Determination of the shelf life of smoked sea bass (*Dicentrarchus labrax* lineaus, 1758) marinade stored under refrigerated conditions (4 °C)

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Abstract

Keywords:

Sea bass
Hot smoking
Quality
Shelf life
Marinade

Article history:

Received 03.09.2018
Received in revised form
11.02.2019
Accepted 31.05.2019

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Introduction. In this study, two different marinade (plain and smoked) were prepared with seabass and determination of their shelf-life was aimed by investigating the quality according to organoleptic, chemical and microbiological parameters.

Material and methods. Fishes supplied from an aquaculture facility were brought to the laboratory under cold storage and filleted after cleaning process. First group (Group A: Plain) was put into the marination solution (Fish:solution ratio was 1:2, 1.68% alcohol vinegar, 6.4% salt and 0.01% citric acid) for 5 days. In second group (Group B: Smoked) fish were smoked and air cooled to the ambient temperature. Cold fish fillets were waited in the marination solution (Fish:solution ratio was 1:2, 1.2% alcohol vinegar, 5.5% salt and 0.01% citric acid) for 2 days.

Result and discussion. According to the results in terms of the chemical analyses, while TBA value was determined as $0,26 \pm 0,01$ mgMDA/kg in raw material, at the end of the study this value was determined as $4,38 \pm 0,05$ mgMDA/kg in Group A (5th month) and $3,05 \pm 0,02$ mgMDA/kg in Group B (7th month). According to results in terms of the microbiological analyses, TMAB count was determined as $2,00 \pm 0,04$ CFU/g in raw material but total bacteria and yeast-mold count was remained <10 CFU/g during the storage period. Coliform bacteria were not encountered in the study. For the organoleptic analyses 5 experienced panelists evaluated the products according to colour, odour, texture, flavour and general appreciation. Both groups got high scores in the beginning but Group A got low scores in 5th month and Group B had low scores in 7th month from panelists and lost quality.

Conclusion. As a result, smoked sea bass marinade was appreciated more than plain sea bass marinade and the shelf life of smoked marinade was detected higher than the plain marinade.

DOI: 10.24263/2304-
974X-2019-8-2-9

Introduction

Fish, which is essential for human health and contains a wealth of fatty acids, is a must consume food source for the sake of a healthy and high quality life. EPA and DHA fatty acids are known to have good use for human health. It's stated that, fish consumption has a positive effect on many illnesses like cardiovascular diseases, development of brain and intelligence, strengthening the immune system, hypertension, cholesterol, depression, amnesia, etc., an even various disease may occur when the lack of consumption [1,2] Seabass (*Dicentrarchus labrax* Linnaeus, 1758), which has a high nutrition value, is naturally found in seas around Turkey; besides it has been cultivated with great numbers in recent years. Total amount of sea bass production that has white meat, delicious taste and low fat content was 80.847 tones [3]. One of the most cultivated fish species in Turkey is seabass and not only it is consumed fresh; but it is exported frozen or processed as well.

With processing seafood, many products are on the market and alternatives are being created on fish consumption lately. Marinated products are on demand along with dried, smoked, salted and canned ones. Marinated products are generally processed by a seasoning process with certain amount of salt and acedic acid brine within a certain time. Ripening process in marinates is resulted from opposing influence of salt and acedic acid on fish meat. Salt provides toughness for fish meat; whereas, acid does smoothness. The ripened fish after a certain period is put into marinade containers and assorted sauce, oil, etc., are added on [4]. Then again shelf life of marinades may alter according to the factors like fish variety, acid/salt rate of brine, brining period and storage conditions. Salt and acid have an effect on decreasing bacteria and enzyme activities so shelf life extends [5]. On the other hand, shelf life of marinades can also be as short as 4-5 months due to the factors like organoleptic changes, rancidity and putrefaction [6]. As for smoking process; it expresses a number of methods in order to give fish a different color, smell, taste or flavor. In this context; first of all, the fish is steeped into salty water solution. After that it is smoked into a special oven. The product is dehydrated with the help of salting and drying; it is also made use of antimicrobials and antioxidants' conservativeness on the compound of smoking process [7-10]. Besides having positive effect on shelf life, smoking process provides high acceptability in view of sensitivity. A product is featured in sensitivity thus attracts the attention of customers especially in developed countries [11]

Either of them are fish conservation methods which have been widely used for long in fishery processing technology. In this research an outcome was tried to obtain by using both of the different technologies. Also in this study it is tried to determine the shelf life of seabass, which was marinated in two different methods (plain and smoked), and aimed to analyze the quality changes during stockpiling.

