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**YASEMİN ÇELEBİ SEZER**

**UNIVERSITY OF GAZİANTEP  
GRADUATE SCHOOL OF  
NATURAL & APPLIED SCIENCES**

**AN INVESTIGATION OF CHANGES IN SAFETY AND QUALITY OF  
SUCUK STUFFED INTO ACTIVE CASING FILMS**

**Ph.D THESIS  
IN  
FOOD ENGINEERING**

**BY  
YASEMİN ÇELEBİ SEZER  
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**An Investigation of Changes in Safety and Quality of Sucuk Stuffed into Active  
Casing Films**

**Ph.D Thesis**

**in**

**Food Engineering**

**University of Gaziantep**

**Supervisor**

**Prof.Dr. Huseyin BOZKURT**

**by**

**Yasemin ÇELEBİ SEZER**

**March 2018**



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Name of the student : Yasemin ÇELEBİ SEZER  
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Approval of the Graduate School of Natural and Applied Sciences

  
Prof. Dr. Ahmet Necmeddin YAZICI  
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of  
Doctor of Philosophy.

  
Prof. Dr. Medeni MASKAN  
Head of Department

This is to certify that we have read this thesis and that in our consensus opinion it is  
fully adequate, in scope and quality, as a thesis for the degree of Doctor of  
Philosophy.

  
Prof. Dr. Hüseyin BOZKURT  
Supervisor

Examining Committee Members:

Prof. Dr. Hasan YETİM  
Prof. Dr. Sevim KAYA  
Prof. Dr. Hüseyin BOZKURT  
Assoc. Prof. Dr. Çiğdem AYKAÇ SOYSAL  
Assist. Prof. Dr. Hande DEMİR

Signature  
  
  
  
  


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**Yasemin ÇELEBİ SEZER**

## **ABSTRACT**

### **AN INVESTIGATION OF CHANGES IN SAFETY AND QUALITY OF SUCUK STUFFED INTO ACTIVE CASING FILMS**

**ÇELEBİ SEZER, Yasemin**

**Ph. D. Thesis in Food Engineering**

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Recently, active packaging, can be observed as one of the best strategies to prevent microbial and other spoilages in meat products (such as sucuks) while maintaining them fresh, safe and in a high quality. In this study, effects of nisin, potassium sorbate, chitosan and silver zeolite (AgZeo) incorporated into polyethylene new packed system as antimicrobial case on the microbiological and physicochemical characteristics of heat treated sucuks at 65, 70 and 75°C were followed for 3 days of fermentation and 12 days of storage after the heat treatment. Microbial growth was the slowest in the nisin and chitosan casings where the total viable count of 8.00 log cfu/g was found on day 3. Higher reductions in aerobic plate count (APC) and lactic acid bacteria (LAB) were noted in the sucuks stuffed into chitosan incorporated casings during storage. Color values of heat treated sucuks were not affected negatively by use of antimicrobial casings. The highest ( $P < 0.05$ ) hardness values were 26.00 N in the sucuks stuffed into collagen case at the end of fermentation. Using different antimicrobial casings and storage time influenced significantly ( $P < 0.05$ ) the hardness, gumminess and chewiness value of sucuks. The lowest tryptamine, putrescine, tyramine and histamine values were 12.54, 160.77, 309.81 and 46.62 mg/kg in sucuk stuffed into chitosan case at the end of fermentation respectively. Cadaverine, tryptamine, putrescine, tyramine and histamine formations in heat treated sucuks decreased ( $P < 0.05$ ) by incorporation of chitosan, nisin, AgZeo and potassium sorbate. These results showed that antimicrobial cases could be used for the production of heat treated sucuks to improve its safety and quality.

**Keywords:** Heat treated sucuks, Antimicrobial case, nisin, silver zeolite, chitosan

## ÖZET

# AKTİF KILIF İLE DOLDURULAN SUCUKLARIN KALİTE VE GÜVENLİK DEĞİŞİMLERİNİN İNCELENMESİ

**ÇELEBİ SEZER, Yasemin**

**Doktora Tezi, Gıda Müh. Bölümü**

**Tez Yöneticisi: Prof. Dr. Hüseyin BOZKURT**

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Son zamanlarda sucuk gibi et ve et ürünlerinin mikrobiyolojik ve diğer bozulmalara karşı önlemede en iyi yöntemlerinden biri olarak kullanılan aktif ambalajlama teknolojisi, ürünleri daha taze, güvenli ve kaliteli olarak sunmaktadır. Bu çalışmada, nisin, potasyum sorbat, kitosan, gümüş zeolit (AgZeo) gibi antimikrobiyal madde içeren polietilen içerikli yeni aktif kılıflar ile doldurulan sucukların, 3 günlük fermentasyon ve ısıtma işlemi sonrası (65, 70 ve 75°C'de) 12 günlük depolama süresince gerçekleşen mikrobiyolojik ve fizikokimyasal değişimleri incelenmiştir. Fermentasyonun 3. gününde toplam canlı sayımı olarak mikrobiyolojik büyüme en yavaş ( $P < 0,05$ ) nisin ve kitosan içerikli kılıflarda 8 log kob/g olarak bulunmuştur. Depolama boyunca mikrobiyolojik azalmanın en yüksek ( $P < 0,05$ ) olduğu örnek kitosan ilave edilmiş kılıflardır. Sucukların renk değerleri üzerine aktif kılıf kullanımının olumsuz etkisi görülmemiştir. En yüksek sertlik değeri (26 N) fermentasyon bitiminde kolajen kılıflarda tespit edilmiştir. Farklı aktif kılıf kullanımının ve değişen depolama zamanının sucukların sertlik, yapışkanlık ve çiğneme değerleri üzerinde önemli ( $P < 0,05$ ) derecede etkisi gözlemlenmiştir. Fermentasyon sonunda en düşük tryptamin, putresin, tyramin ve histamin değerleri sırasıyla 12,54, 160,77, 309,81, 46,62 mg/kg olarak kitosan içerikli kılıflar ile doldurulan sucuklarda tespit edilmiştir. Kılıflara kitosan, nisin, AgZeo and potasyum sorbat ilavesi, kadaverin, tryptamin, putresin, tyramin ve histamin formülasyonlarında önemli derecede azalmaya neden olmuştur. Bu sonuçlar, antimikrobiyal kılıfların, güvenliği ve kaliteyi arttırmak için ısıtma işlemi görmüş sucuk üretiminde kullanılabileceğini göstermektedir.

**Anahtar kelimeler:** Isıtma işlemi görmüş sucuk, Antimikrobiyal kılıf, Nisin, Gümüş zeolit, kitosan



*To My Parents...*

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## LIST OF ABBREVIATIONS

APC	Aerobic Plate Count
LAB	Lactic Acid Bacteria
TBARS	Thiobarbituric Acid Reactive Substances
MDA	Malondialdehyde
cfu	colony-forming unit
N	Newton
mJ	Millijoule
AgZeo	Silver Zeolit

## CHAPTER I

### INTRODUCTION

#### 1.1 General Introduction

Quality and safety of food supply has become popular due to growing concerns recalls and ingredient sourcing problems over consumers (Balasubramanian et al., 2009). Producers has started to add some antimicrobial chemicals into the food. Incorporation of compounds directly into food is an established application with several disadvantages. Incorporation of antimicrobials directly into food ensures prevention of some undesired microorganisms growth. However, the alive microorganisms will continue to grow, when antimicrobials get finished (Balasubramanian et al., 2009). Nowadays consumers are demanding of food products less processed and containing minimum chemical (Barros et al., 2010). To tide over these negative aspects, novel technologies, namely “Active Packaging” was discovered. Active packaging has been described as “a kind of packaging that modifies the status of the packaging to enlarge the shelf-life or develop sensory or safety features while preserving the properties of food” (Vermeiren et al., 1999). Antimicrobial package is one the main application of the active packaging (Quintavalla and Vicini, 2002; Joerger, 2007). Multilayer active packaging systems including the antimicrobial substances may be adjusted to lose slowly their activities during the storage period (Ishitani, 1995). Consequently, shelf life of packaged products is significantly prolonged because of the fact that the antimicrobial contents added into packaging ingredients can control the microbial infection (Soysal et al., 2015).

The United States Food and Drag Administration (FDA) and World Health Organization (WHO) describes that nisin is a bacteriocin known as generally regarded as safe (GRAS). It is produced by specific species of *Lactococcus lactis* subsp. *lactis*. Nisin is effective on gram-positive bacteria, but not effective on

gram negative bacteria, mold and yeast. It has been observed to be influential in preventing pathogenic and spoilage bacteria in meat and meat products (Barros et al., 2010).

Potassium sorbate that is effective against mould, yeast and bacteria, is a commonly employed antimicrobial compound (Pranoto et al., 2005). Chitosan has also antimicrobial activities against Gram positive/negative bacteria, molds and yeasts (Siripatrawan and Noipha, 2012). It is acquired from deacetylation of chitin that is a functional biopolymer. Further, chitosan is known as friendly packaging material for environment due to its nontoxic, biocompatible and biodegradable properties (Pereda et al., 2011). Similarly, chitosan could be employed as an active antimicrobial film because of its antimicrobial properties and great film-forming capacities (Fan et al., 2009). One of the most common antimicrobial agents incorporated into plastics is silver substituted zeolite (AgZeo). Having high antimicrobial activity of Ag-ions can inactivate microorganisms with the aid of a sequence of metabolic enzymes (Kerry et al., 2006).

Sucuk is known as a dry and fermented meat product which is consumed commonly in Middle East, Balkans, Caucasus and especially in Turkey (Ercoşkun et al., 2010). Sucuks are extremely sensitive to oxidation of lipid and microbial deterioration, which cause formation of rancid flavor and different kinds of spoilage (Bozkurt, 2006). In recent years heat process is the practice of option to which increasing the quality attributes of sucuks. Additionally, heat treatment has been an important step of sucuk production even though heat treatment is not a part of manufacturing technology of traditional sucuk (Ercoşkun et al., 2010). Accordingly, the extension of shelf life can be supplied by inhibiting bacterial growth and heat treatment (Siripatrawan and Noipha, 2012). Hence, multilayer films as a casing containing antimicrobial substances will be good choice for preventing microbial growth and extending shelf life such as sucuks (Soysal et al., 2015).

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Sucuk

Sucuk is a dry, spiced, fermented sausage which is highly consumed in Turkey and also in countries located in Middle East, Balkans and Caucasus. Sucuk was identified in the “Divan-ı Lugatı Türk” (Compendium of the languages of the Turks, originally written by Mahmud of Kashgar in 1072) (Atalay, 1991). Sucuk is a kind of sausage manufactured from a mixture of meat (sheep, beef), fat (beef and sheep tail fat), garlic, salt, sugar and seasonings and spices and this mixture is stuffed into a casing where fermentation is achieved until a dry product is acquired (Turkish Standards Institute, TSI, 2007).

During the fermentation, the lactic acid bacteria (LAB) or Gram-positive, catalase-positive cocci (GCC) cause biochemical changes in fermented sausages such as; glycolysis (Viallon et al., 1996) proteolysis and lipolysis (Tabanelli et al., 2012). These stages influence quality properties of the products (Tabanelli et al., 2012) .

Lipids in dry sucuks are existed in fat tissue (tail fat) and muscle tissue. Tail fat has primarily of triglycerides, while lean meat has about 7% lipids. The lipids in muscle tissue are intramuscular fat, which occurs 62%-80% triglycerides and 16%-34% phospholipids. While phospholipids are available in lower quantities than triglycerides, they are more sensitive to oxidation and lipolysis because they are present in high amount in polyunsaturated fatty acids (PUFA) (Gokalp et al., 2002). Free fatty acids (FFAs) are the major materials for lipid oxidation; so, lipolysis is a important stage for flavour production. For all that, endogenous enzymes, such as lipases and phospholipases are thought to be the principal cases of free fatty acid (FFA) release, bacterial lipase efficiency cannot be disregarded. Lactic acid bacteria (LAB) and Coagulase-negative *Staphylococci* (CNS) are commonly used as starter

cultures in fermented meats. Lipases have been extracted, purified and characterised in *Lactobacillus plantarum*, *Staphylococcus xylosus* and *S. warneri*. Also, lipolytic efficiency of these microorganisms has been defined in fermented sucuks, particularly in *Staphylococcus* strains (Dalmış and Soyer, 2008).

Dry fermented sausages are sensitive to lipid oxidation that can change their sensory characteristics, by accumulation of materials such as aldehydes, n-alkenals and dienals which are caused a rancid taste and odor. Moderate oxidation of FFAs is ensured of lipolysis and is responsible for flavour formation (Lorenzo et al., 2016). Volatile compounds such as linear alcohols, acids, aldehydes, esters and ketones have been derived from FFA autoxidation. Generally, five to nine-carbon aldehydes can maintain the oil and lipid odors of fermented meats, and long chain aldehydes are associated with a laurel odor. For instance, hexanal, which is a characteristic product of linoleic acid oxidation, contributes to a fresh cut grass odor; heptanal contributes to a nut odour; and nonanal contributes to the citrus, laurel and carnation odors. Although, methyl ketone existing from microbial incomplete  $\beta$ -oxidation is one of the major participants to a characteristic fermented flavour. However, high amount lipid oxidation is the reason of quality degradation in fermented sucuks, resulting in rancidity, discolouration, drip loss, loss of nutrient content and meat protein oxidation (Falowo et al., 2014).

Spoilage of sausages is mostly associated with microorganisms. *Staphylococcus aureus* is one of the reasons of food poisoning when present, because of, wrong hygienic applications, in the plant on the surface of material that is in touch with the food surface. The surfaces of meat products were found to be contaminated with foodborne pathogens after the cooking process due to microbial accumulation on the surface of the equipment. Also, meat products were frequently deteriorated by the growth of spoilage bacteria, for example *Brochothrix thermosphacta* and *Lactobacillus* spp. which generate an off-flavor, gas and discoloration and *Leuconostoc* spp. which reason aropy slime creation (Borch, et al., 1996).

Mold and yeast growth is requested related to meat products. *Debaryomyces hansenii* and *Candida utilis* have for example been found to positively influence on the aroma formation and to stabilise the red color of fermented sausages (Dura, et al., 2004).

But, in most cases yeast growth in meat products is undesired and may reason deteriorate of the product such as by gas-swelling of packaged meat products; by slime formation and discolouration on the surfaces of sausages; and by producing off-flavours (Fleet, 1992).

The safety and quality of products are connected with the microflora of the fermented product. It is hard to acquire fermented sucuks of a standard high quality (Gökalp, 2002), mostly because of changes in technological, safety and hygienic requirements and manufacturing technology. Heat treatment is the practice for selection of increase in the safety and quality attributes of meat products in comparison to non-thermal techniques such as irradiation and high pressure operating (Thippareddi and Sanchez, 2006). The producing technology of conventional sucuk has been changed, and heat treatment has become a part of production while thermal processing is not a part of traditional sucuk processing. Heat treatment in sucuk production is used to inhibit pathogens, to prolong shelf life, shorten manufacture time and reduce production costs. This application is generally performed in a sealed oven by steam injection to increase temperature and relative humidity, and sucuks are kept under these status for a time that is enough to inhibit all pathogens in advanced food processing plants. Also, in small-scale plants, heat process under different status is performed based on the decisions of tradesmans (Erçoşkun et al., 2010).

After heat treatment, the microorganisms that survived from pasteurization can grow and deteriorate the product during storage. *Bacillus thermosphacta*, *Enterococcus faecalis*, *L. sakei*, *Leuconostoc mesenteroides subsp. mesenteroides*, *Leuconostoc carnosum* and *Carnobacterium divergens* are commonly known having negative impact on the aroma, flavour, texture, and color of sliced and vacuum packed meat products. Also, the growth of heterofermentative bacteria can cause for the packaging to swell or rupture because of the existence of CO<sub>2</sub>. Bacterial activity is affected by the availability of the sugars that are incorporated with the brine during tumbling, resting, or cooling for a long time. LAB and *B. thermosphacta*, which can contaminate the fresh meat, from the handling-related procedures during operation or from the medium (Comi and Iacumin, 2012).

Recently, active packaging, can be observed as one of the best strategies to prevent microbial spoilage in food products while maintaining them fresh, safe and in more quality. A kind of antimicrobial food packaging films have been improved by an incorporation of different antimicrobial compounds to different packaging materials (Robertson, 2006). For this reason, the use of active packaging might control deterioration of meat and meat products such as sucuks.

## **2.2 Active Packaging**

Active packaging is a popular food packaging type that has been offered as a reply to the changes in consumer requests. According to choosed defination in European FAIR-project CT 98-4170 has been presented as a “kind of packaging that modifies the condition of the packaging to prolong shelf-life or improve safety or sensory characteristics while maintaining food quality” (Vermeiren, et al., 1999).

Active food packaging can provide different functions that is not obtained in conventional packaging systems. The active attributes might include antimicrobial activity, emission of flavours and ethanol, scavenging of oxygen, ethylene or moisture. Microbial contamination reduces shelf life of food and increases the risk of foodborne illnesses. Refrigeration, drying, irradiation, freezing and modified atmosphere packaging are traditional techniques for preserving foods. However, some of these methods can not be used for several food products, such as fresh and processed meats and meat products (Quintavalla and Vicini, 2002).

Active packaging extends the shelf life of foods, while improving their nutritional level, preventing the growth of spoilage and pathogenic microorganisms, prohibiting migration of contaminants. Active packaging technologies include some physical, chemical, or biological features that modify reactions between a film, product, and/or headspace of the film to get a requested effect. Active packaging is consisted of two kind of systems; pads and sachets that are place to packages, and active ingredients which are incorporated inside of packaging materials (Robertson, 2006; Pavelková and Flimelová, 2012). Antimicrobial packaging is that one of the active packaging type is a new packaging system. Antimicrobial packaging is a popular type of active packaging system that that can release antimicrobial compounds for increasing the quality and safety of products during storage.

### **2.2.1 Antimicrobial Packaging**

Antimicrobial packaging, attention is given as an instrument for the addition and/or slow release of antimicrobials, the expansion of the bacterial delay phase, the slowing of the growth rate of microorganisms, and the protection of food quality and safety (Han, 2000; Guerra et al., 2005).

The antimicrobial materials used for antimicrobial packaging can be categorized depending on the material base as either 1) organic or inorganic or 2) chemical agents or natural agents or probiotics. The following food-grade antimicrobial agents can be used for antimicrobial food packaging systems; organic acids and their salts [benzoic acid, acetic acid, benzoic anhydride, sodium benzoate, potassium sorbate, sorbic anhydride, alkyl (ethyl, methyl, propyl) paraben, fatty acids (palmitoleic acid, lauric acid, glycerol mono-laurate)], metals (silver, copper, zirconium, titanium oxide), chelating agent (EDTA), enzymes (lysozyme, peroxidase, glucose oxidase), polypeptide (lactoferrin), bacteriocin (nisin, pediocin, lacticins), antioxidants, chitosan, antibiotics, sanitizing gas, fungicides, sanitizers, plant spice/spice extracts, plant volatiles, phenolics, plant essential oils (EOs) (cinnamon, oregano, lemongrass), nitrites and sulphites and probiotics (Franssen and Krochta, 2003; Han and Floros, 2007; Lee, et al., 2008; Lee and Yam, et al., 2008). Thus, the use of packaging films containing antimicrobial agents may be more efficient with controlled migration of the compound into the food.

Antimicrobial agents included in packaging materials can control microbial contamination by reducing the growth rate and maximum growth population and/or prolonging the delayed phase of the target microorganism or by inactivating microorganisms by contact (Kerry, et al., 2006).

The antimicrobial classes listed are organic acids, acid anhydrite, enzymes, alcohol, bacteriocins, polysaccharides and chelating agents. Examples of concentrated antimicrobial materials in the form of concentrated (e.g. AgIONe, AgION Technologies LLC, USA) extracts [Nisaplin (Nisin), Integrated Ingredients, USA] and films (Microgarde Rhone-Poulenc, USA) are also presented (Kerry, et al., 2006). Bacteriocins are proteinaceous toxic made by bacteria to inhibit the growth of similar or closely related bacteria (Deshmukh and Thorat, 2013). Nisin, the bacteriocin

produced by *Lactococcus lactis* subsp. *Lactis* are used as food preservatives in various countries (Benkerroum, 2000). Nisin incorporated films were reported to have an antimicrobial activity for Gram positive bacteria, such as *B. thermosphacta*, *L. helveticus*, *Listeria monocytogenes*, *Myotis flavus* and *Pediococcus pentosaceus* (Daeschel et al., 1992; Siragusa et al., 1999; An et al., 2000) and thus, the deteriorating foods have been shown to extend shelf life by suppressing the growth of degradation bacteria. In addition, nisin is non-toxic, heat-stable and digestive protease sensitive (Guerra and Pastrana, 2002).

Potassium sorbate is a widely utilised antimicrobial and antioxidant ingredient. Potassium sorbate, a microbial inhibitor, can be used to prevent the formation of biogenic amines in foods. Potassium sorbate is effective against yeast, mould and many bacteria (Shalaby, 1996).

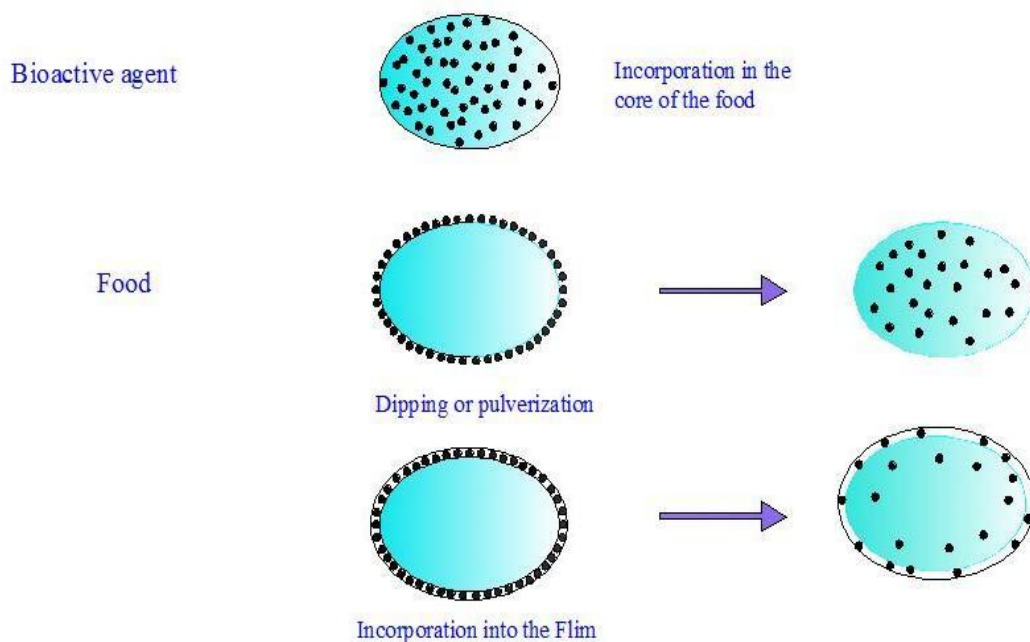
Silver substituted zeolites (AgZeo) are the most commonly used as polymer agents for food processings, especially in Japan. Sodium ions available in zeolites are substituted by silver ions, which are antimicrobial against a wide range of microorganisms. These substituted zeolites are added into polymers such as polyethylene, polypropylene, nylon and butadiene styrene at levels of 1-3%. Commercial examples of silver substituted zeolites contain Zeomic, Apacider, AgIon, Bactekiller and Novaron (Brody et al., 2001).

Chitosan is widely provided from the exoskeleton (food waste) of crustacean shells. It is a linear polysaccharide occurring (1, 4)-linked 2-amino-deoxy- $\beta$ -D-glucan, and deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature just after cellulose. Chitosan indicates to be nontoxic, biodegradable, biofunctional, biocompatible and has antimicrobial properties. Antimicrobial properties of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms. Chitosan, a high molecular mass linear copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked by  $\beta$  (1-4), obtained by the partial hydrolysis of the N-acetyl groups of chitin, is a well-known antimicrobial biopolymer, active against a wide range of microorganisms (yeasts, moulds and bacteria) (Dutta et al., 2009).

Especially, a grade of polymerization of at least seven monomeric units is required to get important antimicrobial impact, and highly deacetylated chitosan indicated higher antimicrobial affect than chitosan with primarily acetylated amino groups (Aider, 2010).

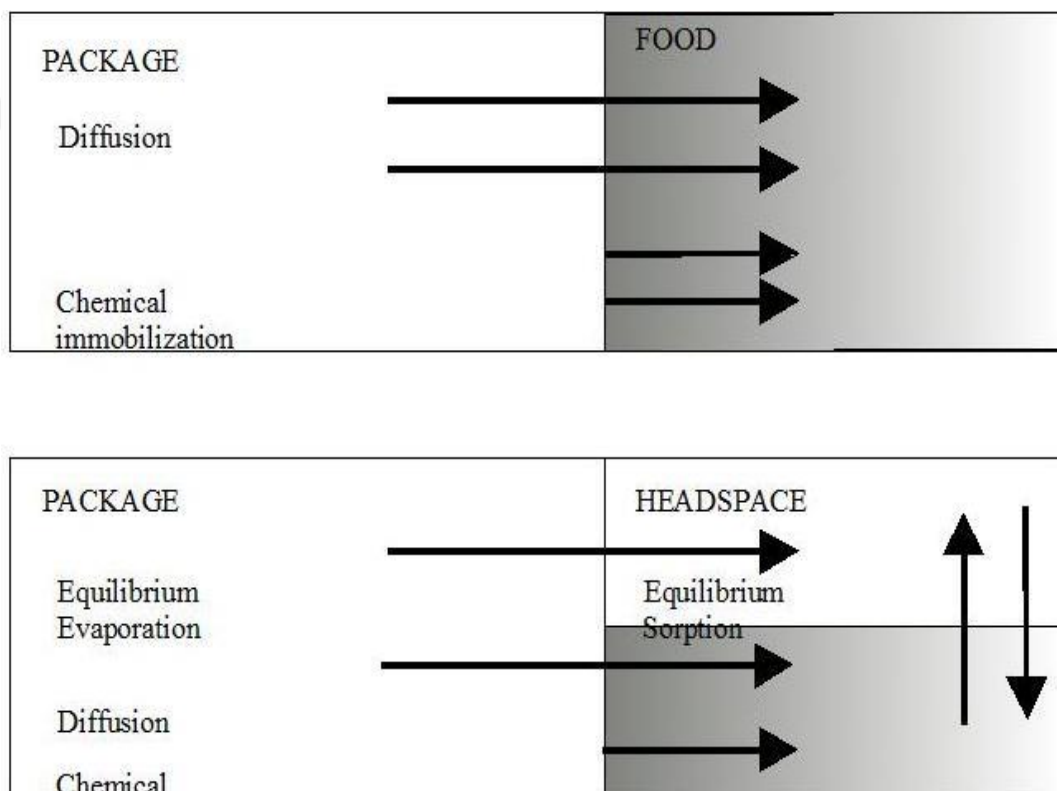
### 2.2.2 Developing Antimicrobial Packaging Systems

Antimicrobial packaging substances may be improved and used because they can prevent growth of the microorganisms and thus increase the shelf life of perishable products and extend the safety and quality of packaged products (Han, 2005). Firstly, antimicrobial packaging system could be more effective by maintaining high concentrations on food surfaces with a low migration of active substances (Figure 2.1). Secondly, the agent will generally not be a direct additive to the food product. Thirdly, the direct incorporation of bactericides or growth inhibitors into meat preparations may cause the active ingredients to be partially inactivated by the food ingredients and therefore it is expected that they will have only a limited effect on the surface flora (Coma, 2008).



**Figure 2.1** Different incorporation methods of additives in food products (incorporation) into the foodstuff, dipping or pulverization, and finally incorporation into a film and consequences. The black points correspond to an antimicrobial substances.

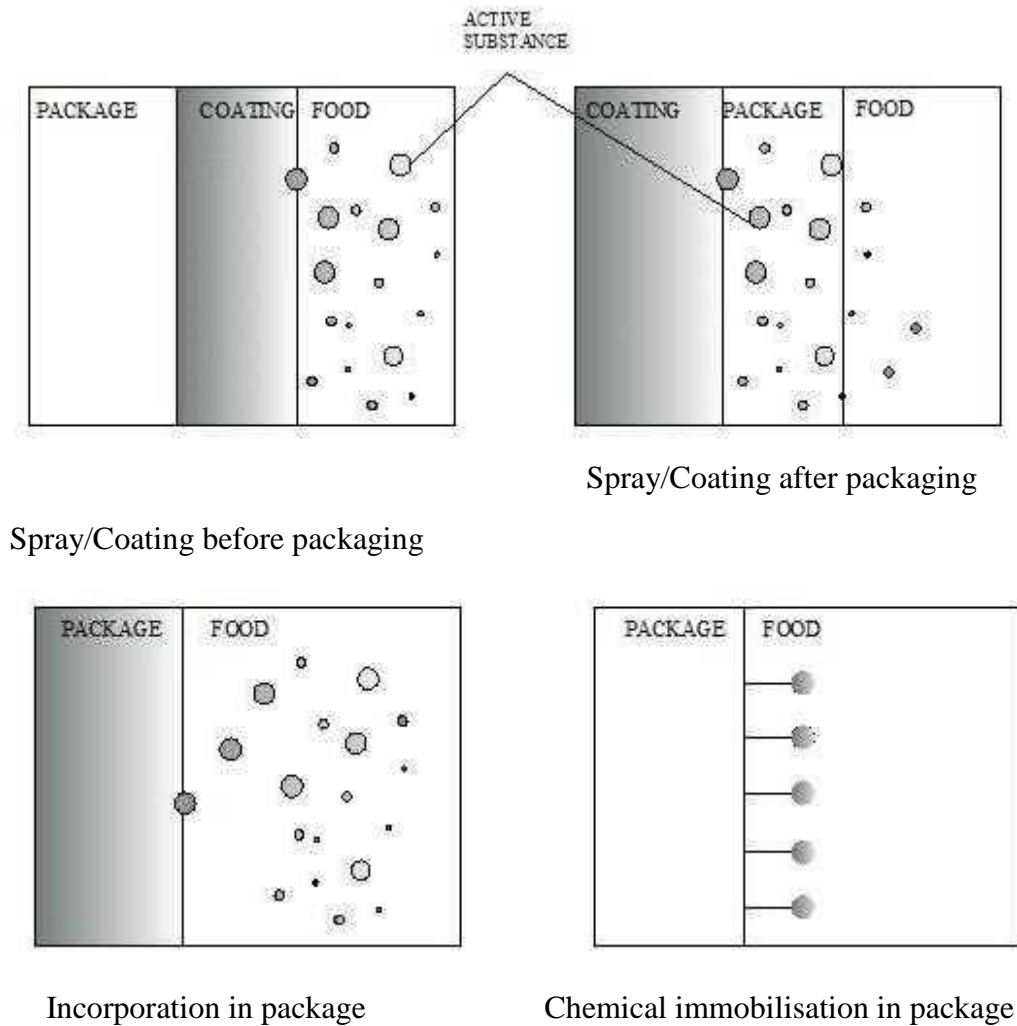
Most food packaging systems indicate a package/food system or a package/headspace / food system (Figure 2.2). The package/food system is a solid food product that is in contact with the packaging material or a low viscosity or liquid foodstuff without headroom void. Individually packaged, ready-made meat products, "sous-vide " baked goods and delicatessen products are good examples. Diffusion among the packaging material and the food and divide at the interface are the main immigration phenomena in this system. Antimicrobial agents can be initially incorporated into the packaging material and migrate to food by diffusion and partitioning (Han, 2000).



**Figure 2.2** Food packaging technics and relative behaviour of active compounds (Han, 2000).

Package/headspace/food systems have been displayed in flexible packaging in cups and packed with cardboard. Evaporation or balanced distribution between head space, packaging material and/or food of a substance should be considered as part of the main migration mechanisms to estimate the interface distribution of the substance. In these systems, a volatile active substance can be used because it can pass through the space between the package and the food and through the air gaps.

Besides diffusion and balanced absorption, some antimicrobial packages use covalently immobilized antibiotics or active moieties such as fungicides or amine groups. This case uses surface inhibition of microbial growth by immobilizing non-food antimicrobial material without diffusional mass transfer (Han, 2000; Quintavalla and Vicini, 2002).



**Figure 2.3** Migration of active compounds in different methods of antimicrobial packaging technics (Han, 2000).

The antimicrobial films have three basic categories:

**1. Direct incorporation of the antimicrobial agent into the packaging film:** Such systems are often developed by adding the antimicrobial compound by extrusion press when film or co-extrusion is produced (Figure 2.3). A disadvantage is that the high temperatures and shear associated with the extrusion process can cause degradation of the antimicrobial adding mixtures (Coma, 2008).

**2. Coating of the packaging with a matrix that acts as a carrier for the antimicrobial agent:** An alternative to the incorporation of antimicrobial compounds during extrusion is to apply the antimicrobial additives as a coating. In this method, the antimicrobial additive is applied as a coating (Figure 2.3). Moreover, the coating can be applied at a later stage, thereby minimizing the exposure of the product to contamination (Coma, 2008).

**3. Immobilisation:** Some antimicrobial packaging systems use covalently immobilized antimicrobial agents that suppress microbial growth (Sebti et al., 2002; Coma et. al., 2008).

### **2.2.3 Factors for designing the antimicrobial packaging system**

According to Han (2000), several factors must be considered in the design or model of antimicrobial film in or pack:

#### **a. Chemical nature of films/coatings**

The choice of antimicrobial agent is often limited by the thermal stability of the component during extrusion or the incompatibility of the component with the packaging material. For example, 1% potassium sorbate in LDPE film inhibits yeast growth on agar plates. LDPE resin and potassium sorbate can be mixed, rolled and pelletized to produce a masterbatch. These pellets can be added to LDPE resin. The master batch should be produced at low temperature to prevent the heat dissipation of potassium sorbate (Han and Floros, 1997). Another study (Weng and Hotchkiss, 1993), nevertheless, it has found that relatively polar sorbate, benzoate and propionate should not coincide with apolar LDPE. Acid anhydrides are thought to be more suitable than free acids and their salts because of their low polarity. Now the antimicrobial activity, effective activity of the antimicrobial substances used after casting (extrusion) and conversion processes (lamination, printing, drying). The effects of adhesives and solvents must be quantitatively characterized.

#### **b. Characteristics of antimicrobial substances and foods**

Food ingredients have a significant impact on the effectiveness and release of antimicrobials. The physico-chemical properties of foods can alter the activity of antimicrobial substances. For example, the pH of the food can affect the ionisation of

the most active chemicals (decomposition/association) and alter the antimicrobial activity of organic acids and their salts. The antimicrobial activity and chemical stability of the active ingredients involved may also be affected by the water activity of the foodstuff. In addition, each food has its own microflora. The release kinetics of antimicrobial agents should be designed to keep the concentration above the critical inhibitory concentration compared to possible contaminating microorganisms.

The growth prevention practice and kinetics are the capital factors to be thought in planning the antimicrobial packaging system. The mathematical model of microbial growth can be developing from the inhibition kinetics and activity. The release kinetics of antimicrobial compounds has to be planned to provide the concentration above the critical inhibitory concentration in accordance with the growth kinetic studies. Because foods have different physicochemical and biological properties such as pH, water activity, carbon and nitrogen source, partial pressure of oxygen, and temperature, they ensure different environmental cases to bacteria and contained antimicrobial compounds. For example, the pH of food influences the microflora and growth ratio of target microorganisms and changes the ionization of most active chemicals, which could alter the antimicrobial activity. Water activity changes the antimicrobial activity and chemical stability of added active compounds as well as microflora. Oxygen in the package headspace can be used by aerobic microorganisms, and the oxygen permeability of the packaging ingredients can change the headspace oxygen formation. Therefore, it is necessary to examine the pH and water activity of the food, oxygen permeability of the packaging material, and microbial profile to design antimicrobial packaging systems (Smith et al., 1989).

### **c. Storage temperature**

Storage temperature can affect the antimicrobial activity of chemical preservatives. Often the increased storage temperature can accelerate the migration of active materials from film/coating layers, while cooling can slow migration rates. It is anticipated that the temperature conditions during production and distribution will determine the effect of active compounds on the antimicrobial activity.

### **d. Mass transfer coefficients**

The simplest system is the release of the active substance from the package by diffusion. A multilayered design has the advantage that the antimicrobial can be

added to a thin layer and controlled migration by the thickness of the film layer or coating. In practice, a matrix consisting of several layers is used to control the release rate of the active substance. The mass transfer model of the transition phenomenon can be used to define the film/coating layer and the fresh concentration profile over time. Han (2000) summarized traditional modes of mass transfer and his own proposed models that could be used to describe the migration of active ingredients through single, double or triple layer food packaging systems. Using the mass transfer models it is possible to calculate the storage times at which the active substance concentration is kept above the critical activity concentration, so that the food safety shelf life can be calculated.

#### **e. Physical properties of packaging materials**

When antimicrobial activity is incorporated into packaging materials to decrease microbial deterioration, it may impact general physical attributes and process/machinability of the packaging materials. Material features of packaging materials contain mechanical properties as burst strength, tensile strength, tearing resistance, elongation, stiffness, and physical properties as water absorptiveness, oxygen (and other gases) and water vapor permeability, wettability, grease resistance, haze, gloss, transparency, brightness and others. The activity of the packaging materials must be ensured with the incorporation of the active compounds, while the materials include more heterogeneous concentrations. In the case of plastics, the active substrates are often very-low-molecular-weight materials compared to the dimension of the polymeric construction and are incorporated in small levels. In antimicrobial packaging system, the chemicals will locate in the amorphous structural areas of the polymer and may not influence the mechanical strength of the polymeric packaging materials. Han and Floros (1997) found no important alteration in tensile attributes before and after incorporating potassium sorbate into LDPE film. Considering the big size of the amorphous area of the polymeric materials to the relatively small size of the antimicrobial agent, a large amount of the antimicrobial substance may be required to indicate any activity on the tensile strength of the packaging materials. But, after the amorphous area of the polymers and the porous area of papers are fed by a high amount, the tensile strength of the antimicrobial materials could be negatively impacted. Moreover mechanical strength alters, incorporation of antimicrobial

compounds often decreases optical features of plastic films like transparency. The transparency of LDPE films reduced with rising potassium sorbate concentration (Han and Floros, 1997). This may effect in important adversely in utilizing this antimicrobial plastic film for see-through packaging.

#### **2.2.4 Antimicrobial packaging applications for meat and meat products**

As indicated by Quintavalla and Vicini (2002), the use of triclosan for food contact applications has recently been demonstrated by the European Food Scientific Committee as a quantitative restriction on monomers for food contact materials and on the tenth additional list of additives, allowed on immigration of 5 mg/kg of foods. For non-volatile bioactive substances, the bioactivity of these film categories is based on the diffusion of biocide into food. To produce an antimicrobial food packaging system, Choi et al. (2005) determined the diffusivity of potassium sorbate included in a K-carrageenan film and investigated the effect of pH and temperature on this diffusivity. Silver-substituted zeolite has been developed as the most common antimicrobial agent associated with plastics in Japan. Zeolite, in which some of its surface atoms are switched by silver, is laminated as a thin layer (3-6  $\mu\text{m}$ ) on the surface of the food contact polymer and is visible. Silver ions, which restricted a broad range of metabolic enzymes, have powerful antimicrobial activity with a broad spectrum (Quintavalla and Vicini, 2002). Bacteriocins can be coated or adsorbed onto polymer surfaces. Samples contain nisin/methylcellulose layers for polyethylene films and nisin layers for poultry based on an adsorption of nisin on acrylics, ethylene vinyl acetate, polyamide, polyethylene, polypropylene, polyvinyl chloride and polyester (Chen and Williams, 2005). Ming et al. (1997) performed bacteriocins to the inner surface of plastic vacuum packaging bags. Materials coated with nisin and pediocin using chilled conditions under ham, Turkey breast meat and they inhibit the growth of *L. monocytogenes* on beef under refrigerated conditions. Skandamis and Nychas (2002) indicated the efficiency of volatile composition of essential oils with the use of MAP conditions. These authors, placed in a package that does not come into contact with paper and petals impregnated with pure essential oil, showed longer shelf life of meat samples enriched with volatile oregano essential oil (*Origanum vulgare*) compounds. Volatile compositions of this oil affect the growth and metabolic activity of meat microorganisms in meat stored in the modified

atmosphere. Whereas, this inhibition is not stronger since it directly participates in the direct meat surface between pure meat and microorganisms.

Ouattara et al. (2000) conducted a study to evaluate the inhibition of surface degradation bacteria in processed meats following administration of chitosan-treated antimicrobial films. Antimicrobial films were prepared by incorporating acetic or propionic acid into a chitosan matrix with or without lauric acid or cinnamaldehyde added and bologna was applied onto normal cooked ham or pastrami. Packages were opened during storage and the amount of antimicrobial agents left in the chitosan matrix was measured. Propionic acid released faster than matrix acetic acid. The strongest inhibition was observed as a greater consequence of antimicrobial activity under these conditions with films containing arsenic (bologna) and cinnamaldehyde on which acid release was slower (Table 2.1).

