Chemical and microbiological changes of salted Caspian Kutum (*Rutilus frisii kutum*) roe

Pourashouri P.[1*]; Yeganeh S.[2]; Shabanpour B.[1]

Received: September 2013 Accepted: February 2014

Abstract

Salted Kutum *roe* was prepared by soaking in brine to a traditional procedure. Proximate composition (moisture, protein, lipid, pH, ash and salt content), total volatile basic nitrogen (TVB-N), fatty acid profiles and microbiological characteristics of raw and salted roe of Caspian white fish (*Rutilus frisii kutum*) were determined. The results indicated that protein and moisture contents were significantly higher in raw roe compared to salted one (28.81 and 61.07% to 23.99 and 51.57%, respectively). Significant differences in ash and salt contents were obtained among different treatments. TVB-N in all samples was less than 25mg/100g. The gas chromatography (GC) analysis showed that the major saturated fatty, monounsaturated fatty acids and poly unsaturated fatty acids were 16:0, 18:1n-9, and 22:6n-3, respectively. Aerobic plate count (APC) and Total Coliform Count (TC) in all samples were significantly higher (p<0.05) in raw roe (5.33 logCFU/g and 210 MPN/g) compared to salted roe (1.23 log CFU/g and 1.2 MPN/g). None of the samples contained *Escherichia coli*, *Salmonella*, *Clostridium perfringens* or yeast.

Keywords: *Rutilus frisii kutum*, Roe, Salting, Microbial population

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

1-Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

2-Faculty of Animal Sciences and Fisheries, Department of Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran. P.O.Box 578.

*Corresponding author's email: Pourashouri.p@gmail.com

Introduction

Roe of many fishes are consumed as food worldwide, either as a whole sac or as loose eggs (Scano *et al*., 2009). Roe, as referred to the oocytes when they are gathered in skeins, and incorrectly named as eggs, the non-fertilized oocytes are processed as a by-product collected from the female fish captured before spawning or during the slaughtering of farmed fish (Mahmoud *et al*., 2008). Caviar, ikura and tobikoare the well-known roe products in some countries. Salted-dried (karasumi) and salted–fermented (karashi mentaiko) are the most popular form in many middle and far eastern countries and a few European countries (e.g. Greece, Spain, Italy, and Norway) (Bledsoe *et al*., 2003; Bekhit *et al*., 2008). Recently, these products have grown in the world and currently enjoy expanding international and domestic markets (Mahmoud *et al*., 2008).

Many procedures are employed to process and preserve this highly perishable foodstuff rich in health-beneficial n-3 polyunsaturated fatty acids (PUFA) (Scano *et al*., 2009). Salting and drying are two methods used by many ancient civilizations for food preservation, while nowadays they are used not only to preserve food but also to give them a special flavor (Rodrigo *et al*., 1998; Katselis *et al*., 2005; Gallart-Jornet *et al*., 2007; Bekhit *et al*., 2008;)

The potential health benefits related to fish and fish-derived products are due to the presence of nutrients such as proteins, minerals, vitamins, and n-3 polyunsaturated fatty acids (n-3 PUFA) (Bledsoe *et al*., 2003; Rosa *et al*., 2009), thatwhich are important for human health (Rosa *et al*., 2009). Furthermore, fish roe has a high percentage of phosphatidylcholine (PC) that is effective for the improvement of learning ability and the lowering of plasma lipids (Shirai *et al*., 2006).

The Kutum (*R. frisii kutum*) (also known as Caspian White Fish or Mahi-Sefid in Persian) is the most popular and highly valued fish among the Iranian seafood products particularly in the Guilan and Mazandaran provinces in north of Iran. Several types of roe called as Ashpal or Ashbal, are used inthese areas, which are consumed as grilled, cured, salted, or mixed with other ingredients. Carp roe is less common and barbel roe is also occasionally used. In this study we investigated the proximate composition and effects of salting on chemical and microbiological characteristics of Kutum roe.

