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The effect of replacing *Lactuca sativa* and *Cornus mas L.* on the chemical characteristics of fish sausage

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ABSTRACT

Currently, meat products are considered one of the most consumed foods in the world. The food industry is not only responsible for providing good food for the general public, but paying attention to nutritional and health-giving components such as reducing the consumption of nitrites and preservatives such as ascorbic acid is also a challenge. It is great for experts and activists in this field. This research was done to investigate the replacement of nitrite and ascorbic acid in the formulation of fish sausage. The samples are in the form of 5 treatments including C (control sample without lettuce powder and blueberry powder), T1(1%lettuce powder and 5% blueberry powder), T2 (2%lettuce powder and 5%blueberry powder), T3 (4%lettuce powder and 5%blueberry powder) and T4 (6%lettuce powder and 5% blueberry powder) blueberries) were produced. Recently, researchers are looking for the use of natural substitutes instead of nitrite in the meat industry to maintain the quality of the products to an acceptable level and to prevent their early spoilage; in this regard, many researchers use turned to plant materials as good sources of antioxidants and antimicrobials. Because blueberries are a rich source of phenolic compounds, anthocyanin, and vitamin C, they can be considered a good source of natural antioxidants. Nitrate accumulation in vegetables such as lettuce is higher than in other vegetables. It is different in different organs of vegetables, so lettuce is a good source of nitrate supply. According to the results obtained from the chemical characteristics, the T4 treatment (fish sausage sample containing 6%lettuce powder and 5% blueberry powder) was in the standard range in all the chemical characteristics of the treatment.

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1- Introduction

Meat and meat products are one of the most important food groups for providing the protein needed by the body. Sausages and sausages are among the most famous meat products that are loved by millions of consumers around the world [1]. Due to variety in processing, taste and suitable and numerous packages are increasing. Various chemicals are used to increase the shelf life and maintain the quality of meat products, one of the most widely used of which is nitrate and nitrite salts. Table salt adds flavor and color to food. Nitrate and nitrite are preservatives that cause pink color. The reduction of nitrite in meat emulsions is desirable because of the mentioned negative effects, but it causes the development of the lipid oxidation reaction. This reaction is one of the most destructive reactions that cause unpleasant odors and tastes The color of desirable pigments (hemoglobin and myoglobin) in meat products is lost. Additives are added to the product to maintain stability, maintain nutritional value, prevent spoilage, adjust pH, increase flavor, or create desired color [2]. Or the oxidation of fats is the most important type of chemical spoilage in meat products [3]. Studies conducted on the nutritional value of blueberries show that this fruit can play an essential role in preventing diseases such as vitamin C deficiency. Fresh blueberry fruits contain vitamin C about twice as much as fruits that have high vitamin C (such as oranges) and also contain sugar, anthocyanin, organic acids and tannins] 4,5,6,7[QThe main side of nitrate that enters the human body is from the consumption of vegetables [8]. Lettuce [1]One of the most important vegetables is salad, which is consumed daily and has a great genetic tendency to accumulate nitrates, and through this, some nitrates enter the human body [9,10].

Dietary fiber is considered as an important factor in improving the nutritional value of food products]11[.Plants are good sources of natural antioxidants and antimicrobial substances and can be considered a good alternative to synthetic preservatives [12]. Recently, researchers are looking for the use of natural alternatives instead of nitrite in the meat industry in order to maintain the quality of the product to an acceptable level and to prevent their early spoilage; In this regard, many researchers have turned to the use of plant extracts as good sources of antioxidants and antimicrobials [13]. Among the researches that have been carried out in this field, we can mention Parsi et al.'s research [14]. who came to the conclusion that the addition of tomato pomace powder as a non-meat protein source on the chemical characteristics of sausages concluded that the addition of tomato pomace powder to the sausage formulation caused a significant increase in the amount of protein and ash and a decrease in pH. Abolhassanzadeh and colleagues [15] [in A study by replacing nitrite with black seed oil on the oxidative stability of cocktail sausage concluded that the replacement of black seed oil in the two concentrations used caused a decrease in the values of peroxide and thiobarbituric acid indices in treatments with reduced nitrite compared to the control.