Vermeiren et al. (2002) indicated that 1.0% triclosan film has a potent antimicrobial effect in vacuum-packed conditions simulated in vitro against food pathogen *Listeria monocytogenes*. However, the triclosan film did not effectively decrease growth of degradation bacteria (*Enterobacteriaceae*, *Pseudomonas* spp. and aerobic mesophilic bacteria) and *L. monocytogenes* on packaged chicken breast packed with refrigerated vacuum stored at 7°C (Table 2.1).

Ha et al. (2001) investigated the effect of grapefruit seed extract (GFSE) on a natural antimicrobial agent (0.5% or 1% concentration) by coextrusion or solution coating on natural cationic polyethylene (PE) films. The microbial status of fresh chopped beef quality [color (L, a, b), TBARS and pH]. The antimicrobial activity of the prepared multilayer films was evaluated by an agar diffusion method. It has been noted that coating of GFSE with the aid of a polyamide binder of the PE film produces more antimicrobial activity than coextrusion with GFSE. By use of the agar diffusion test, the film co-extruded with 1% w/w GFSE showed antimicrobial activity against *Metaphycus flavus*, which exhibits activity against various microorganisms such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, a film coated with only 1% GFSE. GFSE-added multilayer PE film decreased the growth of aerobic and coliform bacteria in film-wrapped beef and stored at 30°C for up to 18 days compared to controls (Table 2.1). Additionally, Ha

et al. (2001) found that the film coated with GFSE at high concentration (1%) had a more pronounced effect on inhibition of bacterial growth than the other films tried. They suggested that GFSE coated films were better than extruded films together to maintain the chemical quality (TBARS) of the packaged beef. The beef color was not affected by the packaging process. These authors reported that GFSE level used (0.5% and 1%) did not significantly effect in film effectiveness for protection of cattle quality (Ha et al., 2001).

Scannell et al. (2000) studied the immobilization of bacteriocin residues and lactis to packaging components of 3147. The plastic film [PE/polyamide (70:30)] composed a stable bond with nisin, in contrast to lacticin 3147, and continued activity for a 3 month period both at room temperature and under refrigerated storage conditions. Antimicrobial packaging decreased the population of lactic acid bacteria (60% N<sub>2</sub> : 40% CO<sub>2</sub>) stored in refrigerated condition, thus extending the product shelf life. Bioactive additions adsorbed with nisin decreased the levels of *L. innocua* and *S. aureus* in hams (Table 2.1).

Natrajan and Sheldon (2000) conducted a study to evaluate the potential use of packaging materials that has nisin-containing formulations to the surfaces of fresh poultry products. The efficacy of native coated (100 µg / ml) polymeric films with varying hydrophobicity [polyvinyl chloride (PVC), linear low density polyethylene (LLDPE) and nylon] has been evaluated in the inhibition of *Salmonella typhimurium* on the skin of fresh broiler drumstick skin. It has been concluded that packaging films coated with nisin are effective in reducing *S. typhimurium* on the skin surface of fresh broilers (Table 2.1).

Antimicrobial packs have relatively small trading successes, except that in Japan Ag-substituted zeolite is the most extensive antimicrobial agent added into plastics. Ag-ions inhibit a number of metabolic enzymes and possess strong antimicrobial activity (Vermeiren et al., 1999). An example of an antimicrobial film is a silver-substituted zeolite film that slowly releases silver ions from the plastic film. Because silver-zeolite films are expensive, they are usually produced as thin layers laminated on the inner surface of a food contact film. These silver-zeolite films are effective against a wide range of bacteria, yeast and molds (Ishitani, 1995).

Boluda-Aguilar et al. (2010) recorded two types of bioactive substances: with action by contact (Alphasan, Articoat, chitosan, DMC, HS, Mirenat-N, Mirenat-NS, silver ions) and others with action in phase gas (essential oils of carvacrol, thyme, rosemary, oregano and ethanol). Moreover, different environments have been studied for the antimicrobial agent to be incorporated into active packaging such as solid absorbent envelopes (zeolite), paper and biopolymer film (polylactic acid, PLA). The more effective antimicrobial agents are zeolite with mirene, thyme, carvacrol and Ag-ION.

Triclosan is the antimicrobial agent used by Microban (UK) (2, 4, 40-Trichloro-20-hydroxydiphenylether). This chemical compound, which is more than 20 years old, is used effectively in personal hygiene products such as toothpaste, mouthwash, deodorant and soap, as well as in an antibacterial agent in the hospital environment. This protection is achieved by combining triclosan with any of the major polymers (e.g., PE, PP and PVC). Triclosan fills the empty areas of the polymer and migrates to the surface to start working against any developing bacteria. During the washing, the molecules closest to the surface are cleaned, but protective for other molecules (Cutter, 1999). Vermeiren et al. (2002) indicated that a 1% triclosan film on *L. monocytogenes* may have a strong antimicrobial effect in vitro experiment but did not effectively reduce film degradation bacteria and reported growth of *L. monocytogenes* in refrigerated packaged chicken breasts stored at 7°C.

Siragusa et al. (1999), added nisin in a liquid form, into a polyethylene-based plastic film, used for vacuum packaging of beef carcasses (Table 2.1). When incorporated into the plastic formulation, they observed that activity of nisin is retained and the conditions used to produce film do not remove antimicrobial activity. Moreover, after 20 days of storage, the microbial load on the meat was lower than the samples treated with the native solution. It maintains activity against *Lactobacillus helveticus* and *Brochothrix thermosphacta* inoculated with carcass surface, as mention. The first reduction of the 2 log<sub>10</sub> cycle of *B. thermosphacta* is observed with impregnated packaged beef in the first 2 days of storage at 4 °C. *B. thermosphacta* populations from impregnated plastic wrapped samples are significantly lower, after 20 days of refrigerated storage at 4°C (Siragusa et al., 1999).

Marcos et al. (2007) examined the capacity of enterococci produced by *Enterococcus faecium* to control *L. monocytogenes* growth in cooked ham (Table 2.1). In fact, cooked ham is heat treated to destroy pathogens, but is then exposed to the environment during stripping, slicing and repacking operations and causes post-processing contamination. Bacteriocin is included in alginate, zein or polyvinyl alcohol based bioprosthesis products. These authors indicate that influential treatment at 6°C storage is the vacuum packaging with alginate films having 2000 AU cm<sup>2</sup> of enterocins. Reduction of cooked ham pathogen strains can still be improved by combining both technologies. The authors noted that such antimicrobial packaging should undergo a high pressure treatment to reduce the vaccinated levels of *Listeria* strains during 60 days of storage at 6 °C (Marcos et al., 2007).

Millette et al. (2007) examined the trapping of alginate matrices to protect bacteriocin against harmful agents in fresh cattle and they interpreted the potential of this system to control *S. aureus* on round beef steak in storage at 4°C. The authors monitor that nisin entrapped in palmitoylated alginate films authorize the discharge and the preservation of nisin activity during storage. The results show that the 1000 IU/ml nisin concentration of the filament reduced 1.86 log CFU cm<sup>2</sup> the level of *S. aureus* on the surface of the beef after 7 days of storage.

Spices are rich in phenolic compounds, such as flavonoids and phenolic acids, which display an extensive of biological impacts, including antimicrobial and antioxidant features (Suppalku et al. 2003; Matan et al., 2006). Direct combination of essential oils into food such as meat yield decrease of the bacterial population but may vary the sensory features of incorporated food. This incorporation of essential oils to edible films may be particularly interesting and Seydim and Sarikus (2006) showed that oregano and garlic oil have, for example, an effective in whey protein-based films against *S. aureus*, *S. enteritidis*, *L. monocytogenes*. Additionally, Tornuk et al. (2018) a new technic was analysed for grafting of nanoclays (montmorillonite (MMT) and halloysite (HNT)) with essential oil constituents (thymol (THY), eugenol (EUG) and carvacrol (CRV)) using Tween 80 as surfactant and then the nanoclay substances were incorporated into LLDPE pellets (5 wt%) to produce active nanocomposite films using a twin screw extruder. They found that the films had strong in vitro antibacterial activity against pathogenic bacteria such as *Salmonella*

*typhimurium*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* whereas their effect against lactic acid bacteria (*Lactobacillus rhamnosus* and *Lb. casei*) was restricted (Table 2.1).

Basil (*Ocimum basilicum*) is a popular culinary plant and has been widely used for many years in the aroma of essential oils, confectionery and baked goods, seasonings, sausages and meats (Suppalku et al., 2003). Suppalku et al., (2003) indicate that basil essential oil has potential use in food preservation, especially with food processing antimicrobial packs. Further research on the antimicrobial activity of the major components is needed to assess the usefulness of packaged foods such as meat and poultry meat in extending shelf life. Ouattara et al. (2002) and Lacroix et al. (2004) evaluated the combined effect of applying gamma irradiation and cross-linked film coatings containing spice powders to reduce microbial growth associated with shredded cattle contamination. Casein and whey are combined with cross-linked films based on protein isolate, thyme, rosemary and sage spice. In addition, the specificity of enzymes should be noted carefully, because of the antimicrobial activity is very delicate to its environment and substrates. Han (2005) gives an example of lysozyme activity, which can be significantly influenced by temperature and pH. In addition, these biocides, direct meat products have been included (eg, ham or sausages), but can be incorporated into a packaging material (Han, 2005).

Chitosan is composed mainly of 1, 4-linked 2-amino-2-deoxy- $\beta$ -D-glucoside and inhibits some Gram-negative and especially Gram-positive bacteria, although it is more active against degradation. Wang et al. (2007) acquired a rise in chitosan-based film antibacterial activity against *S. aureus* or *E. coli* development by combining aminopolysaccharide with the rectorite nanoparticles used for meat preservation. The chitosan around the layered silicate demonstrates that it may be more likely to block the growth of the bacteria because of the increase in the positive charge density in each unit volume with the chitosan chains accumulated between the interstitial layers of the rectorite.

According to another study, fresh oysters and chopped cattle are wrapped in antimicrobial films coated with a bacteriocin (nisin or lactis NK24) added into a polyamide binding layer. Packaged foods are stored at 3°C and 10°C and coliform

bacteria are displayed with changes in total aerobic bacterial counts and appropriate qualities to identify the effect of preservation of bacteriocin-coated films on shelf life and prolongation of shelf life. Compared to flat low density polyethylene film, plastic films with bacteriocin obtain slowed microbial growth on packaged oysters and chopped beef at both temperatures and contribute to preservation of chemical quality to some extent and prolongation of shelf life. While the effect of antimicrobial films on suppression of coliform bacteria growth is more pronounced than 3°C at 10°C, the effects on total aerobic bacteria are constant at both temperatures. There is no difference in the quality of food between the two types of antimicrobial films (Kim et al., 2002).

Polyethylene (PE) and polyethylene oxide (70% PE and 30% PEO; PE + PEO) blend; PE and nisin (PE + nisin); PE, nisin, and EDTA (PE + nisin + EDTA) blend; and PE, PEO with nisin (PE + PEO + nisin) are used by Cutter et al. (2001). Of the polymers surveyed, PE and PE + PEO do not display any antimicrobial activity to *Brochothrix thermosphacta*. Beef surfaces are experimentally inoculated with 3.50 log<sub>10</sub> cfu/cm<sup>2</sup> of *B. thermosphacta*, vacuum packaged with each of the five polymers and held at 4°C for 21 days. After 3 days at 4°C, *B. thermosphacta* is reduced > 1.70 log<sub>10</sub> by PE + nisin and > 3.50 log<sub>10</sub> with PE + nisin + EDTA or PE + PEO + nisin. By 21 days at 4°C, *B. thermosphacta* is decreased to 0.30 log<sub>10</sub> cfu/cm<sup>2</sup> when used with PE + PEO + nisin. It appears that PE + PEO + nisin or PE + nisin + EDTA were more effective for decreasing *B. thermosphacta*, as compared to polymers formed of PE + nisin (Cutter et al., 2001).

Poultry meat samples were inoculated *Listeria monocytogenes* (Ming, et al., 1997) and they reported that the bags incorporated with pediocin powder completely inhibit the growth of the inoculated *L. monocytogenes* at 4 ° C at 12 ° C. Application of bactericides for food packaging films is an effective approximation to decrease *L. monocytogenes* contamination in meat and poultry (Ming, et al., 1997).

Pediocin is antimicrobial peptide produces by *Pediococcus sp* and investigations reveal their ability to inhibit the growth of certain pathogenic bacteria (Appendini and Hotchkiss, 2002). Santiago-Silva et al. (2009) developed and evaluated the antimicrobial efficacy of the films produced with pediocin for sliced ham protection. Antimicrobial films are combined with pediocin (25% and 50%) in a cellulose-based

emulsion in this study. The antimicrobial effect of the films to *Listeria innocua* or *Salmonella sp.* on sliced ham is tested by a coercion test in which the slices were immersed in a 0.1% peptone solution containing approximately  $10^6$  CFU/mL of *L. innocua* or *Salmonella sp.* The survey is set to overlap between ham slices and films (control, 25% and 50% of pediocinum). The systems are packaged in vacuum and stored at 12 °C. Ham slices are analyzed for *L. innocua* and *Salmonella sp.* counts at 0, 3, 6, 9, 12 and 15 storage days. Antimicrobial films prevent *L. innocua* growth more effectively. They found that 50% pediocin film shows a 2-day cycle reduction compared to control treatment after 15 days of storage and 25% and 50% pediocin films have similar performance on *Salmonella sp.* inhibition; both provided 0.5 log cycle decrease after 12 days of storage, in relation to the control. Therefore, the films added with pediocin display potential use as one hurdle technology incorporated in the storage period between others good manufacturing practices for preservation of sliced ham (Santiago-Silva et al. 2009) (Table 2.1).

In another study, bacteriocins had been applied to cooked and dry cured ham spiked with enterocin A and B and cucumber, *L. monocytogenes*, *Salmonella enterica* and *S. aureus* were subjected to a high pressure of 600 MPa (Appendini and Hotchkiss, 2002). Prior to pressurization, some significant reductions in *L. monocytogenes* and *S. aureus* counts have been observed in dry cured ham. After the pressure, *Salmonella* and *L. monocytogenes* were not detected in the 25 gr cooked and dry cured ham and remained at that level throughout the storage (57 days at 4 ° C + 63 days at 15 ° C). However, the levels of *S. aureus* have fallen below the limit of detection (1 log cfu / g) in some parts of the population. Later, when storage conditions were made at a malicious temperature, the ability of *S. aureus* to grow was due to the bacteriocin and meat product used. Therefore, after the storage is finished, all dried curing ham pieces can inhibit only growth in cooked ham while *S. aureus* counts <1 log cfu / g (Anna et al.2008) (Table 2.1).

Gregory and James (1993) studied that a method of administering organic acids in a calcium alginate gel was surveyed to prevent bacteria contaminating sterile lean beef tissue surfaces. The treated specimens were incubated at 5°C under controlled humidity conditions up to 7 days and living populations of pathogens were identified. For the count of *L. monocytogenes*, alginate/lactic acid treatment decreased the log<sub>10</sub>

numbers to 1.8 units/0.96 for alginate-free acid treatment when collected on tryptic soy agar. Log<sub>10</sub> reductions with acetic acid were 1.51 and 2.33 for acetic acid treatment with alginate/acetic acid only. *S. typhimurium* was decreased to 2.11 log<sub>10</sub> units and 1.11 log<sub>10</sub> for alginate/lactic acid and acid treatments, respectively. Both Gram positive and negative organisms were less obstructed by acetic acid procedure (Gregory and James, 1993) (Table 2.1).

Joerger et al. (2009) indicate that the ethylene copolymer film is coated with chitosan by affixing the polymer to the film corona treated surface and analyzed for compound film antimicrobial activity. The film was activated to bacteria in 0.625 mM phosphate buffer; counts of *Escherichia coli* 25922 and *Listeria monocytogenes* Scott A reduced by 5 and 2-3 log<sub>10</sub>, respectively, after 24 hours exposure. The activity against bacteria in the chitosan-coated film buffer rised when the silver ions were added to the films as shown in the buffer in *Escherichia coli* O157:H7 DD3795 completely killed and the chicken isolate *Stenotrophomonas maltophilia* in the buffer within 2 hours. The film is efective in 0.5% buffered peptone water to a pH of about 7.0 % against *L. monocytogenes* Scott A. Tests on beef and poultry exams revealed antimicrobial activity against *Escherichia coli* O157: H7 and *L. monocytogenes* Scott A against colony forming units of approximately 2 and 1-2 log<sub>10</sub> reductions, respectively. Antimicrobial activity against *L. monocytogenes* Scott A film also surveyed on Turkey breast and about 1.7 log<sub>10</sub> units after 10 days and after 15 days at 4 °C 1.2 log<sub>10</sub> reduction was achieved. Exposure to chitosan-coated film and 350 MPa pressure, 55 ° C or 1% sodium diacetate, provides a synergistic effect (Joerger et al., 2009).

In another study, polyethylene antimicrobial active films having triclosan (0, 2000 and 4000 mg/kg) at several levels were applied for extruded food (Camilloto et al., 2009). The activities of the films were interpreted against the development of *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa* using agar diffusion test. The mechanical properties of the films were also assessed using the Universal Test Machine (Instron). Incorporation of triclosan did not affect the mechanical features of antimicrobial films compared to the control film. The average film thickness was 82.0 µm and the tensile strength and fracture elongation were 30.3 N and 46.2%, respectively. Films

having triclosan demonstrated an antimicrobial effect against *E. coli* and *S. aureus* in vitro and both were inhibited. However, the result was not watched for *L. innocua*, *S. choleraesuis*, and *P. aeruginosa*, but a drop in colony density around the film was observed for both incubation temperatures ( $7 \pm 2^\circ\text{C}$  and  $35 \pm 2^\circ\text{C}$ ). Sliced ham packed with antimicrobial films indicated a decrease of 1.5 log cycles compared to ham in contact with a control film after 12 days storage at  $7 \pm 2^\circ\text{C}$  for *E. coli* and *S. aureus*. Antimicrobial films carry application potential as active packaging materials because they have been shown to be effective against some pathogenic microorganisms that can be transmitted by food (Camilloto et al., 2009) (Table 2.1).

Nisin incorporating antimicrobial packaging system has been used to prevent the spread out of the aerobic plate count (APC) and lactic acid bacteria (LAB) of beef burger stored at refrigerator ( $4^\circ\text{C}$ ) and increase its shelf life and quality (Ferrocinoa et al., 2016). Nowadays, packaging with nanoscale silver coating has been performed to fresh pork sirloin and the growth microorganisms in meat) is retarded (Kuulialaa et al., 2015) (Table 2.1).

Biji et al. (2015) studied that application of nisin and pediocin on cellulose casing for preventing the growth of *Listeria monocytogenes* on beef, ham and turkey meat. It was also detected that lysozyme and nisin in corn zein and soy-protein films importantly prevented *Escherichia coli* and *Lactobacillus plantarum* on laboratory condition. Additionally, lactic acid bacteria (LAB) isolated from fish and other aquatic animals are used to prevent *Listeria monocytogenes* and different foodborne spoilage bacteria in fish and other muscle based products (Boulares et al., 2011). Another example is adding carvacrol, thymol and eugenol with nanoclays into linear low-density polyethylene (LLDPE) to increase the shelf life of packaged Turkish type fermented sucuk (Tornuk et al., 2015).

Jonaidi Jafari et al. (2017) examined influences of chitosan coating by ethanolic extract of propolis on the quality of refrigerated chicken fillet. Microbial analysis found that coating had an important decreasing influence on growth of microorganisms during storage (12 days at  $4^\circ\text{C}$ ). Additionally, the increase of TBARS, total volatile nitrogen and peroxide value of samples coated by chitosan and ethanolic extract of propolis was lesser than control group. Chitosan and propolis can be used to increase the shelf life of chicken fillet and protect food quality.

Adzaly et al. (2016) improved the trading applicability of a novel casing formed from chitosan including cinnamaldehyde (2.2%, w/v), glycerol (50%, w/w) and Tween 80 (0.2% w/w) under fermented sausage processing status. Before and after cooking, both casings are checked for mechanical, barrier, and other features. The chitosan casing had a better ( $P \leq 0.05$ ) barrier to water, oxygen, liquid smoke, and UV light than collagen casing. Additionally, the chitosan casing had higher ( $P \leq 0.05$ ) tensile strength, lower ( $P \leq 0.05$ ) elongation at break and tensile energy to break, and better ( $P \leq 0.05$ ) transparency whereas a similar water solubility to the collagen casing.

Marcos et al. (2013) reviewed *Listeria monocytogenes* contaminated on the surface of sliced fermented sausages with no added sodium salt. The pathogen was gradually inactivated during the storage (90 days). Antimicrobial packaging of fermented sausages with polyvinyl alcohol (PVOH) films including nisin induced a more evident degradation of *L. monocytogenes* counts during refrigerated storage. Combination of antimicrobial packaging with High Pressure Processing (HPP) did not perform *L. monocytogenes* growth compared to only antimicrobial packaging. The lack of impact of HPP on *L. monocytogenes* was connected to a protective influence applied by the low water activity of the product and its lactate amount. These results express that antimicrobial packaging with incorporation of nisin could be used as an effective method to decrease the values of *L. monocytogenes* in sliced fermented sausages with no added sodium salt.

### **2.3 Research Objectives and Tasks**

As a result, there is a little information exist about the antioxidant and antimicrobial packing of foods, although there is no information about antioxidant and antimicrobial packaging of the sucuk in the literature.

Meat products especially sucuk is sensitive to both microbial spoilage and oxidative deterioration. Their preservation properties may have been enhanced by the use of packaging that has antimicrobial and antioxidant properties, which may be provided by the incorporation of both antimicrobial and antioxidant additives in the polymer matrix. Therefore, in this study, antimicrobial cases (multilayered active film) incorporated nisin, chitosan, potassium sorbate or silver ions (AgZeo) were

employed. The purpose of this study was to evaluate the effectiveness on the microbial and physicochemical characteristics of sucuks stuffed with multilayer film incorporation of antimicrobial compounds (chitosan, nisin, potassium sorbate and AgZeo) during fermentation and storage after the application of different heat treatment temperatures (65, 70 and 75°C) conditions. Also, two different control groups composed of multilayer plastic case without antimicrobial compounds and collagen casing only were prepared for the comparison of the samples evaluated.



**Table 2.1** Examples of application of antimicrobial films incorporated with antimicrobial agents for preserving fresh meat and processed meat products

Antimicrobial substances	Products	Carrier film	Effects	Reference
Nisin	Beef	PE	Reduction of <i>B. Thermosphacta</i>	Siragusa et al. (1999)
Nisin	Fresh poultry (broiler skin)	PVC, LLDPE and Nylon	Inhibition of <i>S. Typhimurium</i>	Natrajan, Sheldon (1995)
Nisin, lacticin	Beef	LDPE, PA	Inhibition of total aerobes and coliform bacteria	Kim et al. (2002)
Nisin, EDTA	Beef	PE	Inhibition of <i>B. Thermosphact</i>	Cutter et al. (2001)
Nisin, EDTA, citrate	Chicken	Acrylics, PVA/PE	Inhibition of many gram-pos. bacteria	Natrajan, Sheldon (2000)
Nisin, lauric acid	turkey bologna	Soy protein	Inhibition of <i>L. Monocytogenes</i>	Dawson et al. (2002)
Nisin, lacticin	Ham stored in MAP(60% N <sub>2</sub> :40% CO <sub>2</sub> )	PE/PA(70:30)	Reduction of <i>L. Innocua</i>	Scannel et al. (2000)
Nisin, sakacin, Potassium lactate	Cooked ham	PET/PE	Inhibition of Salmonella	Anna et al. (2008)
Nisin and pediocin	Beef, Ham and turkey meat	Nisin and Pediocin on cellulose casing	Inhibition of <i>Listeria monocytogenes</i>	Biji et al., (2015)
Nisin	Beef burger	Nanoscale silver coating	Inhibition of aerobic plate count (APC) and lactic acid bacteria (LAB)	Ferrocinoa et al. (2016)
Pediocin	Poultry and meat products	Casings, plastic bag	Inhibition of <i>L. Monocytogenes</i>	Ming et al. (1997)
Pediocin	Sliced ham	Cellulose	Reduction of Salmonella and <i>L. Innocua</i>	Santiago-Silva et al. (2009)

**Table 2.1 (Cont.)** Examples of application of antimicrobial films incorporated with antimicrobial agents for preserving fresh meat and processed meat products

Antimicrobial substances	Products	Carrier film	Effects	Reference
Trichlosan	Sliced ham	PE	1.5 log reduction of <i>E. coli</i> and <i>S. aureus</i>	Camilloto et al. (2009)
Trichlosan	Refrigerated packaged chicken breast	Film	Inhibition of <i>L. monocytogenes</i>	Vermeiren et al. (2002)
Trichlosan	Food-borne pathogenic bacteria	Plastic matrix	Inhibition of <i>S. aureus</i> , <i>Shigella</i> , and <i>S. Typhimurium</i>	Cutter (1999)
Amino polysaccharide	Meat products	Chitosan based film	Inhibition of <i>S. aureus</i> and <i>E. coli</i>	Wang et al. (2007)
Chitosan	Turkey breast	Ethylene copolymer film	Inhibition of <i>L. monocytogenes</i> and <i>E. coli</i> O157:H7	Joerger et al. (2009)
Chitosan	Chicken fillet	Chitosan coating incorporated with ethanolic extract of propolis	Increase the shelf life	Jonaidi Jafari et al. (2017)
Organic acid/cinnamaldehyde	Processed meat	Chitosan	Inhibition of Enterobacteriaceae and <i>Serratia liquefaciens</i>	Ouattara et al. (2000)
Organic acid	Beef carcass	Alginate	Reduction of <i>L. monocytogenes</i> , <i>S. typhimurium</i> and <i>E. coli</i> O157:H7	Gregory and James (1993)
Grape fruit seed extract	Fresh minced meat	Multilayered PE films	Inhibition of spoilage bacteria	Ha et al. (2001)
Tocopherol	Beef	LDPE	Inhibition of <i>L. monocytogenes</i>	Moore et al. (2003)
Carvacrol, Thymol and Eugenol	Turkish type fermented sucuk	Nanoclays into linear low-density polyethylene (LLDPE)	Increase the shelf life	Tornuk et al. (2015)

## CHAPTER 3

### MATERIAL AND METHODS

#### 3.1 Materials

Nisin and chitosan were provided from Handary SA (Belgium) whereas potassium sorbate and silver zeolite (AgZeo) were taken from Aldrich (Chemical Company, Inc., USA), respectively.

Fresh boneless lamb meat (shoulder) and beef meat (whole brisket), commercial virgin olive oil, artificial collagen case, spices, salt, tail fat and sugar were obtained from Coskoy Company in Osmaniye. A mixture of *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Staphylococcus carnosus* and *Staphylococcus xylosus* was provided from Biocarna (Wiesby, Germany). These mixtures used as starter cultures.

#### 3.2 Film Preparation

All the multilayer films were produced by NAKSAN Plastic Company in Gaziantep. The active low density polyethylene (LDPE) master batch was produced by mixing antimicrobial substances (potassium sorbate, chitosan, silver substituted zeolite or nisin) and polyethylene pellets. The mixture of 2% antimicrobial agent, 2% ethylene vinyl acetate copolymer (EVA), 2% orevac as tie layer and 94% polyethylene pellets were prepared by utilization of twin screw extruder (Randal, UK) for master batches. The control (LDPE/polyamide/LDPE) and multilayer antimicrobial films (LDPE-Antimicrobial/PA/LDPE) having the uniform thicknesses (70 mm) were fabricated by utilization a blown film extrusion process. After that, films were fastened to get ready bags with 15 cm width and 25 cm length in hygienic situation (Soysal et. al., 2015). Finally, the bags were sealed in size of artificial collagen casing for stuffing of sucuk dough.

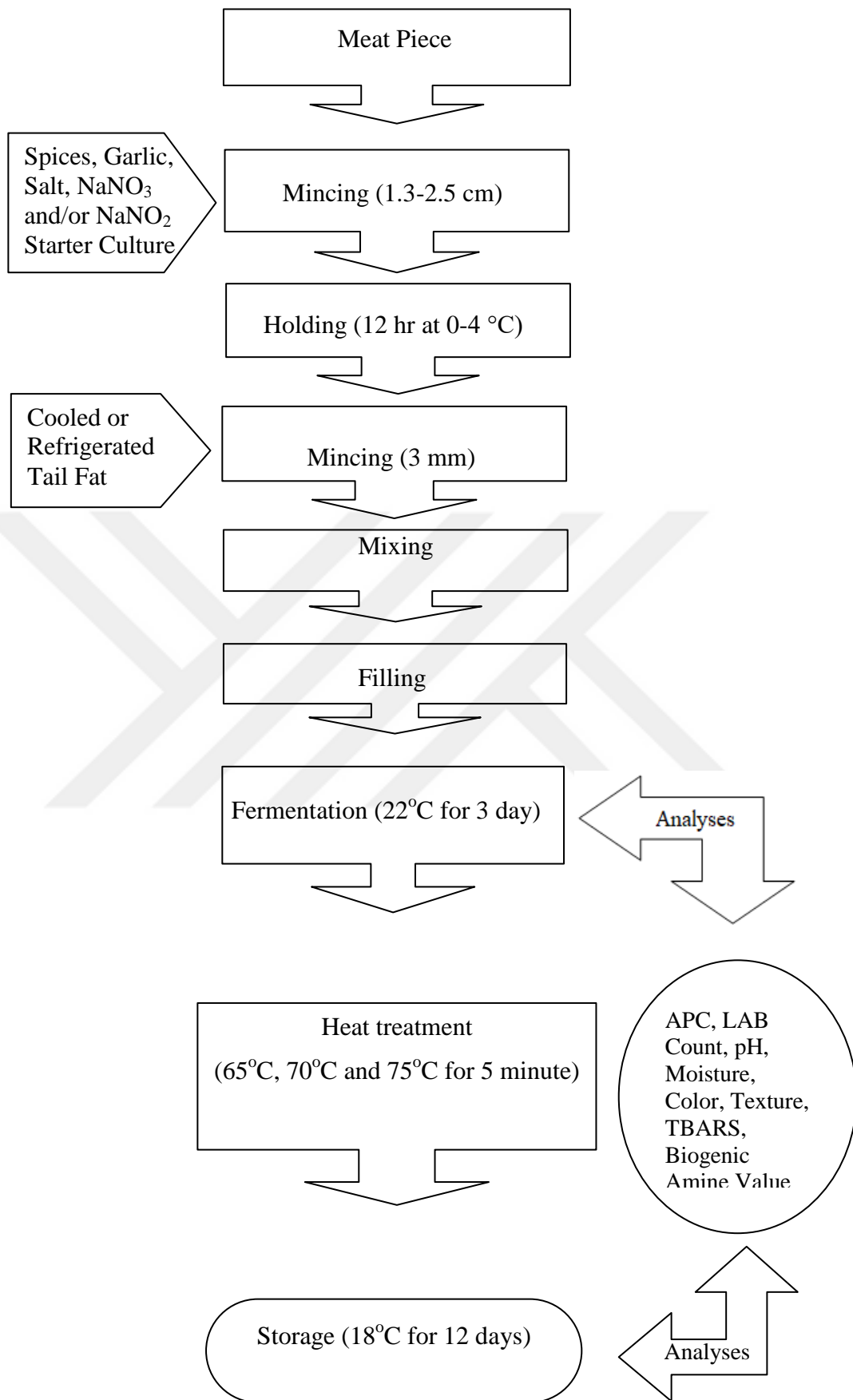
### 3.3 Sucuk Preparation

Sucuks were produced in Coskoy Food Company (Osmaniye). The mixtures of beef and lamb with tail fat, olive oil, starter culture, spices, salt, clean dry garlic and sugar were used for sucuk dough from according to the following proportions; 450 g beef (about 6% fat), 450 g lamb (about 8% fat), 200 g tail fat, 2.1 g olive oil, 11.42 g all spice, 11 g red pepper, 5.5 g black pepper, 18 g salt, 20.76 g garlic, 4.4 g sugar, 5.5 g cumin, 1.1 g cinnamon, 0.48 g clove. 50 mg/kg nitrite and 100 mg/kg nitrate were used as curing agents (Bozkurt and Erkmen, 2004). The meat was chopped in the grinder (OEM, 750 W and China). The other ingredients except the tail fat were added and mixed with chopped meat in a cutter for 15 min at 4°C and at the same time starter culture was added as a 20 g commercial culture mixture (*Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Staphylococcus carnosus* and *Staphylococcus xylosus*) per 100 kg of meat. Sucuk dough was rested for 12 h at 0-4°C and then the chopped-refrigerated tail fat was put into the sucuk dough in a cutter. Then, artificial collagen casings (35 Q, Yildiz Food Company, Turkey) of 38 mm diameter and multilayer antimicrobial cases of 38 mm diameter were filled with sucuk dough under hygienic conditions by the filling machine (Xiaojin, GZY3600, China) at 4°C (Bozkurt, 2006). Artificial collagen cases and multilayer case without antimicrobial compounds were control groups.

Sucuks were prepared at 2 different times (as two replicates) using different cases. Therefore, each of about 100 g in weight, forty four sucuks were prepared and fermented for 3 days at 22 °C. After that, they were heated until the center temperature reach to 65°C, 70 °C or 75 °C and hold at those temperatures for 5 minutes in an oven (Charan, KBO 3000, Turkey). Then, obtained sucuks were stored at 18 °C for 12 days (Figure 3.1).

### 3.4 Sampling and Sample Preparation

The samples of sucuks were taken from each batch on the 0<sup>th</sup> and 3<sup>th</sup> days of fermentation for analysis. After fermentation, the samples were taken from heat treated sucuks starting from 0<sup>th</sup> days of heat treatment with the interval of 3 days.



**Figure 3. 1** A flow chart of fermented and heat processed Turkish sucuk producing

Two different control groups composed of multilayer plastic case without antimicrobial compounds and collagen casing only were prepared for the comparison of the samples evaluated. The lactic acid bacteria count (LAB), aerobic plate counts (APC), pH values, moisture values, 2-thiobarbituric acid reactive substances (TBARS) values, biogenic amine contents, color and texture parameters (cutting scores, flavor and color) were determined and all analyses were fulfilled in duplicate. A 25 g of sucuk sample homogenized in a stomacher bags containing 225 ml 0.1% peptone water for microbiological analysis (Bozkurt and Erkmen, 2002). Then, in order to perform color and texture analysis, sucuk samples about 2 cm length were cut. The remaining about 60 g sucuk samples were cut into small pieces and homogenized. For TBARS and biogenic amines, the residue of sucuk samples was stored till the analysis at refrigerator (-20°C).

### **3.5 Microbiological Analysis**

#### **3.5.1 Aerobic Plate Count**

APC was performed as the Spread Plate Method on aerobic Plate Count Agar (Merck, Darmstadt, Germany). After making dilution series from sample, 0.1 ml pipetted out from the appropriate desired dilution series onto the center of the surface of on plate count agar plate. The sample was spread evenly over the surface of agar using the sterile glass spreader, carefully rotating the petridish underneath at the same time. After that, the plates were incubated at 37°C for 48 hours (Erkmen, 2000).

#### **3.5.2 Lactic Acid Bacteria**

The counts of LAB were fulfilled as the Spread Plate Method on MRS sharp agar (Merck, Darmstadt, Germany). After making dilution series from sample, 0.1 ml pipetted out from the appropriate desired dilution series onto the center of the surface of an MRS sharp agar plate. The sample was spreaded evenly over the surface of agar using the sterile glass spreader, carefully rotating the petridish underneath at the same time. After that, the plates were incubated at 30°C-35 °C for 48 hours.

Then, morphological characteristic and typical colony types associated with each growing medium were examined visually for all plates and these data are described as a logarithm of colony forming units per gram (log cfu/g).

### **3.6 Chemical analysis**

#### **3.6.1 Determination of pH**

The value of pH was determined by use of 10 g homogenized sample made up to 100 ml distilled water by using a pH meter at room temperature (20°C) (Thermo Scientific, Orion 2 Star) (AOAC, 1990). The pH meter was calibrated by use of calibration solutions at pH 4.0, 7.0 and 10.0.

#### **3.6.2 Determination of Moisture (%)**

The moisture content of sucuks was analyzed using the oven method at 105 ±2°C until constant weight reached (AOAC, 1990).

#### **3.6.3 Determination of 2-thiobarbituric acid reactive substances (TBARS) value**

TBARS values of the sucuk samples were analyzed by use of a spectrophotometric method (Bozkurt and Erkmen, 2004). After homogenization of sucuk samples, 2 g sucuk sample were received. TBARS was extracted twice with 10 ml of 0.4 M perchloric acid. Extracts were accumulated and completed to 25 ml with 0.4 M perchloric acid and centrifuged for 5 min at 1790xg. After that, 1 ml of extract was dumped into a glass stoppered test-tube. TBA reagent (5 ml) was incorporated and the extract was warmed in a boiling water bath for 35 min. After that refrigeration in fountain water, the absorbance of the sample was commented against the appropriate blank at 538 nm. A calibration curve was arranged using 1, 1, 3, 3-tetraethoxypropane (TEP) (Bozkurt and Erkmen, 2004).

#### **3.6.4 Determination of biogenic amines**

The contents of biogenic amines were examined by a chromatographic method (Bozkurt and Erkmen, 2002). The HPLC system had a quadratic gradient pump (Ultimate, 3000 Pump), anionex Ultimate 3000 Diode Array Dedector, an Ultimate 3000 Column Compartment, and a computer containing a Chromomelon package

program. The HPLC column was Spherisorb ODS2, 10  $\mu\text{m}$ , 200x 4.60 mm, (Phenomenex, Torrance, CA, US).

Ammonium formate solution (0.4 M) was made up by dissolving 12.61 g ammonium formate in 500 ml of ultra pure water and then straining with a 0.45  $\mu\text{m}$  Millipore filter. Acetonitrile was straining with a 0.45  $\mu\text{m}$  Millipore filter. Ammonium formate and acetonitrile were utilized as the LC mobile phases. A gradient elution program was utilized with mobile phases of acetonitrile (solvent A) and 0.4 M ammonium formate (solvent B), starting with 50% solvent A and 50% solvent B and finishing with 90% solvent A and 10% solvent B after 20 min. The flow rate was 1.0 ml/min. 2 g homogenized sucuk sample were received and then extracted with 10 ml of 0.4M perchloric acid. Samples were accumulated and completed to 25 ml with 0.4M perchloric acid and centrifuged for 5 min at 1790xg.

1 ml of the extracted sample done alkalized by addition of 200  $\mu\text{l}$  of 2 N NaOH solution; 300  $\mu\text{l}$  of saturated sodium bicarbonate were mixed as buffer. 2 ml of dansyl chloride solution was joined to each sample and incubated for 75 min at 40°C. Remaining dansyl chloride was suspended by adding 100 ml of 25% ammonia. After waiting 30 min at room temperatures, the sample was completed to 5 ml with acetonitrile, centrifuged for 5 min at 1790xg. The supernatant was filtered (0.45  $\mu\text{m}$ ), and then 20  $\mu\text{l}$  was injected onto the HPLC.

### **3.7 Determination of color**

After sampling for microbial analysis, sucuks were hold at room temperature (20°C) about 30 min for conditioning. After that, cut surface color was measured freshly at room temperature about 20°C in triplicate. The measurement of color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) were carried out by use of Konica Minolta Chroma meter CR 400 (The illuminants: C (Y:93.6 x:0.3132 y:0.3193),  $D_{65}$ , (Y:93.6 x:0.3157 y:0.3321); aperture: r=15 mm).  $L^*$ ,  $a^*$ , and  $b^*$  values express lightness ( $L^*$ ), greenness ( $-a^*$ ) or redness ( $+a^*$ ), and blueness ( $-b^*$ ) or yellowness ( $+b^*$ ), respectively.

### **3.8 Texture profile analysis (TPA)**

Texture profile analysis (TPA) was analysed as expressed by Bozkurt and Bayram (2006) using Brookfield Texture Analyzer (CT3, Load cell: 4500 g) to detect

hardness, adhesiveness, gumminess, chewiness. For TPA, about 38 mm diameter of sucuk samples were cut into cylinders with height about 20 mm and held for equilibration to room temperature. The conditions were: cylinder probe (50.88 mm diameter and 20 mm length); test speed 1 mm/s; pre-test speed 2 mm/s, post-test speed 1 mm/s; compression (strain) 25%; and 25 kg load cell. The data calculation was performed using the Texture Pro CTV1.4 Built 17.

