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Original article

Assessment of chemical, microbiological and sensory quality of Sous vide cooked *Luciobarbus esocinus* (Heckel, 1843) during chilled storage

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Summary. The aim of this study was to investigate chemical, microbiological and sensory quality of sous-vide cooked *Luciobarbus esocinus* (Heckel, 1843) during chilled storage. Moisture, ash, fat, protein, salt, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARs), counts of aerobic mesophile bacteria, aerobic psychrophile bacteria and yeast-mold were determined in the raw material. The sensorial, chemical and microbiological changes of products obtained under the cold storage were analyzed in 3 days interval with three replicates. TermsTVB-N value in group C was determined as 11.06 mg/100 g, in the S1 group as 10.40 mg/100 g, and in the S2 group as 11.99 40 mg / 100 g ($p<0.05$). At the beginning of storage, TBARs value was found to be 1.58 MDA/kg in traditionally processed fish, 1.42 MDA/kg in S1 samples, and 1.28 mg MDA/kg in S2 samples. As. The average count of aerobic mesophile bacteria of S1 group was 3.65 ± 0.03 log CFU g^{-1} on the 1st day. On the 15th day of the storage, however, it exceeded the limit value. For the S2 samples, this was 3,17 ±0,60 log CFU g^{-1} on the 1st day while on the 18th day, it exceeded the limit value. Aerobic psychrophile bacteria count was 3.45 ± 0.13 log CFU g^{-1} in C group, 3.08 ± 0.58 log CFU g^{-1} in S1 group and 2.14 ± 0.99 log CFU g^{-1} in S2 group. Yeast mold count was 3.93 ± 0.29 log CFU g^{-1} in the C group, 4.09±0.08 log CFU g^{-1} in the S1 group, and 2,81±0,47 log CFU g^{-1} in the S2 group. It was observed that products prepared according to the sous-vide method are liked considerably. In conclusion; the results suggested that the C application would be a promising approach to limit microbial growth and provide food safety for sous-vided samples. It was determined that application of sous-vide technology had a positive affect upon shelf life of fish products.

Key Words: *Luciobarbus esocinus*, Sous-Vide, Cold Storage, Shelf Life

Introduction

Due to changing living standards and evolving food technology, the storage of ready-to-eat foods, which can be ready for consumption in a short time and can be stored at low temperatures, have gained importance (1).

Sous vide, a French term for "under vacuum", is defined as foods that are cooked under low temperature and for a long time in vacuum packed bags (2). Sous vide technology was first developed in 1970 by a chef in France, George Pralus, who applied low heat to raw foods. Then, in 1971, Ready cooked the food in heat-stable vacuum packed bags, and the technique was tested at temperatures for different durations. Sous-vide packaging was first used in France, Belgium, America, Canada and Singapore (3).

In sous vide cooking, which is applied under controlled conditions of temperature and time, food and auxiliary products (sauce-spices) are cooked in-

side heat-stable vacuum packed bags. Then, following the heat application, the temperature is rapidly reduced, and the food is stored under cold conditions (1-4 ˚C). This process is also referred to as lapping, vacuum cooking, cooking in vacuum packed bags or baking-cooling in vacuum packed bags. In this technique, oxidative and aerobic bacteria development provides the blocking effect on the products. Moreover, pasteurization provides microbial protection effect. This method is increasing its popularity due to factors such as easy procurement and presentation, availability of foods with high organoleptic properties as ready-to-eat foods, prevention of water loss in foods due to evaporation during cooking, and prevention of flavor loss during the heat application (4, 5).

Currently, many scientific studies are being conducted that apply this technology on various foods at different temperatures and for different durations to develop additives and other methods that can be used along with sous vide technology (2, 6-9).

In the study it was aimed to determine the shelf life of sous-vide cooked *Luciobarbus esocinus* (Heckel, 1843) during cold storage.

Material and Methods

Fish fillets and sous-vide cooking (SV)

The material of the study is *Luciobarbus esocinus* (Heckel, 1843), a fish species found in the Keban Dam Lake. The weights of fish ranged from 3-5 kg and a total of 40 kg of fish were used. The study was carried out with two parallels and three replications.

