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STORAGE STABILITY OF RAW CHICKEN DRUMSTICKS PACKED WITH ANTIMICROBIAL PACKAGE FILMS

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ENGİN DİRİCAN

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Storage Stability of Raw Chicken Drumsticks Packed with Antimicrobial Package Films

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Food Engineering

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Supervisors

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by

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Engin DİRİCAN

ABSTRACT

STORAGE STABILITY OF RAW CHICKEN DRUMSTICKS PACKED WITH ANTIMICROBIAL PACKAGE FILMS

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Antimicrobial packaging is a packaging system which inhibits the microorganisms and pathogens causing food spoilage by controlled release, prevents spoilage and increases the shelf life of foods.

In this study, storage stability of raw chicken drumsticks packed with antimicrobial package films was studied. For this purpose, raw chicken drumsticks were packed under vacuum with antimicrobial packaging films containing chitosan, nisin, silver-loaded zeolite (AgZeO) or potassium sorbate (PS) and stored at +4 °C for a week. In addition raw chicken drumsticks were packed with control films (without additive) under vacuum and stored at same conditions to compare the effect of antimicrobial packaging. Since microbiological growth and lipid oxidation are the most important factors in deterioration of raw chicken drumsticks, the samples were taken daily and their microbiological count (total aerobic bacteria, total coliform, and total mold and yeasts) and 2-thiobarbituric acid reactive substances (TBARS) values, changes in pH, color, and hardness values were determined during the storage period.

Total aerobic mesophilic plate counts (APC) of samples packed in chitosan, nisin, AgZeO and PS containing films were 0.99, 0.97, 0.51, and 0.06 log, respectively lower than those of samples packed in control alone. The corresponding total coliform counts were 0.75, 0.68, 0.28, and 0.25 log lower. Mold and yeast count were lower by 1.02, 0.87, 0.86, and 0.25 log for samples packaged in chitosan, PS, nisin, and AgZeO-containing films, respectively. Samples packaged in chitosan, PS, AgZeO, and nisin containing films had lower TBARS values by 3.69, 1.72, 0.59, and 0.48 mg/kg, respectively lower than those of samples packed in control alone. The pH, color and hardness of the samples were not affected significantly by use of the different packaging films.

Therefore, active films containing chitosan or nisin can be suggested as suitable packaging materials for chicken drumstick to increase safety and quality.

Key words: Raw chicken drumstick, Chitosan, Nisin, Silver-loaded zeolite, Potassium sorbate

ÖZET

ANTİMİKROBİAL FİLMLERLE PAKETLENMİŞ TAZE TAVUK BUTUNUN DEPOLANMA DAYANIKLILIĞI

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Antimikrobiyal paketleme gıdaların bozulmasına neden olan mikroorganizmaları ve patojenleri etkisiz hale getiren, bozulmaları engelleyen, gıdanın raf ömrünü uzatan paketleme sistemidir.

Bu çalışmada, antimikrobiyal vakum paketlemenin tavuk butlarının dayanımına etkisi çalışılmıştır. Bunun için tavuk butları kitosan, nisin, gümüşlenmiş zeolit ve potasyum sorbat içeren dört ticari antimikrobiyal filmle vakum altında paketlendi ve +4°C'de bir hafta depolandı. Antimikrobiyal filmin etkisini anlamak için tavuk butları aynı zamanda katkısız kontrol filmle vakum altında paketlenerek aynı şartlarda depolandı. Tavuğun bozulmasında mikrobiyolojik üreme ve yağların oksitlenmesi en önemli etkenlerdendir. Bu sebeple depolama sürecinde günlük numune alınarak tavukların mikrobiyal (toplam bakteri, toplam koliform ve küf-maya) sayımları ve 2-tiyobarbitürik asit reaktif maddeleri (TBARS) değerleri, pH, renk ve sertlik değişimleri belirlendi.

Kitosan, nisin, gümüşlenmiş zeolit ve potasyum sorbat içeren antimikrobial filmlerde toplam bakteri sayımı, katkısız paketlenmiş kontrol numunesinden sırasıyla 0,99; 0,97; 0,51; ve 0,06 log daha düşüktür. Ayrıca toplam koliform sayıları da; 0,75; 0,68; 0,28; 0,25 log daha düşüktür. Toplam küf ve maya değerleri, kitosan, PS, nisin ve AgZeO içeren filmlerle paketlenmiş numuneler için sırasıyla 1,02; 0,87, 0,86; ve 0,25 log düşük bulunmuştur. Kitosan, PS, AgZeO ve nisin içeren filmlerle paketlenmiş numuneler kontrole göre sırasıyla 3,69; 1,72; 0,59 ve 0,48 mg/kg daha düşük TBARS değerlerine sahiptirler. Numunelerin pH, renk ve sertlik değerleri farklı ambalaj filmlerin kullanılmasıyla önemli ölçüde etkilenmedi.

Bu nedenle, kitosan ya da nisin içeren aktif filmler tavuk butlarının güvenliğini ve kalitesini artırmak için uygun ambalaj malzemeleri olarak önerilebilir.

Anahtar Kelimeler: Tavuk butu, kitosan, nisin, gümüşlenmiş zeolit, potasyum sorbat

To Specially my wife

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LIST OF SYMBOLS/ABBREVATION

APC	Total aerobic mesophilic plate count
FDA	Food and Drug Administration
HPMC	Hydroxypropyl methyl cellulose
LDPE	Low density polyethylene
MA	Methacrylic acid
MC	Methyl cellulose
MDA	Malondialehyde
N	Newton
NA	Nutrient Agar
PDA	Potato Dextrose Agar
PE	Polyethylene
PS	Potassium sorbate
SCF	Scientific Committee for Food

CHAPTER 1

INTRODUCTION

Packaging can be identified active when it gives any preferred function in food preservation other ensuring inert barrier to outer conditions (Hutton, 2003). Active package is a kind of package that changes condition of packaging food to improve shelf-life, to increase safety and sensory characteristics that providing quality of packaged food (Vermeiren et al., 1999).

Usually, active packaged food may provide some functions such as scavenging oxygen, moisture or ethylene, emission of ethanol, flavors and presenting antimicrobial activity (Quintavalla and Vicini, 2002). The aim of adding antimicrobials in the food packaging film is to retard spoilage and to lower contamination with pathogenic microorganisms. Antimicrobial compounds adding in the packaging materials can be configured to quietly release while food transportation and storage (Ishitani, 1995). Consequently, microbial quality of food products may be drastically advanced and shelf life considerably extended.

Antimicrobial agent has various activities and affects microorganisms differently. These are because of the difference in antimicrobial mechanisms and physiologies of microorganisms. Addition of antimicrobial agents into the packaging films is a convenient method of obtaining active packages with antimicrobial activity. There are studies reported on literature which show effect of antimicrobial agents incorporated into film on specific microorganism. Commonly studied agents are: nisin (Bari et al., 2005; Pranoto et al., 2005; Economou et al., 2009; Ercolini et al., 2010; La Storia et al., 2012), chitosan (Chen et al., 1999; Quattara et al., 2000; Chounou et al., 2013), potassium sorbate (Weng and Hotchkiss, 1993; Han and Floros, 1997; Pranoto et al., 2005), silver substituted zeolite (Ishitani, 1995; Brody et al., 2001), triclosan (Cutter, 1999; Vermeiren et al., 2002), and essential oils (Hong et al., 2000; Oussalah et al., 2004).

Although these antimicrobial packaging materials have antimicrobial activity against several individual microorganisms in laboratory conditions, they may lose some of their activity due to some reasons; entrapping of the active sites of the antimicrobial agents with the other constituents of the film and/or processing conditions (temperature etc.) of the packaging substance, that producing such a substance with antimicrobial activity has become to be unfeasible. Hence there was a need to check their activities on food systems that effectiveness of them has been still uncertain.

Consumption of poultry meat has risen up in the last two decades; therefore control of microbial growth and contamination of chicken carcasses is of important concern for the food industry. Microbial growth which decreases the shelf-life of chicken and rises risk of food borne illness happens mainly on surface, because of post-processing handling; tries that are provided to increase safety and to retard spoilage. As a result, improving techniques to increase shelf-life and total full safety/quality show main task of poultry processing industry (Economou et al., 2009).

The purpose of study was to determine effect of various antimicrobial-vacuum packaging (chitosan, nisin, PS, and AgZeo containing films) on the storage stability of raw chicken drumsticks during refrigerated storage.

CHAPTER 2

LITERATURE REVIEW

2.1. Food packaging

2.1.1. History of food packaging

It is believed that modern food packaging has started in 19th century, finding of canning by Nicholas A., Samuel C. Prescott and William L. Underwood worked to set up fundamental rules of bacteriology as related to canning processes after introduction of food microbiology by Pasteur and colleagues in 19th century (Wilson, 2007).

3-peace tin-plated steel cans, glass bottles and wooden crates have been used for food and beverage dissemination at the beginning of the 20th century. Lots of package innovations used while the interval among World War I and II that contain aluminum foil, electrically powered package machinery, plastics like polyethylene, polyvinylidene chloride, aseptic package and flexible package. Many of these innovations used to protect military foods from excessive condition in places where war was happening (Lord, 2008).

The other important innovations in this century contain active packaging and intelligent or smart packaging. Multilayer block plastic cans, microwave suspectors, dispensing closures, gas barrier films for main cuts of meat, modified atmosphere packaging, are the important examples of developments for ease feature into the 21st century. In addition, some of these developments are connected with nanotechnology that future can lie in development of barrier and structural/mechanical features of packaging substances and improvement of sensing technologies (Brody et al., 2008).

2.1.2. Roles of food packaging

Main acts of food packaging are to preserve foods from externally effects and damage, to include food, to ensure consumers with ingredient and nutritional knowledge (Coles, 2003; Robertson, 2006; Marsh and Bugusu, 2007). This function includes delay of deterioration, increasing of shelf life and protection of quality and safety of packaging food. The secondary roles of the food packaging are traceability, convenience and the tamper indication. The aim of the food packaging is to satisfy both producer and consumers by including food in cost effective way, sustains safety of food and also to make environmental effect minimum.

Packaging preserves foods from environmental effects like heat, light, moisture, oxygen, pressure, microorganism, dirty particles and dusty fragments. Most of these factors cause decay of beverages and foods (Marsh and Bugusu, 2007). Extending shelf life includes delay of enzymatic, microbial and biochemical reactions through different strategies like control of moisture, temperature and removal of oxygen. To prevent recontamination exact integration of product, process, packaging and transport have play very crucial role (Robertson, 2006).

2.2. Active packaging

Active package is a kind of package which is innovative concept developed to modify actively the internal environment by continuously interacting with the food over the stipulated shelf-life or improves safety or sensory features of product (Suppakul et al., 2003).

Active packaging can modify the environment inside the package, thus altering the state of the food system or headspace to improve food quality through improve of shelf life, continue of microbial safety or enhancement of sensory qualities (De Kruijf et al., 2002; Han, 2003; Suppakul et al., 2003). Due to the increased desire for high quality, natural, safe products by humans, the popularity of active packaging has increased (Quintavalla and Vicini, 2002; Han, 2003; Cha and Chinnan, 2004; Özdemir and Floros, 2004). Today, three groups of active packaging methods used to protect and enhance the quality and safety of food which are absorbers, releasing systems and other systems. Unpreferred combinations like oxygen, carbon dioxide, ethylene, excessive water, etc., are cleaned off by absorbing systems. Packaged

substances which include protective adding or emitting active compounds to packaged food or onto head-space or packaged by to release systems. The cooling and heating packaged substances may be showed as other packaged systems (Ahvenainen, 2003).

2.3. Antimicrobial packaging

Fresh foods spoil easily because of the presence of intensive and extensive spoilage or pathogenic organisms. Antimicrobial agents are related to wide range of industrial sectors such as: environmental, food, synthetic textiles, packaged, healthcare, medical also structure and decoration. This may be divided in the two kinds as organic and inorganic. Compared with inorganic antimicrobial agents, organic antimicrobial substances are frequently unstable especially at high temperatures and/or pressures. This situation presents potential obstacle for product formulation. As a result, inorganic substances such as metal and metal oxides have fascinated a lot of attention over past decade because of their capability to withstand harsh process conditions (Zhang et al., 2007).

Antimicrobial packaging is a kind of active packaging (Appendini and Hotchkiss, 2002). To increase shelf-life and to improve product quality and safety, antimicrobial packaging material has to sustain lag phase and decrease growth rate of microorganisms.

The raised request for safe and less processed fresh product have intensified studies at the antimicrobial packaging which including antimicrobial agents can be more effective. The little movement of agents from packaging material to surface of product limited functional effect on food surface. On the other hand, for extended periods of time they remain at high concentrations (Quintavalla and Vicini, 2002; Pranoto et al., 2005).