Materials and Methods

All analytical grades of chemicals and reagents were purchased from Merck (Darmstadt, Germany) or Sigma (St Louis, MO). Kutum was caught in the southwestern coast of Caspian Sea (Anzali port) by beach seine. The average weight of fish specimens and ovaries were 1800±20g and 185±5g respectively. All ovaries were in final stage of maturity. Dried Rose madder (*Rubia tinctorum*) plant, whose root is a source of red dye, used in food processing, was purchased from the local shops.

Preparation of salted roes

Roes were collected from 24 freshly killed fishes. The whole ovaries were divided in two parts; the first part was packed in aseptic bags, placed in ice (ice/ roe ratio 2/1) and immediately trandferred to the laboratory within 1h for further analyzes. The other

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

parts, were used for salting; salted roes were prepared according to the traditional recipe. In this method, a mixture of 70g salt and 5 g madder for each sample were prepared and covered each ovary wrapped in paper and completely immersed in brine saturated with salt The experiments were carried out in triplicate and samples were stored at 20°C for 45 days.

Chemical composition

Moisture, protein and lipid contents

The moisture content of roe samples was determined by drying samples to constant weight at 102-105˚C for 20 to 24 h according to the AOAC standard method (AOAC, 1995). The crude protein content of roe samples was measured using the Kjeldahl method. Approximately 2g of roe sample was used for protein content determination by using a factor 6.25 following the AOAC standard method (AOAC, 1995). The roe lipids were extracted using a chloroform and methanol mixture (ratio 1:1) according to the protocol of Bligh and Dyer (Bligh and Dyer, 1959).

pH value, salt content and ash determination

Samples of raw and salted fish roes (10 g) were homogenized in sterile blenders with 10 mL of distilled water to make a thick slurry. The pH of this slurry was measured by a calibrated portable digital pH meter (Beckman Φ40, Krefeld, Germany) (Kung *et al*., 2008). NaCl content was determined by the volumetric method of Volhard .The homogenized roe samples was titrated with 0.1 M $AgNO_3$, using 10 % w/v K_2CrO_4 solution as an indicator. The salt content was calculated as percentage of the sample. Ash was determined after heating the samples overnight at 550˚C (AOAC, 1995).

Determination of total volatile basic nitrogen (TVB-N)

The TVB-N content of the Kutum roe samples were measured by the method of Conway's dish. Each ground sample (10 g) of roes was extracted with 20 mL of 6% trichloroacetic acid (TCA) and filtered. The TCA extracts of the roe samples were absorbed by boric acid and then titrated with 0.02 N HCl. The TVB-N content was expressed in mg/100 g of fish roes (Kung *et al.,* 2009).

Fatty acid analysis

The total lipids (TL) were extracted from each sample by the method of Bligh and Dyer. Fatty acid methyl esters were prepared by transmethylation using 2M KOH in methanol and n-heptane. Extracted lipids (10mg) were dissolved in 2 mL heptane followed by 4 mL of 2M methanolic KOH. The tube vortex and centrifuged at 4000 rpm ×g unit for 10 minutes (Shirai *et al*., 2006). The heptane layer was taken for GC analyses. Fatty acids were measured by the gas chromatograph (DANI 1000, DANI Instruments SpA, Italy) equipped with a fused silica capillary SGE column (30m×0.25 mm ID). The carrier gas was helium with a split injection of 40:1. The temperature profiles were as follows: initial temperature, 175˚C; heating rate, 1˚ C/min; final temperature, 220 ˚C (final time, 20 min); injector temperature, 250 ˚C, and detector temperature, 270 ˚C. The fatty acids were identified by comparison of the retention times with those of standard purified fatty acids (Shirai *et al*., 2006).

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Microbiological analysis

Aerobic Plate Count (APC)

A 25 g portion of each test sample was homogenized at high speed for 2 minutes in a sterile blender with 225 mL of sterile potassium phosphate buffer (pH 7.0). The sterile blender was prepared by autoclaving for 15 minutes at 121^{o}C. The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 mL aliquots of the diluted homogenates were inoculated into an aerobic plate count (APC) agar (Difco, Detroit, MI, USA) containing 0.5% NaCl. Bacterial colonies were counted after incubating the plates at 35 ^{o}C for 48 hours. The bacterial numbers in the roe samples were expressed as $\log_{10}$ colony- forming units (CFU/g) (Huang *et al*., 2010).