Material and method

100 Seabass (*Dicentrarchus labrax* Linnaeus, 1758) whose approximate lengths are 28.53 ± 0.12 cm and weights are 277.35 ± 2.45 g were brought to laboratory to under cold storage and these fish were supplied from Kuzey Su Ürünleri Fish Farm which is situated between Samsun-Sinop provinces in December 2017. After removing guts, beheading and cleaning, they were filleted without scaling. Before the process, fillets were steeped into the icy salted water (20%, 30 mins) until emptying their blood, later that they were washed off in order to leak all the extra water. All the fillets were divided into two groups. The first fillets

group (Group A) was plain marinated bass and the other group (Group B) was hot smoked. In order to maturing fish; a solution which is derived from diluting industrial marinade brine was used. On the other hand, smoking process was completed in semi traditional smoking oven in Sinop University Faculty of Fisheries Processing Laboratory using wood shavings by beech tree.

Fish processing

For plain seabass marinade (Group A), the fish was stored for 6 days in refrigerator circumstances. The rate of fish to brine is 1/2 and the brine consists of 1.68% alcohol vinegar, 6.4% salt and 0.1% citric acid. At the end of marinating, the fish were taken out of brine and were put to draining.

For second group (Group B) marinade; the fish was aligned onto baking trays then smoked. Smoking oven was adjusted to 60°C and the fish was kept in smoking oven for 45 min. as 20 min. pre-drying and 25 min. smoking process. After smoking process ends, the fish was taken to a colder place to cool off. After that it was put into marinade brine (F:B=1/2, 1.2% alcohol brine+5.5% salt+0.1 citric acid) and kept for 3 days in refrigerator. After the maturing process; the fish in both groups was aligned into marinade containers. Then the containers were filled with sunflower oil and covered. Consequently, the marinades were all stored in refrigerator.

Analyzing methods

The study was executed as 2 groups; on the other hand, executed as 2 recurrence analyses and 2 parallel analyses. During storage period the products' qualities were examined also it was tried to figure out the shelf life in refrigerator circumstances by the help of monthly sensory, chemical, physical and microbiologic analysis.

TVB-N amounts of fresh samples and products were modified by Antonacopoulos and determined by Lücke-Geidel method [12]. Quality classification of fish in TVB-N value was done according to Varlık et al. [13]. Amount of TBA was done according to Erkan and Özden [14]. Quality of fish in TBA value was evaluated according to Varlık et al [13] and determination of salt and vinegar was made according to Varlık et al. [15]. In pH measurement the fish was added into pure water as 1:1; then, measured with WTW Multi 340i portable pH-meter probe [16]. Color was analyzed with Minolta "CR-400 Chromometer" brand device according to CI L* a* b* scale and values were given as L* a* b*. White platform of the device was used for calibration [17]. Water activity was measured in Novasina brand automatic water activity measurement device.

The microbiologic analyses are bacterial counts which are total mesophilic aerobic bacteria, total psychrophile aerobic bacteria, yeast-mold bacteria and total coliforms. The exterior surfaces of packages were cleansed with 70% ethyl alcohol then opened with sterilized knife. 10 grams of fish samples were taken into stomacher bags with sterilized spatula and homogenized with adding in 90 ml peptone solution near the flame [18]. 10^{-1} - 10^{-6} rated dilutions were prepared using 1ml homogenization and 9ml physiological salty water solution (0.85% NaCl). Plate Count Agar was used in order to count the total mesophilic aerobic and psychrophile bacteria. The fish was incubated at 37°C for 2 days for petris mesophilic count and at 7°C for 10 days for psychrophile bacteria count in microbiologic analysis which was made with pour plate technique [19].

