# The Quality Changes of Trout (*Oncorhynchus mykiss* W., 1792) with a Vegetable Topping During Frozen Storage (-18°C)

\*Bahar Tokur<sup>1</sup>, Şükran Çaklı<sup>2</sup>, Abdurrahman Polat<sup>1</sup>

<sup>1</sup>Çukurova University, Faculty of Fisheries, 01330, Balcali, Adana, Turkey <sup>2</sup>Ege University, Faculty of Fisheries, 35100, Bornova, İzmir, Turkey \*E mail: b tokur@vahoo.com

Özet: Sebze sosu ile kaplanmış alabalık (Oncorhynchus mykiss *W.,* 1792)'ın dondurarak depolama (-18°C) boyunca kalitesindeki değişimler. Sebze püresi ile kaplanmış alabalık filetoları (TFVT)' nın besinsel, kimyasal ve duyusal kalitesi üzerine - 18 °C' de dondurarak depolamanın etkisi belirlenmeye çalışılmıştır. Ayrıca kontrol grubu olarak sebze püresi ile kaplanmamış alabalık filetosu (TF)' da değerlendirilmiştir. Depolama sonucunda, TFVT' de methionin ve TF' de histidin amino asidi hariç diğer tüm amino asitler yavaşça azalmıştır. Tiyobarbiturik asit (TBA) değeri (mg malonaldehit/kg) taze iken 0.20 mg malonaldeyhit/kg iken, depolama sonucunda SPAF' de 0.50 mg malonaldeyhit/kg' a TF' de ise 1.30 mg malonaldeyhit/kg' a önemli bir şekilde artmıştır (p<0.05). Toplam uçucu bazik azot (TVB-N) değerinde (mg N/100 g) TFVT' de önemli bir değişim görülmemiştir (p>0.05). TF' de ise, TVB-N değeri (p<0.05) depolamanın 5. ayında önemli bir şekilde artmıştı, daha sonra yavaşça azalmış ve sabit kalmıştır. Myofibriller ve sarkoplazmik protein çözünürlüğü her iki grupta da önemli bir şekilde azlamıştır (p<0.05). Duyusal değerlendirimede; renk koku, tat, doku yapısı ve genel kabul edilebilirlik TFVT' de önemli bir değişim göstermezken tüm bu parametrelerin skorları TF' de azalmıştır. Buna rağmen ne TFVT' de ne de TF' de depolama boyunca panelistler tarafından verilen skorlar kabul edilemez sınırına ulaşmamıştır.

Anahtar Kelimeler: Alabalık, sebze püresi, amino asit, myofibriler protein, sarkoplazmik protein, duyusal değerlendirme, dondurarak depolama.

Abstract: Trout fillets with a vegetable topping (TFVT) were examined to determine the effect of frozen storage at -18 °C on the nutritional, chemical and sensory qualities. Trout fillets without vegetable topping (TF) were also evaluated as a control group. The amounts of amino acids in both groups decreased slightly except for methionine in TFVT and histidine in TF at the end of the storage. The Thiobarbutiric acid (TBA) value (mg malonaldehyde/kg) was 0.20 mg malonaldeyhde/kg as fresh, while it significantly increased to 0.50 in TFVT and to 1.30 in TF at the end of the storage (p<0.05). Total volatile basic nitrogen (TVB-N) value (mg N/100 g) did not show any significant (p>0.05) change in TFVT. In TF, TVB-N value (p<0.05) increased slightly and remained almost constant. The solubility of myofibrillar and the sarcoplasmic proteins significantly decreased in both groups (p<0.05). In terms of sensory evaluation, color, odor, flavor, texture and general acceptability, was scores did not show any significant change in TFVT while the scores for these attributes decreased in TF, but neither TFVT nor TF was scored negatively by the panelists during frozen storage.

Key Words: Trout, vegetable topping, amino acids, myofibrillar protein, sarcoplasmic protein, sensory evaluation, frozen storage.

## Introduction

The important factors affecting the quality of fish and fishery products during frozen storage are known to be the lipid oxidation and protein denaturation. Many studies have shown that the development of lipid oxidation in trout muscle takes place more slowly than in other fatty fish due to the presence of antioxidants such as carotenoids and naturally occurring antioxidant mechanism in a certain time after frozen storage (Andersen et al 1990, Gobantes et al 1998, Han and Liston 1989, Nilsson and Ekstrand 1995, Jensen et al 1998). However, a prolonged time in frozen storage has been reported to cause lipid oxidation (Ingemansson et al 1993, Bjerkeng and Johnsen 1995) and lead to undesirable textural changes in trout muscle (Refsgaard et al 1998). The changes in textural properties of fish muscle could result from aggregation and denaturation of proteins, mainly myofibrillar proteins which cause a decrease in protein solubility during frozen storage (Chang and Regenstein 1997, Saeed and Howell 2004).

The fish sarcoplasmic proteins which are soluble in neutral salt solutions of low ionic strength account for 20-50% of total ordinary muscle proteins (Nakawaka et al 1988) and include many enzymes such as hydrolases, oxidoreductases, transferases, lysas, and isomerases (Haard 1995). Many of these enzymes play vital roles in postharvest fish quality. They cause not only decomposition of fish and fishery products (Haard 1990, Medina et al 1999, Benjakul and Bauer 2000, Fik and Surówka 2004) but are also involved in antioxidant mechanisms (Kishi et al 1991, Han and Liston 1989). For these reasons, the determination of changes in the solubility of sarcoplasmic proteins as much as the solubility of myofibrillar proteins during frozen storage will also be impotant. However, the studies about myofibrillar and sarcoplasmic protein solubility in trout muscle during frozen storage have not been recorded.