### **3.9 Sensory Analysis**

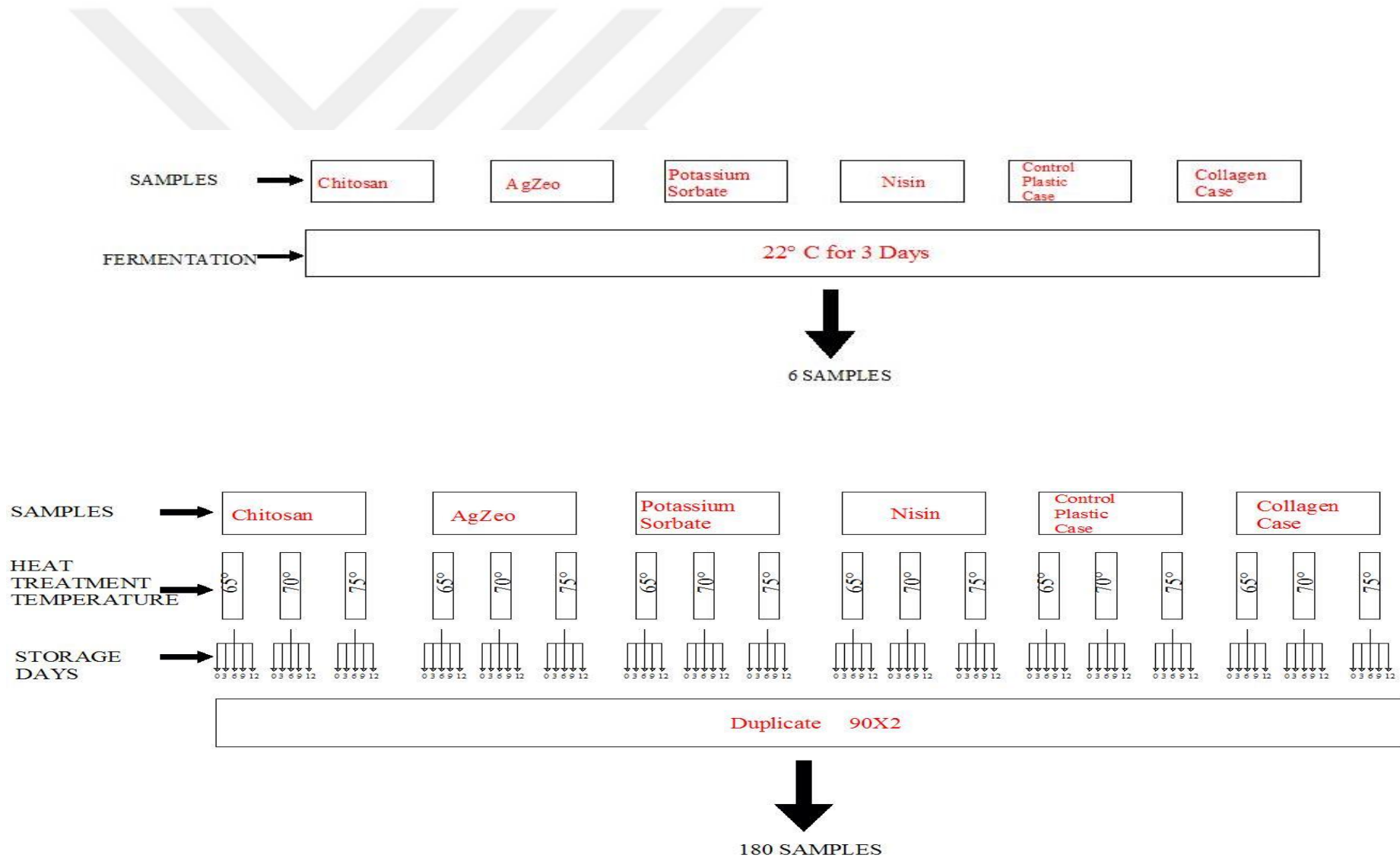
The sensory parameters (ease of cutting, flavor and color) of 25 g of sucuk were investigated as twice at each sampling time, by a panel of 10 untrained panelists. Three sessions were conducted for sensory analysis. Panelist tested 3 samples and gave scores as 1 (worst) to 10 (best) for flavor and color of each sample. Cutting score (scale of 10 (best) to 1 (worst)) was examined by panelists by evaluating whether the sucuk was well cut or stuck to the knife. In addition, the overall sensory properties were studied from this formula (Bozkurt and Bayram, 2006) as;

$$\text{Overall Sensory Quality} = (\text{flavor} \times 0.50) + (\text{color} \times 0.25) + (\text{cutting} \times 0.25) \quad (1)$$

### **3.10 Statistical analysis**

The moisture, pH, color, biogenic amine formation, TBARS value, microbiological counts (aerobic plate count, total lactic acid bacteria) and texture attributes were analyzed with one-way ANOVA to detect important differences at  $P < 0.05$  using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests was also performed on the different antimicrobial casings during fermentation and storage.

Sucuks were fermented at 22 °C for three days. After fermentation, three different heat temperatures (65, 70 and 75 °C) were applied on the sucuks and samples were analyzed on during storage (0., 3., 6., 9. and 12. days). Sucuks were prepared at 2 different times (as two replicates) using different cases (Figure ).



**Figure 3. 2** Experimental design of the study

## CHAPTER 4

### RESULT AND DISCUSSION

#### 4.1 Microbial Changes

##### 4.1.1 Fermentation

Results for the aerobic plate count (APC) and lactic acid bacteria (LAB) counts of sucuk packed with different packaging systems kept 3 days at 22°C for fermentation are given in Table 4.1. Initial APC and LAB counts were 7.10 and 6.86 log cfu/g in sucuk dough, respectively. On the other hand during fermentation, APC and LAB counts increased significantly ( $P < 0.05$ ) because of the high RH (85–95%) and optimum growth temperature (22°C) suitable for most of microorganisms compared to first day of analysis. At the end of 3 days of fermentation, the lowest APC and LAB were found in sucuks stuffed into antimicrobial cases. Microbial growth was the slowest in the nisin and chitosan casings where the total viable count was around 8.00 log cfu/g on day 3 compared to control groups. Results are not similar to Kristo et al. (2008) reported that nisin coated sodium caseinat films inhibited pathogen fully. Nisin incorporated antimicrobial packaging system has been used to prevent the spread out of the aerobic plate count (APC) and lactic acid bacteria (LAB) of beef burger stored at refrigerator (4°C) and increase its shelf life and quality was obtained (Ferrocinoa et al., 2016). According to another study, chitosan film incorporated with potassium sorbate had antimicrobial effect against *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* (Pranoto et al., 2005). The highest APC and LAB counts were seen in control plastic and collagen case samples, respectively. There were significant differences ( $P < 0.05$ ) between all samples during fermentation (Table 4.1).

**Table 4.1** Changes in APC (log cfu/g) and LAB count (log cfu/g) of sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

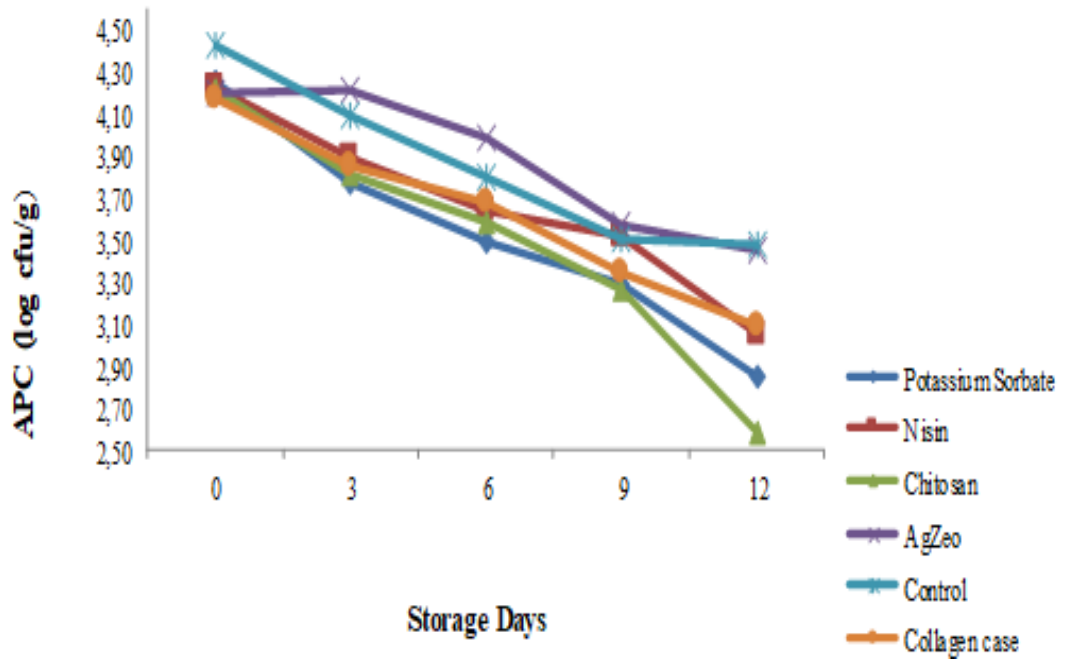
Parameters	Fermentation Time (day)	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
APC (log cfu/g)	0	7.10 ±0.50a,B	7.10 ±0.5a,B	7.10±0.50a,B	7.10±0.50a,B	7.10±0.50a,B	7.10±0.50a,B
	3	8.64±0.05a,b,A	8.9±0.04a,A	8.44±0.59b,A	8.00±0,56c,A	8.01±0.01 c,A	8.2±0.03b,c,A
LAB (log cfu/g)	0	6.86 ±0.08 a,B	6.86±0.08a,B	6.86±0.08a,B	6.86±0.08a,B	6.86±0.08 a,B	6.86±0.08a,B
	3	8.80 ±0.07 a,A	8.53±0.03a,A	7.9±0.55 b,A	7.30±0.51c,A	7.57±0.06b,c,A	7.92±0.03b,A

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha=0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the fermentation time at  $\alpha=0.05$  level in each column.

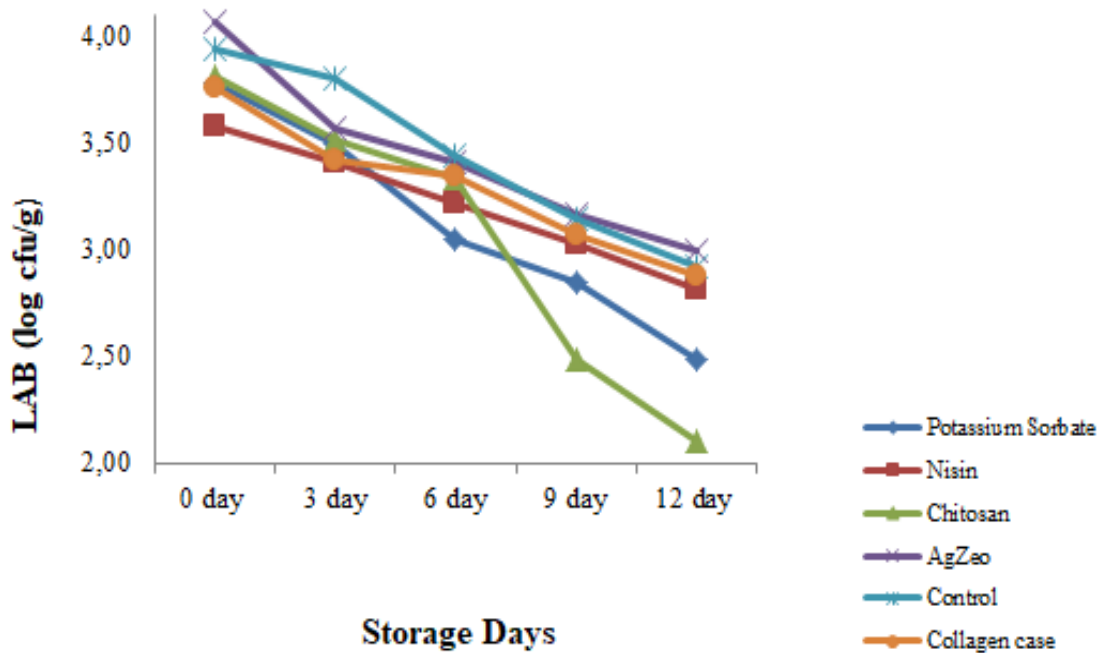
#### 4.1.2 Storage

Changes in APC and LAB (log cfu/g) counts in stuffed into antimicrobial (nisin, potassium sorbate, chitosan and AgZeo), control plastic and collagen cases and heat treated sucuks at 65 °C, 70 °C and 75°C were observed during storage period, and their results are shown in Table 4.2 and 4.3. To determine the effect of antimicrobial cases on APC and LAB counts, further statistical analysis were also employed.

For heat treated sucuks at 65°C, there were no significant differences ( $P > 0.05$ ) on APC among sucuks stuffed into active cases at the beginning of storage. After the heat treatment at 65°C, APC reduced to about 4.18 and 4.43 log cfu/g and LAB counts decreased about 3.58 and 4.07 log cfu/g (Figure 4.1 and Figure 4.2). It was observed in many studies that reductions in APC and LAB level were about 3– 5 log units after the heat treatment (Dalmış and Soyer, 2008; Erçoşkun et al., 2010). The samples stuffed into plastic cases (control) only had the highest LAB and APC at the beginning of storage. At the end of storage period APC varied between 2.59 log cfu/g (in chitosan incorporated case) and 3.48 log cfu/g (in control plastic case) at the end of storage. Similarly, the highest LAB count as 3.00 log cfu/g was observed in sucuk stuffed into AgZeo whereas the lowest LAB count as 2.11 log cfu/g was in sucuk stuffed into chitosan at the end of storage (Table 4.3). Park et al. (2010) reported that chitosan could be migrated from LDPE films to inactivate bacterial population. They also concluded that if the concentration of chitosan is higher than 1.4%, both gram negative and gram positive bacteria are constricted.



**Figure 4.1** Changes in Aerobic Plate Count (APC) of heat treated sucuks at 65°C during storage



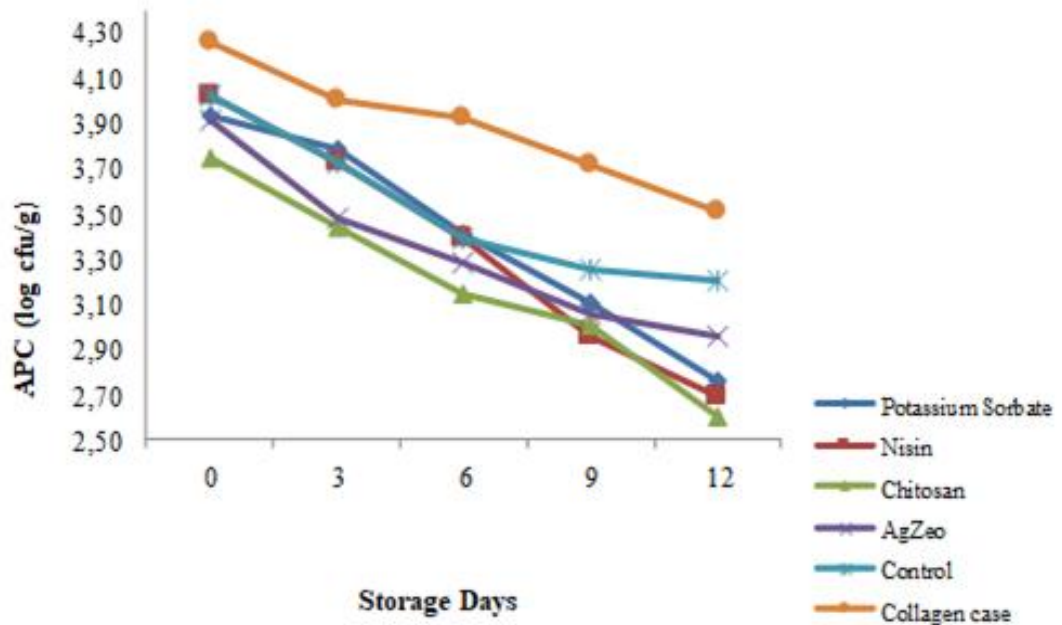
**Figure 4.2** Changes in Lactic Acid Bacteria (LAB) counts of heat treated sucuks at 65°C during storage

APC reduced to about 3.74 log cfu/g, LAB counts decreased to about 3.23 log cfu/g after the heat treatment at 70°C (Figure 4.3 and Figure 4.4.). Furthermore, APC

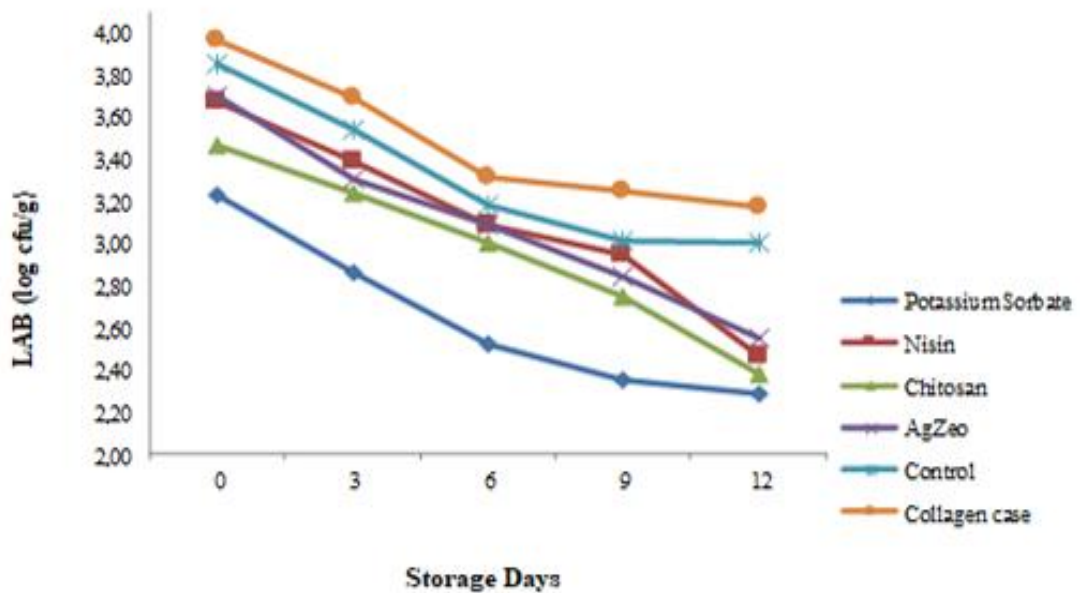
varied between 2.60 log cfu/g (in chitosan incorporated case) and 3.51 log cfu/g (in collagen case) on last day of storage. Also, there was no significant difference ( $P > 0.05$ ) between APC of potassium sorbate and AgZeo sucuk samples. Similarly, the highest LAB count was 3.17 log cfu/g in sucuk stuffed into collagen case whereas the lowest LAB count was 2.29 log cfu/g in the potassium sorbate at the end of storage (Table 4.3). Additionally, there was no significant difference ( $P > 0.05$ ) between LAB counts of potassium sorbate and nisin on 12<sup>th</sup> days of storage (Table 4.3). Several authors reported that nisin and potassium sorbate are active against fungi, yeasts and many bacteria (Pranoto et al., 2005; Bhatia and Bharti, 2015; Soysal et al. 2015). Biji et al. (2015) studied application of nisin and pediocin on cellulose casing for prevention of growth of *Listeria monocytogenes* on beef, ham and turkey meat. It was also detected that lysozyme and nisin in corn zein and soy-protein films importantly prevented *Escherichia coli* and *Lactobacillus plantarum* on laboratory condition. Nguyen et al. (2008) studied the effect of nisin-including bacterial cellulose films on *L. monocytogenes* on the surface of frankfurters and smoked salmon. They found that the nisin-including bacterial cellulose films (2500 IU/ml) were detected to be efficient in decreasing growth of *L. monocytogenes* during the refrigerated storage period. Ercolini et al. (2010) studied effect of nisin contained antimicrobial packaging for the beef storage. They found that count of lactic acid bacteria decreased significantly by use of antimicrobial packaging. Also, count of enterobacteria has been found to decrease about 1-3 log cycles by use of antimicrobial packaging between 22 and 32 storage days at 1°C (Ercolini et al., 2010). As expected higher reductions in APC and LAB counts were monitored in the sucuks stuffed into chitosan incorporated cases during storage for both heat process temperatures 65°C and 70°C. Similarly, Jonaidi Jafari et al. (2017) examined influences of chitosan coating with ethanolic extract of propolis on the quality of refrigerated chicken fillet. Microbial analysis found that coating had an important decreasing influence on growth of microorganisms during storage (12 days at 4 °C).

After the heat treatment at 75°C, APC reduced to about 3.72 log cfu/g, LAB counts decreased to about 3.09 log cfu/g (Figure 4.5 and 4.6). Collagen and control plastic case had the highest APC at the beginning of storage (Figure 4.5). After the storage, APC varied between 2.39 log cfu/g (in AgZeo incorporated case) and 2.77 log cfu/g (in potassium sorbate incorporated case). Nowadays, packaging with nanoscale silver

coating has been performed to fresh pork sirloin and the growth of the meat microorganisms is retarded (Kuulialaa et al., 2015).



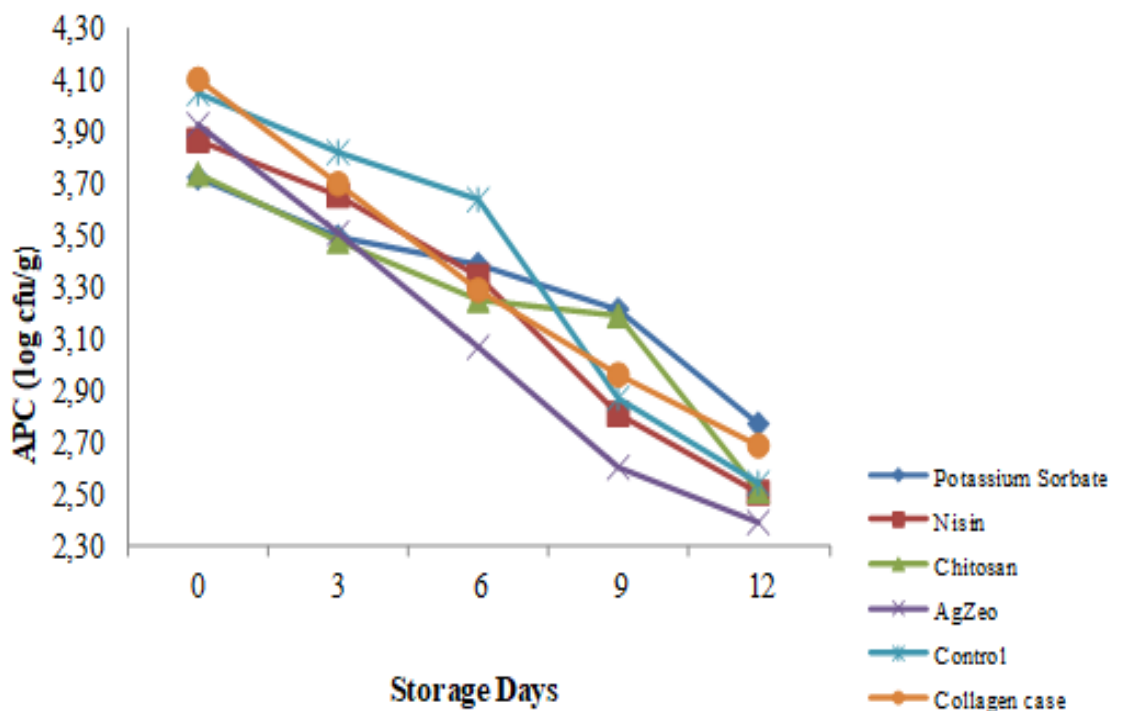
**Figure 4.3** Changes in Aerobic Plate Count (APC) of heat treated sucuks at 70°C during storage



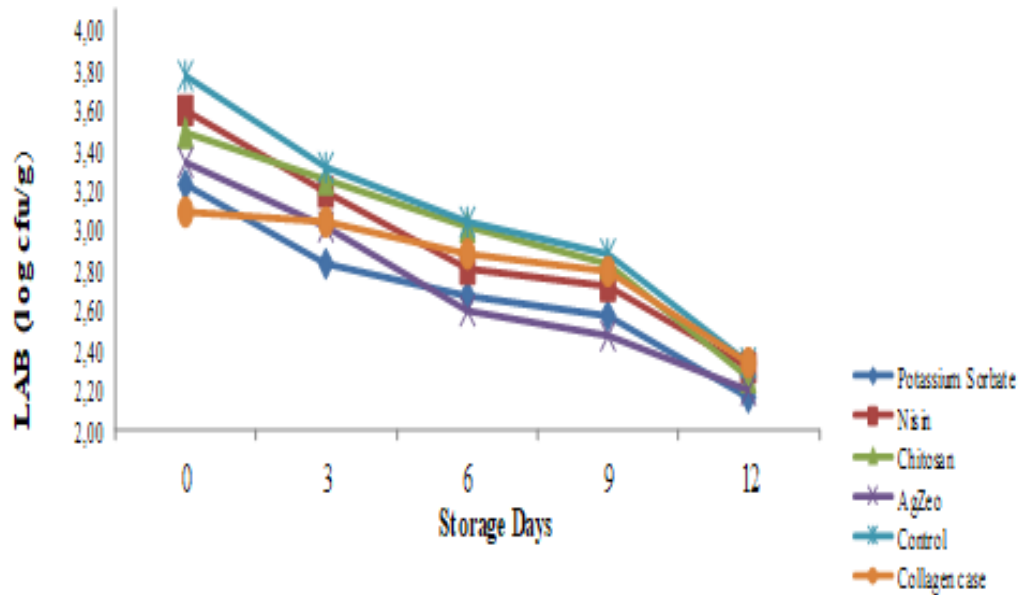
**Figure 4.4** Changes in Lactic Acid Bacteria (LAB) counts of heat treated sucuks at 70°C during storage

Boluda-Aguilar et al. (2010), studied fresh loin packed with zeolite bags including an antimicrobial agent (essential oil of thyme). They reported that during 3 weeks of

storage, the zeolite and thyme caused a reduction in microbial count of 2 log cfu/g compared to control plastic case. It was determined in many studies that reductions in APC and LAB level were about 3– 5 log cycles after heat treatment (Dalmış and Soyer, 2008; Erçoşkun et al., 2010). Our results were similar for APC and LAB counts by other studies results that LAB and APC increased during the fermentation and reduced after heat treatment (Erçoşkun et al., 2010). Similarly, the highest LAB count was 2.33 log cfu/g in sucuk stuffed into collagen case whereas the lowest LAB count was 2.15 log cfu/g in sucuk stuffed into chitosan at the end of storage (Table 4.3). Several authors reported that chitosan has antimicrobial activities against fungi, yeasts and bacteria (Aymerich et al., 2008; Soysal et al. 2015). The antimicrobial attribute of chitosan is well accepted and probably because of the interaction of the positively charged chitosan with negatively charged ruins on the cell surface of numerous fungi and bacteria, inducing wide cell surface changes and changing cell permeability (Park et al., 2010; Soysal et al., 2015).



**Figure 4.5** Changes in Aerobic Plate Count (APC) of heat treated sucuks at 75°C during storage



**Figure 4.6** Changes in Lactic Acid Bacteri (LAB) counts of heat treated sucuks at 75°C during storage

Moreover, one way ANOVA and multiple range test were employed to define the effect of different heat treatment (65°C, 70 °C and 75°C) on APC and LAB (Table 4.2 and 4.3). Generally, APC and LAB counts were affected by heat treatment ( $P < 0.05$ ) during storage (Table 4.4). Also, using different heat process can affect microbial quality of sucuks.

## 4.2 pH

### 4.2.1 Fermentation

pH values of sucuks stuffed into active cases (potassium sorbate, nisin, chitosan, AgZeo), control plastic case and artificial collagen case before the heat treatment were reduced from an initial value of 5.10 to 4.70 during 3 days of fermentation (Table 4.5). Acids produced by lactic acid and other acid-producing bacteria could be the reason of this reduction (Erçoşkun et al., 2010). The highest ( $P < 0.05$ ) pH value was observed as 4.93 in AgZeo incorporated case while the lowest pH value was 4.70 in collagen case at the end of fermentation (Table 4.5). There were significant differences ( $P < 0.05$ ) between control samples and antimicrobial cases (incorporated AgZeo, chitosan, potassium sorbate and nisin) at the end of fermentation (Table 4.5). Fermented sucuks must have a pH value between 4.7 and 5.4 according to the Turkish Food Codex (2000). In this study, pH values of all of sucuks were in this

**Table 4.2** Changes in aerobic plate count (APC) (log cfu/g) heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
(APC) (log cfu/g)	65°C	0	4.18 ±0.14 c,A	4.43±0.07a,b,A	4.26±0.05b,c,A	4.22±0.03b,c,A	4.25±0.07b,c,A	4.20±0.07b,c,A
		3	3.86 ±0.07 c,d,B	4.09 ±0.01 b,B	3.78 ±0.01 d,B	3.89 ±0.01 c,B	3.81±0.05c,d,B	4.22±0.02 a,B
		6	3.68 ±0.12 b,c,B	3.80±0.007 b,C	3.50 ±0.02 d,C	3.64±0.05b,c,d,C	3.59±0.01c,d,C	3.99±0.07 a,C
		9	3.35 ±0.07 a,b,C	3.51±0.16a,b,D	3.29 ±0.15 b,C	3.52 ±0.03 a,b,C	3.27±0.03b,D	3.57±0.04 a,D
		12	3.09 ±0.12 b,C	3.48±0.00a,D	2.85 ±0.07 c,D	3.05 ±0.07 b,c,D	2.59 ±0.15 d,E	3.46±0.02 a,D
	70°C	0	4.26 ±0.12 a,A	4.02±0.17a,b,A	3.93±0.07a,b,A	4.02 ±0.03 a,b,A	3.74 ±0.26 b,A	3.91±0.27 b,A
		3	4.00 ±0.16 a,A,B	3.72±0.22a,A,B	3.78 ±0.01 a,A	3.72 ±0.10 a,A,B	3.44 ±0.24 b,A	3.48±0.19 b,A
		6	3.92 ±0.24 a,A,B	3.39±0.09 a,B	3.41±0.09a,A,B	3.39 ±0.12 a,B	3.14 ±0.22 a,B	3.28 ±0.13 a,B
		9	3.71 ±0.15 a,A,B	3.25±0.09a,b,B	3.10±0.14b,B,C	2.95 ±0.01 b,C	3.00 ±0.21 b,B	3.05±0.25a,b,B
		12	3.51 ±0.12 a,B	3.20±0.18a,b,B	2.76±0.05b,c,C	2.69 ±0.12 c,C	2.60 ±0.11 c,C	2.95±0.20b,c,C
	75°C	0	4.10 ±0.02 a,A	4.05 ±0.07 a,A	3.72 ±0.04 d,A	3.87 ±0.03 b,c,A	3.74±0.09c,d,A	3.93±0.01a,b,A
		3	3.70 ±0.11 a,b,A	3.82 ±0.05 a,B	3.49 ±0.12 b,B	3.65 ±0.09 a,b,B	3.48±0.04 b,B	3.51 ±0.21 b,B
		6	3.29 ±0.13 b,c,B	3.64 ±0.04 a,C	3.39 ±0.01 b,B	3.34 ±0.12 b,C	3.25±0.07b,c,C	3.07 ±0.10 c,C
		9	2.96 ±0.05 b,C	2.87 ±0.03 b,D	3.21 ±0.04 a,C	2.81 ±0.05 b,D	3.19 ±0.01 a,C	2.60 ±0.14 c,D
		12	2.69 ±0.12 a,b,D	2.54±0.05 b,c,E	2.77 ±0.02 a,D	2.50 ±0.02 b,c,E	2.51±0.02b,c,D	2.39 ±0.12 c,D

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.3** Changes in lactic acid bacteria count (LAB) (log cfu/g) heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
LAB (log cfu/g)	65°C	0	3.76±0.06 <sup>b,c,A</sup>	3.94 ±0.08 <sup>a,b,A</sup>	3.78±0.01 <sup>b,c,A</sup>	3.58 ±0.12 <sup>c,A</sup>	3.81 ±0.04 <sup>b,A</sup>	4.07 ±0.09 <sup>a,A</sup>
		3	3.42±0.10 <sup>c,B</sup>	3.80 ±0.007 <sup>a,B</sup>	3.50±0.02 <sup>b,c,B</sup>	3.41 ±0.05 <sup>c,B</sup>	3.5 ±0.04 <sup>b,c,B</sup>	3.57 ±0.04 <sup>b,B</sup>
		6	3.35±0.07 <sup>a,b,B</sup>	3.44 ±0.05 <sup>a,C</sup>	3.05±0.07 <sup>c,C</sup>	3,22 ±0.03 <sup>b,C</sup>	3.34±0.05 <sup>a,b,B</sup>	3.41 ±0.05 <sup>a,C</sup>
		9	3.07±0.03 <sup>a,b,C</sup>	3.15 ±0.02 <sup>a,D</sup>	2.85 ±0.07 <sup>c,D</sup>	3.03 ±0,04 <sup>b,D</sup>	2.49 ±0,01 <sup>d,C</sup>	3.17 ±0.03 <sup>a,D</sup>
		12	2.88±0.02 <sup>a,b,D</sup>	2.92 ±0.03 <sup>a,b,E</sup>	2.49 ±0.01 <sup>c,E</sup>	2.82 ±0.03 <sup>b,E</sup>	2.11 ±0.15 <sup>d,D</sup>	3.00 ±0.01 <sup>a,E</sup>
	70°C	0	3.97±0.11 <sup>a,A</sup>	3.85 ±0.16 <sup>a,b,A</sup>	3.23 ±0.07 <sup>c,A</sup>	3.67±0,03 <sup>a,b,c,A</sup>	3.47±0,04 <sup>b,c,A</sup>	3.70±0.26 <sup>a,b,c,A</sup>
		3	3.69±0.15 <sup>a,A,B</sup>	3.54 ±0.21 <sup>a,A,B</sup>	2.86±0.02 <sup>b,A,B</sup>	3.39 ±0.12 <sup>a,A,B</sup>	3.24 ±0.22 <sup>b,A</sup>	3.31 ±0.18 <sup>b,A</sup>
		6	3.32±0.21 <sup>a,B</sup>	3.18 ±0.08 <sup>a,B</sup>	2.52±0.02 <sup>a,B,C</sup>	3.09 ±0.05 <sup>a,B,C</sup>	3.00 ±0.21 <sup>a,B</sup>	3.09 ±0.13 <sup>a,B</sup>
		9	3.25±0.13 <sup>a,B</sup>	3.01 ±0.08 <sup>a,B</sup>	2.35 ±0.06 <sup>b,C</sup>	2.95 ±0.001 <sup>a,C</sup>	2.75 ±0.19 <sup>a,B</sup>	2.84 ±0.24 <sup>a,B</sup>
		12	3.17±0.20 <sup>a,B</sup>	3.00 ±0.17 <sup>a,b,B</sup>	2.29 ±0.01 <sup>c,C</sup>	2.47 ±0.10 <sup>c,D</sup>	2.38±0.10 <sup>c,B,C</sup>	2.55±0.18 <sup>b,c,B,C</sup>
	75°C	0	3.09±0.01 <sup>b,A</sup>	3.77 ±0.01 <sup>a,A</sup>	3.22±0.056 <sup>e,A</sup>	3.60 ±0.02 <sup>b,A</sup>	3.48 ±0.04 <sup>c,A</sup>	3.33 ±0,02 <sup>d,A</sup>
		3	3.04±0.05 <sup>b,A</sup>	3.31 ±0.07 <sup>a,B</sup>	2.83 ±0.07 <sup>c,B</sup>	3.19 ±0.01 <sup>a,B</sup>	3.25 ±0.07 <sup>a,B</sup>	3.01 ±0,021 <sup>b,B</sup>
		6	2.87±0.03 <sup>a,b,B</sup>	3.04 ±0.05 <sup>a,C</sup>	2.66±0.09 <sup>c,d,B,C</sup>	2.80 ±0.04 <sup>b,c,C</sup>	3.01±0.02 <sup>a,b,C</sup>	2.59 ±0.15 <sup>e,C</sup>
		9	2.79±0.01 <sup>a,B</sup>	2.87 ±0.03 <sup>a,D</sup>	2.56 ±0.09 <sup>b,C</sup>	2.72 ±0.06 <sup>a,C</sup>	2.83±0.02 <sup>a,D</sup>	2.46 ±0.06 <sup>b,C</sup>
		12	2.33±0.07 <sup>a,C</sup>	2.32 ±0.04 <sup>a,E</sup>	2.15 ±0.07 <sup>b,D</sup>	2.30 ±0.05 <sup>a,D</sup>	2.25±0.03 <sup>a,b,E</sup>	2.19 ±0.05 <sup>a,b,D</sup>

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

interval. Ensoy (2004) found that pH levels of sucuks produced through traditional method were between the range of 5.16-5.26. Similar studies showed that before heat treatment the pH level were 6.06 in the first day of fermentation (Erçoşkun, 2010).

**Table 4.4** The effect of different heat treatment degrees on Aerobic Plate Counts(APC) and Lactic Acid Bacteria (LAB) counts (log cfu/g)

Parameter	Storage Time	Heat Treatment Temperature	CASE TYPE					
			Collagen case	Control Plastic case	Potassium Sorbate	Nisin	Chitosan	AgZeo
APC (log cfu/g)	0	4.18±0.14 a	4.43±0.07 a	4.26±0.05 a	4.22±0.03a	4.22±0.07 a	4.20±0.08a	4.18±0.14 a
		70°C	4.26±0.02 a	4.02±0.01 b	3.93±0.07b	4.02±0.03 b	3.74±0.1b	3.91±0.02b
		75°C	4.10±0.03 a	4.05±0.07 b	3.72±0.04c	3.87±0.03 c	3.74±0.09b	3.93±0.01b
	3	65 °C	3.86±0.07 b	4.09±0.02 a	3.78±0.01a	3.89±0.02 b	3.81±0.04 a	4.22±0.02a
		70°C	4.00±0.01 a	3.72±0.05 b	3.78±0.01a	3.72±0.10 a	3.44±0.00b	3.48±0.06b
		75°C	3.705±0.10b	3.82±0.05 b	3.49±0.12b	3.65±0.09 a	3.48±0.04b	3.51±0.21b
	6	65 °C	3.68 ±0.05 a,b	3.80±0.01 b	3.50±0.09a	3.64±0.12 b	3.59±0.11b	3.99±0.08c
		70°C	3.92±0.13 b	3.39±0.04 a	3.41±0.01a	3.39±0.12 a	3.14±0.07 a	3.28±0.10b
		75°C	3.29±0.07 a	3.64±0.16 a	3.39±0.15a	3.34±0.03 a	3.25±0.03 a	3.07±0.04a
	9	65 °C	3.35±0.07 b	3.51±0.16 a	3.29±0.15a	3.52±0.03 a	3.27±0.03 a	3.57±0.04a
		70°C	3.71±0.01 a	3.25±0.09 b	3.10±0.14a	2.95±0.05 b	3.05±0.07b	3.05±0.07b
		75°C	2.96±0.05 c	2.87±0.03 c	3.21±0.04a	2.81±0.05 c	3.1±0.01a,b	2.6±0.14 c
12	65 °C	3.09±0.12 a	3.48±0.00 a	2.85±0.07a	3.05±0.07 a	2.59±0.15 a	3.46±0.02a	
	70°C	3.51 ±0.03 a,b	3.20±0.12 b	2.76±0.05a	2.69±0.12 b	2.61±0.01 a	2.94±0.08b	
	75°C	2.69±0.12 b	2.54±0.05 b	2.77±0.02a	2.5±0.02 b	2.51±0.02 a	2.39±0.12c	
LAB (log cfu/g)	0	65 °C	3.76±0.06 a	3.94±0.08 a	3.78±0.01a	3.58±0.12 a	3.81±0.04 a	4.07±0.09a
		70°C	3.97±0.03 a	3.85±0.08 a	3.23±0.07b	3.67±0.03 a	3.47±0.07b	3.70±0.07b
		75°C	3.09±0.05 b	3.77±0.01 a	3.22±0.01b	3.60±0.02 a	3.48±0.04b	3.35±0.02c
	3	65 °C	3.42±0.10 a	3.80±0.01 a	3.5±0.02 a	3.415±0.05a	3.52±0.03 a	3.57±0.04a
		70°C	3.69±0.05 a	3.54±0.09 b	2.86±0.02b	3.39±0.12 a	3.24±0.05b	3.31±0.00b
		75°C	3.04±0.07 b	3.31±0.07 b	2.83±0.05b	3.19±0.01 a	3.25±0.07b	3.01±0.02c
	6	65 °C	3.35±0.07 a	3.44±0.05 a	3.05±0.07a	3.22±0.03 c	3.34±0.05b	3.41±0.05c
		70°C	3.32±0.01 a	3.19±0.08 b	2.52±0.02b	3.09±0.05 b	3.00±0.08 a	3.09±0.05b
		75°C	2.87±0.09 b	3.04±0.05 b	2.66±0.03b	2.80±0.05a	3.01±0.02 a	2.59±0.15a
	9	65 °C	3.07±0.03 a	3.15±0.02 a	2.85±0.07a	3.03±0.04 a	2.49±0.02 c	3.17±0.03a
		70°C	3.25±0.00 a	3.01±0.08 b	2.35±0.06b	2.95±0.00 a	2.75±0.03 a	2.84±0.03b
		75°C	2.79±0.09 b	2.87±0.03 b	2.56±0.01a	2.72±0.06 b	2.83±0.02b	2.46±0.09c
12	65 °C	2.88±0.02b	2.92±0.03 b	2.49±0.02a	2.82±0.03 a	2.11±0.15 a	3.00±0.01c	
	70°C	3.17±0.02a	3.00±0.07 b	2.29±0.01b	2.47±0.1 b	2.38±0.07 a	2.55±0.09b	
	75°C	2.33±0.07c	2.32±0.03a	2.15±0.07b	2.30±0.05 b	2.25±0.03 a	2.19±0.04a	

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.5** Changes in pH values and moisture content of sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Parameter	Fermentation Time (Days)	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
pH	0	5.1±0.4a,A	5.1±0.4a,A	5.1 ±0.4a,A	5.1±0.4a,A	5.1±0.4a,A	5.1±0.4a,A
	3	4.7 ±0.3b,A	4.8±0.3aA	4.89 ±0.34a,A	4.83±0.34a,A	4.9 ±0.34a,A	4.9±0.34a,A
Moisture (%)	0	59.6±4.2aA	59.6±4.2a,A	59.6±4.2a,A	59.6 ±4.2a,A	59.6 ±4.2a,A	59.6±4.2a,A
	3	52.9±4.2aA	58.7±4.1a,A	58.9±4.16a,A	58.2±4.12a,A	58.4±4.1a,A	58.6±4.1a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha=0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the fermentation time at  $\alpha=0.05$  level in each column.

