

Proximate and Fatty Acid Composition of Salted Caspian Kutum (*Rutilus frisii*) Roes Influenced by Storage Temperature and Vacuum-Packaging

¹Majid Taghiof, ²Mehdi Nikoo and ¹Khalil Pourshamsian

¹Department of Chemistry, Islamic Azad University, Tonekabon Branch, 46817 Tonekabon, Iran

²School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China

Abstract: In this study Caspian kutum (*Rutilus frisii*) roe was dry-salted and stored at ambient temperature and non-packed (S1), refrigerated-stored and non-packed (S2), ambient temperature and vacuum-packed (S3), refrigerated-stored and vacuum-packed (S4) and the changes in proximate and fatty acid composition were evaluated at days 5, 60 and 120 of storage. Salted roe contained 60-65% moisture, 18-22% protein, 4-7% lipid and 3-4.6% ash. A slight decrease in lipid content was observed during storage while moisture, protein and ash remained unchanged. Palmitic acid (C16:0, 14.36-21.35%) and oleic acid (C18:1, 26.23-43.9%) were the most abundant fatty acids of salted roe. S4 samples had the highest contents of docosahexaenoic acid (EPA; C22:6 n-3), eicosapentaenoic acid (EPA; C20:5 n-3) and omega-3 fatty acids. Therefore, refrigerated-storage and vacuum-packaging could better protect omega-3 fatty acids in salted roe.

Key words: Salting % Roe % Storage % Fatty Acid Profile % Caspian Kutum

INTRODUCTION

Seafood plays an important role in human nutrition, disease prevention and health promotion [1]. Fish roe products are excellent and valuable products enjoy high demand in international and domestic markets [2]. Fish roe contains albumins (11%), ovoglobulin (75%) and collagen (13%) and is a valuable source of nutritive lipids, especially phospholipids and long chain unsaturated fatty acids [3]. Polyunsaturated fatty acids are highly important for human health as these fatty acids have beneficial role on reducing atherosclerosis, prevention and treatment of numerous disorders like cardiovascular disease and others. Caviar from sturgeon species is the most consumed fish roe product which is produced from sturgeon roe after the eggs have been graded, sorted, singled-out, salted or brined and cured. Collapse in sturgeon stocks in the natural habitat has diverted the attention to produce caviar from other fish species such as catfish, salmon, lumpfish, flying fish, herring, mullet and cod [2]. In Italy salted-and dried mullet roe is called *bottarga* which is sold as a vacuum-packed whole ovary [4]. Dried and salted tuna roe product is a typical fish-based food in the Mediterranean area of Spain [5].

As fresh fish roe is highly perishable, it is commonly processed to be offered for sale [6]. Salting is a traditional preservative method of fish in many countries to increase the shelf life of food product [7]. The preservative effect of salt is mainly due to the decrease in water activity which has suppressive effects on growth of many spoilage organisms [8]. Salting is also a preliminary step of other processing techniques such as smoking, drying and marinating. For salting, dry salt (dry-salting method) or salt solutions (brine-salting method) are used as salting agents [9]. Natural brine (or blood brain) formed during dry salting and brine solution was reported to be important for the development of the characteristic organoleptic properties of salted fish during ripening [10]. Kutum *Rutilus frisii* is a commercially important fish available in the southern waters of the Caspian Sea and some lakes of Turkey with high market acceptance [11]. The eggs from female fishes are processed into "*eshpel*" (a traditionally dry-salted roe product) which has high demand in local market [12]. Production of *eshpel* is based on experience and sold as whole ovary while stored at ambient temperatures. Little is known about the changes in omega-3 essential fatty acids and nutritional quality of *eshpel* that take place during salting and subsequent

storage at ambient temperature. Information on the effects of refrigerated storage and vacuum packaging of salted kutum roe governing its fatty acid composition is limited. Therefore the objective of this study was to evaluate the changes in proximate and fatty acid composition of salted kutum roe as influenced by refrigerated-storage and vacuum-packaging.

