

## Microbiological Quality and Shelf-life of Sausage Treated with Chitosan

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Geliş Tarihi / Received : 15.02.2011

### ABSTRACT

In this study, the microbiological quality and shelf-life of sausage treated with different concentrations of chitosan was investigated. Sausage samples obtained from a local producer were dipped into 0.25%, 0.5% and 1% chitosan solutions prepared with 1% acetic acid. The samples were drained, vacuum packed and stored at 4°C a period of sixty days. The sausages were evaluated for sensorial properties and microbiological counts (aerobic mesophilic bacteria, total psychrotrophic bacteria, lactic acid bacteria, molds and yeasts) on days 1, 5, 10, 15, 30 and 60 of storage. All samples vacuum packed were stored. All the three different chitosan application (0.25%, 0.5% and 1.0%) did not cause undesirable alteration in sensory properties of sausages. Even more, treated samples had a brighter color than the control group. The first alterations indicating the spoilage were observed at the 20<sup>th</sup> storage day in the untreated samples (control group). However, an abnormal changes were not determined on the samples treated with chitosan, even on the last day of storage. The counts of all determined microbiological indicators were significantly affected by the treatment with chitosan (P<0.05). The results indicated that the application of chitosan on the sausage surface by dipping improves the microbiological quality and extends the shelf-life, which could an alternative to chemical protective additives.

**Key Words:** Chitosan, antimicrobial effect, sausage, storage, shelf-life

### ÖZET

#### KİTOSAN İLE MUAMELE EDİLEN SOSİSLERİN RAF ÖMRÜ VE MİKROBİYOLOJİK KALİTESİ

Bu çalışma kitosanın farklı konsantrasyonları ile muamele edilen sosislerin mikrobiyolojik kalitesi ve raf ömrü araştırılmıştır. Yerel bir üreticiden temin edilen sosis örnekleri % 1,0'lik asetik asit içinde hazırlanmış % 0,25, % 0,5 ve % 1,0 kitosan solüsyonlarına daldırılmıştır. Bütün örnekler süzöldükten sonra vakum paketlenmiş ve 4°C'de 60 gün süreyle muhafaza edilmiştir. Sosisler depolamanın 1, 5, 10, 15, 30 ve 60. günlerinde duyu özellikleri ve mikroorganizma sayıları (aerobik mezofil toplam bakteri, psikrotrofik bakteri, laktik asit bakterileri, küf ve maya) bakımından analiz edildi. Her üç farklı kitosan uygulaması (% 0,25, % 0,5 ve % 1,0) sosislerin duyu özelliklerinde istenmeyen bir değişime neden olmamıştır. Hatta muamele görmüş örnekler muamele edilmeyenlere göre daha parlak

bir renge sahip olmuştur. Muamele görmeyen örnekler (kontrol grubu) muhafazanın yirminci günü bozulma belirtileri göstermiştir. Bununla birlikte kitosanla muamele edilen örneklerde muhafazanın son gününde bile anormal değişiklikler gözlenmemiştir. Örneklerdeki bakteri sayıları kitosan ile muamele ile önemli derecede etkilenmiştir ( $P<0.05$ ). Elde edilen bulgulara göre sosis yüzeylerinin daldırılarak kitosanla muamele edilmesinin mikrobiyolojik kaliteyi iyileştirdiği ve raf ömrünü uzattığı sonucuna varılmıştır. Bu anlamda kitosan kimyasal koruyucu katkı maddelerine bir alternatif olarak düşünülmelidir.

**Anahtar Kelimeler:** Kitosan, antimikrobiyel etki, sosis, muhafaza, raf ömrü

### Introduction

Sausage is exposed to high temperature during the heating process. But this temperature is not enough to inactivate all of the microorganisms present. Sausages are also recontaminated with spoilage bacteria during the processing stages after cooking. The excessive proliferation of present microflora in the sausage content or on the surface during storage causes economic losses because of spoilage and deterioration. Especially, lactic acid bacteria are considered to be a major component of the microbial population found on vacuum-packaged sausages (Özdemir, 1997). Lactic acid bacterial growth on the surface of the sausages produces undesirable sensory attributes (Korkeala and Björkroth, 1997). Therefore, various methods have been used to prevent the growth of lactic acid bacteria and other spoilage microorganisms and to extend the shelf-life of vacuum-packaged sausages.

