EFFECT OF EDIBLE FILM AND ALLICIN ON SHELF LIFE OF CHICKEN MEAT

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ABSTRACT

EFFECT OF EDIBLE FILM AND ALLICIN ON SHELF LIFE OF CHICKEN MEAT

Chicken meat is an important food for healthy nutrition due to its nutritional value and is consumed by most populations of society such as growing children, and the elderly. However, chicken meat is a perishable food, and edible coatings and bioactive ingredients are used to prevent this. In this study, the antibacterial, antimicrobial, and biodegradable properties of chitosan and the antimicrobial, antifungal, and antioxidant properties of allicin were used to investigate the effect on the shelf life of chicken drumsticks. The treatment of chicken drumsticks with chitosan and allicin solutions prepared at different concentrations K1A1(1% Chitosan + 10% Allicin), K1A2(1% Chitosan + 20% Allicin), K2A1(2% Chitosan + 10% Allicin), K2A2(2% Chitosan + 20% Allicin) and with solutions containing only different concentrations of chitosan (%1 Chitosan, %2 Chitosan) was evaluated by storing them at 4 ˚C for the determined shelf life of the microbiological properties, sensory properties, chemical, and oxidative deterioration parameters. In samples of both groups, the total viable, *Enterobacteriaceae* and *Pseudomonas* bacteria counts were significantly reduced compared to the control group. The most efficient result in inhibiting the total viable count was given by K2A1 treatment, which extended the shelf life of chicken drumsticks. In the TBA results on the 10th day of storage, it was found that the chicken drumsticks coated with 1% chitosan were the most successful group in inhibiting lipid oxidation with a value of 0.33 mg MDA/kg. After this result, the most successful result was the K1A2 sample with a value of 0.55 mg MDA/kg.

Keywords: *Allicin, Antimicrobial, Antioxidant, Chitosan, TBA, Total viable*

ÖZET

YENİLEBİLİR KAPLAMA VE ALLİSİNİN TAVUK ETİNİN RAF ÖMRÜNE ETKİSİ

Tavuk eti besleyici değeri sebebiyle sağlıklı beslenme için önemli bir gıdadır ve büyüme çağındaki çocuklar ve yaşlılar gibi toplumun çoğu popülasyonu tarafından tüketilen bir gıdadır. Ancak tavuk eti kolay bozulabilen bir gıdadır ve bunun önüne geçmek için yenilebilir kaplamalardan ve biyoaktif bileşenlerden yararlanılmaktadır. Bu çalışmada, kitosanın antibakteriyel, antimikrobiyal, biyouyumlu ve biyolojik olarak parçalanabilir özellikleri ve allisinin antimikrobiyal, antifungal ve antioksidan özellikleri kullanılarak tavuk bagetlerdeki raf ömrüne etkisi incelenmiştir. Tavuk bagetlerin, farklı konsantrasyonlarda hazırlanan kitosan ve allisin çözeltileriyle K1A1(1% Kitosan + 10% Allisin), K1A2 (1% Kitosan + 20% Allisin), K2A1(2% Kitosan + 10% Allisin), K2A2(2% Kitosan + 20% Allisin) ve sadece kitosanın farklı konsantrasyonlarını içeren çözeltileriyle (%1 Kitosan, %2 Kitosan) kaplanması, mikrobiyolojik özellikleri, duyusal özellikleri, kimyasal parametleri, oksidatif bozulma parametreleri belirlenen raf ömrü boyunca 4 ˚C'de saklanarak değerlendirilmiştir. Kitosan ve allisin ile kaplanan numunelerde ve kitosan çözeltisiyle kaplanmış numunelerde kontrol grubuna kıyasla toplam canlı, *Enterobacteriaceae, Pseudomonas* bakteri sayımlarında, bakteri sayısı önemli ölçüde azalmıştır. Toplam canlı gelişimini engellemede en etkili sonuç, tavuk bagetlerin raf ömrünü uzatan K2A1 örnek grubuyla elde edildi. Depolamanın 10. günündeki TBA sonuçlarında, %1 kitosanla kaplanmış tavuk bagetlerin 0.33 mg MDA/kg değeriyle lipid oksidasyonunu engellemede en başarılı grup olduğu bulunmuştur. Bu sonuçtan sonra en başarılı sonuç 0.55 mg MDA/kg değeriyle K1A2 örneği olmuştur.

