

**Araştırma Makalesi**

**EFFECTS OF CHITOSAN ON THE MICROBIOLOGICAL QUALITY OF  
READY TO COOK MEATBALL\***

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**Hazır Köftelerin Mikrobiyolojik Kalitesi Üzerine Kitosanın Etkisi**

**Özet.** Bu çalışma, değişik seviyelerdeki kitosanın hazır köftelerin mikrobiyolojik kalitesi üzerine etkisinin araştırılması amacıyla gerçekleştirilmiştir. Farklı seviyelerde kitosan (0, 50, 100, 250 ve 500 mg/kg) içeren köfte hamurundan şekillendirilen köfte örnekleri polietilen filmle kaplandıktan sonra 4°C'de 8 gün boyunca muhafaza edilmiştir. Muhafaza periyodu sırasında örnekler duyuşsal özellikleri ve mikroorganizma sayıları yönünden analiz edilmiştir. Kontrol grubuyla karşılaştırıldığında kitosan ilavesi kullanılan miktara bağılı olarak mikrobiyel üremeyi yavaşlatmış ve raf ömründe artışa neden olmuştur. Sonuçlar hazır köftelerin mikrobiyolojik kalitesini artırmak ve raf ömrünü uzatmak için 100 mg/kg'dan az olmamak üzere kitosan ilavesinin doğal bir katkı olarak yararlı olabileceğini göstermektedir.

**Anahtar Kelimeler:** Kitosan, köfte, kalite, mikrobiyoloji

**Abstract.** The aim of this study was performed to investigate the effects of various levels of chitosan on the microbiological quality of meatballs. Meatball samples shaped from batter containing different levels of chitosan (0, 50, 100, 250 and 500 mg/kg) were wrapped with polyethylene film and stored at 4°C for 8 days. Organoleptic properties and microbial counts of meatball samples were periodically analyzed. Compared to control group, chitosan addition to meatballs delayed microbial growth and increased the spoilage period depending on the level used. The results indicated that chitosan addition at level of 100 mg/kg and over is to be useful as a natural preservative to improve the microbiological quality and extend the shelf-life of ready to cook meatballs.

**Key Words:** Chitosan, meatball, quality, microbiology

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### Introduction

The growing consumer demand for food without chemical preservatives has focused efforts in the discovery of new natural additives. As exhibiting antimicrobial activity against a range of foodborne filamentous fungi, yeast and bacteria, chitosan is one of the new generation food additives has attracted attention as a potential food preservative of natural origin (5).

It's a natural biopolymer derived by deacetylation of chitin, is a major component of the shells of crustaceans such as crab, shrimp, and crawfish. During the past several decades, chitosan has been receiving increased attention for its commercial applications in biomedical, food and chemical industries (28). Various experimental animal studies have shown that chitosan has beneficial effects like dietary fibers (9, 26, 33). It could not be degraded in the human intestine due to the absence of enzymes such as chitosanase. In this context, chitosan may behave as dietary fibres which are excreted without any degradation in the intestine (25). Chitosan is water insoluble but is readily soluble in dilute organic acid, and becomes a polycationic polymer, which possesses film forming property. Due to this feature, chitosan can be used as coating material on food such as fresh fruits and vegetables to preserve the quality and extend shelf life during storage (1, 2, 13, 25).

Chitosan also prevents microbial activity, targeting the different groups of microorganisms such as bacteria, fungi and yeasts (8, 14, 16, 34). It's antimicrobial mechanism appears to derive in part from ionic interaction between the cationic groups of the chitosan molecules and the anionic groups of the microbial cell membrane, which can rupture the cell membrane (11). 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 (3). According to some theories oligomeric, chitosan binds DNA and prevent mRNA synthesis by penetrating into the microorganism (12). Muzzarelli et al. (15) reported that microbial cells exposed to N-carboxybutyl chitosan underwent marked morphological alterations in examination by electron microscopy. Microbial susceptibility depends on type of microorganism, molecular weight, concentration and deacetylation degree of chitosan; temperature and pH of medium (8, 16-18, 27).