The fresh fish brought to the laboratory were washed with potable water. After that, the fish was filleted with the help of suitable tools and knives. The fillets were washed and then processed.

5% dry salted fillets were stored at +4 ˚C for 4 hours. After the salting process was completed, each fillet was placed in a polypropylene (PP) heat resistant bag and samples were vacuum packaged. The fish fillets were sous vide processed in a sous-vide machine at 43 ˚C for 20 minutes and 56 ˚C for 10 minutes (Fig. 1). As a consequence, three groups (C, S1 and S2) were obtained as presented in Figure 2.

Storage conditions and analysis

All of the fish fillets processed in the study were kept at 2±1 ˚C. In this study was determined nutrient compositions of raw material and processing samples were determined during storage. The chemical parameters, microbiological and sensorial analyses were performed three days intervals on three groups. Whereas the study was conducted as three parallel analyses, the experiment repeated two times.

Figure 1. Sous-vide cooking of samples

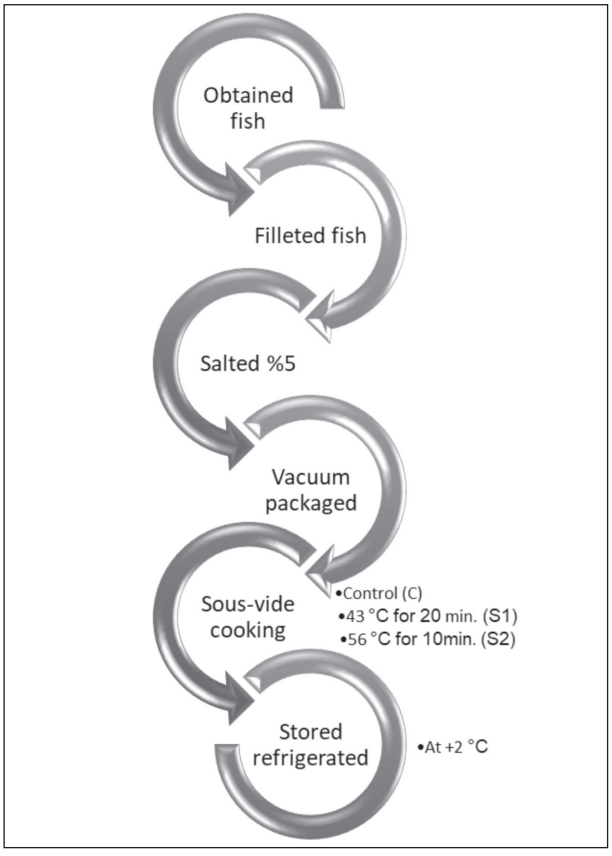

Figure 2. Process of sous vide

Physicochemical Analysis

The moisture content of fish samples was in accordance with method 950.46 (10). The crude protein (method 928.08), crude oil (method 960.39) and crude ash (method 920.153) of fillet samples were measured in accordance with the standard methods (10). The pH values were determined on processed fillets with a pH meter (Thermo Scientific Orion 3-Star). The sample was homogenized in distilled water in the ratio 1:10 (wt/vol) ratio, and a pH meter was employed for the measurement (10). The total volatile basic nitrogen (TVB-N) content was determined according to the method of Antonacopoulos & Vyncke (11). Salt value was analyzed according to the method of Tolgay & Tetik (12). The value of thiobarbituric acid (TBARs) was analyzed according to the method of Tarladgis et al. (13) for samples to evaluate the oxidation changes during storage, and the results are expressed as the TBARs value in milligrams of malondialdehyde per kilogram of the fillet.

Microbial Analysis

The samples were analyzed aerobic bacteria (AB), psychrophile bacteria (PB), and yeast and mold from each of different groups. During storage, fish samples from the three trials of each of the treatments were collected for microbiological analysis.