The frozen storage of trout in facilitating the distribution and keeping the quality for longer storage periods due to the increase of trout production, is necessary and inevitable. Recently, there has been an increasing demand for new products produced from trout. As ready- to-cook fish dishes, the frozen fillets of Alaska pollock, haddock, hake and hoki with different type of vegetable topping have been greatly sold in European markets. In this study, such products of trout fillet with vegetable topping were evaluated according to their nutritional, chemical and sensory quality changes during twelve months frozen storage (-18°C).

## Materials and Methods

Rainbow trout (Oncorhyncus mykiss, W. 1792), 300 g mean weight and 8 months old, used in this study were supplied by the Freshwater Research Center, Faculty of Fisheries, University of Çukurova. They were gutted and behead, the bones and skin were removed in the laboratory at 15°C temperature. The potatoes, carrot, peas, green pepper, tomatoes, parsley, egg, corn meal, wheat meal, milk, lemon juice and different kinds of spices were used as dressing on trout fillet. The potatoes were boiled for 15 min and the carrots and peas for 20 min to prevent enzymatic discoloration during frozen storage. All vegetable and spices were homogenized in a kitchen blender until a puree was obtained. The approximately 150 g trout fillets were placed in aluminum dishes with a capacity of 200 g and topped with 50 g vegetable mixture. Then, the trout fillet topping with the vegetable mixture (TFVT) and without the vegetable mixture (TF) as a control group were quick frozen at - 40 °C and stored - 18 °C for twelve months. The vegetable mixture topped on the trout fillet was removed before the analyses for the assessment of nutritional, chemical and sensory qualities during frozen storage. The proximate, chemical and sensory analyses were conducted in triplicate and the amino acid was done dublicate.

The crude protein content was determined by Kjeldahl's method (AOAC 1984). Lipids were determined by the method of Bilgh and Dyer (1959). Water content and crude ash were determined in an oven at 103°C and 550°C respectively until the weight became constant. The carbohydrate content was analyzed by the method of Dubois et al (1956).

The sample was hydrolyzed with 6M HCl at 110 °C under a nitrogen atmosphere for 24 h. The amino acids were determined using Eppendorph LC 3000 Amino Acid Analyzer (Eppendorf-Biotronic), according to the standard program (AOAC 1984).

Thiobarbituric acid (TBA, mg malonaldehyde/kg) was determined spectrophotometrically according to the procedure described by Tarladgis et al (1960). Total volatile basic nitrogen (TVB-N, mg N/100 g) was determined according to Antonocopoulos (1973). pH values were determined for the homogeneous mixtures of fish and distillated water (1:10, w:v) using a digital Mettler Toledo pH meter (Santos et al 1981). The extraction of myofibrillar was performed with 0.02 M NaHCO<sub>3</sub> and 5 % NaCl and the extraction of sarcoplasmic proteins was performed with 0.02 M NaHCO<sub>3</sub> and distillated water according to Dyer et al (1950). The protein content of the extract was analyzed by the biuret method (Snow 1950). Bovine serum albumin was used as the standard. The protein solubility in samples was expressed as a percentage.

Sensory evaluation of TFVT and TF was conducted by eight trained persons. The panel rated the fillets on a 1 to 9 Hedonic scale where 1 is equal to "Dislike extremely," 5 is equal to "Neither like nor dislike" and 9 is equal to "Like extremely" for color, odor, flavor, texture, and general acceptability (Paulus et al 1979). Before being presented to the panelist, TFVT and TF were cooked in an oven at 225 °C for 45 min.

Data were analyzed by the general linear model (GLM) by means of the analysis of variance (MANOVA) using the SPSS 10,0. for Windows. Duncan multiple range was used to find the significant differences between storage periods. Independent sample T test was used to determine the differences between TFVT and TF.

### Results

The contents of moisture, crude protein, lipid, crude ash, and carbohydrate of the trout fillet with vegetable topping (TFVP) were found to be 74.64%, 18.47%, 3.48%, 1.23% and 2.4%, while the contents of moisture, crude protein, lipid, crude ash, and carbohydrate of trout fillets (TF) were found to be 73.08%, 22.96%, 2.71%, 1.23%, and 0,568, respectively.

The amino acids composition was used for the determination of the nutritional qualities of trout fillet with vegetable topping (TFVT) and fillet (TF) during frozen storage (Table 1).

Table1. The amino acids composition of TFVT and TF during frozen storage at -18°C (g amino acids/16 g N)

A	İnitial	4 <sup>th</sup> m	onth	12 <sup>th</sup> month		
Amino Acids	TF	TFVT	TF	TFVT	TF	
Aspartic acids	9.31	8.55	8.63	8.59	7.93	
Threonine*	4.09	3.78	3.82	3.73	3.57	
Serine	5.22	4.95	4.63	4.77	4.19	
Glutamic Acid	8.02	7.97	7.56	7.66	6.79	
Proline	3.41	3.89	2.98	3.26	2.74	
Glycine	4.23	4.40	4.15	3.97	3.50	
Alanine	3.51	3.47	3.31	3.36	2.94	
Valine*	3.90	4.02	3.84	3.75	3.52	
Methionine*	2.43	2.65	2.47	2.59	2.27	
Isoleucine*	3.27	3.25	3.06	3.15	2.90	
Leucine*	6.50	6.32	6.01	6.20	5.56	
Tyrosine	2.15	2.24	2.03	2.02	1.88	
Phenylalanine*	3.57	3.61	3.60	3.56	3.26	
Histidine	2.03	1.85	1.70	2.00	2.07	
Lysine*	8.03	7.31	7.54	7.72	6.94	
Arginine	5.06	4.70	4.77	4.73	4.15	
E/NE	0.79	0.74	0.76	0.76	0.77	