2- Materials and methods

Ingredients needed to make sausage First, the fish were washed well with cold water, and then the abdominal contents, head, tail, and bones were slowly separated. Then, the skin of the fish was gently taken by hand and filleting was done. The fish fillets were washed 3 times with cold water. During the washing of the fillets, water-soluble compounds, fat, enzymes, sarcoplasmic proteins and odor generating substances and

1 .Lettuce sativa

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compounds were removed. The flavoring agent is separated from the fish. Of course, washing the fish meat will not affect its amino acids. The minced fish fillets were minced. Then the minced fillets were stored in the refrigerator at a temperature of 4 degrees Celsius. For the preparation of lettuce powder, a few fresh and healthy lettuce plants They were completely washed and cleaned. After dewatering, the lettuces were chopped and all parts of the lettuce were used for this experiment. Chopped lettuces were placed in a home dehydrator for 14 hours until their juice was completely dry to be

In order to prepare blueberry powder, the core of blueberries was completely separated from its fruits. After core extraction, the blueberries were placed in a fruit dryer for 18 hours and dried completely.

The desired spices included ginger, cloves, nutmeg, coriander seeds, lemon, garlic, black pepper, and salt. The minced fish meat was sent to the laboratory in a container, wrapped in cellophane, and in a Uniolite box with ice to prevent the meat from spoiling. was taken away

2-2- How to prepare fish sausage

50% fish sausage formulation:

Liquid oil (16%), starch (2.4%), wheat gluten (1.3%), milk powder (0.9%), sodium polyphosphate (0.5%), table salt (1.2%).), mixed spices (0.6%), ascorbic acid (0.05%), sodium nitrite (0.05%) and crushed ice (16%).

First, the minced fish meat was divided into 5 equal parts. Then, the required ingredients were ready to be added to the minced meat according to their percentage and weighed on a digital laboratory scale (AND brand, model 600Gf). During production, with a digital thermometer The temperature was measured regularly. The desired temperature for the production of sausages was 4 degrees Celsius. In this research, we are looking for the elimination or reduction of nitrite from meat products by making changes in the meat processing system. 5 different formulations based on The ingredients in fish sausage were prepared and in them formulas containing nitrite and low nitrite and the use of natural cranberry color and antioxidant were mentioned.

Control treatment (C): no substitution of lettuce and blueberries

Treatment (T1): Contains 1% lettuce powder and 5% blueberry powder

Treatment (T2): Contains 2% lettuce powder and 5% blueberry powder

Treatment (T3): contains 4% lettuce powder and 5% blueberry powder

Treatment (T4): Contains 6% lettuce powder and 5% blueberry powder

Minced fish meat was poured into the cutter machine, and the ingredients of the formulation were added little by little to the desired dough, and crushed ice was added to the dough little by little while stirring until a completely uniform and soft dough was obtained. Then the produced sausage dough was removed from the machine. The cutter was transferred to the filling machine and under proper pressure, the dough was filled in polyamide covers with a diameter of 2 cm and closed with cotton thread from both ends of the produced sausages with a length of 10 to 12 cm. Performing these steps regularly, the ambient temperature was measured with a digital thermometer (4°C). In order to heat the product to form a suitable emulsion, the produced sausages were placed in a pot with a temperature of 60 degrees Celsius for 2 hours and the water temperature was regularly controlled with a digital thermometer. After the end of the heating process, the temperature of the products slowly and in During 45 minutes using cold water, they reached about 20 degrees. Then the samples were kept in the refrigerator at 4 degrees Celsius until the

appropriate time for sampling (2, 4 and 21 days after production).