2.3.1. Types of antimicrobial food packaging

2.3.1.1. Usage of sachets/pads including volatile antimicrobial agents

Sachets or pads are the most important and accomplished commercial utilization of antimicrobial packaging. They may be enclosed loose or connected to the interior of package. There are three types (Appendini and Hotchkiss, 2002):

-Oxygen absorbers

-Moisture absorbers

-Ethanol vapor generators.

a. Oxygen absorbers: These systems consume oxygen from package headspace and oxygen which attends through package wall. For preventing growth of aerobic bacteria and molds into dairy and bakery products, oxygen scavenging is an effective way (Suppakul et al., 2003). Iron or ascorbic acid was used since they can oxidize and consume the available oxygen in the package. Iron powder may decrease the oxygen concentration into headspace to smallest than 0.01%. A drawback of iron-based scavengers is which generally may not pass metal detectors. However ascorbic acid, ascorbate salts or catechol may be used as alternative to iron-based absorbers, they do not use widely today (Robertson, 2006).

b. Moisture absorbers: Based on the respiration in horticulture produce, melting of ice, temperature fluctuations in food package with high equilibrium relative humidity, or drip of tissue fluid from cut meats and produce, liquid water may be accumulative in packaging. The main objective of scavenging systems is to decrease water activity of package, which prevents the growth of microorganisms on foodstuff. In most of foods, such as tomato, cheeses, meats and spices, pouches including sodium chloride and desiccant have been successfully utilized for moisture control (Suppakul et al., 2003).

c. Ethanol vapor generators: Since sachets including encapsulated ethanol set free its vapor into the packaged headspace in ethanol generating system, it sustains the preservative effect into product (Suppakul, 2003). That is only effective in products

with low water activity (a_w< 0.92), and delays molds in bakery and dried fish products (Appendini and Hotchkiss, 2002).

2.3.1.2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers

Antimicrobials can be associated into polymers by two manners:

1. Associated of agents into the polymer.

2. Associated of antimicrobial materials in the solvents spreading to the surface of film.

Thermal polymer processing techniques, extrusion and injection molding, can be used by thermally stable antimicrobials. Solvent compounding can be most suitable technique for their associate into polymers for heat sensitive antimicrobials such as enzymes and volatile compounds (Appendini and Hotchkiss, 2002).

Antimicrobial chemicals entered in the packaging substance can be volatile or non-volatile. If they were non-volatile, antimicrobial packaging substances could touch food surface. Antimicrobial agents may spread to the surface in this way. At this point, surface properties and diffusion kinetics can be important. Due to the surface concentration needed to be sustained at less inhibitory concentration at the food surface, the diffusion of antimicrobial from film could happen at appropriate rate.

Packaged systems set free volatile antimicrobials were improved. They were carbon dioxide, sulfur dioxide, allylisothiocyanate and chlorine dioxide which may penetrate bulk matrix of food. Polymer cannot want to contact product in this case (Appendini and Hotchkiss, 2002).

2.3.1.3. Coating or adsorbing antimicrobials to polymer surfaces

Antimicrobial agents are responsive to high temperature cannot be used in polymer processing. For this reason, whose are frequently covered on substance after forming or to add pouring films. Nisin/methylcellulose coatings applied on polyethylene films (Appendini and Hotchkiss, 2002) are examples. Protein has enough improved

capacity for adsorption because of their amphiphilic structure. Figure 2.1 indicate effect of covering location on diffusion of antimicrobial agents.

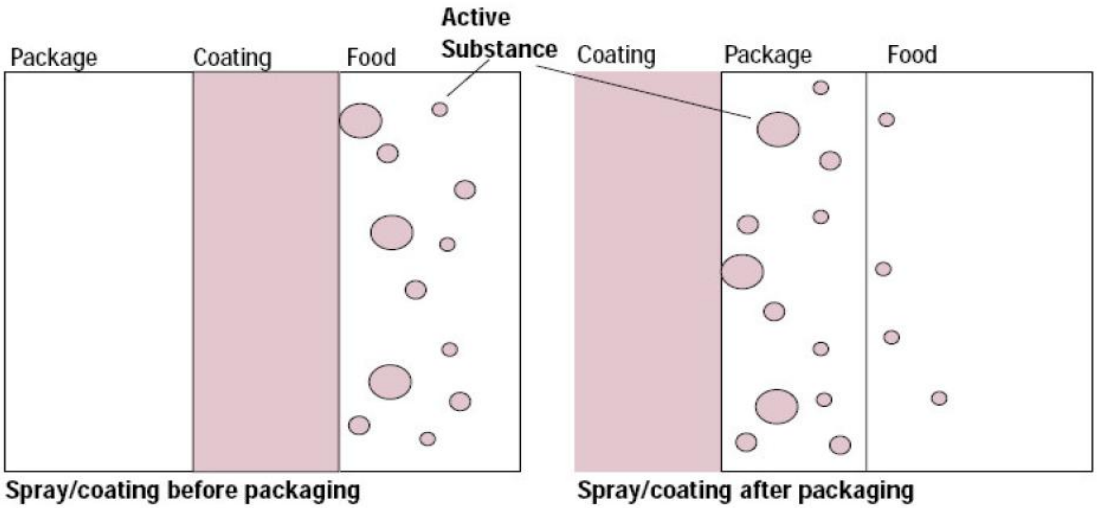


Figure 2.1.The effect of the coating location on the diffusion of antimicrobial agents

2.3.1.4. Immobilization of antimicrobials to polymers by ion or covalent linkages

The kind of fixing happens with presence of functional groups on both antimicrobial and polymer. Peptides, enzymes, polyamines and organic acids are good examples for antimicrobials with functional groups. In addition there are many examples of polymers used for food packaging that having functional groups. Table 2.1 shows antimicrobials which covalently immobilized.

Table 2.1Antimicrobials covalently/ionically immobilized in polymer supports (Appendini and Hotchkiss, 2002).

Functional support	**Antimicrobials**
Ionomeric films	Benomyl Benzoyl chloride Bacteriocin
Polystyrene	Lysozyme Synthetic antimicrobial peptides
Polyvinyl alcohol	Lysozyme
Nylon 6,6 resins	Lysozyme

2.3.1.5. Use of polymers that are naturally antimicrobial

Cationic polymers like chitosan and poly-L-lysine are naturally antimicrobial and were used in films and coatings (Appendini and Hotchkiss, 2002). Chitosan was confirmed as food ingredient from FDA recently; for this reason, the use of chitosan for new product improvement is popular as inherent antimicrobial agent (Ahvenainen, 2003).

Generally, antimicrobial agents combined into plastics one of them is the silver-substituted zeolite which was improved in Japan. Silver-ions, that inhibit large area of metabolic enzymes, have strong antimicrobial activity with wide spectrum. The zeolite, that have a lot of its surface atoms replaced by silver, which is laminated as thin layer (3–6 mm) on surface of food interact polymer and shows to set free silver ions as aqueous solution from food enters the exposed cavities of porous structure (Ishitani, 1995).

There are different antimicrobial agents used to develop antimicrobial packaging in different polymer systems. Table 2.2 shows some examples of antimicrobial food packaging applications.

Some of traditional antimicrobial films, many functional groups which get antimicrobial activity were immobilized onto surface of polymer films by modified chemical techniques to avoid the transfer of antimicrobial materials from the polymer to food (Haynie, 1995).

Table 2.2 Applications of antimicrobial food packaging (Quintavalla and Vicini, 2002).

Antimicrobial agent	Packaging material	Substrate
Acetic acid	Chitosan Chitosan	Water Bologna, cooked ham, pastrami
Benzoic acid	PE-co-MA	Culture Media
Benzoic anhydride	LDPE	Culture Media
Sodium benzoate	MC/chitosan	Culture Media
p-aminobenzoic acid	WPI	Culture Media
Lactic acid	Alginate Corn zein film	Lean beef muscle Culture Media
Propionic acid	Chitosan	Water Bologna, cooked ham, pastrami
Sorbic acid	WPI	Culture Media
Sorbic acid anhydride	PE	Culture Media
Potassium sorbate	MC/HPMC/fatty acid MC/palmitic acid Starch/glycerol MC/Chitosan LDPE	Culture Media Culture Media Culture Media Culture Media Cheese

LDPE: Low density polyethylene, MC: Methyl cellulose, HPMC: Hydroxypropyl MC, PE: Polyethylene, MA: Methacrylic acid, WPI: Whey protein isolate.

2.5. Shelf life of chicken meat

Especially in developing countries like Turkey, chickens ensure nutrition for the family, a small cash flow reserve for times of celebrations or need and in some areas contribute to religious ceremonies and recreation (Roberts, 1995). As a result of this, chicken meat largely consumed in the world, so that shelf life of the chicken meat is very important.

Since the chicken meat is one of the most consumed poultry meat in the world, the procedures applied in the production, packaging, storage and market distribution of meat products have to insure their hygienic quality and health safety until the expire date that should be properly and visibly indicated on each package. To observe the manufacturer's instructions relative to storage conditions is very important in retail trade. According to the literature it is well known and practice that poultry meat is liable to quick deterioration when kept under inadequate storage conditions (Wang et al., 2004). As a result, poultry meat and meat products are main reason of alimentary infections. Since poultry meat isn't consumed raw, epidemics occur as the result of secondary contamination in the course of production, storage or preparation (Mulder, 1999; Kozačinski et al., 2012).

The microflora of poultry is transferred from the primary production areas to production lines and even further, by subsequent contamination. The results of the previous studies about etiology of food poisoning indicated that contamination with pathogenic bacteria primarily refers to *Salmonella* spp., *Campylobacter* spp., *Staphylococcus* spp., *Listeria* spp., then *Yersinia enterocolitica*, *Escherichia coli* and *Clostridium perfringens* (Kozačinski et al., 2012).

Prolonging the shelf life of poultry products are the main relate for the poultry industry. The shelf life of poultry relates on various parameters, mostly primary bacterial loads, storage temperature and gaseous environment around the product (Wang et al., 2004). Thus, any method that should control these elements can be the key for the increase of shelf life of poultry products.

The most of the studies were involved in chicken breast, since the breast meat and the leg meat have some differences; in this study raw chicken drumstick has been

studied. Another point of the study was to present the applicability of the packaging films produced in industrial scale.

2.6. Antimicrobials Used in the Study

2.6.1. Chitosan

For preserving the quality and safety of foods for assuring shelf-life extension, packaging is very important. The non-biodegradability of much synthetic polymer based packaging, also increased environmental relate by consumers and government bodies, has paved the way for different approaches. That situation has led to focus on packaging substances obtained from inherently happening polymers (Koide, 1998).

Chitin and its main derivative chitosan are between the novel families of biological macromolecules. Chitin, chitosan and derivatives used in lots of area. Potential and regular applications of chitin and chitosan are calculated to more than 200 (Kumar, 2000). This large area of applications includes food, biotechnology, biomedicine, agriculture, cosmetics, etc.

Chitosan is a linear polysaccharide derived from crustacean chitin and composed of randomly distributed glucosamine and N-acetylated glucosamine (acetylamino-2-deoxy-D-glucopyranose) units linked by β-(1-4) glycoside bonds (Figure 2.2). While chitin is broadly acetylated, chitosan comply with deacetylated form (Koide, 1998).

Chitin and chitosan are also biodegradable, bioresorbable and chitosan exhibits bioactive features either in its polymeric or oligomeric form (Begin and Van Calsteren, 1996; Tsai et al., 2000; Coma, 2002; Coma et al., 2003). Chitosan binds to negatively charged molecules like fats and lipids because of its positive charge on amino group in acidic conditions (Jumaa and Müller, 1999; Muzzarelli et al., 2000).

So that its capability to form active edible or biodegradable films (Arai et al., 1968), chitosan coating may be expected to restrict prime contamination onto food surfaces and reduce environmental effect.

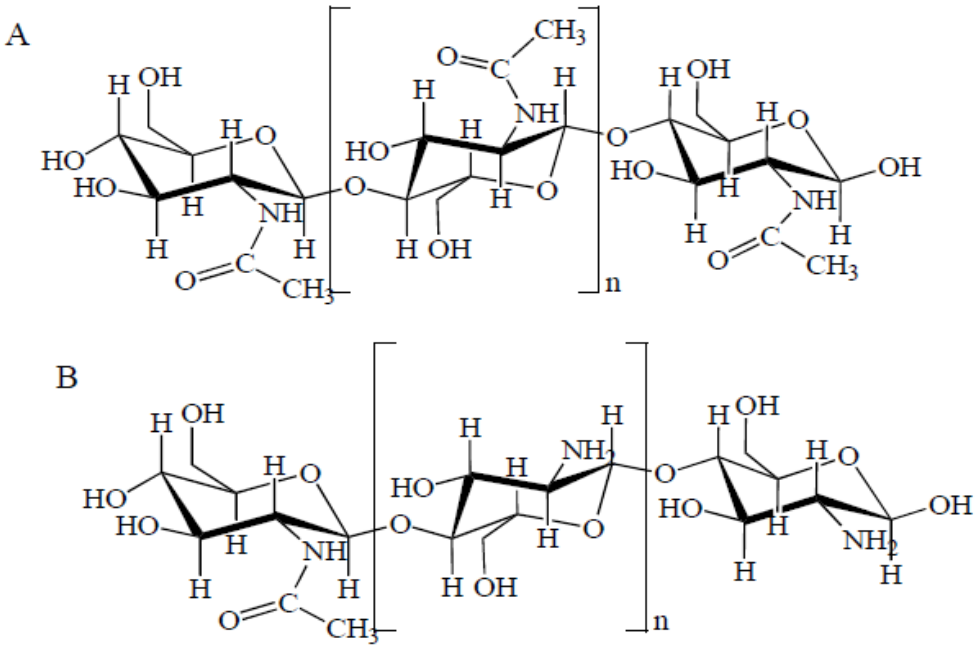


Figure 2.2. Chemical structure of 100% acetylated chitin (A) and chitosan (B).