Anahtar Kelimeler: *Allisin, Antimikrobiyal,Antioksidan, Kitosan, TBA, Toplam canlı*

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LIST OF ABBREVIATIONS

K1A1 : 1% Chitosan + 10% Allicin

K1A2 : 1% Chitosan + 20% Allicin

K2A1 : 2% Chitosan + 10% Allicin

K2A2 : 2% Chitosan + 20% Allicin

TVC : Total Viable Count

OECD : Organisation for Economic Co-operation and Development

USDA : United States Department of Agriculture

EFSA : European Food Safety Authority

TBARS : Thiobarbituric Acid Reactive Substance

TCA : Trichloroacetic Acid

TAMB : Total Aerobic Mesophilic Bacteria

CFU : Colony Forming Unit

GRAS : Generally Recognized as Safe

GMP : Goods Manufacturing Practices

FCM : Food Coating Materials

FCA : Food Contact Materials

Mcg : Microgram

IU : International Unit

CHAPTER 1

INTRODUCTION

Today, the food industry is gradually changing according to consumer demands. In our country, people's economic access to foods with high protein value rapidly decreases. That's why meat products are not preferred much. As a result, blood levels decrease, the immune system decreases, and diseases proliferate. At this point, chicken meat comes into play and its consumption has increased in recent years due to its high nutritional value, ideal fatty acid profile, and high protein value. Protein is also important for bodybuilders because it helps develop muscle and repair damaged muscle cells. Essential amino acids and other nutrients may be obtained from various protein sources, including casein protein and plant-based protein powder. However, animal-based protein sources are often more efficient than plant-based ones for stimulating muscle protein synthesis. Therefore, chicken meat is a significant source of protein for bodybuilders. However, animal-based protein sources are generally more effective than plant-based protein sources and better stimulate muscle protein synthesis. Therefore, chicken meat is also an important source of protein for bodybuilders (Siddiqui et al. 2024).

Although chicken meat has a lot of positive effects, the product's shelf life in terms of quality and sensory properties decreases due to the presence of microorganisms such as Salmonella, Campylobacter jejuni, Listeria monocytogenes, and lipid oxidation in poultry meat. In this case, studies on the shelf life of chicken meat using the edible coating method have come to the fore. The edible coating on chicken meat has multiple benefits. Reduced oxidation reaction, elimination of off taste or odor, increased shelf life, improved antioxidant activity, and textural and nutritional properties are just a few examples. Biopolymers of many kinds, including proteins, polysaccharides, lipids, and composites, are used to make edible coatings. An edible covering made of polysaccharides can enhance the mechanical qualities of food by obstructing the transfer of moisture and oxygen. The ingredients utilized in polysaccharide-based coatings include cellulose, pectin, chitosan, carrageenan, starch, and alginates. Waxes, fatty acids, and resins are utilized as oil-based edible films, whereas whey, gluten, soy, and zein are used as protein-based edible films. Chitosan, which is biodegradable in nature and can be

compressed with many substances such as minerals, antimicrobial agents, and food vitamins, is used as a coating in many foods, especially fruits and vegetables. Antioxidant qualities that are hypoglycemic and hypolipidemic protect coated foods from microbial spoilage (Priya, Thirunavookarasu, and Chidanand 2023). Plant-derived essential oils possess antibacterial and antioxidant characteristics. Integrating essential oils into edible coatings is one of the ongoing research topics in the food industry. Allicin is one of the important compounds that is extracted from white garlic. Strong antioxidant qualities and inhibitory actions against various microorganisms, including viruses, bacteria, fungi, and protozoa, are its key features. Combining allicin and edible coating materials is an effective method for reducing microbial activity in food products. In addition, the edible coating needs to be approved for safety before it can be used commercially because it comes into direct contact with food. The use of some approved natural plant extracts and essential oils may be harmful and allergenic to humans. Consequently, the toxicity and allergenic properties of essential oils and extracts used with edible coatings are very important and regular testing and precautions are necessary.

This project aims to examine the effects of edible coating application and garlic extract on chicken drumsticks with the help of microbiological and chemical analyses. Although chicken drumsticks have an average shelf life of 7-8 days, if the necessary cold chain is not provided, the shelf life on the market shelves decreases to 4-5 days. To solve this problem, food providers and consumers have increasing demands on food manufacturers. At this stage, edible coatings are an example of innovative approaches used recently. By adding essential oils and biopolymer substances with antioxidant and antimicrobial properties, functional products and innovative foods emerge. In this study, different percentages of chitosan were used to examine its adhesion to chicken drumsticks and its activity on shelf life, and the effect of the combination of allicin and chitosan with these different percentages was examined separately.

