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Original article

Production of fresh fish sausages containing natural preservatives (*Laurus nobilis L.*) it's nutritional composition and oxidative stability

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Summary. *Aim*: The aim of this study was develop the fresh fish sausage containing natural protective from rainbow trout (*Oncorhynchus mykiss*) and it's was investigate the nutritional composition and oxidative stability during storage at 2°C. *Method*: For this purpose were stored the fresh trout sausages added 2% *Laurus nobilis* extract and 4% *Laurus nobilis* extract for 10 days. Three different sausage formulations were analyzed for nutritional composition and lipid oxidation products (total volatile base nitrogen (TVB-N) thiobarbituric acid (TBA), peroxide value (PV) and free fatty acids (FFAs)). *Results*: The formulated fresh trout sausages determined 68.11-68.26% moisture, 18.06-18.25% protein, 6.12-6.15% lipid, 3.14-4.28% ash as "good quality" and " might buy". According to oxidative stability results the highest levels of TVB-N TBARS, PV and FFAs were related to the control group and the least amount of these parameters was determined in *Lauris nobilis* extract (LE) applications. *Conclusion*: Based on the this datas, it can be deduced that the LE successfully improve the oxidative stability of the fresh trout sausages in the cold storage

Keywords: *Oncorhynchus mykiss*, Fresh fish sausage, *Laurus nobilis*, Natural preservative, Lipid oxidation

Introduction

All over the country, fast food is becoming more and more popular and fast food business is growing. Fish sausage, a product of new generation fast food, is popular especially among young generation due to its delicious taste, unique texture and color, and high nutritional quality. Its popularity is increasing among the working people as it is easy to prepare. The sausages are produced in different ways, dried, cured and cooked. Fresh sausages are not heat treated and they are sold in a raw state. In basis, fresh sausages are not cured. In many countries of the world they are produced on demand in butcher shops. At a cheaper price, people can obtain an excellent taste and quality nutrition from fish sausage, which is made with minced fish. Fish sausages are also seasoned with various spices to ensure that they have tasty flavors (1). On the other hand, one disadvantage fish sausages are that they have a limited shelf life and are vulnerable to deterioration. Therefore, increasing the shelf-life of fish sausages will reduce losses due to spoilage, thus, yielding remarkable economic results, and will make it possible to disseminate the product to distant and new markets (2). Recently, a lot of concerns have been voiced about whether synthetic food preservatives are safe due to their potential toxicity. For this reason, the use of natural protectives to stabilize fatty food has come to the agenda of nutritionists and food experts who attach importance to health (3,4).

Laurus nobilis L., also known as laurel, is an evergreen tree or large shrub belonging to the family Lauraceae. It is native to southern Europe and the Mediterranean region, so, it is grown in many countries in this region. Other terms used for Laurus include sweet bay, bay laurel, Grecian laurel, true bay, and bay tree (5). Throughout history, people have used Laurus leaves for the treatment of epilepsy, neuralgia, and parkinsonism. Besides, Laurus leaves are commonly used

in different cuisines in combination with various herbs to add flavor to soups, brines, sausages, fish recipes, and meat dishes (6). The antimicrobial and the antioxidant properties of laurel essential oil and its extracts have been reported by several studies. Its main components are 1,8-cineole, α-pinene, β- pinene, sabinene, limonene, and linalool (5,6).

In this study, it was focused on determining the nutritional value of fresh sausage produced from rainbow trout and their investigating oxidative stability by supporting with natural preservative (*Lauris nobilis*). We knowledge, this reserach is the first study made with natural preservative on the fresh fish sausage.

Materials and Methods

Reagents

Laurus nobilis L. extract (pure %100) was obtained from a commercial company (1001 Natural). The other ingredients for the sausage were obtained from local markets.

Oncorhynchus mykiss

From a local market in Elazığ, a city in Eastern Anatolia, Turkey, fresh *Oncorhynchus mykiss* were purchased. For fresh sausage production, three fish, which weighed around 5-6 kg, were used. Firstly, the fish were put on ice in polystyrene to be transported to the laboratory. Then, they were cleaned, skinned, and filleted under aseptic conditions.

Formulation and sausage process

Fresh sausages were prepared based on the method proposed by Veloso et al. (7), but with some changes. The formulations were used 2 kg trout minced for each treatment. The ingredients used for the preparation of sausages were determined with respect to preliminary trials: trout fillets (80%), sunflower oil (6%), potato starch (6%), sausage seasoning (1%), salt (NaCl)+sodium nitrite (1%), stabiliser (0.25% containing sodium tripolyphosphate), and antioxidant (0.25% containing sodium erythorbate and ascorbic acid), milk powder 0.5%, ice (5% for control group, 3% for LE2 group, 1% for LE4 group) and *Lauris nobilis* extract (0% for control group, 2% for LE2 group, 4% for LE4 group).