The preparing process of chitin and chitosan from raw material is shown in Figure 2.3.

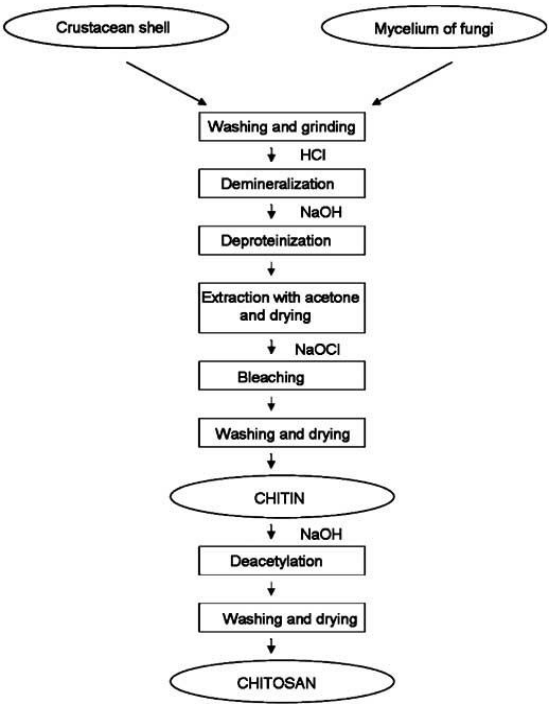


Figure 2.3. Preparing process of chitin and chitosan from raw material

2.6.2. Nisin

Food spoilage generally begins on food surfaces because of presence and growth of pathogenic or spoilage organism. For preserving food surfaces from spoilage, frequently antimicrobial materials incorporated by dipping, dusting and spraying (Hotchkiss, 1995; Nicholson, 1998). However, incorporated antimicrobial substances show loss in antimicrobial activity because of decrease of active concentration, resulting from to contact with food components and diluted diffusion in the bulk foods (Greer, 1982). To overcome these problems, controlled release of antimicrobial agents from food packaging substances to the food surface have been attempted (Vojdani and Torres, 1990).

Nisin (Figure 2.4) is a bacteriocin produced by *Lactococcus lactis*. It has big potential for application in antimicrobial packaging (Cutter and Siragusa, 1997; Nicholson, 1998). Nisin is a natural antimicrobial agent; due to this situation it may endure activity loss during thermal processing and exhibition to acidic environments that plays a great role for adding in food packaging substance.

The convenience of nisin as a food preservative linked with below properties:

-Nisin is non-toxic, produces strains of *L. lactis* are regarded as safe.

-Nisin is not used clinically.

-There is no apparent cross-resistance in bacteria which can effect antibiotic therapeutics

-Nisin is fastly digested.

Nisin was sold under the trade name of Nisaplin. It includes about 2.5% nisin, the balance containing of milk and milk solids obtained from fermentation of modified milk medium by nisin producing strains of *L. lactis* since 1953. The product is standardized to an activity of one million international units per gram.

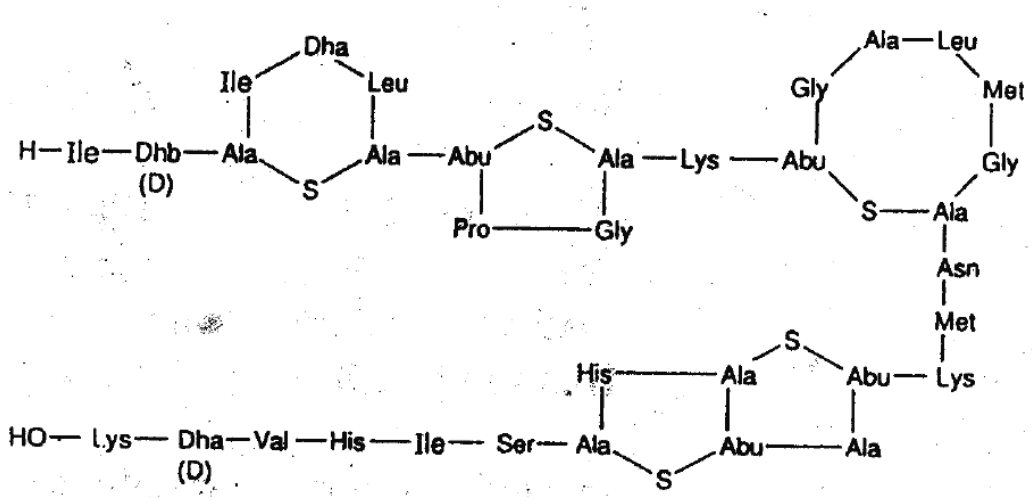


Figure 2.4. The chemical structure of Nisin

2.6.3. Potassium sorbate

Potassium sorbate is a chemical that when dissolved in water ionizes to form sorbic acid (Wine Lab, 2000), which is effective against yeasts, molds, and selective bacteria. Potassium sorbate is mainly used as preventatives in food which may keep effectively activity of mould, yeast and aerophile bacteria. Restrain growth and reproduction of pernicious microorganisms like *Pseudomonas, Staphylococcus* and *Salmonella* action to keep growth is more powerful than killing. The preservative efficiency of sorbic acid (potassium sorbate) is 5-10 times of sodium benzoate.

Potassium Sorbate is one type of non saturated fatty acid compounds. It may be absorbed by human body fastly, and then transferred into CO_2 and H_2O, further no remaining in body.

2.6.4. Silver-loaded zeolite

The discovery of zeolites dates back to the 18th century (Bozoğlu, 2010). They were first discovered in 1756 by a Cronstedt, who named them from two Greek words for ‘boiling stone’ (‘zein’–boiling and ‘lithos’–stone) (Breck, 1974; Thompson, 1998). Following their discovery zeolites were found to be characterized by the following properties (Van Bekkum, 2001):

-Catalytic properties;

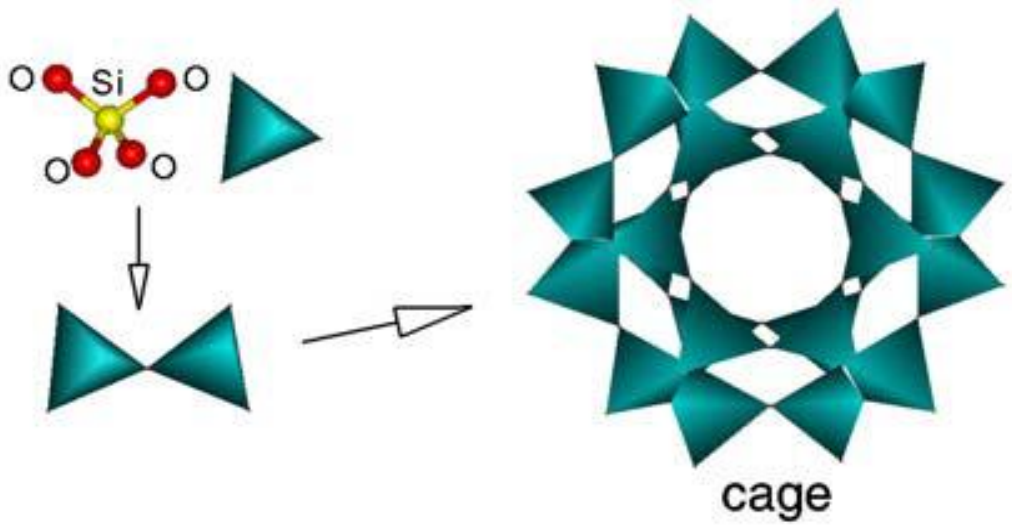
-High hydration properties;

-Stable crystal structure when dehydrated;

-Low density and high void volume when dehydrated;

-Cation exchange and sorption properties.

Zeolites are crystalline hydrated alumino silicates whose framework structure consists of cavities or pores that are occupied by cautions or water molecules. Both the caution and the water molecule have considerable freedom of movements and this permits ion exchange and reversible dehydration (Occelli and Kessler, 1997). The zeolite structure is made up of a central atom commonly silicon or aluminum surrounded by four oxygen atoms (Figure 2.5).



A **B**

Figure 2.5. Tetrahedra framework structure of zeolites (lwww.bza.org/zeolites.html)

To generate antimicrobial properties silver has been used in different polymers. Many commercial filler like zirconium phosphate, titanium dioxide and some zeolites were used as silver carriers. From these, silver-loaded zeolites are one of the most frequently used antimicrobial additives in food packaging substances (Sanchez-Valdes, 2011).

CHAPTER 3

METERIALS AND METHODS

3.1. Materials

Antimicrobial packaging films used in the study (containing chitosan, nisin, PS and the AgZeO) were obtained commercially from Naksan Plastic Company, Turkey. Raw chicken drumsticks were obtained from a national chicken meat producer. All used chemicals were reagent grade.

3.2. Packaging and Storage of Raw Chicken Drumsticks

Fresh chicken drumsticks were transferred to chilled conditions to the laboratory and stored under refrigeration at 4°C. All raw chicken drumsticks were collected from the same batch at the plant, with all of them supplied from the same farm. Fresh raw chicken drumsticks were packaged under vacuum with each of antimicrobial film.

In addition raw chicken drumsticks were packed with control films without additive under vacuum and stored at the same conditions to compare the effect of antimicrobial packaging. Samples were taken daily and their microbiological analysis (total aerobic bacteria, total coliform and total mold and yeasts), TBARS (thiobarbituric acid reactive substances) values, changes in pH, color and hardness values were determined during storage period.

Samples for the antimicrobial and control films were taken 0, 1, 2, 3, 4, and 6^{th} days of storage and after removal of the films. Three replicates of samples for each storage day were taken and analyzed at 4°C for a week.

3.3. Microbiological Analysis

3.3.1. Preparation of media and solutions

Potato dextrose agar (PDA) was prepared for mold and yeast count. Nutrient Agar (NA) was prepared for total aerobic mesophilic plate count (APC). Violet red bile agar (VRBA) was prepared for total coliform count.

All agar media were sterilized in an autoclave at 121°C for 15-20 min. And then they were poured into sterile petri dishes under the septic condition.

Peptone water is used for sample and dilution waters. Peptone was readily powdered for mixing with distilled water and it was weighed on sensitive balance 0.1% peptone water was used during preparation of dilution waters, culture solution and preparation of sample waters. Simply 1 g of peptone powder was mixed with 1000 ml of distilled water in a clean balloon, then distributed to bottles and test tubes in required amount and sterilized at 121°C for 20 min in an autoclave.

Dilution water is used for diluting the sample of food products that is expected to include microorganisms in it. In order to prepare sterile dilution water, first of 9 ml 0.1% peptone water was put into test tubes in aseptic conditions and mouths of tubes were closed by the lids. Which were sterilized in autoclave at 121°C for 20 min. Test tubes were stored at refrigerator for further use. For homogenization of solid food samples 225 ml 0.1% peptone water was poured into 250 ml flask, a magnetic stirrer rod was put into the flask too and the mouths of flasks were closed by cotton and aluminum foil and tied. After closing flasks, they were sterilized in autoclave at 121°C for 20 min. Flasks were stored in the refrigerator for further use.

3.3.2. Sampling and Analysis

25 g of raw chicken drumsticks sample was weighed aseptically, transported to a flask including 225 mL of sterile water and stirred for 15 min at room temperature. For microbial enumerations, 0.2 mL samples of serial dilutions were diffused on the surface of dry media. Total aerobic mesophilic plate count (APC) was determined using NA after incubation at 37°C for 2 days.

Total mold and yeast count was determined by PDA after incubation at 25°C for 5 days.

For total coliform, 0.2 mL sample was inoculated into VRBA and incubated at 37°C for 2 days. Big purple colonies of haloes were counted. Total plates were examined visually for similar colony kinds and morphological properties incorporated with each growth medium.

Microbiological data are displayed as a logarithm of colony forming units per gram of sample (log cfu/g) (Hasapidou and Savvaidis, 2011).

3.4. Chemical Analysis

Chicken drumstick samples (10 g) were homogenized thoroughly with 90 mL distilled water and pH of homogenate was determined by pH meter (Chounou et al., 2013).