The growing consumer demand for foods without chemical preservatives has focused efforts in the discovery of new natural additives. Chitosan is one of the new generation food additives and has been accepted as a potential food preservative of natural origin (Devlieghere et al., 2004). Chitosan is a natural non-toxic biopolymer derived from the deacetylation of chitin, a major component of the shells of crustacea such as crabs, shrimps and crawfish (Shahidi et al., 1999; Rinaudo, 2006). Deacetylated chitin, or chitosan, is comprised of chains of D-glucosamine. Chitosan, included to the GRAS (Generally Recognized As Safe) category by the FDA, is known to possess numerous

technological and physiological properties useful in foods. In addition to its lack of toxicity and allergenicity, its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications in food processing fields. Chitosan readily soluble in dilute organic acid by gel forming. Coating with chitosan gel of food items such as fresh fruits, vegetables and egg contributes to preserve the quality and extend shelf-life (Butler et al., 1996; Chien et al., 2007; Kim et al., 2007). Chitosan exhibits antimicrobial activity against a range of microorganisms in vitro conditions (Choi et al., 2001; Gerasimenko et al., 2004; Liu et al., 2001; No et al., 2002; Roller and Covill, 1999; Tikhonov et al., 2006; Tsai and Su, 1999; Zheng and Zhu, 2003). The spectrum of microbial susceptibility depends on a number of factors such as type of microorganism, molecular weight, deacetylation degree of chitosan; temperature and pH of medium (Chen and Hwa, 1996; Gerasimenko et al., 2004; No et al., 2002; No et al., 2006; Omura et al., 2003). On the other hand, bacteria in different growth stages have different sensitivity to chitosan (Liu et al., 2006). Minimum inhibitory concentration of chitosan has a wide range between 0.01% - 1% (Rhoades and Roller, 2000; Tsai and Su, 1999; Tsai et al., 2000).

Antimicrobial efficacy of chitosan is not always realized in foods due to the highly reactive nature of the polycationic chitosan, which interacts readily with proteins, fats and other anionic substances in foods (Rhoades and Roller, 2000). Nevertheless, some successful applications in food have been reported (Darmadji and Izumimoto, 1994; Georgantelis

et al., 2007; Juneja et al., 2006; Kanatt et al., 2008; Lin and Chao, 2001; Quattara et al., 2000; Roller et al., 2002; Sagoo et al., 2002; Youn et al., 1999; Youn et al., 2004). The antimicrobial activity of chitosan is due to the great number of cationized amines present in chitosan that can potentially interact with the negatively charged residues of macromolecules at the microbial cell surface. This ionic interaction results in subsequently inhibition of microbial growth through different mechanisms, including rupture the cell membrane (Andres et al., 2007; Helander et al., 2001; Liu et al., 2004; Li et al., 2007). Muzzarelli et al. (1990) reported that microbial cells exposed to N-carboxybutyl chitosan underwent marked morphological alterations in examination by electron microscopy. In addition, chitosan can act as a chelating agent for certain metals and in this way it could also interfere in the formation of toxins and in microbial growth (Cuero et al., 1991). According to some theories, oligomeric chitosan binds DNA and prevent mRNA synthesis by penetrating into the microorganism (Jung et al., 1999; Sudarshan et al., 1992).

This study was carried out to assess the feasibility of using different concentrations of chitosan to improve the microbiological quality and extend the shelf-life of sausage.

## Materials and Methods

### Production of experimental sausage

Sausages were prepared as in a local meat processing factory. In each trial, the basic material (10 kg) consisting of 90% lean beef meat and 10% chicken meat was ground twice in a meat grinder through a 3-mm plate, respectively. Chilled ground meat mixture was transferred in a cutter and chopped by the simultaneous addition of sodium chloride (250 g), sodium nitrite (1.25 g), sodium tripolyphosphate (30 g) and one-third of the total amount of finely crushed ice (100 g). After 6–8 minutes, fat (1.2 kg), then starch (50 g), spice mix (60 g), sodium ascorbate (3 g) and the remaining two-thirds of the total ice (200 g) are added. The mix spice contained coriander, ginger, black pepper, red pepper, and garlic.

Chopping is then continued until the batch is finely comminuted. The final mixture was transferred to a commercial sausage filling machine and stuffed into the permeable synthetic casings with 21 mm calibre. The sausages dried (50°C for 15 min), smoked (65°C for 15 min), and cooked to 72°C of internal temperature using a smokehouse. The cooked sausages were showered with cold water for 5 min, dried at room temperature for 30 min and kept at 4°C overnight.