A kitchen meat mincer (Russell Hobbs 23480-56/RH) with a pore size of 3.0 mm was used to mince the trout fillets. Following that, salt, stabiliser, sodium nitrite and half of the ice were added, which were then mixed for 2 min at a higher speed. Afterward, potato starch, other half of the sunflower oil, antioxidant and the other half of the ice were added. Finally, sausage seasoning was added to the mixture, then mixed at a low speed for one minute. Subsequently, the mixture was filled in natural casings obtaining fresh trout sausages with 3 cm diameter and 50 g per unit. Fresh trout sausages were packed in plastic bags without vacuum and stored at 2°C for 10 days. Two samples were taken from each batch in duplicate for analysis.

Nutritional Composition

Nutrition composition of sausages were performed according to AOAC (8) methods. The moisture content of fresh sausages was measured by drying the fish sample at 105°C until a constant weight was obtained. Crude protein content was performed by Micro-Kjeldahl system (6.2 x N). Fat content was determined using the Soxhlet system. Ash content the samples were determined by dry ashing in a furnace at 550-600°C.

Lipid oxidation analysis

TVB-N (Total Volatile Bases Nitrogen) value was performed in accordance to, in duplicate, the method of (9).

TVB-N datas was calculated using the following equation

TVB-N (mg N/100 g) =

[volume HCL (mL)×normality of HCL×14× extraction volume TCA×100] / (25 × sample weight)

The lipid oxidation was measured by the thiobarbituric acid reactive substances (TBARS) method, in duplicate, according to Tarladgis et al (10). For the calculation of the values of TBARS, a standard curve for thiobarbituric acid (TBA) reagent was obtained, and the results were stated as equivalent mg of malonaldehyde/kg sausage sample.

Measurements of the peroxide value (PV) (11) were carried out of the fresh trout sausages. The peroxide value was transformed from mmol/kg to meq oxygen/kg by multiplying it with 2.

The hydrolytic degradation of sausage samples was explained by percentage of free fatty acids (FFAs),

which was stated using traditional titration method defined by AOCS (12).
The following equation was employed to calculate the amount of FFAs:
FFA = V×28.2×100/ W×1000
V; volume of sodium hydroxide, and W; sample fat content.

Statistical analysis

SPSS statistical package program was employed for the statistical analysis of research data (IBM, version 22.0, USA). All data were expressed as mean ± standard deviation (mean± SD). To determine the statistical significance ($p<0.05$), analysis of variance (ANOVA) was performed. Two-way ANOVA was used with treatment and storage time as main effects for the analysis of the storage data. Finally, Duncan's multiple range test was employed for the comparison of means (13).

Results and Discussion

The nutritional compositions of trout sausages are presented in fig 1. The moisture contents, protein and lipid of samples were no found to be significantly different($p>0.05$) but ash content of treatment groups increased when compared to control group ($p<0.05$). The high value of ashes in the sausages might have occurred by the extract adding in the formulation.

Similar results were recorded by Veloso et al (7), Raju et al (14), Oliveira Filho et al (15), and Egbal and Ghada (16). The Turkey meat and meat products legislation does not prescribe the ratio of nutritonal composition for fresh sausage, therefore there is no way to check the composition with any set limits.

TVB-N is another index used to determine the quality of fish and fish products and it is related to spoilage by the activity of endogenous enzymes and bacterial growth. TVB-N is composed of ammonia and primary, secondary and tertiary amines derived from the deterioration of nucleotides and from the deamination of amino acids by microorganisms (17-19).

The acceptability limit value of the TVB-N is 30mg N/100g (20,21). In accordance with statistical analysis, significant difference ($p<0.05$) was found between groups starting from the day 4 of chilled storage. Lowest TVB-N value was determined in sausage samples applied with 4% *Lauris nobilis* extract during the storage (Fig. 2). Higher TVB-N values were observed in control samples as per LE treated sausage samples (LE2 and LE4) throughout the chilled storage. Though the control group arrived the acceptability limit of 30 mgN/100g at the end of the 10 days, LE2 and LE4 groups were still under this limit. This demonstrates that bay leaf extract (LE) applications had a significant impact on the reduce of TVB-N values in trout sausages by phenolic features. LE4 group, which containing a higher rate of *Lauris nobilis* extract, showed the highest antioxidant activity, LE2 group indicated the lowest antioxidant activity along the chilled storage. Antioxidant activity of *Laurus nobilis* was previously