Salmonella spp.

A 25g of sample and 225 mL buffered peptone-water were homogenized using a filter Stomacher. The analysis depends on the principles of pre-culturing for 18-24 hours in Buffered Peptone-Water at 35-37 ^{o}C, the selective culturing for 24 hours in Selenite Cystine Broth at a temperature of 35 ^{o}C, and the identification of the suspected colonies through the current biochemical and serological tests (Valcheva *et al.*, 2011).

C. perfringens

A 10 g of sample and 100 mL buffered peptone-water were homogenized and further 10-fold dilutions' were made. The analysis was on incubation for 24 hours in Sulphite polimyxin sulphadiazine agar at a temperature of 35-37 ^{o}C and the identification of the Clostridiae through the biochemical test (Huang *et al*., 2010).

Total Coliform and E. coli

Analyses of total coliform and *E. coli* in roe samples were conducted by using three tubes according to the most probable number (MPN) method (Kung *et al.*, 2009). Lauryl sulphate tryptose broth (LST broth) and brilliant green lactose bile (2 %) broth (BGLB broth) were used for presumptive and confirmed tests for total coliform, respectively. *E. coli* was determined by the LST broth and *E. coli* (EC) broth. Cultures that showed positive production of gas were then confirmed by eosin methylene blue agar (EMBA) (Huang *et al*., 2010).

Yeast

A 10 g of sample and 100ml peptone-water were homogenized and further 10-fold dilutions' were made. The analysis depends on incubation for 5 days in Dichloran Rose Bengal Chloramphenicol agar at a temperature of 25 ^{o}C; yeasts colonies were counted (Altug and Bayrak, 2003).

Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/g). All plates were visually examined for the typical colony types and morphological characteristics associated with each growth medium. All counts were performed in triplicate.

Statistical analyses

Experiments were performed in triplicates and they were expressed as mean ± standard deviation. Data were evaluated by software program SPSS version 12.0. Data were subjected to T-test. The level of significance was set at $p<0.05$.

Results

Chemical composition

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Table 1 shows the results of proximate composition (moisture, crude protein, fat, ash and pH) of raw and salted Kutum roes. The moisture contents varied significantly from 61.1 to 51.5% (raw and salted roes, respectively). The mean protein contents of raw and salted roes were 28.8 and 24.0 % respectively which was in good agreement with 28.5% (trout roe) (Mahmoud *et al*., 2008).

Table 1: Proximal composition of Raw and Salted roe of Kutum

Sample	Protein	Lipid	Moisture	Ash	Salt	pH
Raw Roe	28.81±1.1a*	6.84±2.2a	61.07±0.3a	1.3±1.8a	0.35±0.08a	7.49±0.05a
Salted Roe	23.99±0.5b	6.4±0.7a	51.57±0.6b	17.74±1.1b	5.13±0.3b	5.1±0.09b

§ Mean ±Standard deviation and data have expressed as % of wet matter.
* a –b Values in the same column with different letters are statistically different ($p< 0.05$).

There was no significant difference between lipid values. Lipid contents of raw and salted samples were 6.84% and 6.4%, respectively ($p>0.05$). Ash content of brined roe (Table 1) was much higher than that of raw samples. Water losses associated with brining resulted in increased ash content. Marked differences were found for pH of the samples. Significant decrease was observed in the pH value of salted roe, indicating the effect of salting process. Salt content in salted roe (5.1%) was significantly higher than raw sample (0.3%), which was the result of salting process. The average of TVB-N value of the raw samples (10.9 mg/100gr) was significantly higher than salted roe (5.1 mg/100gr) (Figure 1); both levels were under determined levels (30 mg/100gr).

