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Chemical quality and microbiological content of Kutum (*Rutilus frisii kutum*) roe processed in different brine concentration during storage

Parastoo Pourashouri[1*], Bahareh Shabanpour[2], Zeinab Noori Hashem Abad[3]

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Abstract

Caviars represent the best-known form of fish roe products. The conventional method of roe processing includes saturated brine salting. However, despite the importance of these products, there is relatively little technical information available about their chemical composition, product quality and food safety attributes.

Three experimental treatments were provided with kutum roe brined in 10, 18 and 24% sodium chloride solutions for 14 days (24°C). Then, the brined-roes were removed from the solution and stored at 4°C for 90 days in refrigerator. The contents of proximate compositions, salt, volatile base nitrogen (VBN), total psychrotrophic bacteria and histamine forming bacteria, color were measured. Sampling was carried out at the first and at the end of days 30, 60 and 90 of storage period.

The samples brined in 10% solution putrefied during the brining and removed from study. The moisture and total volatile nitrogen content of 24% brined roes were lower than 18% treatment. The pH and histamine forming bacteria number at the end of storage and total psychrotrophic bacteria number after 60 days of storage were higher. The increase of L* value and the decrease of a* value in samples of brine 18% were observed on days 60 and 90 of storage, but this increase was induced only on the day 90 for samples of brine 24%.

18%brined roe showed acceptable chemical and microbial results in refrigerated condition, and 24% brine roe appeared optimal during storage period.

Keywords: Kutum, roe, Shelf life, Brine concentration

Introduction

Marine by-products have been reported as good sources of nutraceuticals as well as functional food ingredients (Rao, 2014). Fish eggs (roes) are highly perishable with short shelf-life and hence to be processed immediately (Narsing Rao *et al*., 2012). The considerable quantity (about 27% of the total body weight) of fish roes could be produced during spawning season. Roes are rich in polyunsaturated fatty acids (PUFA), amino acids and proteins depending on the variety of fish (RAO, 2014; Balaswamy *et al*., 2007; Lapa-guimarães *et al*., 2011). Caviar is a processed food originated from the aquatic animal's roes that salted and cured after separation of connective tissues. Sturgeon fish caviar is a well-known product traditionally comes from Caspian Sea littoral states (Bledsoe *et al*., 2003). Many other fish species (e.g., catfish, salmon, lumpfish, flying fish, herring, capelin, mullet and cod) have been used for making different kinds of caviar and consumed in the worldwide (Lapa-guimarães *et al*., 2011; Bledsoe *et al*., 2003; Shin *et al*., 2007).

Salting is one of the preserving techniques of fish and fishery products. This method has been used over the centuries (Chaijan, 2011). It mainly causes to the reduction of water activity and thus inhibits the growth of spoilage microorganisms (Goulas and Kontominas, 2005). Two main types of salting methods include dry and wet salting. The wet salting allows fish and roe to immerse in a strong brine or pickle. Higher brine concentration lead to increase the water phase salt content of fish products (Chaijan, 2011).

In northern fish markets of Iran, a large part of Kutum (*Rutilus frisii kutum*) roes are an underutilized by-product which is removed

1, 2 and 3. Assistant professor, Professor and PhD student, Department of Seafood Processing, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
(*-Corresponding Author: Pourashouri.p@gmail.com)
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during processing. The roes are highly perishable. Preparation of traditional salted roe from fully developed kutum gonads (i.e. Ashbal) for storing at room temperature and its physicochemical properties were studied earlier (Pourashouri *et al.*, 2015). The traditional processing affected the proximate and fatty acid composition. However, the fresh roes were found to be more acceptable than heavy salted roes in terms of healthy product. On the other hand, it was reported that the light salt processing (3.5- 4.8%, w/w) did not affect the proximate composition, total amino acids and fatty acids composition compared to fresh roes (Balaswamy *et al*., 2007). The kutum roes containing 61-63% moisture, 28-29% protein and 1.3% ash. The lipid content is 6-7%, which following the composition: 4.37% docosahexaenoic acid and 5.13% eicosapentaenoic acid (Pourashouri *et al.*, 2015).

Some studies showed that light brine salting promotes better yield and water holding capacity than saturated brines (Martinez-alvarez *et al*., 2005). Furthermore, to the best of our knowledge, no work was carried out previously by different salt concentrations on chemical and microbiological properties of kutum roe pickle during storage. The objective of the present work was to study the effect of different brine concentrations (10, 18 and 24%) on kutum roes and assess the physicochemical and microbiological properties and shelf-life during storage.