3- Chemical tests

The amount of carbohydrates in the homogenized sample was determined according to standard 2303 [16]. To measure the amount of peroxide, it was done according to standard 19197 [17]. To measure the pH level, first 15 grams of each product sample was well homogenized in 150 ml of distilled water by a homogenizer and then kept in a stationary state for 2 minutes. Then with a digital pH meter according to the national standard of Iran 1028 It was measured [18]. To determine the amount of nitrite of the samples according to standard 2303, it was done [19]. To determine the amount of fat according to standard 742, it was calculated using the following equation, in which, M_1 Weight of empty balloon, M_2 The total weight of the balloon and oil and W is the weight of the sample [20].

$$\frac{M1-M2}{IN} \times 100 = \text{percentage of fat}$$

To determine the amount of ash according to standard 744, it was calculated based on the following equation, where M_1 The total weight of the porcelain plant and the sample after the furnace, M_2 The weight of the plant and W is the weight of the sample [21].

$$\frac{M1-M2}{IN} \times 100 = \text{percentage of ash}$$

To determine the moisture content of the sausage samples according to the 745 standard, the percentage of the weight of the sausage sample, before and after baking, was calculated based on the initial weight of the sample [22].

To measure the amount of protein in the samples according to the 942 standard, it was calculated by the Keldall method and based on the following equation, where V_1 The amount of milliliters of hydrogen chloride available, V2 is the amount of milliliters of sodium hydroxide used for the sample, W is the amount of the sample, and H represents the amount of moisture [23].

$$\text{amount of protein} = \frac{(V1 - V2) \times 0.0014 \times 5.7 \times 100}{W(100 - H)}$$

In order to determine the amount of thiobarbituric acid in sausage samples, it was done according to standard 10494 [24].

4- Statistical analysis method

Data analysis was carried out in the form of a completely randomized design, in which there were 2 treatments of lettuce powder in 4 levels and blueberry powder in 1 level, and each of the desired tests was performed in 3 repetitions. Also, comparison of treatment means was done using Duncan's multi-range test at 99% confidence level. For this purpose, SPSS version 26 statistical software was used.

5- Results and discussion

1-5-carbohydrate

On the fourth day and the third week after production, a significant difference was observed between the treatments ($P<0.01$) that the minimum amount. This parameter is related to the control and its maximum value was obtained in the fourth treatment (Table 1).This increase in carbohydrates is due to the presence of lettuce and blueberry powder. Since the nature of vegetables is cellulose and cellulose is considered a type of carbohydrate, therefore, with the increase in the amount of lettuce powder, the amount of carbohydrates in the treatments has also increased.. Farkhandeh et al. [25] in the study of the effect of nisin and tomato pomace powder as a substitute for nitrite observed that in all treatments the amount of carbohydrates increased compared to the control sample.

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Table 1: The number of carbohydrates in the samples during 21 days of storage at 4 degrees Celsius (Mean± standard deviation)

Day21	Day4	Treatments
3.87 ± 0.01^{bE}	3.90 ± 0.02^{aE}	C
4.59 ± 0.01^{aD}	4.55 ± 0.02^{bD}	T1
4.68 ± 0.01^{BC}	4.69 ± 0.01^{BC}	T2
4.97 ± 0.02^{aB}	4.95 ± 0.01^{bB}	T3
5.16 ± 0.01^{aA}	5.15 ± 0.02^{aA}	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments Different lowercase letters in each row (same treatments) indicate significant differences between experimental days (p<0.01)

5-2-Peroxide

The results of peroxide measurement in Table No. 2 indicate the significance of the effect of different levels of lettuce powder and one level of blueberry powder and time (P<0.01). On the 4th and 21st days after production, with the increase in the percentage of lettuce powder, the amount of peroxide index has increased during the 21 days of storage. In the research of Khodaei and Khani [26], it was observed that all treatments showed lower values of peroxide index than the control sample during the storage period of the product. Also, with the passage of time, this index started to increase and reached its maximum value on the 35th day after production, which is according to the research results .Antioxidants can slow down this process. The findings of the present research are consistent with the results of the research of Ma'rafian et al. [27] in the investigation of the antioxidant effect of mint essential oil in heated sausage. Considering that the acceptable peroxide value for fatty foods such as sausage is meq$o_2$25/kg is determined [28] And the fact that the amount of peroxide in all the samples is lower than the limit until the end of the storage time can be due to the prolongation of the oxidation period and the decrease in the formation of hydroperoxides according to the pH level, the nature and amount of fats and the presence of antioxidant compounds [29] [, As a result, all production treatments are acceptable in terms of peroxide number index.