CHAPTER 2

LITERATURE REVIEW

2.1. Chicken Meat

2.1.1. Chicken Meat Consumption

The connection between meat consumption and health has reached a critical point. Although red meat is now popular, people's economic struggles make this difficult. Therefore, its moderate energy content, highly digestible proteins with good nutritional value, unsaturated fats, B group vitamins, and minerals, and chewability properties in the mouth due to low connective tissues, make poultry meat stand out and be preferred over red meat consumption. The fact that poultry is cheaper in low-income countries and is seen as a healthy food option has increased the interest in white meat. In addition, poultry consumption has increased rapidly because of the worldwide prevalence of nutrition-related diseases such as obesity and cardiovascular diseases. Authorities have warned consumers to prioritize white meat in their daily eating habits (Keskin and Demirbaş 2012).

According to OECD data, the per capita consumption of poultry meat in main countries in 2022 is given in Figure 1, and Israel has the highest rate. The USA and Malaysia follow with similar results at around 50%. The general world chicken consumption rate is the same as the previous year, at 14,9 kg/year. USDA data shows global chicken meat exports increased by 1.8% compared to the previous year, reaching 13.5 million tons in 2022. Brazil ranks first in exports with a share of 32.9%, and the USA ranks second with 24.5%. Türkiye ranked fourth in world chicken meat exports with a share of 4.3% in 2022 (Gülaç 2023).

According to the OECD-FAO Agricultural Outlook study, the world's meat supply is expected to grow to 374 million tons by 2030. The report also stated that, mostly as a result of rising affluence and population, the average global intake of beef protein during the next ten years is predicted to rise by 14% by 2030. China is expected to contribute the

most to the overall growth in meat production, with the United States and Brazil following closely after. The increase in global meat production is largely due to growth in poultry production. In high-income countries, white meat is preferred because it is quicker to cook and healthier, while in low-income countries, individuals prefer poultry because it is cheaper than red meat. Poultry is projected to provide 41% of global protein from meat sources by 2030. This represents a 2% increase from the baseline period. For these reasons, worldwide per capita meat consumption is predicted to increase by 0.3% per year. By 2030, the retail weight equivalent (raw) will be 35.4 kilograms. Increased per capita consumption of poultry meat accounts for more than half of this increase (Outlook 2021).

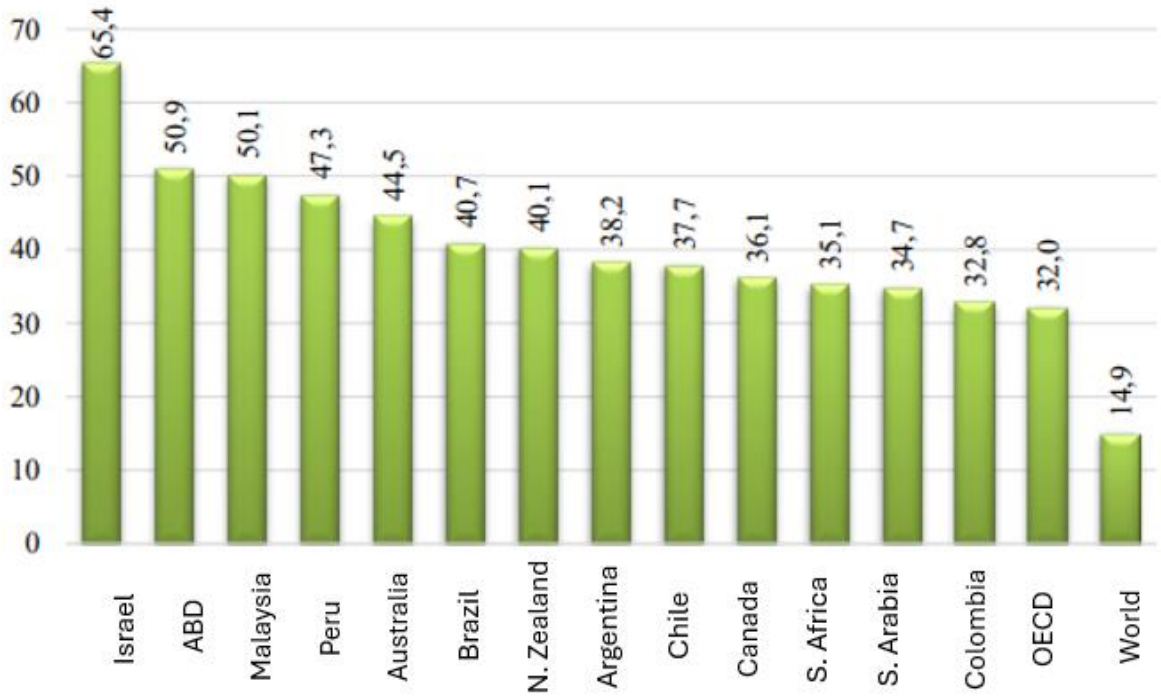

Figure 1. Poultry meat consumption per capita in 2022 by country (kg/year) (Source: Gülaç 2023)