\*Essential amino acid for human; Tryptophan was not determined

The main amino acids in trout fillet were found to be aspartic acid, glutamic acid, leucine and lysine. The amount of

amino acids decreased slightly in both TFVT and TFt except for methionine in TFVT and histidine in TF at the end of the storage periods (-18°C) and the decrease in amino acids content in TF was higher than those in TFVT. The E/NE ratio of trout muscle was found to be 0.79 at the beginning of storage, while this value slightly decreased to 0.76 in TFVT and 0.77 in TF at the end of storage.

The effect of vegetable topping on trout fillet TVB-N, TBA, pH, myofibrillar and sarcoplasmic protein contents during frozen storage are presented in Table2.

Table2. TBA (mg malonaldehyde/kg), TVB-N (mg N/100 g), pH, and the solubility of myofibrillar and sarcoplasmic protein (%) in TFVT and TF during frozen storage (-18°C)<sup>1,2,3</sup>

	TBA		TVB-N		рН		Myofibrillar protein (%)		Sarcoplasmic protein (%)	
	(mg malonaldehyde/kg)		(mg N/100 g)							
Months	TFVT*	TF	TFVT*	TF	TFVT*	TF	TFVT*	TF	TFVT*	TF
Fresh	$0.20 \pm 0.008^{aef}$	0.20±0.008ª	11.67±0.66ª	11.67±0.66ª	6.70±0.01ª	6.70±0.01ª	100ª	100ª	100ª	100ª
1	0.17±0.003b	0.18±0.007 ª	1189±0.51ª	13.39±0.48 <sup>b</sup>	6.68±0.02 <sup>b</sup>	6.55±0.01 <sup>b</sup>	99.67±0.71ª	94.73±2.69 <sup>b</sup>	162.84±2.22 <sup>b</sup>	174.01±0.41 <sup>b</sup>
2	-	-	11,16±0.04ª	14.62±0.57 <sup>b</sup>	$6.57 \pm 0.02^{cd}$	6.51±0.01°	98.75±0.87ª	76.89±5.29°	-	109.02±2.29°
3	0.16±0.009cb	0.46±0.024b	-	-	6.59±0.01°	6.51±0.01 <sup>cd</sup>	96.05±0.05b	96.20±1.99 <sup>ba</sup>	101.68±0.56ca	102.93±0.67d
4	0.12±0.006d	0.31±0.005°	11.18±0.01ª	11.14±0.05ª	6.47±0.01d	6.48±0.00 <sup>cd</sup>	105.60±0.53℃	82.69±2.64d	100.47±2.64ca	107.73±1.59°
5	0.10±0.003e	0.49±0.016b	16.63±0.07b	20.85±1.07°	6.46±0.00d	6.51±0.01 <sup>cd</sup>	104.25±1.20℃	83.79±1.20 <sup>dh</sup>	81.79±2.80d	93.91±3.63e
6	0.14±0.003 <sup>cd</sup>	$0.77 \pm 0.022^{df}$	14.43±0.77°	18.02±0.57de	6.47±0.00 <sup>d</sup>	6.52±0.02 <sup>cd</sup>	94.10±0.51d	90.46±0.46e	96.56±3.40ae	30.99±1.28 <sup>f</sup>
7	0.13±0.008d	0.82±0.118d	11.69±0.31ª	18.27±0.44 <sup>de</sup>	6.48±0.01d	6.51±0.00 <sup>cd</sup>	83.48±0.03e	81.26±1.85d	82.96±5.96d	93.21±1.83e
8	0.18±0.022 <sup>ef</sup>	0.64±0.021e	11.15±0.38ª	17.78±0.21ef	6.46±0.01d	6.50±0.01 <sup>cd</sup>	84.82±2.04e	52.3±0.12 <sup>f</sup>	94.32±0.91°	83.08±1.83 <sup>g</sup>
9	0.20±0.046e	0.66±0.035e	9.53±0.65d	15.59±0.32 <sup>g</sup>	6.47±0.01de	6.47±0.02 <sup>d</sup>	69.20±1.87 <sup>f</sup>	64.12±2.32 <sup>g</sup>	94.09±3.20e	95.39±82.65e
10	0.17±0.013 <sup>fbc</sup>	0.72±0.036 <sup>f</sup>	11.47±0.45ª	16.72±0.70eg	6.48±0.02 <sup>def</sup>	$6.41{\pm}0.03^{df}$	72.10±1.639	86.45±0.75 <sup>th</sup>	56.28±6.70 <sup>f</sup>	79.71±8.95 <sup>h</sup>
11	0.18±0.009 <sup>f</sup>	0.94±0.0219	11.05±0.50ª	17.54±1.07º	6.47±0.00 <sup>fg</sup>	6.33±0.01ef	71.10±1.019	47.95±4.10 <sup>i</sup>	87.07±0.519	88.23±3.02 <sup>i</sup>
12	0.50±0.015 <sup>fg</sup>	1.30±0.095 <sup>h</sup>	11.06±0.45ª	17.87±0.48e	6.48±0.019	6.28±0.03e	66.04±2.96 <sup>h</sup>	52.12±0.71 <sup>j</sup>	59.31±1.44 <sup>f</sup>	79.80±09 <sup>ij</sup>
				4						

