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INVESTIGATION OF THE PHYSICOCHEMICAL, ORGANOLEPTIC AND DIETARY PROPERTIES OF TROUT FERMENTED SAUSAGES

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Abstract. Decrease in nitrosamine and optimal smell and flavor gives credit to such meat products as fermented sausages for their considerably optimal storage period and better nutritional values. Replacing red meat with fish and using fat replacer and probiotics in such products have played a great role in the production of such a highly functional food. Therefore, the focus of this study is mainly on the production of fermented trout sausages applying *lactobacillus Rhamnosus* and *Plantarum*, substituting part of its fat with inulin (2% of the total fat of the formulation) while examining the physicochemical, textural, dietary, and sensory properties and comparing with control sample, too. The effect of the inulin present in formulation, the type of probiotic strains, and life time (30 days) influencing the physicochemical and textural properties and nitrosamine samples were compared in three ten-day periods as independent valuables completely randomized factorial design. Sensory evaluation was also performed at the end of the 30-day maintenance period. Results suggest that samples with inulin content have experienced less moisture loss during storage. Little fat was also observed in probiotic content samples at the end of the storage. The pH value in the probiotic samples, as compared to the control sample, shows more decline. Inulin content samples caused a marked decline in lightness and an increase in redness. Meanwhile, probiotic presence has caused more declines in lightness intensity in samples. Inulin content samples show more hardness as compared to high fat samples and the probiotics present after pH decline to isoelectric point caused an increase in intensity and hardness of protein fibers. Simultaneously, along with an increase in fat, cohesiveness increased. Nitrosamine content in probiotic samples was lower than the one in test sample much as there was an increase seen in all samples. Despite a little more odor being present, sensory analysis was in favour of the test samples. Other samples demonstrated little difference in sensory evaluation.

Keywords: Fermented sausage, Inulin, Nitrosamine, Sensory analysis, Trout.

Introduction. Formulation of the problem

Meat and meat products are considered as a major source of protein in human diet, so its appropriate consumption can maintain an important supply of nutrient food. Such factors as sensory, dietary, economic ones play a major function in general meat consumption [1]. Fermented sausages are major meat products which have a majority share in the world food production. Their special properties include better storage period, boosted nutritional value for the decline in nitrosamine and improvement of odor and fragrance as a result of bacterial metabolite during the fermentation process [2]. However, like sausage products, meat is considered to be low with respect to health parameters for the presence of nitrates, salt, and high fat content ruins the image of meat as functional food [3]. High fat content in such meat products is responsible for a variety of properties in different sausage products. First, they are nutritionally and physiologically a good source of fat soluble proteins and energy. Second, fat can play a dominant role in dispensing sweet fragrance, flavor, a good oral sensation, texture, and general consumer's acceptability. Finally, fat granules are technologically influential in reducing moisture in the inner layer of the sausage [4-5]. To reduce concerns about consumption of such meat products, researchers have modified sausage processing. Replacing (red) meat with fish applying fat substitutes along with probiotic strains in such products has almost led to a highly profitable product [6-7].

Fish as a rich source of easily digestible proteins with highly biologic values can provide the body with vitamins, minerals, and necessary fatty acids [8]. High rate of spoilage in fish makes it preferably a freshly-served diet especially in sea shores and nearby fishing areas. Using fermentation technology as an alternative strategy to lengthen the storage period of a product can give it added value and enhance its optimal qualities specifically its taste and flavor. Due to high spoilage rate in fish, lactic fermentation can be a significant method to preserve sea products in developing countries [9].

The trout fish meat is a good source of iron for hematopoiesis which, in turn, boosts immune system against microbes. The iron content in trout is easily absorbed by the digestive system and better consumed in the body. Such iron content helps boost vegetable iron content absorption much easier resulting in little iron deficiency in blood. Research suggests Omega-3 in salmon can considerably reduce high blood pressure, regulate heartbeat rate, and slow epileptic attacks. Salmon, as a rich protein source, can be easily digested and absorbed by digestive system, totally consumed in the body. Zinc in salmon helps child growth and is highly influential during puberty stage. It also prevents shortness of stature. Salmon contains considerable amounts of vitamin B group which help regulate the activity of the nerve cells resulting in a healthy nervous system. Skin benefits from vitamin B contents, too. These, too, are effective in hematopoiesis.

