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RESEARCH ARTICLE

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 [DN_1], after mixing [DN_2], after filling [DN_3], after ripening [DN_4]) 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 DN_4. 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)*

Türk Sucuğu Üretiminde Kitosan Kullanımı ve Kalite Üzerine Etkileri

Özet

Bu çalışma, Türk Sucuğu üretiminde doğal bir polisakkarit olan kitosanın farklı oranlarda (%0.05, %0.1, %0.5 ve %1) kullanılarak ürünün kalitesi üzerine etkilerini araştırmak amacıyla yapıldı. Kontrol grubuna %0.05 oranında nitrat ilave edildi. Deneysel sucuk üretiminin dört ayrı aşamasında (ette [DN_1], karışım sonrası [DN_2], dolum sonrası [DN_3] olgunlaştırma sonrası [DN_4]) ve depolamanın 1, 7, 15, 30 ve 60. günlerinde mikrobiyolojik analizler (Toplam aerobik mezofilik bakteri, *Enterobacteriaceae*, koliform, *Escherichia coli,* sülfit-indirgeyen clostridia ve küf-maya sayımı) yapıldı. DN4 aşamasında duyusal nitelikleri (lezzet, renk, görünüm ve tekstür) değerlendirildi. Türk sucuğu üretiminde düşük oranlarda (%0.05, %0.1 ve %0.5) kitosan ilavesinin mikrobiyolojik ve duyusal kaliteyi olumlu yönde etkileyebileceği, yüksek oranlarda kitosan (örn., %1) uygulamalarının ise duyusal kaliteyi olumsuz yönde etkilediği belirlendi.

Anahtar sözcükler: *Antimikrobiyel etki, Kalite, Kitosan, Türk sucuğu*

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 micro-organisms 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 poly-saccharide composed of randomly distributed β-(1-4)-

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linked D-glucosamine (deacetylated unit) and N-acethly-D-glucosamine (acetylated unit) [3-6]. 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, -NH_2 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 pollycathyonic structure of chitosan, it absorbs electronegative charged substances in the cell and it kills the cell by deforming its activities [8-10]. In various studies over the antibacterial effect of chitosan on different foods in other countries were evaluated [4,6,7,11-13]. This study aims to investigate the effect of chitosan- a natural polysaccharide in the production of Turkish sausage- on the quality of the product.

MATERIAL and METHODS

Chitosan

Chitosan (CAS No: 9012-76-4, 75-85% deasetilasion 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) [9].

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 formulization 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 homogeneous 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. TAMB, *Enterobacteriaceae*, coliform and *E. coli* counts (bioMerieux) was performed in the TEMPO® system. Tempo TVC medium cards were used for the analysis of TAMB 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 50°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 (DN_1), after the mixture (DN_2), after the filling (DN_3), after-ripening (DN_4) 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 DN_3 ($P<0.05$). A similar situation was also observed in DN_4 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 TAMB 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*