TBARS values of chicken samples were defined using spectrophotometric method (Bozkurt, 2006). 2 g of samples were taken and TBARS was extracted twice with 10 mL of 0.4 M perchloric acid. The extract was diluted to 25 mL with 0.4 M perchloric acid. The extracted solution was centrifuged for 5 min at 1790×*g*. After centrifugation, 1mL of extract was poured into glass stoppered test tube, 5 mL of TBA reagent was added and the mixture was heated in boiling water-bath for 35 min. After cooling the tube in tap water, the absorbance of the sample was read against the appropriate blank at 538 nm. A calibration curve was prepared by malondialehyde (MDA). TBARS value was displayed as mg of MDA/kg sample.

3.5. Physical Analysis

Color of the samples was measured by Hunter lab Colour Flex (A60-1010-615 Model Calorimeter, Hunter lab, Reston, VA). The instrument was standardized each time with a white and black ceramic plate (L_0 = 93.01, a_0 = -1.11 and b_0 = 1.27). The changes in skin color (Hunter L*, a* and b*) of the drumsticks packed with active and control films were determined from the same areas of the samples after removal of the films, in all measurements (Bozkurt, 2006).

Hardness values of the samples were determined by TA-XT2 Texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). A needle (1.8 mm in diameter) probe was used to measure the hardness of the meat. Hardness of samples was recorded after penetration of the needle to a depth of 5 mm sample at speed of 1 mm/s. Data collection and calculation were done by Texture Expert Exceed Version 2 V3 (Stable Micro Systems, 1998). The hardness values were given as the mean of six measurements and expressed as Newton (N). All hardness values were determined from the same points of the samples.

3.6. Statistical Analyses

Analysis of Variance (ANOVA) was performed for each response (mold and yeast count, APC, total coliform count, pH, TBARS, L*, a*, b*, and hardness) to determine significant differences using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests were also carried out to distinguish examined groups.

CHAPTER 4

RESULTS AND DISCUSSION

In the experiment, major modes of deterioration parameters (microbial, chemical and physical changes) were followed for the evaluation of the applicability of active packaging on storage of chicken drumsticks.

4.1. Microbiological Results

APC, total coliform and mold and yeast counts for the chicken drumstick samples packed with five different films were determined as a function of storage time. These analyses are used in poultry industries as general indicators of processing hygiene, storage quality and potential shelf-life.

Changes in APC (log cfu/g) in chicken drumstick packaged with the active and control films during the 6 days of storage at 4°C are shown in Figure 4.1. Chicken drumsticks packaged with active films had lower ($P<0.05$) APC than the sample packed with control films. APC of all samples increased ($P<0.05$) during storage and at the end of storage period, APC of samples ranged from 4.69 to 5.73 log cfu/g. Similar results were reported by Kim and Marshall (1999) that APC in chicken drumsticks increased to about 6 log cfu/g after 6 days of storage at 4°C. In our samples, increase in APC packed with chitosan was about 0.5 log cfu/g whereas 1.54 log cfu/g in control films during storage period. The increase in APC values were very low when compared with the data reported by Del Rio et al. (2007) in which 6 log cfu/g increase in APC of chicken drumstick samples (from about 3.20 to 9.20 log cfu/g) after 5 days of storage at 3°C. Cox et al. (1998) reported that spoilage of poultry generally occurs when total APC exceed 7 log cfu/g. In this study, after 6 days of storage APC of all drumsticks samples were lower than 6 log cfu/g.

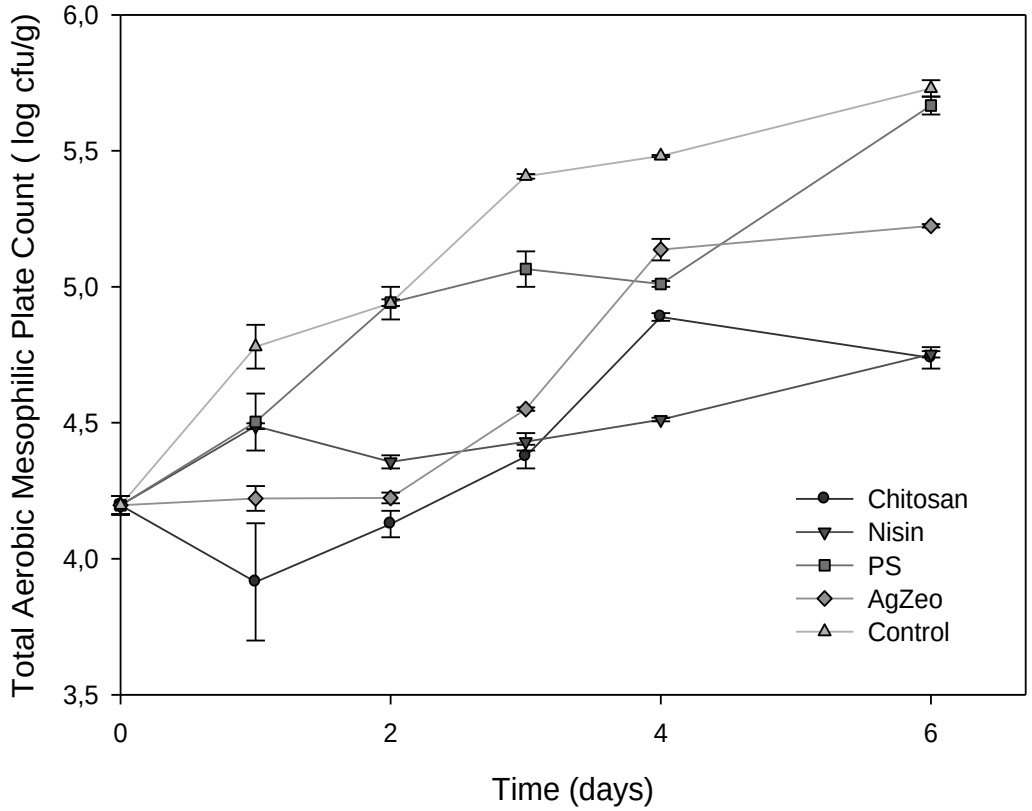


Figure 4.1.Total aerobic bacteria counts of samples

Changes in total coliform count in chicken drumstick packaged with control and active films are shown in Figure 4.2. During the storage period, changes in total coliform count was statistically different ($P<0.05$) in all samples. Total coliform count increased ($P<0.05$) during the 6 days of storage period at 4°C from 3.19 log cfu/g to the counts ranging from 3.74 to 4.59 log cfu/g. Similar results were also reported in the literature. Kim and Marshall (1999) observed that total coliform count was increased in chicken drumsticks during the storage periods. Del Rio et al. (2007) found that total coliform count increased from about 2.10 log cfu/g to about 7 log cfu/g during the storage of 5 days at 3°C. In our study, the increment rate of total coliform count was lower (as about 1.4 log cfu/g) than the results of Del Rio et al. (2007). Álvarez-Astorga et al. (2002) found that contamination level of drumsticks with coliforms was around 3.56 log cfu/g. They also reported that levels of coliforms in chicken skin varied between 2.7 log cfu/g and 4.9 log cfu/g. No reduction of *E.*

coli cells was observed in LDPE film containing chitosan polymer or oligomer, in a cast corn zein film containing lysozyme, and in a LDPE film coated with nisin.

In our study, use of active films containing chitosan and nisin decreased ($P<0.05$) total coliform count of chicken drumstick when compared with the control sample. The lowest ($P<0.05$) total coliform count was observed in the chicken drumstick packed with chitosan whereas the highest ($P<0.05$) in chicken drumstick packed with control.

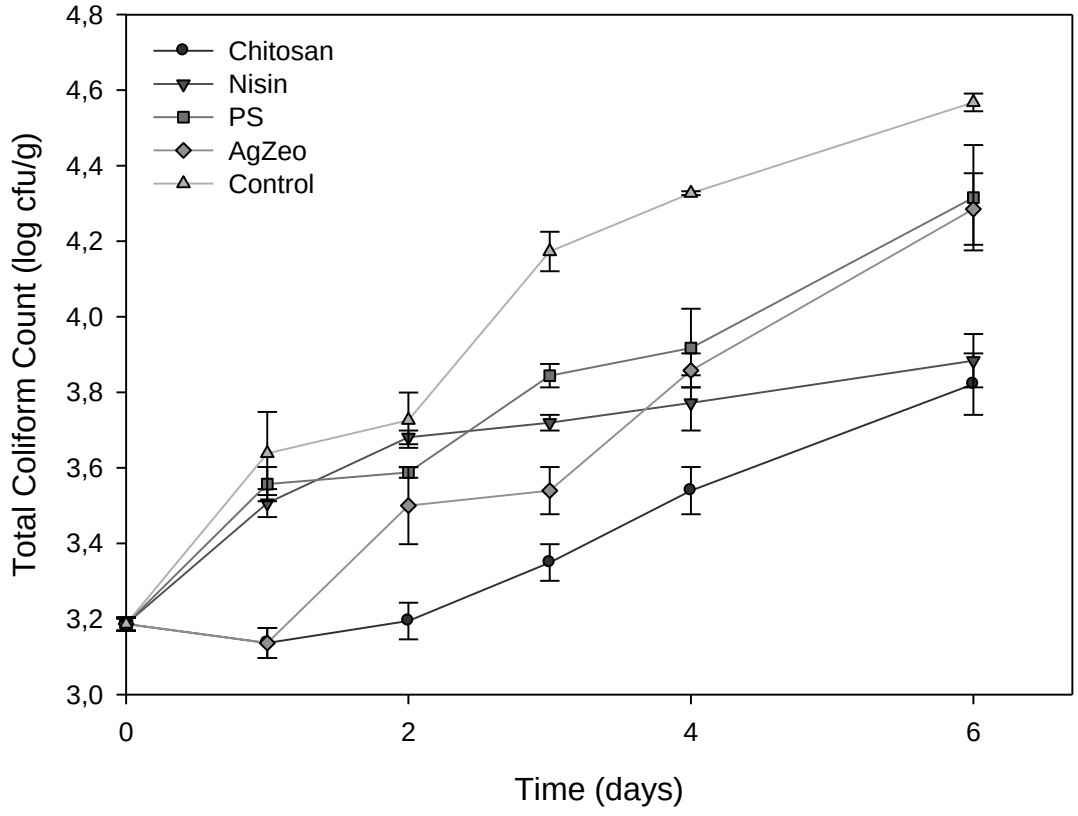


Figure 4.2.Total coliform counts of samples

One-way ANOVA showed that mold and yeast counts were affected from both storage time and incorporation of antimicrobial agents into the plastic films. Mold and yeast count increased ($P<0.05$) from 5.49 to the counts ranging from 5.81 to 6.87 log cfu/g after 6 days of storage. Difference in mold and yeast counts of the samples packed with antimicrobial (except AgZeO film) and control films was observed after the 1st day of storage. However, the difference became more pronounced after 2

days. As shown in Figure 4.3, the drumsticks packed with active films had lower ($P<0.05$) mold and yeast counts than those with control films. Increment rate of mold and yeast count during the storage periods was the highest in drumstick packed with control film whereas; it was the lowest in the samples packed with chitosan film.