MATERIALS AND METHODS

Roe Samples and Salting Protocol: Twenty samples of kutum roe (average weight 100-200 g) were purchased from local fish markets in Sari (Mazandaran, Iran). All samples were placed in ice with roe to ice ratio of 1:2 (w/w) and transported to laboratory within 1 h. For salting, the traditional method of salting kutum roe was followed. The roe were subjected to dry-salting for 5 days at 20°C in a plastic container. The roe to salt ratio was 1:1 (w/w). Samples of salted roe were then divided into four homogenous groups and named as S1, S2, S3 and S4. S1 group stored at ambient temperature and non-packed, S2 group refrigerated-stored and non-packed, S3 group stored at ambient temperature and vacuum-packed and S4 group refrigerated-stored and vacuum-packed. Samples were removed at days 5, 60 and 120 of storage and subjected to analysis.

Proximate Composition: Moisture was determined by drying the samples in an oven (Heraeus, D-63450, Hanau, Germany) at 105°C to a constant weight [13]; lipid was extracted according to Bligh and Dyer [14]. Ash was determined by incineration in a muffle furnace (Isuzu, Tokyo, Japan) at 600°C for 3 h [13]; crude protein was determined by the Kjeldahl method ($N \times 6.25$) using an automatic Kjeldahl system (230-Hjeltec Analyzer, Foss Tecator, Höganäs, Sweden) [13].

Lipid Extraction: Lipid was extracted according to the method of Bligh and Dyer [14]. Fifty g of sample were homogenized in a blender for 2 min. with a mixture of 50 ml chloroform and 100 ml methanol. Then 50 ml of chloroform were added and further homogenized for 30 sec. Finally 50 ml of distilled water were added to the mixture and blended for 30 sec. The homogenate was centrifuged (Avanti J-E, Beckman Coulter, Inc., USA) at 3000 rpm for 15 min at 4°C. Supernatant was then transferred into a separating flask and the lower phase (chloroform phase) was drained off into a 250 ml Erlenmeyer flask containing 4 g anhydrous sodium sulfate and shaken vigorously. The solution was then filtered through a Whatman No. 4 filter paper into a round-bottom flask. Rotary evaporator

(Rotavapor R-114, Büchi, Flawil, Switzerland) was used for solvent evaporation at 25°C.

Fatty Acid Analysis: Fatty acid methyl ester was prepared as follows: Lipid samples (1 g) were diluted with 2 ml of 2 M potassium hydroxide in methanol followed by the addition of 7 ml n-hexane in a sealed tube. The mixture was then shaken using a vortex for 1 min and left for about 20 min. in a water bath (temperature 50-55°C) until it was separated into two phases. From top layer, fatty acid methyl ester was then taken for analysis by using Trace GC (Thermo Finnigan, Italy). The GC conditions were as follows: capillary column (Bpx-70, 60 m, 0.32 mm, i.d. 0.25 µm); the split ratio of 90:1; injection port temperature of 250 °C; flame ionization detector temperature of 270 °C. Oven temperature was set at 195 °C for 75 minutes. Flow rate of carrier gas (helium) was 1 mL min⁻¹ and the makeup gas was N₂ (30 ml/min). The sample size injected for each analysis was 1 µL. The data are expressed as g/100 g of total fatty acids.

RESULTS AND DISCUSSION

Proximate Composition: Proximate composition of salted kutum roe is shown in Table 1. Moisture accounts for 60-65% of the salted roe while protein, lipid and ash contents were 18-22%, 3.4-7.2% and 3-4.6% respectively. The content of protein in salted kutum roe is similar to protein content of skipjack, tongol and bonito in the ranges of 18.16-20.15% [3] and white sturgeon caviar in the ranges of 23.9-25.4% [15]. In dried and salted hake and ling roe, protein content were reported to be 39.1% and 43.6% respectively [16]. The protein content of different wild sturgeon species (great sturgeon, Russian and stellate sturgeon) and farmed species (Siberian and stellate sturgeon and paddle fish) caviar was between 26.21-31.13% [17]. The lipid content of salted kutum roe is lower than that of wild and farmed sturgeon species studied by Gessner *et al.* [17] in the ranges of 10.9-19.41% and dried and salted hake and ling roes with lipid content of 14.13 and 14.8% respectively [16]. However its lipid content was similar to lipid content of tuna species roe (3.29-5.68%) studied by Intarasirisawat *et al.* [3]. At the fifth day of storage, lipid content of salted kutum roe was between 5 to 7.2% and S4 samples had the highest value (Table 1). A slight decrease in lipid content of all salted roe samples was observed during storage and its level reached 3.4-4.7% at the end of storage period, however the difference between samples was not significant. The contents of protein, ash and moisture did not change significantly during storage among different samples.