Due to high microbial contamination risk and suitable composition, meat and meat products are often exposed spoilage and cause to microbial food poisoning easily. In this respect, safety of production and storage of these products are to be highly important for consumer health and food safety. Applications of chitosan as a potential natural antimicrobial agent have been studied in some meat products. It was also reported that chitosan improved the microbiological quality and extended the shelf-life of products (4, 22, 23, 29, 32). Meatballs have serious potential risk of microbial contamination during the production steps and considerably microbial load before cooking.

This study was performed to investigate the effects of various levels of chitosan on the microbiological quality and shelf-life of meatballs.

## Materials and Methods

### Materials

Chitosan (MP Biomedicals) from a crustacean shell with a molecular weight (MW) of 120 kDa and deacetylation degree (DD) of 91%, was used. Stock solution of chitosan (1%) was prepared in 1% acetic acid.

Meatball samples were prepared by conventional methods. Ground beef and lamb were incorporated with NaCl, spice (powdered black pepper, cumin powder, powdered red pepper, pimento) and other ingredients (crumbled bread, onion, garlic, soy protein, parsley, water), and kneaded with hand. The batter was divided into five batches. The first batch was separated as a control. The other four batches were supplemented with chitosan to have a final concentration of 50 mg/kg, 100 mg/kg, 250 mg/kg and 500 mg/kg, respectively. Each batch were homogeneously mixed and processed into meatballs (25-30 g) by hand. The raw meatballs were placed on polystyrene plates and wrapped with polyethylene film, and than stored at 4°C for 8 days.

### Analyses

During refrigerated storage, meatballs were periodically analysed for their organoleptic properties and microbial colony counts.

For sensory evaluation, color, brightness and smell of samples were visually observed during storage period. The darkening and paleness in color, unpleasant odor were accepted as the beginning of spoilage.

For microbiological analyses, 10 g sample of each meatball sample was homogenised 1:10 (w/v) with sterile 0,1 % peptone water for 2 min in Stomacher Lab-Blender (Seward). Serial 10-fold dilutions were prepared from the same solution and pour-plated (0,1 or 1,0 ml) into selected growth media. Aerobic mesophilic bacteria were enumerated in standard plate count agar (Merck, 1.05463) after incubation at 37 °C for 48 h, total coliform in Violet Red Bile Agar (Oxoid, CM 107) incubated for 24 h at 37 °C, *Pseudomonas* spp in *Pseudomonas* CFC Agar (Oxoid - CM 559) incubated for 48 h at 25°C and molds and yeasts in Yeast Extract Glucose Chloramphenicol Agar (Merck, 1.16000) incubated for 5 days at 22°C (7, 10).

The experimental meatball trials were repeated three times at different days. Colony counts were converted to logarithmic values. 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 (24).

## Results and Discussion

Food additives should not cause undesirable alterations in the organoleptical and textural properties of the food in addition to their main functions. In this study, chitosan addition caused no abnormal colour, odor, taste, consistency and appearance of the product. These results are in agreement with findings of Youn et al. (32) in a similar study that chitosan addition was not caused any undesirable changing on appearance, taste and scent of food.

Chitosan retarded microbial growth (except mould and yeast) depending on the level used (Table 1-4). In the microbiological analysis of the meatball samples, it was observed that the microbial counts increased gradually compared to the control group during the storage period. Inhibition effect of chitosan was stronger on the total aerobic mesophile bacteria, and colony counts of samples containing chitosan except 50 mg/kg were significantly lower than control group at last day of storage.

**Table 1:** Colony counts of total aerob mesophile bacteria in meatballs during refrigerated storage ( $\log_{10}$  cfu/g  $\pm$  SD).<sup>1</sup>

**Tablo 1:** Soğuk depolama sırasında köftelerdeki toplam aerobik mezofil toplam bakteri sayıları ( $\log_{10}$  kob/g  $\pm$  SD).<sup>1</sup>