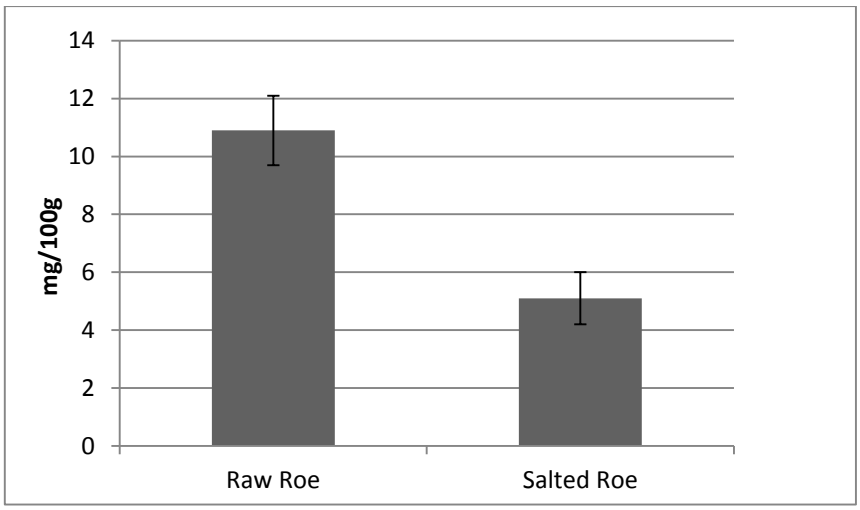

Figure 2: Comparison of TVB-N [1] measured in raw and salted roe of Kutum

[1] Mean values from three independent determinations. Standard deviations are expressed by bars.

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Fatty acid composition

Fatty acid composition of Kutum roe are presented in Table 2. C16:0, C16:1, C18:0, C18:1 and C22:6 were the main fatty acids of roe. Monounsaturated fatty acids (MUFA) represented the most dominant class of total fatty acids (49.58%) followed by saturated fatty acids (27.93%).

Table 2: Fatty acid composition of total lipid (TL) in raw and salted Kutum roe (%).

Fatty acid	Raw Roe	Salted Roe
[a]SFA		
14:0	[d] 2.09±0.3	0.62±0.1
15:0	0.68±0.00	–
16:0	18.26±0.3	18.93±0.6
17:0	0.85±0.05	–
18:0	6.05±0.3	7.48±0.1
20:0	0.00	0.00
∑	27.93	27.3
[b]MUFA		
14:1n-5	0.00	0.14±0.02
15:1	0.37±0.01	–
16:1n-7	12.02±0.5	4.52±0.3
17:1n-7	1.48±0.03	–
18:1n-9	27.15±1.3	12.67±1.3
18:1n-7	7.39±0.5	6.18±0.5
20:1n-9	1.18±0.07	0.39±0.08
∑	49.58	23.9
[c]PUFA		
18:2n-6	1.33±0.02	2.11±0.4
18:3n-3	0.63±0.08	0.35±0.05
20:2n-6	0.46±0.04	0.33±0.02
20:3n-3	0.00	0.00
20:4n-6	1.63±0.1	4.74±0.6
20:5n-3	4.37±0.3	7.6±0.5
22:6n-3	5.17±0.9	21.62±1.4
∑	13.59	36.75
PUFA/SFA	0.49	1.34

[a] SFA, saturated fatty acids.
[b] MUFA, monounsaturated fatty acids.
[c] PUFA, polyunsaturated fatty acids.
[d] Samples were analyzed in triplicate and results are expressed as mean value ±SD (p<0.05).

According to the results of the analyses of fatty acids, there was significant difference between SFA and MUFA values (p<0.05). The percentage of MUFA differed from 49.58 to 23.9 % in raw and salted samples. The predominant MUFA in both samples was oleic acid (C18:1n9, 27.15 and 12.67%). A significant difference was found between the processing and the MUFA; showing a decreasing trend (Fig. 2). The samples had < 40% PUFA (13.6 and 36.7%, raw and salted roe), eicosapentaenoic acid (EPA) (C20:5n3) had the highest percentage in raw and salted

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Kutum roe (5.1 and 21.6%, respectively). Results showed that salted roe had higher percentage of DHA and EPA compared to raw samples.