Tablo 1. *Sucuk örneklerindeki mikrobiyolojik analiz aşamaları ve günleri*

Group	TAMB ** (log_{10} cfu/g±SD						*Enterobacteriaceae* (log_{10} cfu/g±SD					
	DN3 *	DN4	7	15	30	60	DN3	DN4	7	15	30	60
Control	6.08±0.26^{a}	7.29±0.22^{a}	6.77±0.46^{a}	6.21±0.42^{a}	5.52±0.47^{a}	4.86±0.52^{a}	5.08±0.49	4.80±0.40^{a}	4.15±0.59^{a}	3.54±1.29^{a}	1.38±0.92	<1.00
%0.05 Chi	6.04±0.25^{ab}	7.13±0.19^{ab}	6.65±0.24^{ab}	6.12±0.30^{ab}	5.43±0.46^{ab}	4.66±0.33^{a}	4.95±0.35	4.56±0.27^{ab}	3.92±0.49^{a}	2.84±1.30^{ab}	1.36±1.42	<1.00
%0.1 Chi	5.97±0.24^{ab}	6.96±0.20^{bc}	6.31±0.37^{bc}	5.75±0.33^{bc}	4.99±0.34^{bc}	4.29±0.40^{a}	4.83±0.34	4.38±0.31^{ab}	3.55±0.65^{ab}	2.12±1.27^{ab}	<1.00	<1.00
%0.5 Chi	5.89±0.23^{ab}	6.72±0.32^{cd}	6.25±0.52^{bc}	5.56±0.25^{c}	4.77±0.24^{c}	3.99±0.62^{bc}	4.74±0.31	4.24±0.26^{bc}	3.37±0.65^{ab}	2.08±0.98^{ab}	<1.00	<1.00
% 1 Chi***	5.77±0.19^{b}	6.48±0.31^{d}	5.92±0.35^{c}	5.35±0.37^{c}	4.52±0.35^{c}	3.30±1.21^{c}	4.67±0.28	3.91±0.51^{c}	2.91±1.02^{b}	1.86±0.76^{b}	<1.00	<1.00
Group	**Coliform (log_{10} cfu/g±SD)**						***E. coli* (log_{10} cfu/g±SD)**					
	DN3	**DN4**	**7**	**15**	**30**	**60**	**DN3**	**DN4**	**7**	**15**	**30**	**60**
Control	3.84±0.40	3.46±0.47	2.52±0.76^{a}	1.94±0.69	<1.00	<1.00	3.12±0.60	2.07±0.70	1.28±0.62	<1.00	<1.00	<1.00
%0.05 Chi	3.72±0.55	3.29±0.64	2.32±0.75^{ab}	1.55±0.61	<1.00	<1.00	2.96±0.65	2.23±0.66	1.42±0.55	<1.00	<1.00	<1.00
%0.1 Chi	3.67±0.46	3.10±0.65	2.25±0.79^{ab}	1.38±0.54	<1.00	<1.00	2.95±0.67	2.11±0.73	1.17±0.39	<1.00	<1.00	<1.00
%0.5 Chi	3.58±0.55	2.71±0.80	1.99±0.97^{ab}	<1.00	<1.00	<1.00	2.91±0.60	1.99±0.83	1.16±0.36	<1.00	<1.00	<1.00
% 1 Chi	3.57±0.55	2.47±0.81	1.75±0.81^{b}	<1.00	<1.00	<1.00	2.84±0.57	1.94±0.75	<1.00	<1.00	<1.00	<1.00
Group	**Sülfite-reducing Clostridia (log_{10} cfu/g±SD**						**Mold-Yeast (log_{10} cfu/g±SD)**					
	DN3	**DN4**	**7**	**15**	**30**	**60**	**DN3**	**DN4**	**7**	**15**	**30**	**60**
Control	2.99±0.51^{a}	2.03±1.06^{a}	<1.00	<1.00	<1.00	<1.00	4.89±0.80	5.27±0.66	4.83±0.68	4.17±0.96	2.64±1.90	2.56±1.83
%0.05 Chi	2.08±0.55^{b}	1.60±0.66^{ab}	<1.00	<1.00	<1.00	<1.00	4.96±0.77	5.35±0.67	5.02±0.72	4.15±1.15	2.67±1.90	2.55±1.81
%0.1 Chi	2.12±0.40^{b}	1.37±0.44^{ab}	<1.00	<1.00	<1.00	<1.00	4.93±0.86	5.25±0.81	5.00±0.75	4.09±1.62	2.81±2.00	2.72±1.91
%0.5 Chi	2.11±0.40^{b}	1.34±0.43^{ab}	<1.00	<1.00	<1.00	<1.00	4.69±0.79	4.92±0.70	4.66±0.73	3.27±1.81	2.09±1.69	2.01±1.58
% 1 Chi	2.02±0.59^{b}	1.15±0.37^{b}	<1.00	<1.00	<1.00	<1.00	4.69±0.75	4.82±0.70	4.46±0.71	3.25±1.78	2.08±1.68	1.99±1..55