Material and method

Preparation of samples

The roes of sixty kutum (*Rutilus friisi kutum*) were obtained from a local fish market (March 2013, Bandar-Anzali, Gilan Province) immediately after dressing of live fish. The roes (350± 40 g) were thoroughly cleaned to remove adhering fat deposits, blood vessels and washed in fresh water. Roes were not separated from skin (a sac which covers roes). Each roes (as a replicate) were subjected separately to the pre-treatments and then soaked in one of the brines consisted of 10, 18 and 24% sodium chloride solution in plastic containers (2 weeks). The containers were kept at room temperature (24± 2°C); solid to liquid ratio was maintained at 1:4 (w/w) (Balaswamy *et al*., 2010). Subsequently, the roe was allowed to drain using plastic baskets for 1 h, wrapped in polyethylene bags and stored in refrigerator (4± 1°C) for 90 days. Sampling was carried out on days of 1, 30, 60, and 90 of the storage.

Chemical analyses

The moisture, crude protein and ether extract contents of the brined-roe were measured according to the AOAC (1990). The pH was measured by using single electrode of a digital pH meter (Metrohm 713 pH meter, Germany) (AOAC, 1990). The amount of salt present in the samples was determined by silver nitrate titration for the chloride ion (Hwang *et al*., 2012). Total volatile basic-nitrogen (TVB-N) was determined in two steps, according to the method of Howgate (1976). At first, to obtain protein-free extracts of brined-kutum roe, 10 g of sample were homogenized with 20 ml 5% trichloroacetic acid for 1 min using an Ultra-Turrax apparatus. The homogenate was centrifuged (1200 x g, 4 min, 18°C) and the extract filtered through filter paper. The precipitate was washed twice with 10 ml 5% TCA, centrifuged and filtered again. The extracts were collected and diluted to 50 ml with 5% TCA in a volumetric flask and kept refrigerated at 4°C until required for further analysis. Then, the deproteinized kutum roe extracts (20 ml each) were steam distilled using a Kjeldahl instrument and the ammonia collected in 4% boric acid containing methyl red/bromocresol green (indicator). The solution was titrated with 0.02 M HCl solution and quantified by mg TVB-N/100g of tissue.

Color measurements

Color measurements of samples were objectively secured using Lovibond (CAM system500). Samples were placed in Petri to occupy the center of the dish. The unit was calibrated using a standard plate supplied by the manufacturer. Individual measurements

were conducted using 5 different roe samples, from which mean measurements were statistically computed. Color measurements employed CIE (Commission Internationale d'Eclairage of France) color system using L* (lightness), a* (redness), and b* (yellowness) color values (Tahergorabi *et al*., 2012).

Microbiological analysis

Changes in bacterial population of the brined-kutum roes were monitored at the same time with chemical analyses. To enumerate the bacterial population, 25 grams of the brined roe was homogenized by a stomacher (P.B.I. Milan, Italy) at high speed for 4 min in 225 ml of sterile saline phosphate buffer (0.05 M, pH 7.0). Serial dilution was made and diluted bacteria then spread onto agar plate. Total psychrotrophic bacteria were enumerated on plate count agar (Merck, Germany) at 4°C for 5 days. Enumeration of coliforms and *Escherichia coli* were carried out respectively on violet red bile agar (Merck, Germany) and MacConkey agar (Merck, Germany) and the plates were incubated in anaerobic jars (Anaerocult A; Merck, Darmstadt, Germany) at 37°C for 48 h. For quantitative detection of histamine-forming bacteria, 0.1 ml aliquots of the appropriate dilutions was spread on a specific medium introduced by Niven et al (1998) and consisted of 0.5% tryptone, 0.5% yeast extract, 2.7% L-histidine.2HCL, 0.5% NaCl, 0.1% CaCO3, 2.0% agar, and 0.006% bromocresol purple (pH 5.3). Histamine-forming bacteria cultured on the agar plates for 4 days at 35°C. After counting the number of colonies on each plate, the number so obtained was multiplied by the inverse of the dilution and the result was stated as the number of colony forming unit (cfu) in 1 gram of the sample (Downes *et al*., 2001).

Statistical analysis

The data were subjected to a completely randomized design with repeated measures. Comparison of means was performed using a Tukey method. All statistical analyses were performed with the SAS system (2003) with the significance level set at $\alpha = 0.05$ and the variability was expressed as standard error of mean (SEM).