### Chitosan

Chitosan extracted from crab shells (Sigma, C 3646, minimum 85% deacetylated) was used. Stock solutions of chitosan (0.25%, 0.5% and 1.0%) were prepared in 1% acetic acid (w/v).

### Treatment with chitosan

After peeling, sausages were divided into five equal groups in order to prepare the experimental treatments. Sausages of three groups were immersed in three different chitosan solutions with concentrations of 0.25%, 0.5% and 1.0% for 15 minutes. The other two groups of sausages were dipped in tap water (water control) and 1.0% acetic acid solution (acetic acid control), respectively, for same periods. After treatments, the samples were drained, vacuum-packaged and stored in a refrigerator (4 °C) for 60 days.

### Sampling and microbiological analysis

The sausages were evaluated microbiologically on the 1, 5, 10, 15, 30 and 60 days of the storage. A piece of sausage representing each group was cut into a sterile bag and mixed. The 10 g samples taken from this mixtures was transferred into a sterile stomacher bag with 90 mL of 0.1% peptone water (Oxoid CM 9) and homogenised in a stomacher (Labblender 400) for 2 min. Serial decimal dilutions were prepared using the same diluent and duplicate 0.1 mL or 1 mL inoculum of appropriate dilutions were plated into the following selected growth media (Harrigan, 1998). Aerobic mesophilic bacteria were enumerated in standard plate count agar (Difco, 247940). Pour plates were incubated at 37 °C for 48 h. Total psychrotrophic bacteria were enumerated in standard plate count agar (Difco,

247940). Pour plates were incubated at 7 °C for 10 days. Lactic acid bacteria were enumerated in de-Man Rogosa Sharpe Agar (Oxoid, CM 0361). Pour plates, with overlay, were incubated at 37 °C for 48 h. Molds and yeasts were enumerated on Potato Dextrose Agar (Merck, 1.10130) adjusted to pH 3.5 with tartaric acid. Spread plates were incubated at 25°C for 5 days. Average results of duplicate measurements, are expressed as log<sub>10</sub> colony forming units (cfu)/g.

#### **Sensory evaluation**

An experienced eight-member panel was used to evaluate the effectiveness of chitosan on the sensorial characteristics of sausages. Testing was initiated after the panelists agreed on the specifications. After treatment and draining, the samples were served to each panelist separately. Water was provided between samples to cleanse the palate. Panelists were asked to evaluate them for color, consistency, flavor and odor on a nine-point Hedonic scale, with 9 being extremely good and 1 being extremely poor (Watts et al., 1989).

#### **Determination of spoilage**

The sausages were visually evaluated for signs of deterioration on the microbiological analysis days. The point of spoilage was defined by an unacceptable off-odor/off-flavor or appearance (Borch et al., 1996).

#### **Statistical analysis**

Experimental studies were repeated three times on different times. All data were analysed statistically by one-way of analyses of variance (Anova) and differences among groups were examined for level of significance by Duncan's multiple range test (SAS Institute, 1991).

### **Results and Discussion**

Protective food additives should not cause any undesirable sensorial changes to the product. In our study, the sausage samples were examined sensorially. The panelists detected no significant differences among the treatments with respect to color, consistency, flavor and odor. However, the panelists described the sausages treated with chitosan as brighter

reddish. Jo et al. (2001) studied the quality properties of cooked pork sausage prepared with the addition of a water-soluble chitosan oligomer (0.2%) and reported that chitosan has an enhancer effect on the brightness of sausage surface. Roller et al. (2002) also reported that the addition of chitosan to sausages would not lead to off-odors and that the appearance would not be rendered objectionable, either of which could potentially lead to rejection by the consumer. Similar findings have been reported by Lin and Chao (2001) who studied with reduced-fat Chinese-style sausage. Darmadji and Izumimoto (1994) determined that chitosan had a good effect on the development of the red color of meat during storage. These results were in accordance with our data.

The most expected effect of chitosan is to extend the shelf-life of the product. In our study, during the period of cold storage the sausages produced experimentally were examined for signs of deterioration. In control group, the formation of slime on sausage surface, discoloration (opacity), sourish odor and taste and a fuzzy liquid accumulation in the package were observed on the twentieth storage day. The degree of these changes increased gradually until the sixtieth day of storage. Similar undesirable changes in the acetic acid-treated sausages were determined on the thirtieth day. However the chitosan-treated sausages were acceptable during 60 days of storage. In these samples, fluid accumulation, appearance of slime and abnormal taste changes were not observed in a significant level even at the end of 60 days. Similar results were obtained from other studies on the improvement of shelf-life in meat and meat products using chitosan. Sagoo et al. (2002) determined that shelf-life of the sausage was extended from seven days to fifteen days with chitosan. Youn et al. (1999) reported that sausage containing chitosan (0.2%) had improved shelf-stability. Kanatt et al. (2008) reported that mixture of chitosan and mint extract enhanced the shelf-life of pork cocktail salami stored at 0-3 °C.