All colonies that were observed in petri was regarded as "total bacteria" and counted standardly; then, the result was given as LogKOB/g at the end of the incubation [20]. PDA

(Potatos Dextroz Agar) feedlot was used for yeast and mold counting. In the pour plate technique analysis, yeast-mold number was counted and specified after 3 days of incubation at 28°C and the results were given as LogKOB/g [13,21,22]. VRBA (Violet Red Bile Agar) (Lab M 31) feedlot was used for total coliform bacteria counting. Petri boxes were turned upside down then incubated for 1 day at 35°C after feedlots were frozen [21]. A group of 5 experienced panelists were chosen for sensory analysis and they were given forms in order to evaluate the products. The sensory evaluation form was modified according to the chart, which is expressed for marinated products, notified by Varlık *et al* [13] and used by Schormüller [23]. The products were evaluated by giving them ranks between 0-5 from the points of color, smell, taste, texture and general liking (0-1: inconsumable, 1-2: Bad, 2-3: Nat bad, 3-4: Good, 4-5: Very good) and those which ranked under 2 points were specified as inconsumable.

Medians and standard errors of the results were analyzed by one-way analysis of variance and tukey's test with the help of Microsoft Excel 2010 and Minitab 17 packaged software [24].

Results and discussion

Now that the products keep their chemical and microbiologic quality during storage period, shelf life of them was determined by sensorial perception. The beginning of the experiment expresses the first day of study while the end it expresses the 5th month for Group A (plain seabass marinade) and 7th month for group B (smoked seabass marinade).

Chemical and physical analysis results

The TVB-N, TBA, pH analyses results and water activity(*aw*) values of both samples were given in Figure 1. In the present study, TBA value of fresh samples were found as 0.26 ± 0.01 mg MDA/kg. After marination of fresh samples, A and B groups were also examined with regard to TBA values. Due to the applied long time marination process to group A, Fish oils were partially degradation and the TBA value was determined as 3.52 ± 0.09 increasingly in this group at the end of the marination process. In group B, due to the antioxidant effect of smoke compounds TBA value of group B showed less increased than group A and it determined as 1.61 ± 0.13 mg MDA/kg.

During storage time, it was observed that there were increases for both groups but it was also detected that this increase could not surpassed over 7-8 mg MDA/kg value which is the minimum consumable rate. The value differences between the groups were found significant both for the beginning and the end of the study ($p < 0.05$).

The change of fat rancidity depending on time was examined with TBA analysis. The analysis results were evaluated by considering the criteria that were displayed by Schormüller [25] and were stated by Varlık *et al*. [13]. Baygar *et al*. confirmed in his study that TBA value of crude material as 0.30 while the value of material in marinate (that is scaled) as 4.25 mg MDA/kg [26]. Alparslan *et al*. stated that seabass marinade did not exceed 8 mg MDA/kg which is the highest consumable limit at the end of 90 days of storing period [27]. Likewise, Kaya detected that seabass marinade did not exceed the limit of TBA value during 200 days of storing period [28].

Balıkçı observed that TBA value of both plain and dill added smoked mackerel marinade increases during 9 months of storing time though the value kept under specified limits [29]. Günlü stated that TBA values of products increase when he examined the quality changes of

seabass after smoking; however, none of the party's TBA value exceeds the consumable limit during storing period [30]. The results are compatible with present study. The reason is Group A spent more time in maturing brine; thus, there was more deterioration of fat. It could also be said that the reason why Group B has less TBA value during storing period was originated from smoking process. Goulas and Kontominas indicated in their study on mackerel that in the course of storing, the low value of TBA is caused by phenolic matters in smoke compounds and these matters have anti-oxidative effect [31].

According to TVB-N analysis, TVB-N value of fresh seabass was determined as 17.11 ± 0.28 mg/100g and this TVB-N value was identified as 11.66 ± 0.44 mg/100g for group A, 9.97 ± 0.33 mg/100g for group B after marination proces decreasingly. The reason for this decrease in TVB-N values can be said that to be the decrease in the pH value due to fish meat waits in brine water.

Since both marinate groups partially lost their acidic properties over time, TVB-N values increased somewhat in both groups at the end of the experiment and TVB-N values of Group A and Group B were determined as 17.41 ± 0.17 , 17.63 ± 0.22 mg/100g respectively. The difference between two groups was found significant ($p < 0.05$).