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

*DN *: Stage TAMB ** : Total Aerobic Mesophlic 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 DN_3 in terms of the number of sulfite reducing clostridia (*Table1*; 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 DN_4. 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 DN_4 stage*
Tablo 2. *DN_4 aşamasında sucuk örneklerinde duyusal analizler*

Group	Flavor	Color	Textur	Appearance
Control	7.81±0.29^{a}	7.89±0.23^{a}	7.36±0.42^{bc}	7.72±0.20
%0.05 Chi	7.89±0.36^{a}	8.03±0.22^{a}	7.72±0.29^{a}	8.20±0.34
%0.1 Chi	7.86±0.35^{a}	8.06±0.33^{a}	7.75±0.29^{a}	8.36±0.33
%0.5 Chi	7.81±0.22^{a}	7.78±0.37^{a}	7.36±0.42^{ab}	8.06±0.27
% 1 Chi	6.97±0.22^{b}	7.42±0.34^{b}	7.30±0.40^{c}	7.58±0.31

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 micro-organisms 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 DN_3 to DN_4. Due to the start of fermentation in sausages from DN_3, 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 DN_4 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 deacetylation degree of chitosan and the environment pH.

It has been determined that the number of *Enterobacteriaceae* from DN_3 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*. Darmadji 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 [5,7,30] 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 [4] suggested that the chitosan improve the sensory quality attributes on meat. Jo et al.[32] 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[33] 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.

REFERENCES

1. Çon AH, Gökalp HY: Afyon'da büyük kapasiteli et işletmelerinde üretilen sucuk örneklerinin bazı mikrobiyolojik özelliklerinin periyodik olarak belirlenmesi. *Turk J Vet Anim Sci,* 26, 11-16, 2002.

2. Gökalp HY, Kaya M, Zorba Ö: Et Ürünleri İşleme Mühendisliği. İkinci Baskı, s. 253-254, Atatürk Universitesi Yayınları, No: 786, Ofset Basımevi, Erzurum, 1997.

3. Shahidi F, Arachchi JKM, Jeon Y: Food applications of chitin and chitosans. *Trends Food Sci Technol,* 10, 37-51, 1999.

4. Darmadji P, Izumimoto M: Effect of chitosan in meat preservation. *Meat Sci,* 38, 243-254, 1994.

5. Rhoades J, Roller S: Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. *Appl and Environ Microb*, 66, 80-86, 2000.

6. Oh H II, Kim YJ, Chang EJ, Kim JY: Antimicrobial characteristic of chitosans against food spoilage microorganisms in liquid media and mayonnaise. *Biosci Biotech Biochim*, 65 (11): 2378-2383, 2001.

7. Wang GH: Inhibition and inactivation of five species of foodborne pathogens by chitosan. *J Food Protect*, 55, 916-919, 1992.

8. Tsai GJ, Su WH: Antibacterial activity of shrimp chitosan against *Escherichia coli*. *J Food Protect,* 62, 239-943, 1999.

9. No HK, Park NY, Lee SH, Meyers SP: Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int J Food Microbiol*, 74, 65-72, 2002.

10. Zheng LH, Zhu JF: Study on antimicrobial activity of chitosan with different molecular weights. *Carbohyd Polym*, 54, 527-530, 2003.

11. Devlieghere F, Vermeulen A, Debevere J: Chitosan: antimicrobial activity, interactions with 4 food components and applicability as a coating on fruit and vegetables. *Food Microbiol*, 21, 703-714, 2004.

12. Altieri C, Srocco C, Sinigaglia M, Del Nobile MA: Use of chitosan to prolong mozzarella cheese shelf life. *J Dairy Sci*, 88, 2683-2688, 2005.

13. Georgantelis D, Ambrosiadis I, Katikou, P, Blekas G, Georgakis, SA: Effect of rosemary extract, chitosan and a-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. *Meat Sci*,76, 172–181, 2007.