In the present study, number of aerobic mesophilic microorganisms in all groups increased gradually during the period of cold

storage (Table 1; Fig 1). However, the increase in the number of microorganisms in the treated samples with chitosan was significantly ( $P < 0.05$ ) less than those treated with only water. Similar results were also observed for psychrotrophic bacteria (Table 2; Fig 1). The results of the present study are in agreement with other studies (Sagoo et al., 2002; Darmadji and Izumimoto, 1994; Soutos et al., 2008; Roller et al., 2002; Georgantelis et al., 2007). Some authors reported that the total counts for sausages with chitosan were significantly lower than in the control sausages in the early days of storage but there is no significant differences between the chitosan-treated and control sausages in the subsequent days of storage (Roller et al., 2002; Youn et al., 1999). In contrast with our findings, Jo et al. (2001) reported that the chitosan oligomer addition (0.2%) did not reduce the number of microorganisms in the emulsion-type sausage during the 21-day storage. In the above study resulted with ineffectiveness or inadequacy, chitosan was added to the sausage formulations as an ingredient. The application of chitosan onto the end product surfaces by dipping may be more effective to prevent microbial growth.

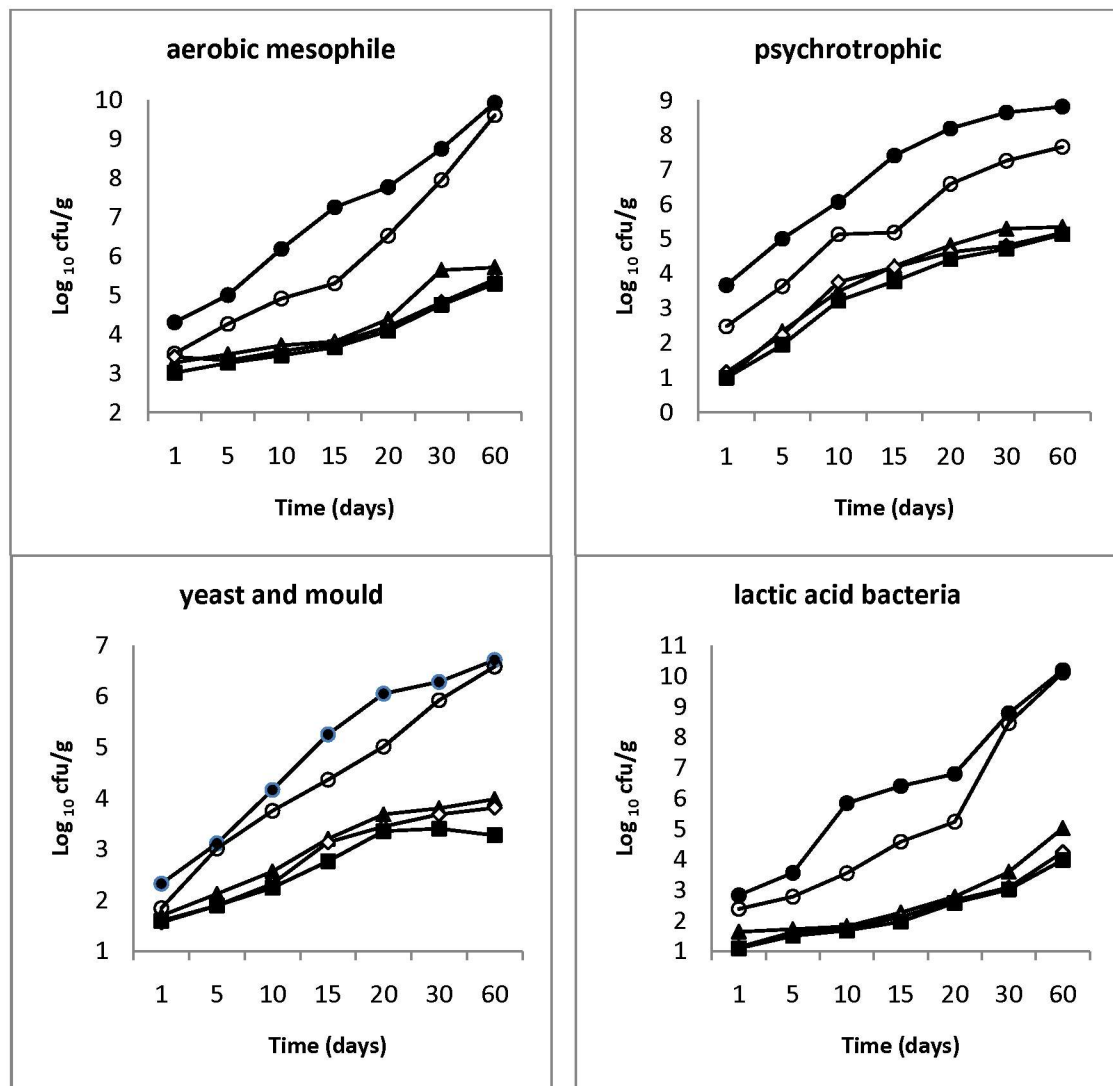
In the present study, there was no significant differences in the aerobic total microbial counts among sausages treated with different concentrations of chitosan during the storage period (except for 10 and 30 days of storage for psychrotrophics). However, other studies showed significant differences in reduction of microorganisms with decreasing concentration of chitosan. Sagoo et al. (2002) determined that total viable counts was reduced by approximately 2 log cfu/g in sausages dipped in chitosan solution (1%), but lower concentrations (0.5%) of chitosan failed to inhibit microbial growth in the sausages. Darmadji and Izumimoto (1994) reported that total microbial growth in refrigerated beef patties was inhibited in the presence of 1.0% chitosan, but not 0.5 or 0.2% chitosan.