Results and discussion

In the present experiment, NaCl concentrations of brine noticeably affected the roe shelf life; as the samples brined in 10% NaCl-solution putrefied during the brining with the change of their appearance and stench and so removed from the study. The results of the two other experimental treatments (18 and 24% brine concentration) during the storage on moisture, and chemical characteristics of salted kutum roe are presented in Table 1.

Table 1- Changes of moisture, dry and salt contents of salted kutum roe during storage

treatment	Day 1	Day 30	Day 60	Day 90
Moisture (%)				
18%	0.57 ± 64.50	0.85 ± 64.00	0.53 ±63.62	1.28 ± 63.45
24%	0.98 ± 62.04	0.51 ± 62.16	0.57 ± 61.92	0.41 ± 61.74
Salt (%)				
18%	0.10 ± 3.35	0.06 ± 3.37	0.07 ± 3.47	0.02 ± 3.52
24%	0.03 ± 4.15	0.03 ± 4.18	0.05 ± 4.23	0.03 ±4.26
Lipid (%)				
18%	0.57 ± 6.24	2.78 ± 6.29	0.60 ±6.24	1.28 ± 6.07
24%	0.98 ± 6.25	0.18 ± 6.63	2.07 ± 6.19	0.15 ± 6.16
Protein (%)				
18%	0.64 ± 22.56	0.23 ± 22.42	0.21 ± 22.53	0.19 ± 22.47
24%	0.32 ± 22.41	0.23 ± 22.57	0.07 ± 22.38	0.17 ± 22.35

[a,b] Different letters within each column represent significant differences($p<0.05$).
[A,B] different letters within each row represent significant differences ($p<0.05$).

The protein and lipid content of the samples were not affected by brine concentration and/or storage time (Table 1). In contrast to the moisture, samples of 24% brine treatment

had a greater salt content than 18% brined-roes. According to the authors' observation on putrefied samples, it found that the light NaCl concentration is not suitable for salting of kutum roe, and had no positive effect as a preservative on crude roe. In agreement with our results and findings of Shabanpour *et al* (2017), which suggested higher percentage of pure and mixed salt for preserving of trout roe (5.5 %). Salt penetrates into the roe by dialysis and water diffuses out of the roe by the osmotic pressure. They have shown that higher protein aggregating which could led to dehydration of more salted samples and so decrease water holding capacity (Shabanpour *et al.*, 2017). Moreover, lower moisture content in the samples of brine 24% could be explained by dynamic mutual diffusion process (Madadlou *et al.*, 2007) induced between the brine salt and roe moisture according to their gradient differences. Therefore, higher simultaneous uptake of NaCl in the concentrated brine increased the salt content in roes and thus led to water diffusion out through them (Madadlou *et al.*, 2007).

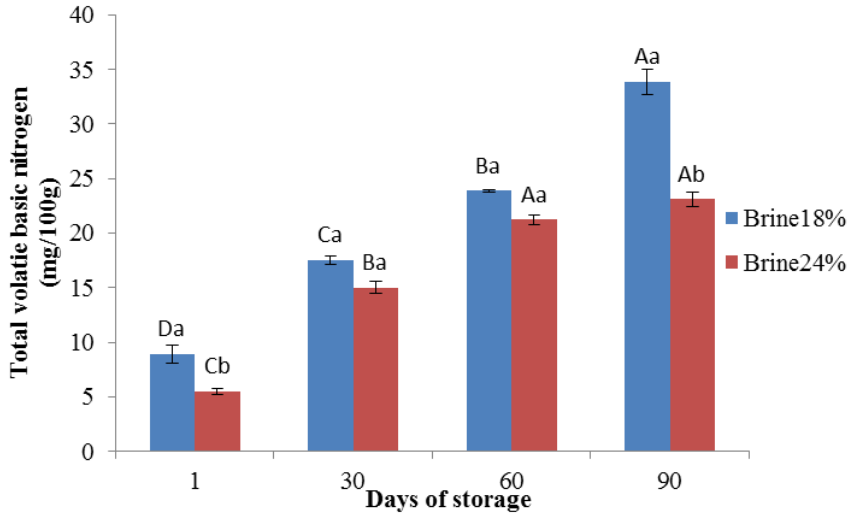

Fig. 1. Total volatile basic nitrogen (mg/100g) value of salted kutum roe during storage. Error bars indicate SEM. Different small letters are significantly different (P< 0.05) between treatments. Different capital letters are significantly different (P< 0.05) during storage.