Fish muscle (10 g) were aseptically added in 90 ml of sterile Peptone water and then stomached (Stomacher 400) for 3 min. Moreover, decimal dilutions were made, and then 0.1 ml of each dilution was pipetted on to the surface of plate count agar (Merck 1.05463) plates for determination of AB and PB, incubated at 30±1°C for three days and at 5±1°C for seven days (14). AnB were determined in Brewer Anaerob Agar (Merck 1.05410) plates incubated at 30±1 °C for three days that an anaerobic atmosphere kit (Anaerogenk, Oxoid) was placed together with the plates inside the anaerobiosis jar. Potato Dextrose Agar medium (Merck 1.10130) was used to count the number of yeast and fungi colonies after the cultivated plaques were incubated at 25±1 °C for 4-5 days (14).

Sensory Analysis

Ten experienced panellists, all of whom academic staff trained were marked to evaluate the sample's overall acceptability on a 1-5-point hedonic scale ranging from very poor (1 point) to very good (5 points). In sensory descriptors for fillet samples, were used to evaluate the quality of samples during storage (15).

Statistical Analysis

All analyses were conducted in triplicate, and data were reported as the mean ± standard deviation. The chemical, microbial and sensorial data obtained during storage of samples of fish were statistically analyzed. For statistical analysis, the IBM SPSS®22 (SPSS Inc., Chicago, IL, USA) statistical computer program package was used. The statistical significance of differences between groups and storage days was determined using ANOVA variance analysis (16).

Results and Discussion

Chemical analysis

Nutrient composition

The nutrition data of the raw material used in the study and the experimental samples are given in Table 1.

Moisture

The statistical analysis showed that the difference in moisture content among the groups was not significant (p> 0.05). In a study on sous vide processed trout, Garcia-Linares et al. (2004) found the moisture content of the traditionally processed trout was 66.60%. On the other hand, they found that the moisture content of the trout cooked at 90 °C for 10 minutes was 69.85%. In another study, the moisture content of traditionally processed whiting (*Merlangius merlangus*) was determined as 69.19%, while that of the whiting cooked at 70 °C for 10 minutes in vacuum packed bags was found to be 69.83% (17). In a study conducted to determine the microbiological

Table 1. Mean nutrition values (%) of fillet specimens prepared in the study n:3

Groups	Moisture	Crude Protein	Crude Fat
Raw material	68.06±0.32	16.93±0.45	7.75±1.20
C	68.72±0.95	14.77±0.83	9.79±0.63
S1	70.09±0.57	14.10±2.26	9.11±0.27
S2	70.11±0.74	19.26±0.49	9.00±0.19

safety and sensory properties of sous vide processed salmon, Gonzalez et al. (4) found that the moisture content of raw salmon was 66.66% while that of sous vide processed salmon was 61.44%. The data obtained from these studies are consistent with the data obtained in the present study.

Protein

In the present study, it was found that the differences in protein values among the groups were not statistically significant ($p > 0.05$). In a study on sous vide cooked trout, Garcia-Linares et al. (18) found that the protein of traditionally processed fish was 18.13% while that of trout cooked at 90° C for 10 minutes was 20.15%. In another study conducted to determine the shelf life of sous vide cooked whiting, Mol et al. (17) found that the crude protein was 20.35% while the protein of the whiting cooked at 70 °C for 10 minutes was 22.26%. Another study reported that raw salmon had 18.13% protein, 15.49% protein on the third day of storage, and 20.43% protein on the 21st day of storage. The differences between the data obtained in the present study and the data from the above-mentioned studies can be attributed to the differences in the raw material used.

Lipid

In the present study, the fat content was determined as 9.79 ± 0.63% in the C group, 9.11% ± 0.27% in the S1 group and 9.00 ± 0.19% in the S2 group. There were no statistically significant differences in fat content among samples ($p > 0.05$).