Analysis of recent research and publications

Picard et al [10] stated that another strategy in processing high quality sausages is the employment of probiotics in fermentation. Probiotics enjoy anti-carcinogens that precede the process by neutralizing the toxicity of Geno toxins present in the intestine. Abrahamse et al [11] explained in their research that consumption of probiotics may finally result in the production of a wide range of fermenting inhibitors and antimicrobial components. Among the inhibitors produced through these bacteria are antibiotics, diacetyl, acetoin, acetaldehyde, free fatty acids, ammoniac, and bacteria catalyzing enzymes, ethanol, peroxide hydrogen, bacteriocines, and amino acids. Antimicrobials produced by these bacteria play a major role in health and increasing the half-life in such products. Using nitrates in (red) meat products in order to improve the color and storage period seems inevitable. However, one of the most important factors in nitrosamine present in in meat products is the usage of nitrate salt in such products [12-13]. It seems that using fish as a substitute for red meat in sausages reduces nitrate consumption and also the present probiotics may help prevent such a compound them from forming. According to the study by Xiao et al [14], lactobacillus pentose R3 inoculated in dry fermented sausages was able to reduce the amount of nitrosamine content compared with control sample. It is also believed that sausage structure can protect probiotics while passing through digestive system [15]. Moosavi-Nasab et al [8] reported the residual nitrite level of the lantern fish sausages was obtained at zero and at the end of storage. Also, results from a research that was done by Jahreis et al [16] on some volunteers suggest that daily consumption of 50 grams of probiotic sausages by some volunteers could boost the host's immune system much as there was no considerable decline in cholesterol and blood serum triglyceride.

The purpose of this research was to produce fermented Trout sausage by inoculating two types of probiotics (lactobacillus *Rhamnosus* and lactobacillus *Plantarum*) and replacing part of the fat in the formulation with fat substitutes, followed by the physicochemical, organoleptic and textural properties of the product was studied. Nitrosamine levels of sausage samples were also evaluated in comparison with the control sample. For this purpose, it is necessary to achieve the following **objectives:**

– Study of changes in physicochemical properties of samples in relation to fat, protein, moisture and pH;

– Study of textural properties of the samples in terms of chewiness, springiness, hardness and cohesiveness with respect to the presence of fat substitutes and comparison with control samples;

– Study of sensory properties of fish sausage samples and evaluation of its general acceptance in comparison with the control sample;

– Evaluation of nitrosamine content in Trout sausage samples due to fermentation by probiotics in comparison with control sample without probiotics over time;

Research materials and methods

Fermented Sausages Process

Each batch contains 69% minced trout fish, 18% ice, 6% sunflower seed oil, 2% soybean protein concentrate, 2% modified cornstarch, 1.75% salt, 0.3% polyphosphate sodium, 0.8% spice, 0.2% nitrate salt, 0.02% acid ascorbic, and 0.1% monosodium glutamate. Separate batches containing 2% inulin of whole fat were prepared and 0.1 to 0.5 g/kg of *lactobacillus Rhamnosus* and *lactobacillus Plantarum* were separately inoculated in each. Then the fermentation was allowed within 24 hours at 20°C temperature so a pH of 5 was achieved, and finally, the thermal process occurred at 60°C cooking temperature. An inulin free batch and a starter as control sample was provided [7,17].

Preparing Probiotic Strains

The lactobacillus strains being observed, i.e. *Plantarum 299V* and *Rhamnosus GG* extracted from lyophilized powder, each contained 10^9 CFU/g of bacteria cultured in MRS Broth medium for 72 hours at 37°C and then it was centrifuged at 6000g at 4°C for 15 minutes: moist concentrated cells were achieved.

Chemical Properties Measures

The pH is directly measured with Hanna pH meter in a 1:1 sample in water solution. Protein based on 6.25 coefficients was determined through azote with Kjeldahl method. The moisture was also measured based on BOE meat analysis standard in a way that the difference between samples weight at 100°C were measured until a stationary weight was reached [4,17]. Lipid content was measured with a solvent in a constant reflex system through Soxhlet extractor [18].