Chitosan level	Storage Period (4 °C)				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day
0 mg/kg (Control)	6,13 $\pm$ 0,20 <sup>a2</sup>	6,23 $\pm$ 0,23 <sup>a</sup>	6,75 $\pm$ 0,11 <sup>a</sup>	7,29 $\pm$ 0,23 <sup>a</sup>	8,12 $\pm$ 0,08 <sup>a</sup>
50 mg/kg	6,08 $\pm$ 0,20 <sup>a</sup>	6,19 $\pm$ 0,29 <sup>a</sup>	6,53 $\pm$ 0,25 <sup>a</sup>	7,20 $\pm$ 0,22 <sup>a</sup>	7,74 $\pm$ 0,25 <sup>ab</sup>
100 mg/kg	6,04 $\pm$ 0,21 <sup>a</sup>	6,08 $\pm$ 0,34 <sup>a</sup>	6,33 $\pm$ 0,31 <sup>a</sup>	7,06 $\pm$ 0,18 <sup>a</sup>	7,49 $\pm$ 0,24 <sup>b</sup>
250 mg/kg	6,03 $\pm$ 0,22 <sup>a</sup>	6,00 $\pm$ 0,32 <sup>a</sup>	6,15 $\pm$ 0,25 <sup>a</sup>	6,77 $\pm$ 0,07 <sup>ab</sup>	7,28 $\pm$ 0,16 <sup>bc</sup>
500 mg/kg	6,01 $\pm$ 0,22 <sup>a</sup>	5,99 $\pm$ 0,32 <sup>a</sup>	6,01 $\pm$ 0,32 <sup>a</sup>	6,47 $\pm$ 0,08 <sup>b</sup>	6,87 $\pm$ 0,09 <sup>c</sup>

<sup>1</sup> Initial count: 5,97  $\log_{10}$  cfu/g.

<sup>2</sup> Means in a column with different letters are significantly ( $P < 0,05$ ) different from one another

**Table 2:** Colony counts of coliform in meatballs during refrigerated storage ( $\log_{10}$  cfu/g  $\pm$  SD).<sup>1</sup>  
**Tablo 2:** Soğuk depolama sırasında köftelerdeki koliform sayıları ( $\log_{10}$  kob/g  $\pm$  SD).<sup>1</sup>

Chitosan level	Storage Period (4 °C)				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day
0 mg/kg (Control)	5,35 $\pm$ 0,22 <sup>a 2</sup>	5,84 $\pm$ 0,06 <sup>a</sup>	6,33 $\pm$ 0,13 <sup>a</sup>	6,89 $\pm$ 0,04 <sup>a</sup>	7,38 $\pm$ 0,30 <sup>a</sup>
50 mg/kg	5,28 $\pm$ 0,18 <sup>a</sup>	5,73 $\pm$ 0,11 <sup>ab</sup>	6,12 $\pm$ 0,19 <sup>ab</sup>	6,69 $\pm$ 0,12 <sup>ab</sup>	6,98 $\pm$ 0,09 <sup>ab</sup>
100 mg/kg	5,20 $\pm$ 0,15 <sup>a</sup>	5,61 $\pm$ 0,11 <sup>abc</sup>	5,92 $\pm$ 0,03 <sup>bc</sup>	6,43 $\pm$ 0,12 <sup>b</sup>	6,78 $\pm$ 0,07 <sup>b</sup>
250 mg/kg	5,11 $\pm$ 0,08 <sup>a</sup>	5,48 $\pm$ 0,12 <sup>bc</sup>	5,74 $\pm$ 0,06 <sup>bc</sup>	6,05 $\pm$ 0,09 <sup>c</sup>	6,47 $\pm$ 0,12 <sup>bc</sup>
500 mg/kg	5,11 $\pm$ 0,11 <sup>a</sup>	5,30 $\pm$ 0,12 <sup>c</sup>	5,61 $\pm$ 0,10 <sup>c</sup>	5,89 $\pm$ 0,04 <sup>c</sup>	6,19 $\pm$ 0,10 <sup>c</sup>

<sup>1</sup> Initial count : 4,98  $\log_{10}$  cfu/g.

<sup>2</sup> Means in a column with different letters are significantly ( $P<0,05$ ) different from one another

**Table 3:** Colony counts of *Pseudomonas* spp in meatballs during refrigerated storage ( $\log_{10}$  cfu/g  $\pm$  SD).<sup>1</sup>

**Tablo 3:** Soğuk depolama sırasında köftelerdeki *Pseudomonas* spp sayıları ( $\log_{10}$  kob/g  $\pm$  SD).<sup>1</sup>