The interaction of brine concentration× storage day was significant for TVB-N and its content in brined-roes increased during storage (P< 0.05). Amount of TVBN in 18% brined samples was higher (8.93 to 33.83 mg/100g) than 24% brined samples (5.46 to 23.1 mg/100g). The TVB-N has been used as quality indicators for aquatic protein sources and includes several compounds such as ammonia, and also mono-, di-, and tri-methylamine, which can be formed by bacterial or endogenous enzymatic (Lapa-guimarães *et al.*, 2011). According to the literature processing, condition and length of the storage have a significant effect on TVB-N content of fresh crude roe (Lapa-guimarães *et al.*, 2011; Kung *et al.*, 2009). Periago *et al.*(2003) reported that the amount of tuna fish roe TVB-N doubled during the 8 weeks of refrigeration at 4°C. Although, it has not been reported a safe range for the amount of TVB-N in fish roe product (Lapa-guimarães et al., 2011), the European Community has determined the maximum limit of TVB-N for consumption of 35 mg of TVB- N per 100 g of fish muscle. Furthermore, The Chilean Official Organization established the maximum TVB-N level in salted and dried fish products at 150 mg N/100 g of sample (Lapa-guimarães et al., 2011). There are relationship between amount of volatile nitrogen base and increasing of pH and bacterial activity (Shabanpour *et al.*,

2015).

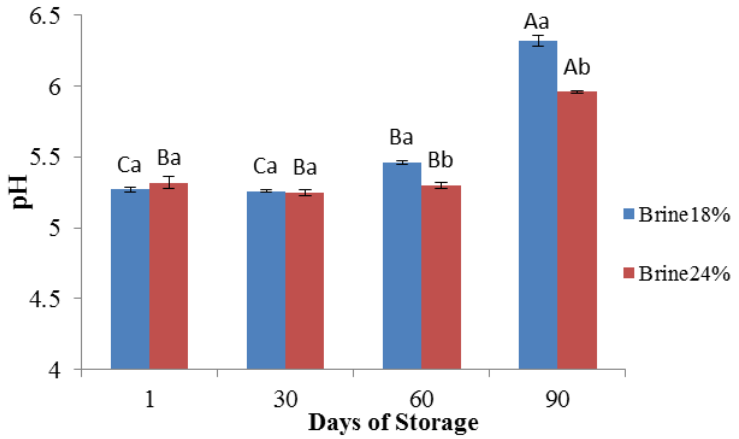

Fig. 2. pH of salted kutum roe of salted kutum roe during storage.
Error bars indicate SEM. Different small letters are significantly different ($P < 0.05$) between treatments.
Different capital letters are significantly different ($P < 0.05$) during storage.

The effects of treatments on pH value and microbial content of salted kutum roe are shown in Fig. 2- 4. The pH value of samples of brines 18% and 24%, significantly increased on days 60 and 90 of storage, respectively. It was increased progressively during the storage of the samples. In 18% brined samples pH increased from 5.27 to 6.32 while in 24% brined samples, pH increased from 5.33 to 5.96. The percentage of salt in 18% and 24% brined samples at the end of storage was 3.52 and 4.26%, respectively. This was presumably due to the production of volatile basic components, such as ammonia, trimethylamine. By the addition of salt, pH reduction which is the result of increasing the ionic strength of the solution inside of the cells occurs (Goulas and Kontominas, 2005). Shabanpour *et al.* (2015) reported pH value of dry-salted roe of rainbow trout by 3.5 % pure salt reached to 7.5 after 60 days of storage. They proposed higher percentage (5.5%) of salt for preserving of salted roe. It's previously reported that the pH value of brined roe has an increasing trend to the basic scale during long-cold storage, which is depending on brine concentration (Inanli *et al.*, 2010).

The sensitivity of microorganisms to NaCl concentrations was found to be different because of static or cidal effects of salt ion osmotic pressure (Hwang *et al.*, 2012; Bassin *et al.*, 2011).