Texture Analysis

A British made Model *Perten.TVT 6700* texture analyzer was employed for texture analysis to measure the hardness, springiness, Chewiness and cohesiveness. Pieces of 2×2×1.5 sausages were prepared. In this analysis, a 75mm probe with an approximate 5g propelling force was used [4].

Color Analysis

To determine such color factors as redness (a*), yellowness (b*), and lightness (L*), samples were analyzed through image J software. The illumination of the compartment was performed by using six fluorescent lamps with 8 W (white color). The compartment have camera Canon model, EOS 1000D, the camera was placed at a distance of 30 cm from the samples, and camera was connected to computer by a USB port. The Illustration was performed by EOS utility with 1880 pixels × 2816 pixels resolution.

Nitrosamine Analysis

To analyze nitrosamine, first, it should be extracted from the sample sausages. Two hundred grams of sausages were mixed with 100ml distilled water plus 120gr Sodium Chloride in a 1000ml Erlenmeyer flask till the mixture is boiled on the same steam. Next, 400ml distilled water was transferred to a cold flask of 40ml dichloromethane, mixed with 3-ml sulfuric acid while 80gr table salt was added to be pushed through a separatory funnel. Finally, a filter (0.22-mm pore size) was used; the mixture was condensed under vacuum operator to reach 0.5ml and be transferred to GC-MS (Gas chromatograph mass spectrometers). Column type was DB-5 and the column length was 30 m with 0.25 mm inside caliber. The detecting system was functional at 70 eV. Thermal processing was set at 50 to 250°C with a 6 degree increase [14].

Sensorial Analysis

Ten male and female panelists, equal in number, participated to help analyzing fish sausages through 7-point Hedonic method. Samples were tested to analyze characteristics such as appearance, texture, i.e. chewiness and crispiness, sensory acceptability on day 20 and each parameter was rated from 1 to 7, i.e. from low to excellent [19].

Statistical analysis

The effect of the inulin present in formulation, the type of probiotic strains, and life time influencing the physicochemical properties and nitrosamine samples were compared in three ten-day periods as independent valuables (Table 1) completely randomized factorial design. Statistical analysis was carried out using SPSS (Version 9.0.) followed by Duncan's multiple range tests. The test was used to compare the differences among means. The results are presented as means ± SD with the significance level set at $p < 0.05$ under varying storage periods. And for comparing the groups, an independent sample t test was used to determine the differences.

Table 1 – The name of treatment tested in the study

Treatments	Inulin Content	Probiotic Strains	Period (Day)
Control (Group)	0	0	Zero - 30
1	2% Total Fat	L.Rhamnosus	Zero - 30
2	0	L.Rhamnosus	Zero - 30
3	2% Total Fat	L.Plantarum	Zero - 30
4	0	L.Plantarum	Zero - 30

Results of the research and their discussion

Physicochemical Properties

In this research, such factors as fat content, protein, moisture, and the fish sausage pH were examined in a 30-day period. Samples with inulin, as fat substitutes, demonstrated a rather meaningful difference in fat counts than other samples. Fat content in the samples fermented with two different probiotic strains, however, failed to show a meaningful difference. Due to possible activity of probiotic strains in sausages, at the end of the storage period as compared to the first day, there was a decline observed in fat content. The results corresponded to some researchers' previous works [18,20]. However, some other results suggest an increase in fat content during a 63-day time due to decline in induced moisture. As for the protein content, there was no meaningful difference evident in any of the samples; however, due to moisture decline, it increased within a 30-day period [4]. There is a meaningful decrease in moisture over time. Such a decrease was more evident in fat-reduced samples. This was possible for the activity of probiotics present in sausages: during the activity of probiotics and lowering the pH to the isoelectric point of the proteins, it becomes more compact and water is expelled between its chains, reducing moisture during shelf life. The decrease in moisture completely corresponds to pH so that in lower pH values, moisture, due to little concentration of protein strains, creates little linkage with them, hence acceleration in moisture loss [7]. For their chemical structure, inulin content samples absorb more moisture, but there was no meaningful difference evident. The pH in samples absolutely depends on the presence of inulin for two reasons: acid nature and the passing of time. The pH values increase for the decrease in probiotics activities i.e. experienced a drop from 5.2 to 4.6 (Table 2). The probiotic strains, however, did not cause much meaningful difference in pH amount [22].