Garcia-Linares et al. (18) reported the fat content is traditionally processed trout as 13.71% and in sous vide processed trout (cooked at 90 °C for 10 minutes) as 7.45%. Mol et al. (17) reported 6.26% fat content in traditionally processed whiting and 5.91% in whiting cooked at 70 °C for 10 minutes. In another study, it was found that the fat content of sous vide processed trout was 19.66% on the 3rd day of storage at 2 °C, 15.33% on the 21st day, and 15.88% on the last (45th) day of storage (4). The difference between the present study and the studies as mentioned above in terms of fat content may be attributed to the fish species and regional differences.

Salt Content

Salt is the most important additive for fish storage. Since high contents of salt decrease protein solubility, they cause the products to be harder.

The average salt contents determined in the present study were 4.44% in C group, 6.39% in S1 group and 6.10% in S2 group. A thorough search of the relevant literature yielded no studies that reported salt contents.

pH

In the present study, different temperature and time were applied. The difference in pH values during the storage period of the samples was found to be significant ($p<0.05$).

In a study conducted to determine the shelf life of sous vide processed bonito (*Sarda sarda*) Cosansu et al. (19) reported that pH value was 6.07 in raw bonito. They also found that, in sous vide processed bonito, pH value was 6.02% on the 1st day of storage, and 6.69% on the last (42nd) day of storage. In another study, it was found that the pH value of smoked salmon was 6.24±0.01 and that there was not much change after the sous vide process (4). In a study conducted to determine the shelf life of sous vide processed whiting (*Merlangius merlangus*), Mol et al. (17) found the pH of the raw fish to be 6.18. They also found that in the heat-treated samples, pH value was 6.27 on the 1st day of storage and 6.69 on the last (42nd day) of storage.

TVB-N Value

Total volatile basic nitrogen (TVB-N) analysis is one of the methods used to determine the deterioration in and quality of fisheries. This method is also applied in determining the level of quality of chilled fisheries (20). The quality classification of fish according to TVB-N content is as follows: "very good up to 25 mg/100 g, good up to 30 mg/100 g, marketable up to 35 mg/100 g, spoiled up to 35 mg/100 g and over". For the determination of freshness of the fish, this value is accepted as 30 mg / 100g (21; 22; 23).

In the present study, at the beginning of the storage, the average TVB-N value in group C was determined as 11.06 mg/100 g, in the S1 group as 10.40 mg/100 g, and in the S2 group as 11.99 40 mg / 100 g. An increase in TVB-N was observed during stor-

Table 2. Values of pH, TVB-N and TBARs samples during storage days

	Groups	Storage Days						
		0.	3.	6.	9.	12.	15.	18.
pH	Control	6.78±0.09	6.79±0.01	6.80±0.04	6.78±0.09	6.79±0.01	–	–
	43 °C	6.60±0.06	6.53±0.12	6.53±0.09	6.60±0.06	6.53±0.12	6.58±0.09	–
	56 °C	6.81±0.02	6.80±0.08	6.85±0.08	6.81±0.02	6.80±0.08	6.85±0.08	6.81±0.02
TVB-N (mg 100g^{-1})	Control	11.06±0.77	12.13±0.82	13.00±0.36	15.43±1.67	19.74±1.53	–	–
	43 °C	10.40±0.71	12.01±0.72	13.39±0.56	14.18±0.99	18.20±0.96	20.92±1.08	–
	56 °C	11.99±1.30	13.58±0.14	14.51±0.45	15.49±0.16	18.69±1.03	20.23±1.54	25.39±0.65
TBARs (mg MA kg^{-1})	Control	1.58±0.25	1.80±0.61	1.72±0.23	2.49±0.16	2.48±0.41	–	–
	43 °C	1.42±0.13	1.73±0.52	1.84±0.65	2.55±0.10	2.40±0.47	3.75±0.32	–
	56 °C	1.28±0.06	1.72±0.06	2.24±0.08	2.35±0.32	2.55±0.11	2.54±0.33	3.52±0.44

age Table 2. The statistical analysis performed showed that the difference in the TVB-N content among the groups was significant ($p<0.05$) during the storage period.