None of these samples contained *Coliforms* or *E. coli*. The total counts of psychotropic bacteria in 18% brined roe during 30 days of cold storage was markedly higher than in the 24% brined roe (3.39 and 2.24 log cfu/g, respectively). For 18% treatment, after 90-day-storage, psychrotrophic bacteria was enhanced (3.48 to 4.42 $\log^{10}$ cfu/g) compared with the 24% brined sample (2.07 to 3.74 log cfu/g), respectively. At the end of storage, there were no significant difference of psychrotrophic bacteria between two treatments (4.42 & 3.74 logcfu/g, respectively). The accepted amount of psychrotrophic bacteria in caviar 5 log cfu/g have been determined (Iranian National Standards, 1995). According the results, the total bacterial counts in all samples were within acceptable limits (less than 5 log cfu/g). Shabanpour *et al* (2015) found that salted roe by higher percentage of salt (5.5%) (5.17 log cfu/g) had lower psychrotrophic bacteria than 3.5% dry-salted roe. According to Shabanpour *et al* (2015) as a result of osmotic exposure of water and salting in effect limited bacterial growth and increased the shelf life of salted roe. Inanli *et al* (2011) reported that the adding

of acetic acid on salted rainbow trout roe had significant effect on reducing of psychotrophic bacteria.

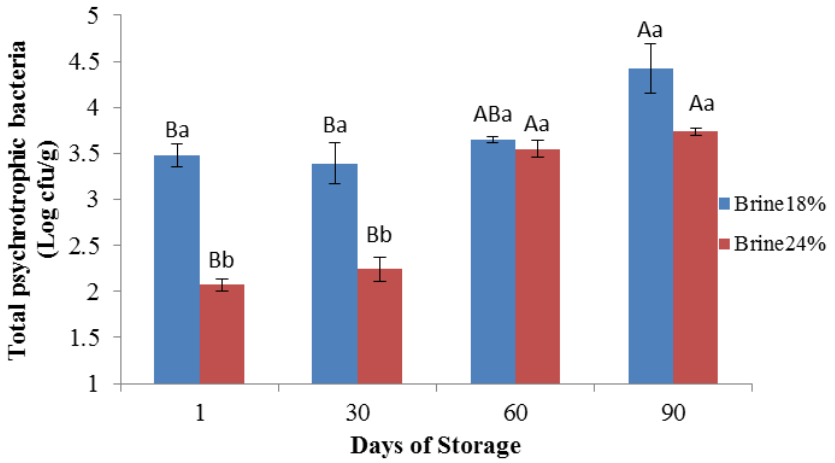

Fig. 3. Total psychrotrophic bacteria (Log_{10} cfu/g) of salted kutum roe during storage. Error bars indicate SEM. Different small letters are significantly different ($P< 0.05$) between treatments. Different capital letters are significantly different ($P< 0.05$) during storage.

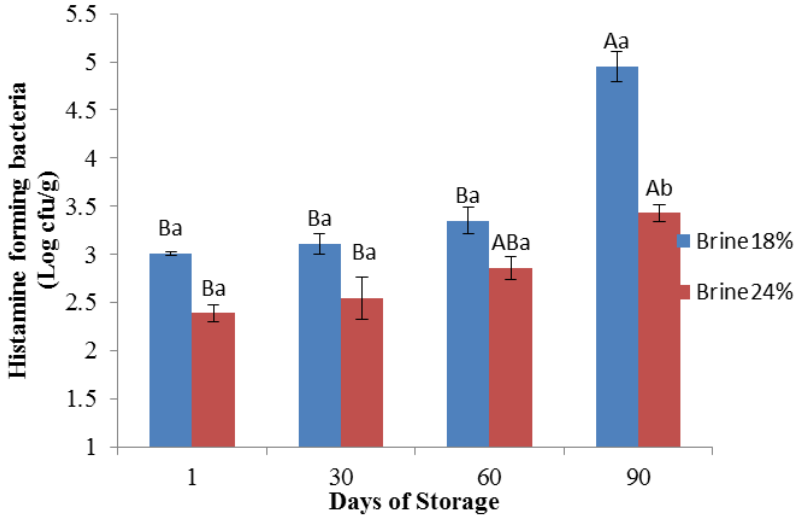

Fig. 4. Histamin forming bacteria (Log_{10} cfu/g) of salted kutum roe during storage. Error bars indicate SEM. Different small letters are significantly different ($P<0.05$) between treatments. Different capital letters are significantly different ($P<0.05$) during storage.