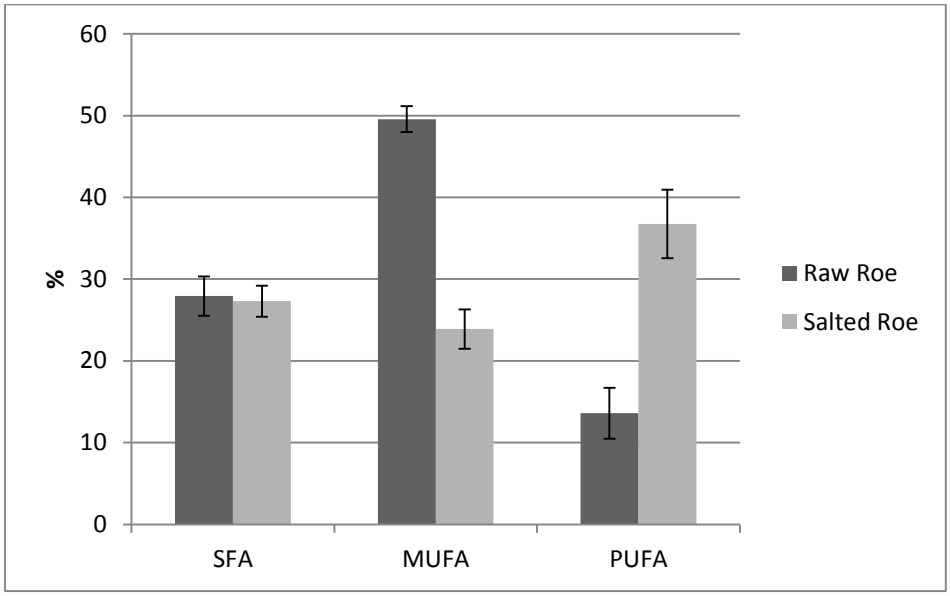

Figure 2: Values of the total Saturated Fatty acid (SFA), Monosaturated Fatty Acid (MUFA), and Polyunsaturated Fatty acid (PUFA) measured in raw and salted roe of Kutum.

[1] Expressed as percentage of total fat.

Microbiological analysis

The content of aerobic plate count (APC), total coliform (TC) and *E. coli*, *Salmonella*, *C. perfringens*, fungi and yeast counts which were obtained in this study are summarized in Table 3.

Table 3: The results of microbiological analysis of raw and salted Kutum roe.

Sample	APC (log cfu/g)	TC (MPN/g)	*E.coli* (MPN/g)	*Salmonella* (cfu/g)	*C. perfringens* (cfu/g)	Fungi (cfu/g)	Yeast
Raw Roe	[§] (5.33 ±1.5) a	(210±0.0) a	0	ND	ND	0	4×10^2
Salted Roe	(1.23 ±0.05) b	(1.2 ±2.07) b	0	ND	ND	0	ND

[1] Means ± SD. Values are average of triplicate (n=3) analyses ± standard deviation. Means within rows followed by different letters denote significant differences (p<0.05). ND: Not detected

None of the raw and salted roe samples contained *E. coli*, *Salmonella and C. perfringens*. The mean value of APC level in raw roe (5.33×10^3 cfu/g) was significantly higher than salted roe (1.23×10^3 cfu/g). The average content of

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Coliforms in raw roe was 210 mpn/g which was significantly higher than salted samples (1.2 mpn/g). The mean content of yeasts in raw roe was 4×10^2 cfu/g, but not detected in salted roe.

Discussion

Salting leads to a reduction in moisture that has been attributed to the denaturing effect of salt on proteins. High brine concentration caused dehydration of the samples, due to the difference in solute concentration between the brine solution and roe water. Jittinandana and others (2002) indicated that denaturation of muscle protein facilitated diffusion of water from fish. Loss of the protein content is one of the main biochemical changes during salting (Aristoy and Toldra, 1991). Sodium chloride increased protein solubility (Martınez-Alvarez and Gomez-Guillen, 2005); According to Jittinandana and others (2002) soaking in high brine solution may have caused salting-out of proteins that led to precipitation and dehydration of proteins and, in turn, exclusion of water molecules; that is dependent on salting time and salt concentration (Aristoy and Toldra, 1991; Andersen *et al*., 2007).