One of the analyses which is considered a freshness indicator of seafood is TVB-N analysis. The results were evaluated by regarding the criteria like; samples containing 25mg/100g TVB-N as “very good”, 30mg/100g TVB-N as “good”, 35mg/100g TVB-N as “marketable”, and samples containing more than 35mg/100g TVB-N as “spoiled” [13]. Baygar et al., found the TVB-N value of fresh sample as 16.89 mg/100g, and stated that the sharp fall of TVB-N amount was caused by pH decrease which was affected from acetic acid used in the brine [32]. Kaya, stated that TVB-N value of fresh seabass as 15.24 ± 0.02 mg/100g decreases down to 13.12 ± 0.58 mg/100g for plain marinades on marination day, 13.33 ± 0.11 mg/100g for sauced marinades; he also stated that these values do not exceed the consumable limit rates in time [28]. Present study shows similarity to previous researchers' results.

In present study, pH value of fresh seabass was measured as 6.47 ± 0.01 . After marination process, pH value of Group A and B were determined as 4.17 ± 0.02 and 4.38 ± 0.03 respectively resulting from acidic treatment.

pH value of fresh fish is expected to be between 6-6.5 with the changes depending on the specie, size, season and rigor mortis [13]. Kaya, determined the fresh seabass pH value as 6.04 ± 0.04 [28], whereas Taşkaya et al. determined as 6.45. pH interval of marinated products is very significant [33]. pH interval is supposed to be between 4-4.5 for marinated products [5,13]. Therefore, it is important both to prevent bacterial growth and to emerge some flavoring matters. Likewise, Alparslan et al. detected the fresh pH value as 6.35 for seabass while marinated product's pH value was as 4.32 [27].

Kaya, measured the beginning pH value of plain seabass marinade as 3.83 ± 0.05 while Çakır; found the beginning pH value of anchovy marinade as 3.75 [28,34]. Seeing that the acid rate that researchers used at their researches is more, those pH values are below from present study. Most researchers stated that the beginning pH values are between 4-4.8 for marinated products [32,35,36]. Even though the research results share similarity to present study, it is considered that the low rate of acid in the brine affected pH value.

When the water activity is investigated, the values which were measured for Group A and B found respectively as 0.932 ± 0.002 and 0.921 ± 0.001 at the beginning of the experiment and as 0.940 ± 0.001 and 0.931 ± 0.001 at the end of the experiment; the differences between them were found significant ($p < 0.05$).

Işıdan detected the a_w value of fresh fish in his shad marinade as 0.995 ± 0.001 but it was 0.953 ± 0.001 at the beginning of the experiment [37]. Giuffrida et al., detected that a_w value of his seabass marinade decreased after marinating and during storing time [38]. Previous

researches indicate increases in a_w value for the products originating from the water holding capacity of salt; so the results show similarity to present study.

As it is known, a_w values decreased due to brine and smoking effects so the water, which is necessary for microorganism growth, removed partially. On the contrary, Huss et al., stated that falling of a_w value, along with pH, restricts or stops bacterial growth after marinating [39].

Figure. 1. The TVB-N, TBA and pH analysis results and water activity values of group A and group B

Monthly analyses were conducted in order to determine the percent of salt and percent of vinegar (total acidity) in fish meat. The salt and percent of vinegar (total acidity) analyses results in sea bass marinade samples were given in Figure 2.

After marination process, Salt and vinegar contents of Group A and B increased to $\%3.73 \pm 0.17$, $\%3.02 \pm 0.05$ and $\%1.03 \pm 0.01$, $\%0.86 \pm 0.01$ respectively ($p < 0.05$). As the marinated samples lost their water during storage, it was observed that salt content increased and as the marinated samples waited in package with oil, vinegar content declined in both groups.

Also, in which there were irregular rises and falls of acidity-salt values, it is considered that keeping the fish in brine before process as well as the acidity-salt rate in the maturing brine could affect these values. Smoked product's being low in acidity and salt values than Group A can be caused by smoking process in addition to the reason above

When salt amounts were examined both for Groups A and B on the last day of the experiment, they were detected as 4.00 ± 0.02 , 3.45 ± 0.03 respectively, As of vinegar amounts were detected as 0.60 ± 0.03 , 0.43 ± 0.02 respectively. The difference between them was found statistically significant ($p < 0.05$).

Kaya, detected the beginning value of acidity and salt of seabass marinade as 1.97 ± 0.12 and 5.09 ± 0.04 and also stated that those values declined on account of time [28]. Çakır, detected the beginning (control) value of acidity and salt for anchovy marinade in plastic container respectively as 4.50 ± 0.02 and 0.85 ± 0.01 he also stated that there were irregular increases in acidity and salt values because of time [34].

