Use of Chitosan in Turkish Sausage (Sucuk) Production and Effects on Quality [1]

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[1] This study is summarized from the doctorate thesis with the same name
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Summary

This study aims to investigate the effect of chitosan, natural polysaccharide, use in different proportions (0.05%, 0.1%, 0.5% and 1%) on the quality of Turkish sausage production. In the study, as a control group, the first group was added only 0.05% nitrate. Microbiological analysis (total aerobic mesophilic bacteria, Enterobacteriaceae, coliform and Escherichia coli, sulphite-reducing clostridia, mold-yeast count) was done in the four different stages of experimental sausage production, (meat [DN1], after mixing [DN2], after filling [DN3], after ripening [DN4]) and on the 1, 7, 15, 30 and 60 days of the storage. Sensory qualities of experimental sausage samples (flavor, color, appearance and texture) were evaluated in the DN4. It was then determined that a little amount of chitosan addition (0.05%, 0.1% and 0.5%) into the production of Turkish sausage affected the microbiological and sensory quality positively. However, addition of much larger amounts (such as 1%) affected the sensory quality in a negative way. Moreover, it was determined that higher amounts of chitosan applications (0.5% and 1%) created technological problems.

Keywords: Antimicrobial effect, Quality, Chitosan, Turkish sausage (sucuk)

INTRODUCTION

Meat content has great importance for human nutrition because of the nutrients it contains. Human beings have always sought ways to make meat more durable and to process it through different aroma to increase its flavors because it has been known for ages that meat is also a good condition for the microorganisms to grow and develop 1. Turkish sausage, which has the most production rate in Turkey among the meat products, is a fermented spicy product with a medium acetic taste, which is air-dried and not fumed 2.

Food additives used for preservation are supposed to be preventive of the growth and development of microorganisms and pathogen bacteria causing food spoilage. Moreover, they should not affect human health adversely and have toxic characteristics. For this reason, consumers demand food without chemical additives 3. That’s why; recently, additives with natural origin or antimicrobial activity such as chitin, chitosan, and their derivatives have increasingly become important. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-
linked D-glucosamine (deacetylated unit) and N-acetyl-
D-glucosamine (acetylated unit) 8. Chitosan has attracted
great attention in food industry as protective additive
because it retains fat and water and because it has the
capability to create color and increase the durability as
well as having antibacterial and antifungal properties 7.
Its antibacterial property is explained in terms of different
mechanisms. In the first mechanism, -NH₂ groups of
chitosan turn into -NH⁺3 groups in an acetic environment
and cell membrane gets damaged as a result of the
electrostatic interaction between the -NH⁺3 groups and
negatively charged phosphoryls and phospholipids, the
components of cell membranes of bacteria. In the second,
the chitosan molecule penetrating into second cell
connects with DNA and kills the cell by blocking its protein
synthesis. In the third, because of polycathonic structure of
the chitosan molecule penetrating into cell and it kills the cell by deforming its activities 8-10. In various studies over the antibacterial eff ect of chitosan on
different foods in other countries were evaluated 4,6,7,11-13.

**Use of Chitosan in Turkish Sausage**

**MATERIAL and METHODS**

**Chitosan**

Chitosan (CAS No: 9012-76-4, 75-85% deasetylation
degree, medium molecular weight (MMW)) was obtained
from the firm Sigma-Aldrich. Chitosan solutions were
obtained from the process in which chitosan was dissolved
in magnetic mixer (Heidolph MR 3002) in 1% acetic acid
(Merck 1.000631000) 8.

**Experimental Sausage Production**

In the preparation of sausage batter (paste), 90% of
beef and 10% of grease (tail fat) was used. Proportions of
additives and spices used in the formulation were in
accordance with the standard proportions mentioned in
Production Regulation (EBK in Turkish) 14. The obtained
mixture was divided into 5 groups of 2 kg each. Nitrate
with the proportion of 0.05% was added into only the
first group to evaluate it as a control group. 0.05% (0.05%
Chi), 0.1% (0.1% Chi), 0.5% (0.5% Chi) and 1% (1% Chi)
(respectively) chitosan proportion solved in the solution of
1% acetic acid was added into the other four groups. The
mixtures were mixed again in order to obtain a homo-
genous mixture and sausage batter (paste) was obtained.
Sausage batter (paste) made ready for filling were filled
into the natural intestinal casing. After ripening process,
the samples were stored at 4°C.