The effectiveness of chitosan in suppressing the lactic acid bacteria growth were also evaluated. The changes in the numbers of lactic acid bacteria in sausage samples during cold storage are given in Table 3 and Fig 1. The number of lactic acid bacteria in the control group was 2.84 log cfu/g at the beginning and reached to a high level (10.2 log cfu/g) on the last day of storage. Treatment with chitosan retarded the lactic acid bacteria growth and lowered the maximum growth levels in the sausages. Colony count of lactic acid bacteria were found between 3.99-5.03 log cfu/g at the end of storage. At the end of the storage, inhibition effect on the lactic acid bacteria of chitosan at 0.5 and 1.0% were significantly stronger than at 0.25% ( $P < 0.05$ ). These results indicating inhibition of lactic acid bacteria are in an agreement with results reported by Sagoo et al. (2002) who found that treatment with chitosan at a concentration of 1% significantly reduced lactic acid bacteria count in both skinless and standard sausages during storage at 7 °C for 18 days. Soutos et al. (2008) investigated the effect of chitosan (0.5% and 1%) added on microbiological properties of fresh pork sausages stored at 4°C for 28 days and reported that lactic acid bacteria counts were approximately 1 and 1.5 log cfu/g lower in the samples containing 0.5% and 1% chitosan, respectively, than in the control samples. Contrary to our findings, Roller et al. (2002) determined that lactic acid bacteria counts in the sausages containing chitosan (0.6%) stored at 4°C were similar to the control. Quattara et al. (2000) studied the efficacies of the antimicrobial films prepared by incorporating acetic or propionic acid into a chitosan matrix for inhibiting bacterial growth, and reported that lactic acid bacteria in vacuum-packaged processed meats during refrigerated storage were not affected by the antimicrobial films.

**Table 1.** Numbers of aerobic mesophilic bacteria in sausages during cold storage (log cfu/g  $\pm$  SD).**Table 1.** Soğuk depolama sırasında sosislerdeki aerobik mezofilik bakteri sayıları (log kob/g  $\pm$  SD).