Figure 2. Salt (%) and total acidity (%) analysis results of group A and group B

The color analysis results were given in Table 1. The quantitative differences of changes in products' physical appearances were revealed with color analysis. Each value (L^* , a^* , b^*), which was measured in the device, reveals a different color notion and the changes during storing. When the results which were acquired by the measurements from 2 different spots of fish fillets were examined and L^* (brightness) value of fresh fish was determined as 41.43 ± 1.23 . L^* values of Group A and B were determined respectively as 78.50 ± 0.58 and 67.36 ± 0.81 on the first day of the experiment ($p < 0.05$). The reason might be the decolorizing feature of acid in the ripening brine.

a^* value is 1.64 ± 0.45 in fresh sample and it was found at the beginning of the experiment as -3.58 ± 0.24 for Group A and as 4.87 ± 0.49 for Group B ($p < 0.05$). While b^* value of fresh fish is -3.16 ± 0.38 , this value is detected at the beginning of the experiment as 2.64 ± 0.70 for Group A and 24.84 ± 0.59 for Group B. The measured a^* and b^* values were higher for both groups than fresh sample, in other words it was observed that there were changes in product color. Especially, emerging highly positive of b^* (24.84 ± 0.59) value which was measured in Group B proved that the product was yellowish which is desired to be so for smoked products. This proves that the smoke demonstrably affects the color. The differences between two groups was found significant ($p < 0.05$).

L* (brightness) value is measured between 0-100 and it expresses the color of a product is getting from dark to light as it gets closer to 100. a* value, which is measured in coloring device, is depicted by greenish color when it is negative; whereas it is depicted by reddish color when it is positive. b* value is depicted by bluish color when it is negative; whereas it is depicted by yellowish color when it is positive.

Bilir, measured the beginning L*, a* and b* values respectively as 41.63, 0.64 and 9.8 and detected that L* value increases, a* value comes out positive, and b* value is higher for fish meat after ripening, in his marinade study made by sardine [40]. Kadak, calculated the fresh L* value as 40.01, a* value as 0.29, and b* value as 9.14 of chitosan added anchovy marinade. He found these values on the first day when he marinated the fish respectively as 63.65, 1.71, and 12.22. These changes in fish meat are accordant with the data of present study [41].

Table 1

Color analysis results of group A and group B

Storage time (Month)		A	B
Fresh Fish		41.43±1.23 ^{Aa}	41.43±1.23 ^{Aa}
First day		78.50±0.58 ^{Abc}	67.36±0.81 ^{Bb}
1st		75.11±0.96 ^{Ac}	71.76±0.55 ^{Bc}
2nd		76.92±1.23 ^{Ac}	72.98±1.04 ^{Bc}
3rd	L*	78.34±0.44 ^{Abc}	69.88±0.53 ^{Bbc}
4th		82.22±0.24 ^{Ab}	69.73±0.30 ^{Bbc}
5th		67.52±1.29 ^{Ad}	62.75±0.24 ^{Bd}
6th		-	58.82±1.45 ^e
7th		-	60.13±0.31 ^{de}
Fresh Fish		1.64±0.45 ^{Aa}	1.64±0.45 ^{Aa}
First day		-3.58±0.24 ^{Abd}	4.87±0.49 ^{Bbc}
1st		-3.61±0.13 ^{Abd}	3.72±0.34 ^{Bcd}
2nd		-1.05±1.15 ^{Ac}	1.24±0.44 ^{Aa}
3rd	a*	-1.45±0.24 ^{Abc}	2.58±0.2 ^{Bad}
4th		-3.24±0.11 ^{Abcd}	4.93±0.07 ^{Bbc}
5th		-3.92±0.29 ^{Ad}	4.10±0.23 ^{Bbcd}
6th		-	5.68±0.60 ^b
7th		-	5.16±0.16 ^{bc}
Fresh Fish		-3.16±0.38 ^{Aa}	-3.16±0.38 ^{Aa}
First day		2.64±0.70 ^{Abc}	24.84±0.59 ^{Bbc}
1st		1.00±0.49 ^{Ac}	23.70±0.81 ^{Bc}
2nd		4.97±0.81 ^{Abd}	23.91±1.04 ^{Bc}
3rd	b*	6.90±0.35 ^{Ad}	27.17±0.33 ^{Bb}
4th		9.28±1.03 ^{Ad}	24.60±0.19 ^{Bbc}
5th		5.62±0.74 ^{Abd}	23.28±0.33 ^{Bc}
6th		-	24.09±0.47 ^c
7th		-	25.26±0.20 ^{bc}

Values with different letters in the row (→) (A, B,...) and in the column (↓) (a, b,...) differ significantly ($p < 0.05$).