Chitosan level	Storage Period (4 °C)				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day
0 mg/kg (Control)	5,26 $\pm$ 0,12 <sup>a 2</sup>	5,65 $\pm$ 0,13 <sup>a</sup>	6,51 $\pm$ 0,26 <sup>a</sup>	7,26 $\pm$ 0,27 <sup>a</sup>	7,81 $\pm$ 0,06 <sup>a</sup>
50 mg/kg	5,13 $\pm$ 0,15 <sup>a</sup>	5,49 $\pm$ 0,18 <sup>ab</sup>	6,31 $\pm$ 0,25 <sup>a</sup>	6,92 $\pm$ 0,27 <sup>ab</sup>	7,49 $\pm$ 0,24 <sup>ab</sup>
100 mg/kg	5,09 $\pm$ 0,16 <sup>a</sup>	5,37 $\pm$ 0,06 <sup>ab</sup>	5,92 $\pm$ 0,08 <sup>ab</sup>	6,54 $\pm$ 0,21 <sup>bc</sup>	7,38 $\pm$ 0,20 <sup>ab</sup>
250 mg/kg	4,92 $\pm$ 0,06 <sup>a</sup>	5,21 $\pm$ 0,10 <sup>ab</sup>	5,66 $\pm$ 0,08 <sup>bc</sup>	6,24 $\pm$ 0,21 <sup>cd</sup>	6,07 $\pm$ 0,08 <sup>bc</sup>
500 mg/kg	4,85 $\pm$ 0,08 <sup>a</sup>	5,08 $\pm$ 0,15 <sup>b</sup>	5,26 $\pm$ 0,15 <sup>c</sup>	5,80 $\pm$ 0,26 <sup>d</sup>	6,64 $\pm$ 0,10 <sup>c</sup>

<sup>1</sup> Initial count : 4,47  $\log_{10}$  cfu/g.

<sup>2</sup> Means in a column with different letters are significantly ( $P<0,05$ ) different from one another

**Table 4:** Colony counts of yeast and mould in meatballs during refrigerated storage ( $\log_{10}$  cfu/g  $\pm$  SD).<sup>1</sup>

**Tablo 4:** Soğuk depolama sırasında köftelerdeki küf ve maya sayıları ( $\log_{10}$  kob/g  $\pm$  SD).<sup>1</sup>

Chitosan level	Storage Period (4 °C)				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day
0 mg/kg (Control)	4,17 $\pm$ 0,26 <sup>a 2</sup>	4,62 $\pm$ 0,35 <sup>a</sup>	5,02 $\pm$ 0,26 <sup>a</sup>	5,42 $\pm$ 0,31 <sup>a</sup>	5,73 $\pm$ 0,20 <sup>a</sup>
50 mg/kg	4,13 $\pm$ 0,27 <sup>a</sup>	4,52 $\pm$ 0,30 <sup>a</sup>	4,90 $\pm$ 0,33 <sup>a</sup>	5,31 $\pm$ 0,32 <sup>a</sup>	5,59 $\pm$ 0,30 <sup>a</sup>
100 mg/kg	4,10 $\pm$ 0,29 <sup>a</sup>	4,49 $\pm$ 0,41 <sup>a</sup>	4,83 $\pm$ 0,44 <sup>a</sup>	5,23 $\pm$ 0,33 <sup>a</sup>	5,50 $\pm$ 0,28 <sup>a</sup>
250 mg/kg	4,05 $\pm$ 0,30 <sup>a</sup>	4,42 $\pm$ 0,41 <sup>a</sup>	4,78 $\pm$ 0,39 <sup>a</sup>	5,15 $\pm$ 0,37 <sup>a</sup>	5,43 $\pm$ 0,26 <sup>a</sup>
500 mg/kg	4,05 $\pm$ 0,29 <sup>a</sup>	4,37 $\pm$ 0,32 <sup>a</sup>	4,71 $\pm$ 0,37 <sup>a</sup>	5,05 $\pm$ 0,29 <sup>a</sup>	5,29 $\pm$ 0,26 <sup>a</sup>

<sup>1</sup> Initial count : 3,92  $\log_{10}$  cfu/g.