Storage period (4 °C)	Water control	Acetic acid control	Chitosan (0.25%)	Chitosan (0.5%)	Chitosan (1.0%)
Day 1	4.31 $\pm$ 0.22 <sup>a</sup>	3.51 $\pm$ 0.33 <sup>ab</sup>	3.28 $\pm$ 0.35 <sup>b</sup>	3.43 $\pm$ 0.87 <sup>ab</sup>	3.02 $\pm$ 0.37 <sup>b</sup>
Day 5	5.01 $\pm$ 0.24 <sup>a</sup>	4.27 $\pm$ 0.29 <sup>b</sup>	3.49 $\pm$ 0.37 <sup>c</sup>	3.33 $\pm$ 0.36 <sup>c</sup>	3.27 $\pm$ 0.37 <sup>c</sup>
Day 10	6.19 $\pm$ 0.30 <sup>a</sup>	4.92 $\pm$ 0.20 <sup>b</sup>	3.72 $\pm$ 0.15 <sup>c</sup>	3.57 $\pm$ 0.27 <sup>c</sup>	3.46 $\pm$ 0.33 <sup>c</sup>
Day 15	7.26 $\pm$ 0.37 <sup>a</sup>	5.31 $\pm$ 0.31 <sup>b</sup>	3.82 $\pm$ 0.18 <sup>c</sup>	3.77 $\pm$ 0.16 <sup>c</sup>	3.67 $\pm$ 0.11 <sup>c</sup>
Day 20	7.78 $\pm$ 0.12 <sup>a</sup>	6.54 $\pm$ 0.26 <sup>b</sup>	4.39 $\pm$ 0.37 <sup>c</sup>	4.20 $\pm$ 0.29 <sup>c</sup>	4.09 $\pm$ 0.26 <sup>c</sup>
Day 30	8.76 $\pm$ 0.16 <sup>a</sup>	7.96 $\pm$ 0.25 <sup>b</sup>	5.65 $\pm$ 0.19 <sup>c</sup>	4.83 $\pm$ 0.16 <sup>d</sup>	4.76 $\pm$ 0.15 <sup>d</sup>
Day 60	9.94 $\pm$ 0.15 <sup>a</sup>	9.62 $\pm$ 0.31 <sup>a</sup>	5.72 $\pm$ 0.24 <sup>b</sup>	5.41 $\pm$ 0.40 <sup>b</sup>	5.30 $\pm$ 0.35 <sup>b</sup>

<sup>a, b, c, d</sup> Means with different letters within a same row are significantly different from one another (P<0.05)

**Figure 1.** Growth of microorganisms in the sausages stored at 4 °C for 60 days. Sausages were dipped in tap water (●), 1.0% acetic acid (○), 0.25% chitosan (▲), 0.5% chitosan (◇) and 1.0% chitosan (■).

**Şekil 1.** Altmış gün süreyle 4°C’de muhafaza edilen sosislerdeki mikroorganizma gelişimi.

**Table 2.** Number of psychrotrophic bacteria in sausages during cold storage (log cfu/g  $\pm$  SD)  
**Tablo 2.** Soğuk depolama sırasında sosislerdeki psikrotrofik bakteri sayısı (log kob/g  $\pm$  SD).

Storage period (4 °C)	Water control	Acetic acid control	Chitosan (0.25%)	Chitosan (0.5%)	Chitosan (1.0%)
Day 1	3.67 $\pm$ 0.21 <sup>a</sup>	2.48 $\pm$ 0.12 <sup>b</sup>	1.00 $\pm$ 0.00 <sup>c</sup>	1.16 $\pm$ 0.15 <sup>c</sup>	1.00 $\pm$ 0.00 <sup>c</sup>
Day 5	5.01 $\pm$ 0.19 <sup>a</sup>	3.64 $\pm$ 0.27 <sup>b</sup>	2.34 $\pm$ 0.15 <sup>c</sup>	2.23 $\pm$ 0.08 <sup>cd</sup>	1.95 $\pm$ 0.05 <sup>d</sup>
Day 10	6.07 $\pm$ 0.21 <sup>a</sup>	5.14 $\pm$ 0.29 <sup>b</sup>	3.49 $\pm$ 0.50 <sup>c</sup>	3.76 $\pm$ 0.16 <sup>c</sup>	3.22 $\pm$ 0.48 <sup>c</sup>
Day 15	7.41 $\pm$ 0.30 <sup>a</sup>	5.19 $\pm$ 0.10 <sup>b</sup>	4.22 $\pm$ 0.30 <sup>c</sup>	4.19 $\pm$ 0.27 <sup>c</sup>	3.78 $\pm$ 0.45 <sup>c</sup>
Day 20	8.19 $\pm$ 0.30 <sup>a</sup>	6.59 $\pm$ 0.43 <sup>b</sup>	4.82 $\pm$ 0.12 <sup>c</sup>	4.62 $\pm$ 0.35 <sup>c</sup>	4.42 $\pm$ 0.25 <sup>c</sup>
Day 30	8.65 $\pm$ 0.23 <sup>a</sup>	7.26 $\pm$ 0.27 <sup>b</sup>	5.30 $\pm$ 0.37 <sup>c</sup>	4.81 $\pm$ 0.25 <sup>cd</sup>	4.72 $\pm$ 0.24 <sup>d</sup>
Day 60	8.82 $\pm$ 0.10 <sup>a</sup>	7.66 $\pm$ 0.22 <sup>b</sup>	5.35 $\pm$ 0.44 <sup>c</sup>	5.18 $\pm$ 0.39 <sup>c</sup>	5.14 $\pm$ 0.38 <sup>c</sup>