Table 2: The amount of peroxide in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
1.47 ± 0.03^{aD}	0.78 ± 0.02^{bC}	C
1.77 ± 0.02^{aA}	1.09 ± 0.01^{not}	T1
1.70 ± 0.01^{aB}	0.92 ± 0.02^{not}	T2
1.62 ± 0.02^{BC}	0.84 ± 0.01^{bB}	T3
1.53 ± 0.03^{aD}	0.71 ± 0.02^{bD}	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments
Different lowercases letters in each row(same treatments)indicate significant differences between experimental days (p<0.01) .

3-5- pH

The pH measurement results in Table No. 3 show the significance of the effect of different levels of lettuce powder and one level of blueberry powder and time (P<0.01).

On the 4th and 21st day after production, the pH value of the treatments has decreased compared to the control sample, but during the 21 days of storage, the pH value of the same treatments has increased compared to each other at all levels, which can probably be due to the increase in the number of gram-negative bacteria such as Enterobacteriaceae, Pseudomonas, molds, and yeasts, which cause the breakdown of proteins, the formation of ammonia, and the increase of pH [30]. On the 4th day after production, with an increase in the percentage of lettuce powder, the pH has also increased, but on the 21st day after production, with an increase in the percentage of lettuce powder, the pH has decreased, which can probably beDue to the production of organic acids by Bacteria by consuming sugars and It is also the oxidation of fat and the creation of acidic compounds that cause the pH of the product to decrease [31].

Table 3: The amount of pH in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
6.87 ± 0.01^{aA}	6.64 ± 0.01^{not}	C
5.73 ± 0.02^{aB}	5.16 ± 0.01^{bD}	T1
5.68 ± 0.03^{BC}	5.24 ± 0.02^{bCD}	T2
5.64 ± 0.02^{BC}	5.34 ± 0.01^{bBC}	T3
5.71 ± 0.01^{aB}	5.45 ± 0.02^{bB}	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments
Different lowercases letters in each row(same treatments)indicate significant differences between experimental days (p<0.01).

4-5-Nitrite

The results of the present research indicated that the amount of nitrite measured every 3 days in all treatments is lower than the control sample and with the increase in the percentage of lettuce, the amount of remaining nitrite has also increased (P<0.01)) The percentage of lettuce powder with nitrite remaining in the sausages is much lower than the control sample (Table 4). On the other hand, with the passage of time, the amount of nitrite remaining in all samples decreased and on the 21st day after production, the amount of nitrite remaining was the lowest. The residue is ripe, which is probably due to the conversion of nitrite to other nitrogenous compounds and its binding to myoglobin and other organic compounds (such as fats and proteins) during the storage period. In another research, it was observed that naturally, due to the substitution of rosemary essential oil and beetroot powder instead of 60 ppm nitrite, lower nitrite levels were obtained compared to the control sample [26].

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Table 4: The amount of nitrite in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Day2	Treatments
30.36±0.71^{that}	105.71±0.61^{not}	118.32±1.01^{aA}	C
.131±0.35^{bE}	3.78±0.98^{aE}	4.26±.031^{aE}	T1
2.85±0.31^{cD}	5.67±0.31^{bD}	7.28±0.23^{aD}	T2
6.07±0.61^{bC}	12.98±1.06^{BC}	16.14±0.31^{BC}	T3
10.97±.040^{cB}	26.87±1.12^{bB}	30.70±0.72^{aB}	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments Different lowercases letters in each row(same treatments)indicate significant differences between experimental days (p<0.01).