Table 2– Physicochemical analysis results of fish sausage's samples during storage

Chemical	**pH**				**Protein (%)**				**Fat (%)**				**Moisture (%)**			
	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
Sample	Day				Day				Day				Day			
control	5.2±0.00 aA	5.2±0.02 aA	5±0.01 aB	5.1±0.02 aA	12.56±0.12 cB	12.6±0.11 bB	12.62±0.19 bB	12.69±0.16 aB	14.4±0.23 aA	14.4±0.18 aA	14.4±0.08 aA	14.3±0.23 aA	63.2±0.23 aA	62.3±0.18 bA	60.1±0.23 cA	57.6±0.16 dD
T1	5.15±0.01 aA	5.05±0.012 bC	4.8±0.03 cC	4.6±0.01 dD	12.35±0.13 cD	12.4±0.08 bD	12.41±0.23 bD	12.45±0.19 aE	9.5±0.2 aB	9.4±0.19 aB	9.1±0.09 bB	9.1±0.16 bB	62.5±0.08 aB	61.9±0.13 bA	60.9±0.25 cA	58.2±0.23 dC
T2	5.2±0.04 aA	5.1±0.05 bB	5.1±0.02 bA	4.8±0.02 cB	12.44±0.08 cC	12.48±0.19 bC	12.49±0.08 bC	12.53±0.08 aD	14.1±0.12 aA	14.2±0.23 aA	14.2±0.09 aA	14.1±0.11 aA	62.9±0.19 aB	62±0.26 bA	60.3±0.2 cA	59.2±0.15 dA
T3	5.2±0.00 aA	5.1±0.02 bB	4.9±0.01 cD	4.6±0.01 dD	12.56±0.16 cB	12.56±0.16 cB	12.59±0.21 bB	12.62±0.23 aC	9.7±0.09 aB	9.5±0.08 bB	9.5±0.02 bB	9.4±0.08 cB	62.8±0.2 aB	60.2±0.09 bC	58.2±0.14 cC	57.9±0.11 dC
T4	5.2±0.05 aA	5.1±0.00 bB	4.9±0.06 cD	4.75±0.0 dC	12.9±0.23 bA	12.91±0.21 bA	12.92±0.23 bA	12.96±0.29 aA	14.2±0.14 aA	14.1±0.09 aA	14.1±0.23 aA	14.1±0.19 bA	63.5±0.18 aA	61.3±0.23 bB	59.4±0.19 cB	58.6±0.08 dB

Small letters show a meaningful difference for period and capital letters show a difference in treatments.

Colorimetry

During storage period, light intensity (L) experienced a little decline. This was related to weight loss and more thickening due to water loss (Olivares et al., 2010). Inulin increase and fat decline coincided with decline in samples light intensity. These observations to results reached by Olivares [4] reported that the addition of inulin and fat decline in Frankfurter sausages caused a decrease in light intensity in sample sausages and an increase in factor (a), i.e. redness. Berizi et al. [23] reported that sample sausages with 6% inulin content experienced little light intensity as compared to control sample.

Table 3. Colorimetry analysis results of fish sausage's samples during storage

Color Analysis	**L^***				**a^***				**b^***			
	0	10	20	30	0	10	20	30	0	10	20	30
Sample	Day				Day				Day			
Control	59.2±1.02 aA	59.2±0.89 aA	58.4±1.32 bA	58.1±1.13 cA	2.8±0.25 cB	2.8±0.45 cC	3±0.21 bC	3.4±0.17 aC	12.1±0.92 bA	12.2±0.74 aA	12.3±0.78 aA	12.1±1.02 bA
T1	54.7±0.58 aC	54.6±1.12 aC	54.1±0.98 aC	53.6±0.88 bD	3.1±0.36 bA	3.1±0.15 bB	3.2±0.32 bB	3.6±0.23 aB	10.3±0.88 bC	10.4±0.62 bD	10.6±0.75 aD	10.5±0.95 aD
T2	57.4±0.23 aB	57.3±0.71 aB	56.6±0.56 bB	56.2±0.35 bB	2.8±0.37 bB	2.9±0.16 bC	3.2±0.34 aB	3.3±0.44 aC	11.2±1.02 cB	11.4±1.02 bB	11.6±1.11 aB	11.6±0.88 aB
T3	54.9±1.03 aC	54.8±1.13 aC	54.2±1.12 bC	54.2±1.23 bC	3.2±0.31 dA	3.4±0.52 cA	3.6±0.91 bA	3.8±0.45 aA	10.6±0.74 cC	10.8±0.95 bC	11±0.95 aC	11.1±0.44 aC
T4	57.1±1.21 aB	57±0.98 aB	56.5±1.09 bB	56.2±1.11 bB	2.8±0.27 bB	2.8±0.45 bC	2.9±0.78 bC	3.2±1.01 aD	11.1±0.63 cB	11.3±1.00 bB	11.4±0.85 bB	11.6±1.02 aB