<sup>2</sup> Means in a column with different letters are significantly ( $P<0,05$ ) different from one another

In this study, we observed that chitosan inhibited the microbial growth but not caused serious decrease in microbial count. Nevertheless, the similar studies reported more effective reduction and inhibition than ours. Darmadji ve Izumimoto (4) reported that chitosan at a concentration of 0,5-1,0% reduced 1-2 log cfu/g on growth of spoilage bacteria such as *Bacillus subtilis*, *Pseudomonas* in minced beef patties stored at 4°C for 10 days. Sagoo et al. (23) reported more effective reduction, 0,3-0,6% concentrations of chitosan in an unseasoned minced pork mixture reduced yeast and moulds, lactic acid bacteria and total viable count by up to 3 log cfu/g in the first 3 days of storage at 4°C. Ouattar et al. (19) applied the antimicrobial films onto vacuum-packaged processed meats and reported that *Enterobacteriaceae* and *Serratia liquefaciens* was delayed or completely inhibited as a result of film application. Similar findings obtained on the fresh pork sausages dipped 1,0% of chitosan; 1-3 log cfu/g reduction of microbial count determined at the end of the storage of 18 days at 7°C. Youn et al. (31) reported inactivation up to 2 log cfu/g of the total microbial flora in sausage meat following the addition of 0,35 or 0,5% chitosan. In our study, the detection of slowing down of microbial growth rather than microbial reduction may have caused as a result of the use of different molecular weight and deacetylation degree of chitosan.

In the present study, chitosan was ineffective on yeast and mould. There was no significant differences in the yeast and mould count among the groups during the storage period. Different findings were reported involved with the effect of chitosan on mould and yeasts. Roller and Covill (21), who investigated the antimicrobial properties of chitosan, reported that the presence of chitosan in apple juice (pH 3,4) at levels ranging from 0,1 to 5 g/l inhibited growth at 25°C of all spoilage yeasts. Fang et al. (6) observed that 0,1-5 mg/ml. of chitosan addition (pH 5,4) inhibited the growth of *Aspergillus* spp. and prevented also aflatoxin production. Cuero et al. (3) also reported that, chitosan inhibited growth of moulds and decreased the production of mycotoxin in the rate of 90%. Contrary to these findings, Tsai et al. (29) observed that the effect of chitosan on fungi was lower than bacteria and no antifungal activity was seen against *Aspergillus fumigatus* or *A. parasiticus* even at 2000 ppm. Roller et al. (22) reported that at low chitosan concentrations, no inhibition of growth was observed. Rodríguez et al. (20) reported that treatment of chitosan as edible film suppressed the growth of *Alternaria* sp, *Penicillium* sp and *Cladosporium* sp in pizzas. However, they observed no effect when added into pizza batter. In our study, the poor effect may be due to direct incorporation of chitosan into processed meats.

In this present study, meatballs with chitosan were showed signs of spoilage more late than control. The control group without chitosan was spoiled on 6<sup>th</sup> day of storage; whereas signs of spoilage were observed in samples containing 50-100 mg/kg chitosan on 8<sup>th</sup> day of storage. Moreover, in samples containing 250-500 mg/kg chitosan, no spoilage was detected during 8 days refrigerated storage. The effects of chitosan on inhibiting growth of spoilage microorganisms were also investigated by other researchers. Our results are in agreement with findings of Darmadji and

Izumimoto (4) in a similar study where concentration of 0,5-1,0% chitosan delayed spoilage on meatballs. Therefore, Sagoo et al. (23) reported that treatment with chitosan increased the shelf-life of raw sausages stored at chill temperatures from 7 to 15 days; similar with our findings, they observed that chitosan addition as a preservative food additive was quite effective on shelf-life of chilled meat products. Tsai et al. (29) studied the effect of chitosan on fishery products. They determined that treatment with concentration of 1,0% chitosan to fish fillets delayed the increase of basic volatile nitrogen, inhibited the microbial growth and increased the shelf-life from 5 to 9 days. Youn et al. (32) observed that chitosan concentrations up to 0,1% extended the shelf-life and decreased the oxidation on spice mixed meat. Youn et al. (31) reported that sausage containing chitosan (0,2%) had improved shelf-stability.

The results of this study indicates that addition of chitosan at level of 100 mg/kg and over to meatballs as a natural and an alternative of preservative food additives may improve their microbiological quality and extend shelf-life depending on the concentration used. Nevertheless, the studies involved the effects of chitosan on the microbiological quality, especially on foodborne pathogens should be supported. Successful applications of chitosan to inhibit foodborne pathogens in fresh meat would provide meaningful economic and food safety benefits.

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