5-5-fat

The amount of fat in the treatments every 2 days is slightly lower than the control treatment (P<0.01). In fact, with the increase in the percentage of lettuce powder and the presence of blueberry powder in the treatments, the fat of the product has also decreased (Table 5), which is probably due to the use of plant materials and the reduction of the cholesterol level of the product. Garcia et al [32] observed that with Increasing the percentage of dietary fiber in the sausage sample has decreased the fat content of the sample compared to the control sample.

Table 5: The amount of fat in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
40.27±0.39^{A}	39.97±0.28^{A}	C
39.25±0.55^{B}	39.50±0.55^{AB}	T1
39.14±0.37^{B}	38.98±0.11^{BC}	T2
38.73±.061^{BC}	38.58±0.03^{C}	T3
37.96±.085^{C}	38.35±0.05^{C}	T4

. (P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments

5-6-ash

The results of ash measurement are shown in Table No. 6. On the 4th day after production, there was a significant difference between the control treatments andT1 Not observed (P>0.01). On the 21st day after production, there was a significant difference between the treatmentsC AndT1 Not observed (P>0.01).

Table 6: The amount of ash in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
3.82±0.02^{D}	3.82±0.02^{D}	C
3.82±0.11^{D}	3.82±0.11^{D}	T1
3.98±0.05^{C}	3.98±0.05^{C}	T2
4.13±0.04^{B}	4.13±0.04^{B}	T3
4.25±0.05^{A}	4.25±0.05^{A}	T4

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. (P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments

In every 2 days, with the increase in the level of lettuce powder, the amount of ash has also increased compared to the control sample. The reason for this is the higher amount of lettuce and blueberry powder, which were not present in the control sample. Also, this issue can be related to the preparation stages and the washing of minerals during the preparation stages [33]. In a survey, it was found that the addition of wheat germ flour causes a significant increase in the amount of sausage ash, which is consistent with the research results [34].

7-5-humidity

The results of moisture measurement are shown in Table No. 7, which indicates the significance of the effect of different levels of lettuce powder and one level of blueberry powder and time (P<0.01).

Table 7: The amount of moisture in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
61.27 ± 0.89^{not}	62.67 ± 0.59^{aAB}	C
61.88 ± 0.84^{not}	63.54 ± 0.58^{aA}	T1
61.05 ± 1.77^{not}	$62.51\pm.054^{aAB}$	T2
$60.60\pm.099^{aA}$	$62.26\pm.048^{aB}$	T3
$58.23\pm.080^{aB}$	$59.34\pm.058^{BC}$	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments
Different lowercases letters in each row(same treatments)indicate significant differences between experimental days (p<0.01).

Polysaccharides, especially fibers, have a high capacity to absorb and retain water. The decrease in moisture in sausage samples is due to the addition of lettuce powder. In a research, it was also observed that the amount of moisture increased with the addition of potato dietary fiber [1].

8-5-Protein

On the 4th and 21st day after production in all treatments, the amount of protein (Table 8) has decreased compared to the control sample (P<0.01). Also, no significant difference was observed between the 4th and 21st day after production in the same treatments, which is probably because the amount of protein in the treatments did not change.

In a study, they observed that by increasing the amount of wheat germ flour, protein increased in the composition of sausage flour, which is probably due to the high protein content of wheat germ flour [34], which is not according to the research results.

While in a research with the addition of potato dietary fiber, the amount of protein has slightly decreased due to the fact that wheat flour has higher amounts of protein than potato dietary fiber [1], which is in accordance with the results of the research.

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Table 8: The amount of protein in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
51.92 ± 0.52^{A}	52.03 ± 0.20^{A}	C
50.05 ± 0.35^{B}	50.63 ± 0.20^{B}	T1
49.75 ± 0.37^{B}	49.70 ± 0.35^{BC}	T2
$46.11\pm.5.32^{C}$	49.00 ± 0.35^{CD}	T3
$47.25\pm.0.10^{C}$	47.60 ± 0.05^{D}	T4

. (P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments

5-9-Thiobarbituric acid

The amount of this parameter has decreased on the 4th day after production (Table 9) compared to the control sample (P<0.01). This decrease can be due to the breakdown of malondialdehyde by Pseudomonas and Enterobacteriaceae, which are able to produce carbonyl compounds such as malondialdehyde. aldehyde, or due to the higher oxidation of malondialdehyde to other compounds such as alcohols and acids that do not react with thiobarbituric acid [35], this change may also be due to the reaction of malondialdehyde with proteins and sugars] 36 [.