Small letters show a meaningful difference for period and capital letters show a difference in treatments

In other researches, Ghassaabnejad et al. [24], studying the effects of inulin addition to ewe kefir milk, reported a decline in light intensity had occurred due to the presence of acid lactic bacteria and protein decomposition and as a result of Maillard reaction. Bolenz et al. [25] found that the inulin present in milk chocolate is responsible for more water intake resulting in a decline in light diffraction that reduces chocolate lightness, which corresponds to other researchers' findings [26]. Due to probable absence of myoglobin pigments in fish sausages, redness or factor (a) did not experience a remarkable change over time and during treatments; however, following a decline in fat percentage, factor (b) or yellowness declined a bit, corresponding to researchers' findings.**Texture**

Analysis Characteristics

Over time, the hardness of trout sausages experienced a little increase. One reason, say, was the decline in wetness and the body of the samples [8]. Samples with more fat content were softer than inulin ones (Table 4). Chewiness increased following fat decline [23,27]. Low fat content samples enjoyed little springiness; however, over time, there was little change in values the same as what Liaros et al. [20] and Moosavi-nasab et al. [8] discovered. Cohesiveness in reduced fat content samples was higher than in control samples and high fat content samples. Berizi et al. [23] reported that due to high water intake, inulin substitution may weaken the 3D structure of emulsion of the sausage which in turn increases in cohesiveness.

Table 4- Texture profile analysis (TPA) results of fish sausage's samples during storage

Textural Analysis	Hardness (N)				Chewiness (N.mm)				Springiness (cm)				Cohesiveness (Ratio)			
	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
Sample	Day				Day				Day				Day			
Control	32.6.2± 2.35 cC	32.9± 1.21 cB	33.6± 1.1 bB	34.1± 1.8 aB	12.5± 0.72 cC	12.6± 0.61 bC	12.62± 0.29 bB	12.69± 0.16 aB	0.96± 0.03 aA	0.95± 0.08 aA	0.97± 0.08 aA	0.96± 0.03 aA	0.14± 0.03 cB	0.15± 0.08 bB	0.16± 0.03 bB	0.2 ± 0.06 aB
T1	35.6 ± 0.92 cA	35.9± 1.52 cA	36.5± 1.12 bA	37± 2.01 aA	13.9± 0.43 cB	13.9± 0.98 cB	14.1± 0.33 bA	14.45± 0.49 aA	0.86± 0.02 aD	0.82± 0.09 bC	0.88± 0.09 aB	0.88± 0.06 aB	0.17± 0.08 cA	0.17± 0.03 cA	0.19± 0.05 bA	0.24± 0.03 aA
T2	30.2 ± 0.77 bD	30.9± 1.05 bC	32.1± 2.02 aC	33.2± 0.9 aC	12.4± 0.78 cC	12.48± 0.39 bC	12.49± 0.98 bB	12.53± 1.08 aB	0.91± 0.12 bC	0.96± 0.03 aA	0.94± 0.09 aA	0.96± 0.01 aA	0.11± 0.09 aC	0.12± 0.06 aC	0.11± 0.02 aC	0.13± 005 aD
T3	34.5 ± 1.35 cB	35.2± 2.16 bA	36.9± 1.87 aA	37.2± 0.56 aA	14.2± 0.66 bA	14.5± 1.16 aA	14.5± 0.21 aA	14.6± 0.23 aA	0.85± 0.09 cD	0.9 ± 0.08 aB	0.91± 0.02 aB	0.89± 0.08 bB	0.19± 0.02 cA	0.18± 0.09 cA	0.22± 0.04 bA	0.24± 0.01 aA
T4	29.8 ± 1.11 cD	30.5± 1.23 cC	31.6± 0.88 bD	32.4± 1.23 aC	12.9± 0.73 bC	12.91± 0.51 bC	12.92± 0.83 bB	12.96± 0.79 aB	0.92± 0.14 aB	0.91± 0.09 aB	0.91± 0.03 aB	0.89± 0.09 bB	0.13± 0.08 bB	0.14± 0.03 bC	0.17± 0.09 aB	0.17± 0.08 aC