Mol et al. (17) found that the TVB-N value was 11.64 mg/100 g in traditionally processed whiting and 9.62 mg / 100 g in the whiting cooked at 70 °C for 10 minutes.

In the present study conducted with *Luciobarbus esocinus*, a freshwater species, the TVB-N value showed an overall increase in all groups during storage, and the TVB-N value did not exceed the consumption limit value in all samples throughout storage (Table 3).

Thiobarbituric Acid (TBARs) Number

Number of TBARs gives information about rancidity in fish meat. When TBARs exceeds 4 mg of malonaldehyde/kg, rancidity begins. Many studies indicate that there is a correlation between the TBARs value and sensory test in terms of rancidity in the fish rich in fat content and emphasizes that this is an important method in detecting lipid oxidation in the fisheries (24). In a very good material, the number of TBARs should be less than 3 and in good material, it should not be more than 5. The consumption limit value is between 7 and 8 (23).

In the present study, at the beginning of storage, the average TBARs value was found to be 1.58 MDA/kg in traditionally processed fish, 1.42 MDA/kg in S1 samples, and 1.28 mg MDA/kg in S2 samples.

Cosansu et al. (19) determined the TBARs value of sous vide processed bonito as 3.04. They also found that the TBARs value of the bonito cooked at 70 °C for 10 minutes was 2.93. In a study conducted to de-

Table 3. Mean values (log CFU g^{-1}) and standard deviation for microbiological values of sous-vided fish during 18 days of storage at 2 ± 1 °C.

	Groups	Storage Days						
		0.	3.	6.	9.	12.	15.	18.
Aerobic Mesophile bacteria	Control	3.99±0.43	4.35±0.11	4.50±0.04	5.06±0.20	7.02±0.57	–	–
	43 °C	3.65±0.03	4.46±0.31	4.31±0.51	4.79±0.66	5.19±0.78	6.84±0.53	–
	56 °C	3.17±0.60	3.24±0.03	3.86±0.03	4.52±0.44	4.83±0.60	5.23±0.37	6.25±0.66
Aerobic Psychrophile bacteria	Control	3.44±0.13	2.57±0.51	3.35±0.44	3.81±0.57	6.41±0.10	–	–
	43 °C	3.08±0.58	2.96±0.52	3.68±0.09	4.04±0.50	4.82±0.67	6.63±0.04	–
	56 °C	2.14±1.30	2.31±0.86	3.75±0.17	4.34±0.57	4.20±0.19	4.55±0.36	5.65±0.43
Yeast-mold	Control	3.23±0.29	3.99±0.08	4.20±0.13	4.48±0.11	5.94±0.55	–	–
	43 °C	4.09±0.08	4.17±0.20	4.60±0.59	4.89±0.49	5.40±1.11	7.19±0.41	–
	56 °C	2.81±0.47	4.29±0.09	4.98±0.53	5.14±0.42	5.57±0.17	5.31±0.28	5.75±0.08

termine the shelf life of sous vide processed whiting, Mol et al. (17) assessed the TBARs value of traditionally processed whiting as 1.09 and the TBARs value of heat-treated samples as 1.59. These TBARs data are similar to the one obtained in the present study.

During storage in the present study, the TBARs content increased. In addition, it was found that the TBARs levels of samples did not exceed the consumption limit value during storage (Table 3).

Statistical analysis showed that the difference among the groups in terms of numbers of TBARs was not significant ($p > 0.05$) during storage.

Changes in microbiological quality

Counts of aerobic mesophile bacteria

It is known that the bacterial load in the fisheries found in freshwaters is extremely low. However, the bacterial load in the species in coastal areas where pollution is high can be higher. Determination of the total counts of mesophilic aerobic bacteria is widely used to determine the freshness of fisheries. 6–7 log CFU g^{-1} mesophilic aerobic bacteria for fisheries is considered as a limit value (20).