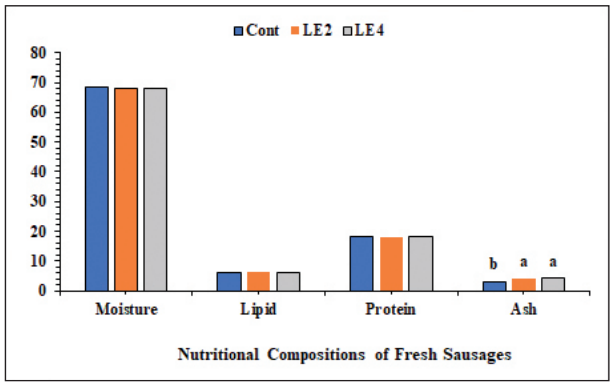

Fig 1. Nutritional Composition of Produced Fresh Sausages from *Oncorhynchus mykiss* **Cont:** Control; **LE2:** 2% *Lauris nobilis* extract; **LE4:** 4% *Lauris nobilis* extract

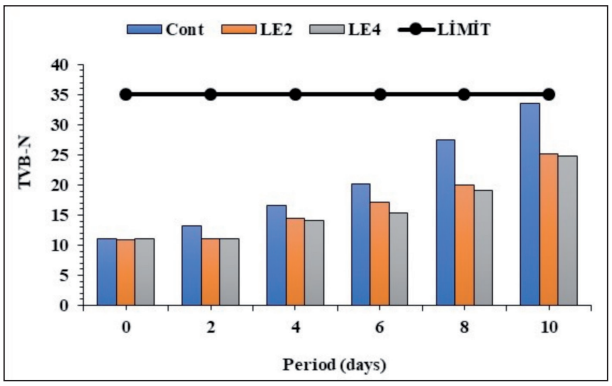

Fig 2. Changes in TVB-N values (mgN/100g) of fresh trout sausages during chilled storage **Cont:** Control; **LE2:** 2% *Lauris nobilis* extract; **LE4:** 4% *Lauris nobilis* extract

noticed studies (22,23). A similar results of the TVB-N value rise along the chilled storage was announcemented by some researcher (24,25).

Peroxide value is a determine of oxidation ratio of lipids and is used for determine the freshness of fish and fish products. Oxidative rancidity reason to off-flavor and odour breakdown in seafood and limits the shelf life of seafood products (3,26). The level of PV (meq O2kg-1) in 2°C fresh trout sausages are demonstrate in Fig.3. The initial PV values were found as 2.12 meq O2kg-1 in control, 2.13 meq O2kg-1 in LE2 and 2.05 meq O2kg-1 in LE4.

As display in Fig.3, the Peroxide value of all sausage samples rised with different rates during the chilled storage period ($p<0.05$). The rising in the PV was partly owing to the dehydration of sample and boosting the oxidation of unsaturated fatty acids (27). There were a significant differences between the Control and treatment groups ($p<0.05$). It is known LE has an important effect on oxidation due to antioxidant and oxygen barrier properties (28). Pending the storage period, LE4 group (4% *Lauris nobilis* applicated fresh sausages) indicated significantly lowest peroxide value than the other groups ($p<0.05$). This rate *Lauris nobilis* extract significantly reduced the lipid oxidation. Varlık et al., (28) recommended 2 meq O2kg-1 and 8-10 meq O2kg-1 to be upper limits for the qualification of the "good quality" and "acceptability level", respectively. In day 10, the PV of control group and LE2 group overruned the acceptability level. Shelf life of control and LE2 groups ended after 8 days. LE4 group were lower than proposed limits during day 10. Application of fresh trout sausage with 4% *Lauris nobilis* extract (LE4) significantly decreased the peroxide value and extend shelf life ($p<0.05$)

Thiobarbituric acid Reactive substances (TBARS) is reported indicators to demonstrate the degree of lipid oxidation during the seafood storage (3). TBARS values were expressed as milligrams of malonylaldehyde content per kilogram, was shown in Fig. 4. The results displayed that TBARS value of all groups rised continually at during storage period ($p<0.05$). This study conclusions are parallel to the findings of López-Caballero et al., (30), Fan et al. (18), Krkić et al., (31). The increase in this index during chilled storage may be owing to a partial dehydration of the samples and an increment in the unsaturated fattyacids oxidation. According to Shormuller (32), a level of 7 mgMDA/kg of fish muscle is generally considered as spoiled. In this study, TBARS value in control and LE2 group samples overruned this limit on days 10, while in LE4 group this concentration keeped below this limit at during storage period. These results are in lined with findings of Emir Çoban et al. (4) who reported that TBARS values of Carp sausages (*Cyprinus carpio*) added with propolis extract reduced as compared to control during chilled storage. Emir Çoban (4), who found the increase of TBARS value in storage time. These results may be declared that the antioxidant activity of *Lauris nobilis* extract is related to the antioxidant activity of phenolic compounds through mechanisms such as radical chains formation prevention.