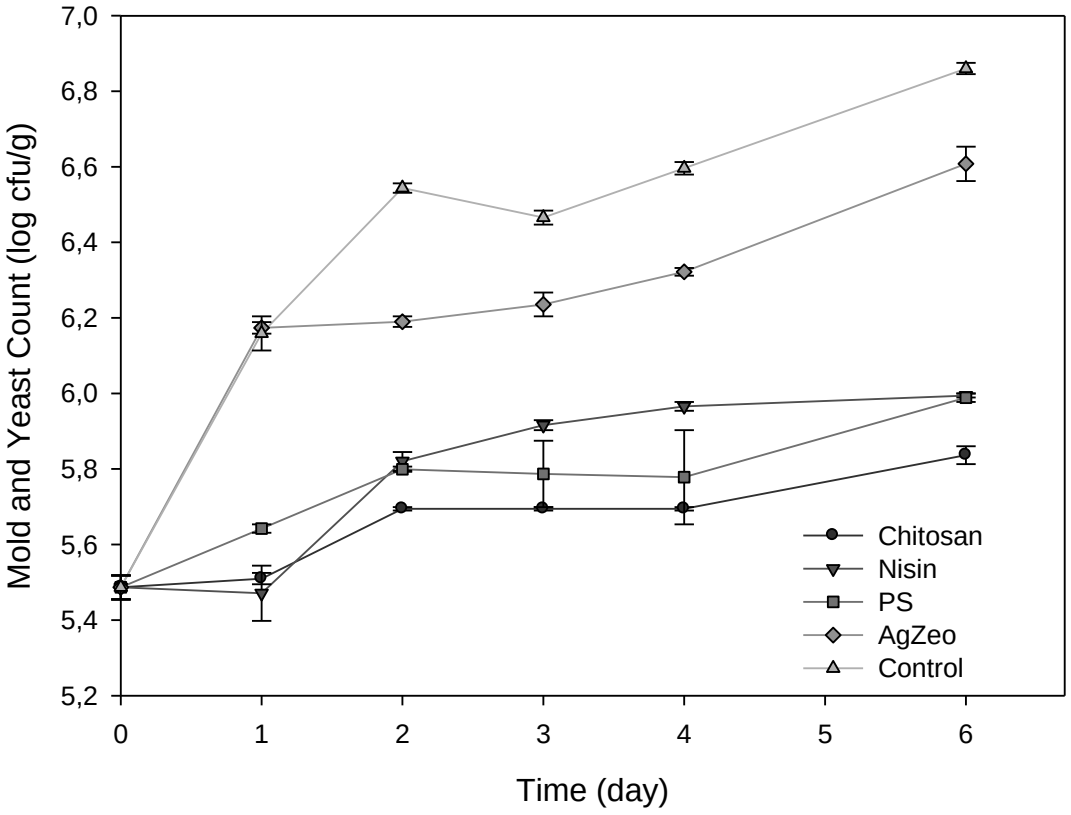


Figure 4.3. Mold and yeast counts of samples

Mold and yeast counts in drumstick packed with nisin and PS were not statistically different ($P>0.05$) after 1st day of storage period. As a result, samples packaged with antimicrobial agents especially with chitosan, nisin and PS had low mold and yeast count after 6 days of storage. The efficiency of using chitosan incorporated polyethylene extruded films on the inhibition of the radial growth of *Aspergilla niger* had been reported by Martínez-Camacho et al. (2013). There are conflicting results in the literature about the activity of PS that some reports suggesting incorporation of PS into polyethylene films (0.4-mm thick) lowered the growth rate of mold and yeast. Some authors expressed that using low density polyethylene films (0.05-µm thick) containing 1.0 % w/w sorbic acid were unable to suppress mold growth.

Moreover, ethylene vinyl alcohol/linear low-density polyethylene (EVA/LLDPE) film (70 mm thick) impregnated with 5.0% w/w PS was unable to inhibit the growth of microorganisms on cheese to extend its shelf life. Therefore, it is clear that the effect of the agents was strictly related with the presence of other components in the film material and the composition of them. Since the films used in this study produced industrially and it was possible to say that these antimicrobials chitosan, nisin and PS, and the vacuum presenting a depressing effect on the mold and yeast growth.

The complexity of the microbial flora in chicken drumsticks makes it difficult to reduce microbial growth by incorporation of natural antimicrobials into films. However, the results of the present study indicated that active films have an inhibitory effect on microbial growth in chicken drumstick (Table 4.1) when compared with the control. Table 4.1 shows the number of microorganisms decreased in the log cfu/g by use of active films after 6 days of storage. As shown from Table 4.1, chitosan was the most effective active film on the APC, total coliform and mold and yeast counts. APC and total coliform had similar sensitivities to active films in the order of chitosan>nisin>AgZeO>PS after 6 days. The number of APC (5.73 log cfu/g) reduced by 0.06 to 0.99 log cfu/g and the number of total coliform (4.57 log cfu/g) reduced by 0.25 to 0.75 log cfu/g. Effect of active films on mold and yeast counts was in the order of chitosan>PS>nisin>AgZeOfor 6 days. The number of mold and yeast (6.86 log cfu/g) decreased by 0.25 to 1.02 log cfu/g at 4°C in 6 days.

Table 4.1.Reduction in microbial counts (in log cfu/g) of samples packed with active films compared to control sample after 6 days of storage.

	Chitosan±SE	Nisin±SE	PS±SE	AgZeo±SE	P value
APC	0.99±0.009	0.97±0.019	0.06±0.060	0.51±0.024	<0.01
Mold and yeast	1.02±0.009	0.86±0.020	0.87±0.004	0.25±0.030	<0.01
Total coliform	0.75±0.104	0.68±0.047	0.25±0.163	0.28±0.118	<0.05

4.2. Chemical Changes

It was observed that there was not any significant difference between the pH values of the samples during the storage period (Table 4.2). The change in pH was higher in control from 6.71 to 6.29 during the studying period than those of the other samples as on the average from 6.62 to 6.58.

Initial TBARS value of the drumstick was 1.35 mg/kg sample. The proposed limit for TBARS value in the meat products is 2 mg/kg above which rancid off-flavors become sensorially detectible. After 2 days of storage, the TBARS values of the samples packed using chitosan films were much lower than those of other samples (Figure 4.4). Although an increasing trend of TBARS values of the all samples were detected, there was no significant difference between the control samples and the samples packed with chitosan and AgZeo, up to 2 days ($p<0.05$). TBARS values of the all samples packaged with active films were lower and significantly different than those of the control films at the fourth day of the storage. Except samples packed with chitosan, all other samples exceeded the limit. The higher susceptibility of drumstick meat towards oxidation has been explained by the higher absolute content of polyunsaturated fatty acids in this muscle. Even after 6 days of storage, chitosan was found to be the best packaging film for the drumsticks in terms of lipid oxidation. TBARS values of control samples decreased after reaching a maximum value. This pattern, which has been observed by other researchers, has been attributed to initial formation of malonaldehyde and its possible decomposition during later stages of storage or its reaction with proteins through a Maillard-type reaction. However, increasing trend of TBARS for samples packed with nisin, PS, and chitosan films were approximately constant up to 6 days storage, there was no difference ($P>0.05$) at the sixth day of storage, except samples packed with chitosan films. Thus, chitosan incorporation might be more helpful to descend the lipid oxidation of the drumsticks during the storage period. The above results are in agreement with those of Georgantelis et al. (2007) who reported a reduction in TBARS values for pork sausages by 73% for samples containing 1% chitosan (0.25 mg MDA/kg) as compared to control samples (0.96 mg MDA/kg after 10 days of

storage at 4°C). Darmadji and Izumimoto (1994) reported that TBARS values of beef containing 1% chitosan remained unchanged after 10 days of storage at 4°C (0.5 mg MDA/kg), whereas respective values of control samples increased sharply.

Since the lipid oxidation is a major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce undesirable off-odors and flavors, it is possible to state that for this aspect chitosan was the best antimicrobial-antioxidant agent for preparation of the active packaging film for chicken drumstick with the application of vacuum which was also helpful to descent the oxidation reaction.

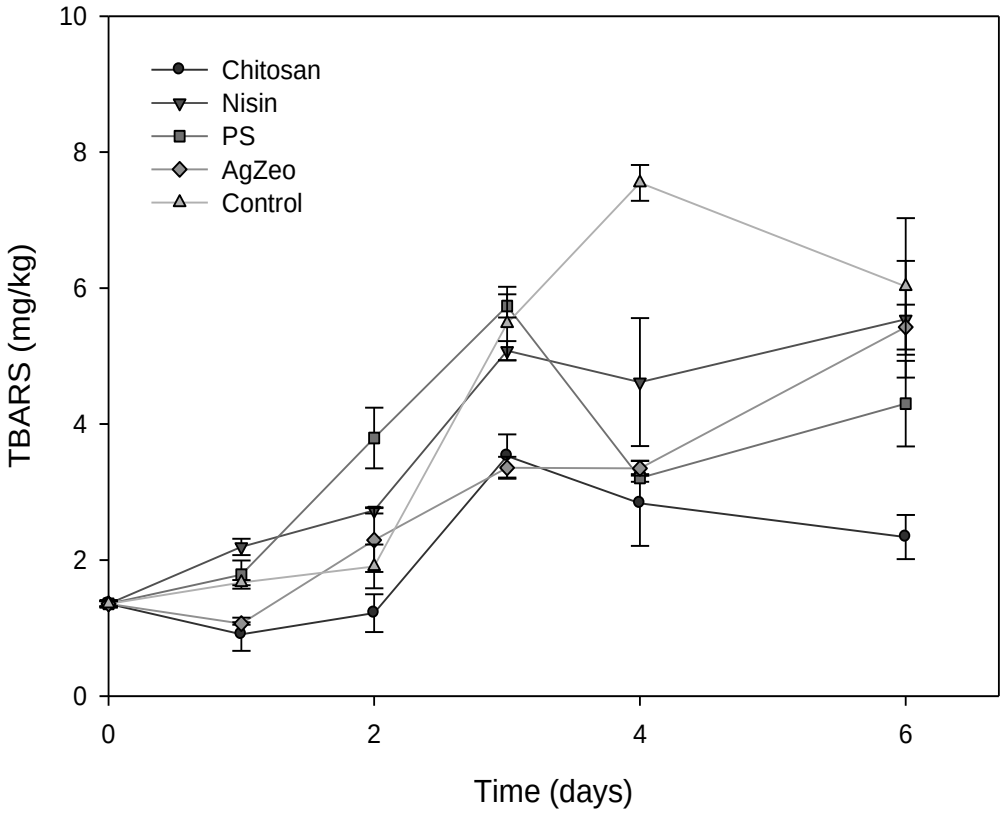


Figure 4.4.TBARS values of samples

4.3. Physical Changes

The changes in skin color (Hunter L*, a* and b*) and texture of chicken drumsticks were determined during storage period.

It was observed that all active packed samples represented a decreasing trend for L* values of the sample packed with PS which then increased to a maximum value (Table 4.2). However, it was possible to state that there was no any significant change in L*-values of all samples ($p>0.05$). This finding is in agreement with results of Mexis et al. (2012) obtained for color parameters of antioxidant added chicken meat. They reported that L*, a* and b* color parameter values of the samples remained unaffected ($p > 0.05$) as a function of storage time for samples containing the citrus extract and/or O_2 absorber. Sante and Lacourt (1994) suggested that application of vacuum protected the color of the samples due to the low rate of myoglobin oxidation. Similarly Chouliara et al. (2007) reported a decrease in L* values in chicken breast meat as a function of storage time in samples containing oregano oil. Hunter a* and b*values are given in Table 4.2. It has been detected that the redness (a*) and yellowness (b*) of the samples packed with control films had the lowest value. Use of active films improved the surface color of the chicken drumstick during studied storage period.

It was possible to state that the incorporation of the antimicrobial agents into the films and storage time affect ($p<0.05$) the hardness value (Table 4.2). The hardness values of the samples packed with chitosan and AgZeO were not different from each other ($p>0.05$). The change in the hardness values was the highest ($p<0.05$) for the samples packed with control film whereas the lowest was with the PS film. There was a significant difference ($p<0.05$) between the samples packed with all activated films and control film up to 4^{th} days of storage (except PS film). However hardness value of PS films was not affected with the storage while the other films affected significantly.

Table 4.2. Changes in physical characteristics (pH, color and hardness) of chicken drumsticks packaged with active and control films.

Property	Time (d)	Chitosan±SE	Nisin±SE	PS[1]±SE	AgZeo[2]±SE	Control±SE
pH						
	0.00	6.74±0.18aA	6.74±0.18aA	6.74±0.18aA	6.74±0.18aA	6.74±0.18aA
	1.00	6.61±0.18aA	6.73±0.18aA	6.47±0.17aA	6.67±0.18aA	6.72±0.19aA
	2.00	6.48±0.19aA	6.74±0.19aA	6.65±0.20aA	6.80±0.19aA	6.56±0.18aA
	3.00	6.65±0.17aA	6.52±0.19aA	6.55±0.19aA	6.73±0.18aA	6.60±0.18aA
	4.00	6.79±0.19aA	6.65±0.19aA	6.67±0.19aA	6.75±0.19aA	6.67±0.18aA
	6.00	6.63±0.20aA	6.42±0.18aA	6.64±0.19aA	6.75±0.19aA	6.41±0.19aA
L*-value						
	0.00	76.60±1.00abA	76.60±1.00aA	76.60±.00abA	76.60±1.00aA	76.60±1.00abA
	1.00	74.09±0.86acA	75.30±1.43abcA	74.43±0.39abA	75.91±0.68abA	73.68±1.36bA
	2.00	71.95±2.30cA	72.42±0.70bA	74.12±1.23aA	73.18±0.89cA	74.82±1.04abA
	3.00	79.79±0.77bA	76.21±0.74aAB	74.63±1.39abB	76.31±0.45abAB	75.52±1.76abB
	4.00	72.33±0.95acA	77.42±0.32aBC	75.96±1.02abBC	74.81±0.78abAB	78.88±1.47bC
	6.00	74.93±1.26acAB	74.29±1.03abB	77.66±0.58bB	73.86±0.83bA	75.79±0.38abAB
a*-value						
	0.00	3.64±0.27abA	3.64±0.27abA	3.64±0.27aA	3.64±0.27aA	3.64±0.27aA
	1.00	4.12±0.46abA	4.01±0.35abcA	5.21±0.21aA	3.60±0.22bA	4.65±0.27aA
	2.00	4.13±0.91abA	4.67±0.26abcA	4.04±0.36aA	4.18±0.36abcA	4.92±0.32aA
	3.00	3.31±0.15aA	5.10±0.36bB	3.85±0.09aAB	4.0±0.07abAB	3.27±0.76aA
	4.00	5.37±0.25bA	3.44±0.65aB	4.87±0.39aA	4.96±0.30bA	3.15±0.54aB
	6.00	3.93±0.87abA	4.98±0.57bA	4.22±0.50aA	5.13±0.47cA	4.20±0.97aA
b*-value						
	0.00	11.22±0.27aA	11.22±0.27abA	11.22±0.27abA	11.22±0.27aA	11.22±0.27aA
	1.00	9.08±0.70abA	8.81±0.22bAB	8.34±0.69cdAB	6.99±0.58bB	8.48±0.50abAB
	2.00	7.82±0.69bA	7.87±0.75bA	10.25±0.79acAB	10.20±0.58acAB	12.09±0.97aB
	3.00	8.27±0.42abAB	13.18±0.26aC	7.20±0.34cAB	9.65±0.84abcBC	4.59±1.16bA
	4.00	8.49±0.92abA	7.93±0.16bA	8.80±0.58acdA	8.39±0.83bcA	9.36±0.79aA
	6.00	8.65±0.46abAB	13.67±0.23aC	12.93±0.84bC	11.57±0.78aBC	7.87±1.58abA
Hardness (N)						
	0.00	2.71±0.08aA	2.71±0.08aA	2.71±0.08aA	2.71±0.08aA	2.71±0.08aA
	1.00	3.28±0.09bA	4.58±0.13bB	2.74±0.08aC	3.22±0.08bA	6.49±0.19bD
	2.00	3.23±0.09bcA	3.75±0.11cB	2.72±0.08aC	3.94±0.11cBD	4.20±0.12cD
	3.00	3.17±0.09bcA	2.20±0.06dB	2.64±0.13aC	3.35±0.10bA	3.80±0.11dD
	4.00	2.40±0.07dA	2.04±0.06dB	1.90±0.05bB	2.34±0.07dA	2.69±0.08aD
	6.00	2.99±0.09cA	3.32±0.10eB	2.32±0.07cC	2.78±0.08aA	2.46±0.07bC