Table 1: Proximate composition of salted kutum roe as a function of storage temperature and vacuum packaging

	Day 5				Day 60				Day 120			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Protein	21.70	20.50	21.60	18.10	18.90	19.30	18.10	18.70	20.30	19.30	19.10	19.70
Lipid	5.80	5.00	6.20	7.20	5.60	4.60	5.20	5.40	4.10	3.40	4.70	4.20
Moisture	60.30	62.40	64.10	63.20	62.20	61.70	64.50	63.80	59.10	60.40	62.80	61.20
Ash	3.50	4.66	3.50	3.25	3.85	3.40	4.08	3.75	3.10	3.92	3.07	3.43

*S1: ambient temperature and non-packed, S2: refrigerated-stored and non-packed, S3: ambient temperature and vacuum-packed, S4: refrigerated-stored and vacuum-packed

Table 2: Fatty acid composition (g/100 g of total fatty acids) of salted kutum roe as a function of storage temperature and vacuum packaging

	Day 5				Day 60				Day 120			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
C14:0	2.46	2.56	1.59	1.49	1.82	2.03	1.95	1.72	3.40	2.10	2.12	1.83
C16:0	17.04	17.14	15.33	14.36	20.26	19.27	18.47	16.89	21.35	17.82	19.59	17.51
C16:1	11.15	11.91	10.09	8.75	13.51	13.21	12.50	10.31	11.69	12.10	13.63	10.80
C18:0	5.35	5.02	4.44	4.70	6.22	4.89	4.63	5.99	6.41	6.48	4.38	5.24
C18:1	40.18	35.56	35.83	26.23	40.51	37.34	34.93	31.40	43.90	38.84	39.88	35.22
C18:2	0.38	0.86	0.51	1.29	1.00	1.19	1.35	1.59	1.61	0.98	0.76	1.22
C18:3	0.29	0.35	0.88	0.42	0.33	0.54	0.54	0.48	0.90	1.76	1.79	1.84
C20:1	2.73	1.13	2.02	1.78	1.42	1.66	1.48	1.78	1.35	1.16	1.04	1.82
C20:5	0.94	1.57	2.74	3.98	2.20	3.18	3.12	2.72	0.70	1.91	2.37	3.53
C22:6	0.61	3.42	5.00	7.26	3.56	3.13	3.01	5.65	0.58	2.24	2.27	5.81

*S1: ambient temperature and non-packed, S2: refrigerated-stored and non-packed, S3: ambient temperature and vacuum-packed, S4: refrigerated-stored and vacuum-packed

Table 3: Major class of fatty acids (g/100 g of total fatty acids) of salted kutum roe as a function of storage temperature and vacuum packaging

	Day 5				Day 60				Day 120			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
~SFA	24.85	24.72	21.29	20.55	28.30	26.19	25.05	24.60	31.16	26.40	26.09	24.58
~MUFA	54.06	48.60	47.94	36.76	55.44	52.21	48.91	43.49	56.94	52.10	54.55	47.84
~PUFA	2.22	6.20	9.13	12.95	7.09	8.04	8.02	10.44	3.79	6.89	7.19	12.40
~N-3	1.84	5.34	8.62	11.66	6.09	6.85	6.67	9.85	2.18	5.91	6.43	11.18
~N-6	0.38	0.86	0.51	1.29	1.00	1.19	1.35	1.59	1.61	0.98	0.76	1.22
N-3/n-6	4.84	6.20	16.88	9.03	6.09	5.75	4.94	6.19	1.35	6.03	8.46	9.16