It is well known that FFA is a result of th enzymatic decomposition of lipid in frozen fish (33). The

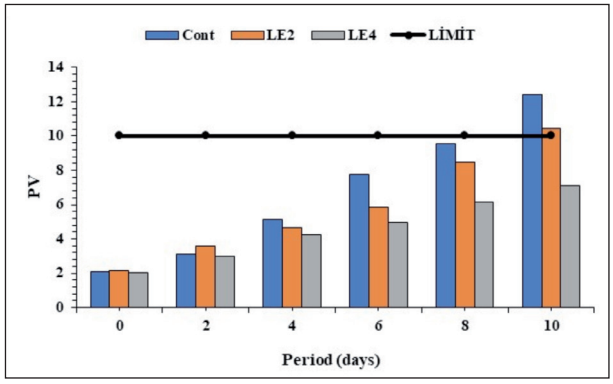

Fig 3. Changes in PV values (meq O2kg-1) of fresh trout sausages during chilled storage **Cont:** Control; **LE2:** 2% *Lauris nobilis* extract; **LE4:** 4% *Lauris nobilis* extract

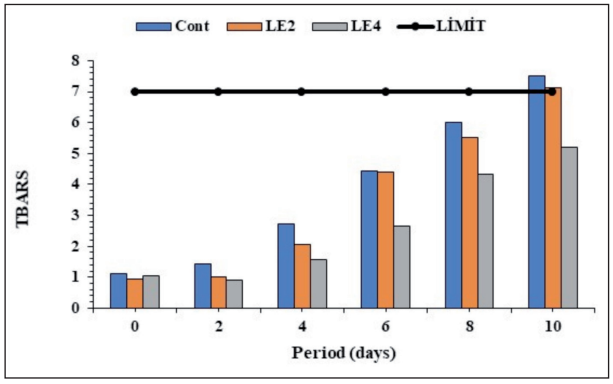

Fig 4. Changes in TBARS values (mgMDA/kg) of fresh fish sausages during chilled storage **Cont:** Control; **LE2:** 2% *Lauris nobilis* extract; **LE4:** 4% *Lauris nobilis* extract

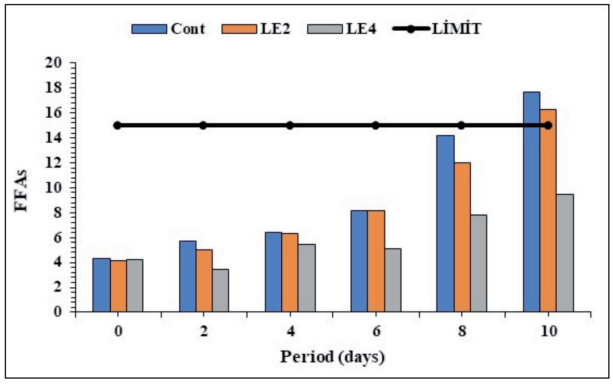

Fig 5. Changes in FFAs values (mg/g oleic acid) of sausages during chilled storage **Cont:** Control; **LE2:** 2% *Lauris nobilis* extract; **LE4:** 4% *Lauris nobilis* extract

acceptable limit for FFA is 15 mg/g (as oleic acid). In the initial FFA values were 4.3 mg/g, 4.1, 4.2 for control, LE2and LE4 group respectively (Fig.5). Significantly higher FFA value ($p<0.05$) was determined in the control group until days 10. Throughout storage, the lower FFA was found for LE extract-applicated groups ($p<0.05$). This is because *Lauris nobilis* extract (bay leaf) blocked oxidation in fresh trout sausages. The antioxidant features of LE are due to their high content of phenolic compounds (34,28). Similar results were recorded by some researchers (5,23,35,36). At day 10, the rising in FFA content of 4% *Laurus nobilis* extract added fresh trout sausages were low. On the other hand, free fatty acid value of control group and LE2 group arrived the acceptible limit value (15 mg/g).

Conclusion

It could be finalized from this study that the fillet of trout can be used for preparation of fresh sausage with good nutritive value and flavour acceptability. In addition, this study has demonstrated that *Lauris nobilis* extract added fresh trout sausages have a positive effect on lipid oxidations parameters of sausages during storage. The best overall quality was ensured from LE4 samples. The fresh trout sausages suplemented with natural preservative could safely be stored in 2°C during 10 days. That's why, a better chance could be submitted at favorable market times.

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