Evaluation of Properties and Characteristics of Functional Trout Sausages Containing Probiotics and Fat Substitutes

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Abstract
Decrease in nitrosamine and optimal smell and flavour gives credit to such meat products as fermented sausages for their considerable optimal storage period and better nutritional values. Replacing red meat with fish and using replacer fat 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 Lactobacillus plantarum, substituting part of its fat with inulin while examining the physicochemical, textural, dietary, and sensory properties. The results showed that samples containing inulin lost less moisture over time (less than 4%). The fat content of the samples did not differ significantly over time and was almost the same. However, the pH of the samples containing probiotics decreased more than the control sample and reached about 4.6-4.8. 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 odour being present, sensory analysis was in favour of the test samples. Other samples demonstrated little difference in sensory evaluation.

Keywords: Fermented sausage, Inulin, Lactobacillus plantarum, Nitrosamine, Trout

Introduction
Among meat products, we can mention fermented sausages, which have a significant share in the world because they have special characteristics such as higher shelf life, better nutritional value due to nitrosamine reduction and more suitable flavour which are mainly due to bacterial metabolites produced during fermentation (Xu et al., 2010). However, sausages are rarely considered a health product because the presence of nitrate, salt and high levels of fat destroy the image of a healthy food (Vuyst et al., 2008). The high fat content in these products is responsible for creating various characteristics in different types of sausages such as source of fat-soluble vitamins, energy source, distribution agent and creating aroma, taste, mouthfeel, texture and overall acceptance by the consumer (Olivares et al., 2010; Rokni, 2008). In this regard, the use of fish meat instead of red meat, the use of fat substitutes and the use of probiotic
strains in various types of sausages has been able to produce a profitable and functional product to a large extent (Alinezhad, 2011; Attar et al., 2016). Trout meat is a good source of iron that is effective in preventing iron deficiency anemia. Salmon omega-3 fats can also regulate blood pressure and heart rate. Its protein is easily digested and absorbed in the gastrointestinal tract. Also, the element zinc in it has an important effect on the growth of children and in regulating the sexual maturity of adolescents, and its consumption during growth prevents short stature. Trout contains a significant amount of B vitamins, which is also effective in regulating the activity of nerve cells and hematopoiesis. The presence of probiotics in fermented sausages in addition to anti-carcinogenic properties by producing a variety of inhibitory fermentative compounds play an essential role in the health and increase the half-life of these products and can prevent the formation of nitrosamines during storage of these products (Wang et al., 2015; Xiao et al., 2018). According to the above, in this study, the properties of Trout fermented sausage with probiotics and the replacement of part of its fat with inulin were investigated.

**Methods and methods**

After producing sausages with specific formulation, separate batches were prepared with inulin at the rate of 2% of the total fat and the compact and moist cells of two starters *Lactobacillus Rhamenusus* and *Lactobacillus plantarum* were inoculated with 0.1 g/kg. Then, for 24 h at 20 °C, fermentation was allowed to reach a pH of about 5 and finally heat treatment at 60 °C at room temperature. A batch without inulin and starter was prepared as a control sample and its characteristics were examined over a period of 30 days (Attar et al., 2016; Dinçer et al., 2017). Table (1) shows the desired treatments in terms of the amount of fat substitutes and probiotics used as independent variables.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inulin (%)</th>
<th>Probiotic</th>
<th>Time (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0-30</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2% of total fat</td>
<td><em>Lactobacillus rhamnosus</em></td>
<td>0-30</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0</td>
<td><em>Lactobacillus rhamnosus</em></td>
<td>0-30</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2% of total fat</td>
<td><em>Lactobacillus Plantarum</em></td>
<td>0-30</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0</td>
<td><em>Lactobacillus Plantarum</em></td>
<td>0-30</td>
</tr>
</tbody>
</table>