The presence of histamine-forming bacteria of 18% brined-roe significantly increased at the end of storage and was higher than 24% brined-roe (4.95 & 3.43 logcfu/g, respectively). Histamine-forming bacteria numbers of 24% brine samples on day 90 increased compared to the first of storage (2.39 to 3.43 logcfu/g). In this study, the effect of different brine concentrataions on HFB was also determined. The content of the histamine-forming bacteria in the 24% brined samples was less than 18% samples. This was according of Tsai *et al.*, (2007), reported that NaCl concentrations of 1.5% and 3.5% had a stimulatory effect on histamine formation, whereas concentrations of NaCl in excess of 7.5% inhibited its growth and histamine formation. Taylor and Speckard (1983) report that 0.5-2.0% NaCl did not inhibit the growth of *M. morganii* and *K.pneumoniae* or inhibit

their histamine production. Periago *et al.* (2003) reported the presence of total aerobic bacteria and histamine forming bacteria in salted (up to 15%) tuna roe. Also, Hwang *et al.* (2012) cultured, isolated and identified some of histamine forming bacteria in salted escolar roe products. Kung *et al.* (2015) have been isolated some histamine forming bacteria of various dried-salted fish products such as salted sardine and Spanish anchovies.

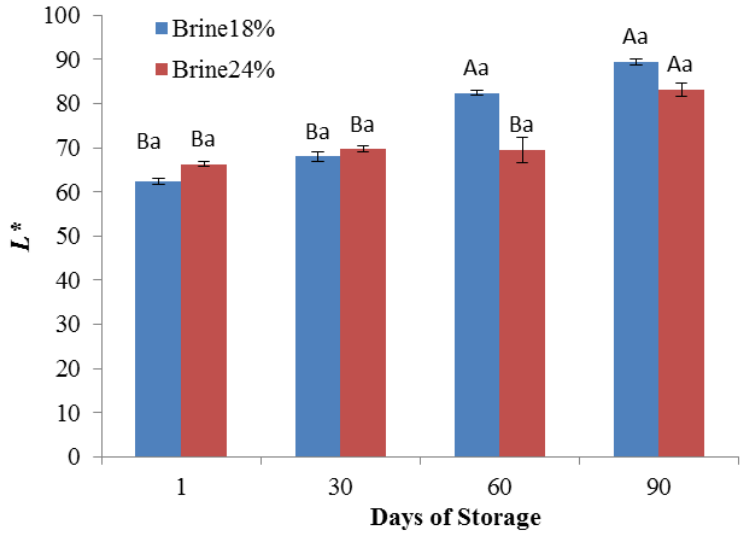

Fig. 5. Lightness (L*) of salted kutum roe during storage.
Error bars indicate SEM. Different small letters are significantly different ($P < 0.05$) between treatments.
Different capital letters are significantly different ($P < 0.05$) during storage.

The effects of experimental treatments on the color change of salted kutum roe during storage are presented in Figures 5-7. The interaction of brine concentration× storage was significant for color values. In storage period, L* value significantly increased in 18% brined roe (62.38 to 82.42 and 89.38). From the result, the lightness (L*) of salted roe in both treatments was found in the ranges of 62.38–89.38 during 90 days of storage (Fig. 5). No differences in L* value of roe treated with 18 and 24 % brine at the same time ($p>0.05$) were noticeable. However, the L* value tended to increase with increasing time for both concentration after 2 months of storage.

The redness-greenness (a*) of salted roe during storage are shown in Fig. 6. The results showed that a* value of in the ranges of 11.76 and 12 (18 and 24% treatment, respectively) at the first of storage, which then decreased throughout the storage (3.44 and 4.51) ($p<0.05$). This index in 24 % brined roe was higher than 18% brined samples at the end of storage. Thereafter, no differences in the a* value between salted roe prepared by both concentration were observed ($p>0.05$). For the yellowness–blueness (b* value) (Fig. 7), no significant differences was between treatments and during storage ($p>0.05$). The decrease in a*value at the end of the storage was possibly due to the excessive oxidation of both lipid resulting in the discolouration of roe samples (Chaijan, 2011). Shabanpour *et al* (2017) reported the redness value of rainbow trout roe decreased during storage and it was related to lipid oxidation and decrease of colorant. They also showed higher lightness in 5.5 of dry-salted treatments. The yellowness of dry-salted rainbow trout roe decresed during storage, that it was different with the current study which there is no significant diffrenes in this index.

Color measurement is an important quality parameter in processed fish products and effective on consumer preference. Carotenoids, such as lutein, astaxanthin, canthaxanthin, zeaxanthin, β- carotene, and β-

cryptoxanthin are the main source of the pigments in fish roe (Bekhit *et al.*, 2009), which could be affected by fish species, diet, age and maturity stage (Bledsoe *et al.*, 2003). On the other hand, some of carotenoids (e.g., Lutein) appear yellow at low concentrations and orange-red at high concentrations (William & Kalpana, 2014).