Table 9: The amount of TBA in the samples during 21 days of storage at 4 degrees celsius(Mean± standard deviation)

Day21	Day4	Treatments
0.35 ± 0.01^{aE}	0.19 ± 0.01^{not}	C
0.45 ± 0.00^{aA}	0.17 ± 0.00^{bB}	T1
0.44 ± 0.01^{aB}	0.17 ± 0.00^{bB}	T2
$0.41\pm.001^{BC}$	0.17 ± 0.00^{bB}	T3
$0.37\pm.000^{aD}$	0.16 ± 0.00^{bC}	T4

(P< 0.01)Different capital letters in each column (same says)indicate significant differences between treatments
Different lowercases letters in each row(same treatments)indicate significant differences between experimental days (p<0.01).

6-Conclusion

According to the results obtained from the chemical characteristics, treatment T4 (fish sausage sample containing 6% lettuce powder and 5% blueberry powder) has the highest values in carbohydrate, nitrite and ash characteristics, which is due to the higher percentage of lettuce powder compared to Other treatments, this conclusion was predictable. But in other chemical characteristics such as peroxide, fat, moisture, protein, thiobarbituric acid and pH, it had lower values compared to other treatments. In general, in all the chemical characteristics of the T4 treatment, it was in the standard range. Also, taking into account the health-giving properties of the natural plant compounds used on the one hand, as well as the losses caused by synthetic preservatives for human health, on the other hand, it is possible to replace lettuce powder and Blueberry powder, while maintaining the desired chemical properties, suggested that it can be an effective step to improve the quality and health characteristics of meat products.

7- Resources

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تاثیر جایگزینی کاهو و زغال اخته بر ویژگی های شیمیایی سوسیس ماهی

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چکیده

درحال حاضر فرآورده‌های گوشتی یکی از پرمصرف‌ترین غذاها در دنیا محسوب می‌شوند. در این راستا صنایع غذایی نه تنها وظیفه تهیه غذای مطلوب برای عموم مردم را دارد، بلکه نگاهی نیز به مولفه‌های تغذیه‌ای و سلامتی بخش مانند کاهش مصرف نیتریت و نگهدارنده ها داشته که چالش بزرگی برای متخصصین و فعالان در این زمینه می‌باشد. این تحقیق با هدف بررسی جایگزینی نیتریت در فرمولاسیون سوسیس ماهی انجام شد. نمونه ها در قالب ۵ تیمار شامل: C(نمونه شاهد بدون پودر کاهو و پودر زغال اخته)،T1(۱ درصد پودر کاهو و ۵ درصد پودر زغال اخته)،T2 (۲ درصد پودر کاهو و ۵ درصد پودر زغال اخته)، T3(۴ درصد پودر کاهو و ۵ درصد پودر زغال اخته) و T4(۶ درصد پودر کاهو و ۵ درصد پودر زغال اخته) سپس نمونه ها را در یخچال با دمای ۴ درجه سانتی گراد تا زمان نمونه برداری (۴و۲۱ روز پس از تولید) نگهداری و برخی از ویژگی‌های شیمیایی شامل کربوهیدرات، پراکسید، پی ایچ، نیتریت، چربی، خاکستر، رطوت، پروتئین و تیوباربیوتوریک اسید ارزیابی شدند. با عنایت به نتایج کسب شده از لحاظ تمامی ویژگی های شیمیایی، تیمار T4 در محدوده استاندارد قرار داشت که می‌توان این تیمار را برای بالا بردن سطح سلامت سوسیس ماهی پیشنهاد کرد.