<sup>1</sup>Data are expressed as mean ±Standard Deviation (n=4<sup>a</sup>, n=2<sup>b</sup>); <sup>2</sup>Means within the same row showing with\* are statistically different at p<0.05; <sup>3</sup>The means within the same column having different superscript are significantly different at p<0.05 during frozen storage

The increase in the TBA (mg malonaldehyde /kg) values of both TFVT and TF were found to be significant during the time of frozen storage (p<0.05). The TBA value was 0.20 mg malonaldehyde /kg at the beginning of the storage and increased to 0.50 in TFVT and 1.30 in TF at the end of storage. This increase in TBA value of TF during the time of storage was significantly higher than the value of TFVT (p<0.05).

The change in the TVB-N value of TFVT was not found to be significant (p>0.05) during the time of frozen storage, while it was found to be significant in TF, and the TVB-N value of TF increased significantly from 11.67 mg N/100 g to 17.87 mg N/100 g (p<0.05) at the end of the storage time. The topping of trout fillet with the vegetable mixture significantly affected the TVB-N value of trout fillet (p<0.05).

The pH value of both TFVT and TF decreased significantly during frozen storage (p<0.05). The decrease in the pH of TFVT was found to be significantly lower than that of TF (p<0.05).

The solubility of myofibrillar proteins both in TFVT and TF was found to decrease throughout the frozen storage (p<0.05). At the end of the storage period, the solubility of myofibrillar proteins was found to be 66.06 % in TFVT and 52.12 % in TF. The topping of trout fillet with vegetable mixture did significantly increase the solubility of myofibrillar protein (p<0.05).

The solubility of sarcoplasmic proteins in TF significantly decreased to 59.31 % for TFVT and to 79.80 % for TF at the end of the storage period. The topping of trout fillet with vegetable mixture was found to be significantly reduce the solubility of the sarcoplacmic proteins (p<0.05).

The color, odor, flavor, texture and general acceptability

were used for the assessment of sensory quality in TFVT and TF.

The sensory scores of TFVT did not change significantly throughout the twelve months storage (p>0.05), while the sensory scores of TF did decline (p<0.05). At the end of the storage period, the scores of odor, flavor, texture and general acceptability were found to be 8.0, 8.0, 8.40, 7.80 and 8.40 for TFVT, and 5.40, 5.20, 5.40, 4.60, and 5.20 for TF, respectively. The topping of trout fillet with vegetable mixture was found to effect the sensory scores (p<0.05).

Around the end of the storage period, although no rancid flavor was estimated in trout fillet in TFVT, a slight rancid flavor in the vegetable mixture was recognized by the panelists. A slight rancid, fishy and metal like flavor was characterized in at the 6<sup>th</sup> months after frozen storage. However, the scores did not exceed acceptable levels.

The loss of moisture and sticky texture in TFVT was identified around the end of the storage periods, while the loss of moisture in TF was estimated after 6<sup>th</sup> months frozen storage. In addition, a more sticky structure was recognized in TF. But the sensory scores in both TFVT and TF did not exceed acceptable levels.

#### Discussion

The results of proximate composition in trout fillet are supported by Weatherup and McCracken (1999) and Gokoglu et al (2004) with a little difference. The proximate composition of rainbow trout fillets can be influenced by changes in feeding regime, diet composition, weight, sexual maturity and starvation (Weatherup and McCracken 1999, Rasmussen 2001).

The findings in the present study for the main amino acids were supported by Iwasaki and Harada (1985), Farmanfarmaian and Sun (1999) and Beklevik et al (2005). A decrease in the amount of many amino acids of fish muscle during frozen storage was also observed by Beklevik et al (2005) who studied the changes in amino acid composition in sea bass fillets during nine months frozen storage (-18 C). Similarly, Castrillón et al (1999) observed the greatest decline in the amount of S-amino acids followed by histidine, tyrosine, leucine, lysine and phenylalanine in sardine (Clupea pilchardus) throughough storage at -20°C. However, Wesselinova (2000) reported that the essential amino acids of scad, mackerel, rock cod, sea bream and belted bonito filleted remained unchanged and slight deviation were obtained in the other amino acids during 12 months at -35°C. In the present study, a decrease in the amino acid content in TF was higher than that in TFVT. According to Jiang and lee (1985), the higher free amino acids in fish muscle could be caused by higher protein denaturation during frozen storage (-20 °C). Similar explanations were also reported by Ruiz-Capillas and Moral (2001) and García-Arias et al (2003). These investigations could describe why the lower myofibrillar protein solubility in TF rather than those in TFVT had the lower amino acids content in TF than those in TFVT at the end of frozen storage in the present study. Although decreases were found in the amino acid contents and in the ratio of E/NE in both two groups, they were still relatively close to the values given for amino acid requirements for humans (Jhaveri et al 1984, Mclarney et al 1996, Farmanfarmaian and Sun 1999).