Lipid content in raw and proceeded Kutum roe was about 6% and it ranged from 3% in pacific herring (*Clupea pallasi*) roe (Rodrigo *et al*.,1998), 12% in *Acipenser huso* (Altug & Bayrak, 2003) , 14.5% in Ikura (salted salmon roe), 3.7% in Tarako (salted pollock roe), 3.2% in Tobiko (salted flyingfish roe), 3% in Kazunoko (salted herring roe) (Shirai *et al*.,2006), 25.7% in salted mullet roe (Lu *et al*., 2006) and 9.8% white fish (*Coregonus albula*) roe (Kaitaranta,1980).

In the present study, the ash content of salted Kutum roe was higher than salted hake and ling roe (*Molva molva*) (6.9%) and mullet roe (2.6%) (Rodrigo *et al*., 1998). This is mainly affected by salting process, which remains a high amount of minerals to the roe (Rodrigo *et al*., 1998). High brine concentration increased water phase salt content of samples, and it corresponded with increased product ash content (Jittinandana *et al.,* 2002).

Marked differences were found for pH of the samples. Significant decrease was observed in the pH value of salted roe, indicating the effect of salting process. In other studies pH of salted roes ranged between 5.5-5.86; as the result of salting process (Tsai *et al*., 2005; Celik *et al*., 2012).

The properties of fish and fish products vary as a result of alterations in water and salt concentration. Diffusion has been known as the most important mass transfer mechanism of sodium and chloride. Differences in osmotic pressures between cells and salting agent cause the abruption of salting agent into fish and excretion of water out of fish (Gallart-Jornet *et al*., 2007).

TVB-N is one of the most widely indicators of fish quality and spoilage (Kung *et al*., 2008). According to the quality categorization of fish and fish products proposed by Lang (1983), TVB-N value should be lower than 25 mg/ 100 g, for ‘‘high quality”, less than 30 mg/100g for ‘‘good quality”, lower than 35 mg/100 g for ‘‘limit of acceptability” and more than 35 mg/100 g for ‘‘spoilt” (Özyurt *et al*., 2009). In both group samples were under determined levels (30 mg/100gr). Based on

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

this category, salted and raw samples may be placed in high quality group. The TVB-N content of salted roe was nearly half of the level of raw samples, indicating that the addition of salt could apparently have some inhibitory effects on spoilage of salted roe samples; it was in agreement with Kung *et al* (2008).

The main fatty acids (SFA) of roe were found similar pattern in Kutum roe by Ghomi and Nikoo (2010). Most of the studies have shown that saturated fatty acids of fish and fish products are between 20 and 30%, which it was in agreement with these studies (Rincón-Cervera *et al*., 2009; Rosa *et al*., 2009; Shin *et al*., 2010). Palmitic acid had highest proportions. In 15 previous studied species, the predominant SFA was palmitic acid (Rincón-Cervera *et al*., 2009) and this fatty acid is required for fish growth and formation of roe in females (Huynh *et al.*, 2007). Rincón-Cervera *et al*. (2009) reported oleic acid as the main component of MUFA in 13 species. Brined roe had less lipid content which is possibly due to decreasing of the MUFA through processing, PUFA percentage of salted roe increased. Scano *et al*. (2009) suggested that the fatty acid profiles of salted and dried mullet roe were not affected by the processing.

Some reports have indicated that the fatty acid composition of fish roe is impacted by diet but does not depend on the level of maturation (Shirai *et al*., 2006). Analysis of fatty acid composition of salted roe of salmon, pollock, flying fish, and herring represented that DHA value was higher than EPA (17.4, 22.2, 27.9 and 22.6%, respectively). Mahmoud *et al* (2008) studied fatty acid profile of rainbow trout (*Oncorhynchus mykiss*) roe and they indicated that the PUFA of rainbow trout roe was about 40-50% of total fatty acid content which was higher than that of raw and salted Kutum roe. Ghomi and Nikoo (2010) suggested the Kutum roe as a good source of PUFA can be used in food products.