14. Et ve Balık Kurumu (EBK), İmalat Dairesi İşletme ve İmalat Yönetmeliği, Yönetmelik Sıra no: 23, 1988.

15. bioMérieux Inc: TEMPO TVC (Total Viable Count) is an automated test for use with TEMPO, for the enumeration of viable aerobic mesophilic flora in 40-48 hours in food products. bioMeriéux, TEMPO® TVC, 2007.

16. bioMérieux Inc: TEMPO EB (*Enterobacteriaceae*) is an automated test for use with TEMPO, for the enumeration of enterobacteria in 22-27 hours in food products. bioMeriéux, TEMPO® EB, 80 003, 2007.

17. bioMérieux Inc: TEMPO TC (Total coliforms) is an automated test for use with TEMPO, for the enumeration of total coliforms in 24 hours in food products. bioMeriéux, TEMPO® TC, 2006.

18. bioMérieux Inc: TEMPO EC (*E. coli*) is an automated test for use with TEMPO, for the enumeration of *Escherichia coli* in 24 hours in foodproducts. bioMeriéux, TEMPO® EC, 80 004; 2006.

19. ISO 15213. Horizontal Method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. 2003.

20. Food and Drug Administration, Bacteriological Analytical Manual, Yeasts, Molds and Mycotoxins, 2001.

21. Stone H and Sidel JC. Sensory Evaluation Practices. *Food Sci and. Technol*, *Academic Pres*. Inc. London. 1985.

22. SPSS, Statistical Package for the Social Sciences Version 10.0 SPSS Inc, Chicago, 2000.

23. Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, Rhoades J, Roller S: Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *Int J Food Microbiol*, 71, 235-244, 2001.

24. Fernandes JC, Tavaria FK, Soares JC, Ramos OS, Monteiro MJ, Pintado ME, Malcata FX: Antimicrobial effects of chitosans and chito-oligosaccharides upon *Staphylococcus aureus* and *Escherichia coli* in food model systems. *Food Microbiol*, 25, 922-928, 2008.

25. Roller S, Sagoo S, Board R, Mahony TO, Caplice E, Fitzgerald G, Fogden M, Owen M, Fletcher H: Novel combinations of chitosan, carnocin and sulphite fort he preservation of chilled pork sausages. *Meat Sci*, 62, 165-177, 2002.

26. Sagoo S, Board R, Roller S: Chitosan inhibits growth of spoilage microorganisms in chilled pork products. *Food Microbiol*, 19, 175-182, 2002.

27. Youn SK, Her JH, Kim YJ, Choi JS, Park SM, Ahn DH: Studies on the improvement of shelf life in spicy beef meat using chitosan. *J Korean Soc Food Sci Nutr*, 33 (1): 207-211, 2004.

28. Soultos N, Tzikas Z, Abrahim A, Georgantelis D, Ambrosiadis I: Chitosan effects on quality properties of Greek style fresh pork sausages. *Meat Sci*, 80, 1150-1156, 2008.

29. Tao W, Linchun M: Application of chitosan to maintain the quality of kamaboko gels made from grass carp (*Ctenopharyngodon idellus*) during storage. *J Food Process Pres*, 33, 218-230, 2009.

30. Babiker EE: Effect of chitosan conjugation on the functional properties and bactericidal activity of gluten peptides. *Food Chem*, 79, 367-772, 2002.

31. Juneja VK, Thippareddi H, Bari L, Inatsu Y, Kawamoto S, Friedman M: Chitosan protects cooked ground beef and turkey against *Clostridium perfringens* spores during chilling. *J Food Sci*, 71 (6): 236-240, 2006.

32. Jo C, Lee JW, Lee KH, Byun MW: Quality properties of pork sausage prepared with water-soluble chitosan oligomer. *Meat Sci*, 59, 369-375, 2001.

33. Mahan FI: Kitosanla kaplanmış soyulmuş sosislerin mikrobiyolojik kalitesi ve raf ömrünün araştırılması. *Yüksek Lisans Tezi*, İstanbul Univ Sağlık Bil Ens, İstanbul, Türkiye, 2007.