#### 4.2.2 Storage

At all heat treatment temperatures (65°C, 70°C and 75°C) no significant differences were observed between pH values of samples (Table 4.6).

pH values of the control samples (plastic case and collagen case) decreased from 4.99 to 4.87 whereas pH values of the heat treated sucuks at 65°C stuffed into antimicrobial cases (potassium sorbate, nisin, AgZeo and chitosan) ranged between 5.03 and 4.70 at the end of the storage. Although pH of the control samples (plastic case and collagen case) decreased from 5.15 to 4.81 while pH values of the heat treated sucuks at 70°C stuffed into antimicrobial cases (potassium sorbate, nisin, AgZeo and chitosan) ranged between 5.15 and 4.84 at the end of the storage. Finally, pH value of the control samples (plastic case and collagen case) decreased from 5.25 to 4.87 whereas pH values of the heat treated sucuks at 75°C stuffed into antimicrobial cases (potassium sorbate, nisin, AgZeo and chitosan) ranged between 5.19 and 4.78 at the end of the storage (Table 4.6). Additionally, pH values of all sucuks were not affected ( $P > 0.05$ ) by heat treatment (Table 4.8). Similarly, Khajehali et al. (2011) reported that they were impacted neither by nisin nor the MAP (Modified Atmosphere Packaging) states. Ensoy (2004) reported that pH levels of heat-treated sucuks were between the range of 5.22-5.31. Ercoşkun et al. (2010) explored in their study on different fermentation variations influence above quality attributes of heat-treated sucuks. In sucuks manufactured by traditional method before heat treatment, the pH value were 6.06 in the first day and 4.87 in the fifth day. After heat treatment, the pH value was 6.18 in the first day and reduced to 5.25 in fifth day, in respect of, sucuks made through traditional method the pH value

provided at 5.01. Lekjing (2016) discovered on chitosan coatings for influences on quality and shelf life of cooked pork sausages stored at  $4 \pm 2^\circ\text{C}$ . The primary pH values were in the range of 6.23 to 6.35. Through 5 days of storage, the actual applications reduced the pH values, likely because of the available of acidified chitosan. These results agree with those found by Lin and Chao (2001) for reduced-fat sausage (including chitosan), and by Giatrakou et al. (2010a, b) for ready to cook chicken (including chitosan). Korkeala et al. (1990) reported that the pH of cooked sausages reduced from 6.3 to about 5.8 with storage at 2, 4 or  $12^\circ\text{C}$ , and pH was determinative of deterioration.

### **4.3 Moisture**

#### **4.3.1 Fermentation**

During the first 3 days of fermentation, moisture content did not change significantly ( $P > 0.05$ ) except the sucuk stuffed into collagen case which had the lowest moisture content with 52.91 %, below the critical limit of the Turkish Standard (TSI, 2002) (Table 4.4). Hampikyan (2009) reported that the moisture levels examined for nisin-coated fermented sausage were parallel to those provided for the non-nisin coated control. Wang (2000) found that nisin had no influence on moisture levels of sausages compared to control (0 to 5 days). Erçoşkun et al. (2010) discovered that moisture amount during the first 5 days of fermentation reduced importantly from an initial value of 57.58–39.63% .

#### **4.3.2 Storage**

Collagen case samples had lower moisture content ( $P < 0.05$ ) than those of other antimicrobial cases for all temperatures ( $65^\circ\text{C}$ ,  $70^\circ\text{C}$  and  $75^\circ\text{C}$ ) (Table 4.7). However, there were no significant differences ( $P > 0.05$ ) between antimicrobial cases during storage all heat treatment levels ( $65^\circ\text{C}$ ,  $70^\circ\text{C}$  and  $75^\circ\text{C}$ ) (Table 4.7). Moisture content of sucuk samples reduced in the range of 8.91% by the heat treatment at  $65^\circ\text{C}$ . According to literature, heat treatment reduced the moisture value of the sucuks in the range of 6.3–0.3% for 0<sup>th</sup> and 5<sup>th</sup> days of fermentation, respectively (Erçoşkun et al., 2010).

**Table 4.6** Changes in pH of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
pH	65°C	0	5.15±0.36a,A	5.02±0.35a,A	5.00±0.33 a,A	5.02 ±0.13a,A	5.03±0.35a,A	5.01±0.35a,A
		3	5.00±0.35 a,A	5.00±0.35a,A	4,99±0,33 a,A	5,00 ±0,20 a,A	4.89±0.34a,A	4.82±0.34a,A
		6	4.95±0.35 a,A	4.88±0.34a,A	4.87±0.32 a,A	4.85 ±0.30a,A	4.88±0.34a,A	4.76±0.33a,A
		9	4.91±0.35 a,A	4.80±0.33a,A	4.83±0.33 a,A	4.81 ±0.19 a,A	4.85±0.34a,A	4.75±0.33a,A
		12	4.91±0.20 a,A	4.77±0.20a,A	4.83±1.19 a,A	4.80 ±0.30 a,A	4.80±0.20a,A	4.70±0.19a,A
	70°C	0	4.94±0.34 a,A	4.99±0.33a,A	5.03 ±0.33a,A	5.15 ±0.33 a,A	5.04±0.21a,A	5.03±0.27a,A
		3	4.93±0.34 a,A	4.99±0.33a,A	5.02 ±0.33a,A	5.13 ±0.33 a,A	5.00±0.28a,A	4.97±0.46a,A
		6	4.93±0.35 a,A	4.98±0.33a,A	5.00 ±0.33a,A	5.08 ±0.33 a,A	4.93±0.13a,A	4.87±0.39a,A
		9	4.89±0.34 a,A	4.93±0.33a,A	4.96 ±0.33 a,A	5.04 ±0.32 a,A	4.92±0.13a,A	4.85±0.32a,A
		12	4.87±0.20 a,A	4.90±0.19a,A	4.96 ±0.19 a,A	5.03 ±0.19 a,A	4.90±0.26a,A	4.84±0.26a,A
	75°C	0	5.25±0.44 a,A	5.16±0.29a,A	5.19 ±0.36a,A	5.14 ±0.32 a,A	5.15±0.21a,A	5.18±0.30a,A
		3	5.02±0.35 a,A	4.92±0.48a,A	4.93 ±0.41a,A	4.97 ±0.21a,A	4.95±0.28a,A	4.77±0.33a,A
		6	5.00±0.29 a,A	4.90±0.34a,A	4.89 ±0.48 a,A	4.88 ±0.35 a,A	4.92±0.35a,A	4.71±0.26a,A
		9	4.93±0.28 a,A	4.87±0.34a,A	4.87 ±0.22 a,A	4.84 ±0.27 a,A	4.89±0.41a,A	4.75±0.13a,A
		12	4.92±0.21 a,A	4.87±0.13a,A	4.85 ±0.27 a,A	4.83 ±0.13 a,A	4.85±0.27a,A	4.78±0.27a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.7** Changes in moisture of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Heat Treatment Temperature	Storage Time (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Moisture	65°C	0	44.45±2.1b,A	59.3 ±4.19 a,A	58.7 ±4.15 a,A	58.76±1.66a,A	58.95±2.50a,A	58.9 ±3.33 a,A
		3	42.31±2.99b,A	59.3 ±3.35 a,A	58.1±4.10 a,A	57.7 ±2.44 a,A	58.94±3.58a,A	58.45 ±5.78 a,A
		6	39.22±2.21b,A	58.72±2.49a,A	57.93±4.09a,A	57.65±3.66a,A	58.21±1.65a,A	57 ±4.83 a,A
		9	32.25±0.91b,B	58.2 ±4.9 a,A	57.5±4.07 a,A	57.61±2.44a,A	55.2 ±1.56 a,A	56.56 ±3.99 a,A
		12	27.13±1.53b,B	56.13±3.96a,A	56.67±2.40a,A	54.85±3.56a,A	55.06±3.11a,A	56.21 ±3.17 a,A
	70°C	0	44 ±3.11 b,A	57.13±4.03a,A	56.9 ±2.41 a,A	57.89±4.91a,A	56.98±4.02a,A	57.29 ±5.67 a,A
		3	41.96±1.18b,A	56.4 ±5.58 a,A	55.49±4.70a,A	56 ±3.16 a,A	55.68±3.14a,A	56.36±4.78a,A
		6	34.75±2.94b,B	55.71 ±4.72 a,A	55.47±2.35a,A	55.84±3.15a,A	55.74±3.15a,A	55.42±2.35a,A
		9	32.18±1.82b,B	54.5 ±3.08 a,A	54.45±2.32a,A	55.07.±5.45a,A	54.45±3.54a,A	54.85±1.55a,A
		12	26.07±1.84b,C	53.01 ±1.49 a,A	53 ±3.74a,A	54.22 ±3.06a,A	53.77±1.52a,A	54.4 ±1.53 a,A
	75°C	0	42.07±3.56b,A	56.43±3.19 a,A	55.44±3.92a,A	57.1 ±3.63 a,A	56.1±2.38a,A	56.49±3.35a,A
		3	39.49±2.79b,A	56.27±5.57 a,A	55.6 ±4.71 a,A	56 ±2.37 a,A	55.6±3.14a,A	56.63±4.00a,A
		6	38.74±2.30b,A	56.04±3.96 a,A	54.58 ±5.4a,A	55.91±3.95a,A	55.54±3.92a,A	56.38±3.18a,A
		9	30.64±1.73b,B	55.65±3.93 a,A	54.46±2.46a,A	55.4 ±3.13 a,A	54.4±4.61 a,A	55.69±1.57a,A
		12	25.76±1.09b,B	54.31±1.52 a,A	53.4 ±3.02 a,A	55.14±1.55a,A	54.11±3.05a,A	54.59±3.08a,A

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-C: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

Moisture content of the sample stuffed into collagen case decreased ( $P < 0.05$ ) from 44.45 % to 27.13 % whereas moisture content of the other samples ranged between 59.30 % and 54.85 % at the end of the storage for heat processing at 65°C (Table 4.7).

Moisture content of the sucuks stuffed into collagen case after heat treatment at 70°C reduced ( $P < 0.05$ ) from 44 % to 26.07% while moisture content of the sucuks stuffed into other cases ranged between 57.89% and 53.01% during storage (Table 4.7).

After the heat treatment at 75°C moisture content of the collagen case samples decreased ( $P < 0.05$ ) from 42.07 % to 25.76% whereas moisture content of the other samples ranged between 57.10% and 53.40% from day 0 to day 12 of storage (Table 4.5). Kurt and Zorba (2011) and Bilenler et al. (2017) reported that moisture content reduced with increasing storage time. According to statistical analysis moisture content were not affected ( $P > 0.05$ ) by different heat treatment (Table 4.8).

#### **4.4 TBARS**

##### **4.4.1 Fermentation**

Quality of sucuk affected by lipid oxidation is one of the major factors. Lipid oxidation of sucuk samples as the mg TBARS values per kg of product are given and the initial TBARS value of the sucuk dough was about 1.10 mg/kg sample. The proposed limit for TBARS value is 2 mg/kg in meat products (Byun et al., 2003). Before the heat treatment, TBARS values of the sucuk samples did not change significantly ( $P > 0.05$ ) up to 3 days of fermentation except control plastic samples (Table 4.7). Additionally, significant difference ( $P < 0.05$ ) was observed between those of the control plastic and samples stuffed into active cases during fermentation. Soysal et al. (2015) found that chicken drumsticks packed with active film incorporated with nisin, potassium sorbate and chitosan had lower TBARS value compared to control plastic films. Control plastic cases had the higher TBARS value ( $P < 0.05$ ) than those of other sucuk at the last day of fermentation. There were no significant differences ( $P > 0.05$ ) between sucuks stuffed into active cases (Table 4.9).

**Table 4.8** The effect of different heat process temperatures on pH values and moisture content

Parameters	Storage Time (Days)	Heat Treatment Temperature	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
pH	0	65 °C	5.15±0.36b	5.02±0.35a	5.00±0.33 a	5.02±0.13 a	5.03±0.35a	5.01±0.35a
		70°C	4.94±0.34a	4.99±0.33a	5.03±0.33 a	5.15±0.33a	5.04±0.21a	5.03±0.27a
		75 °C	5.25±0.44b	5.16±0.29a	5.19±0.36 a	5.14±0.32a	5.15±0.21a	5.18±0.30a
	3	65 °C	5.00±0.35 a	5.00±0.35 a	4.99±0.33 a	5.00±0.20 a	4.89±0.34 a	4.82±0.34 a
		70°C	4.93±0.34 a	4.99±0.33 a	5.02±0.3 3a	5.13±0.33 a	5.00±0.32 a	4.97±0.46 a
		75 °C	5.02±0.35 a	4.92±0.48 a	4.93±0.41 a	4.97±0.21 a	4.95±0.28 a	4.77±0.33 a
	6.	65 °C	4.95±0.35 a	4.88±0.34 a	4.87±0.33 a	4.85±0.3 a	4.88±0.34 a	4.76±0.33 a
		70°C	4.93±0.35 a	4.98±0.33 a	5.00±0.33 a	5.08±0.33 a	4.93±0.13 a	4.87±0.39 a
		75 °C	5.00±0.29 a	4.9±0.34 a	4.89±0.48 a	4.88±0.35a	4.92±0.3 a 5	4.71±0.26 a
	9	65 °C	4.91±0.35 a	4.80±0.33 a	4.83±0.33 a	4.81±0.19 a	4.85±0.34 a	4.75±0.33 a
		70°C	4.89±0.34 a	4.93±0.33 a	4.96±0.33 a	5.04±0.32 a	4.92±0.13 a	4.85±0.32 a
		75 °C	4.93±0.28 a	4.87±0.34 a	4.87±0.22 a	4.84±0.27 a	4.89±0.41 a	4.75±0.13 a
12	65 °C	4.91a±0.20	4.77a±0.20	4.83±1.19 a	4.80a±0.30	4.80a±0.20	4.70a±0.19	
	70°C	4.87a±0.20	4.90a±0.19	4.96±0.19 a	5.03a±0.19	4.90a±0.26	4.84a±0.26	
	75 °C	4.92a±0.21	4.87a±0.13	4.85±0.27 a	4.83a±0.13	4.85a±0.27	4.78a±0.27	
Moisture	0	65 °C	44.45±2.51a	59.3±4.19 a	58.7±4.15 a	58.76±1.66 a	58.95±2.50a	58.9a±3.33
		70°C	44±3.11 a	57.13±4.03a	56.9±2.41 a	57.89±4.91 a	56.98±4.02a	57.29a±5.67
		75 °C	42.07±3.56a	56.43±3.19a	55.44±3.92a	57.1±3.63 a	56.1±2.38 a	56.49a±3.35
	3	65 °C	42.31±2.99a	59.3±3.35 a	58.1±4.10 a	57.7±2.44 a	58.94±3.58a	58.45±5.78a
		70°C	41.96±1.18a	56.4±5.58 a	55.49±4.70a	56±3.16 a	55.68±3.14a	56.36±4.78a
		75 °C	39.49±2.79a	56.27±5.57a	55.6±4.71 a	56±2.37 a	55.6±3.14 a	56.63±4 a
	6	65 °C	39.22±2.21a	58.72±2.49a	57.93±4.09a	57.65±3.66 a	58.21±1.65a	57±4.83 a
		70°C	34.75±2.94a	55.71±4.72a	55.47±2.35a	55.84±3.15 a	55.74±3.15a	55.42±2.35a
		75 °C	38.74±2.30a	55.04±3.96a	54.58±5.40a	55.91±3.95 a	55.54±3.92a	55.38±3.18a
	9	65 °C	32.25±0.91a	58.2±4.9 a	57.57±4.07a	57.61±2.44 a	55.2±1.56 a	56.56±3.99a
		70°C	32.18±1.82a	54.5±3.08 a	54.45±2.32a	55.07±5.45 a	54.45±3.54a	54.85±1.55a
		75 °C	30.64±1.73a	55.65±3.93a	54.46±2.46a	55.4±3.1 3 a	54.4±4.61 a	55.69±1.57a
12	65 °C	27.13±1.53a	56.13±3.96a	56.67±2.40a	54.85±3.56 a	55.06±3.11a	56.21±3.17a	
	70°C	26.07±1.84a	53.01±1.49a	53.00±3.74a	54.22±3.06a	53.77±1.52a	54.4±1.53 a	
	75 °C	25.76±1.09a	54.31±1.52a	53.40±3.02a	55.14±1.55 a	54.11±3.05a	54.59±3.08a	

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.9** Changes in 2-thiobarbituric acid values (TBARS) sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Fermentation Time (Days)	CASE TYPE					
	Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
0	1.1±0,07 a,A	1.10 ±0,07 a,A	1.10 ±0.07 a,A	1.1±0.07 a,A	1.1±0.07 a,A	1.1 ±0.07a,A
3	1.25±0.01 a,A	1.40 ±0.08b,A	1.30 ±0.09 a,A	1.35±0.07a,A	1.3±0.09 a,A	1.38±0.09a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha=0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the fermentation time at  $\alpha=0.05$  level in each column.

#### 4.4.2 Storage

After the heat treatment at 65°C, there were no significant differences ( $P > 0.05$ ) between TBARS values of all samples. TBARS values of sucuks stuffed into potassium sorbate, nisin, control plastic case and collagen case decreased importantly ( $P < 0.05$ ) during storage (Table 4.10). Although, Sureshkumar (2010) investigated the influence of nisin on quality of buffalo meat sucuks stored at  $35 \pm 2^\circ\text{C}$  and 70–80% RH. They found that TBARS levels rised during storage period. The rises in TBARS levels during storage is in agreement with the results of Karthikeyan et al. (2000) in caprine keema and Sebranek et al. (2005) in pork sausage.

Also, sucuks stuffed into chitosan had the lowest TBARS value (1.51 mg/kg) whereas control plastic case had the highest TBARS value (1.74 mg/kg) at the beginning of storage for 70°C. Chitosan and AgZeo sucuk samples had lower TBARS values ( $P < 0.05$ ) than those of other samples stored for 3 days. Chitosan film including pomegranate rind powder extract (PRP) was used as an active packaging compound for pork meat patties stored at  $4 \pm 1^\circ\text{C}$  for 20 days by Yu-yue et al. (2014). Their findings proposed that lipid oxidation in pork meat patties could be reduced by the use of chitosan-based film. The technique by which this inhibition took place was expected to be connected with chelation of free iron, which released from hemoproteins of meat during storage (Yu-yue et al., 2014). TBARS values of heat treated samples in collagen case at 70°C were lower ( $P < 0.05$ ) than those of other sucuk samples at the end of storage. Additionally, TBARS values of sucuks stuffed into potassium sorbate, nisin, control plastic case and collagen case decreased gradually ( $P < 0.05$ ) during storage (Table 4.10). Georgantelis et al. (2007) found that decrease in TBARS contents for sausages prepared from porks by 73% for samples including 1% chitosan (0.25 mg MDA/kg) as compared with control samples (0.96 mg MDA/kg) after 10 days of storage at 4 °C. In other study on minced meat using together with chitosan and lysozyme, the shelf life of the sample rised by preventing the growth of *B. cereus*, *E. coli* and *Pseudomonas fluorescens* while a major decrease was declared in the *S. aureus* counts. Lipid oxidation was reduced by use of this combination (Rao et al., 2008).

After the heat treatment at 75°C, there were significant differences ( $P < 0.05$ ) between TBARS values of sucuk samples except only 6 day of storage. Also, 1.55 mg/kg was

the lowest TBARS value found in sucuks stuffed into nisin while 1.99 mg/kg was the highest in control plastic case samples at the first day of storage. Similarly, Lu et al. (2010) studied effect cinnamon and nisin in alginate–calcium coating on fresh fish fillets quality at 4°C. They reported that TBARS values of alginate–calcium coating with 10 µL/mL cinnamon and 2000 IU/mL nisin and 150 µg/mL EDTA gave better influences than fish fillets treated with only alginate–calcium coating. Furthermore, sucuks stuffed into collagen case had lower TBARS values than those of other samples after 12 days of storage. TBARS values of sucuks decreased gradually ( $P < 0.05$ ) during storage except sucuks stuffed into potassium sorbate, AgZeo and chitosan (Table 4.10). Hsu and Sun (2006) examined effects of different additives (salt, phosphates, potassium sorbate and sodium erythorbate) on quality of meat ball. They found that TBARS values of samples rised with potassium sorbate (Hsu and Sun, 2006). According to another study on the sucuks that use of potassium sorbate decreased TBARS values (Bozkurt and Erkmén, 2002). On the other hand, it was found that TBARS values were affected insignificantly ( $P > 0.05$ ) by heat process at different temperatures (65 °C, 70 °C and 75°C) (Table 4.11).

## **4.5 Color**

### **4.5.1 Fermentation**

The changes in  $L^*$ ,  $a^*$  and  $b^*$  values of sucuk samples during the fermentation are given in Table 4.12. The lightness ( $L^*$ ) value of sucuk dough was about 54.01 and reduced ( $P > 0.05$ ) to about 51.00 during the fermentation period (Table 4.12). Similarly, the reduction in  $L^*$  values of traditional sucuks were reported by Kayaardı and Gök (2004) and Bozkurt and Bayram (2006). The lowest lightness ( $L^*$ ) value was 51.00 in sucuks stuffed into nisin case whereas the highest ( $P > 0.05$ ) lightness ( $L^*$ ) value was 53.75 in sucuks stuffed into with collagen case (Table 4.12). Redness ( $a^*$ ) value of samples rised insignificantly ( $P > 0.05$ ) from an initial value of 11.72 to 12.92 during 3 days of fermentation (Table 4.12). The rise in redness value during fermentation may be because of the formation of nitrosomyoglobin, depend on the typical red color of this type of meat product (Wirth, 1986).

**Table 4.10** Changes in 2-thiobarbituric acid values (TBARS) of heat treated sucuks at 65°C, 70°C and 75°C stuffed into antimicrobial, control and collagen cases during storage

Heat Treatment Degrees	Storage Time (Days)	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
65°C	0	1.50 ±0.08 a,A	1.54 ±0.10 a,A	1.52 ±0.10 a,A	1.53 ±0.04 a,A	1.49±0.06a,A	1.66±0.09a,A
	3	1.41 ±0.10 a,A,B	1.40 ±0.07 a,A,B	1.21 ± 0.08 a,B	1.38 ±0.05a,A,B	1.47±0.08a,A	1.51±0.14a,A
	6	1.28 ±0.07 a,B,C	1.29 ±0.05 a,B	1.21 ±0.08 a,B	1.36 ±0.08 a,A,B	1.45±0.05a,A	1.24±0.1a,A
	9	1.21 ±0.03 a,C,D	1.26 ±0.10 a,B	1.21 ±0.08 a,B	1.27 ±0.05 a,B,C	1.20±0.03a,A	1.18±0.08a,A
	12	1.07 ±0.06 a,D	1.17 ±0.08 a,B	1.19 ±0.05 a,B	1.18 ±0.07 a,C	1.12±0.06a,A	1.15±0.06a,A
70°C	0	1.53±0.10 b,A	1.74 ±0.12 a,A	1.62 ±0.06 a,b,A	1.72 ±0.17 a,A	1.51±0.10b,A	1.58±0.11b,A
	3	1.3±0.09 b,c,A,B	1.65 ±0.11 a,A	1.33 ±0.11 b,c,B	1.5 ±0.12 a,b,B	1.32±0.09c,A	1.35±0.09c,A
	6	1.32 ±0.09 a,B	1.35 ±0.09 a,B	1.19±0.05 a,A,B	1.36 ±0.05 a,B,C	1.24±0.08a,A	1.27±0.09a,A
	9	1.25 ±0.08 a,B	1.27 ±0.09 a,B	1.18±0.05 a,A,B	1.25 ±0.03 a,C,D	1.23±0.08a,A	1.23±0.08a,A
	12	0.84 ±0.03 b,C	1.19 ±0.05 a,B	1.06 ±0.07 a,B	1.15 ±0.04 a,D	1.16±0.04a,A	1.08±0.04a,A
75°C	0	1.64 ±0.13 b,c,A	1.99 ±0.11 a,A	1.85 ±0.13 a,b,A	1.71 ±0.10 a,b,c,A	1.55±0.07 c,A	1.76±0.10b,c,A
	3	1.63 ±0.11 a,A	1.55 ±0.15 a,b,B	1.42 ±0.12 b,c,B	1.59 ±0.06 a,b,A	1.32±0.07c,d,A	1.36 ±0.09 d,A
	6	1.39 ±0.08 a,B	1.25 ±0.08 a,C	1.28 ±0.12 a,B	1.39±0.09 a,B	1.29±0.09a,A	1.19 ±0.06 a,A
	9	1.31 ±0.07 a,B	1.10 ±0.07 b,C	1.19 ±0.54 a,b,B	1.26 ±0.07 a,b,B,C	1.25±0.10a,b,A	1.14±0.03a,b,A
	12	0.80 ±0.03b,C	1.19 ±0.05 a,B	1.06 ±0.07 a,B	1.15 ±0.04 a,D	1.16±0.04a,A	1.08±0.04a,A

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.11** The effect of different heat treatment degrees on 2-thiobarbituric acid values (TBARS) values

Storage Time (Days)	Heat Treatment Temperature	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
0. Day	65 °C	1.50±0.08 a	1.54±0.10 b	1.52±0.10a	1.53±0.04a	1.49±0.06a	1.66±0.09 a
	70°C	1.53±0.10 a	1.74 ±0.12 a,b	1.62±0.06a	1.72±0.17a	1.51±0.10a	1.58±0.11a
	75 °C	1.64±0.13 a	1.99±0.11 a	1.85±0.13a	1.71±0.10a	1.55±0.07a	1.76±0.10a
3. Day	65 °C	1.41±0.10 a	1.40±0.07 a	1.21±0.08a	1.38±0.05a	1.47±0.08a	1.51±0.14a
	70°C	1.38±0.09 a	1.65±0.11 a	1.33±0.11a	1.5±0.12 a	1.32±0.09a	1.35±0.09a
	75 °C	1.63±0.11 a	1.55±0.15 a	1.42±0.12a	1.59±0.06a	1.32±0.07a	1.36±0.09a
6. Day	65 °C	1.28a±0.07	1.29a±0.05	1.21±0.08a	1.36±0.08a	1.45±0.05a	1.24a±0.10
	70°C	1.32a±0.09	1.35a±0.09	1.19±0.05a	1.36±0.05a	1.24±0.08a	1.27a±0.09
	75 °C	1.39a±0.08	1.25a±0.08	1.28±0.12a	1.39±0.09a	1.29±0.09a	1.19a±0.06
9. Day	65 °C	1.21±0.03 a	1.26±0.10 a	1.21±0.08a	1.27±0.05a	1.20±0.03a	1.18±0.08a
	70°C	1.25±0.08 a	1.27±0.09 a	1.18±0.05a	1.25±0.03a	1.23±0.08a	1.23±0.08a
	75 °C	1.31±0.07 a	1.10±0.07 a	1.19±0.54a	1.26±0.07a	1.25±0.10a	1.14±0.03a
12. Day	65 °C	1.07 ±0.06 a,b	1.17±0.08a	1.19±0.05a	1.18±0.07a	1.12±0.06a	1.15±0.06a
	70°C	0.84±0.03 a	1.19±0.05 a	1.06±0.07a	1.15±0.04a	1.16±0.04a	1.08±0.04a
	75 °C	1.25±0.05 b	1.05±0.02 a	1.19±0.06a	1.19±0.03a	1.15±0.06a	0.93±0.05a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

The lowest redness ( $a^*$ ) value was 11.82 in sucuks stuffed into potassium sorbate case whereas the highest ( $P > 0.05$ ) redness ( $a^*$ ) value was 12.92 in collagen case sample. Before the heat treatment, yellowness ( $b^*$ ) value of sucuks increased significantly ( $P < 0.05$ ) up to third day except potassium sorbate and chitosan samples (Table 4.12). The  $b^*$  values of sausages are associated with oxidation process. Potassium sorbate and chitosan have antimicrobial and antioxidant properties that may have prevented the rate of oxidation during fermentation (Fernández-López and

Vicente, 2000). Similar results were also reported by Kayaardı and Gök (2004). There were no significant differences ( $P > 0.05$ ) between b values of sucuk during fermentation (Table 4.12).

**Table 4.12** Changes in color values sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Parameters	Fermentation Time (Days)	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
L*	0	54.01±3.81a,A	54.01±3.81a,A	54.01±3.81a,A	54.01±3.81a,A	54.01±3.81a,A	54.01±3.81a,A
	3	53.75±3.80a,A	53.25±3.76a,A	52.19±3.69a,A	51.00±3.60a,A	51.85±3.66a,A	52.10±3.68a,A
a*	0	11.78±0.83a,A	11.78±0.83a,A	11.78±0.83a,A	11.78±0.83a,A	11.78±0.83a,A	11.78±0.83a,A
	3	12.92±0.91a,A	12.86±0.90a,A	11.82±0.97a,A	12.85±0.90a,A	12.59±0.89a,A	12.71±0.89a,A
b*	0	8.50 ±0.60 a,B	8.50 ±0.60 a,A	8.50 ±0.60 a,A	8.50 ±0.60 a,B	8.50 ±0.60 a,A	8.50 ±0.60 a,B
	3	10.49±0.74b,A	9.90±0.7a,b,A	9.93 ±0.70 a,A	9.91 ±0.70 a,A	9.25±0.65 a,A	8.50±0.60 a,A

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha = 0.05$  level in each row. A-C: Different capital letters indicate a statistical difference between the storage time at  $\alpha = 0.05$  level in each column.

#### 4.5.2 Storage

For all heat treatment (65°C, 70°C and 75°C), there were no significant differences ( $P > 0.05$ ) between L\* values of sucuk samples (Table 4.13). The lowest lightness (L\*) values were 55.63, 56.64 and 54.99 in sucuks stuffed into nisin, potassium sorbate and chitosan case whereas the highest lightness (L\*) value was 57.93, 57.64 and 56.91 in sucuks stuffed into collagen cases after heat treatment at 65, 70 and 75, respectively, at the end of storage. Samples stuffed into control plastic case and collagen case had insignificantly ( $P > 0.05$ ) higher L\* values than those of antimicrobial cases (Table 4.13). This information is in agreement with color results declared by Soysal et al. (2015). They noticed no important change ( $P > 0.05$ ) in the L\* values of samples packed with nisin and potassium sorbate. At all temperatures, there were no significant differences ( $P > 0.05$ ) between a\* values of sucuk samples. The lowest redness (a\*) values were 11.43, 12.53 and 11.65 in sucuks stuffed into AgZeo, chitosan and nisin cases whereas the highest redness (a\*) value was 12.98, 13.63 and 13.21 in sucuks stuffed into collagen cases for heat treatment at 65, 70 and 75°C, respectively at the end of storage (Table 4.13). The a\* values of all samples

increased insignificantly ( $P > 0.05$ ) during storage for heat treatment at 70°C. Moreover,  $a^*$  values of heat treated sucuks at 65°C and 75 °C increased insignificantly ( $P > 0.05$ ) except sucuk stuffed into chitosan and nisin (Table 4.13). Additionally, color values of all sucuks were not gradually ( $P > 0.05$ ) affected by increased heat treatment temperature (Table 4.14). Changes in color values of samples were probably because of microbial spoilage and lipid oxidation. During storage, the discoloration of control samples may be due to the accumulation of hydrogen peroxide produced by lactic acid bacteria (Cayré et al., 2005). Similar results were also reported by Kayaardı and Gök (2004) and Bozkurt and Bayram (2006).

The  $b^*$  values of sucuks stuffed into nisin decreased significantly ( $P < 0.05$ ) during storage for heat treatment at 65, 70 and 75°C (Table 4.13). This effect could be due to the antimicrobial attributes of nisin (Martí'nez et al., 2006).

Consequently, use of antimicrobial substances in sucuk case and storage period did not cause any significant ( $P > 0.05$ ) changes in color values of heat treated sucuks. Similarly, different amounts of nisin (16, 31, and 63 g/ml) were microencapsulated into alginate-cellulose nanocrystal beads to inhibit *Listeria monocytogenes* in ready to eat (RTE) ham by Huq et al. (2014). They reported that microencapsulated nisin beads haven't negative effect on the color values of RTE ham during storage. Khajehali et al. (2011) studied the influences of nisin (30 ppm) and modified atmosphere packaging (MAP) on the color levels of emulsion type sausage. The sausages were stored at 3°C for 42 days. Similar to our results, no significant differences ( $P > 0.05$ ) in the  $a^*$  parameter (redness) and  $b^*$  (yellowness) were found either during the storage or during the packaging conditions.

The  $a^*$  value associated with the degree of the oxidation process ( $P > 0.05$ ). This could be because of the available of nitrite, which reacts with muscle pigment myoglobin to produce the requested red pigment, nitrosomyoglobin, which cannot act as catalyst of lipid oxidation (Khajehali et al., 2011). Harms et al. (2003) and Rubio et al. (2008) provided same findings for cured pork and dry fermented sausage as well. Blacha et al. (2013) did not find any  $L^*$  differences between high-oxygen and vacuum packaged poultry patties and pork cutlets, respectively.

**Table 4. 13** Changes in color values (L\*, a\*, b\*) of heat treated sucuks stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Heat Treatment Temperature	Storage Time (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
L*	65°C	0	55.54 ±3.14 a,A	55.17 ±3.90 a,A	55.16 ±3.90 a,A	54.75 ±1.54 a,A	55.85 ±2.36 a,A	55.14 ±3.19 a,A
		3	56.16 ±3.97 a,A	55.33 ±3.13 a,A	55.19 ±3.90 a,A	55.26 ±2.34 a,A	56.01 ±3.40 a,A	55.31 ±5.47 a,A
		6	57.32 ±3.24 a,A	55.96 ±2.37 a,A	55.31 ±3.91 a,A	55.53 ±3.53 a,A	56.36 ±1.56 a,A	56.94 ±4.84 a,A
		9	57.35 ±1.62 a,A	56.46 ±4.79 a,A	56.27 ±3.97 a,A	55.57 ±2.35 a,A	56.51 ±1.59 a,A	57.37 ±4.05 a,A
		12	57.93 ±3.27 a,A	56.59 ±4.00 a,A	56.28 ±2.38 a,A	55.63 ±3.61 a,A	56.59 ±3.15 a,A	57.52 ±3.14 a,A
	70°C	0	55.69 ±3.93 a,A	55.28 ±3.90 a,A	55.01 ±2.33 a,A	55.65 ±4.80 a,A	53.94 ±3.81 a,A	54.02 ±3.81 a,A
		3	55.85 ±3.94 a,A	55.82 ±3.94 a,A	55.27 ±4.69 a,A	56.65 ±3.14 a,A	55.65 ±3.93 a,A	55.72 ±3.9 a,A
		6	56.42 ±3.98a,A	56.32 ±3.98 a,A	56.10±2.38a,A	56.87 ±3.20 a,A	56.14a,A±3.96	56.22 ±3.97 a,A
		9	56.66±4a,A	56.63±4 a,A	56.6±2.40 a,A	56.95±5.60a,A	56.44±3.9 a,A	56.57±4 a,A
		12	57.64 ±2.44a,A	57.03±2.41a,A	56.64 ±4 a,A	57.39±3.13a,A	56.66 ±2.40 a,A	56.66±2.40a,A
	75°C	0	55.01 ±4.73 a,A	55.74 ±3.15 a,A	54.565 ±3.89 a,A	54.27 ±3.49 a,A	55.07 ±2.35 a,A	53.16 ±3.25 a,A
		3	55.83 ±3.96 a,A	56.07 ±5.55 a,A	55.14 ±4.73 a,A	54.65 ±2.30 a,A	55.16 ±3.12 a,A	54.73 ±3.93 a,A
		6	56.04 ±3.37 a,A	56.09 ±3.98 a,A	55.77 ±5.40 a,A	54.83 ±3.87 a,A	55.19 ±3.90 a,A	54.87 ±3.09 a,A
		9	56.86 ±3.11 a,A	56.25 ±3.97 a,A	56.13 ±2.54 a,A	55.95 ±3.16 a,A	55.27 ±4.68 a,A	55.62 ±1.68 a,A
		12	56.91 ±2.41 a,A	56.31 ±1.58 a,A	56.57 ±3.20 a,A	54.99 ±1.54 a,A	55.44 ±3.11 a,A	56.12 ±3 a,A
a*	65°C	0	14.74 ±0.83 a,A	14.54 ±1.02 a,A	15.00 ±1.06 a,A	15.74 ±0.43 a,A	15 ±0.63 a,A	14.52 ±0.82 a,A
		3	13.81 ±0.97 a,A	13.67 ±0.76 a,A	14.46 ±1.02 a,A,B	15.3 ±0.64 a,A	14.32 ±0.87 a,A,B	14.46 ±1.43 a,A
		6	13.79±0.78a,b,A	13.56 ±0.57 a,b,A	13.71 ±0.96 a,b,A,B	15.16 ±1 a,A	13.09 ±0.37 b,B,C	13.88 ±1.17 a,b,A,B
		9	13.29±0.37a,b,c,A	12.55 ±1.06 a,b,c,A	13.69 ±0.96 a,b,A,B	14.23 ±0.60 a,A	11.94 ±0.33 b,c,C	11.65 ±0.82 c,B
		12	12.98±0.73 a,b,A	12.4 ±0.87 a,b,A	12.21 ±0.51 a,b,B	13.74 ±0.89 a,A	11.64 ±0.65 b,C	11.43 ±0.64 b,B