Carotenoid is known to be a powerful guencher of singlet oxygen activity and a strong scavenger of oxygen free radicals could be protecting trout lipid tissue from peroxidation (Burton and Ingold 1984, Frankel 1991, Nilsson and Ekstrand 1995). In addition to carotenoid, two types of protection factors against lipid peroxidation in trout white muscle cytocol were also observed (Han and Liston 1989). For this reason, the TBA value (mg malonaldehyde/kg) measuring the oxidative stability of muscle was estimated to be develop very slowly in trout (Bjerkeng and Johnsen 1995, Andersen et al 1990, Clark et al 1999, Nilsson and Ekstrand 1995). Similarly, TBA value of TFVT and TF in the present study displayed a slight increase. However, the range of TBA value of TF increased faster than TFVT. These results indicated that the topping of trout fillet with vegetable mixture may be protecting the lipid oxidation because of preventing it to be exposed to air and light of fillets.

The TVB-N value is widely used as an initial freshness indicator of fish and fishery products. However, many studies showed that the TVB-N value for fish and fishery products was not stable during frozen storage and could be changed according to species, processing methods, and storage temperature (Rehbein and Oehlenschlager 1982, Ben-gigirey et al 1999, Suvanich et al 2000, Tokur et al 2004). For these reasons, TVB-N value might not be a good quality indicator for frozen fish. The TVB-N (mg N/100 g) value did not show any significant (p>0.05) change in TFVT but it was found at a

higher value (p<0.05) at 5<sup>th</sup> and 6<sup>th</sup> months than others. In TF, TVB-N value significantly (p<0.05) increased by the 5<sup>th</sup> month of storage then slightly decreased and almost remained at a constant level. These data showed that the topping of trout fillet with vegetable mixture prevent rise of TVBN-N value in trout fillet.

A decrease in pH of both TFVT and TF was observed. However, an increase in pH value of fish was observed in many studies during frozen storage (Vareltzis et al 1997, Simeonidou 1997, Tokur et al 2004). The pH value of the fish in the stage of rigor mortis and post mortem has been reported ranging between 6.2 and 6.9 due to the production of lactic acids depending on species, age, catching season, sex and feeding status (Love 1979, Rustad 1992, Ingolfsdottir 1996). The findings in our study on pH value might be causing the production of lactic acids in trout muscle.

A reduction in the solubility of proteins extracted in 5% NaCl was reported by many researchers during the frozen storage (Jiang and Lee 1985, Tejeda et al 1996, Del Mazo et al 1997). Similarly, a decrease in the solubility of myofibrillar proteins extracted in 5% NaCl was found in TFVT and TF throughout the frozen storage. However, the reduction of solubility did not change regularly. These fluctuations during frozen storage could be caused by interactions of proteins with other compounds such as fatty acids, decomposition products of lipids and amine compounds (Saeed and Howell 2002, Siddaiah et al 2001, Careche et al 1999). A gradual decrease was observed in the myofibrillar protein solubility in TFVT throughout frozen storage, while a sharp decrease was observed in the myofibrillar protein solubility in TF at 10th month after storage. These data indicate that the topping of trout fillet with vegetable mixture might be preventing against protein denaturation and aggregations during the frozen storage.

The studies about the changes in sarcoplasmic proteins during the frozen storage have been limited (Awad et al 1969, Yowell and Flurkey 1986, LeBlanc and LeBlanc 1989). LeBlanc and LeBlanc (1989) investigated the sarcoplasmic proteins of cod fillets (Gadus morhua) using electrophoresis and HPLC on four different storage temperatures (-30 °C, -22 °C, -15 °C and -12 °C). At the end of the study, they concluded that sarcoplasmic proteins undergo protein insolubilizations during frozen storage, especially during frozen storage at -12 °C. Sikorski et al (1975) reported that the solubility of sarcoplasmic proteins in mince codfish with added formaldehyde declined after 7 days at -20 °C. Similarly, Verma et al (1995) found a decrease in the solubility of water soluble protein in oil sardine mince (Sardinella longiceps) during frozen storage. Similar results have been observed by Namulema et al (1999) in Nile perch (Lates niloticus) stored at -13 and -27 °C. Similarly, a decline in the protein solubility of TFVT and TF was observed in the present study at the end of the twelve months frozen storage. However, the solubility of sarcoplasmic proteins increased up to 4 months of storage. Hashimoto et al (1979) studied sarcoplasmic proteins in sardine (Sardinops melanosticta) white and dark muscle in

stages of pre-rigor, rigor, post-rigor and frozen. They reported that the sarcoplasmic protein solubility in dark muscle of sardine increased to 37% after two week at - 80 °C. They noted that the reason of these increase in the solubility of sarcoplasmic proteins was the utilization sarcoplasmic proteins during decomposition of myofibrillar proteins such as myosin and troponin. In the present study, a higher solubility in sarcoplasmic proteins in TF than those of TFVT was found.

At the end of the twelve month frozen storage periods, the color of TFVT was observed to be similar to the initial quality. But, a slight turn into yellow was observed in the vegetable mixture on the fillet. The reason of this might have been the oxidation of chlorophyll pigment in peas (Groeschel et al 1966, Gökmen et al 2005). In TF, some dark regions on the fillet surface were observed after 6 months storage periods. The levels of dark region of filet surface of TF attracted special attention during nine months of storage. It is well known that the reason of this dark region on the surface of filet is the loss of moisture in these regions of fillets.