**Microbiological Analysis**

Ten g mixer (Stomacher Lab. IUL) from the samples in
aseptic conditions in a laboratory was weighed into a special
sterile bag and 90 ml of dilution fluid Maximum Recovery
Diluent (Merck 1.12535) was added on samples and the
mixture was homogenized. An automated TEMPO system
was used for counting (total aerobic mesophilic bacteria,
*Enterobacteriaceae*, coliform, *E. coli*) of microorganisms in
food quality indicator. TAMn, *Enterobacteriaceae*, coliform
and *E. coli* counts (bioMerieux) was performed in the
TEMPO system. Tempo TVC medium cards were used for
the analysis of TAMn and in 30°C for 40 h 15. Tempo EB
medium cards were used for *Enterobacteriaceae* counting
and in 35°C for 22-27 h 16. Tempo TC medium cards were
used for coliform count for 22-27 h in 30°C 17 and Tempo
EC medium cards were used to count *E. coli* for 22-27 h
at 37°C 18 after incubated. Tempo cards were evaluated
automatically by a reader. Sulfide Iron Agar (Merck 1.10864)
was inoculated for sulfite-reducing clostridia count and a
cooled (up to 5°C) Sulfide Iron Agar was added in order to
obtain a secondary layer with 10 ml and it was incubated
at 37°C for 48±2 h 19. For mold-yeast count, Dichloren Rose
Bengal Chloramphenicol Agar (DRBC, Merck 1.00466) was
incubated at 25°C for 5 days 20. A scale of hedonic type was
used for sensory evaluation. Samples were evaluated by
a testing panel in terms of color, flavor, appearance and
texture 21.

**Statistical Analysis**

SPSS/PC version 10.0 program was used in making statistical accounts 22.

**RESULTS**

Chitosan in different proportions was added to our
traditional product, Turkish sausage in order to increase
the quality and shelf life. On the meat used in the
production of sausage (DN1), after the mixture (DN2), after
the quality and shelf life. On the meat used in the
production of sausage (DN1), after the mixture (DN2), after
the filling (DN3), after-ripening (DN4) and microbiological
analysis on the 1, 7, 15, 30 and 60 days of the storage
(*TAMB*, *Enterobacteriaceae*, coliform *E. coli*, sulfite-reducing
clostridia and mold-yeast count) were performed. The
stages and the days of microbiological analysis of sausage
samples are shown in Table 1.

Statistically significant differences between groups were observed in point of the TAMB number in DN1 (P<0.05). A similar situation was also observed in DN1, and it has been determined that 0.05% Chi group has similar number of TAMB with control group, the number of TAMB decreases depending on the increase of chitosan application and there are differences between the groups (P<0.05). Given
the storage period, the lowest number of TAMn was found in 1% Chi group (Table 1). Significant differences between
groups were determined in point of the number of
*Enterobacteriaceae*. During this period, it has been observed that 0.05% Chi and 0.1% Chi groups of containing similar
numbers of *Enterobacteriaceae* group microorganisms, 0.5% Chi group showed similarities with other chitosan treated
Table 1. Microbiological analysis stages and days in sausage (sucuk) samples