<sup>a, b, c, d</sup> Means with different letters within a same row are significantly different from one another (P<0.05)

**Table 3.** Number of lactic acid bacteria in sausages during cold storage (log cfu/g  $\pm$  SD)  
**Tablo 3.** Soğuk depolama sırasında sosislerdeki laktik asit bakterileri sayısı (log kob/g  $\pm$  SD).

Storage period (4 °C)	Water control	Acetic acid control	Chitosan (0.25%)	Chitosan (0.5%)	Chitosan (1.0%)
Day 1	2.84 $\pm$ 0.12 <sup>a</sup>	2.39 $\pm$ 0.26 <sup>b</sup>	1.64 $\pm$ 0.16 <sup>c</sup>	1.14 $\pm$ 0.14 <sup>d</sup>	1.10 $\pm$ 0.10 <sup>d</sup>
Day 5	3.57 $\pm$ 0.24 <sup>a</sup>	2.79 $\pm$ 0.17 <sup>b</sup>	1.73 $\pm$ 0.17 <sup>c</sup>	1.63 $\pm$ 0.22 <sup>c</sup>	1.51 $\pm$ 0.33 <sup>c</sup>
Day 10	5.85 $\pm$ 0.13 <sup>a</sup>	3.56 $\pm$ 0.16 <sup>b</sup>	1.82 $\pm$ 0.16 <sup>c</sup>	1.75 $\pm$ 0.22 <sup>c</sup>	1.68 $\pm$ 0.28 <sup>c</sup>
Day 15	6.41 $\pm$ 0.27 <sup>a</sup>	4.59 $\pm$ 0.22 <sup>b</sup>	2.27 $\pm$ 0.31 <sup>c</sup>	2.12 $\pm$ 0.31 <sup>c</sup>	1.97 $\pm$ 0.22 <sup>c</sup>
Day 20	6.81 $\pm$ 0.06 <sup>a</sup>	5.24 $\pm$ 0.26 <sup>b</sup>	2.79 $\pm$ 0.07 <sup>c</sup>	2.67 $\pm$ 0.16 <sup>c</sup>	2.59 $\pm$ 0.14 <sup>c</sup>
Day 30	8.79 $\pm$ 0.20 <sup>a</sup>	8.47 $\pm$ 0.46 <sup>a</sup>	3.60 $\pm$ 0.21 <sup>b</sup>	3.09 $\pm$ 0.15 <sup>c</sup>	3.03 $\pm$ 0.15 <sup>c</sup>
Day 60	10.20 $\pm$ 0.44 <sup>a</sup>	10.12 $\pm$ 0.28 <sup>a</sup>	5.03 $\pm$ 0.25 <sup>b</sup>	4.25 $\pm$ 0.39 <sup>c</sup>	3.99 $\pm$ 0.22 <sup>c</sup>

<sup>a, b, c, d</sup> Means with different letters within a same row are significantly different from one another (P<0.05)

Lactic acid bacteria, especially *Lactobacillus* spp. are commonly the cause of spoilage in vacuum-packaged cooked meat products (Borch et al., 1996; Korkeala and Bjorkroth, 1997). Antibacterial substances active against lactic acid bacteria are therefore of particular interest. The counts of lactic acid bacteria in the water control and acetic acid control samples were determined over 6 log cfu/g on the days observed the first alterations indicating the spoilage. However, lactic acid bacteria in the samples treated with chitosan did not reach this level in any stage of storage. Based on these findings, the above-mentioned improving effects of chitosan on the shelf-life of sausages may be correlated with the inhibition of lactic acid bacteria.

Yeast and mold counts in the sausage during cold storage are shown in Table 4 and Fig. 1. In the sausages with treated chitosan, yeast and mold counts were considerably lower than in the water control at all sampling days. 1.0% concentration of chitosan was relatively more effective than the others in suppressing the yeast and mold growth in the sausages. Sagoo et al. (2002) reported a similar sensitivity to chitosan for yeast and molds. They determined that the yeast and mold counts in the sausage dipped in 1.0% chitosan solution were reduced approximately 2 log cfu/g at the end of 18 storage days at 7 °C. However, in contrast to our findings, Roller et al. (2002) reported that chitosan (0.6%) incorporated into the sausages did not reduce significantly the yeast and molds counts during storage at 4°C.