Decomposition products from lipid, protein and non protein nitrogen compounds can cause undesirable changes in flavor (Girard and Nakai 1991, Refsgaard 1998, Jo and Ahn 1999, Girard and Durance 2000). The formation of aldehydes and ketones has been reported to cause rancid off-flavors, and can affect the sensory attributes even in a small amount (Reffsgaard et al 1998, Undeland and Lingnert 1999). Rancidity measured by TBA in the present study was supported with flavor in both TFVT and TF.

After the long time of frozen storage, the fish muscle lost its natural fibril, firmness and juicy structure due to denaturation of myofibrillar proteins (Shenouda 1980, Haard 1990, De Koning and Mol 1991, Haard 1992, Mackie 1993, Careche and Li-Chan 1997). In this study, the protein denaturation was supported by the textural changes in TF but this denaturation was not observed in sensory assessment in TFVT although a decline was estimated in the protein solubility. The results of these data indicate that the solubility of protein should not be used alone for the assessment of texture quality of trout fillet.

The results of this study, the topping of trout fillet with vegetable mixture was found to have a significant effect on chemical quality parameters and lessen the loss of chemical and nutritional quality of trout fillets during frozen storage. With respect to sensory quality, the quality of TFVT remained fairly close to the initial fresh quality throughout the frozen storage. This finding indicated that the production at such a product from the trout fillet can improve its nutritional, chemical and sensory quality during frozen storage and prove a much longer frozen storage period than the trout stored as an only fillet.

#### References

- A.O.A.C. 1984. Official Methods of Analysis 14 th Ed. Association of Official Analytical Chemists. Washington. DC. USA.
- Andersen, H.J., G. Bertelsen, A.G. Christophersen, A.Ohlen, and L.H. Skibsted. 1990. Development of rancidity in salmonid steaks during retail display. Z. Lebensm. Unters. Forsch, 191:119-122.

- Antonocopoulus, N. 1973. Bestmmung des flüchtigen basenstickstoofs, pp. 224-225. In W Ludorf. and V. Meyer (eds.), Fische und Fischerzeugnisse. AulageVerlag Paul Parey.
- Awad, A., W.D. Powrie, and O.R. Fennema. 1969. Deterioration of fresh-water whitefish muscle during frozen storage at −10 °C. J. Food Sci., 34:1.
- Beklevik, G., A. Polat, and F. Ozoğul. 2005. Nutritional value of sea bass (*Dicentrarchus labrax*) fillets during frozen (-18 °C) storage. Turkish Journal of Veterinary & Animal Sciences, 29:891-895.
- Ben-gigirey, B., J.M.V.B. Sousa, T.G. Villa, J. Barros-velazquez. 1999. Chemical changes and visual appearance of albacore tuna as related to frozen storage. J. Food Sci., 64(1):20-24.
- Benjakul, S., and F. Bauer. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. J. Sci. Food Agric., 80:1143-1150.
- Bjerkeng, B., and G. Johnsen. 1995. Frozen storage quality of rainbow trout (Oncorhynchus mykiss) as affected by oxygen, illumination, and fillet pigment. J. Food Sci., 60(2): 284-288.
- Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can.J. Bochem. Physiol., 37: 911-917
- Burton, G.W., and K.U. Ingold. 1984. β-carotene: an unusual type of lipid antioxidant. Science, 224: 569-573.
- Careche, M., A.M. Herrero, A. Rodriguez-Casado, M.L.D. Del Mazo, and P. Carmona. 1999. Structural changes of hake (*Merluccius merluccius* L..) fillets: Effect of freezing and frozen storage. J. Agric. Food Chem. 47:952-959
- Careche, M., and E.C.Y. Li-Chan. 1997. Structural changes in cod myosin after modification with formaldehyde or frozen storage. J. Food Sci., 62: 717-723.
- Castrillón, A.M., E. Alvarez-Pontes, M.T. Garcia Arias, and P. Navarro. 1995. Influence of frozen storage and defrosting on the chemical and nutritional quality of sardine (*Clupea pilchardus*). J. Sci Food and Agric., 70(1):29-34.
- Chang, C.C., and J.M. Regenstein. 1997. Textural changes and functional properties of cod mince proteins as affected by kidney tissue and cryoprotectants. J Food Sci 62:299-304.
- Clark, T. H., C. Faustman, W.K.M. Chan, H.C. Furr, and J.W. Reisen. 1999. Canthaxanthin as an antioxidant in a liposome model system and in minced patties from rainbow trout. J. Food Sci., 64 (6): 982-986.
- De Koning, A.J., and T.H. Mol. 1991. Quantitative quality test for frozen fish : soluble protein and free fatty acid content as quality criteria for hake (Merluccius capensis) stored at - 18 °C. J. Sci. Food Agric., 54: 440-58.
- Del Mazo, M., P. Torrejon, M. Careche, M. Tejeda. 1997. Characteristics of the salt soluble fraction of hake fillets stored at -20 and -30 ℃. Presented at the 27<sup>th</sup> Western European Fish Technologists' Association (WEFTA) Meeting, Madrid, Spain.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and selected substances. Anal. Chem., 28: 350-356.
- Dyer, W.J., H.V. Fench, and J.M. Snow. 1950. Proteins in fish muscle. 1. Extraction of protein fraction in fresh fish. J. Fish Res. Bd. Can. 7:585
- Farmanfarmaian, A., and L.Z. Sun. 1999. Growth hormone effects on the essential amino acid absorption, muscle amino acid profile, n-retention and nutritional requirements of striped bass hybrids. Genetic Analyses: Bimolecular Engineering, 15, 107-113.
- Fik, M., and K. Surówka. 2004. Autoproteolysis rate of rainbow trout muscle proteins. Nahrung, 48(2): 104-109.
- Frankel, E.N. 1991. Recent advances in lipid oxidation. J. Sci. Food Agric., 54:495-511.
- García-Arias, M.T., E. Alvarez-Pontes, M.C. García-Fernández, F.J Sánchez-Muniz. 2003. Freezing/defrosting/frying of sardine fillets. Influence of slow and quick defrosting on protein quality. J. Sci. Food Agric., 83(6):602-608.
- Girard, B., and T. Durance. 2000. Headspace volatiles of sockeye and pink salmon as affected by retort process. J. of Food Sci. 65(1):34-39.
- Girard, B., and S. Nakai. 1991. Static headspace gas chromatographic method for volatiles in canned salmon. J. of Food Sci., 56(5):1271-1274
- Gobantes, I. I, G. Choubert, J.C. Milicua, and R. Gomez. 1998. Serum carotenoid concentration changes during sexual maturation in farmed rainbow trout (*Oncorhynchus mykiss*). J Agric Food Chem., 46(2):383-387
- Gökmen, V., K.S. Bahçeci, A. Serpen, and J. Acar. 2005. Study of lipoxygenase and peroxidase as blanching indicator enzymes in peas: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. LWT-Food