Small letters show a meaningful difference for period and capital letters show a difference in treatments

Nitrosamine Content

Nitrosamines in meat products take different forms: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) nitroso dibothylamine (NDBA), and nitrosopyrolydine (NPYR) are the most common nitrosamines in meat products. All the nitrosamines of all samples were analyzed in this research. The nitrosamine present in control sample was meaningfully higher than the ones in other samples. This means that the presence of probiotics inhibited the formation of nitrosamines. Probably, it was due to the formation of organic acids that such event failed to occur. Although nitrosamine increased over time, the inulin probiotic samples could almost keep the amount at a constant level. Over time, probiotic samples containing inulin were more effective in decreasing nitrosamine than those missing it (Fig 1). These findings correspond to other researchers' results studying lactobacillus strains used in fermented sausages [14, 28].

Sensory Analysis. In sensory analysis, excluding the distinct odor in the control sample, testers gave better marks comparing it to other properties. Crispiness, mouth feel, and the appearance of the control sample scored higher. However, inulin content samples inoculated with aroma and scent strains, scored better. Probably, the activity of probiotic strains could relatively remove the fishy flavor. Samples with lower fat content gained weaker oral sensation and chewiness for they were apt to more hardness. Provided scores, however, were so near. The results are shown in figure 2.

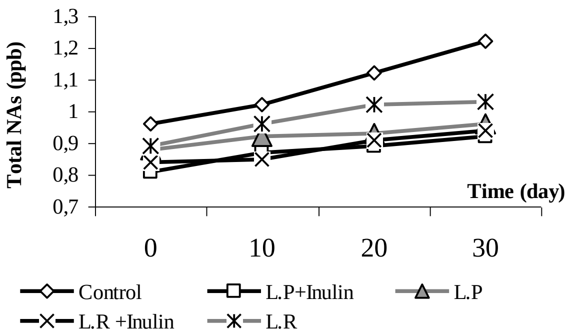

Fig. 1. Nitrosamine content in fish sausage's samples during storage

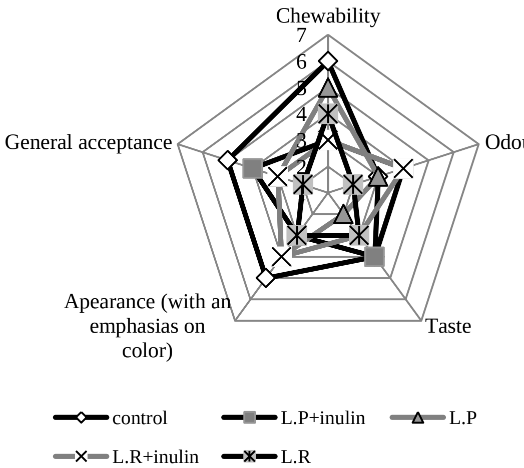

Fig. 2. The results of sensory evaluation of fish sausage's samples

Conclusion

Generally, the effectiveness of inoculation of probiotics on trout sausages, also replacing part of fat in sausages formulated with inulin and comparing it with control sample showed that samples with inulin content have lost less moisture during storage period. The fat in probiotic content samples was low at the end of storage period. The pH in probiotic sausages as compared to control sample showed fewer declines. Inulin content samples increased the lightness intensity and redness. Meanwhile, the presence of probiotics decreased the intensity of lightness in the samples. Inulin content samples demonstrated more hardness than the fattier samples. The presence of probiotics increased the protein fibers and made them denser due to pH decline to isoelectric point. Cohesiveness, too, increased as fat declined. Nitrosamine present in probiotic content samples as compared to control sample increased; however, all samples increased over time. Sensory analysis voted for control samples despite fishy odor was more evident. Other samples in the sensory analysis did not demonstrate much sensory difference.

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