*S1: ambient temperature and non-packed, S2: refrigerated-stored and non-packed, S3: ambient temperature and vacuum-packed, S4: refrigerated-stored and vacuum-packed

Fatty Acid Composition: Fatty acid compositions of salted roe are shown in Table 2 and 3. Palmitic acid (C16:0, 14.36-21.35%), palmitoleic acid (C16:1, 8.75-13.63%) and oleic acid (C18:1, 26.23-43.9%) were the most abundant fatty acids of salted roe. Monounsaturated fatty acids (MUFA) were the most dominant class of fatty acids followed by saturated (SFA) and polyunsaturated fatty acids (PUFA). The contents of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were 0.7-3.98 and 0.58-7.26% respectively. Salted roe contained higher amounts of N-3 fatty acids (1.84-11.66%) compare to n-6 fatty acids (0.38-1.61%) giving n-3/n-6 ratio of 1.35 to 16.88. Ecological studies have found a negative correlation between the risk of developing heart diseases and fish

consumption because of their long chain omega-3 fatty acids [18]. In humans many chronic diseases such as cardiovascular, inflammatory and autoimmune diseases are associated with high intake of n-6 fatty acids and instead increased levels of omega-3 fatty acids such as EPA and DHA in diets and lower n-6/n-3 ratio have beneficial health effects [19].

There was a slight increase in the content of saturated fatty acids during storage and samples refrigerated-stored and vacuum-packed (S4) had the lowest SFAs content. Similar to SFAs, the content of MUFAs increased slightly in all samples. In S4 samples, MUFAs was 36.76% after 5 days of storage; however it increased to 43.49 and 47.84% at days 60 and 120 post

storage (Table 3). The content of PUFAs in the samples refrigerated-stored and vacuum-packed (10.44-12.95%) was significantly higher than other samples. In samples refrigerated-stored and non-packed (S2) the content of PUFAs at the fifth day of storage (6.20%) was lower than the samples stored at ambient temperature and vacuum-packed (9.13%), however at days 60 and 120 of storage, S2 and S3 samples had similar content of PUFAs. In order to evaluate the effects of storage temperatures (ambient or refrigerated temperatures) and vacuum-packaging on fatty acid composition of salted kutum roe, samples were stored under different storage conditions and the results indicated that S4 samples which stored as refrigerated and vacuum-packed contained highest amounts of docosahexaenoic acid (EPA; C22:6 n-3), eicosapentaenoic acid (EPA; C20:5 n-3) and omega-3 fatty acids (Table 2). Although information on the effects of storage temperatures and packing on omega-3 fatty acids of fish roe is limited, however studies on fish fillets have shown the positive effects of vacuum-packaging and lower storage temperature on the quality and shelf life of products. Vacuum-packaging of n-3 enhanced farmed rainbow trout resulted in lower oxidation compare to non-vacuum packaging; but it did not affect fatty acid composition [7]. Frangos *et al.* [8] reported that combination of salting, oregano oil extract and vacuum-packing resulted in a better quality and significant shelf-life extension of refrigerated trout fillets compared to the control sample, kept under aerobic conditions. Similarly vacuum-packed gravled rainbow trout product had better quality and longer shelf life when stored at lower temperature [20].

In conclusion the results of this study showed that refrigerated-storage and vacuum-packaging is better protection for omega-3 fatty acids in salted kutum roe and found to be optimal storage conditions for kutum salted roe product.

ACKNOWLEDGMENTS

This study was financially granted by Research Affair of Islamic Azad University, Tonekabon Branch.