Science and Technology, 66(2): 187-192.

- Gokoglu N., P.Yerlikaya, and E. Cengiz. 2004. Effects of cooking methods on the proximate composition and mineral content of rainbow trout (Onchorhynchus mykiss), Food Chemistry, 84, 19-22.
- Groeschel, E.C., A.I. Nelson, and M.P. Steinberg. 1966. Changes in color and other characteristics of green beans stored in controlled refrigerated atmospheres. J. Food Sci., 31:488.
- Haard, N.F. 1990. Biochemical reactions in fish muscle during frozen storage, pp 176-209. In: E. G. Bligh (Eds.), Seafood Science and Technology. Fishing News Books.
- Haard, N.F. 1995. Composition and nutritive value of fish proteins and other nitrogen compounds, pp 77-116. In: E. Ruiter, Fish and Fishery Products. CAB International.
- Cab Int., T.J. Han, and J.Liston. 1989. Lipid peroxidation protection factors in rainbow trout (*Salmo gairdnerii*) muscle cytosol. J. Food Sci., 54(4):809-813.
- Hashimoto, K., S.Watabe, M. Kono, and K. Shiro. 1979. Muscle protein composition of sardine and mackerel. Bulletin of the Japanese Soci. of Sci. Fisheries, 45(11): 1435-1441.
- Ingemansson, T., A. Petterson, P. Kaufmann. 1993. Lipid hydrolysis and oxidation related to astaxanthin content in light and dark muscle of frozen stored rainbow trout (Oncorhynchus mykiss). J. Food Sci., 58:513-518.
- Ingolfsdottir, S., G. Stefansson, and K. Kristbergsson. 1996. Seasonal variations in physicochemical and textural properties of north Atlantic cod Gadus morhua mince.J. of Aquatic Food Technology, 7(3):39-61.
- Iwasaki, M, and R. Harada. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. J. of Food Sci., 50: 1585-1587.
- Jensen, C., E. Birk, A. Jokumsen, L.H. Skibsted, and G. Bertelsen. 1998. Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. Z. Lebensm Unters Forsch A, 207:189-196.
- Jhaveri,S.N., P.A. Karakoltsidis, J. Montecalvo, and S.M Constantinides. 1984. Chemical composition and protein quality of some southern New England marine species. J. of Food Sci., 49:110-113.
- Jiang, S., and T. Lee. 1985. Changes in free amino acids and protein denaturation of fish muscle during frozen storage. J. Agric. Food Chem., 33:839-844.
- Jo, C., and D.U. Ahn. 1999. Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/ gas chromatography. J. of Food Sci. 64(4):641-643
- Kishi, H., H. Nozawa, and N. Seki. 1991. Reactivity of muscle transglutaminase on carp myofibrils and myosin B. Bulletin of Japanese Society of Scientific Fisheries, 57: 1203-1210.
- LeBlanc, E.L., and R. J. LeBlanc. 1989. Separation of cod (*Gadus morhua*) fillet proteins by electrophoresis and HPLC after various frozen storage treatments. J. Food Sci., 54: 827-834.
- Love, R.M. 1979. The Post-mortem pH of cod and haddock and its seasonal variation. J. Sci. Food Agric., 30:433-438
- Ludorf, W., V. Meyer, 1973. Fische und Fischerzeugnisse. Paul Parey Verlag. Mackie, I.M. 1993. The effects of freezing on flesh proteins. Food Rev. Int.,
- 9:575-610. Mclamey, M., P.L. Pellett, and V.R. Young. 1996. Pattern of amino acid requirements in human: An interspecies comparison using published amino
- acid requiremt recommendation. The Journal of Nutrition, Bethesda, 1871-1883. Medina, I., S. Saeed, and N. Howell. 1999. Enzymatic oxidative activity in
- sardine (Sardine pilchardus) and herring (Clupea harengus) during chilling and correlation with quality. European Food Research and Technology. 210(1):34-38.
- Nakawaka, T., S. Watabe, and K. Hashimoto. 1988. Electrophoretic analysis of sarcplasmic proteins from fish muscle on polyacrylamide gels. Nippon Suisan Gakkaishi, 54(6): 993-998.
- Namulema, A., J.H. Muyonga, and A.N. Kaaya. 1999. Quality deteriotion in frozen Nile perch (*Lates niloticus*) stored at –13 °C and – 27 °C. Food Research Int., 32:151-156
- Nilsson, K., and B. Ekstrand. 1995. Frozen storage and thawing methods affect biochemical and sensory attributes of rainbow trout. J. Food Sci., 60(3): 627-630.
- Paulus, K., R. Zacharias, L. Robinson, and H. Geidel. 1979. Kritische

betrachtungen zur "Bewetenden prüfung mit skale" als Einem Wesentlichen Verfahren der Sensorichen Analyse. LWT, 12(1): 52-61.