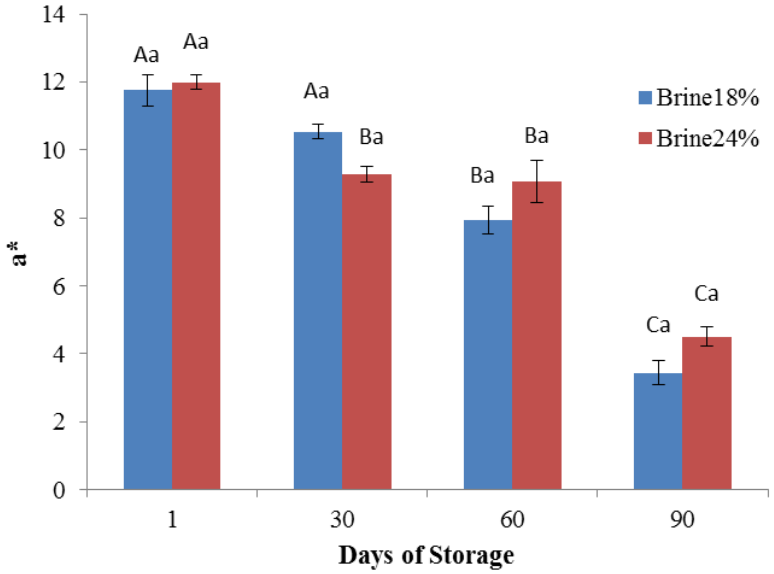

Fig. 6. Redness (a*) of salted kutum roe during storage.
Error bars indicate SEM. Different small letters are significantly different ($P < 0.05$) between treatments.
Different capital letters are significantly different ($P < 0.05$) during storage.

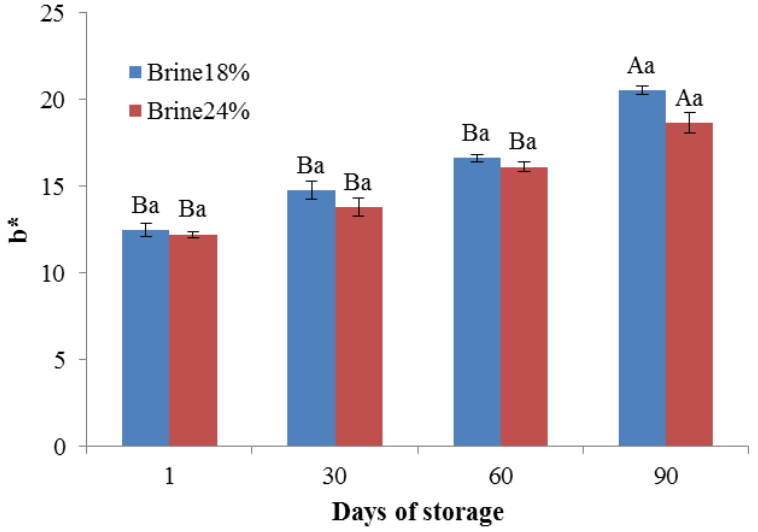

Fig. 7. Yellonewss (b*) of salted kutum roe during storage.
Error bars indicate SEM. Different small letters are significantly different ($P<0.05$) between treatments.
Different capital letters are significantly different ($P<0.05$) during storage.

Table 2. Color parameters of salted kutum roe during storage

treatment	Storage	ΔE	C^*_{ab}	h^*_{ab}
18%	1	3.62 ± 0.04^{De}	2.46 ± 0.08^{Dd}	180.81 ± 0.51^{Dc}
	30	3.69 ± 0.04^{Cd}	2.51 ± 0.05^{Cc}	180.95 ± 0.15^{Cc}
	60	3.85 ± 0.04^{Bb}	2.53 ± 0.05^{Bc}	181.12 ± 0.56^{Bab}
	90	3.92 ± 0.07^{Aa}	2.63 ± 0.06^{Aa}	181.40 ± 0.40^{Aa}
24%	1	3.67 ± 0.04^{Ce}	2.46 ± 0.04^{Bd}	180.79 ± 0.45^{Abc}
	30	3.70 ± 0.05^{Bc}	2.44 ± 0.04^{Bd}	180.97 ± 0.81^{Abc}
	60	3.71 ± 0.05^{Bc}	2.53 ± 0.05^{Ac}	181.05 ± 0.78^{Abc}
	90	3.89 ± 0.07^{Ab}	2.56 ± 0.05^{Ab}	181.33 ± 0.35^{Aab}