Tsai *et al.* (2005) reported that higher salt content (>5%) had some inhibitory effects on bacterial growth which results are in agreement. Besides, the positive effect of salt on microbial growth (Wheaton and Lawson, 1985), rose madder (*Rubia tinctorum*) have antimicrobial effects on some bacteria (Mehrabian *et al*., 2000). APC is a criteria for determination of the general microbiological quality of the product (FAO, 1992). Coliforms in raw roe was higher than salted samples. The existence of coliform in processed food products indicates that the processing have been conducted under inefficient hygienic conditions (ICMSF, 1987). These samples revealed that salt had some inhibitory effect on coliform bacterial growth. Some studies proved that most bacteria responsible for fish meat spoilage are stressed by salt content above 1% and would die or at least would be stopped further development, as the salt content increase from 6% to 8% (Wheaton and Lawson, 1985; Kung *et al*., 2008). During the process of excerption of the roe from the fish, the bacteria on the surface of the fish may be transferred to the roe.

The yeast was not detected in salted roe. Among the micro-organisms, the yeasts are known to be causing the food spoilage and organoleptic spoilage that were visually noticed depending on the time and

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

production intensity. This can affect the commercial quality of the product in a negative manner (Altug and Bayrak, 2003).

This study represents a report on the proximate composition and quality changes of raw and salted Kutum roe. Despite the increasing levels of TVN in raw samples, a decrease occurred for the salted samples. Although TVB-N values seems to be a good spoilage factor for both samples, but to draw a better conclusion these values should be interpreted in combination with quality changes (lipid oxidation,…) and shelf storage life of the products are required. The total count and APC of raw samples significantly were higher than that of salted samples representing the preservative effect of salting. Overall most of the results obtained in this study indicate that salting might be a suitable procedure for preserving quality of Kutum roe. It had some inhibitory effects on bacterial growth. Therefore, further research is suggested to investigate the effects of salting on quality and sensory changes during storage.

References

Altug, G. and Bayrak, Y., 2003. Microbiology analysis of caviar from Russia and Iran. *Food Microbiology*, 20, 83-86.

Andersen, E., Andersen, M.L., and Baron, C.P., 2007. Characterization of oxidative changes in salted Herring (*Clupea harengus*) during ripening. *Journal of Agriculture and Food Chemistry*, 55, 9545-9553.

AOAC, 1995. Official methods of analysis of AOAC International, Sixteenth ed, AOAC International, Arlington, VA.

Aristoy, M.C. and Toldra, F., 1991. Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *Journal of Agriculture and Food Chemistry*, 39, 1792–1795.

Bekhit, A.A., Morton, J.D., Dawson, C.O. and Sedcole, R., 2008. Optical properties of raw and processed fish roe from six commercial New Zealand species. *Journal of Food Engineering*, 91, 363-371.

Bledsoe, G.E., Bledsoe, C.D. and Rasco, B., 2003. Caviars and fish roe products. *Critical Reviews in Food Science and Nutrition*, 43, 317-356.

Bligh, E.G. and Dyer, W.J.A, 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 900-917.

Celik, U., Altinelataman, C., Dincer, T. and Acarch, D., 2012. Comparison of fresh and dried flathead grey mullet (*Mugil cephalus*) caviar by means of proximate composition and quality changes during refrigerated storage at 4±2 ℃. *Turkish Journal of Fisheries and Aquatic Sciences*, 12, 1-5.

FAO, 1992. Manual of food quality control, Vol. 4, Rev.1. Microbiological analysis. Food and Agricultural Organization of the United Nations, Rome. 344P.

Gallart-Jornet, L., Barat, J.M., Rustad, T., Erikson, U., Escriche, I. and Fito, P., 2007. A comparative study of brine salting of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*). *Journal of Food Engineering*, 79, 261–270.

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

Huynh, M.D., Kitts, D.D., Hu, C., and Trites, A.W., 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. *Comparative Biochemistry and Physiology*, 46, 504-11.

ICMSF, 1987. Microorganisms in foods, Volume, 1.University of Toronto Press, Toronto.

Jittinandana, S., Kenney, P.B., Slider, S.D., and Kiser, R.A. 2002. Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. *Journal of Food Science*, 67, 2095-2099.

Kaitaranta, J., 1980. Lipids and fatty acids of whitefish (*Coregonus albula*) flesh and roe. *Journal of Science Food and Agricultural*, 31(**12**), 1303-1308.