- Rasmussen, R.S. 2001. Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. Aquaculture Research, 32:767-786.
- Refsgaard, H.H.F., P.B. Brockhoff, and B. Jensen. 1998. Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar*) during frozen storage. J. Agric. Food Chem., 46:3473-3479
- Rehbein, H., and J. Oehlenschlager. 1982. Zur zusammensetzung der tvb-n fraktion (flüchtige basen) in sauren extrakten und alkalischen destillaten von seefischfilet. Archiv Für Lebensmittelhygiene, 33:44-48.
- Ruiz-Capillas, C., A. Moral. 2001. Changes in free amino acids during chilled storage of hake (*Merluccius merluccius* L.) in controlled atmospheres and their use as a quality control index. Eur Food Res Technol., 212: 302-307.
- Rustad, T. 1992. Muscle chemistry and the quality of wild and farmed cod, pp. 19-27 In: H.H. Huss, M. Jakobsen and J. Liston (Eds) Quality Assurance in the Fish Industry. Elsevier Sci. Publ. B.V.
- Saeed, S., and N.K. Howell. 2002. Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus*). J. Sci. Food Agric., 82:579-586.
- Saeed, S., N.K. Howell. 2004. Rheological and differential scanning calorimetry studies on structural and textural changes in frozen Atlantic mackerel (Scomber scombrus) J.Sci. Food Agric., 84:1216-1222.
- Santos, C.L.D., D. James, F. Teutscher. 1981. Guidelines for chilled fish storage experiments. FAO. Fish Tech. Paper 210s.
- Shenouda, S.Y.K. 1980. Theories of protein denaturation during frozen storage of fish flesh. Adv. Food Res., 26: 275-311.
- Siddaiah, D., G.V.S. Reddy, and T.C. Chandrasekhar. 2001. Changes in lipids, proteins and kamaboko forming ability of silver carp (*Hypophthalmichthys molitrix*) mince during frozen storage. Food Research International 34:47-53
- Sikorski, Z.E., S. Kostuch, and I. Kolosziejska. 1975. Protein denaturation in frozen fish. Nahrung, 19(9-10):997-1003.
- Simeonidou, S., A. Govaris, and K. Vareltzis. 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake (*Merluccius mediterraneus*). Z. Lebensm Unters Forsch A., 204:405-410.
- Snow, J.M. 1950. Proteins in fish muscle. ii. Colorimetric estimation of fish muscle protein J. Fish Res. B.D. Can. 7:10
- Suvanich, V., M.L. Jahncke, and D.L.Marshall. 2000. Changes in selected chemical quality characteristics of channel catfish frame mince during chill and frozen storage. Journal of Food Sci., 65(1):24-29
- Tarladgis B., B.M. Watts, and M. Yonathan. 1960. Distillation method for determination of malonaldehyde in rancid food, J. Of American Oil Chemistry Society, 37 (1), 44-48.
- Tejeda, M., M. Careche, P. Torrejon, M.L. Del Mazo, M.T. Solas, M.L. Garcia, and C. Barba. 1996. Protein extracts and aggregates forming in minced cod (Gadus morhua) during frozen storage. J. Agric. Food Chem. 44:3308-3314
- Tokur, B., A. Polat, G. Beklevik, S.Özkütük. 2004. The quality changes of tilapia (Oreochromis niloticus) burger during frozen storage. European Food Research and Technology 218 (5): 420-423
- Undeland, I., and H.Lingnert. 1999. Lipid oxidation in fillets of herring (*Clupea harengus*) during frozen storage. influence of prefreezing storage. J. Agric. Food Chem., 47: 2075-2081.
- Vareltzis, K., D. Koufidis, E. Gavriilidou, E. Papavergou, and S. Vasiliadou. 1997. Effectiveness of a natural Rosemary (*Rosmarinus officinalis*) extract on the stability of filleted and minced fish during frozen storage. Z. Lebensm Unters Forsch A., 205: 93-96.
- Verma, J.K., L.N. Srikar, N.S. Sudhakara, and J. Sarma. 1995. Effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine (*Sardinella longiceps*) mince. Food Res. Int., 28(1):87-90.
- Weatherup, R.N., and K.J. McCracken. 1999. Changes in rainbow trout, Onchorhynchus mykiss (Walbaum), body composition with weight. Aquaculture Research, 30:305-307.
- Wesselinova, D. 2000. Amino acid composition of fish meat after different frozen storage periods. J. Aquatic Food Prod. Tech., 9(4): 41-48
- Yowell, K., W.H. Flurkey. 1986. Effect of freezing and microwave heating on proteins from cod fish fillets. Analysis by SDS polyacrylamide gel electrophoresis. J.Food Sci., 51:508.