The counts of aerobic mesophile bacteria determined during the production and storage of experimentally prepared *Luciobarbus esocinus* fillets are shown in Table 4. The average mesophilic aerobic bacteria count of the control group was 4.00 ± 0.43 log CFU g^{-1} for the 1st day. On the 12th day of the storage, however, it exceeded the limit value. The average mesophilic aerobic bacteria count of S1 group was 3.65 ± 0.03 log CFU g^{-1} on the 1st day. On the 15th day of the storage, however, it exceeded the limit value. For the S2 samples, this was 3,17 ±0,60 log CFU g^{-1} on the 1st day while on the 18th day, it exceeded the limit value. When the increase in the count of mesophilic bacteria due to deterioration of the products during storage is statistically analyzed, it can be seen that the difference among the groups in terms of total aerobic mesophilic counts is not significant ($p > 0.05$).

Garcia-Linares et al. (18) conducted a study to evaluate the microbiological safety of rainbow trout treated with sous vide at a different time and temperature conditions when stored at 2 °C and 10 °C. The trout was subjected to three different heat treatments 15 minutes at 90 °C, 5 minutes at 90 °C, 10 minutes at 70 °C and were stored at 2 °C and 10 °C. They found that the mesophilic bacteria had a low rate of increase in the products stored at 2 °C.

Counts of aerobic psychrophilic bacteria

The total number of aerobic psychrophilic bacteria determined the end of incubation at 5 ± 1°C of the samples of sous vide cooking is given in Table 4. In the present study, the average aerobic psychrophilic bacteria count was 3.45 ± 0.13 log CFU g^{-1} in C group, 3.08 ± 0.58 log CFU g^{-1} in S1 group and 2.14 ± 0.99 log CFU g^{-1} in S2 group. These counts increased during storage. When the data were analyzed statistically, it was found that the difference between the groups in terms of total aerobic psychrophilic counts was not significant ($p > 0.05$).

In a study by Gonzalez et al. (4), salmon was heat-treated in three groups (5 minutes at 65 °C, 10 minutes at 90 °C, and 15 minutes at 90 °C). Later on, it was stored at 2 °C and 10 °C. They found that psychrophilic bacteria showed a low increase in the group stored at 2 °C.

Counts of yeast-mold

In the present study, the average yeast-mold count was 3.93 ± 0.29 log CFU g^{-1} in the C group, 4.09±0.08 log CFU g^{-1} in the S1 group, and 2,81±0,47 log CFU g^{-1} in the S2 group.

An increase in the yeast-mold counts was observed due to deterioration during storage.

When the data obtained from the study are analyzed statistically, it can be seen that the difference between the groups in terms of yeast-mold counts is not significant ($p > 0.05$).

Sensory Evaluation

Correctly performed sensory analyzes can provide an objective and reliable quality parameter. However, although sensory analyzes are of great importance in decision making, they are subjective, so it is difficult to obtain accurate results. Therefore, such evaluations should be supported by other methods. If a product which is acceptable in terms of quality parameters is considered unacceptable in terms of sensory properties, this product is accepted to be non-consumable (25, 26).

The researchers evaluated the *Luciobarbus esocinus* samples in terms of color, odor, appearance, flavor and brittleness during storage. It was determined that the control group became non-consumable on the 12th day of storage, S1 (cooked at 43 ˚C for 20 minutes) on the 15th day of storage, and S2 (cooked at 56 ˚C for 10 minutes) on the 15th day. Therefore, the samples were evaluated only in terms of color, odor and appearance. The changes in sensory evaluations during the storage period are presented in Figure 3.

The average color scores of the 1st day were 4.82 ± 0.10 in the C group, 4.62 ± 0.15 in the S1 group and 4.89 ± 0.09 in the S2 group. The average odor scores of the 1st day were 4.57±0.08, 4.95±0.08 an 4.95±0.08, respectively.

When the samples are examined in terms of average flavor scores, S2 group received the highest scores from the 1st day.

In consequence of sensory evaluations, S2 group samples received the highest score (4.94±0.05) on the 1st day in terms of general appreciation. When the data obtained in the study are analyzed statistically, it can be seen that the difference between the groups was not significant in terms of general appreciation (p> 0.05).