<table>
<thead>
<tr>
<th>Group</th>
<th>DN3</th>
<th>DN4</th>
<th>7</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>DN3</th>
<th>DN4</th>
<th>7</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.08±0.26a</td>
<td>7.29±0.22a</td>
<td>6.77±0.46a</td>
<td>6.21±0.42a</td>
<td>5.52±0.47a</td>
<td>4.86±0.52a</td>
<td>5.08±0.49</td>
<td>4.80±0.40a</td>
<td>4.15±0.59a</td>
<td>3.54±1.29a</td>
<td>1.38±0.92</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>0.05 Chi</td>
<td>6.04±0.25a</td>
<td>7.13±0.19a</td>
<td>6.65±0.24a</td>
<td>6.12±0.30a</td>
<td>5.43±0.46a</td>
<td>4.66±0.33a</td>
<td>4.95±0.35</td>
<td>4.56±0.27a</td>
<td>3.92±0.49a</td>
<td>2.84±1.30a</td>
<td>1.36±1.42</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>0.1 Chi</td>
<td>5.97±0.24a</td>
<td>6.96±0.20c</td>
<td>6.31±0.37c</td>
<td>5.75±0.33c</td>
<td>4.99±0.34c</td>
<td>4.29±0.40c</td>
<td>4.83±0.34</td>
<td>4.38±0.31a</td>
<td>3.55±0.65a</td>
<td>2.12±1.27b</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>0.5 Chi</td>
<td>5.89±0.23a</td>
<td>6.72±0.32a</td>
<td>6.25±0.52a</td>
<td>5.56±0.25a</td>
<td>4.77±0.24a</td>
<td>3.99±0.62a</td>
<td>4.74±0.31</td>
<td>4.24±0.26a</td>
<td>3.37±0.65a</td>
<td>2.08±0.98a</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>%1 Chi</td>
<td>5.77±0.19a</td>
<td>6.48±0.31a</td>
<td>5.92±0.35a</td>
<td>5.35±0.37a</td>
<td>4.52±0.35a</td>
<td>3.30±1.21a</td>
<td>4.67±0.28</td>
<td>3.91±0.51a</td>
<td>2.91±1.02b</td>
<td>1.86±0.76a</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Coliform (log_{10} cfu/g±SD)</th>
<th>E. coli (log_{10} cfu/g±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DN3</td>
<td>DN4</td>
</tr>
<tr>
<td>Control</td>
<td>3.84±0.40</td>
<td>3.46±0.47</td>
</tr>
<tr>
<td>0.05 Chi</td>
<td>3.72±0.55</td>
<td>3.29±0.64</td>
</tr>
<tr>
<td>0.1 Chi</td>
<td>3.67±0.46</td>
<td>3.10±0.65</td>
</tr>
<tr>
<td>0.5 Chi</td>
<td>3.58±0.55</td>
<td>2.71±0.80</td>
</tr>
<tr>
<td>%1 Chi</td>
<td>3.57±0.55</td>
<td>2.47±0.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Sulfite-reducing Clostridia (log_{10} cfu/g±SD)</th>
<th>Mold-Yeast (log_{10} cfu/g±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DN3</td>
<td>DN4</td>
</tr>
<tr>
<td>Control</td>
<td>2.99±0.51a</td>
<td>2.03±1.06a</td>
</tr>
<tr>
<td>0.05 Chi</td>
<td>2.08±0.55a</td>
<td>1.60±0.66a</td>
</tr>
<tr>
<td>0.1 Chi</td>
<td>2.12±0.40a</td>
<td>1.37±0.44a</td>
</tr>
<tr>
<td>0.5 Chi</td>
<td>2.11±0.40a</td>
<td>1.34±0.43a</td>
</tr>
<tr>
<td>%1 Chi</td>
<td>2.02±0.59a</td>
<td>1.15±0.37a</td>
</tr>
</tbody>
</table>

Different letters (a-c) within a same column (different batches) differ significantly (P < 0.05).

DN*: Stage  TAMB**: Total Aerobic Mesophilic Bacteria  Chi***: Chitosan; cfu: colony forming units.
groups. During this period, 1% Chi group does not form a statistically significant difference with 0.5% Chi but there are statistical differences with the other groups (Table 1, P<0.05). On the 7 day of the storage, significant differences were observed between groups in terms of the number of coliform (Table 1, P<0.05). During this period, the lowest number of coliform was found in 1% Chi group. On the 15 day, 0.05% Chi group and 0.1% Chi group produce similar number of the coliform group of bacteria but 0.5% and 1% Chi groups were not reproductive. On the 7 day, no E. coli increase could be detected in 1% Chi group. On the 15th, E. coli production completely stopped in all groups (Table 1). Statistically differences between control group and the groups in which chitosan was applied were found in DN1 in terms of the number of sulphite reducing clostridia (Table 1; P<0.05). But from the 7 day of the storage onwards the growth of sulphite-reducing clostridia in all the groups could not be observed (Table 1). In spite of an increase in the number of mold-yeast growth in all groups in the storage period, a specific reduction was determined (Table 1).

Sensory analysis of samples (taste, color, appearance and texture) was also evaluated in DN4. Sensory analysis of sausage samples after ripening is shown in Table 2.

That group of 1% Chi from sausage samples was statistically different from other groups in terms of flavor, color and texture (P<0.05). Differences between the groups in appearance are not statistically significant (P>0.05).

Table 2. Organoleptic analysis of sausage samples in DN4 stage

<table>
<thead>
<tr>
<th>Group</th>
<th>Flavor</th>
<th>Color</th>
<th>Textur</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.81±0.29</td>
<td>7.89±0.23</td>
<td>7.36±0.42</td>
<td>7.72±0.20</td>
</tr>
<tr>
<td>%0.05 Chi</td>
<td>7.89±0.36</td>
<td>8.03±0.22</td>
<td>7.72±0.29</td>
<td>8.20±0.34</td>
</tr>
<tr>
<td>%0.1 Chi</td>
<td>7.86±0.35</td>
<td>8.06±0.33</td>
<td>7.75±0.29</td>
<td>8.36±0.33</td>
</tr>
<tr>
<td>%0.5 Chi</td>
<td>7.81±0.22</td>
<td>7.78±0.37</td>
<td>7.36±0.42</td>
<td>8.06±0.27</td>
</tr>
<tr>
<td>%1 Chi</td>
<td>6.97±0.22</td>
<td>7.42±0.34</td>
<td>7.30±0.40</td>
<td>7.58±0.31</td>
</tr>
</tbody>
</table>