A: Plain sea bass marinade

B: Hot smoked sea bass marinade.

Microbiological analysis results

In present study, the products' monthly Total Mesophilic Aerobic Bacteria, Total Psychrophilic Aerobic Bacteria, Total Coliform Bacteria and Total Yeast-Mold Bacteria Loads were examined. According to the microbiological analysis results, the total mesophilic bacteria number was detected as 2.00 ± 0.04 LogCUF/g; however, not a single bacterial reproduction was observed during storing time (<10 CUF/g). Due to the lethal effect of the smoke on the bacteria and the preventing effect of the marination process on the growth of bacteria, The microbiological load remained below the limit values in the study. Coliform group bacteria were not detected at present study.

Kaya, examined the products in seabass marinade microbiologically during storing time (200 days), and hereby he stated that the products did not exceed the consumable limit (10^6 CUF/g) [28]. Balıkçı, reported that smoked mackerel marinade did not exceed the limit value of total mesophilic aerobic bacteria count during storing time and he also stated that this prevents the bacterial growth as a result of marinating and smoking processes [29].

Sen and Temelli, stated that they marinated anchovy and microorganism count decreased after marinating [42]. Kılınç and Çaklı, reported that yeast and mold growth was not observed in tomato sauce marinated sardine and also Olgunoğlu reported the same for marinated anchovy [43,44].

Sensory analyses results

At present study, in which the quality of seabass marinade under refrigerator conditions examined, shelf life of the product was determined as a result of sensory analyses and these results were given in Figure 3. At first, the products in both groups with high ratings were liked and consumed by panelists. A difference was detected between groups owing to the storing time. Group A at the end of 5th month and Group B at the end of 7th month got low ratings from the panelists and lost their qualities. Throughout the study, unpleasant changes in the color and smell of the product were not noted; on the other hand, it was stated by the panelists that some deterioration occurred in the texture and the taste disappeared in the 4th month for Group A and in the 6th month for Group B. Therefore, shelf life of Group A was determined as 4 months and Group B as 6 months. Throughout the study, the most favorite group is the seabass marinade which was marinated by smoking; in other words, Group B. The differences between two groups was found significant ($p < 0.05$).

Thereby, even if parameters of a product quality are acceptable, it is stated as inconsumable when it loses its sensory features [45]. Kaya, stated that the product did not spoil for 200 days but there were only some quality losses in the texture in his plain marinade done by seabass [28]. Balıkçı, stated that there were losses on smell, taste and texture of the products, which were prepared both plain and dilled smoked mackerel marinade, but they were in good quality during 9 months of storing time [29]. Işıdan, examined the quality changes during storing time in 3 different shad marinades (brine, oiled and vacuum packaged). According to his findings, the vacuum packaged product was spoiled on 7th month in sensory perspective; on the other hand, the products which were kept in brine or oil were in better condition on the 7th month [37]. Özoğlu et al. stated that sensory features (taste, smell, etc.) of smoked anchovy marinade declined due to storing time, furthermore their shelf lives are 6 months [46]. Even the previous researches resemble to present study, it can be said that shortness of shelf life is resulted from acid-salt rate in the brine and waiting time.

Figure 3. Sensory analyses result of group A and group B

Conclusion

Today, sensory features are at the top when it comes to a product's approval. Even though the product has good quality, when it is not qualified with sensory properties the product is neither preferred nor considered as tradable. It can be said that the results of present study have similar outcomes. Chemical and microbiological values of seabass marinade were between consumable limits; however, since the products have lost their sensory quality, the examination was terminated. According to the findings, shelf life of Group A noted as 4 months, Group B as 6 months. The most favorite group of the study is Group B.

Acknowledgements. This work was supported by Sinop University Scientific Research Projects Unit (SÜF-1901-15-05 Project code).

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