[1]PS: potasyumsorbate, [2]AgZeo: silver substituted zeolite.
Different capital letters indicate a statistical difference at á = 0.05 level in hardness values of each packaging system at each time.
Different lower-case letters indicate statistical difference at á =0.05 level in hardness value in storage time for each packaging system.

4.4. Effect of film type on the quality parameters of the drumsticks at the end of storage

At the end of storage, total aerobic bacteria, mold and yeast and total coliform counts of samples packed with active films were lower than the sample packed with control (Figure 4.5).

These results show that the antimicrobial packaging materials suppressed the growth of microorganisms. The highest value was found in control packed sample at the end of storage. However the lower value was found in chitosan-packed sample at the end of storage.

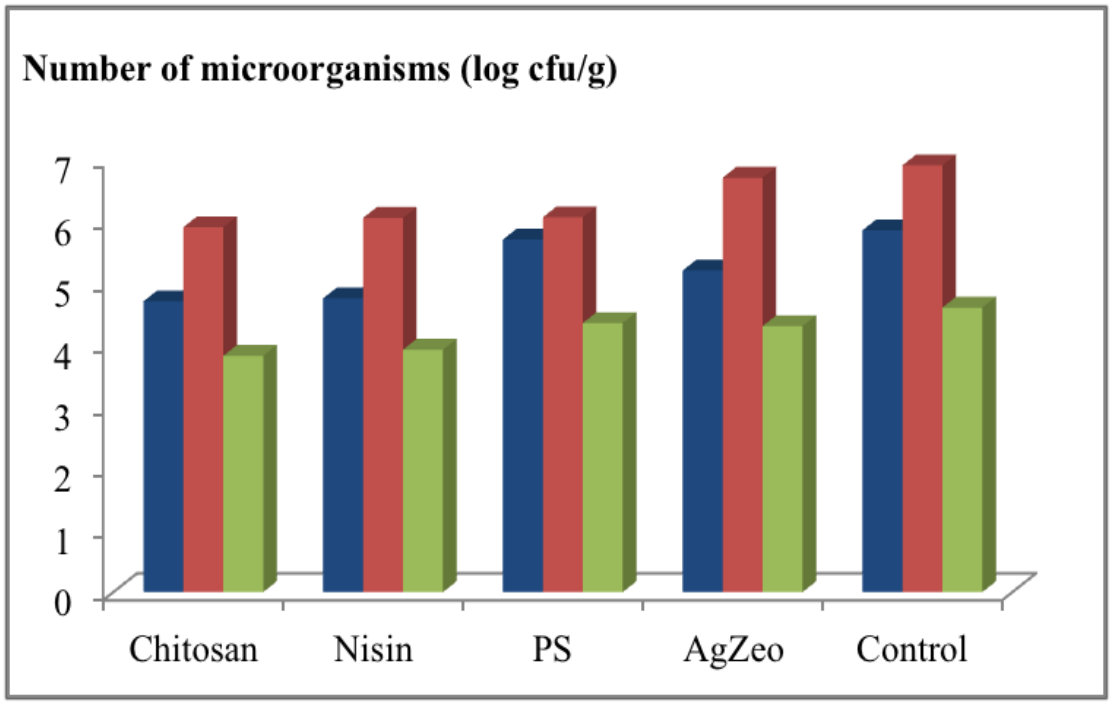


Figure 4.5. Total aerobic bacteria count, Mold and Yeast count and Total coliform count values of samples at the end of storage

At the 6th day of storage, APC of samples packaged with nisin and chitosan films were not statistically different ($P>0.05$), but they had lower ($P<0.05$) APC than the other samples. Similar results were also found by Siragusa et al. (1999) and Park et al. (2010). Siragusa et al. (1999) highlighted the incorporation of nisin into low density polyethylene (LDPE) film for controlling food spoilage and enhancing product safety. Since nisin, bacteriocin, inhibits gram-positive food borne pathogens and spoilage microorganisms. Park et al. (2010) observed that chitosan could be released from LDPE films to inhibit bacterial growth. Also, they found that higher chitosan concentrations (1.4%) inhibited both gram positive and gram negative bacteria. The antibacterial property of chitosan is well known and its most accepted microbial inhibition mechanism is the interaction of positively charged chitosan with negatively charged residues on the cell surface of many fungi and bacteria, causing extensive cell surface alterations and altering cell permeability.

When pH values of samples at the end of storage were compared it was observed that there was not any important difference between the samples (Figure 4.6). It was examined that the pH value of packed samples with chitosan at the end of the storage was 6.63, with nisin 6.43, with PS 6.64, with AgZeo 6.75 and with control 6.41.The highest pH value was found in AgZeo packed sample and lowest pH value was found in control packed sample at the end of storage.

Mean TBARS values of samples at the end of the storage packed with chitosan was 2.34, with Nisin was 5.54, with PS was 4.30, with AgZeo was 5.43 and with control was 6.21 at the end of storage. It was observed that all antimicrobial packaged samples had lower TBARS value than the control sample at the end of storage (Figure 4.7).

As shown from Figure 4.7, highest TBARS value was observed for the chicken drumstick packed with control film without additive and lowest TBARS value was observed with chitosan film. This could be due to the antioxidant effect of chitosan.

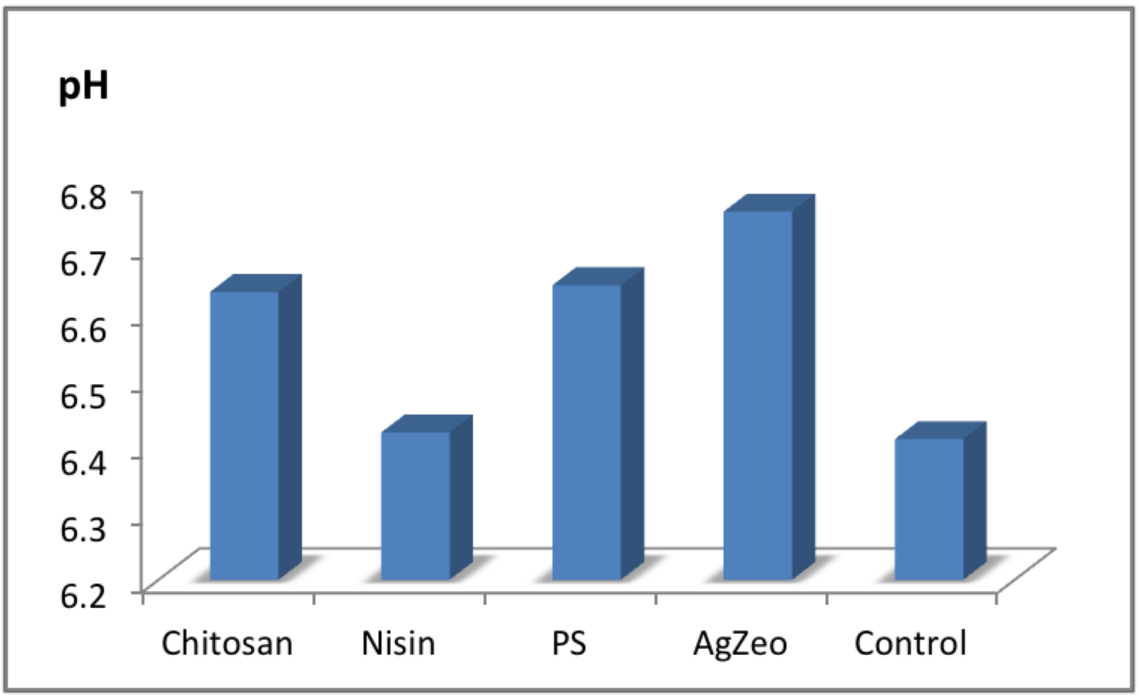


Figure 4.6. pH values of samples at the end of the storage

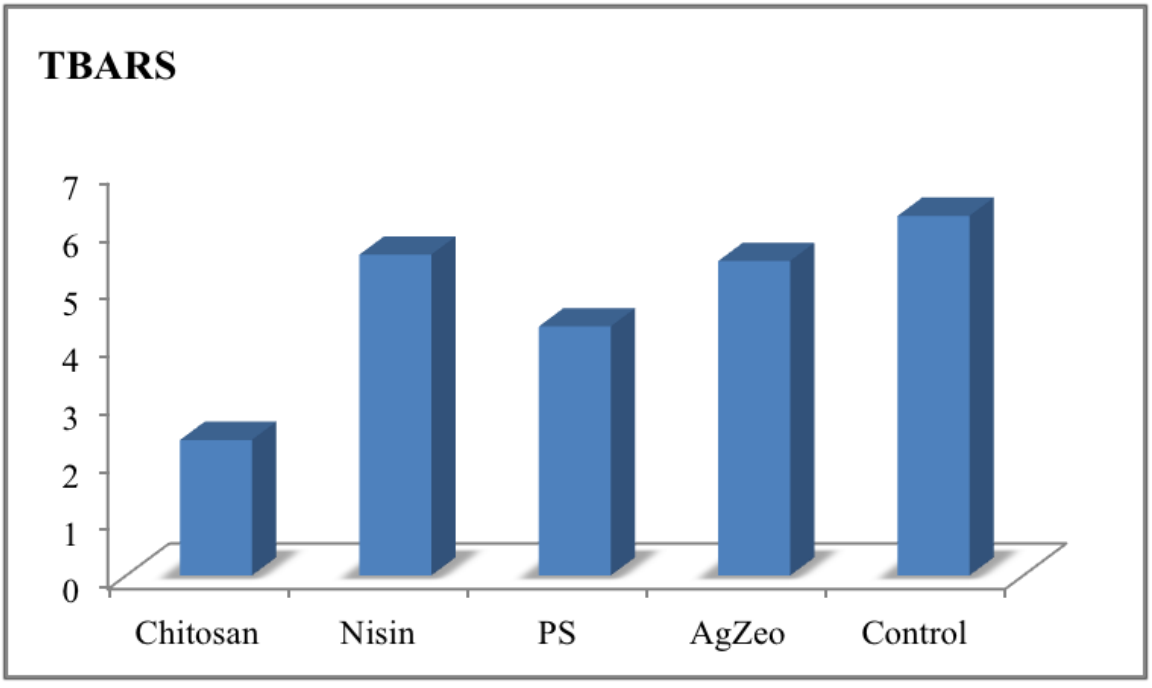


Figure 4.7. TBARS values of samples at the end of the storage

Figure 4.8 indicates effect of antimicrobial packaging on color parameters of samples. At the end of the storage period, it was observed that L*, a* and b* values are quite close to each other in all samples.

L*-value of sample packed with PS-containing film was found to be the highest and with AgZeo-containing film to be the lowest. Sample packed with AgZeo-containing film had highest a*-value and that of chitosan-containing film had lowest. b*-value was found to be the highest in sample packed with nisin-containing film and control sample to be lowest.