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-C: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Tablo 4.13 (Cont.)** Changes in color values (L\*, a\*, b\*) of heat treated sucuks stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Heat Treatment Temperature	Storage Time (Days)	CASE TYPE						
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo	
a*	70°C	0	14.80 ±1.04 a,A	14.27 ±1.00 a,A	14.12 ±0.59 a,A	14.27 ±1.21 a,A	14.02 ±0.99 a,A	14.15 ±1.00 a,A	
		3	14.52 ±1.02 a,A	14.06 ±0.99 a,b,A	14.04 ±1.19 a,b,A	13.77 ±0.77 a,b,A	13.77 ±0.97 b,A,B	13.94 ±0.98 a,b,A,B	
		6	13.92 ±0.98 a,A	13.76 ±0.97 a,A	13.51 ±0.57 a,A	13.69 ±0.73 a,A	12.89 ±0.91 a,A,B	13.42 ±0.94 a,A,B	
		9	13.83 ±0.97 a,A	13.65 ±0.96 a,A	13.31 ±0.55 a,A	13.10 ±1.19 a,A	12.70 ±0.89 a,A,B	13.20 ±0.93 a,A,B	
		12	13.63 ±0.57 a,A	13.61 ±0.57 a,A	13.28 ±0.78 a,A	13.03 ±0.68 a,A	12.53 ±0.53 a,B	13.04 ±0.55 a,B	
	75°C	0	14.12 ±1.19 a,A	13.33 ±0.75 a,A	13.45 ±0.95 a,A	14.9 ±0.94 a,A	13.88 ±0.58 a,A	13.95 ±0.82 a,A	
		3	14.05 ±0.99 a,A	13.85 ±1.37 a,A	13.16 ±1.11 a,A	14.55 ±0.61 a,A	13.62 ±0.77 a,A	13.83 ±0.97 a,A	
		6	13.97 ±0.82 a,A	13.69 ±0.96 a,A	13.07 ±1.29 a,A	12.66 ±0.89 a,B	13.44 ±0.95 a,A	13.61 ±0.76 a,A,B	
		9	13.45 ±0.76 a,A	13.52 ±0.95 a,A	12.24 ±0.55 a,A	12.41 ±0.70 a,B	12.32 ±1.04 a,A	13.75 ±0.38 a,A,B	
		12	13.21 ±0.56 a,A	11.84 ±0.33 b,A	11.73 ±0.66 b,A	11.65 ±0.32 b,B	11.68 ±0.66 b,A	11.79 ±0.66 b,B	
	b*	65°C	0	16.55 ±0.70 a,B	12.74 ±0.90 a,A	12.41 ±0.87 a,A	13.09 ±0.37 a,A	12.67 ±0.53 a,A	13.05 ±0.74 a,A
			3	14.93 ±1.05 a,A	12.83 ±0.72 a,b,A	12.12 ±0.85 b,A	11.92 ±0.51b,A,B	12.43 ±0.75 b,A,B	12.56 ±1.24 b,A
6			15.85 ±0.89 b,A	13 ±0.55 a,A	12.04 ±0.85 a,A	11.95 ±0.76 a,A,B	12.13 ±0.34 a,A,B	11.34 ±0.96 a,A	
9			15.49 ±0.43 a,A	12.47 ±1.05 b,c,A	11.24 ±0.79 b,c,A	10.84 ±0.48 c,B,C	12.21 ±0.34 b,c,A,B	12.79 ±0.90 b,A	
12			12.43 ±0.93 a,A	10.28 ±0.72 b,B	10.46 ±0.44 b,A	10.20 ±0.66 b,C	11.15 ±0.63 b,B	11.54 ±0.65 b,A	
70°C		0	14.89 ±1.05 a,A	13.30a,A±0.94	12.12 ±0.51 a,A	12.40 ±1.05 a,A	12.40 ±0.87 a,A	13.20 ±0.93 a,A	
		3	14.78 ±1.04 a,A	11.86b,A,B±0.83	11.57 ±0.94 b,A	11.90 ±0.64 b,A	11.36 ±0.80 b,A	11.73 ±0.82 b,A	
		6	14.45 ±1.02 a,A	11.75 ±0.83 b,c,A,B	11.33 ±0.48 b,A	11.36 ±0.52 c,A,B	10.58 ±0.74 b,A	10.90 ±0.77 b,c,A	
		9	14.24 ±1.00 a,A	11.02 ±0.77 b,B	11.28 ±0.47 b,A	10.92 ±0.99b,A,B	10.02 ±0.70 b,A	10.60 ±0.74 b,A	
		12	14.14 ±0.60 a,A	10.76 ±0.45 b,c,B	11.01 ±0.81 b,A	10.02 ±0.67 b,B	10.19 ±0.43 b,c,A	10.28 ±0.43 c,A	
75°C		0	16.37 ±1.20 a,A,B	13.90 ±0.74 a,b,A	12.55 ±0.82 b,A	12.99 ±0.78 a,b,A,B	12.97 ±0.55 a,b,A	11.97 ±0.68 b,A,B	
		3	15.26 ±1.07 a,A,B	13.11 ±1.37 a,b,A	11.74 ±1.06 b,A	12.26 ±0.55 a,b,A	12.89 ±0.70 b,A	11.58 ±0.84 b,A	
	6	14.89 ±0.80 a,B	12.96 ±0.91 a,A	11.62 ±1.16 a,b,A	11.50 ±0.81 a,b,A,B	12.46 ±0.81 a,b,A,B	10.65 ±0.60 b,A,B		
	9	14.18 ±0.84 a,A,B	12.43 ±0.87 b,A	11.12 ±0.50 b,c,A	11.03 ±0.62 b,c,B	11.55 ±1.09 b,A,B	10.28 ±0.29 c,B		
	12	13.57 ±0.68 a,A	11.49 ±0.32 b,A	10.43 ±0.59 b,c,A	10.73 ±0.30 b,c,B	10.11 ±0.57 c,B	10.08 ±0.57 c,B		

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-C: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

Additionally higher  $a^*$  values were observed in high-oxygen compared to vacuum packaged turkey and pig meat (Blacha et al., 2013) and in pork sausages (Martínez et al., 2006). Blacha et al. (2013), Orkusz et al. (2013) and Deus et al. (2017) showed the opposite influences with higher  $a^*$  value in vacuum packaged goose and turkey meat, respectively. Azlin-Hasim et al. (2015, 2016) and Deus et al. (2017) reported that there were no influences on the  $L^*$  values of chicken or turkey meat using nanosilver film. The findings of the nanosilver coated film primarily agree with Azlin-Hasim et al. (2015, 2016) who only reported importantly higher  $a^*$  values of the nanosilver wrapped chicken meat 3<sup>rd</sup> and 6<sup>th</sup> day of storage, respectively. However, Deus et al. (2017) found that the nanosilver coated film importantly reduced the  $a^*$  values of the turkey meat on days 1, 4 and 8. Duan et al. (2010) determined that it had no influences of chitosan coating process on  $L^*$ ,  $a^*$ , and  $b^*$  values of cold stored fish products during storage. However, Mastromatteo et al. (2010) investigated using of lysozme, nisin and EDTA combined for providing high quality of packed ostrich patties. They found that high amount of nisin and lysozme could cause the biggest color loss. Additionally, gelatin-based antimicrobial edible coating containing nisin, EDTA and potassium sorbate improved the shelf life of chilled chicken was reported by Liang et al. (2011). The chilled chicken was stored at 4°C. They also found that sample color values was developed during early storage period by a gelatin-based antimicrobial edible coating containing nisin and potassium sorbate. Additionally, goat sausage was made with the aim of measuring the influence of including of chitosan on quality and shelf life by Do Amaral et al. (2016). They prepared sausages including 2% chitosan with different fat values (5%, 12.5% and 20%, w/w) and stored at refrigerated temperature for 15 days. Do Amaral et al. (2016) found that the incorporation of chitosan resulted in the lowest  $L^*$  and  $b^*$  levels, while control sausages (no chitosan added) had importantly ( $P < 0.05$ ) higher levels. This effect could be due to the antioxidant attributes of chitosan. But, normally the  $b^*$  values rise during storage with the violence of the oxidation process that inclines to increase the yellowness of sausages induced by rancidification (Fernández-López and Vicente, 2000). Sayas-Barberá et al. (2011) found same results in pork model burgers, pointing out that the increase in  $L^*$  values during the 0<sup>th</sup> day could be connected with oxidation increasing metmyoglobin formulation. Also, Do Amaral et al. (2016) reported that during storage, the lightness ( $L^*$ ) and

redness ( $a^*$  values) of fresh goat sausages reduced importantly ( $P < 0.05$ ), residual lower in samples with incorporated chitosan when compared with the corresponding sausages used as a control. This influence may be expressed by the fact that chitosan exists antioxidant attributes which may contribute to preserve redness in muscle foods, for its capability to treat as a chelator on transition metal ions which catalyze oxidative reagents of myoglobin (Zimoch et al., 2016). According to Georgantelis et al. (2007) chitosan could be chelating iron ions of meat hemoproteins during heat treatment or storage. Similar results were also provided for pork meat products with chitosan (Sayas-Barberá et al., 2011). Lopez-Caballero et al. (2005) reported higher  $L^*$  value of patties coated with chitosan than in the control group at an initial phase of storage, then detected a rise in the  $L^*$  value of chitosan coating group during storage. Siripatrawan and Noipha (2012) observed rises in  $L^*$  and  $b^*$  values in chitosan wrapped pork sausages during storage and additionally observed lower changes in color due to chitosan wraps, and these lower color varies were expressed by antioxidant and antimicrobial attributes of chitosan.

**Table 4.14** The effect of different heat process temperature on color values ( $L^*$ ,  $a^*$ ,  $b^*$ )

Parameter	Storage Time (Days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
$L^*$	0	65 °C	55.54±3.14a	55.17±3.90a	55.16±3.90a	54.75±1.54a	55.85±2.3a	55.14±3.1a
		70°C	55.69±3.93a	55.28±3.90a	55.01±2.33a	55.65±4.80a	53.94±3.8a	54.02±3.8a
		75°C	55.01±4.73a	55.745±3.1a	54.56±3.89a	54.27±3.49a	55.07±2.3a	53.16±3.2a
	3	65 °C	56.16±3.97a	55.33±3.13a	55.19 ±3.9a	55.26±2.34a	56.01±3.4a	55.31±5.4a
		70°C	55.85±3.94a	55.82±3.94a	55.27±4.69a	56.65±3.14a	55.65±3.9a	55.72±3.9a
		75°C	55.83±3.96a	56.07±5.55a	55.14±4.73a	54.65±2.30a	55.16±3.1a	54.73±3.9a
	6	65 °C	57.32±3.24a	55.96±2.37a	55.31±3.91a	55.53±3.53a	56.36±1.5a	56.94±4.8a
		70°C	56.42±3.98a	56.32±3.98a	56.10±2.38a	56.87±3.20a	56.14±3.9a	56.22±3.9a
		75°C	56.04 ±3.37a	56.09±3.98a	55.77±5.40a	54.83±3.87a	55.19±3.9a	54.87±3.0a
	9	65 °C	57.35±1.62 a	56.46±4.79 a	56.27±3.97a	55.57±2.35a	56.51±1.5a	57.37±4.0a
		70°C	56.66±4.00 a	56.63±4.00 a	56.62±2.40a	56.95±5.60a	56.44±3.9a	56.57±4.0a
		75°C	56.86±3.11 a	56.25±3.97 a	56.13±2.54a	55.95±3.16a	55.27±4.6a	55.62±1.6a
	12	65 °C	57.93±3.27 a	56.59±4.00 a	56.28±2.38a	55.63±3.61a	56.59±3.1a	57.52±3.1a
		70°C	57.64±2.44 a	57.03±2.41 a	56.64±4.00a	57.39±3.13a	56.66±2.4a	56.66±2.4a
		75°C	56.91±2.41 a	56.31±1.58 a	56.57±3.20a	54.99±1.54a	55.44±3.1a	56.125±3 a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.14 (Cont.)** The effect of different heat process temperature on color values (L\*, a\*, b\*)

Parameter	Storage Time (Days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
a*	0	65 °C	14.74±0.83a	14.54±1.02a	15±1.06 a	15.74±0.43a	15±0.63 a	14.52±0.8a
		70°C	14.80±1.04a	14.27±1.00a	14.12±0.59a	14.27±1.21a	14.02±0.9a	14.15±1.0a
		75 °C	14.12±1.19a	13.33±0.75a	13.45±0.95a	14.9±0.94 a	13.88±0.5a	13.95±0.8a
	3	65 °C	13.81±0.97a	13.67±0.76a	14.46±1.02a	15.3±0.64 a	14.32±0.8a	14.46±1.4a
		70°C	14.52±1.02a	14.06±0.99a	14.04±1.19a	13.77±0.77a	13.77±0.9a	13.94±0.9a
		75 °C	14.05±0.99a	13.85±1.37a	13.16±1.11a	14.55±0.61a	13.62±0.7a	13.83±0.9a
	6	65 °C	13.79±0.78a	13.56±0.57a	13.71±0.96a	15.16±1.00a	13.09±0.3a	13.88±1.1a
		70°C	13.92±0.98a	13.76±0.97a	13.51±0.57a	13.69±0.73a	12.89±0.9a	13.42±0.9a
		75 °C	13.97±0.82a	13.69±0.96a	13.07±1.29a	12.66±0.89a	13.44±0.9a	13.61±0.7a
	9	65 °C	13.29±0.37a	12.55±1.06a	13.69±0.96a	14.23±0.60a	11.94±0.3a	11.65±0.8a
		70°C	13.83±0.97a	13.65±0.9a	13.31±0.55a	13.1±1.19 a	12.70±0.8a	13.20±0.9a
		75 °C	13.45±0.76a	13.52±0.95a	12.24±0.55a	12.41±0.70a	12.32±1.0a	13.75±0.3a
12	65 °C	12.98±0.73a	12.4±0.87 a	12.21±0.51a	13.74±0.89a	11.64±0.6a	11.43±0.6a	
	70°C	13.63±0.57a	13.61±0.57a	13.28±0.78a	13.03±0.68a	12.53±0.5a	13.04±0.5a	
	75 °C	13.21±0.56 a	11.84±0.33a	11.73±0.66a	11.65±0.32a	11.68±0.6a	11.79±0.6a	
b*	0.	65 °C	16.55±0.70a	12.745±0.9a	12.415±0.8a	13.09±0.37a	12.67±0.5a	13.05±0.7a
		70°C	14.89±1.05a	13.30±0.94a	12.12±0.51a	12.40±1.05a	12.40±0.8a	13.20±0.9a
		75 °C	16.37±1.20a	13.90±0.74a	12.55±0.82a	12.99±0.78a	12.97±0.5a	11.97±0.6a
	3.	65 °C	14.93±1.05a	12.83±0.72a	12.12±0.85a	11.92±0.5 a	12.43±0.7a	12.56±1.2a
		70°C	14.78±1.04a	11.86±0.83a	11.57±0.94a	11.90±0.64a	11.36±0.8a	11.73±0.8a
		75 °C	15.26±1.07a	13.11±1.37a	11.74±1.06a	12.26±0.55a	12.89±0.7a	11.58±0.8a
	6.	65 °C	15.85±0.89a	13.00±0.55a	12.04±0.85a	11.95±0.76a	12.13±0.3a	11.34±0.9a
		70°C	14.45±1.02a	11.75±0.83a	11.33±0.48a	11.36±0.52a	10.58±0.7a	10.90±0.7a
		75 °C	14.89±0.80a	12.96±0.91a	11.62±1.16a	11.50±0.81a	12.46±0.8a	10.65±0.6a
	9.	65 °C	15.49±0.43a	12.47±1.05a	11.24±0.79a	10.84±0.48a	12.21±0.3a	12.79±0.9a
		70°C	14.24±1.00a	11.02±0.77a	11.28±0.47a	10.92±0.99a	10.02±0.7a	10.60±0.7a
		75 °C	14.18±0.84a	12.43±0.87a	11.12±0.50a	11.03±0.62a	11.55±1.0a	10.28±0.2a
12.	65 °C	12.43±0.93a	10.28±0.72a	10.46±0.44a	10.20±0.66a	11.15±0.6a	11.54±0.6a	
	70°C	14.14±0.60a	10.76±0.45a	11.01±0.81a	10.02±0.67a	10.19±0.4a	10.28±0.4a	
	75 °C	13.57±0.68a	11.49±0.32a	10.43±0.59a	10.73±0.30a	10.11±0.5a	10.08±0.5a	

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

## 4.6 Texture Profile Analysis

### 4.6.1 Fermentation

Texture profiles of sucuks (adhesiveness, hardness, chewiness, cohesiveness and gumminess) were observed during the fermentation and their findings are shown in Table 4.15. Hardness value of all sucuk samples increased ( $P < 0.05$ ) during fermentation (Table 4.15), due to the coagulation of protein at low moisture content and pH (Bozkurt and Bayram, 2006). Sucuks stuffed into collagen case had higher

hardness value than those of other sucuks ( $P < 0.05$ ) (Table 4.15). The highest hardness value was 26.00 N in the sucuks stuffed into collagen case whereas the lowest hardness value was 14.00 N in the AgZeo case at the end of fermentation ( $P < 0.05$ ). Additionally, there were no significant differences ( $P > 0.05$ ) between hardness values among antimicrobial cases (Table 4.15).

The gumminess and chewiness values of sucuks stuffed into cases incorporated with active compounds (nisin, potassium sorbate, AgZeo and chitosan), control plastic cases and artificial collagen case increased ( $P < 0.05$ ) from 5.31 N to 17.68 N and from 3.34 mJ to 12.38 mJ during fermentation, respectively (Table 4.15). Similarly, an increase in hardness, chewiness and gumminess in fermented sausages reported by Gou et al., 1996. At the same time there were significant differences ( $P < 0.05$ ) both between gumminess and chewiness values of all samples on day 3. Additionally, hardness, gumminess and chewiness values of the sucuks stuffed into nisin and potassium sorbate incorporated cases were not different ( $P > 0.05$ ) from each other in the fermentation period. Adhesiveness values reduced importantly ( $P < 0.05$ ) from -4.00 mJ to -6.70 mJ during fermentation (Table 4.15). Thus, sucuks were cut easily by this reason; it would be more suitable (Bozkurt and Bayram, 2006). There were no important differences ( $P > 0.05$ ) between adhesiveness values of all samples. Additionally, cohesiveness and springiness values of all sucuks were not influenced ( $P > 0.05$ ) by use of active case during fermentation (Table 4.15). However, Barbut (2005) reported that reduce in springiness in salami type products with reducing pH has been found.

#### **4.6.2 Storage**

The highest hardness value was seen in the sucuks stuffed into collagen case whereas the lowest hardness value was observed in the sucuks stuffed into chitosan case ( $P < 0.05$ ) both after heat treatment at 65°C and end of the storage (Table 4.16). These values were 42.88, 19.59 and 91.58, 42.88 N after heat treatment at 65°C and end of the storage respectively. Additionally, there were no significant differences ( $P > 0.05$ ) between in the sucuks stuffed into nisin, AgZeo and control plastic at the end of storage. Furthermore, the hardness values increased by heat treatment at 65°C, 70°C and 75°C during storage (Table 4.16).

**Table 4.15** Changes in texture properties of sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Parameters	Fermentation Time (Days)	CASE TYPE					
		Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Hardness (N)	0	8.85±0.63a,A	8.85±0.63a,B	8.85 ±0.63 a,B	8.85 ±0.63 a,B	8.85±0.63a,B	8.85 ±0.63a,B
	3	26.00 ±1.83 a,B	17.00 ±1.20 b,A	19.00 ±1.34 b,A	17.00 ±1.20 b,A	15±1.06b,A	14.00 ±0.98 b,A
Gumminess (N)	0	5.31 ±0.37 a,B	5.31 ±0.37 a,B	5.31 ±0.37 a,B	5.31 ±0.37 a,B	5.31±0.37a,B	5.31 ±0.37 a,B
	3	17.68 ±1.25 a,A	12.24 ±0.86 b,c,A	12.96 ±0.91 b,A	13.49 ±0.95 b,A	10.50±0.74c,d,A	9.10 ±0.64 d,A
Chewiness (mJ)	0	3.34 ±0.23 a,B	3.34 ±0.23 a,B	3.34 ±0.23 a,B	3.34 ±0.23 a,B	3.34 ±0.23a,B	3.34 ±0.23 a,B
	3	12.37 ±0.87 a,A	8.69±0.61c,A	9.33 ±0.57 b,c,A	10.38 ±0.48 b,A	8.19 ±0.57 c,A	6.82 ±0.48 d,A
Adhesiveness (mJ)	0	-4.00 ±0.28 a,A	-4.00 ±0.28 a,A	-4.00 ±0.28 a,A	-4.00 ±0.28 a,A	-4.00 ±0.28 a,A	-4.00 ±0.28 a,A
	3	-5.00 ±0,35 a,B	-6.00 ±0.40 a,B	-6.60 ±0.46 a,B	-6,70 ±0,43 a,B	-6.70 ±0.47 a,B	-6.50 ±0.45 a,B
Springiness (mm)	0	0.63±0.04 a,A	0.63±0.04a,A	0.63 ±0.04 a,A	0.63 ±0.04 a,A	0.63±0.04a,A	0.63±0.04a,A
	3	0.70±0.04a,A	0.71±0.05a,A	0.76 ±0.01 a,A	0.77 ±0.02 a,A	0.78±0.04a,A	0.75±0.03a,A
Cohesiveness	0	0,6 ±0,04a,A	0,60±0,04a,A	0,60 ±0,04 a,A	0,60 ±0,04 a,A	0,60±0,04a,A	0,60±0,04a,A
	3	0,68±0,04a,A	0,72±0,04a,A	0,72 ±0,04a,A	0,71 ±0,04 a,A	0.70±0.04a,A	0.65±0.04a,A

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the fermentation time at  $\alpha= 0.05$  level in each column.

These changes may be coagulation of protein at low moisture and pH (Bozkurt and Bayram, 2006). However, Abdulhameed et al. (2016) explained the changes in texture of chicken sausage during superheated steam cooking. Chicken sausages were cooked at temperature ranging from 150–200°C with treatment times ranging from 2–6 mins. It could be estimated that the hardness reduced with both time and temperature. The first hardness value was 5.69 kgf and this reduced to 3.53 kgf at 200°C (6 min). The reduce in the hardness value could be associated with the softening in the connective tissue induced by the conversion of collagen to gelatin which developed meat tenderness (Dhanapal et al., 2012). Similarly García-Segovia (2008) reported that the hardness value reduced as the temperature and time rised because of starch gelatinization during the cooking operation.

Sucuks stuffed into collagen case had the highest hardness value (58.35 N) while sucuks stuffed into potassium sorbate had the lowest hadness value (22.59 N) after heat treatment at 70°C. At the end of storage, 95.00 N was the highest hardness value in collagen case sample, 44.00 was the lowest hardness value in chitosan samples (Table 4.16) There were significant differences ( $P < 0.05$ ) between sucuks stuffed into nisin, chitosan, control plastic and collagen case (Table 4.16). Similarly, Do Amaral et al. (2016) incorporated 2% chitosan and different fat values (5%, 12.5% and 20%, w/w) in goat sucuks and stored at 4°C for 15 days. Results provided from the goat sausage texture indicated that the incorporation of chitosan rised the hardness values ( $5.99 \pm 0.07$  to  $7.27 \pm 0.08$ ) when compared with their control sausages, without chitosan and the same value of fat ( $5.66 \pm 0.25$  to  $5.81 \pm 0.49$ ). Concerning cooked goat sausage, the sausages with chitosan indicated higher hardness values than the control. This influence can be due to the fact that chitosan have the capability to act as a binder, thus promoting the formation of a stronger gel favoring as a more stable structure. After 15 days of refrigeration storage, the hardness of cooked goat sausage increased significantly ( $P < 0.05$ ) in all batches, with this rise being importantly higher in the samples with chitosan than in the control. This attitude can be expressed not only by the slight drying of the product during storage but additionally because of the stabilization of chitosan linkages with matrix ingredients at refrigerated temperature (Ganhão et al.2010). Youn et al. (1999) found that the hardness of the sausage rised by an increase of molecular weight of chitosan, and especially, at the highest molecular weight (120,000) rised viscosity which may

reason negatively influence on processing. However, García et al. (2010) in pork sausage found that chitosan addition did not impact significantly the texture profile results.

There were no significant differences between heat treated sucuks at 75°C stuffed into chitosan and potassium sorbate beginning of the storage. However, Thomas et al. (2007) investigated that shelf stable pork sausages were improved using hurdle technology (low pH, low water activity, vacuum packaging and post package reheating) and stored at 37±1°C. Dipping in potassium sorbate solution previous to vacuum packaging was investigated too. Hurdle process reduced the hardness by 27%, while dipping in K-sorbate reduced it by 28.6% compared to the control. The better fat and water holding due to higher pH and lack of reheating easily conducted to the importantly higher hardness and cohesiveness in the control sausages. It might be due to the formation of good quality gel matrix as a result of decreased muscle protein denaturation. Likewise, the highest hardness value (67.63 N) was seen in the sucuks stuffed into collagen case whereas the lowest hardness value (23.36 N) was monitored in the sucuks stuffed into AgZeo case on the (P< 0.05) beginning of storage. Additionally, there were no significant differences between heat treated sucuks at 75°C stuffed into chitosan and AgZeo on 12 day storage. At the end of storage, 100.00 N was the highest hardness value in collagen case sample, 52.02 N was the lowest hardness value in nisin samples (Table 4.16).

Gumminess is the force essential to disintegrate a semi-solid state sample until swallowing and chewiness is described as the product of hardness and cohesiveness (Do Amaral et al., 2016). The highest gumminess value was found in the sucuks stuffed into collagen case whereas the lowest gumminess value was observed in the sucuks stuffed into chitosan case (P< 0.05) after heat treatments at 65°C and on 12 days of storage (Table 4.17). These values were 33.23, 16.21 and 65.02, 31.94 N. There were no significant differences (P> 0.05) between gumminess values of heat treated sucuks at 65°C stuffed into nisin and AgZeo beginning of storage (0 day). Moreover, at the end of storage, there were no significant differences (P> 0.05) between gumminess values of sucuks stuffed into AgZeo and potassium sorbate (Table 4.17).

**Table 4.16** Changes in hardness of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	StorageTime (Days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Hardness (N)	65°C	0	42.8 ±2.25 a,C	24.62 ±1.41 b,c,B	27.41 ±1.38 b,C	21.11 ±1.97 c,d,C	19.59 ±1.31 d,B	23.47 ±1.27 b,c,d,C
		3	65.46±2.90 a,B	26.16 ±1.80 b,c,B	31.24 ±2.09 b,C	26.53 ±1.25 b,c,B	22.84 ±1.89 c,B	30.37 ±1.00 b,B
		6	81.00±4.82 a,A	40.41 ±1.14 b,A	37.73 ±2.67 b,B	27.06 ±1.22 c,B	39.09±11.05 b,A	30.72 ±1.07 c,B
		9	91.00 ±2.37a,A	42.55 ±1.60 b,A	41.63 ±2.43 b,c,B	40.73 ±1.28 b,c,A	39.25 ±1.10 b,c,A	35.90 ±2.39 c,B
		12	91.58 ±3.80a,A	46.27 ±2.72 b,c,A	52.17 ±2.13 b,A	44.80 ±2.14 b,c,A	42.88 ±1.25 c,A	50.98 ±2.84 b,c,A
	70°C	0	58.35 ±1.25a,C	33.34 ±2.35 b,C	22.59 ±1.58d,D	28.48 ±2.19 b,c,d,C	24.79 ±1.53 c,d,C	30.30 ±2.71 b,c,C
		3	76.88 ±1.74a,B	37.93±3.51b,c,B,C	31.48 ±2.70c,d,C	40.72 ±3.55 b,B	32,57 ±1.42 c,d,B	30.45 ±1.22 d,C
		6	80.87 ±6.21a,B	38.72 ±3.30b,c,B,C	43.11 ±1.29b,B	40.81 ±17.31 b,c,B	35.22 ±1.92 b,c,B	33.79 ±1.11 c,B,C
		9	85.31±5.26 a,A,B	42.51 ±2.78 b,c,B	47.24 ±2.04b,A,B	46.56 ±13.16 b,B	41.79 ±2.18 b,c,A	38.34 ±3.95 c,B
		12	95.00 ±7.17a,A	67.68 ±1.14 b,A	50.04 ±3.38c,d,A	58.55 ±16.56 c,A	44.83 ±1.20 d,A	50.12 ±2.35 c,d,A
	75°C	0	67.63 ±5.38a,B	35.80 ±2.25 b,B	31.54 ±2.30 b,c,C	26.27 ±1.72c,d,A	30.93 ±1.12 b,c,D	23.36 ±1.88 d,E
		3	74.23±2.49 a,B	35.94 ±3.55 b,c,B	39.21 ±3.72 b,c,C	32.71±1.87 b,c,A	40.48 ±2.87 b,C	31.01 ±2.93 c,D
		6	95.33±5.62 a,A	53.86 ±3.80 b,A	51.16 ±5.64 b,B	37.68 ±2.64 c,B	52.11 ±3.84 b,B	40.01±2.63 c,C
		9	97.78±5.31 a,A	56.56 ±3.99 b,A	53.45 ±2.18 b,B	49.94 ±2.28 b,B	58.09 ±4.29 b,B	55.46 ±1.68 b,B
		12	100 ±2.42 a,A	60.50 ±1.11 c,d,A	67.97±38,44 b,c,A	52.02 ±1.71 d,C	72.41 ±5.96 b,A	74.98 ±4.42 b,A

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4. 17** Changes in gumminess of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	StorageTime (Days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Gumminess (N)	65°C	0	33.23 ±1.87 a,C	20.43 ±1.44 b,c,C	22.47 ±1.58 b,D	19.21±0.54 c,d,B	16.26 ±0.57 d,B	19.21 ±1.08 c,d,B
		3	48.77 ±3.44 a,B	21.58 ±1.22 b,c,B	25.31±1.78 b,C,D	21.89±0.92 b,c,B	18.38 ±1.12 c,B	21.89 ±2.1 b,B
		6	59.53 ±3.36 a,A	32.33 ±1.37 b,B	29.99 ±2.12 b,B,C	21.92 ±1.39c,A	31.27 ±0.88 b,A	21.92 ±1.85 c,B
		9	65.97 ±1.86 a,A	32.34 ±2.74 b,B	31.63 ±2.23b,c,B	32.17±1.36b,c,A	31.00±0.87 b,c,A	32.17 ±2.27 c,A
		12	65.02 ±3.67 a,A	34.47 ±2.43 b,c,A	38.86 ±1.64 b,A	33.15 ±2.15 b,c,A	31.94 ±1.80 c,A	33.15 ±1.87 b,A
	70°C	0	44.05 ±3.10a,B 0	27.74 ±1.96 b,C	19.09 ±0.81 d,D	27.74 ±1.35 b,c,B	20.45 ±1.44 c,d,C	25.60 ±1.81 b,c,d,C
		3	56.51 ±3.99 a,A	31.07 ±2.19 b,c,B	25.97 ±2.20 c,d,C	31.07 ±0.75 d,B	26.55 ±1.87 c,d,B	24.97 ±1.76 b,C
		6	58.63 ±4.14 a,A	31.30 ±2.21 b,c,B	34.27 ±1.45 b,B	31.30 ±0.77 c,B	28.70 ±2.02 b,c,B	27.03 ±1.91 b,c,B,C
		9	61.00 ±4.31 a,A	33.22 ±2.34 b,c,B	37.09 ±1.57 b,A,B	33.22 ±1.28 c,B	33.22 ±2.34 b,c,A	30.67 ±2.16 b,c,B
		12	66.50 ±2.82 a,A	51.77 ±2.19 b,A	39.28 ±2.77 c,A	50.42 ±2.86 c,A	35.41 ±1.50 c,A	39.34 ±1.66 b,A
	75°C	0	53.09 ±4.50 a,B	19.98 ±1.13 b,D	26.65 ±1.88 b,c,C	30.43 ±1.93 c,d,B	26.44 ±1.12 b,c,D	22.59 ±1.34 d,C
		3	57.53 ±4.00 a,B	25.27 ±2.50b,c,d,D	32.34 ±2.74 b,c,C	29.65 ±1.25 c,d,B	34.57 ±1.95 b,C	27.31 ±1.93 d,B
		6	73,40 ±2.98 a,A	31.40 ±2.22 b,C	41.44 ±4.10 b,B	43.62 ±3.08 c,A	44.29 ±3,13 b,B	30.90 ±1.75 c,B
		9	70.40 ±3.36 a,A	43.53 ±3.07 b,B	43.29 ±1.95 b,B	45.81 ±2.59 b,A	47.05 ±3,99 b,B	39.74 ±1.12 b,A
		12	68.50 ±1.90 a,A	58.11 ±1.65 c,A	54.71 ±3.08 b,A	48.40 ±1.36 d,A	58.29 ±3.29 b,A	41.09 ±2.32 b,A

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4. 18** Changes in chewiness of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	StorageTime (Days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Chewiness (mJ)	65°C	0	30.11 ±1.70 a,C	16.58 ±1.17 b,c,C	18.37 ±1.29 b,C	16.16 ±0.45 b,c,C	13.11 ±0.55 d,B	16.16c,d,B±0.61
		3	42.67 ±3.01 a,B	17.51±0.99b,c,B,C	19.86 ±1.40 b,B,C	17.45 ±0.74 b,c,B	14.48 ±0.08 c,B	17.45b,B±0.72
		6	50.45 ±2.85 a,A	26.06 ±1.10 b,B,C	23.39 ±.16b,B	17.31 ±1.10 c,A	23.77 ±0.06 b,A	17.31c,B±0.46
		9	52.69 ±1.49 a,A	22.92 ±1.95 b,c,B	23.57 ±1.66 b,c,B	25.34 ±1.07 b,B	23.56 ±0.66 b,c,A	25.34c,A±0.79
		12	50.15 ±2.83 a,A	23.87 ±1.68 c,d,A	28.03 ±1.18 b,c,A	25.4±1.65b,c,d,A,B	22.68 ±1.28 d,A	25.40b,A±0.43
	70°C	0	33.31 ±2.35 a,B	23.34 ±1.65 b,C	15.30 ±0.44 d,C	23.34 ±0.98 c,B	16.69 ±1.18 c,d,C	21.50 ±1.52 b,c,B
		3	42.38 ±2.99 a,A	25.59 ±1.80 b,B	20.48 ±0.73 c,B	25.59 ±0.44 c,B	20.90 ±1.47 c,B	20.35 ±1.43 b,B
		6	42.36 ±2.99 a,A	24.80 ±1.75 b,c,B	25.96 ±1.10b,A	24.80 ±0.40 c,B	22.42 ±1.58 b,c,B	20.24 ±1.43 b,B
		9	43.23 ±3.05 a,A	25.91 ±1.83 b,c,B	27.86 ±1.18 b,A	25.91 ±1.56 c,B	25.83 ±1.82 b,c,A	22.92 ±1.62 b,B
		12	46.38 ±1.96 a,A	39.54 ±1.67 b,A	29.02 ±1.05 c,A	38.50 ±1.17 c,A	26.60 ±1.12 c,A	29.11 ±1.23 b,A
	75°C	0	52.09 ±2.44 a,A,B	17.48 ±0.86 b,D	22.42 ±1.58 b,c,C	24.53 ±1.56 c,C	22.44 ±0.95 b,c,D	18.75 ±1.11 c,C
		3	50.62 ±2.58 a,B	20.57 ±1.03 b,c,d,D	27.13 ±2.30 b,c,C	23.83 ±1.01 c,d,C	29.17 ±1.65 b,C	22.56 ±1.02 d,B
		6	60.74 ±1.60 a,A	25.48 ±0.80 b,C	34.49 ±3.41 b,B	35.06 ±2.47 c,B	36.37 ±2.57 b,B	24.87 ±0.40 c,B
		9	57.29 ±1.24 a,A,B	33.25 ±1.35 b,c,B	34.36 ±1.55 b,c,B	35.96 ±2.03 c,B	38.52 ±2.30 b,B	31.15 ±0.43 b,c,A
		12	54.02 ±1.29 a,A,B	42.49 ±1.20 c,A	42.67 ±2.41 b,A	35.69 ±1.00 d,B	45.32 ±1.56 b,A	28.56 ±0.61 b,A

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

There were important differences ( $P>0.05$ ) between gumminess values of heat treated sucuks at 70°C stuffed into active cases, control plastic and collagen case at the beginning of storage (0 day). Also, the highest gumminess value (44.05 N) was seen in the sucuks stuffed into collagen case whereas the lowest gumminess value (19.09 N) was monitored in the sucuks stuffed into potassium sorbate case ( $P< 0.05$ ) on first day of storage. At the end of storage, 66.50 N was the highest gumminess value in collagen case sample, 35.41 N was the lowest gumminess value in chitosan samples (Table 4.17). There were significant differences ( $P< 0.05$ ) between sucuks stuffed into collagen cases and active cases (nisin, potassium sorbate, Agzeo, chitosan) (Table 4.17). Likewise, the effect of nisin on the textural attributes of buffalo meat sausage detected by Sureshkumar et al. (2010). They reported that the combination of nisin and BHA had better effect on textural characteristics of meat sausage.

Heat treatment at 75°C, there were significant differences ( $P< 0.05$ ) between gumminess values of sucuks except potassium sorbate and chitosan samples both at the beginning and last day of storage (Table 4.17). Similarly, Do Amaral et al. (2016) reported that the other textural factors in always were not influenced by the incorporation of chitosan. Concerning the other factors of the texture profile, chitosan supplementation increased slightly the values of cohesiveness, chewiness, springiness and gumminess of cooked sausages compared with control, but this trend was not always important. Sucuks stuffed into collagen case had higher ( $P< 0.05$ ) gumminess values than those of other samples at the end of storage. Collagen case sample had 68.50 N gumminess values whereas gumminess values of active cases ranged from 41.09-58.29 N stored after 12 days. Also, heat treated sucuks at 75°C stuffed into AgZeo had the lowest gumminess value (41.09 N). Moreover, the gumminess values of sucuk samples at all temperature (65 °C, 70 °C and 75°C) increased ( $P< 0.05$ ) during storage (Table 4.17).

The highest ( $P< 0.05$ ) chewiness value was found in the sucuks stuffed into collagen case but the lowest ( $P< 0.05$ ) chewiness value was seen in the sucuks stuffed into chitosan case ( $P< 0.05$ ) both after heat treatment at 65°C and on 12 day storage (Table 4.18). These values were 30.11, 13.11 and 50.15, 22.68 mJ after heat treatment at 65°C and end of the storage, respectively. Besides, the chewiness values

of collagen case samples were significantly different ( $P < 0.05$ ) than those of active case samples during storage.

There were important differences ( $P < 0.05$ ) of chewiness values of heat treated sucuks at 70°C stuffed into active cases, control plastic and collagen case at the first day of storage. Sucuks stuffed into collagen case had higher chewiness values ( $P < 0.05$ ) than those of other sucuks during storage. At the end of storage, potassium sorbate and Agzeo sucuk samples had the lowest chewiness value (29.02 mJ). Also, there were no significant differences ( $P > 0.05$ ) the chewiness values of sucuks stuffed into nisin, potassium sorbate and chitosan at the last day of storage. Also, the chewiness values of heat treated samples at 65°C and 70°C samples increased ( $P < 0.05$ ) during storage (Table 4.18).

Additionally, heat treated sucuks at 75°C stuffed into collagen case sample had 52.09 mJ chewiness values, but chewiness values of active cases ranged from 17.48-24.53 mJ at the beginning of storage ( $P < 0.05$ ). At the same time, the chewiness values of collagen case samples were significantly different ( $P < 0.05$ ) from those of active case samples during storage (Table 4.18). Also, there were no significant differences the chewiness values of sucuks stuffed into potassium sorbate and chitosan at the first and last day of storage. Similarly, Hsu and Sun (2006) investigated influences of different additives (potassium sorbate, phosphates, sodium erythorbate and salt) on textural attributes of meat ball. They reported that sodium erythorbate and potassium sorbate had no influence on texture attributes of samples. At the end of storage, 54.02 mJ was the highest chewiness value in collagen case sample, 28.56 mJ was the lowest chewiness value in AgZeo samples (Table 4.18). Also, the chewiness values of heat treated samples at 75°C increased ( $P < 0.05$ ) during storage except collagen case sample. During storage using different antimicrobial cases and storage time influenced significantly ( $P < 0.05$ ) the hardness, gumminess and chewiness value of sucuks (Table 4.16, 4.17 and 4.18). Also, statistical analysis were used to determine the effect of different heat process temperature (65°C, 70°C and 75°C) on hardness, gumminess and chewiness values (Table 4.19, 4.20 and 4.21). Generally, hardness, gumminess and chewiness values were affected by different heat treatment ( $P < 0.05$ ) during storage. First day of storage, hardness values of sucuks stuffed into collagen case increased ( $P < 0.05$ ) by heat treatment. At the same time the hardness,

gumminess and chewiness values of chitosan sucuk samples ( $P < 0.05$ ) increased by use of different heat treatments on the 3<sup>rd</sup> days of storage (Table 4.19, 4.20 and 4.21). Additionally, hardness, gumminess and chewiness values of nisin sucuk samples ( $P < 0.05$ ) increased using different heat temperatures on the 6<sup>th</sup> days of storage (Table 4.19, 4.21 and 4.21). Also, using different heat process can affect hardness, gumminess and chewiness values of sucuks. Similarly, Jin et al. (2017) examined the influence of dry status on physicochemical features of pork sausages during refrigerated storage (4°C for 8 weeks). The dry condition significantly impacted the hardness, gumminess and chewiness values in the sausages ( $P < 0.01$ ). The semi-dry sausages had lower values in hardness, gumminess and chewiness compared to the dry sausages. The dry medium was a first factor influencing texture features of sausages. As the dry operation continues, the moisture in sausages is released then a rise in hardening of dry-cured meat products happens (Arnau et al., 2007). Ruiz-Ramírez et al. (2005) found that hardness of dry-cured ham rises with the reduce in water amount after it achieves around 0.55 in dry matter (kg H<sub>2</sub>O/kg dry matter). Additionally, Arnau et al. (2005) found that drying operation preferably guides to the reduction of moisture content on the surface. Consequently, it was detected that the rise in the levels of gumminess and chewiness is due to the decrease of moisture amount in the sausages through drying.