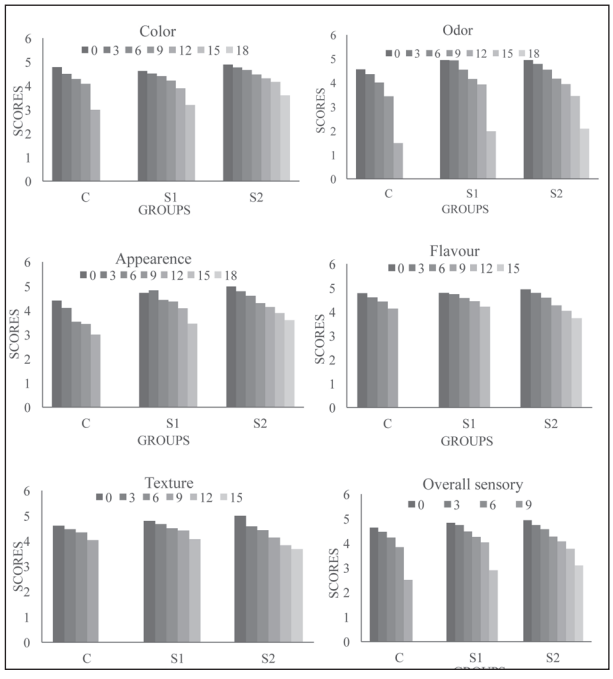

Figure 3. Changes of sensory scores in the of samples during storage at 2 ± 1 ˚C.

Can (27) stored carp cooked at 90 ˚C for 15 minutes as the control group (no sauce) and sauced group at 2 ˚C and 10 ˚C. They found that the shelf life of the group stored at 10 ˚C was 14 days, while that of the group stored at 2 ˚C was 56 days. Diaz et al. (28) found that the shelf life of salmon treated with sous vide technology at 80 ˚C for 43 minutes and stored at 2 ˚C was 25 days. In the present study, the shelf life of the fish sous vide cooked at 43 ˚C and stored at 2 ˚C was found to be 15 days while that of the fish cooked at 56 ˚C was found to be 18 days.

Mol et al. (17) reported a the shelf life of 35 days for the whiting cooked at 70 ˚C for 10 minutes and stored at 4±1 ˚C.

In a study by Gonzalez et al. (4) trout was heat-treated in three groups (10 minutes at 70 ˚C, 5 minutes at 90 ˚C, 15 minutes at 90 ˚C) and stored at 2 ˚C and 10 ˚C. They reported that the heat treatment at 90 ˚C for 15 minutes yielded the best results and recommended storage at low temperature for sous vide processed fisheries. In addition, in another study by Gonzalez et al., salmon was heat-treated in three groups (5 minutes at 65 ˚C, 10 minutes at 90 ˚C and 15 minutes at 90 ˚C), and, similar to the study as mentioned above, reported that heat treatment at 90 ˚C for 15 minutes yielded better results. Both studies found that when the samples are stored at 2 ˚C, they retained their quality for 45 days. Garcia-Linarese et al. (18) processed trout and salmon with sous-vide technology and stored them at 4 ˚C to compare them with traditionally cooked trout and salmon. They found that sous vide technology yielded better results in both types of fish than the traditional method and ensured an increase in the shelf life.

Conclusion

The present study examined the shelf life of *Luciobarbus esocinus*, caught from the Keban Dam Lake and treated with sous vide, at cold storage. Analyzes carried out to investigate the changes in the quality of fish samples revealed that the shelf life of the products was 9 days for the control group, 12 days for the samples sous-vide cooked at 43 ˚C for 20 minutes, and 15 days for the samples sous-vide cooked at 56 ˚C for 10 minutes.

Sous vide increased the flavor of the fish samples examined in the study and facilitated their presentation. Furthermore, it was concluded that the chemical, microbiological and sensory properties of the samples were preserved for longer periods, mainly due to the effect of vacuum packaging. It is thought that the application of sous vide technology to fisheries at different temperature and for varying durations will contribute to the consumption of ready-to-eat food.

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