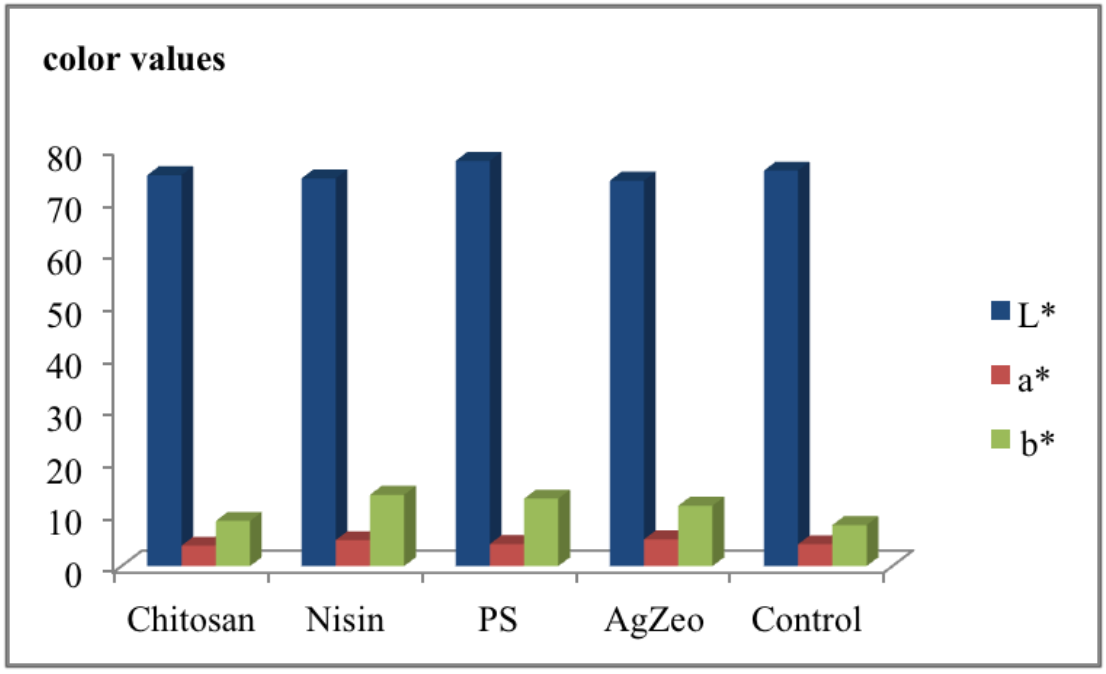


Figure 4.8. Color values of samples at the end of the storage

At the end of the storage period, it was observed that the mean hardness value in the antimicrobial packaging sample with AgZeo was 2.78, with chitosan was 2.99, with nisin was 3.32 and with PS was 2,32. However the hardness value in control was found as 2,46 (Figure 4.9).

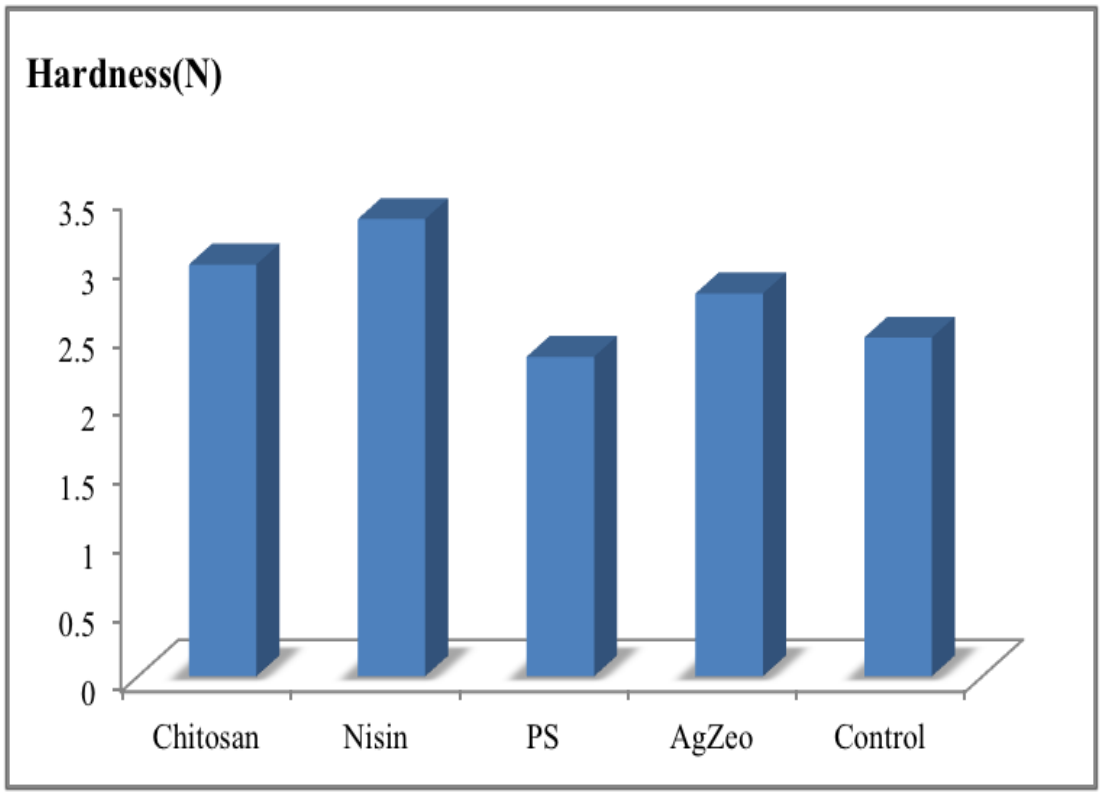


Figure 4.9. Hardness (N) values of samples at the end of storage

CONCLUSION

In this study, it was indicated that levels of APC, total coliform and mold and yeasts in raw chicken drumsticks could be reduced by use of antimicrobial films under vacuum. Of the antimicrobial agents examined in this study, chitosan was the most effective agent compared to the other agents tested. Efficiency of antimicrobial films on APC and total coliform was in the order of chitosan>nisin>AgZeo>PS, and on mold and yeasts was in the order of chitosan>PS>nisin>AgZeO at 4°C within 6 days.

Addition to the microbiological results, use of antimicrobial films protected the physical, chemical and safety parameters of raw chicken drumsticks compared to control samples. The results of this study may be useful for the selection of the suitable antimicrobial packaging for raw chicken drumsticks.

TBARS values found were lower than the values given in literature at the studied temperature since vacuum has been applied to all packaging types and some of the agents incorporated into packaging system having antioxidant property like chitosan.

Application of vacuum with addition of chitosan protected the color values more than other agents because chitosan has antioxidant activity more than others. Antimicrobial agent containing packaging systems improved the red color of the samples during studied storage period related to the surface color of the drumsticks. The incorporation of the antimicrobial agents into polyethylene films did not cause any important changes in the hardness values of the samples.

The results of this study indicated that use of antimicrobial package increase stability of raw chicken drumsticks at +4°C.

REFERENCES

Ahvenainen, R. (2003). Active and intelligent packaging, an introduction. In R. Ahvenainen (Ed.), Novel food packaging techniques (pp. 5–21). Cambridge, UK, Woodhead Publishing Ltd.

Ai, H., Wang, F., Xia, Y., Chen, X., and Lei, C. (2012). Antioxidant, antifungal and antiviral activities of chitosan from the larvae of housefly, Muscadomestica L. *Food Chemistry*, **132**, 493–498.

Álvarez-Astorga, M., Alonso-Calleja, F., Capita, R.C., Moreno, B., and Garcia-Fernández, M.C. (2002). Microbiological quality of retail chicken by-products in Spain. *Meat Science*, **62**, 45–50.

An, D.S., Kim, Y.M., Lee, S.B., Paik, H.D., and Lee, D.S. (2000). Antimicrobial low density polyethylene film coated with bacteriocins in binder medium. *Food Science and Biotechnology*, **9**, 14–20.

Appendini, P., Hotchkiss, J.H. (2002). Review of Antimicrobial Food Packaging, *Innovative Food Science and Emerging Technologies*, **3**, 113.

Arai K., Kinumaki T. and Fujita T. (1968). Toxicity of chitosan. Bull Tokai Reg Fish Res Lab, **56**, 89–92.

Bari, M.L., Ukuku, D.O., Kawasaki T., Inatsu Y., Isshiki K. and Kawamoto S. (2005). Combined efficiency of nisin and pediocin with sodium lactate, citric acid, phytic acid and potassium sorbate and EDTA in reducing the Listeria monocytogenes population of inoculated fresh-cut produce. *Journal of Food Protection*, **68**, 1381-1387.

Begin, A. and Van Calsteren, M.R. (1996). Antimicrobial films produced from chitosan. *International Journal of Biological Macromolecules*, **26**, 63–67.

Bozkurt, H. (2006). Investigation of the effect of sumac extract and BHT addition on the quality of sucuk (Turkish dry fermented sausage). *Journal of the Science of Food and Agriculture*, **86**, 849-856.

Breck, D.W. (1974). In: Zeolite Molecular Sieves, Structure Chemistry and Use, Wiley, New York, NY, **55**, 133.

Brody, A.L., Bugusu, B., Han, J.H., Sand, C.K. and Mctugh, T.H. (2008). *Innovative Food Packaging Solutions, Journal Of Food Scıence*, **73**, 107-116.

Brody, A.L., Stupinsky, E.R., and Kline, L.R. (2001). Antimicrobial Packaging. Active Packaging for Food Applications, Technomic Publisher Co., Inc. Lancaster, USA, **72**, 225.

Broughton, J.D. (2005). Nisin as a food preservative. *Food Australia*, **57**, 525-527.

Byun, J.S., Min, J.S., Kim, I.S., Kim, J.W., Chung, M.S., and Lee, M. (2003). Comparison of indicators of microbial quality of meat during aerobic cold storage. *Journal of Food Protection*, **66**, 1733-1737.

Capita, R., Alonso-Calleja, C., Garcia-Fernandez, M.D., and Moreno, B. (2001). Microbiological quality of retail poultry carcasses in Spain. *Journal of Food Protection*, **6412**, 1961-1966.

Cha, D.S. and Chinnan, M.S. (2004). Biopolymer-based antimicrobial packaging: *A review, Critical Review Food Science Nutr*ition, **44**, 223–237.

Chen, M.J., Weng, Y.M., and Chen, W. (1999). Edible coating as preservative carriers to inhibit yeast on Taiwanese-style fruit preserves. *Journal Food Safety*, **19**, 89-96.

Chouliara, E., Karatapanis, A., Savvaidis, I.N., and Kontominas, M.G. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology*, **24**, 607–617.

Chouliara, I., Savvaidis, I.N., Riganakos, K. and Kontominas, M.G. (2005). Shelf-life extension of vacuum-packaged sea bream (Sparusaurata) fillets by combined γ-

irradiation and refrigeration, microbiological, chemical and sensory changes. *Journal of the Science of Food and Agriculture*, **85**, 779-784.

Chounou, N., Chouliara, E., Mexis, S.F., Stavros, K., Georgantelis, D., and Kontominas, M.G. (2013). Shelf life extension of ground meat stored at 4 °C using chitosan and an oxygen absorber. *International Journal of Food Science and Technology*, **48**, 89-95.

Cohen, J.D., Erkenbrecher, C.W., Haynie, S.L., Kelley, M.J., Kobsa, H., Roe, A.N., and Scholla, M.H. (1995). Process for preparing antimicrobial polymeric materials using irradiation. *United States Patent US*, **5**, 428.

Coles R. (2003). Introduction. In: Coles R., McDowell D., Kirwan M.J., editors. Food packaging technology. London, U.K.: *Blackwell Publishing, CRC Press*., 1–31.

Coma, V., Sebti, I., Pardon, P., Deschamps, A. and Pichavant, F.H. (2001). Antimicrobial edible packaging based on cellulose ethers, fatty acids and nisin incorporation to inhibit Listeria innocuaand Staphylococcus aureus. *Journal Food Protection*, **64**, 470-5.

Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F., and Deschamps, A. (2002). Edible anti-microbial films based on chitosan matrix. *Journal of Food Sciences*, **67**, 1162–1169.

Coma, V., Deschamps, A., and Martial-Gros, A. (2003). Bioactive packaging materials from edible chitosan polymer antimicrobial activity assessment on dairy-related contaminants. *Journal of Food Sciences*, **68**, 2788–2792.

Cox, N.A., Bailey, J.S., and Berrang, M.E. (1998). Bactericidal treatment of hatching eggs I. Chemical immersion treatments and salmonella. *Journal of Applied Poultry Research*, **7**, 347-350.

Cutter, C.N. (1999). The effectiveness of triclosanincorporated plastic against bacteria on beef surfaces. *Journal of Food Protection*, **62**, 474–479.

Cutter, C.N., and Siragusa, G.R. (1997). Growth of Brochothrixthermosphacta in ground beef following treatments with nisin in calcium alginate gels, *Food Microbiology*, **14**, 425–430.