Katselis, G., Koutsikopoulos, C., Rogdakis, Y., Lachanas, T., Dimitriou, E., and Vidalis, K., 2005. A model to estimate the annual production of roe (*avgotaracho)* of flathead mullet (*Mugil cephalus*) based on the spawning migration of species. *Fisheries Research*, 75**,** 138–148.

Kung, H.F., Chien, L.T., Liao, H.J., Lin, C.S., Liaw, E.T., Chen, W.C. and Tsai, Y.H., 2008. Chemical characterization and histamine-forming bacteria in salted mullet roe products. *Food Chemistry*, 110, 480-485.

Kung, H.F., Wang, T.Y., Huang, Y.R., Lin, C.S., Wu, W.S., Lin, C.M. and Tsai, Y.H., 2009. Isolation and identification of histamine-forming bacteria in tuna sandwiches. *Food Control*, 20, 1013–1017.

Lu, J.Y., Ma, Y.M., Williams, C. and Chung, R.A., 2006. Fatty and amino acid composition of salted mullet roe. *Journal of Food Science*, 44(**3**), 676-677.

Mahmoud, K.A.S., Linder, M., Fanni, J. and Parmentier, M., 2008. Characterization of the lipid fractions obtained by proteolytic and chemical extractions from rainbow trout (*Oncorhynchus mykiss*) roe. *Process Biochemistry*, 43, 376-383.

Mehrabian, S., Majd, A. and Majd, I., 2000. Antimicrobial effects of three plants *(rubia tinctorum, carthamus tinctorius and juglans regia)* on some airborne microorganisms.*Aerobiologia*, 16, 455–458.

Özyurt, G., Kuley, E., Özkütük, S. and Öogul, F., 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry*, 114, 505-510.

Rincón-Cervera, M.Á., Suárez-Medina M.D. and Guil-Guerrero, J.L., 2009. Fatty acid composition of selected roes from some marine species. *European Journal of Lipid Science and Technology*, 3, 920–925.

Rodrigo, J., Ros, G., Periago, M.J., Lopez, C. and Ortuiio, J., 1998. Proximate and mineral composition of dried salted roes of hake *(Merltcccircs merluccius,* L.) and ling *(Molva molva,* L.). *Food Chemistry*, 63(**2**), 221-225.

Rosa, A., Scano, P., Paola Melis, M., Deiana, M., Atzeri, A. and Assunta Dess, M., 2009. Oxidative stability of

[DOR: 20.1001.1.15622916.2015.14.1.15.2]

lipid components of mullet (*Mugil cephalus*) roe and its product ‘‘bottarga”. *Food Chemistry*, 115, 891-896.

Scano, P., Rosa, A., Locci, E., Dessi, M.A. and Lai, A., 2009. NMR study of the lipid profile of mullet raw roe and bottarga. *European Journal of Lipid Science Technology*, 111, 505–512.

Shin, J.H., Oliveira, A.C.M. and Rasco, B., 2010. Quality attributes and microbial storage stability of caviar from cultivated white sturgeon (*Acipenser transmontanus*). *Journal Food Sciences*, 75, 43-48.

Shirai, N., Higuchi, T. and Suzuki, H., 2006. Analysis of lipid classes and the fatty acid composition of the salted fish roe food products, Ikura, Tarako, Tobiko and Kazunoko. *Food Chemistry*, 94, 61–67.

Tsai, Y.H., Lin, C.Y., Chang, S.C., Chen, H.C., Kung, H.F., Wei, C.I. and Hwang, D.F., 2005. Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology*, 22, 461-467.

Valcheva, R., Belopopska, P., Mateva, G., Hristova, T., and Daskalov, H. 2011. Distribution and serological typing of *Salmonella* spp. isolates from broiler carcasses in bulgaria. *Bulgarian Journal of Veterinary Medicine*, 14, 31-38.

Valipour, A. and Khanipour, A.A., 2008. KUTUM Jewel of the Caspian Sea. INFRO. Tehran. pp.1-34.

Wheaton, F.W. and Lawson, T.B., 1985. Other preservation methods. In Processing Aquatic Food Products, New York: John Wiley & Sons Press. pp. 273–328.

[DOR: 20.1001.1.15622916.2015.14.1.15.2]