Different letters (a-c) within a same column (different batches) differ significantly (P<0.05)

**DISCUSSION**

Developing technology brings with some dangers to the agenda especially in food industry. Today, some different chemical additives are used in food to fight with microorganisms which are in the group of biological hazards and to create taste, flavor and charm in the product. However, using these additives above the standard limit causes negative consequences on human health. This negativity is brought to the agenda by the researchers investigating only natural origin additives. In recent years chitosan, which is a natural biopolymer in the food industry, has drawn attention. The number of studies related to the use of chitosan in meat and meat products is very low. In this study, the (microbiological, sensory) effects on the quality were investigated by adding chitosan in different proportions to a traditional product of our country, Turkish sausage.

In this study, a certain increase was determined in TAMB number of all groups from DN1 to DN4. Due to the start of fermentation in sausages from DN1 the increase in the number of TAMB has shown that chitosan has no significant inhibitory effect on fermentation of bacteria. As some researchers 4,23,24 expressed, this case can be explained by a reduction of antibacterial activity in the case of pH≥6.0. A certain number of reduction in TAMB number is seen in all groups from DN4 until the 60th day of storage. These results show similarities with the works of some researchers 4,13,25-27. Contrary to the findings of this study, some researchers 28,29 suggested that chitosan has no inhibitory effect on TAMB. These differences are being assumed to cause by the product types used in the studies, the deactylolation degree of chitosan and the environment pH.

It has been determined that the number of Enterobacteriaceae from DN1 decreased in all stages of analysis period (Table 1). This situation was similar to the results of some researchers' works 4,13,28. According to the control group in Greek type sausage with chitosan kept at 4°C for 28 days, a decrease in the number of Enterobacteriaceae has been reported 28. It has been determined that chitosan has inhibitory effect on the coliform and E. coli. Darmadj and Izumimoto 4 have determined that chitosan in meat at a rate of 0.5-1.0% prevents such bacteria causing deterioration like coliform, staphylococcus, pseudomonas. However, some researchers 5,7,28 have reported differences in microbial inhibition concentration of chitosan on E. coli. These differences are thought to stem from the degree of deacetylation and polymerization chitosan used in studies, the experimental incubation temperature, the experimental pH and organic acids used as a solvent. The antibacterial effect of chitosan on sulfide-reducing clostridias was determined (Table 1). Similar situation has been suggested by Juneja et al. 31. The researchers have reported that the use of 3% chitosan decreased the formation of Clostridium perfringes spores at a level of 4-5 log cfu/g compared to the control group. However, it is thought that new researches are absolutely necessary to express this activity. When production and storage period is taken into account, it gives rise to the thought that the chitosan applications may have protective effect against mold and yeast growth, generating major problems especially in the period of Turkish fermented sausages (Table 1) and new researches have to be done in this area.

One of the most important features of the nutrients is undoubtedly sensory qualities. Sensory qualities are important in consumer choice. Therefore, the sensory characteristics of Turkish sausages obtained by chitosan application have been evaluated in the context of the research. The sausage samples in 1% Chi group have taken
the lowest score in terms of flavor and this difference has been found significant statistically. A similar situation has also been identified in terms of color. It has been observed that the sausage samples in 1% Chi group got the highest value in terms of color. As these two sensory characteristics were evaluated together, it was concluded that technology and tastes of Turkish sausage should be taken into account on high-level chitosan applications. Darmadji and Izumimoto suggested that the chitosan improve the sensory quality attributes on meat. Jo et al. have put forward that chitosan has a positive contribution to the formation of color in sausages by the study with pork sausage prepared by adding chitosan oligomers. Mahan reported that no acceptable defect has been determined in flavor, smell and consistency of sausage groups treated with chitosan in three (0.25%, 0.5% and 1%) different concentrations.

Consequently, low rates (0.05%, 0.1% and 0.5%) of chitosan in Turkish sausage production could affect the microbiological and sensory quality positively while high proportions of chitosan (eg. 1%) practices affect sensory quality adversely. It has also been determined that high rates of chitosan (0.5% and 1%) applications created technological problems.

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