Heat treated sucuks at 65°C stuffed into AgZeo had significantly lower ( $P < 0.05$ ) adhesiveness values than those samples stuffed into control plastic case storage on 0 day. The lowest adhesiveness value (-12.5 mJ) was seen in sucuks stuffed into AgZeo while the highest adhesiveness value was in sucuks stuffed into control plastic case at first day of storage (day 0). The adhesiveness values of sucuk samples decreased markedly ( $P < 0.05$ ) during storage except Agzeo sucuk sample. It had great differences in adhesiveness values between all samples from day 0 to day 12 of storage. The lowest adhesiveness value was -40.00 in sucuks stuffed into collagen case whereas the highest adhesiveness value was -10.00 in sucuks stuffed into chitosan (Table 4.22). The adhesiveness values of heat treated sucuk samples at 70°C reduced importantly ( $P < 0.05$ ) during storage except Agzeo and chitosan sucuk samples from day 0 to day 12 of storage. The sucuks stuffed into control plastic case had lower adhesiveness values ( $P < 0.05$ ) than those of sucuks stuffed into AgZeo storage on 0 day.

**Table 4.19** The effect of different heat treatment degrees on hardness values

Parameters	Storage Time (Days)	Heat Treatment Degrees	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Hardness (N)	0	65 °C	42.88±2.25 a	24.62±17.41 a	27.41±1.38a	21.11±0.97 a	19.59±8.31 a	23.47±1.27 a
		70°C	58.35±1.25 b	33.34±2.35 b	22.59±0.58a	28.48±2.19 b	24.79±1.53a,b	30.30±2.71 b
		75 °C	67.63±5.38 c	35.804±2.25 b	31.54±2.30b	26.27 ±1.72 a,b	30.93±1.12 c	23.36±1.88 a
	3	65 °C	65.46±4.29 a	26.16±1.80 a	31.24 ±22.09 a	26.53±11.25 a	22.84±1.89 a	30.37±0.06 a
		70°C	76.88±1.74 b	37.93±3.51 b	31.48±26.7 a	40.72±34.55 c	32.57±18.42b	30.45±1.22 a
		75 °C	74.23±2.49 b	35.94±35.58 b	39.21±33.72 b	32.71±13.87 b	40.43±22.87c	31.01±2.93 a
	6	65 °C	81.00±4.82 a	40.41±17.14 a	37.73±26.67 a	27.06±17.22 a	39.09±1.05 a	30.72±2.07 a
		70°C	80.87±6.21 a	38.72±3.39 a	43.11±18.29 b	40.81±17.31 b	35.22±1.92 a	33.79±1.11 a
		75 °C	95.33±5.62 b	53.86±3.08 b	51.16±50.64 c	37.68±26.64 c	52.11±3.84 b	40.01±2.63 b
	9	65 °C	91 ±2.37 a,b	42.55±36.10 a	41.63±29.43 a	40.73±17.28 a	39.25±1.10 a	35.90±2.39 a
		70°C	85.31±4.26 a	42.51±2.78 a	47.24 ±0.04 a,b	46.56±13.16 b	41.79±2.18 a	38.34±3.95 a
		75 °C	97.78±5.31 b	56.56±3.99 b	53.45±24.18 b	49.99±28.28 b	58.09±4.29 b	55.46±1.68 b
	12	65 °C	91.58±5.80 a	46.27±3.72 a	52.17±22.13 a	44.80±29.14 a	42.88±2.25 a	50.98±2.84 a
		70°C	95.00±6.17 a	67.68±1.14 b	50.04±35.38 a	58.55±16.56 b	44.83±1.68 a	50.12±2.35 a
		75 °C	100±4.42 a,b	60.50±1.11 b	67.97±38.44 b	52.02±14.71 b	72.41±4.96 b	74.98±4.42 b

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.20** The effect of different heat treatment degrees on gummy values

Parameters	Storage Time (Days)	Heat Treatment Degrees	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Gumminess (N)	0	65 °C	33.23±1.87 a	20.43±1.44 a	22.47±1.58 a	19.21±0.54 a	16.26±0.57 a	19.21±1.08 a
		70°C	44.05±3.10 b	27.74 ±1.96 b	19.09±0.81 a	27.74±1.35 b	20.45±1.4 a,b	25.60±1.81 b
		75 °C	53.09±4.50 c	19.98±1.13 a	26.65±1.88 b	30.43±1.93 c	26.44±1.12 b	22.59±1.34a,b
	3	65 °C	48.77±3.44 a	21.58 ±1.22 a	25.31±1.78 a	21.89±0.92 a	18.38±1.12 a	21.89±2.16 a
		70°C	56.51±3.99 b	31.07±2.19 c	25.97±2.20 a	31.07±0.75 b	26.55±1.87 b	24.97±1.76a,b
		75 °C	57.53±4.00 b	25.27 ±2.50 a,b	32.34±2.74 b	29.65±1.25 b	34.57±1.95 c	27.31±1.93 b
	6	65 °C	59.53±3.36 a	32.33±1.37 a	29.99±2.12 a	21.92±1.39 a	31.27±0.88 a	21.92±1.85 a
		70°C	58.63±4.14 a	31.30±2.21 a	34.27±1.45 b	31.30±0.77 b	28.70±2.02 a	27.03±1.91 b
		75 °C	73.40±2.98 b	31.40±2.22 a	41.44±4.10 c	43.62±3.08 c	44.29±3.13 b	30.90±1.75 b
	9	65 °C	65.97±1.86 b	32.34 ±2.74 a	31.63±2.23a	32.17±1.36 a	31.00±0.87 a	32.17±2.27 a
		70°C	61.00±4.31 a	33.22±2.34 a	37.09±1.57b	33.22±1.28 a	33.22±2.34 a	30.67±2.16 a
		75 °C	70.40±3.36 c	43.53±3.07 b	43.29±1.95c	45.81±2.59 b	47.05±3.99 b	39.74±1.12 b
	12	65 °C	65.02±3.67 a	34.47±2.43 a	38.86±1.64 a	33.15±2.15 a	31.94±1.80 a	33.15±1.87 a
		70°C	66.50±2.82 a	51.77±2.19 b	39.28±2.77 a	50.42±2.86 b	35.41±1.50 a	39.34±1.66 b
		75 °C	68.5±1.90 b	58.11±1.65 c	54.71±3.08 b	48.40±1.36 b	58.29±3.29 b	41.09±2.32b,c

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4. 21** The effect of different heat treatment degrees on chewiness values

Parameters	Storage Time (Days)	Heat Treatment Degrees	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Chewiness (mJ)	0	65 °C	30.11±1.70 a	16.58±1.17 a	18.37±1.29 b	16.16±0.45 a	13.11±0.55 a	16.16±0.61 a
		70°C	33.31±2.35 a	23.34±1.65 b	15.30±0.44 a	23.34±0.98 b	16.69±1.18 b	21.50 ±1.52 c
		75°C	52.09±2.44 b	17.48±0.86 a	22.42±1.58 c	24.53±1.56 b	22.44±0.95 c	18.75±1.11 b
	3	65 °C	42.67±3.01 a	17.51±0.99 a	19.86±1.40 a	17.45±0.74 a	14.48±0.08 a	17.45±0.72 a
		70°C	42.38±2.99 a	25.59±1.80 c	20.48±0.73 a	25.59±0.44 b	20.90±1.47 b	20.35±1.43 b
		75°C	50.62±2.58 b	20.57±1.03 b	27.13±2.30 b	23.83 ±1.01 b	29.17±1.65 c	22.56±1.02 b
	6	65 °C	50.45±2.85 b	26.06±1.1 b 0	23.39±1.65 a	17.31±1.10 a	23.77±0.06 a	17.31±0.46 a
		70°C	42.36±2.99 a	24.80±1.75 a	25.96±1.10 a	24.80±0.40 b	22.42±1.58 a	20.24±1.43 b
		75°C	60.74±1.60 c	25.48±0.80 a	34.49±3.41 b	35.06±2.47 c	36.37±2.57 b	24.87±0.40 c
	9	65 °C	52.69±1.49 b	22.92±1.95 a	23.57±1.66 a	25.34±1.07 a	23.56±0.66 a	25.34±0.79 b
		70°C	43.23±3.05 a	25.91±1.83 b	27.86±1.18 b	25.91±1.56 a	25.83±1.82 a	22.92±1.62 a
		75°C	57.29±1.24 c	33.25±1.35 c	34.36±1.55 c	35.96±2.03 b	38.52±2.30 b	31.15±0.43 c
12	65 °C	50.15±2.83 b	23.87±1.68 a	28.03±1.18 a	25.40±1.65 a	22.68±1.28 a	25.40±0.43 a	
	70°C	46.38±1.96 a	39.54±1.67 b	29.02±1.05 a	38.50±1.17 c	26.60±1.12 b	29.11±1.23a,b	
	75°C	54.02±1.29 c	42.49±1.20 c	42.67±2.41 b	35.69±1.00 b	45.32±1.56 c	28.56±0.61a,b	

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

At the end of storage, sucuks stuffed into agZeo and nisin had the lowest adhesiveness values (-24.5). There were no significant differences ( $P > 0.05$ ) between AgZeo sucuk and collagen case samples (Table 4.22).

The lowest adhesiveness value was seen in the sucuks stuffed into collagen case whereas the highest monitored in the sucuks stuffed into nisin case ( $P < 0.05$ ) both after heat treatment at 75°C (0<sup>th</sup> day) and end of the storage (12<sup>th</sup> day) (Table 4.22). Using different antimicrobial cases and storage time affected significantly ( $P < 0.05$ ) the adhesiveness value of all heat treated sucuks at 75°C on the last day of storage (Table 4.22). Adhesiveness value was in parallel to the moisture content during fermentation and storage. Additionally, it was found that adhesiveness values of sucuks were affected significantly ( $P < 0.05$ ) by different heat treatments (65 °C, 70 °C and 75°C) (Table 4.25). Similarly, Chatli et al. (2014) studied decontamination of raw chevon chunks wrapped with bioactive films based on starch-chitosan composite adsorbed with nisin and cinnamaldehyde during storage at 4°C. They reported that textural attributes was better in bio-packaged samples than that of control during storage.

Cohesiveness value of heat treated sucuks at 65°C stuffed into nisin had significantly higher ( $P < 0.05$ ) than those of sucuks stuffed into collagen case after heat treatment (0 day). The lowest cohesiveness value (0.77) was found in sucuks stuffed into collagen case while the highest cohesiveness value was observed in sucuks stuffed into nisin case on the beginning of storage (day 0). It had no significant differences ( $P > 0.05$ ) between cohesiveness values of all heat treated sucuk at 65°C during storage period. The cohesiveness values of heat treated sucuks at 65°C sucuk samples remain constant ( $P > 0.05$ ) during storage except nisin sucuk samples from day 0 to day 12 of storage. Additionally, Jo et al. (2001) reported that the incorporation of chitosan oligomer in the emulsion-type pork sausage did not vary its cohesiveness levels.

Storage time affected insignificantly ( $P > 0.05$ ) the cohesiveness value of sucuks in heat treated at 70°C and 75°C. There were no significant differences ( $P > 0.05$ ) cohesiveness values sucuks after heat treatment at 70°C during storage. At the end of storage, sucuks stuffed heat treated sucuks at 75°C into collagen case had lower cohesiveness value ( $P < 0.05$ ) than those of other sucuks (Table 4.23).

**Table 4.22** Changes in adhesiveness values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Adhesiveness (mJ)	65°C	0	-12.00 ±0.67 b,A	-7.50 ±0.53 a,A	-12.00 ±0.84 b,A	-8.50±0.24 a,A	-8.00 ±0.33 a,A	-12.50b,A±0.70
		3	-19.00 ±1.34 d,B	-8.00 ±0.45 a,A,B	-13.00 ±0.91 c,A	-10.50±0.44 b,B	-8.50 ±0.51 a,b,B	-13.40c,A±1.32
		6	-29.50 ±1.66 d,C	-8.50 ±0.36 a,A,B	-14.00 ±0.98 c,A	-11.00±0.70 b,B	-9.00 ±0.25 a,b,B,C	-13.50 ±1.14 c,A
		9	-37,5 ±1,06 c,D	-9,5±0,80 a,B	-14.50 ±1.02 b,A	-14.00 ±0,6 b,C	-9.50 ±0.26 a,B,C	-13.50 ±0.95 b,A
		12	-40 ±2,26 d,D	-12.50 ±0.88a,b,C	-20.50 ±0,86 c,B	-15.00 ±0,97 b,C	-10.00 ±0.56 a,C	-13.50 ±0.76 b,A
	70°C	0	-10.00±0.7a,b,A	-6.50 ±0.45 a,A	-8 ±0,33 a,b,A	-9 ±0,76 a,b,A	-8.00 ±0.56 a,b,A	-9.00 ±0.63 b,A
		3	-15.50 ±1.09 c,B	-8.00 ±0.56 a,B	-10.5 ±0.89 b,B	-14 ±0.79 c,B	-11.50 ±0.81 b,A	-14.00 ±0.98 c,A
		6	-17.00 ±1.20 a,B	-8.50 ±0.60 a,B	-13.5 ±0.57 a,C	-15 ±0.84 a,B	-12.00 ±0.84 a,A	-15.00 ±1.06 a,A
		9	-19.50 ±1.37 e,C	-8,70 ±0.61 a,B	-15 ±0.63 c,d,C,D	-16 ±1.58 d,B	-12.50 ±0.88 b,A	-16.00 ±1.13 c,A
		12	-19.50±0.82c,d,C	-9.00 ±0.38 a,B	-16.5 ±1.16 b,c,D	-24.5 ±1.38 d,C	-13.50 ±0.57 a,b,A	-24.50 ±1.03 c,d,A
	75°C	0	-18 ±1.52 d,A	-10.5 ±0.59 b,A	-10.00 ±0.70 a,b,A	-8 ±0.51 a,A	-15.5c,A±0.65	-15 ±0.89 c,A
		3	-21.00 ±1.48 d,B	-11.50 ±1.13b,A	-10.5 ±0.89 a,b,A	-8.50 ±0.36 a,A	-16.00c,A±0.90	-17.50 ±1.23 c,A,B
		6	-34.50 ±2.04 d,C	-13.00 ±0,81 a,b,A	-14.50 ±1.43 b,B	-11.00 ±0.77 a,B	-20.50c,B±1.44	-20.00 ±1.13 c,B
		9	-39.50 ±2.23 e,D	-18.00±1.27 b,B	-16.50 ±0.74 b,B	-11.00 ±0.62 a,B	-22.50c,B,C±1.90	-26.50 ±0.75 d,C
		12	-40.50±1.71e,D	-19.00±0.53 b,B	-16.50 ±0.93 a,b,B	-14.50 ±0.41 a,C	-25.00 ±1.41 c,C	-28.50 ±1.61 d,C

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.23** Changes in cohesiveness values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Cohesiveness	65°C	0	0.77 ±0.04 b,A	0.83±0.05a,b,A	0.82±0.05a,b,A	0.91 ±0.02 a,A	0.83±0.03a,b,A	0.81a,b,A±0.04
		3	0.74 ±0.05 a,A	0.82 ±0.04 a,A	0.81±0,05 a,A	0.82 ±0,03 a,A,B	0.80±0,04 a,A	0.80±0.07 a,A
		6	0.73 ±0.04 a,A	0.80 ±0.03 a,A	0.79±0.05 a,A	0.81 ±0.05 a,A,B	0.80±0.01 a,A	0.79±0.06 a,A
		9	0.72 ±0.02 a,A	0.76 ±0.06 a,A	0.76±0.05 a,A	0.79 ±0.03 a,B	0.79±0.02 a,A	0.76±0.05 a,A
		12	0.71 ±0.04 a,A	0.74 ±0.05 a,A	0.74±0.03 a,A	0.74 ±0.04 a,B	0.74±0.04 a,A	0.76±0.04 a,A
	70°C	0	0.75 ±0.06 a,A	0.84 ±0.05 a,A	0.84±0.04 a,A	0.84 ±0.07 a,A	0.82±0.05 a,A	0.84 ±0.08 a,A
		3	0.73 ±0.02 a,A	0.82 ±0.08 a,A	0.82±0.07 a,A	0.79 ±0.04 a,A	0.81±0.05 a,A	0.82±0.06 a,A
		6	0.72 ±0.06 a,A	0.80 ±0.06 a,A	0.79±0.03 a,A	0.78 ±0.04 a,A	0.81±0.04 a,A	0.80 ±0.03 a,A
		9	0.71 ±0.04 a,A	0.79 ±0.04 a,A	0.78±0.03 a,A	0.77 ±0.07 a,A	0.78±0.05 a,A	0.80 ±0.02 a,A
		12	0.70 ±0.05 a,A	0.78 ±0.02 a,A	0.78±0.05 a,A	0.77 ±0.04 a,A	0.76±0.02 a,A	0.78±0.02a,A
	75°C	0	0.78 ±0.06 a,A	0.85 ±0.05 a,A	0.84±0.05 a,A	0.85 ±0.04 a,A	0.85±0.03 a,A	0.86±0.05 a,A
		3	0.77 ±0.05 a,A	0.81 ±0.05 a,A	0.82±0.07 a,A	0.82 ±0.08a,A	0.85±0.04 a,A	0.83 ±0.03 a,A
		6	0.77 ±0.04 a,A	0.78 ±0.04 a,A	0.81±0.08 a,A	0.81 ±0.05a,A	0.85±0.06 a,A	0.82 ±0.05 a,A
		9	0.72 ±0.04 a,A	0.78 ±0.02 a,A	0.81±0.04 a,A	0.81 ±0.05a,A	0.81±0.06 a,A	0.79 ±0.04 a,A
		12	0.68 ±0.03b,A	0.77 ±0.04 a,A	0.80±0.04 a,A	0.80 ±0.02a,A	0.80±0.04 a,A	0.79 ±0.02 a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.24** Changes in springiness values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Heat Treatment Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Springiness (mm)	65°C	0	0.90 ±0.05 a,A	0.81±0.05 a,A	0.81±0.05 a,A	0.84 ±0.02a,A	0.80±0.03a,A	0.80±0.04 a,A
		3	0.87±0.06 a,A,B	0.81 ±0.04 a,A	0.78±0.05 a,A	0.79 ±0.03a,A	0.78 ±0.04 a,A,B	0.78 ±0.07 a,A
		6	0.84 ±0.05 a,A,B	0.80 ±0.03 a,A	0.78±0.05 a,A	0.79 ±0.05a,A	0.76±0.02a,A,B	0.75 ±0.06 a,A
		9	0.79 ±0.02 a,A,B	0.70 ±0.06 a,A	0.74±0.05 a,A	0.78 ±0.03a,A	0.76±0.02 a,A,B	0.74 ±0.05 a,A
		12	0.77 ±0.04 a,B	0.69 ±0.04 a,A	0.72±0.03 a,A	0.76 ±0.05a,A	0.71±0.04 a,B	0.73 ±0.04 a,A
	70°C	0	0.95 ±0.06 a,A	0.84 ±0.06 a,A	0.80±0.03 a,A	0.84 ±0.07 a,A	0.81±0.05 a,A	0.84 ±0.08 a,A
		3	0.86 ±0.02 a,A,B	0.82 ±0.08 a,A	0.78±0.06 a,A	0.81 ±0.05 a,A	0.78±0.04 a,A	0.81±0.06 a,A
		6	0.82 ±0.06 a,A,B	0.79 ±0.06 a,A	0.75±0.03 a,A	0.74 ±0.04 a,A	0.78±0.04 a,A	0.80 ±0.03 a,A
		9	0.79 ±0.04a,B	0.78 ±0.04 a,A	0.75±0.03a,A	0.74 ±0.07 a,A	0.77±0.05 a,A	0.70 ±0.02 a,A
		12	0.76 ±0.05 a,B	0.76 ±0.02 a,A	0.73±0.05 a,A	0.71 ±0.04 a,A	0.75±0.02 a,A	0.75 ±0.02 a,A
	75°C	0	0.98 ±0.08 a,A	0.80 ±0.04 b,A	0.84±0.05 a,b,A	0.83 ±0.05 b,A	0.84 ±0.03 a,b,A	0.87 ±0.05 a,b,A
		3	0.88 ±0.06 a,A,B	0.80 ±0.07 a,A	0.84±0.07 a,A	0.82 ±0.03 a,A	0.84 ±0.04 a,A	0.81 ±0.05 a,A,B
		6	0.83 ±0.04 a,B	0.80 ±0.05 a,A	0.83±0.08 a,A	0.80 ±0.05 a,A,B	0.82 ±0.05 a,A	0.81 ±0.04 a,A,B
		9	0.81 ±0.05 a,B	0.78 ±0.05 a,A	0.79±0.03a,A	0.78 ±0.04 a,A,B	0.81 ±0.06 a,A	0.76 ±0.02 a,A,B
		12	0.78 ±0.03 a,B	0.73 ±0.02 a,b,A	0.78±0.04a,b,A	0.69 ±0.02 b,B	0.77 ±0.04 a,b,A	0.73±0.04 a,b,B

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

Using different antimicrobial cases and storage time had no significant effect ( $P > 0.05$ ) on the springiness values of heat treated sucuks at 65°C and 70°C during storage (Table 4.24). After heat treatment at 75°C, springiness values of sucuks stuffed into collagen case were different ( $P < 0.05$ ) than those of others both at 0 and 12 days of storage. On the other hand, it was found that cohesiveness and springiness values of sucuks were not affected significantly ( $P > 0.05$ ) by heat treatment at 65 °C, 70 °C and 75°C (Table 4.26 and 4.27). However, Feng and Xiong (2002) examined that the quality of the gel matrix had an important part in detecting the textural features of cooked frankfurters. The significantly lower springiness observed in hurdle treated sausages could be associated with their lower elastic properties resulting from decreased fat binding. Gumminess and chewiness indicated the similar tendency as observed for springiness. Furthermore, Voller et al. (1996) detected a decrease in different textural properties of cooked fowl meat gels at high processing temperatures.

#### **4.7 Biogenic Amine Content**

Biogenic amines (BAs) are nitrogenous materials formed in high protein foods. BAs in meat and meat products are important due to their toxicological influence on the intestinal, blood and nervous systems. Usually, most BAs are formed by naturally present decarboxylases of microbial origin. So, BAs are one of the most important index of microbial deterioration. BA's structures could be heterocyclic (e.g. histamine and tryptamine), aliphatic (e.g. putrescine, cadaverine, spermine and spermidine) or aromatic (e.g. tyramine and phenylethylamine) (Santos, 1996). Although the available of BAs in meat and meat products is not an exact criterion for the spread out of spoilage microorganisms, since there might be status where BAs are made by other microorganisms, the BAs value is a good indicator to measure food deterioration (Hernández-Jover et al., 1996).

##### **4.7.1 Fermentation**

Table 4.28 shows the contents of cadaverine, putrescine, histamine, serotonin, tryptamine, spermidine, spermine and tyramine in the sucuks stuffed into antimicrobial (nisin, chitosan potassium sorbate and AgZeo), control plastic and collagen cases during the fermentation.

**Table 4.25** The effect of different heat process temperature on adhesiveness values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Adhesiveness (mJ)	0	65 °C	-12.00±0.67 b	-7.50 ±0.53 a,b	-12.00±0.84 c	-8.50±0.24 a	-8.00±0.33a	-12.50±0.70 b
		70°C	-10.00±0.70 a	-6.50±0.45 a	-8.00±0.33 a	-9.00±0.76 a	-8.00±0.56a	-9.00±0.63a
		75°C	-18.00±1.52 c	-10.50±0.59 c	-10.00±0.70 b	-8.00±0.51 a	-15.5±0.65b	-15.00±0.89 c
	3	65 °C	-19.00±1.34 b	-8.00±0.45 a	-13.00±0.91 b	-10.50±0.44 b	-8.50±0.51 a	-13.40±1.32 a
		70°C	-15.50±1.09 a	-8.00±0.56a	-10.50±0.89 a	-14.00±0.79 c	-11.50±0.81 b	-14.00±0.98 a
		75°C	-21.00±1.48 c	-11.50±1.13b	-10.50±0.89 a	-8.50±0.36 a	-16.00±0.90 c	-17.50±1.23 b
	6	65 °C	-29.50±1.66 b	-8.50±0.36 a	-14.00±0.98 a	-11.00±0.70 a	-9.00±0.25 a	-13.50±1.14 a
		70°C	-17.00±1.20 a	-8.50±0.60 a	-13.50±0.57 a	-15.00±0.84 b	-12.00±0.84 b	-15.00±1.06 a
		75°C	-34.50±2.04 c	-13.00±0.81 b	-14.50±1.43 a	-11.00±0.77 a	-20.50±1.44 c	-20.00±1.13 b
	9	65 °C	-37.50±1.06 b	-9.50±0.80 a	-14.50±1.02 a	-14.00±0.6 b	-9.50±0.26 a	-13.50±0.95 a
		70°C	-19.50±1.37 a	-8.70±0.61 a	-15.00±0.63 a	-16.00±1.58 c	-12.50±0.88 b	-16.00±1.13 b
		75°C	-39.50±2.23 b	-18.00±1.27 b	-16.50 ±0.74 a,b	-11.00±0.62 a	-22.50±1.90 c	-26.50±0.75 c
	12	65 °C	-40.00±2.26 b	-12.50±0.88 b	-20.50±0.86 b	-15.00±0.97 a	-10.00±0.56 a	-13.50±0.76 a
		70°C	-19.50±0.82 a	-9.00±0.38 a	-16.50±1.16 a	-24.50±1.38 b	-13.50±0.57 b	-24.50±1.03 b
		75°C	-40.50±1.71 b	-19.00±0.53 c	-16.50±0.93 a	-14.50±0.41 a	-25.00±1.41 c	-28.50±1.61 c

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.26** The effect of different heat process temperature on cohesiveness values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen case	Control Plastic Cases	Potassium Sorbate	Nisin	Chitosan	AgZeo
Cohesiveness	0	65 °C	0.77±0.04 a	0.83±0.05 a	0.82±0.05 a	0.91±0.02 b	0.83±0.03 a	0.81±0.04 a
		70°C	0.75±0.06 a	0.84±0.05 a	0.84±0.04 a	0.84±0.07 a	0.82±0.05 a	0.84±0.08 a
		75 °C	0.78±0.06 a	0.85±0.05 a	0.84±0.05 a	0.85±0.04 a	0.85±0.03 a	0.86±0.05 a
	3	65 °C	0.74±0.05 a	0.82±0.04 a	0.81±0.05 a	0.82±0.03 a	0.80±0.04 a	0.80±0.07 a
		70°C	0.73±0.02 a	0.82±0.08 a	0.82±0.07 a	0.79±0.04 a	0.81±0.05 a	0.82±0.06 a
		75 °C	0.77±0.05 a	0.81±0.05 a	0.82±0.07 a	0.82±0.08 a	0.85±0.04 a	0.83±0.03 a
	6	65 °C	0.73±0.04 a	0.80±0.03 a	0.79±0.05 a	0.81±0.05 a	0.80±0.01 a	0.79±0.06 a
		70°C	0.72±0.06 a	0.80±0.06 a	0.79±0.03 a	0.78±0.04 a	0.81±0.04 a	0.80±0.03 a
		75 °C	0.77±0.04 a	0.78±0.04 a	0.81±0.08 a	0.81±0.05 a	0.85±0.06 a	0.82±0.05 a
	9	65 °C	0.72±0.02 a	0.76±0.06 a	0.76±0.05 a	0.79±0.03 a	0.79±0.02 a	0.76±0.05 a
		70°C	0.71±0.04 a	0.79±0.04 a	0.78±0.03 a	0.77±0.07 a	0.78±0.0 a 5	0.80±0.02 a
		75 °C	0.72±0.04 a	0.78±0.02 a	0.81±0.04 a	0.81±0.05 a	0.81±0.06 a	0.79±0.04 a
	12	65 °C	0.71±0.04 a	0.74±0.05 a	0.74±0.03 a	0.74±0.04 a	0.74±0.04 a	0.76±0.04 a
		70°C	0.70±0.05 a	0.78±0.02 a	0.78±0.05 a	0.77±0.04 a	0.76±0.02 a	0.78±0.02 a
		75 °C	0.68±0.03 a	0.77±0.04 a	0.80±0.04 a	0.8±0.02 a	0.8±0.04 a	0.79±0.02 a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.27** The effect of different heat process temperature on springiness values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Springiness (mm)	0	65 °C	0.90a±0.05	0.81a±0.05	0.81±0.05 a	0.84±0.02 a	0.80±0.03 a	0.80a±0.04
		70°C	0.95a±0.06	0.84a±0.06	0.80±0.03 a	0.84±0.07 a	0.81±0.05 a	0.84a±0.08
		75°C	0.98a±0.08	0.80a±0.04	0.84±0.05 a	0.83±0.05 a	0.84±0.03 a	0.87a±0.05
	3	65 °C	0.87a±0.06	0.81a±0.04	0.78±0.05 a	0.79±0.03 a	0.78±0.04 a	0.78a±0.07
		70°C	0.86a±0.02	0.82a±0.08	0.78±0.06 a	0.81±0.05 a	0.78±0.04 a	0.81a±0.06
		75°C	0.88a±0.06	0.80a±0.07	0.84±0.07 a	0.82±0.03 a	0.8±0.04 a	0.81a±0.05
	6	65 °C	0.84±0.05 a	0.80±0.03 a	0.78±0.05 a	0.79±0.05 a	0.76±0.02 a	0.75±0.06 a
		70°C	0.82±0.06 a	0.79±0.06 a	0.75±0.03 a	0.74±0.04 a	0.78±0.04 a	0.80±0.03 a
		75°C	0.83±0.04 a	0.80±0.05 a	0.83±0.08 a	0.80±0.05 a	0.82±0.05 a	0.81±0.04 a
	9	65 °C	0.79±0.02 a	0.70±0.06 a	0.74±0.05 a	0.78±0.03 a	0.76±0.02a	0.74±0.05 a
		70°C	0.79±0.04 a	0.78±0.04 a	0.75±0.03 a	0.74±0.07 a	0.77±0.05 a	0.70±0.02 a
		75°C	0.81±0.05 a	0.78±0.05 a	0.79±0.03 a	0.78±0.04 a	0.81±0.06 a	0.76±0.02 a
	12	65 °C	0.77±0.04 a	0.69±0.04 a	0.72±0.03 a	0.76±0.05 a	0.71±0.04 a	0.73±0.04 a
		70°C	0.76±0.05 a	0.76±0.02 a	0.73±0.05a	0.71±0.04 a	0.75±0.02 a	0.75±0.02 a
		75°C	0.78±0.03 a	0.73±0.02 a	0.78±0.04 a	0.70±0.02 a	0.77±0.04 a	0.73±0.04 a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

Tryptamine was not detected in sucuk dough. Its content increased ( $P < 0.05$ ) during fermentation. The highest tryptamine content was 19.70 mg/kg in sucuks stuffed into collagen case while its lowest content was 12.54 mg/kg in sucuks stuffed into chitosan ( $P < 0.05$ ) (Table 4.28). There were no significant differences ( $P > 0.05$ ) observed between sucuks stuffed into potassium sorbate, chitosan and AgZeo (Table 4.18). Sucuks stuffed into nisin had higher ( $P < 0.05$ ) tryptamine than those of sucuks stuffed into other active cases during the fermentation period (Table 4.28).

It was found that sucuk dough had about 33.00 mg/kg putrescine content. Fermentation time and using nisin, chitosan, AgZeo and potassium sorbate included cases had significant effect on ( $P < 0.05$ ) the formation of putrescine. Putrescine concentration rised gradually ( $P < 0.01$ ) from 33 mg/kg to 220.92 mg/kg during the first 3 days of fermentation in collagen case (Table 4.28). Similarly, Bozkurt (2006) found that the formation of putrescine rised gradually up to 159.76 mg/kg during the first 6 days of the fermentation. Furthermore, the lowest putrescine content (160.77 mg/kg) was determined in sucuks stuffed into chitosan ( $P < 0.05$ ) (Table 4.28).

Cadaverine formation increased significantly ( $P < 0.05$ ) up to 105, 113, 115, 118, 125.95 and 128.17 mg/kg during the first 3 days of fermentation in sucuks stuffed into potassium sorbate, nisin, chitosan and AgZeo control plastic and collagen cases, respectively (Table 4.28). Cadaverine levels were different ( $P < 0.05$ ) from each other at the end of fermentation.

Serotonine concentration increased ( $P < 0.05$ ) during the fermentation period and the highest serotonine formation was monitored in the sample stuffed into chitosan. Incorporation of active agents hadn't an important effect ( $P > 0.05$ ) on serotonine formation during fermentation (Table 4.28).

Spermine concentration differed between 25-33 mg/kg at the end of the fermentation. Additionally, Loizzo et al. (2016) found that spermine concentrations of a kind of spreadable salami were  $5.7 \pm 0.05$  mg/kg and  $12.3 \pm 0.04$  mg/kg during 0<sup>th</sup> months, ripening (3 months) respectively. No important differences ( $P > 0.05$ ) were observed between all samples for spermine formation during fermentation (Table 4.28).

**Table 4.28** Changes in biogenic amine content of sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Parameters	Fermentation Time (days)	CASE TYPE					
		Collagen case	Control Plastic Cases	Potassium Sorbate	Nisin	Chitosan	AgZeo
Tyrptamine (mg/kg)	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	3	19.70±1.39 a,A	16.55 ±1.17 b,A	13.10 ±0.92 c,A	15.2±1.07b,c,A	12.54±0.8c,A	13.15 ±0.92 c,A
Putrescine (mg/kg)	0	33.00 ±2.33 a,B	33.00 ±2.33 a,B	33.00 ±2.33 a,B	33.00 ±2.33 a,B	33.00 ±2.33 a,B	33.00 ±2.33 a,B
	3	220.92 ±15.62 a,A	200.44 ±14.17 a,b,A	170 ±12.02 b,A	190 ±13.43 a,b,A	160.77 ±11.36 c,A	180.60±12.77 b,c,A
Cadaverine (mg/kg)	0	22.00 ±1.55 a,B	22.00±1.55 a,B	22.00 ±1.55a,B	22.00 ±1.55 a,B	22.00 ±1.55 a,B	22.00 ±1.55 a,B
	3	128.17 ±9.06 a,A	125.95 ±8.91 a,A	105.00 ±7.42 d,A	113 ±7.99 b,c,A	115.00 ±8.13 b,A	118.04 ±8.34 b,A
Histamine (mg/kg)	0	12.00 ±0.84 a,A	12.00 ±0.84 a,A	12.00 ±0.84a,A	12.00 ±0.84a,A	12.00 ±0.84 a,A	12.00 ±0.84 a,A
	3	104.00 ±7.35 a,B	81.00 ±5.72 b,B	50.00 ±3.53 c,B	60.00 ±4.24 c,B	46.62 ±3.29 d,B	56.00 ±3.95 c,B
Tyramine (mg/kg)	0	65.00 ±4.59 a,B	65.00 ±4.59 a,B	65.00 ±4.59 a,B	65.00 ±4.59 a,B	65.00 ±4.59 a,B	65.00 ±4.59 a,B
	3	320.10±22.63c,A	378.00±26.70 b,A	320.10 ±22.63 c,A	324.38 ±22.93 b,c,A	309.00 ±0.21 c,d,A	313.47±22.16 c,d,A
Serotonine (mg/kg)	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	3	42.84 ±3.02 a,A	41.34 ±2.92 a,A	39.99±2.82a,A	39.80±2.80a,A	43.40 ±3.06a,A	42.46 ±3.00 a,A
Spermidine (mg/kg)	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	3	2.50±0.04 a	1.80±0.05 b	1.10±0.01 c	1.15±0.02 c	0.93 ±0.03c,d	0.75±0.04 d
Spermine(mg/kg)	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	3	33.00 ±2.33 a,A	30.92 ±2.18 a,A	28.20 ±0.05 a,A	29.50±0.08a,A	27.16 ±1.92 a,A	30.71 ±2.17 a,A

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-C: Different capital letters indicate a statistical difference between the fermentation time at  $\alpha= 0.05$  level in each column.

There was no spermidine content in sucuk dough. Fermentation time and using nisin, chitosan, AgZeo and potassium sorbate containing cases had significant effect on ( $P < 0.05$ ) the formation of spermidine. The maximum spermidine concentration was identified ( $P < 0.05$ ) in sucuk stuffed into collagen case, while the lowest was in sucuk stuffed into case produced with incorporation of AgZeo on third day of fermentation. Also, spermidine level of sucuks in active cases ranged from 0.75-1.15 mg/kg at the last day of fermentation (Table 4.28).

Fermentation time and use of active cases had a great effect ( $P < 0.05$ ) on tyramine formation. Its concentration rised ( $P < 0.01$ ) during fermentation, its concentration ranged between 65 and 502 mg/kg at the end of the fermentation (Table 4.28). Sucuks stuffed into chitosan had lower tyramine content (309 ppm) than those of other sucuks at the end of fermentation. The highest concentration of tyramine was seen in collagen case sample as 502 mg/kg (Table 4.28). Kurt and Zorba (2009) found that tyramine level in sucuks rised in the first days of the fermentation. Some researchers (Bover-Cid et al., 2001) reported that tyramine formation increased by growth of lactic acid bacteria. Additionally, the total aerobic viable count could influence tyramine formation (Pätzberger et al., 1997).

Use of different antimicrobial cases and fermentation time affected ( $P < 0.05$ ) histamine concentration. Firstly, sucuk dough had 12 mg/kg histamine content (Table 4.28). Their concentrations rised ( $P < 0.05$ ) to 50.00 mg/kg and 104.00 mg/kg during the first 3 days of fermentation in sucuks stuffed into potassium sorbate and collagen case, respectively (Table 4.28). Parallel findings were observed by Bozkurt and Bayram (2006) and Ruiz-Capillas and Jimenez-Colmenero (2004) that the histamine formation rised during the first days of fermentation. The highest histamine concentration was observed ( $P < 0.05$ ) in sucuk stuffed into collagen case, while the lowest was in sucuk stuffed into chitosan case both at the end of fermentation. Histamine concentration increased ( $P < 0.05$ ) in sucuks stuffed into active cases during fermentation. Generally, histamine values of the samples stuffed into AgZeo and potassium sorbate cases were not different ( $P > 0.05$ ) from each other during fermentation (Table 4.28).

#### 4.7.2 Storage

After the heat treatment at 65°C, sucuks in the nisin case had higher tyryptamine than sucuks stuffed into chitosan, AgZeo and potassium sorbate (Table 4.29). The lowest tyryptamine content (9.21 mg/kg) was seen in collagen case samples storage on 0 day. At the end of storage, 1.49 mg/kg was the lowest tyryptamine content observed in collagen case sucuk samples. Also, sucuks stuffed into potassium sorbate, nisin and AgZeo had lower ( $P < 0.05$ ) tyryptamine content than those of chitosan samples (Table 4.29).

The lowest tyryptamine content was seen in the sucuks stuffed into nisin whereas the highest tyryptamine value was monitored in the sucuks stuffed into collagen case ( $P < 0.05$ ) both after heat treatment at 70°C and end of the storage (Table 4.29).

The lowest tyryptamine content was found in the sucuks stuffed into collagen case whereas the highest tyryptamine value was observed in the sucuks stuffed into control plastic case ( $P < 0.05$ ) both after heat treatment at 75°C and end of the storage (Table 4.20). Films containing potassium sorbate and nisin had significantly lower ( $P < 0.05$ ) tyryptamine than those of other active cases (AgZeo and chitosan) (Table 4.29). Storage time affected significantly ( $P < 0.05$ ) tyryptamine formation in heat treated sucuks at all temperatures (65°C, 70°C and 75°C) (Table 4.32). Kurt and Zorba (2010) studied the influences of nisin and nitrite on biogenic amine content in sucuk using a central composite design of response surface methodology. They found that the important linear relation was observed ( $P < 0.01$ ) between nisin and tryptamine levels. So, tryptamine content reduced with rising nisin or nitrite values. Sirocchi et al. (2013) reported that no significant differences between meat with active package (AP) and meat packed with poly-coupled package (PP) for tryptamine concentration during storage.

Storage time and using different cases had important effect on ( $P < 0.05$ ) putrescine formation in heat treated sucuks at 65°C (Table 4.30). Similarly, Loizzo et al. (2016) reported that putrescine level generally rised with time. They found that putrescine level were  $2.4 \pm 0.02$  mg/kg,  $15.3 \pm 0.08$  mg/kg and 53.3–120.2 mg/kg during 0<sup>th</sup> months, ripening (3 months) and storage (6–15 months), respectively.

