigerator are shown in Table 1. Ismail [19] 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 to 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 first two 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 [27] 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 [12] 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.

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

Variable		Taste	Smell	Texture	Color	Total Acceptance
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	0Cd	0Bd	0Bc	0Bd	0Cd
	%2	1.5Bd	2Ac	2Ac	2Ac	1.5Bc
	4%	3Ad	3Ac	3Ac	3Ac	3Ac
12	Control	0Bd	0Bd	0Bc	0Bd	0Bd
	%2	1ABd	1Ad	1.5Ac	1Ad	1ABc
	4%	1.5Ae	1Ad	1.5Ad	1Ad	1.5Ad
15	Control	0Ad	0Ad	0Ac	0Ad	0Ad
	%2	0Af	0Ae	0Ad	0Ae	0Ad
	4%	0Af	0Ae	0Ae	0Ae	0Ae

Uppercase and lowercase letters between treatments indicate significance at different times

4. Conclusions

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.

6. Reference

1. Agatemor C. Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. Food Chem 2009; 8(3): 195-200.
2. Alipour D, Rouzbehan Y. Effects of ensiling grape pomace and addition of polyethylene glycol on in vitro gas production and microbial biomass yield. Animal Feed Science and Technology 2007; 137 (1-2): 138-149.
3. AOAC. Official Methods of Analysis of AOAC international (17th Ed.) MD, USA: Association of Official Analytical Chemistry 2002.
4. Arvanitoyannis I S and Van Houwelingen-Koukaliaroglou M. Functional Foods: A Survey of Health Claims, Pros and Cons, and Current Legislation. Critical Reviews in Food Science and Nutrition 2005; (45): 385-404.
5. ASTM. Manual on Sensory Testing Methods, American Society for Testing and Materials, 1916 Race Street, Philadelphia, pa. 19103, 1969. pp. 34-42.
6. Baydar N G, Ozkan G, and Sagdic O. Total phenolic contents and antibacterial activities of grape (Vitis vinifera L.) extracts. Food Control 2004; 15 (33): 5–339.
7. Bozan B, Tosun G. and Ozcan D. Study of polyphenol content in the seeds of red grape (Vitis vinifera L.) varieties cultivated in Turkey and their antiradical activity. Food Chemistry 2008; 109: 426-430.
8. Caillet S, Salmieri S. and Lacroix, M. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chemistry 2006; 95: 1-8.
9. Connell J J. Control of fish quality 3rd edn (Oxford: Fishing News Books) 1990.
10. Connell J J. Quality Control In Fish Industry. Torry Advisory Note No. 58. 2002.
11. EU. Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Official Journal L 1991; 268: 0015–0034.
12. Fan W, Chi Y, and Zhang SThe use of a tea polyphenol dips to extend the shelf life of silver carp (Hypophthalmicthys molitrix) during storage in ice. Food Chemistry 2008. 108: 148–153.
13. Gelman A, Glatman L, Drabkin V, and Harpaz S. Effects of storage temperature and preservative treatment on shelf life of the pond raised freshwater fish, silver perch (Bidyanus bidyanus). Journal of Food Protection 2001; 64: 1584–1591.
14. Gram L, and Huss H H. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 1996; 33: 121–137.
15. Harpaz S, Glatman L, Drabkin V, and Gelman A. Effects of herbal essential oils used to extend the shelf life of freshwater-reared Asian sea bass fish (Lates calcarifer). Journal of Food Protection 2003; 66: 410–417.
16. Howgate P. A critical review of total volatile bases and Trimethylamine as indices of freshness of fish. Part 1. Determination. Electronic Journal of Environmental Agricultural and Food Chemistry 2010; 9: 29–57.
17. Hubbs J. Fish microbiological spoilage and safety. Food Science 1991; 5: 160-173.
18. Huss H H. Fresh Fish Quaity and Quality changes; Danish International Development Agency. Rome: FAO 1998. p. 43-45.
19. Ismail M I. Lipid oxidation in some of Malaysian freshwater fish. B Sc thesis, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia Serdang, Selangor, Malaysia. 2000.
20. Jayaprakasha G K, Selvi T, and Sakariah K K. Antibacterial and antioxidant activities of grape (Vitis vinisfera) seed extracts. Food Research International 2003; 36: 117-122.
21. Kammerer D, Claus A, Carle R, and Scheiber A. Polyphenol screening of pomace red and white grape varieties (Vitis viniferal L.) by HPLC–DAD–MS/MS. Journal of Agricultural and Food Chemistry 2004; 52: 4360–4376.
22. Makkar H P S. Quantification of Tannins in Tree Foliage. A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on Use of Nuclear and Related techniques to Develop Simple Tannin Assays for Predicting and Improving the safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage. Joint FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Subprogramme, FAO/IAEA Working Document. IAEA, Vienna, Austria. 2000.
23. Manju S, Srinivasa Gopal, T K, Ravishankar C N. and Jose L. Effects of sodium acetate dip treatment and vacuumpackaging on chemical, microbiological, textural and sensory changes of Pearl spot (Etroplus suratensis) during chill storage. Food Chemistry 2007; 102 (1): 27-32.
24. Negro C, Tommasi L, and Miceli A. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology 2003; 87: 41e44.
25. Ojagh S M, Rezaei M, Razavi S H. and Hosseini S V. Effect of chitosans coating enriched with cinnamon oil on the quality of refrigerated Rainbow Trout. Food chemistry 2010; 120: 193-198.
26. Ozkan G O, Sagdic N G, and Z. Kurumahmutoglu. Antibacterial activities and total phenolic contents of grape pomace extracts. Journal of Food Science and Agriculture 2004; 84: 1807–1811.
27. Ozogul F, Polat A. and Ozogul Y. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (Sardina pilchardus). Food Chemistry 2004; 85: 49–57.
28. Rodriguez M R, Romero Peces R, Chacon Vozmediano J L, Martinez Gascuena J. and Garcia Romero E. Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. Journal of Food Comp 2006; 19: 687-693.
29. Sallam K I, Ishioroshi M. and Samejima K. Antioxidantand

antimicrobial effects of garlic in chicken sausage. Food Science Technology 2004; 37: 849-55.
30. Shittu l A J, Bankole MA , Ahmed T, Bankole M N, Shittu R K, Saalu C L. and Ashiru O A. Antibacterial and antifungal activities of essential oils of crude extracts of Sesamum radiatum against some common pathogenic microorganisms. Iranian Journal of Pharmacology and Therapeutics (IJPT) 2007; 6: 165-170.
31. Shoko T, Soichi T, Megumi M M, Eri F, Jun K. and Michiko W. Isolation and identification of an antibacterial compound from grape and its application to foods. Nippon Nogeikagaku Kaishi 1999; 73: 125-128.
32. Steel R G D and Torrie J H. Principles and Procedures of Statistics, Second Edition, New York: McGraw-Hill. 1980.
33. Suvanich V, Jahncke M L, Marshall D L. Changes in selected chemical quality characteristics of Channel Catfish frame mince during chill and frozen storage. Journal of Food Science 2000; 65 (1), 24-29.
34. Vattem D A, Lin Y T. and Shetty L K. Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bio processing using the food grade fungus Rhizopus oligosporus. Proc. Biochemistry 2004; 39: 1939-1946.
35. Yilmaz Y. and Toledo R T. Major flavonoids in grape seed and skins: antioxidant capacity of catechin, epicatechin and Gallic acid. Journal of Agricultural and Food Chemistry 2004; 52: 255–260.
36. Yin M C. and Cheng W S. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Science 2003; 63: 23–28.