REFERENCES

1. Khoddami, A., A.A. Ariffin, J. Bakar and H.M. Ghazali, 2009. Fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Sardinella lemuru*). World Appl. Sci. J., 7(1): 127-131.
2. Bledsoe, G.E., C.D. Bledsoe and B. Rasco, 2003. Caviars and fish roe products. Crit. Rev. Food Sci. Nutri., 43: 317-356.
3. Intarasirisawat, R., S. Benjakul and W. Visessanguan, 2011. Chemical compositions of the roes from skipjack, tongol and bonito. Food Chem., 124: 1328-1334.
4. Rosa, A., P. Scano, M. Paola, M. Deiana, A. Atzeri and M. Assunta Dessi, 2009. Oxidative stability of lipid components of mullet (*Mugil cephalus*) roe and its products bottarga. Food Chem., 115: 891-896.
5. Periago, M.J., J. Rodrigo, G. Ros, J.J. Rodriguez-Jerez and M. Hernandez-Herrero, 2003. Monitoring volatile and nonvolatile amines in dried and salted roes of tuna (*Thunnus thynnus* L.) during manufacture and storage. J. Food Protection, 66: 335-340.
6. Krizek, M., F. Vacha and T. Pelikanova, 2011. Biogenic amines in carp roe (*Cyprinus carpio*) preserved by four different methods. Food Chem., 126: 1493-1497.
7. Chaijan, M., 2011. Physicochemical changes of tilapia (*Oreochromis niloticus*) muscle during salting. Food Chem., 129: 1201-1210.
8. Frangos, L., N. Pyrgotou, V. Giatrakou, A. Ntzimani and I.N. Savvaiddis, 2010. Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiol., 27: 115-121.
9. Gallart-Jornet, L., T. Rustad, U. Erikson, I. Escriche and P. Fito, 2007. A comparative study of brine salting of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*). J. Food Engin., 79: 261-270.
10. Andesen, E., M.L. Andersen and C.P. Baron, 2007. Characterization of oxidative changes in salted herring (*Clupea harengus*) during ripening. J. Agri. Food Chem., 55: 9545-9553.
11. Ghomi, M.R., M. Sohrabnejad and M. Zarei, 2011. Growth rate, proximate composition and fatty acid profile of juvenile kutum *Rutilus frisii* kutum under light/dark cycles. Jordan J. Biol Sci., 4(1): 37-42.
12. Ghomi, M.R. and M. Nikoo, 2010. Fatty acid composition of kutum *Rutilus frisii* kutum Roe: The Effect of Fish Size. World Appl. Sci. J., 11(4): 470-472.
13. AOAC, 2005. Official methods of analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg.
14. Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

15. Caprino, F., V.M. Moretti, F. Bellagamba, G.M. Turchini, M.L. Busetto, I. Giani, M.A. Paleari and M. Pazzaglia, 2008. Fatty acid composition and volatile compounds of caviar from farmed white sturgeon (*Acipenser transmontanus*). *Analytica Chimica Acta*, 617: 139-147.
16. Rodrigo, J., G. Ros, M.J. Periago, C. Lopez and J. Ortúño, 1998. Proximate and mineral composition of dried salted roes of hake (*Merluccius merluccius*, L.) and ling (*Molva molva*, L.). *Food Chem.*, 63: 221-225.
17. Gessner, J., M. Wirth, F. Kirschbaum, A. Krüger and N. Patrice, 2002. Caviar composition in wild and cultured sturgeons-impact of food sources on fatty acid composition and contaminant load. *J. Appl. Ichthyol.*, 18: 665-672.
18. Afkhami, M., A. Mokhlesi, K. Darvish Bastami, R. Khoshnood, N. Eshaghi and M. Ehsanpour, 2011. Survey of some chemical compositions and fatty acids in cultured common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), Noshahr, Iran. *World Journal of Fish and Marine Sci.*, 3(6): 533-538.
19. Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, 56: 365-379.
20. Lyhs, U., J. Lahtinen, M. Fredriksson-Ahomaa, E. Hyytiä-Trees, K. Elfving and H. Korkeala, 2001. Microbiological quality and shelf-life of vacuum-packaged 'gravad' rainbow trout stored at 3 and 8°C. *Int. J. Food Microbiol.*, 70: 221-230.