**Table 4.29** Changes in tyramine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Tyrptamine (mg/kg)	65 °C	0	9.21 ±0.68 b,A	16.36 ±4.50 a,A	13.91 ±1.03 a,b,A	15.88 ±1.18 a,A	11.91 ±0.89 a,b,A	12.26 ±1.25 a,b,A
		3	6.82 ±0.51 c,B	14.66 ±1.09 a,A	11.82 ±0.88 b,B	10.06 ±0.74 b,B	11.62 ±0.86 b,A	10.57 ±0.78 b,A,B
		6	4.56 ±0.34 c,C	12.44 ±0.92 a,A,B	9.45 ±0.70 b,C	9.15 ±0.68 b,B	8.93 ±0.66 b,B	10.01 ±0.74 b,B,C
		9	2.74 ±0.20 d,D	10.81 ±0.88 a,A,B	7.26 ±0.54 b,D	4.58 ±0.34c,C	8.00 ±0.59 b,B,C	8.18 ±0.60 b,C
		12	1.49 ±0.11 d,E	8.52 ±0.63 a,B	5.07 ±0.37 c,E	4.38 ±0.32 c,C	6.73 ±0.50 b,C	5.28 ±0.39 c,D
	70 °C	0	19.84 ±1.58 a,A	16.84 ±1.55 b,A	12.51 ±0.93 c,A	12.67 ±0.94 c,A	12.83 ±0.94 c,A	13.45 ±0.98 c,A
		3	17.18 ±1.21 a,A,B	15.44 ±1,09 a,A,B	11.33 ±0.84 b,A	10.80 ±0.80 c,B	12.24 ±0.86 b,A	11.13 ±0.78 b,B
		6	14.81 ±1.04 a,B,C	13.10 ±0.92 a,B,C	7.77 ±0.57 c,B	9.47 ±0.70 b,B	9.41 ±0.66 b,B	10.54 ±0.74 b,B
		9	12.89 ±0.91 a,C,D	11.38 ±0.80 a,C	6.89 ±0.51 d,B	7.03 ±0.52 c,C	8.43 ±0.59 b,B,C	8.62 ±0.60 b,C
		12	11.57 ±0.49 a,D	8.97 ±0.38 b,D	6.64 ±0.49 c,d,B	5.81 ±0.43 d,C	7.09 ±0.30 c,C	5.56 ±0.23 d,D
	75 °C	0	4.88 ±0.36 c,A	14.20 ±1.05 a,A	12.49 ±0.92 a,b,A	10.40±0.77 b,A	11.27 ±0.83 b,A	12.56 ±0.93 a,b,A
		3	4.37 ±0.32 c,A	11.55 ±0.85 a,B	11.14 ±0.82 a,A,B	10.25 ±0.76 a,A	9.49 ±0.70 b,B	10.50 ±0.78 a,b,B
		6	3.53 ±0.26 d,B	10.18 ±0.75 a,B,C	9.66 ±0.71 a,b,B,C	8.36 ±0.62 b,c,B	7.52 ±0.56 c,C	7.43 ±0.55 c,C
		9	2.72 ±0.20 c,C	8.29 ±0.61 a,C,D	8.03 ±0.59 a,C	7.74 ±0.57 a,B	7.41 ±0.55 a,b,C	6.27 ±0.46 b,C,D
		12	1.23 ±0.09 d,D	6.31 ±0.47 a,D	4.35 ±0,32 c,D	4.49 ±0.33 c,C	5.50 ±0.40 a,b,D	5.23 ±0.38 b,c,D

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.30** Changes in putrescine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Cases	Potassium Sorbate	Nisin	Chitosan	AgZeo
Putrescine (mg/kg)	65°C	0	240.62±13.61 a,A	220.26 ±15.57 a,b,A	170.15 ±12.03 c,A	190.41 ±5.38 b,c,A	150.93 ±1.18 d,A	170.67 ±1.31 c,A
		3	190.05 ±13.43 a,B	190.08 ±10.75 a,B	140.34±9.92b,B	150.38±6.38b,B	140.82 ±1.10 b,A,B	140.83 ±1.10 b,B
		6	170.57±9.64 a,B,C	170.10 ±7.21 a,B,C	130.39±9.21b,B	130.41±8.30b,C	130.49 ±5.00 b,A,B	130.46 ±3.50 b,B
		9	150.25 ±4.24 a,C	140.8 ±11.94 a,b,C	120.89 ±8.54 b,B	130.02 ±5.51 a,b,C	120.93 ±1.96 b,B	120.49 ±1.92 b,B
		12	150.20 ±8.49 a,C	140.25 ±9.91 a,b,C	120.65±5.11b,B	120.95±7.86b,C	120.23 ±1.91 b,B	120.16 ±1.91 b,B
	70°C	0	221.05 ±16.62 a,A	200.94±13.37 a,b,A	180.20 ±1.35 b,c,A	150.25 ±1.13 d,A	160.85 ±11.39 c,A	180.68±11.8b,c,A
		3	210.06±14.85a,A,B	200.09±14.14a,A	140.43 ±1.07 c,B	140.10 ±1.05 c,A,B	150.61±10.64b,A,B	150.62±10.65b,B
		6	200.50 ±14.17 a,A,B	180.00±12.72 a,A,B	140.05 ±1.04 b,B	130.02 ±2.96 b,A,B	130.21±9.20b, B,C	140.17 ±9.91 b,B,C
		9	190.06 ±13.43 a,A,B	150.58±10.64b,B	130.11 ±1.97 b,B,C	120.51 ±3.93 b,B	120.62 ±8.52 b,C	130.15±9.20 b,B,C
		12	180.00 ±7.63 a,B	150.00 ±6.36 b,B	120.54 ±2.93 c,C	120.20 ±2.90 c,B	110.88 ±4.70 c,C	120.80 ±5.12 c,C
	75°C	0	210.59 ±1.60 a,A	180.30 ±1.36 b,A	170.91 ±1.33 b,A	140.35 ±1.06 c,A	150.93 ±1.18 b,c,A	170.86 ±1.32 b,A
		3	160.15 ±1.20 a,b,B	150.22 ±1.13 a,b,B	140.45 ±1.07 a,b,B	130.94 ±1.03 a,b,B	130.52 ±1,98 b,A,B	160.81 ±1.25 a,A,B
		6	150.77 ±1.17 a,B	150,12 ±1.12 a,B	130.31 ±0.99 a,B	130.81 ±1.02 a,B	130.51 ±1.78 a,A,B	130.94±1.03 a,B,C
		9	150.67 ±1.16 a,B	140.85 ±1.10 a,B	130.28 ±0.98 a,B	130.30 ±0.99 a,B	130.22 ±0.98 a,A,B	130.69 ±1.01 a,D
		12	150.6 ±1.16 a,B	140.29 ±1.06 a,b,B	120.54 ±0.93 b,C	120.33 ±0.91 b,B	120.69 ±0.94 b,B	120.70 ±0.94 b,D

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.31** Changes in cadaverine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Cadaverine (mg/kg)	65°C	0	121.76 ±4.06 b,A	151.95 ±5.31 a,A	107.05 ±3.96 d,A	123.70 ±6.51 b,c, A	118.75 ±6.83 b,c,A	112.13 ±4.34 c,A
		3	115.65 ±4.60 a,A,B	112.1 ±4.34 a,B	105.15 ±4.72 b,A	106.23 ±3.90 b,A	110.56 ±4.22 a,b,A,B	106.52 ±3.92 b,A
		6	98.86 ±4.35 a,b,B,C	99.80 ±4.42 a,b,B,C	94.99 ±4.07 a,A,B	100.71 ±4.49 b,A	105.75 ±4.87 c,A,B	106.39 ±4.91 c,A
		9	89.90 ±6.69 a,b,C,D	94.60 ±7.04 a,b,B,C	78.07 ±5.81 b,B,C	80.78 ±6.01 b,B	100.90 ±7.51 a,A,B	104.75 ±7.79 a,A
		12	74.46 ±5.54 b,D	78.98 ±5.87 a,b,C	76.38 ±5.68 a,b,C	75.17 ±5.59 b,B	91.20 ±6.78 a,B	83.98 ±6.25 a,b,B
	70°C	0	128.19±9.08a,A	125.90±8.93a,A	108.29 ±8.06 b,c,A	122.91 ±9.14 a,b,A	115.23 ±8.15 a,b,A	118.17±8.44a,b,A
		3	121.74±8.60a,A	120.00±8.48a,A,B	94.79 ±7.05 b,A,B	120.10 ±8.93 a,A	116.38 ±8.22 a,b,A	112.13±7.92 a,b,A
		6	120.00±8.48a,A,B	115.05±8.13a,A,B	93.22 ±6.93 b,A,B	112.36 ±8.36 a,b,A,B	111.32 ±7.87 a,b,A	111.99±7.91 a,b,A
		9	118.00 ±8.34 a,B	110.58±7.81a,b,A	89.73 ±6.67 b,B	104.64 ±7.78 a,b,B	106.22 ±7.51 a,b,A	110.27±7.79 a,b,A
		12	115.00 ±4.87 a,B	103.14±4.37 a,b,B	89.25 ±6.64 b,c,B	78.60 ±5.85 c,C	86.01 ±3.65 b,c,B	98.41 ±4.15 a,b,B
	75°C	0	119.91 ±8.92 b,A	153.96±11.46a,A	153.31 ±11.41 a,A	109.21 ±8.12 b,A	156.10 ±11.61 a,A	122.77 ±9.13 b,A
		3	85.81 ±6.38 a,B	101.30±7.54 a,b,B	102.66 ±7.64 a,b,B	106.69 ±7.94 b,A	115.06 ±8.56 b,B	105.42±7.84 a,b,A,B
		6	84.40 ±6.28 a,b,B	81.16 ±6.04 b,C	91.06 ±6.77 a,b,B	97.39 ±7.24 a,b,B	96.92 ±7.21 a,b,B,C	100.35±7.46 a,B
		9	78.82 ±5.86 b,c,B	75.81 ±5.64 b,c,C	70.35 ±5.23 c,C	97.17 ±7.23 a,B	91.87 ±6.83 a,b,D	97.57 ±7.26 a,B
		12	70.31 ±5.23 b,c,B	64.59 ±4.80 b,c,C	59.21 ±4.40 c,C	93.07 ±6.92 a,B	77.96 ±5.80 b,D	78.06 ±5.81 b,C

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

The highest ( $P < 0.05$ ) putrescine content was found in sucuks stuffed into collagen case both storage on 0 and 12 days. Sucuks stuffed into chitosan had lower putrescine content than those of other sucuks at the beginning of storage. At the end of storage, there were no significant differences ( $P > 0.05$ ) between films included potassium sorbate, nisin, Agzeo and chitosan. At the same time active cases films had the lowest ( $P < 0.05$ ) putrescine formation on the last day of storage (Table 4.30).

After the heat treatment  $70^{\circ}\text{C}$ , the highest ( $P < 0.05$ ) putrescine content was determined in sucuks stuffed into collagen case both storage on  $0^{\text{th}}$  and  $12^{\text{th}}$  days. These values were 221.05 mg/kg and 180 mg/kg after heat treatment at  $70^{\circ}\text{C}$  and end of the storage, respectively. Multilayer films including nisin had lower ( $P < 0.05$ ) putrescine level than those of other samples at the beginning of storage (Table 4.30). The highest ( $P < 0.05$ ) putrescine content was found in sucuks stuffed into collagen case both storage on  $0^{\text{th}}$  and  $12^{\text{th}}$  day between films contained AgZeo and potassium sorbate. Additionally, 110.88 mg/kg was the lowest ( $P < 0.05$ ) putrescine content observed in sucuks stuffed into chitosan (Table 4.30).

After heat treatment at  $75^{\circ}\text{C}$ , the maximum putrescine concentration was identified ( $P < 0.05$ ) in sucuk stuffed into collagen case, while the lowest was in sucuk stuffed into case incorporation of nisin at the first and last days of storage (Table 4.30). At the same time, putrescine content of the samples stuffed into AgZeo and potassium sorbate incorporated cases were not different from each other ( $P > 0.05$ ) during storage (Table 4.30). The putrescine content decreased ( $P < 0.05$ ) during storage in heat treated sucuks at 70 and  $75^{\circ}\text{C}$ . Potassium sorbate could be used as a microbial inhibitor to reduce the formation of biogenic amines in meat products (Shalaby, 1996). Several researchers found that the concentration of putrescine is associated with the total aerobic viable count (Bozkurt, 2006a; Kurt and Zorba, 2009). Putrescine is formed due to activity of enterobacter, some lactic acid bacteria and aerobic bacteria (Bover-Cid et al., 2001). Sirocchi et al. (2013) studied effects of essential oils of *Rosmarinus officinalis* incorporated into high density polyethylene on the formation of biogenic amine for fresh meat. While putrescine concentration of meat in active package (AP) was 33.1 mg/kg, their concentration in poly-coupled package (PP) was 62.3 mg/kg at the end of first 7 fermentation day.

Cadaverine formation in heat treated sucuks at 65°C was affected ( $P < 0.05$ ) by storage time. Additionally, Loizzo et al. (2016) measured the biogenic amines in 'Nduja of Spilinga (a kind of spreadable salami) stored during 15 months of shelf-life. They reported that during storage (6–15 months) cadaverine level was 22.6–94.3 mg/kg confirming previous results of most of the studies on cadaverine level in meat products (Suzzi and Gardini, 2003; Ruiz-Capillas and Jimenez-Colmenero, 2004). This effect can be probably related with a more accentuate proteolysis and as well as with a possible external contamination. Control plastic cases had the maximum concentration of cadaverine whereas potassium sorbate films had minimum concentration of cadaverine on the first day of storage. Films including nisin and chitosan were not different ( $P > 0.05$ ) from each other after the heat treatment. At the end of storage, nisin and collagen case samples had lower ( $P < 0.05$ ) cadaverine concentration than those of other samples (Table 4.31).

After the heat treatment at 70°C, the highest cadaverine content was detected in sucuks stuffed into collagen case for both storage on 0<sup>th</sup> and 12<sup>th</sup> days ( $P < 0.05$ ). These values were 128.19 mg/kg and 115 mg/kg after heat treatment at 70°C and end of the storage, respectively. The minimum concentration of cadaverine was 108.90 mg/kg in potassium sorbate films after heat treatment (Table 4.31). At the end of storage, nisin films had lower cadaverine concentration than those of other films ( $P < 0.05$ ). Incorporation of nisin, chitosan, AgZeo and potassium sorbate decreased ( $P < 0.05$ ) cadaverine formation in heat treated sucuks at 70°C during storage (Table 4.19). However, Chantarasataporn et al. (2014) reported that the cadaverine values of minced pork importantly increased ( $P < 0.05$ ) with the use of nanowhiskey chitosan (CSWK) and oligochitosan (OligoCS) as a food preservative.

After the heat treatment at 75°C, sucuks stuffed into nisin, AgZeo and collagen case had lower ( $P < 0.05$ ) cadaverine concentration than those of other sucuks. Incorporation of nisin, chitosan, potassium sorbate and AgZeo decreased ( $P < 0.05$ ) cadaverine formation in in heat treated sucuks at 75°C during storage (Table 4.31). At the end of storage, potassium sorbate films had the lowest ( $P < 0.05$ ) cadaverine formation at the last day of storage. Cadaverine formation in heat treated sucuks at 75°C was affected ( $P < 0.05$ ) by storage time (Table 4.31). Generally, tyryptamine, putrescine and cadaverine values were affected by different heat treatment ( $P < 0.05$ )

during storage (Table 4.32, 4.33 and 4.34). First day of storage, tyryptamine content of sucuks stuffed into nisin, control plastic case and collagen case were changed ( $P < 0.05$ ) by heat treatment whereas putrescine and cadaverine content of all sucuks were affected importantly ( $P < 0.05$ ) by use of heat process (Table 4.32). At the same time, the tyryptamine and cadaverine content of chitosan, control plastic case and collagen case samples ( $P < 0.05$ ) changed by use of different heat treatments from 3<sup>rd</sup> days to 12<sup>th</sup> days of storage (Table 4.32 and 4.34). Also, using different heat process can affect tyryptamine, putrescine and cadaverine levels of sucuks. Additionally, biogenic amine level decreased by use of nisin-producing strains on chill smoked Salmon with vacuum packaged (Duffes et al., 1999). Jastrzębska et al. (2015) studied the effect of meat additives on the amount of biogenic amines. Potassium sorbate, lactic and citric acids, sodium chloride, disodium diphosphate, sodium nitrite, ascorbic acid, sodium metabisulphite, butylated hydroxyanisole, propyl 3,4,5-trihydroxybenzoate (propyl gallate) and  $\alpha$ -tocopherol were added in white and red meat samples. They found that cadaverine and putrescine contents decreased by use of all the additives during storage. Sirocchi et al. (2013) found that cadaverine formation in meat by active packaging (AP) was 513.9 mg/kg, its formation polypropylene was 652.8 mg/kg during 7 days of storage at 4°C.

After heat treatment at 65°C, 70°C and 75°C, there were no significant differences ( $P > 0.05$ ) between serotonin amount in all sucuks. In the same way, storage time could not affected ( $P > 0.05$ ) serotonin formation of sucuks except collagen case samples after heat process at 65°C and 75°C. Additionally, storage time influenced importantly ( $P < 0.05$ ) serotonin formation in heat treated sucuks at 70°C (Table 4.35).

After the heat treatment at 65°C, spermine concentration of sucuks stuffed into potassium sorbate and chitosan had lower ( $P < 0.05$ ) than those of other sucuks (Table 4.36). The maximum spermine content was 31.35 mg/kg in heat treated sucuks at 65°C stuffed into collagen case ( $P < 0.05$ ). Also, spermine level decreased importantly ( $P < 0.05$ ) in nisin, AgZeo and control plastic cases from day 0 to day 3 of storage (Table 4.36).

No important differences ( $P > 0.05$ ) were observed between all heat treated samples at 70 and 75°C for spermine formations during storage.

**Table 4.32** The effect of different heat treatment temperatures on tyrtamine values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Tyrptamine (mg/kg)	0	65 °C	9.21±0.68 b	16.36±4.50 b	13.91±1.03 a	15.88±1.18 c	11.91±0.8a	12.26±1.2a
		70°C	19.84±1.58 c	16.84±1.55 b	12.51±0.93 a	12.67±0.94 b	12.83±0.9a	13.45±0.9a
		75 °C	4.88±0.36 a	14.20±1.05 a	12.49±0.92 a	10.40±0.77 a	11.27±0.8a	12.56±0.9a
	3	65 °C	6.82±0.51 b	14.66±1.09 b	11.82±0.88 a	10.06±0.74 a	11.62±0.8b	10.57±0.7a
		70°C	17.18±1.21 c	15.44±1.09 b	11.33±0.84 a	10.80±0.80 a	12.24±0.8b	11.13±0.7a
		75 °C	4.37±0.32 a	11.55±0.85 a	11.14±0.82 a	10.25±0.76 a	9.49±0.70 a	10.50±0.7a
	6	65 °C	4.56±0.34 a	12.44±0.92 b	9.45±0.70 b	9.15±0.68 a	8.93±0.6a,b	10.01±0.7b
		70°C	14.81±1.04 b	13.10±0.92 b	7.77±0.57 a	9.47±0.70 a	9.41±0.66 b	10.54±0.7b
		75 °C	3.53±0.26 a	10.18±0.75 a	9.66±0.71 b	8.36±0.62 a	7.52±0.56 a	7.43±0.55 a
	9	65 °C	2.74±0.20 a	10.81±0.88b	7.26±0.54 a	4.58±0.34 a	8.00±0.59 b	8.18±0.60 b
		70°C	12.89±0.91 b	11.38±0.80 b	6.89±0.51 a	7.03±0.52 b	8.43±0.59 b	8.62±0.60 b
		75 °C	2.72±0.20 a	8.29±0.61 a	8.03±0.59 a	7.74±0.57 b	7.41±0.55 a	6.27±0.46 a
	12	65 °C	1.49±0.11 a	8.52±0.63 b	5.07 ±0.37 a,b	4.38±0.32 a	6.73±0.5a,b	5.28±0.39 a
		70°C	11.57±0.49 b	8.97±0.38 b	6.64±0.49 b	5.81±0.43 a	7.09±0.30 b	5.56±0.23 a
		75 °C	1.23±0.09 a	6.31±0.47 a	4.35±0.32 a	4.49±0.33 a	5.50±0.40 a	5.23±0.38 a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.33** The effect of different heat treatment temperatures on putrescine values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Putrescine (mg/kg)	0	65 °C	240.62±13.61 c	220.26±15.57 c	170.15±12.03 a	190.41±5.38 c	150.93±1.18 a	170.67±1.31 a
		70°C	221.05±16.62 b	200.94±13.37 b	180.20±1.35 b	150.25±1.13 b	160.85±11.39 b	180.68±11.80 b
		75 °C	210.59±1.60 a	180.30±1.36 a	170.91±1.33 a	140.35±1.06 a	150.93±1.18 a	170.86±1.32 a
	3	65 °C	190.05±13.43 b	190.08±10.75 b	140.34±9.92 a	150.38±6.38 c	140.82±1.1b	140.83±1.1 a
		70°C	210.06±14.85 c	200.09±14.14 b	140.43±1.07 a	140.10±1.05 b	150.61±10.64 c	150.62±10.65 b
		75 °C	160.15±1.20 a	150.22±1.13 a	140.45±1.07 a	130.94±1.03 a	130.52±1 a	160.81±1.25 c
	6	65 °C	170.57b±9.64	170.1±7.21 b	130.39±9.21 a	130.41±8.30 a	130.49±1 a	130.46±1 a
		70°C	200.5c±14.17	180±12.72 c	140.05±1.04 b	130.02±2.96 a	130.21±9.20 a	140.17±9.91 b
		75 °C	150.77±1.17 a	150.12±1.1 a	130.31±0.99 a	130.81±1.02 a	130.5±1.5 a	130.94±1 a
	9	65 °C	150.25±4.24 a	140.8±11.94 a	120.89±8.54 a	130.02±5.51 b	120.93±1.96 a	120.49±1.92 a
		70°C	190.06±13.43 b	150.58±10.64 b	130.11±1.97 b	120.51±3.93 a	120.62±8.52 a	130.15±9.20 b
		75 °C	150.67±1.16 a	140.85±1.10 a	130.28±0.98 b	130.30±0.99 b	130.22±0.98 b	130.69±1.01 b
	12	65 °C	150.2±8.49 a	140.25±9.91a	120.65±5.11 a	120.95±7.86 a	120.23±1.91 b	120.16±1.91 a
		70°C	180.00±7.63b	150±6.36 b	120.54±2.93 a	120.20±2.90 a	110.88±4.70 a	120.80±5.12 a
		75 °C	150.6±1.16 a	140.29±1.06a	120.54±0.93 a	120.33±0.91 a	120.69±0.94 b	120.70±0.94 a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.34** The effect of different heat treatment temperatures on cadaverine values

Parameters	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Cadaverine (mg/kg)	0	65 °C	121.76±4.06 a	151.95±5.31 b	107.05±3.96 a	123.70 ±6.51 b	118.75±6.83 a	112.13±4.34 a
		70°C	128.19 ±9.08 a,b	125.90±8.93 a	108.29±8.06 a	122.91±9.14 b	115.23±8.15 a	118.17 ±8.44 a,b
		75 °C	119.91±8.92 a	153.96±11.46 b	153.31±11.41 b	109.212±8.12 a	156.1±11.61 b	122.77±9.13 b
	3	65 °C	115.65±4.60 b	112.1±4.34 b	105.15±4.72 b	106.23±3.90 a	110.56±4.22 a	106.52±3.92 a
		70°C	121.74±8.60 b	120.00±8.48 c	94.79±7.05 a	120.10±8.93 b	116.38±8.22 a	112.13±7.92 b
		75 °C	85.81±6.38 a	101.30±7.54 a	102.66±7.64 b	106.69±7.94 a	115.06±8.56 a	105.42±7.84 a
	6	65 °C	98.86±4.35 b	99.80±4.42 b	94.99±4.07 a	100.71±4.49 a	105.75±4.87 a,b	106.39±4.91 a,b
		70°C	120.00±8.48 c	115.05±8.13 c	93.22±6.93 a	112.36±8.36 b	111.32±7.87 b	111.99±7.91 b
		75 °C	84.40±6.28 a	81.16±6.04 a	91.06±6.77 a	97.39±7.24 a	96.92±7.21 a	100.35±7.46 a
	9	65 °C	89.90b±6.69	94.60b±7.04	78.07±5.81 b	80.78±6.01 a	100.90±7.51 b	104.75b±7.79
		70°C	118.00c±8.34	110.58c±7.81	89.73±6.67 c	104.64±7.78 c	106.22±7.51 b	110.27b±7.79
		75 °C	78.82a±5.86	75.81a±5.64	70.35±5.23 a	97.17±7.23 b	91.87±6.83 a	97.57a±7.26
	12	65 °C	74.46±5.54 a	78.98±5.87 b	76.38±5.68 b	75.17±5.59 a	91.20 ±6.78 b,c	83.98±6.25b
		70°C	115.00±4.87b	103.14±4.37c	89.25±6.64 c	78.60±5.85 a	86.01±3.65b	98.41±4.15c
		75 °C	70.31±5.23 a	64.59±4.80 a	59.21±4.40 a	93.07±6.92 b	77.96±5.80a	78.06±5.81a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

Moreover, storage time and antimicrobial cases had no important influence ( $P < 0.05$ ) on the formation of spermine for process temperature at 70°C and 75°C (Table 4.36). Chantarasataporn et al. (2014) used nanowhisker chitosan (CSWK) and oligochitosan (OligoCS) as a food preservative on the minced pork. It was reported that spermine was the highest biogenic amine in all products (31–35 mg/kg) after 1 day of storage.

Storage time and using different cases had important effect on ( $P < 0.05$ ) spermidine formation in heat treated sucuks at 65°C (Table 4.37). The highest ( $P < 0.05$ ) spermidine content was observed in sucuks stuffed into collagen case both storage on 0<sup>th</sup> and 12<sup>th</sup> days. Sucuks stuffed into potassium sorbate had lower spermidine ( $P < 0.05$ ) content than those of other sucuks at the first and last days of storage (Table 4.37).

The lowest spermidine content was in the sucuks stuffed into potassium sorbate whereas the highest in the sucuks stuffed into collagen case ( $P < 0.05$ ) both after heat treatment at 70°C and end of the storage (Table 4.37). These values were 2.75 mg/kg, 0.006 mg/kg and 4.35 mg/kg, 0.99 mg/kg after heat treatment at 70°C and end of the storage respectively. Spermidine formation in heat treated sucuk at 70°C was affected ( $P < 0.05$ ) by storage time (Table 4.37). Potassium sorbate is less used in the meat industry. In the case of potassium sorbate computed biogenic amine levels were above 20 mg/kg for all tested products on the 3rd day of the analysis, which demonstrated poor meat quality. It is worth noting the rapid growth of the amount of biogenic amine in all products, which recommends the low protection of meat with this addition (Jastrzębska et al., 2016).

After the heat treatment at 75°C, sucuks stuffed into potassium sorbate had lower ( $P < 0.05$ ) spermidine concentration than those of other sucuks (Table 4.37). At the end of storage, potassium sorbate films had the lowest spermidine formation ( $P < 0.05$ ). Spermidine formation in heat treated sucuks at 75°C was affected ( $P < 0.05$ ) by storage time (Table 4.37). Generally, spermine, spermidine and serotonin values were affected by heat treatment ( $P < 0.05$ ) during storage (Table 4.38, 4.39 and 4.40). Spermine and serotonin content of all sucuks changed ( $P < 0.05$ ) by heat treatment at the beginning of storage.

**Table 4.35** Changes in serotonin values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Serotonin (mg/kg)	65°C	0	40.69 ±2.30 a,A	39.27±2.77a,A	40.59 ±3.02 a,A	40.20 ±2.99 a,A	41.23 ±3.06 a,A	40.33±3 a,A
		3	38.89 ±2.74 a,A,B	38.76±2.19 a,A	38.30 ±2.85 a,A	40.02±2.97a,A	40.97 ±3.04 a,A	39.46 ±2.93 a,A
		6	38.19 ±2.16 a,A,B	38.25 ±1.62 a,A	36.94 ±2.74 a,A	35.48±2.64a,A	38.52 ±2.86 a,A	37.43 ±2.78 a,A
		9	35.15 ±0.99 a,A,B	36.46 ±3.09 a,A	36.29 ±2.70 a,A	35.08±2.61a,A	37.05 ±2.75 a,A	37.07 ±2.75 a,A
		12	34.96 ±1.97 a,B	36.29 ±2.56 a,A	35.63±2.65 a,A	34.02±2.53a,A	35.95 ±2.67 a,A	35.15 ±2.61 a,A
	70°C	0	42.94 ±3.05 a,A	42.44 ±3.92 a,A	38.24 ±2.84 a,A	40.15 ±2.98 a,A	43.50 ±3.19 a,A	42.66a,A ±3.08
		3	40.94 ±2.89 a,A,B	40.81 ±2.88 a,A,B	36.83 ±2.74 a,A	39.80 ±2.96 a,A	43.13 ±3.04 a,A,B	41.54 ±2.93 a,A
		6	40.20 ±2.83 a,A,B	40.27 ±2.84 a,A,B	36.79 ±2.73 a,A	36.50 ±2.71 a,A	40.55a,A,B±2.86	39.41 ±2.78 a,A
		9	37.00 ±2.61 a,A,B	38.38 ±2.71 a,B	36.28 ±2.70 a,A	35.68 ±2.65 a,A	39.01a,A,B±2.75	39.03 ±2.75 a,A
		12	36.80 ±1.56 a,B	38.20 ±1.62 a,B	33.93 ±2.52 a,A	35.25 ±2.62 a,A	37.79a,B±1.60	37.00 ±1.56 a,A
	75°C	0	38.84 ±2.89 a,A	38.18 ±2.84 a,A	37.58 ±2.79 a,A	37.26 ±2.77 a,A	38.31 ±2.85 a,A	39.14 ±2.91 a,A
		3	35.26±2.62 a,B	37.97 ±2.82 a,A	36.26 ±2.69 a,A	37.02 ±2.75 a,A	36.80 ±2.74 a,A	37.34 ±2.77 a,A
		6	35.26±2.62 a,B	36.60 ±2.72 a,A	35.81 ±2.66 a,A	36.17 ±2.69 a,A	36.01 ±2.68 a,A	37.14 ±2.76 a,A
		9	35.02±2.61 a,B	35.60 ±2.65 a,A	34.69 ±2.58 a,A	36.14 ±2.69 a,A	35.75 ±2.66 a,A	36.50 ±2.71 a,A
		12	34.74±2.58 a,B	34.87±2.59 a,A	33.82 ±2.51 a,A	35.12±2.61a,A	35.36 ±2.63 a,A	34.30 ±2.55 a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4. 36** Changes in spermine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Spermine (mg/kg)	65°C	0	31.35 ±2.33 a,A	29.37 ±2.18 a,b,A	25.49 ±1.89 b,A	26.03 ±1.93 a,b,A	25.80 ±1.92 b,A	29.17 ±2.17 a,b,A
		3	26.60±1.97a,A,B	24.79 ±1.84 a,B	23.92 ±1.78 a,b,A	21.61 ±1.60 b,B	22.88 ±1.70 a,b,A	22.56 ±1.67 a,b,B
		6	24.7 ±1.83 a,B	23.45 ±1.74 a,B	21.66 ±1.61 a,A	21.57 ±1.60 a,B	21.51 ±1.60 a,A	22.4 ±1.67 a,B
		9	24.17±1.79 a,B	22.77 ±1.69 a,B	21.47 ±1.59 a,A	21.40 ±1.59a,B	21.38 ±1.59 a,A	21.38 ±1.59 a,B
		12	23.16±1.72a,B	22.39 ±1.66 a,B	21.38 ±1.59 a,A	21.32±1.58 a,B	21.32 ±1.58 a,A	21.32 ±1.58 a,B
		0	27.20±2.01 a,A	26.14 ±1.94 a,A	26.41 ±1.96 a,A	24.38±1.81 a,A	25.31 ±1.88 a,A	23.87 ±1.77 a,A
	70°C	3	25.24±1.87 a,A	23.94 ±1.78 a,A	23.09 ±1.71 a,A,B	24.33±1.81 a,A	22.75 ±1.69 a,A	22.93 ±1.70 a,A
		6	24.19±1.80 a,A	23.56 ±1.75 a,A	21.51 ±1.60 a,B	21.75±1.61 a,A	21.68 ±1.61 a,A	21.54 ±1.60 a,A
		9	23.24±1.73 a,A	23.38 ±1.74 a,A	21.34 ±1.58 a,B	21.55±1.60 a,A	21.38 ±1.59 a,A	21.53 ±1.60 a,A
		12	22.89±1.70 a,A	22.33 ±1.66 a,A	21.33 ±1.58 a,B	21.37±1.59 a,A	21.23 ±1.58 a,A	21.42 ±1.59 a,A
		0	26.20±1.95 a,A	22.39 ±1.66 a,A	22.41 ±1.66 a,A	24.32±1.81 a,A	22.15 ±1.64 a,A	23.12 ±1.72 a,A
	75°C	3	24.18±1.80 a,A	21.36 ±1.59 a,A	21.43 ±1.59 a,A	21.85±1.62 a,A	21.96 ±1.63 a,A	21.36 ±1.58 a,A
		6	22.25 ±1.65a,A	21.35 ±1.58 b,A	21.43 ±1.59 a,A	21.46±1.59b,A	21.53 ±1.60 b,A	21.34 ±1.58 b,A
		9	21.85±1.62 a,A	21.24 ±1.58 a,A	21.42 ±1.59 a,A	21.30 ±1.58a,A	21.38 ±1.59 a,A	21.24 ±1.58 a,A
		12	21.76 ±1.61a,A	21.16 ±1.57 a,A	21.27 ±1.58 a,A	21.22 ±1.57a,A	21.33 ±1.58 a,A	21.22 ±1.58 a,A

a-b: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-B: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.37** Changes in spermidine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Spermidine (mg/kg)	65°C	0	3.62 <sup>a</sup> ,A±0.26	2.77 <sup>b</sup> ,A±0.2	1.29±0.09 d,A	1.25 ±0.09 d,A	1.95±0.14 c,A	1.76±0.13c,A
		3	2.16 ±0.16 a,B	1.44 ±0.10 b,B	0.32±0.02 d,B	0.85 ±0.06 c,B	0.46±0.03 d,B	0.52 ±0.04 d,B
		6	2.01 ±0.15 a,B	1.17 ±0.08 b,B	0.15 ±0.01d,C	0.38 ±0.02 c,C	0.36±0.02 c,B	0.21 ±0.01 c,d,C
		9	1.89 ±0.14 a,B	0.79 ±0.05 b,C	0.11 ±0.008 e,C,D	0.33 ±0.02 d,C,D	0.52±0.03 c,B	0.20 ±0.01 d,e,C
		12	1.37 ±0.10 a,C	0.30 ±0.02 b,D	0.002 ±0.00 c,D	0.22 ±0.02 b,D	0.04 ±0.003 c,C	0.20±0.01b,C
	70°C	0	4.35 ±0.32 a,A	3.44 ±0.25 b,A	2.75±0.20 c,A	2.72 ±0.21 c,A	2.75±0.20 c,A	2.49 ±0.18 c,A
		3	2.54 ±0.18 a,B	2.19 ±0.16 b,B	1.37±0.10 d,B	1.11 ±0.08 d,B	1.74±0.13 c,B	1.21 ±0.09 d,B
		6	1.22 ±0.09 a,C	1.15 ±0.08 a,C	0.73±0.05 b,C	0.85 ±0.06 b,B	0.09 ±0.006 d,C	0.51 ±0.03 c,C
		9	1.13 ±0.08 a,C	0.89 ±0.06 b,C,D	0.37±0.03 c,D	0.04 ±0.003 e,C	0.08 ±0.006 e,C	0.20 ±0.01 d,D
		12	0.99 ±0.07 a,C	0.59 ±0.04 b,D	0.006 ±0.0005 d,E	0.01 ±0.0012 c,d,C	0.08 ±0.0059 c,d,C	0.13 ±0.009 c,D
	75°C	0	3.04 ±0.22 a,A	2.81 ±0.21 a,A	1.13±0.08 c,A	2.35 ±0.17 b,A	2.09 ±0.15b,A	2.19 ±0.16 b,A
		3	2.81 ±0.21 a,A	1.74 ±0.12 b,B	0.80±0.06 d,B	1.18 ±0.08 c,B	1.14 ±0.08c,B	1.14 ±0.08 c,B
		6	2.129 ±0.15 a,B	0.92 ±0.06 b,C	0.75±0.05 b,B	0.77 ±0.05 b,C	0.92 ±0.06b,B	0.78 ±0.05 b,C
		9	1.82 ±0.13 a,B	0.58 ±0.04 b,D	0.06 ±0.0005 c,C	0.16 ±0.01 c,D	0.52 ±0.03b,C	0.47 ±0.03 b,D
		12	1.06 ±0.07 a,C	0.19 ±0.01 b,c,E	0.04 ±0.003 d,C	0.11 ±0.008 c,d,D	0.20±0.01 b,D	0.04 ±0.003 d,E

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-E: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

Additionally, serotonin content of sucuks stuffed into chitosan, potassium sorbate, AgZeo, control plastic case and collagen case ( $P < 0.05$ ) changed from heat treatment during storage (Table 4.38 and 4.40). Also, spermidine content of sucuks stuffed into active cases were affected by heat treatments from 6<sup>th</sup> days to 12<sup>th</sup> days of storage (Table 4.39). Chantararataporn et al. (2014) found spermidine values of minced pork displayed the same levels ( $P > 0.05$ ) on the use of nanowhisker chitosan (CSWK) and oligochitosan (OligoCS).

After the heat treatment at 65°C, the highest ( $P < 0.05$ ) tyramine content was identified in sucuks stuffed into collagen case both at the beginning and end of the storage (Table 4.41). Multilayer films including nisin had lower tyramine level than those of other samples at the beginning of storage. Additionally, it had no significant differences between potassium sorbate and nisin sucuk samples. Also, 103 mg/kg was the lowest tyramine content seen in sucuks stuffed into chitosan ( $P < 0.05$ ) at the last day of storage (Table 4.41). There were no significant difference between films including AgZeo and chitosan (Table 4.41).

After the heat treatment at 70°C, multilayer films contained AgZeo and chitosan had lower tyramine content than those of AgZeo and nisin films. The highest ( $P < 0.05$ ) tyramine content was determined in sucuks stuffed into collagen case both storage on 0<sup>th</sup> and 12<sup>th</sup> days. At the end of storage, sucuks stuffed into nisin had the lowest ( $P < 0.05$ ) tyramine value as 164.12 mg/kg (Table 4.41).

After the heat treatment at 75°C, tyramine content of the samples stuffed into active and control cases were different ( $P < 0.05$ ) from sucuk stuffed into collagen case during storage (Table 4.41). No important differences ( $P > 0.05$ ) were observed between plastic control plastic and collagen case samples with respect to their tyramine formations at the first and last days of storage. Storage time had important influence ( $P < 0.05$ ) on the formation of tyramine for all heat treatment (65°C, 70°C and 75°C) (Table 4.41).

Vinci and Antonelli (2002) reported that the tyramine formation in red meat rised during 36 days of storage at 4°C. In our study, use of antimicrobial cases decreased ( $P < 0.05$ ) the tyramine formation during storage.