Aldemir and Bostan (2009) indicated that chitosan (50-500 mg/kg) added into meatball was no neffective on yeast and molds. This

differences may be related to chitosan application method.

**Table 4.** Number of yeast and mold in sausages during cold storage (log cfu/g  $\pm$  SD)  
**Tablo 4.** Soğuk depolama sırasında sosislerdeki küf ve maya sayıları (log kob/g  $\pm$  SD).

Storage period (4 °C)	Water control	Acetic acid control	Chitosan (0.25%)	Chitosan (0.5%)	Chitosan (1.0%)
Day 1	2.33 $\pm$ 0.23 <sup>a</sup>	1.85 $\pm$ 0.06 <sup>b</sup>	1.70 $\pm$ 0.09 <sup>b</sup>	1.57 $\pm$ 0.27 <sup>b</sup>	1.60 $\pm$ 0.17 <sup>b</sup>
Day 5	3.12 $\pm$ 0.30 <sup>a</sup>	3.02 $\pm$ 0.15 <sup>a</sup>	2.13 $\pm$ 0.15 <sup>b</sup>	1.90 $\pm$ 0.03 <sup>b</sup>	1.90 $\pm$ 0.05 <sup>b</sup>
Day 10	4.17 $\pm$ 0.23 <sup>a</sup>	3.76 $\pm$ 0.19 <sup>b</sup>	2.57 $\pm$ 0.15 <sup>c</sup>	2.33 $\pm$ 0.21 <sup>c</sup>	2.25 $\pm$ 0.05 <sup>c</sup>
Day 15	5.26 $\pm$ 0.32 <sup>a</sup>	4.37 $\pm$ 0.34 <sup>b</sup>	3.21 $\pm$ 0.34 <sup>c</sup>	3.14 $\pm$ 0.48 <sup>c</sup>	2.77 $\pm$ 0.26 <sup>c</sup>
Day 20	6.06 $\pm$ 0.15 <sup>a</sup>	5.02 $\pm$ 0.17 <sup>b</sup>	3.69 $\pm$ 0.16 <sup>c</sup>	3.45 $\pm$ 0.25 <sup>cd</sup>	3.36 $\pm$ 0.10 <sup>d</sup>
Day 30	6.29 $\pm$ 0.27 <sup>a</sup>	5.93 $\pm$ 0.12 <sup>b</sup>	3.81 $\pm$ 0.14 <sup>c</sup>	3.69 $\pm$ 0.22 <sup>cd</sup>	3.41 $\pm$ 0.13 <sup>d</sup>
Day 60	6.72 $\pm$ 0.23 <sup>a</sup>	6.59 $\pm$ 0.26 <sup>a</sup>	3.99 $\pm$ 0.09 <sup>b</sup>	3.82 $\pm$ 0.11 <sup>b</sup>	3.28 $\pm$ 0.26 <sup>c</sup>

<sup>a, b, c, d</sup> Means with different letters within a same row are significantly different from one another (P<0.05)

In the present study, chitosan was dissolved in 1% acetic acid. Acetic acid is also have antimicrobial effects. Potential antimicrobial effect of chitosan solutions may also result in acetic acid used as a solvent. Therefore, a part of sausages was dipped only into 1.0% acetic acid to understand whether the possible effects of chitosan solutions arised from chemicals used to dissolve. Although microbial growth in the sausages treated with only acetic acid were relatively slower compared to the control, the number of microorganisms were obviously higher than in groups with chitosan in all analysis days (Table 1-4; Fig 1). This data demonstrates that the effect of chitosan on the microbial growth is not directly related to acetic acid in which it was dissolved.

### Conclusion

The results obtained in this study indicates that the treatment with chitosan of sausages by dipping inhibits the microbial growth and extends the shelf-life. Chitosan concentration of 0.25% was sufficient in respect to the slowing down of aerobic mesophilic bacteria and psychrotrophic bacteria growth, but higher concentration (0.5 and 1.0%) were needed for the lactic acid bacteria and yeast-mold, respectively. It was concluded that chitosan can be used as an alternative natural preservative in

the sausage. The coating with chitosan of the heat processed meat meat products will provide significant advantages, when also taking into account other capabilities such as reducing effect of oxidation and sensorial attributes as reported in the literature data. However, activity of chitosan or it's combination with other natural preservatives on specific foodborne pathogenic and spoilage microorganisms should be further studied in sausage or other meat products.

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