**Assays**

In this study, physicochemical properties such as pH, protein content were measured by Kajeldal method, moisture content was measured by weight difference and fat content was measured by Soxhlet method according to meat analysis standard (AOAC, 1975; AOCS, 2006). The texture of the samples was evaluated for factors such as hardness, springiness, chewiness and cohesiveness (Olivares et al., 2010). Colorimetric factors such as redness, yellowness and brightness were analyzed using Image J 1.52V software (Huang et al., 2013). The amount of nitrosamine after its extraction was measured by gas chromatography-mass spectrometry GC-MS (Xiao et al., 2018). Sensory evaluation of samples was performed by 7-point hedonic method in terms of characteristics such as appearance, texture (chewability and crispness) and oral sensation (Rubio et al., 2013). In this study, the effect of the presence of inulin in the formulation, type of probiotic strain and shelf life in three 10-day intervals as independent variables in a completely randomized design on physicochemical properties, color, texture and nitrosamine of samples LSD test at 95% level.

**Results and discussion**

Samples containing inulin as a fat substitute had a significant difference (*P*>0.05) in terms of fat content with other samples. But the amount of fat between samples containing inulin and
probiotic strains during the study period was not significantly different at the level of 5% (Liaros et al., 2009; Ndife et al., 2019). Protein content was not significantly different between any of the samples ($P > 0.05$) but increased over a 30-day period due to a slight decrease in moisture relative to the total weight. The moisture content of the samples decreased significantly over time. The pH of the samples was completely dependent on the presence of inulin due to its acidic nature and time. Over time, the pH decreased due to the activity of probiotics, from about 5.2 to about 4.6. During storage, the intensity of Lightness decreased. This was due to weight loss and thickening of the tissue due to water loss. On the other hand, by replacing inulin with fat, the brightness of the samples was significantly reduced ($P > 0.05$). Probably due to the lack of myoglobin pigment in fish sausages, the amount of redness or factor did not differ significantly between treatments at the level of 5%, but with a decrease in the percentage of fat, the amount of factor b or yellowness was reduced to a very small extent (Olivares et al., 2010). The hardness of sausages increased slightly over time. One of the reasons for this was the reduction in moisture and compaction of most samples (Moosavi-Nasab et al., 2018). Fat-containing samples were softer than inulin-containing samples. Chewing also increased with decreasing fat in the samples (Berizi et al., 2017; Selgas et al., 2009). Berizi et al. (2017) reported that inulin replacement due to higher water absorption weakens the three-dimensional structure of the sausage emulsion, which in turn increases coherence. The amount of nitrosamine in the control sample was significantly ($P < 0.05$) higher than other samples and over time increased from 0.96 to 1.22 pbb. However, its comparison with samples containing probiotics showed that the presence of probiotics and their activity in the production of organic acids prevented the formation and increase of nitrosamines over time. Although the amount of nitrosamine in the control sample increased over time, probiotic samples were able to maintain its level at an almost constant level (0.89-0.92 pbb) over time. In sensory evaluation, most evaluators gave higher scores to other characteristics of the control sample than the more specific smell of fish. The fragility, mouthfeel and appearance of the control sample scored higher. However, samples containing inulin inoculated with both strains obtained better aroma.

**Conclusions**

Inulin-containing samples generally lost more moisture over time. Also, the fat of the samples over time did not differ significantly at the level of 5% compared to the first day. The pH of the samples in sausages containing probiotics decreased compared to the control sample. Samples containing inulin decreased light intensity and increased redness. On the other hand, the presence of probiotics also reduced the lightness of the samples. Inulin-containing samples were harder than fat-containing samples. The presence of probiotics also increased the protein filaments and increased the hardness due to lowering the pH to the isoelectric point. Cohesiveness also increased with decreasing fat. The amount of nitrosamine in the samples containing probiotics was lower than the control sample and the samples containing probiotics over time were able to prevent the increase in the amount of nitrosamines and keep its amount almost constant. Sensory evaluation also voted for superiority of control samples, although the smell of fish was slightly more noticeable. Other samples did not differ much in sensory evaluation.

**References**

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