**Table 4.38** The effect of different heat treatment temperatures on serotonin values

Parameter	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Serotonin (mg/kg)	0	65 °C	40.69±2.30 b	39.27±2.77 a	40.59±3.02 a	40.20±2.99 a	41.23±3.06b	40.33±3.00a
		70°C	42.94 ±3.05 c	42.44±3.92 b	38.24±2.84 b	40.15±2.98 a	43.50±3.19c	42.66±3.08b
		75 °C	38.84±2.89 a	38.18±2.84 a	37.58±2.79 b	37.26±2.77 b	38.35±2.85a	39.14±2.91a
	3	65 °C	38.89±2.74 b	38.76±2.19 a	38.30±2.85 b	40.02±2.97 b	40.97±3.04b	39.46±2.93b
		70°C	40.94±2.89 c	40.81±2.88 b	36.83±2.74 a	39.80±2.96 b	43.13±3.04c	41.54±2.93c
		75 °C	35.26±2.62 a	37.97±2.82 a	36.26±2.69 a	37.02±2.75 a	36.8±2.74 a	37.34±2.77a
	6	65 °C	38.19±2.16 b	38.25±1.62 b	36.94±2.74 a	35.48±2.64 a	38.52±2.86b	37.43±2.78a
		70°C	40.20±2.83 c	40.27±2.84c	36.79±2.73 a	36.50±2.71 a	40.55±2.86c	39.41±2.78b
		75 °C	35.26±2.62 a	36.6±2.72 a	35.81±2.66 a	36.17±2.69 a	36.01±2.68a	37.14±2.76a
	9	65 °C	35.15±0.99 a	36.46±3.09 a	36.29±2.70 b	35.08±2.61 a	37.05±2.75b	37.07±2.75a
		70°C	37.00±2.61 b	38.38±2.71 b	36.28±2.70 b	35.68±2.65 a	39.01±2.75c	39.03±2.75b
		75 °C	35.02±2.61 a	35.60±2.65 a	34.69±2.58 a	36.14±2.69 a	35.75±2.66a	36.50±2.71a
	12	65 °C	34.96±1.97 a	36.29±2.56 b	35.63±2.65 b	34.02±2.53 a	35.90±2.67a	35.15±2.61a
		70°C	36.80±1.56 b	38.20±1.62 c	33.93±2.52 a	35.25±2.62 a	37.79±1.60b	37.00±1.56b
		75 °C	34.74±2.58 a	34.87±2.59 a	33.82±2.51 a	35.12±2.61 a	35.36±2.63a	34.30±2.55a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column

**Table 4. 39** The effect of different heat treatment temperatures on spermidine values

Parameter	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Spermidine (mg/kg)	0	65 °C	3.62±0.26 a	2.77±0.20 a	1.29±0.09 a	1.25±0.09 a	1.95±0.14 a	1.76±0.13 a
		70°C	4.35±0.32 b	3.44±0.25 b	2.75±0.20 b	2.72±0.21 b	2.75±0.20 a	2.49±0.18 a,b
		75°C	3.04±0.22 a	2.81±0.21 a	1.13±0.08 a	2.35±0.17 b	2.09±0.15 a	2.19±0.16 a,b
	3	65 °C	2.16±0.16 a	1.44±0.10 a	0.32±0.02 a	0.85±0.06 a	0.46±0.0 a 3	0.52±0.04 a
		70°C	2.54±0.18 a	2.19±0.16 b	1.37±0.10 c	1.11±0.08 a	1.74±0.13 b	1.21±0.09 b
		75°C	2.81±0.21 a	1.74±0.12 a	0.80±0.06 b	1.18±0.08 a	1.14±0.08 b	1.14±0.08 b
	6	65 °C	2.01±0.15 b	1.17±0.08 a	0.15±0.01 a	0.38±0.02 a	0.36±0.02 b	0.21±0.01 a
		70°C	1.22±0.09 a	1.15±0.08 a	0.73±0.05 b	0.85±0.06 b	0.09±0.006a	0.51±0.03 b
		75°C	2.12±0.15 b	0.92±0.06 a	0.75±0.05 b	0.77±0.05 b	0.92±0.06 c	0.78±0.05 b
	9	65 °C	1.89±0.14 a	0.79±0.05 a	0.11±0.008 b	0.33±0.02 c	0.52±0.03 b	0.20±0.01 a
		70°C	1.13±0.08 a	0.89±0.06 a	0.37±0.03 c	0.04±0.003 a	0.08±0.006a	0.20±0.01 a
		75°C	1.82±0.13 a	0.58±0.04 a	0.06±0.0005 a	0.16±0.01 b	0.52±0.01 b	0.47±0.03 b
	12	65 °C	1.37±0.10 a	0.30±0.02 a	0.002±0.00a	0.22±0.02 c	0.04±0.003a	0.20±0.01 b
		70°C	0.99±0.07 a	0.59±0.04 b	0.006±0.00 b	0.01±0.012 a	0.08±0.001b	0.13±0.009b
		75°C	1.06±0.07 a	0.19±0.01 a	0.04±0.003 c	0.11±0.00 b	0.20±0.01 c	0.04±0.003a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column

**Table 4. 40** The effect of different heat treatment temperatures on spermine values

Parameter	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Spermine (mg/kg)	0	65 °C	31.35±2.33 b	29.37±2.18 c	25.49±1.89 b	26.03±1.93 b	25.80±1.92b	29.17±2.17b
		70°C	27.20±2.01 a	26.14±1.94 b	26.41±1.96 b	24.38±1.81 a	25.31±1.88b	23.87±1.77a
		75 °C	26.20±1.95 a	22.39±1.66 a	22.40±1.66 a	24.32±1.81 a	22.15±1.64a	23.12±1.72a
	3	65 °C	26.6±1.97 b	24.79±1.84 b	23.92±1.78 b	21.61±1.60 a	22.88±1.70a	22.56±1.67a
		70°C	25.24±1.87ab	23.9±1.78a,b	23.09±1.71 b	24.33±1.81 b	22.75±1.69a	22.93±1.70a
		75 °C	24.18±1.80 a	21.36±1.59 a	21.43±1.59 a	21.85±1.62 a	21.94±1.63a	21.36±1.58a
	6	65 °C	24.7±1.83 b	23.45±1.74 a	21.66±1.61 a	21.57±1.60 a	21.51±1.60a	22.4±1.67 a
		70°C	24.19±1.80 b	23.56±1.75 a	21.51±1.60 a	21.75±1.61 a	21.68±1.61a	21.54±1.60a
		75 °C	22.25±1.65 a	21.36±1.58 b	21.43±1.59 a	21.46±1.59 a	21.53±1.60a	21.34±1.58a
	9	65 °C	24.17±1.79 b	22.77±1.69 a	21.47±1.59 a	21.40±1.59 a	21.38±1.59a	21.38±1.59a
		70°C	23.24±1.73 b	23.38±1.74 a	21.34±1.58a	21.55±1.60 a	21.39±1.50a	21.53±1.60a
		75 °C	21.85±1.62 a	21.24±1.58 a	21.42±1.59 a	21.30±1.58 a	21.37±1.39a	21.24±1.58a
12	65 °C	23.16±1.72 b	22.39±1.66 a	21.38±1.59 a	21.32±1.58 a	21.32±1.58a	21.32±1.58a	
	70°C	22.89±1.70 a	22.33±1.66 a	21.3±1.58 a	21.35±1.59 a	21.23±1.58a	21.42±1.59a	
	75 °C	21.76±1.61 a	21.16±1.57 a	21.27±1.58 a	21.22±1.57 a	21.33±1.58a	21.22±1.5a	

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column

The maximum tyramine concentration was monitored in the collagen case and the minimum in the potassium sorbate case as their mean values were about 431 and 198 mg/kg, respectively. Similarly, Loizzo et al. (2016) measured the biogenic amines in 'Nduja of Spilinga (a kind of spreadable salami) stored in several packaging materials (i.e. natural casing under vacuum, glass jar, aluminum tube) during 15 months of shelf-life. They found that tyramine contents were  $7.6 \pm 0.01$  mg/kg,  $29.5 \pm 0.07$  mg/kg and 90.2–174.9 mg/kg during 0<sup>th</sup> months, ripening (3 months) and storage (6–15 months), respectively. Gençcelep et al. (2007) observed that presence of starter culture and ripening period had important effects on tyramine, putrescine and cadaverine contents. The permissible maximum level of tyramine in foods is 100-800 mg/kg and 1080 mg/kg is toxic (Shalaby, 1996). In this study, the concentrations were below than this level.

It was found that sucuks stuffed into collagen case had higher histamine values ( $P < 0.05$ ) than those of other sucuks after heat treatment at 65, 70 and 75°C. Collagen case samples had 77.70 mg/kg, 72.70 mg/kg and 68.89 mg/kg at the last day of storage after heat treatment at 65, 70 and 75°C, respectively (Table 4.42). At the same time, sucuks stuffed into chitosan had the lowest histamine values at the beginning of storage after heat treatment. These values were 51.62 mg/kg, 46.72 mg/kg and 42.69 mg/kg after heat treatment at 65°C, 70 °C and 75°C, respectively. Additionally, the histamine formation in heat treated sucuks at all temperature (65 °C, 70 °C and 75°C) decreased significantly ( $P < 0.05$ ) during storage (Table 4.42).

Histamine levels of sucuks were in the order of stuffed into collagen case > control plastic cases > Agzeo in heat processed at 65°C and 70°C whereas its levels were sucuks stuffed into collagen case > control plastic cases > nisin in heat processed at 75°C on 12<sup>th</sup> days of storage (Table 4.42). Nout (1974) reported the allowable maximum histamine contents for sausages ranged from 50 to 100 mg/kg. In this study, histamine content was found in this range both during the fermentation and storage. Sirocchi et al. (2013) studied the performances of the new active packaging system (AP), prepared by essential oils of *Rosmarinus officinalis* at 4% w/w, on chicken meat. They reported that, histamine level in active packed chicken meat was lower than that with poly- coupled packaging in refrigerator at 4°C storage.

**Table 4.41** Changes in tyramine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Tyramine (mg/kg)	65°C	0	513.15 ±19.02 a,A	389.15±7.51 b,c,A	320.15 ±22.63 c,A	324.62 ±9.19 c,A	426 ±6.31 b,A	372 ±8.27 b,c,A
		3	499.15 ±15.29 a,A,B	329.15±8.61 b,B	287.15 ±20.30 c,A,B	294.15 ±12.47 b,c,A	339 ±5.25 b,B	265 ±7.19 d,B
		6	461.15±16.08a,A,B	311.15±13.20b,B	248.72 ±17.58 c,d,B,C	230.45 ±14.66 d,B	282 ±8.21 b,c,C	260 ±7.19 c,B
		9	443.15 ±12.53 a,A,B	299.15±15.38 b,B	223.92 ±15.83 d,C,D	229.14 ±9.72 d,B	252 ±9.18 c,C	258 ±9.19 c,B
		12	431.15 ±14.38 a,B	281.15±9.88 b,B	198.15 ±8.40 c,D	211.15 ±13.73 c,B	103 ± 7.07 d,D	108 ±9.08 d,C
	70°C	0	502.08 ±26.01 a,A	379.06 ±17.06 b,A	331 ±5.39 c,A	322 ±7.29c,A	310.02 ±9.31 d,A	315.66±23.08d,A
		3	488 ±14.50 a,B	318.00 ±12.48 b,B	311 ±4.23 b,B	318 ±6.23 b,B	276.00 ±19.51 c,A,B	283.00±20.01c,A
		6	450 ±11.81 a,C	300±11.21b,B	243 ±6.18 c,C	255 ±7.19 c,C	237.57 ±16.79 c,d,B,C	219.30±15.50 d,B
		9	432±10.54 a,C	288±10.36b,C	229 ±5.17 c,C	2508 ±8.18 b,c,C	212.77 ±15.04 c,C,D	217.99±15.41c,B
		12	420 ±17.81 a,D	270±11.45 b,D	188 ±6.06 d,D	164 ±7.122 e,D	187.00 ±7.93 d,D	200±8.48c,B
	75°C	0	380 ±9.28 a,A	398 ±10.29 a,A	298 ±9.29 b,c,A	287 ±10.2 c,A 1	300 ±15.3 b,A	332 ±10.24 a,b,A
		3	354 ±10.26 a,A	310 ±9.23 a,b,B	243 ±9.18 d,B	258 ±11.19 c,A	265 ±19.19 b,c,B	280 ±11.23 b,A
		6	332 ±11.24 a,A	257 ±9.19 b,B,C	243 ±10.18c,B	251 ±12.18 b,c,A	242 ±20.18 c,B	266 ±9.22 b,A
		9	233 ±12.17 a,A,B	234 ±10.17 a,D	132 ±10.09 c,C	224 ±13.18 b,A	221 ±10.16 b,B	222 ±10.16 b,B
		12	230 ±13.13 a,A,B	222 ±7.16 a,D	130±10.03c,D	132 ±7.09 c,B	155 ±20.11 b,c,C	131 ±10.15 c,B

a-d: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

**Table 4.42** Changes in histamine values of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Parameters	Temperature	Storage Time (days)	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Histamine (mg/kg)	65°C	0	109.00±6.16 a,A	86.00±6.08 b,A	51.62 ±3,65 e,A	61.00±1,72d,A	55.00 ±2.10 e,A	72.65 ±1.25 c,A
		3	103.38±7.31a,B	84.64±4.78 b,A	40.29 ±2,84 d,e,B	52.88±2,24 c,B	45.00 ±1.19 d,B	56.78±1.75 c,B
		6	92.88±5.25a,C	76.76±3.25b,B	18.52 ±1,31 e,C	42.76±2,72 c,C	22.65 ±1.04 d,C	46.36±1.2c,C
		9	88.88±2.51a,D	70.58±5.98 b,B	17.11 ±1,21 e,C	24.52 ±1,04 c,d,D	15.06±0.08 e,C,D	29.15±1.39c,D
		12	77.7±4.39 a,E	55.58±3.9 b,C	15.00 ±0,63 d,C	19.11±1,24c,E	13.00 ±0.09 d,D	20.07 ±1.9c,E
	70°C	0	104.05 ±7.45 a,A	81.10 ±5.92 b,A	50.00 ±1.23 c,d,A	55.00 ±1.16 c,A	46.72 ±3.19 d,A	56.10 ±3.75 c,A
		3	98.38 ±6.95 a,A	79.64 ±5.63 b,A	39.49 ±1.19 d,B	47.58 ±1.34 c,A,B	35.29 ±2.49 d,B	47.88 ±3.38 c,B
		6	87.88 ±6.21 a,B	71.76 ±5.07 b,B	16.60 ±0.77 d,C	40.66 ±0.98 c,B	13.52 ±0,95 d,C	37.76 ±2,67 c,C
		9	83.88 ±5.93 a,B	65.58 ±4.63 b,C	15.17 ±0.59 d,C	21.98 ±0.07 c,C	12.11 ±0.85 d,C	19.52 ±1.38 c,D
		12	72.70 ±3.08 a,C	50.58 ±2.14 b,D	13.35 ±0.08 c,d,C	17.68 ±0.08 c,C	10.00 ±0.42 d,C	14.11 ±0.59 c,D
	75°C	0	100.07 ±2.78 a,A	75.60 ±2.35 b,A	48.87 ±1.33 c,d,A	50.49 ±1.43 c,A	42.69 ±1.41 d,A	53.30 ±1.15 c,A
		3	95.68±2.56 a,A	77.74 ±2.20 b,A	38.47 ±1.24 d,B	45.48 ±1.33 c,A,B	30.87 ±1.25 e,B	44.76 ±1.10 c,B
		6	84.79 ±1.23 a,B	65.64 ±1.99 b,B	15.69 ±0.87 d,C	35.98 ±1.15c,C	12,57 ±0.98 d,C	34.89 ±1.10 c,C
		9	80.88 ±2.98 a,B	60.43 ±1.55 b,B	13.79 ±0.76 d,C	18.79 ±0.90 c,D	10.07 ±0.87 d,C	15.52 ±0.78 c,D
		12	68.89 ±1.99 a,C	44.49 ±1.34 b,C	11,72 ±0.90 d,C	16.67 ±0.80 c,D	9.90 ±0.78 d,C	10.31 ±0.56 d,D

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha= 0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha= 0.05$  level in each column.

Also, using different heat process can affect tyramine and histamine formation of sucuks (Table 4.43 and 4.44).

## **4.8 Sensory Analysis**

Overall sensory values were calculated from an equation proposed by Bozkurt and Erkmen (2004) and the results are shown in Tables 4.45, 4.46 and 4.47.

### **4.8.1 Fermentation**

On the overall sensory quality, statistical analysis was carried out to detect the effect of storage time and active cases. The overall sensory quality of sucuks influenced ( $P < 0.05$ ) by antimicrobial cases at the end of fermentation (Table 4.45). Significant difference ( $P < 0.05$ ) was observed between those of the control plastic samples and the samples stuffed into active cases at the end of fermentation. Furthermore, the most attractive sucuks were found to be collagen cased samples. Also, sucuks stuffed into nisin and AgZeo were popular in 3 days of fermentation (Table 4.45).

### **4.8.2 Storage**

There were significant differences ( $P < 0.05$ ) between all heat treated sucuks at 65°C during storage. After heat treatment, the most admired samples were in sucuks stuffed into AgZeo and nisin while collagen case sample had the lowest sensory values. It was found that sucuks stuffed into AgZeo were the most acceptable ( $P < 0.05$ ) samples at the end of storage (Table 4.46). The sensory properties of sucuks increased significantly ( $P < 0.05$ ) during storage except chitosan and AgZeo samples (Table 4.46).

There were significant differences ( $P < 0.05$ ) between heat treated sucuks at 70°C during storage on day 3, 5 and 15 days (Table 4.46). After heat process (0<sup>th</sup> day), the most preferred samples were sucuks stuffed into AgZeo while control sample had the lowest sensory values. The sensory values of AgZeo samples were higher than those of other samples at the end of storage. The sensory features of heat treated sucuks at 70°C stuffed into potassium sorbate, nisin, control plastic and collagen case risen significantly ( $P < 0.05$ ) during storage (Table 4.46).

**Table 4.43** The effect of different heat treatment temperatures on tyramine values

Parameter	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Tyramine (mg/kg)	0	65 °C	513.15±19 b	389.15±7.51b	320.15±22.6b	324.62±9.19 b	426±6.31 c	372±8.27 c
		70°C	502.08±26 b	379.06±17a	331.00±5.39 c	322±7.29 b	310±9.31a,b	315.6±23 a
		75 °C	380.00±9.28 a	398±10.29 b	298.00±9.29 a	287±10.21 a	300±15.30 a	332±10.24 b
	3	65 °C	499.15±15.29 b	329.15±8.6 b	287.15±20.3 b	294.15 ±12.47 a,b	339.00±5.25 c	265±7.19 b
		70°C	488±14.50 b	318±12.48 a	311.00±4.23 c	318.00±6.23 b	276±19.51 b	283±20.01 a
		75 °C	354±10.26 a	310±9.23 a	243.00±9.18 a	258.00±11.19a	265±19.19 a	280±11.23 a
	6	65 °C	461.15±16.08 b	311.15±13.20 b	248.72±17.58 b	230.45±14.66 a	282±8.21 b	260.00±7.19 b
		70°C	450±11.81 b	300.00±11.21 b	243.00±6.18 a	255.00±7.19 b	237.5±16.79 a	219.30±15.50 a
		75 °C	332±11.24 a	257.00±9.19 a	243.00±10.18 a	251.00±12.18 b	242.0±20.18 a	266.00±9.22 b
	9	65 °C	443.15±12.53 b	299.15±15.38 b,c	223.92±15.83 a	229.14±9.72 a	252±9.18 c	258.00±9.19 b
		70°C	432±10.54 b	288±10.36 b	229.00±5.17 a	250.80±8.18 b	212.7±15.04 a	217.99±15.41 a
		75 °C	233±12.17 a	234±10.17 a	132±10.09 b	224±13.18 a	221 ±10.16 a,b	222.00±10.16 a
	12	65 °C	431.15±14.38 b	281.15 ±9.88 b,c	198.15±8.40 b	211.15±13.73c	103± 7.07 a	108±9.08 a
		70°C	420±17.81 b	270±11.45 b	188.00±6.06 b	164.00±7.12 b	187.00±7.93 c	200±8.48 c
		75 °C	230.00±13.1 a	222±7.16 a	130±10.03 a	132.00±7.09 a	155±20.11 b	131±10.15 b

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.44** The effect of different heat treatment temperatures on histamine values

Parameter	Storage Time (days)	Temperature	CASE TYPE					
			Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
Histamine (mg/kg)	0	65 °C	109.00±6.16 c	86.00 ±6.08 b,c	51.62±3,65 a	61.00±1,72 c	55.00±2.10 c	72.65±1.25 c
		70°C	104.05 ±7.45 a,b	81.10±5.92 b	50.00±1.23 a	55.00±1.16 b	46.72±3.19 b	56.10 ±3.75 a,b
		75°C	100.07±2.78 a	75.60±2.35 a	48.87±1.33 b	50.49±1.43 a	42.69±1.41a	53.30±1.15a
	3	65 °C	103.38±7.31b	84.64±4.78 b	40.29±2,84 a	52.88±2,24 b	45.00±1.19c	56.78±1.75b
		70°C	98.38±6.95 a	79.64±5.6 a 3	39.49±1.19 a	47.58±1.34 a	35.29±2.49b	47.88±3.38a
		75°C	95.68±2.56 a	77.74±2.20 a	38.47±1.24 a	45.48±1.33 a	30.87±1.25a	44.76±1.10a
	6	65 °C	92.88±5.25 c	76.76±3.25 c	18.52±1,31 b	42.76±2,72 b	22.65±1.04b	46.36±1.20c
		70°C	87.88±6.21 b	71.76±5.07 b	16.60±0.77 a	40.66±0.98 b	13.52±0,95 a	37.76 ±2.67 a,b
		75°C	84.79±1.23 a	65.64±1.99 a	15.69±0.87 a	35.98±1.15 a	12.57±0.98a	34.89±1.1a
	9	65 °C	88.88±2.51 c	70.58±5.98 c	17.11±1.21 c	24.52±1.04 c	15.06±0.08c	29.15±1.39c
		70°C	83.88±5.93 b	65.58±4.63 b	15.17±0.59 b	21.98±0.07 b	12.11±0.85b	19.52±1.38b
		75°C	80.88±2.98 a	60.43±1.55 a	13.79±0.76 a	18.79±0.90 a	10.07±0.87a	15.52±078 a
	12	65 °C	77.70±4.39 c	55.58±3.93 c	15.00±0.63 c	19.11±1.24 b	13.00±0.09b	20.07±1.90c
		70°C	72.70±3.08 b	50.58±2.14 b	13.35±0.08 b	17.68±0.08 a	10.00±0.42a	14.11±0.59b
		75°C	68.89±1.99 a	44.49±1.34 a	11.72±0.90 a	16.67±0.80 a	9.90±0.78 a	10.31±0.56a

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

**Table 4.45** Changes in overall sensory quality sucuks stuffed into antimicrobial, control plastic and collagen cases during fermentation

Fermentation Time (days)	CASE TYPE					
	Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3	5.0±0.3a	3.0±0.35c	3.5±0.21b,c	4.00 ±0.28b	3.0±0.21c	4.0±0.28b

a-c: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha=0.05$  level in each row.

There were significant differences ( $P < 0.05$ ) between heat treated sucuks at 75°C during storage. After heat treatment, the most favored samples were sucuks stuffed into AgZeo and chitosan while control plastic and collagen case sample had the lowest sensory values (Table 4.46). The sensory values of chitosan and AgZeo samples were higher than those of other samples at the end of storage. Sucuks stuffed into control plastic and collagen case had the lowest sensory values. The sensory properties of sucuks were increased significantly ( $P < 0.05$ ) during storage except chitosan and AgZeo samples (Table 4.46). The different heat process influenced significantly ( $P < 0.05$ ) sensory values of all sucuks (Table 4.47).

**Table 4.46** Changes in sensory properties of heat treated sucuks at 65, 70 and 75°C stuffed into antimicrobial, control plastic and collagen cases during storage

Temperature	Storage Time (days)	CASE TYPE					
		Collagen case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
65°C	0	2.8±0.15 d,C	4.00 ±0.28 c,C,D	3±0.21d,C,D	5.50 ±0.15 a,C	5.00 ±0.21 b,A	6.00 ±0.34 a,A
	3	2.6±0.18 e,C	3.74 ±0.21 d,D	2.8±0.19d,e,D	5.14 ±0.21 c,C	4.67 ±0.28 b,A	5.61 ±0.55 a,A
	6	3.3±0.19 b,B	4.80±0.20a,b,B,C	3.6±0.25b,B,C	6.60 ±0.42 a,B	6.00±0.16a,b,A	7.20 ±0.61 a,A
	9	4.3±0.12 c,A	5.80 ±0.49 b,c,A	4.6±0.32b,c,A	7.60 ±0.32 a,A	7.00±0.19b,c,A	8.20±0.58a,b,A
	12	3.9 ±0.22 d,A	5.61 ±0.39 c,A,B	4.2±0.17d,A,B	7.70 ±0.50 a,A	7.01 ±0.39 b,A	8.41 ±0.47 a,A
70°C	0	5±0.35 b,B	3.00 ±0.21 d,C,D	4±0.16c,B,C	4.00 ±0.39 c,C	5.00 ±0.35 b,A	6.00 ±0.42 a,A
	3	4.6 ±0.33 c,B	2.80 ±0.19 d,D	3.7±0.32 c,d,C	3.74 ±0.31 c,d,C	4.67 ±0.33 b,A	5.61 ±0.39 a,A
	6	5±0.35a,b,B	3.60 ±0.25 b,B,C	4.8 ±0.20a,b,B	4.80 ±0.20 a,b,B	6.00±0.42a,b,A	7.20 ±0.5 a,A 1
	9	5±0.35 a,B	4.60 ±0.32 a,A	5.8±0.25 a,A	5.80 ±0.16 a,A	7.00 ±0.49 a,A	8.20 ±0.57 a,A
	12	6 ±0.25 c,A	4.20 ±0.17d,A,B	5.6 ±0.39 c,A	5.61 ±0.15 c,A	7.01 ±0.29 b,A	8.41 ±0.35 a,A
75°C	0	4±0.34 b,C	3.00 ±0.16 c,C,D	6 ±0.42 a,A	4.5 ±0.28 b,C	7.00 ±0.3 a,A	6.5 ±0.38 a,A
	3	3.7±0.26c,d,C	2.80 ±0.27 d,D	5.6 ±0.47 b,A	4.20 ±0.17 c,C	6.54 ±0.37 a,A	6.07 ±0.43 a,A
	6	4.8±0.28b,c,B	3.6 ±0.25 c,B,C	7.2±0.71 a,b,B	5.40 ±0.4 a,b,c,B	8.40 ±0.6 a,A	7.80±0.44a,b,A
	9	5.8±0.32b,c,A	4.60 ±0.32 c,A	8.2±0.37 a,B	6.40±0.36a,b,c,A	9.40±0.79a,b,A	8.80±0.24a,b,A
	12	5.6 ±0.24 c,A	4.20 ±0.12 d,A,B	8.4 ±0.47 b,B	6.31c,A±0.17	9.81 ±0.55 a,A	9.11±0.51a,b,A

a-e: Different lower-case letters indicate a statistical difference between the cases type at  $\alpha=0.05$  level in each row. A-D: Different capital letters indicate a statistical difference between the storage time at  $\alpha=0.05$  level in each column.

**Table 4.47** The effect of different heat treatment temperatures on sensory properties

Storage Time (Days)	Temperature	CASE TYPE					
		Collagen Case	Control Plastic Case	Potassium Sorbate	Nisin	Chitosan	AgZeo
0.	65 °C	2.80±0.15 a	4.00±0.28 b	3.00±0.21 a	5.5±0.15 b	5.00±0.21a	6.00±0.34 a
	70°C	5.00±0.35 c	3.00±0.21 a	4.00±0.16 b	4.00±0.39a	5.00±0.35a	6.00±0.42 a
	75°C	4.00±0.34 b	3.00±0.16 a	6.00±0.42 c	4.5±0.28 a	7.00±0.30b	6.5±0.38 a
3.	65 °C	2.61±0.18 a	3.74±0.21 a	2.80±0.19 a	5.14±0.21 <sub>b</sub>	4.67±0.28a	5.61±0.55 a
	70°C	4.67±0.33 c	2.80±0.19 a	3.74±0.32 b	3.74±0.31 a	4.67±0.33a	5.61±0.39 a
	75°C	3.74±0.26 b	2.80±0.27 a	5.61±0.47 c	4.20±0.17 a	6.54±0.37b	6.07±0.43 a
6.	65 °C	3.36±0.19 a	4.80±0.20 b	3.60±0.25 a	6.6±0.42 b	6±0.16 a	7.20±0.61 a
	70°C	5.00±0.35 b	3.60±0.25 a	4.80±0.20 b	4.80±0.20 a	6±0.42 a	7.20±0.51 a
	75°C	4.80±0.28 b	3.60±0.25 a	7.20±0.71 c	5.4 ±0.4a,b	8.40±0.60b	7.80±0.44a,b
9.	65 °C	4.36±0.12 a	5.8±0.49 b	4.60±0.32 a	7.6±0.32 b	7.00±0.19a	8.20±0.58 a
	70°C	5.00 ±0.35 a,b	4.60±0.32 a	5.80±0.25 b	5.80±0.16 a	7.00±0.49a	8.20±0.57 a
	75°C	5.80±0.32 b	4.60±0.28 a	8.20±0.37 c	6.4±0.36a,b	9.40±0.79b	8.80±0.24a,b
12.	65 °C	3.92±0.22 a	5.61±0.39 b	4.20±0.17 a	7.70±0.50 c	7.01±0.39a	8.41±0.47 a
	70°C	6.00±0.25 b	4.20±0.17 a	5.61±0.39 b	5.61±0.15 a	7.01±0.29a	8.41±0.35 a
	75°C	5.61±0.24 b	4.20±0.12 a	8.41±0.47 c	6.3±0.17 b	9.81±0.55b	9.11±0.51 b

Different lower-case letters indicate a statistical difference between the temperatures at  $\alpha=0.05$  level in each each column.

## CHAPTER 5

### CONCLUSION

In this work, a novel active casing, prepared with incorporation of antimicrobial compounds into LDPE, developed to improve the quality characteristics of heat treated sucuks. For this purpose, effects of nisin potassium sorbate, chitosan, silver zeolite (Ag-zeo) incorporated polyethylene antimicrobial, new, case on sensory, microbiological, chemical and physical characteristics of heat treated sucuks in 65, 70 and 75°C were followed for 3 days of fermentation and 12 days of storage after heat treatment.

The results of this study are listed below:

- The findings of this study show that use of active cases reduced the counts of aerobic plate count (APC) and lactic acid bacteria (LAB) more compared to collagen case.
- At all heat treatment temperatures (65°C, 70°C and 75°C) no significant differences were observed between pH values of samples.
- Collagen case samples had the lower moisture content ( $P < 0.05$ ) than other antimicrobial cases for all temperatures. Also, there were no significant differences between antimicrobial cases during storage all heat treatment levels.
- Color values of heat treated sucuks were not different by use of antimicrobial cases.
- TBARS were not influenced importantly ( $P > 0.05$ ) by use of antimicrobial cases.
- Using different antimicrobial cases, fermentation and storage time influenced significantly ( $P < 0.05$ ) the hardness, gumminess and chewiness value of sucuks. ). Additionally, cohesiveness and springiness values of all sucuks

- were not influenced ( $P > 0.05$ ) by use of active case during fermentation and storage. As a result, textural attributes of heat treated sucuks were not affected importantly ( $P > 0.05$ ) by use of nisin, potassium sorbate, chitosan and AgZeo.
- Antimicrobial plastic cases containing nisin, potassium sorbate, chitosan and AgZeo reduced ( $P < 0.05$ ) putrescine, histamine, tyramine and cadaverine concentrations of all heat treated sucuks. . Incorporation of active agents hadn't an important effect ( $P > 0.05$ ) on serotonin and spermine formation during storage.
- According to sensory analyses, sucuks stuffed into nisin and AgZeo cases were the most desirable ( $P < 0.05$ ) at the end of fermentation and storage.

The suggestions of this study are listed below:

- More safer and attractive products can be obtained by use of these antimicrobial cases.
- Consequently, sucuks packaged with minimum processing could be delivered to consumer safe and naturally.
- Therefore, food safety will be provided and shelf life of product can be increased.

## CHAPTER 6

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## RESUME

1. **Name and Surname:** Yasemin ÇELEBİ SEZER

2. **Date of Birth:** 02.01.1988

3. **Academic Title:** Research Assistant

4. **Education:**

DEGREE	FIELD	UNIVERSITY	YEARS
Bachelor	Food Engineerig	Ege University	2005-2009
Master	Food Engineerig	Erciyes University	2009-2012
Doctorate	Food Engineerig	Gaziantep University	2013-2018

5. **Papers**

5.1 **Article exculding technical notes, letter to editor, discussions, event presentations or abstracts published in international periodicals covered by SCI (Science Citation Index)**

Kesmen Z., Çelebi Y., Güllüce A., Yetim H., "Detection Of Seagull Meat In Meat Mixtures Using Real-Time PCR Analysis ", Food Control, vol.34, pp.47-49, 2013.

Süfer, Ö., Çelebi Sezer, Y., Bozok, F., "Convective and microwave drying of mushrooms (A.bisporus and P.ostreatus)", Indian Journal of Pharmaceutical Education and Research, 51(3), Suppl: S389- 392, 2017 (DOI: 10.5530/ijper.51.3s.54).

Çelebi Sezer, Y., Süfer, Ö., Sezer, G., "Extraction of phenolic compounds from oven and microwave dried mushrooms (Agaricus bisporus and Pleurotus ostreatus) by using methanol, ethanol and acetone as solvents", Indian Journal of Pharmaceutical

Education and Research, 51(3), Suppl: S393-397, 2017 (DOI: 10.5530/ijper.51.3s.55).

Çelebi Sezer, Y., Bozkurt, H. “Use of Novel Casing in Sucuk Production: Antimicrobials Incorporated into Multilayer Plastic Film”, *Acta Alimentaria*, 2018, DOI: 10.1556/066.2018.0001.

## **5.2. Other articles published in international peer-reviewed journals**

Öz, A.T., Süfer, Ö., Çelebi Sezer, Y. “Poly (Lactic Acid) Films in Food Packaging Systems”, *Food Science and Nutrition Technology*, 2017, 2(4), 1-5.

## **5.3. Paper presented at international congress, conference or symposium, and published as full text in proceedings**

Kesmen, Z., Celebi, Y., Yetim, H., Detection of seagull meat in meat mixtures using realtime PCR assay. 57th ICoMST International Congress of Meat Science and Technology, 7-12 August, pp:249, 2011, Ghent, Belgium.

Y.Ç.Sezer, Z.Kesmen, A.E.Yetiman, H.Yetim., Evaluation of Competitiveness and Adaptation Ability of Autochthonous Starter Cultures in Sucuk Fermentation. 59th ICoMST International Congress of Meat Science and Technology, 18-23 August, pp:259, 2013, İzmir, Turkey.

Sezer, Y.Ç., Süfer, Ö., “Lactic Starter Cultures Used in Fermented Meat Product, Sucuk”, 2nd International Food Congress, Novel Approaches in Food Industry, NAFI 2014, 26-29 May 2014, Kuşadası, Turkey. (Poster)

Süfer, Ö., Çelebi Sezer Y., “Kerebiç”, The 2nd International Symposium on “Traditional Foods from Adriatic to Caucasus”, 24-26 October 2013, Ohrid, Macedonia. (Poster).

Süfer, Ö., Sezer, Y.Ç., “A Research On Specification of Functional Food Tendencies of Turkish Consumers”, 2014 Annual Conference & Exhibition, Functional Foods, Nutraceuticals, Natural Health Products and Dietary Supplements, 14-17 October 2014, Istanbul, Turkey. (Poster)

Sezer, Y.Ç., Süfer, Ö., “All Aspects of Food Nanotechnology and Current Status in World”, 2014 Annual Conference & Exhibition, Functional Foods, Nutraceuticals, Natural Health Products and Dietary Supplements, 14-17 October 2014, Istanbul, Turkey. (Poster)

Süfer, Ö., Çelebi Sezer, Y., Şahin, T., “An Immigrant Taste: Curd Cheese Dessert with Black Mulberry”, The 3<sup>rd</sup> International Symposium on “Traditional Foods from Adriatic to Caucasus”, 1-4 October 2015, Sarajevo / Bosnia and Herzegovina. (Poster)

Şahin, T., Süfer, Ö., Çelebi Sezer, Y., “Katırcı Halva – Disappering Flavour”, The 3<sup>rd</sup> International Symposium on “Traditional Foods from Adriatic to Caucasus”, 1-4 October 2015, Sarajevo / Bosnia and Herzegovina. (Poster)

Şahin, T., Süfer, Ö., Çelebi Sezer, Y., “The Most Chill Drink of Çukurova: Aşlama”, The 3<sup>rd</sup> International Symposium on “Traditional Foods from Adriatic to Caucasus”, 1-4 October 2015, Sarajevo / Bosnia and Herzegovina. (Poster)

Çelebi Sezer, Y., Süfer, Ö., Sezer, G., Bozkurt, H., “Physico-chemical and Microbiological Quality of Commercial Sucuk Samples”, 26<sup>th</sup> International Scientific Experts Congress on Agriculture and Food Industry, 27-30 September 2015, Sarajevo. (Oral Presentation)

Çelebi Sezer, Y., Sezer, G. “Application of Real Time PCR Techniques in Food Industry” 26<sup>th</sup> International Scientific Experts Congress on Agriculture and Food Industry, 27-30 September 2015, Sarajevo. (Poster)

Süfer, Ö., Çelebi Sezer, Y., Nakilcioğlu Taş, E., “Mathematical Modeling Researches Focused on Baking of Cereal Products”, 15<sup>th</sup> International Cereal and Bread Congress (15<sup>th</sup> ICBC), 18-21 April 2016, İstanbul / Turkey (Oral Presentation).

Süfer, Ö., Çelebi Sezer, Y., Bozok, F., “Convective and microwave drying of mushrooms (*A.bisporus* and *P.ostreatus*)”, The Third International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP 2017), 13-16 April 2017, Girne-Turkish Republic of Northern Cyprus.

Çelebi Sezer, Y., Süfer, Ö., Sezer, G., “Extraction of phenolic compounds from oven and microwave dried mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) by using methanol, ethanol and acetone as solvents”, The Third International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP 2017), 13-16 April 2017, Girne-Turkish Republic of Northern Cyprus.

Çelebi Sezer, Y., Süfer, Ö., “Conventional Drying and Color Changes of Anchovy (*E. encrasicolus*)”, International Conference on Agriculture, Forest, Food Sciences and Technologies (ICAFOF 2017), 15-17 May 2017, Cappadocia, Turkey.

Öz, A. T., Süfer, Ö., Çelebi Sezer, Y., “Poly(lactic) acid films in food packaging systems,” International Conference on Agriculture, Forest, Food Sciences and Technologies (ICAFOF 2017), 15-17 May 2017, Cappadocia, Turkey.

Süfer, Ö., Çelebi Sezer, Y., Gamlı, Ö. F., “Fish drying and mathematical models: A review”, 5th International Conference Sustainable Postharvest and Food Technologies (INOPTTEP 2017), 23-28 April 2017, Vrsac, Serbia.

Çelebi Sezer, Y., Süfer, Ö., Gamlı, Ö. F., “Physical attributes of Turkish sucuks cooked in microwave oven”, 5th International Conference Sustainable Postharvest and Food Technologies (INOPTTEP 2017), 23-28 April 2017, Vrsac, Serbia.

#### **5.4. Paper presented at national congress, conference or symposium, and published as full text in proceedings**

Çelebi Y., Törnük F, Şimşek H, Yetim H., Din ve inançların dünyada et ve et ürünleri tüketimindeki rolü ve gelecekte yaşanabilecek gelişmeler, 7. Gıda Mühendisliği Kongresi, 24-26 Kasım, 2011, Ankara

Sezer Y.Ç., Kesmen Z., Yetiman, A.E., Yetim H., Sucukta Laktik Mikrofloranın Belirlenmesinde Polimeraz Zincir Reaksiyonu- Denatüre Gradient Jel Elektroferez (PCR DGGE) Yönteminin Propidium Monoazide (PMA) ile Birlikte Kullanım, 3. Geleneksel Gıdalar Sempozyumu, 10-12 Mayıs 2012, Konya.

Sezer Y.Ç., Süfer Ö., Yetim H., “Tüm Yörelere Sahiplendiği Ortak Lezzet: Tirit”, 3. Geleneksel Gıdalar Sempozyumu, 10-12 Mayıs 2012, Konya.

Süfer Ö., Sezer Y.Ç., “Bir Karadeniz Klasığı: Kuymak”, 4. Geleneksel Gıdalar Sempozyumu, 17-19 Nisan 2014, Adana.

Sezer Y.Ç., Süfer Ö., “Toprak Güveç (Çömlek) ve Kullanımı”, 4. Geleneksel Gıdalar Sempozyumu, 17-19 Nisan 2014, Adana.

Sezer, G., Sezer Y.Ç. “Moleküler Biyoloji ve Genetik Çalışmalarında Yaygın Kullanılan Polimeraz zincir Reaksiyonu Kullanım Hataları”. 4. Ulusal Moleküler Biyoloji ve Biyoteknoloji Kongresi, 21-24 Ağustos 2015, Afyon.

Sezer Y.Ç., Sezer, G., Bozok, F., Doğan, H.H. Mantar Tozu İlave Edilerek Üretilen Sucukların Mikrobiyolojik ve Kimyasal Özellikleri. X. Türkiye Yemeklik Mantar Kongresi, 20-23 Ekim 2015, Adana.

#### **6. International references**

There are 12 international references.

#### **7. Projects**

Çelebi Yasemin, “Zeytin yaprağı ekstraktı, eldesi, analizi, kullanım alanları”, Ege Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2009, İzmir (Bachelor)

Bilimsel Araştırma Projesi, “Sucuktan izole edilen Laktik asit Bakterilerinin Starter Kültür Olarak Kullanım Potansiyelinin Belirlenmesi”, Erciyes Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği ABD, KAYSERİ (Master).

TÜBİTAK, 107 O 987, Bursiyer, Sucuktan İzole Edilen Fermentatif Bakterilerin Starter Kültür Olarak Kullanım Potansiyelinin Belirlenmesi.

Bilimsel Araştırma Projesi, MF 14.14. “Aktif Film ile Paketlenen Sucuklardaki Değişimlerin İncelenmesi”, Gaziantep Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği ABD, GAZİANTEP (Doctorate).

### 8. Undergraduate and graduate level courses in the last two years

Academic Year	Period	Name of Course	Hours	
			Theoric	Practice
2015 – 2016 and 2016 - 2017	Autumn	GMB 305 Gıda Mikrobiyolojisi Lab 1		2
		GMB 403 Gıda Teknolojisi Laboratuvarı 1		2
	Spring	GMB 302 Gıda Mikrobiyolojisi Lab 2		2
		GMB 404 Gıda Teknolojisi Laboratuvarı 2		2