Darmadji, P., and Izumimoto, M. (1994). Effects of chitosan in meat preservation. *Meat Science*, **38**, 243–254.

De Kruijf, N., Van Beest, M., Rijk, R., Sipilainen-Malm, T., Losada, P.P. and De Meulenaer, B. (2002). Active and intelligent packaging: applications and regulatory aspects, Food Addit. Contam., **19**, 144–162.

Del Rio, E., Panizo-Morán, M., Prieto, M., Alonso-Calleja, C., and Capita, R. (2007). Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. *International Journal of Food Microbiology*, **115**, 268-280.

Devlieghere, F., Vermeiren, L., Bockstal, A., and Debevere, J., (2000). Study on antimicrobial activity of a food packaging material containing potassium sorbate. *ActaAlimentaria*, **29**, 137-146.

Economou, T., Pournis, N., Ntzimani, A., and Savvaidis, I.N., (2009). Nisin–EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chemistry*, **114**, 1470–1476.

Ercolini, D., Ferrocino, I., La Storia, A., Mauriello, G., Gigli, S., Masi, P., and Villani, F. (2010). Development of spoilage microbiota in beef stored in nisin activated packaging. *Food Microbiology*, **27**, 137–143.

Floros, J.D., Dock, L.L. and Han, J.H. (1997). Active packaging technologies and applications. *Food Cosmet Drug Package*, **20**, 7-10.

Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas, G., and Georgakis, S.A. (2007). Effect of rosemary extract, chitosan and alpha-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 degrees C. *Meat Science*, **76**, 172-181.

Greer, G.G. (1982). Mechanism of beef shelf life extension by sorbate. *Journal Food Protection*, **45**, 82-83.

Han, J.H. (2000). Antimicrobial food packaging. *Food Technology*, **54**, 56-65.

Han, J.H. (2003). Antimicrobial food packaging, In Novel Food Packaging Techniques, A. Raija (Ed.), 50–70, CRC Press LLC.

Han, J.H. and Floros, J.D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film and Sheeting*, **13**, 287–298.

Hasapidou, A. and Savvaidis, I.N. (2011). The effects of modified atmosphere packaging, EDTA and oregano oil on the quality of chicken liver meat. *Journal Food Process Preservation*, **19**, 77-169.

Haynie, S.L., Crum, G.A. and Doele, B.A. (1995). Antimicrobial activities of amphiphilic peptides covalently bonded to a water insoluble resin. *Antimicrobial Agents and Chemotherapy*, **39**, 301–307.

Hong, S.I., Park, J.D. and Kim, D.M. (2000). Antimicrobial and physical properties of food packaging films incorporated with some natural compounds. *Food Science and Biotechnology*, **9**, 38 - 42.

Hotchkis, J.H. (1995). Safety considerations in active packaging. In Active Food Packaging, Roone ML (ed). Blackie Academic & Professional: London, 238-255.

Hutton, T. (2003). Food packaging, an introduction. Key topics in food science and technology-Number 7. Chipping Campden, Gloucestershire, UK, Campden and Chorleywood Food Research Association Group (pp. 108).

Ishitani, T. (1995). Active packaging for food quality preservation in Japan. In P. Ackermann, M. Jagerstad and T. Ohlsonn (Eds.), Food and food packaging materials-chemical interactions (pp. 177–188). Cambridge, England, Royal Society of Chemistry.

Jensen, C., Guidera, J., Skovgaard, I.M., Staun, H., Skibsted, L.H., Krogh Jensen, S., Møller, A.J., Buckley, J. and Bertelsen, G. (1997). Effects of dietary alfa-tocopheryl

acetatesupplementation on alfa-tocopherol deposition in porcine m. psoas major and m. longissimusdorsi and on drip loss, color stability and oxidative stability of pork meat. *Meat Science*, **45**, 491-500.

Jumaa, M. and Müller, B.W. (1999). Physicochemical properties of chitosan–lipid emulsions and their stability during the autoclaving process. *International Journal of Pharmaceutics*, **183**, 175–184.

Kim, C.R. and Marshall, C.L. (1999). Microbiological, color and sensory changes of refrigerated chicken legs treated with selected phosphates. *Food Research International*, **32**, 209-215.

Koide, S.S. (1998). Chitin–chitosan: Properties, benefits and risks. *Nutrition Research*, **18**, 1091–1101.

Kozačinski, L., Fleck, Z.C., Kozačinski, Z., Filipovic, I., Mitak, M., Bratulic, M. and Mikus, T. (2012). Evaluation of shelf life of pre-packed cut poultry meat, Veterinarski Arhiv, **82**, 47-58.

Kumar, M.N.V.R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polym*ers, **46**, 1-27.

Labuza, T.P. and Breene, W.M. (1988). Applications of "active packaging" for improvement of shelf-life and nutritional quality of fresh and extended shelf-life foods. *Journal Food Process Preserv*ation, **13**, 1-69.

La Storia, A., Ferrocino, I., Torrieri, E., Di Monaco, R., Mauriello, G., Villani, F., and Ercolini, D. (2012). A combination of modified atmosphere and packaging to extend the shelf-life of beefsteaks stored at chill temperature. *International Journal of Food Microbiology*, **158**, 186-194.

Lord, J.B. (2008). The food industry in the United States. In: Brody AL, Lord J, editors. Developing new food products for a changing market place. 2nd ed. Boca Raton, Fla.: CRS Press., 1–23.

Marsh, K. and Bugusu, B. (2007). Food packaging: roles, materials, and environmental issues. *Journal Food Science*, **72**, 39–55.

Martínez-Camachoa, A.P., Cortez-Rochaa, M.O., Graciano-Verdugob, A.Z., Rodríguez- Félixa, F., Castillo-Ortegac, M.M., Burgos-Hernándeza, A., Ezquerra-Brauera, J.M., and Plascencia-Jatomeaa, M. (2013). Extruded films of blended chitosan, low density polyethylene and ethylene acrylic acid. *Carbohydrate Polymers*, **91**, 666–674.

Mexis, S.F., Chouliara, E. and Kontominas, M.G. (2012). Shelf life extension of ground chicken meat using anoxygen absorber and a citrus extract LWT. *Food Science and Technology*, **49**, 21-27.

Mulder, R.W.A.W. (1999). Hygiene during transport, slaughter and processing. In: Poultry Meat Science. Poultry Science Symposium Series Volume 25 (Richardson and Mead, Eds.). CABI Publishing, 277-285.

Muzzarelli, R.A.A., Frega, N., Miliani, M., Muzzarelli, C. and Cartolari Torres, M. (2000). Interactions of chitin, chitosan, N-lauryl chitosan and N-dimethylaminopropyl chitosan with olive oil. *Carbohydrate Polymers*, **43**, 263–268.

Nicholson, M.D. (1998). The role of natural antimicrobials in food packaging biopreservation. *Journal Plastic Film Sheeting*, **14**, 234 - 241.

Occelli, M.I. and Kessler H. (Eds). (1997). Synthesis of Porous Materials: Zeolites, Clays and Nanostructures. New York, CRC Press, **47**, 234-151.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein based film containig essential oils for the preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry*, **52**, 5598 – 5605.

Ozdemir, M. and Floros, J.D. (2004). Active food packaging technologies, *Crit. Rev. Food Science*, **44**, 185–193.

Ozdemir, M., Yurteri, C.U. and Sadikoglu, H. (1999). Surface treatment of food packaging polymers by plasmas. *Food Technology*, **53**, 54–58.

Padgett, T.M., Han, I.Y. and Dawson, P.L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection*, **61**,1330–1335.

Paik, J.S., Dhanasekharan, M. and Kelly, M.J. (1998). Antimicrobial activity of UV-irradiated nylon film for packaging applications. *Packaging Technology and Science*, **11**, 179–187.

Park, S.I., Marsh, K.S. and Dawson, P. (2010). Application of chitosan-incorporated LDPE film to sliced fresh red meats for shelf life extension. *Meat Science*, **85**, 493–499.

Perrins, C.M. (2003). Firefly encyclopedia of birds, Firefly books, pp. 656

Pranoto, Y., Rakshit, S.K. and Salokhe, V.M. (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate, and nisin. LWT – *Food Science and Technology*, **38**, 859-865.

Quattara, B., Simard, R.E., Piette, G., Begin, A. and Holley, R.A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology*, **62**, 139 – 148.

Quintavalla, S. and Vicini, L. (2002). Antimicrobial Food Packaging in Meat Industry. *Meat Science*, **62**, 373-380.

Rodrigues, E.T. and Han, J.H. (2000). Antimicrobial whey protein films against spoilage and pathogenic bacteria. Proceedings of the IFT Annual Meeting; Dallas, Tex.; June 10-14. Chicago, Ill.: Institute of Food Technologists. p 191.

Rodriguez-Calleja, J.M., Cruz-Romero, M.C. and O'Sullivan, M.G. (2012). High-pressure- based hurdle strategy to extend the shelf-life of fresh chicken breast fillets. *Food Control*, **25**, 516-524.

Roberts, J.A. (1995). Assessing the scavenging feed resource base for sustainable Small holder poultry development. Proceedings ANRPD workshop, Addis Ababa, Ethiopia.pp 40 – 52.

Robertson, G.L., (2006). Food Packaging Principles and Practice, (CRC press, second edition) pp. 292-293.

Rooney, M.L. (1995). Active packaging in polymer films. In M. L. Rooney (Ed.), Active food packaging (pp. 74–110). Glasgow, UK: Blackie Academic & Professional.

Sanchez-Valdes, S., Ramirez-Vargas, E., Ortega-Ortiz, H., Ramos-deValle, L.F., Mendez-Nonell, J., Mondragon-Chaparro, M., Neira-Velazquez, G., Yanez-Flores, I., Meza-Rojas, D.E. and Lozuno-Ramirez, T. (2011). Silver Nanoparticle Deposition on Hydrophilic Multilayer Film Surface and Its Effect on Antimicrobial Activity. *Journal of Applied Polymer Science*, **123**, 2643-2650.

Sante, V. and Lacourt, A. (1994). The effect of dietary a-tocopherol supplementation and antioxidant spraying on color stability and lipid oxidation of turkey meat. *Journal of the Science of Food and Agriculture*, **65**, 503–507.

Shearer, A.E.H., Paik, J.S., Hoover, D.G., Haynie, S.L., and Kelley, M.J. (2000). Potential of an antibacterial ultraviolet-irradiated nylon film. *Biotechnology and Bioengineering*, **67**, 141–146.

Sherwin, C.M. and Nicol, C.J. (1993). Factors influencing floor-laying by hens in modified cages. *Applied Animal Behaviour Science*, **36**, 211-222.

Siragusa, G.R., Cutter, C.N., and Willett, J.L. (1999). Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. *Food Microbiology*, **16**, 229−235.

Suppakul, P., Miltz, J., Sonneveld, K., Bigger, S.W. (2003). Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications, *Journal of Food Science*, **68**, 408-420.

Thompson, R.W. (1998). Molecular Sieves, Science and Technology, Weitkamp, I.J. (Ed). Springer, Berlin, 47-62.

Tsai, G.J., Wu, Z.Y. and Su, W.H. (2000). Antibacterial activity of a chitooligosaccharide mixture prepared by cellulase digestion of shrimp chitosan and its application to milk preservation. *Journal of Food Protectection*, **63**, 747–752.

Van Bekkum, H., Flanigen, E.M., Jacobs, P.A. and Jansen, J.C. (2001). Introduction to zeolite science and practice. In Studies in Surface Science and Catalysis, Elsevier Science Publishers, Amsterdam, **137**, 469 – 472.

Vermeiren, L., Devlieghere, F., Van Beest, M., De Kruijf, N. and Debevere, J. (1999). Developments in the active packaging of foods. *Trends in Food Science & Technology*, **10**, 77–86.

Vermeiren, L., Devlieghere, F., Debvere, J. (2002). Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*, **19**, 163 – 171.

Vojdani, F. and Torres, J.A. (1990). Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: effect of fatty acids. *Journal of Food Science*, **55**, 841–846.

Wang, S.H., Chang, M.H. and Chen, T.C. (2004). Shelf-life and Microbiological Profiler of Chicken Wing Products Following Sous vide Treatment. *International Journal of Poultry Science*, **3**, 326-332.

Weng, Y.M. and Hotchkiss, J.H. (1992). Inhibition of surface moulds on cheese by polyethylene film containing the antimycoticimazalil. *Journal of Food Protection*, **55**, 367–369.

Weng, Y.M. and Hotchkiss, J.H. (1993). Anhydrides as antimycotic agents added to polyethylene films for food packaging. *Packaging Technology Science*, **6**, 123 - 128.

Wilson, C. (2007). Frontiers of intelligent and active packaging for fruits and vegetables, Boca Raton, Fla.: CRC Pres, 360 p.

Zhang, L., Jiang, Y., Ding, Y., Povey, M. and York, D. (2007). Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). *Journal of Nanoparticle Research*, **9**, 479-489.