Different capital letters in each column show significant difference in each treatment ($P < 0.05$)
Different small letters in each column show significant difference between treatment ($P < 0.05$)

Therefore, a part of the change of a* value in the brined roe during the storage period (Fig 6) could be explained by the decrease of carotenoids concentration after their oxidation (William & Kalpana, 2014). However, the higher L* value of 18% brined roe could be due to the positive relation between moisture content and L* value (Bekhit et al., 2009). Regardless of the brine concentration, the ΔE value of salted roe (Table 2) increased significantly with the time of storage. On the other hand, chroma value of the samples (Table 2) also increased significantly ($P<0.05$) with storage. According to the color parameters, 18% brined-roe changed more than 24% brined samples. In general, both treatment at the first of storage showed a less yellowish appearance with in lower h* when compared to the end of storage. Total color differences was higher in 18% treatment in comparison of 24% brined samples ($P<0.05$).

The results obtained in this study demonstrated that 10% brine concentration has no protective effect on crude kutum roe. However, 18% brined roe showed acceptable chemical and microbial results in refrigerated condition, and 24% brine roe appeared optimal during storage period. Future studies on brined kutum roe should investigate the levels of histamine in this product and provide an appropriate packaging method to more control of carotenoids oxidation and microbial growth.

Acknowledgments

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Conflict of Interest

Authors have no conflict of interest to declare.

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کیفیت شیمیایی و بارمیکروبی تخم عمل‌آوری شده ماهی سفید (*Rutilus frisii kutum*) با غلظت‌های مختلف آب نمک در طی نگهداری

پرستو پورعاشوری[1*]- بهاره شعبانپور[2]- زینب نوری هاشم آباد[3]

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چکیده

خاویار به‌عنوان شناخته شده‌ترین شکل از محصولات تخم ماهیان است. تخم ماهی به‌طور سنتی و رایج در آب نمک اشباع نمک سود می‌شود. علیرغم اهمیت این محصولات اطلاعات تکنیکی اندکی در مورد ترکیب شیمیایی، کیفیت محصول و امنیت غذایی این محصولات وجود دارد. تخم ماهی سفید (*Rutilus frisii kutum*) به مدت 14 روز در سه تیمار آزمایشی در محلول‌های 10، 18 و 24 درصد آب نمک قرار گرفت. پس از خروج از آب نمک تخم ماهی سفید به مدت 90 روز در دمای یخچال (4 درجه سانتی‌گراد) نگهداری گردید. pH، ترکیبات تقریبی، نمک و بازهای نیتروژنی فرار (TVN)، باکتری‌های سرمادوست، کلی‌فرم‌ها، باکتری‌های تولیدکننده هیستامین و شاخص رنگ محصول مورد ارزیابی قرار گرفت. آزمایشات در ابتدای تولید و پس از 30، 60 و 90روز نگهداری انجام شد. تیمار 10% در طی مرحله نمک سود فاسد شد و مورد آزمایش قرار نگرفت. میزان رطوبت و TVN در تیمار 24% کمتر از تیمار 18% بود. pH و HFB در انتهای نگهداری و تعداد باکتریهای کل سرمادوست در روزهای 60 و 90 بیشتر بودند. افزایش شاخص *L و کاهش مقدار *a در نمونه‌های 18% در روزهای 60 و 90 مشاهده شد اما این افزایش در تیمار 24% تنها در روز 90 نگهداری مشاهده شد. فرآوری تخم ماهی سفید در آب نمک 18 درصد دارای شاخص‌های شیمیایی و میکروبی قابل‌قبولی در شرایط یخچال بود و تخم نمک سود شده در آب نمک 24 درصد شرایط بهینه را در طی نگهداری نشان داد.

واژه‌های کلیدی: تخم ماهی، ماهی سفید، مدت ماندگاری، غلظت آب نمک‌گذاری

1، 2 و3- به‌ترتیب استادیار، استاد و دانشجوی دکتری، گروه فرآوری محصولات شیلاتی، دانشکده شیلات و محیط زیست، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، ایران
(*- نویسنده مسئول : Email: Pourashouri.p@gmail.com)