The poultry meat industry in Türkiye has been among the rapidly developing sectors since the 1980s. In Türkiye, the number of poultry in 2022 is 367 million, the largest part of which is chicken with 98.5%, and 674 thousand tons of poultry meat was exported, 98.6% of which is chicken meat (Gülaç 2023). Table 1 shows poultry meat consumption per capita (kg) in Türkiye. It is seen that total poultry meat consumption has increased over the years. While the average consumption amount was 22,29 kg in 2017, it has started to decline since 2018 due to changes in the exchange rate, increasing feed

prices, drought, and the events that occurred with the COVID-19 epidemic and started to reach the same level again in 2022. In a study conducted on chicken consumption in Turkey, it was stated that it varies depending on income level, marital status, living in rural or urban areas, and living and eating habits. According to the findings, consumption amounts in the eastern and southeastern regions remain low compared to the western and central regions (Dokuzlu et al. 2013). In the study examining the consumption of rabbit, beef, chicken, turkey, and lamb meat, bodybuilding athletes were asked which meat was the most loved and preferred, and as a result, it was obtained that lean, easy-to-cook white meats such as chicken and turkey were preferred (Siddiqui et al. 2024).

Table 1. Türkiye Poultry Meat Consumption per Capita (kg)

Year	Chicken Meat	Turkey Meat	Total Poultry Meat
2000	9,46	0,28	9,75
2005	13,01	0,58	13,59
2010	18,03	0,42	18,45
2011	18,79	0,46	19,25
2012	18,94	0,51	19,45
2013	18,41	0,43	18,84
2014	19,54	0,52	20,06
2015	20,29	0,6	20,89
2016	20,01	0,51	20,52
2017	21,73	0,56	22,29
2018	20,89	0,73	21,62
2019	20,47	0,56	21,02
2020	20,50	0,59	21,10
2021	20,68	0,51	21,19
2022	21,95	0,54	22,50

Source: Turkish Statistical Institute (TurkSTAT 2024)

Figure 2 shows Türkiye's chicken meat production and consumption between 2019 and 2022, in line with the information provided by TurkSTAT. It is seen that the amount of chicken slaughtered is 2,418 (thousand head) tons in 2022. While the number of slaughtered chickens increased by 8.4% compared to the previous year, chicken meat production increased by 7.7% (Gülaç 2023). The goal of the Poultry Meat Industry, one of the important sectors of the Turkish economy, is to increase chicken meat production to 3.35 million tons in 2025 (Besd-bir 2024b).

A study conducted voluntarily with a face-to-face survey method with students in the Department of Nutrition and Dietetics (median age 20), determined that 98% consumed chicken meat. 85.2% of the students gave a positive answer to whether they were informed about the nutritional value of chicken meat. This study concludes that chicken meat is among the foods that are easy to prepare and is preferred by students (Varol Avcılar, Karataş, and Yılmaz 2022).

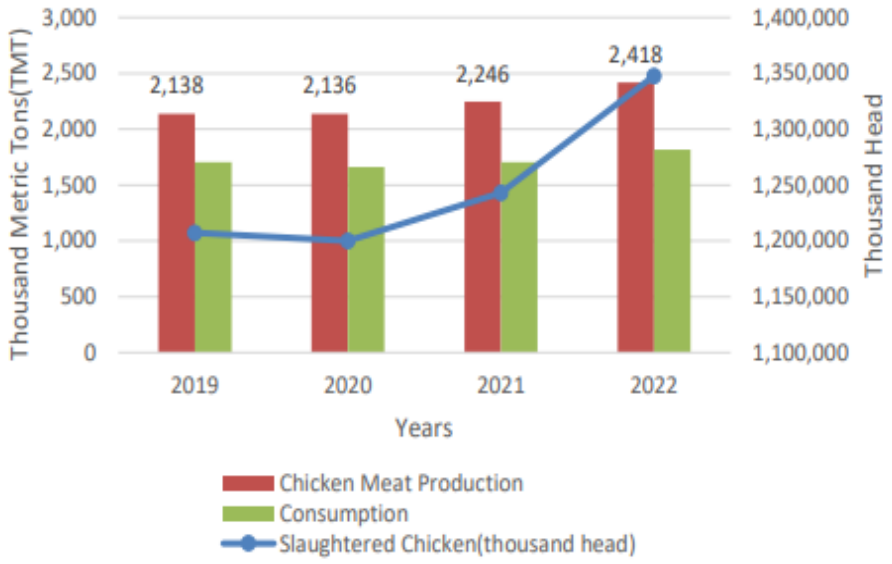

Figure 2. Slaughtered Chickens and Chicken Meat Production versus Chicken Meat Consumption (USDA 2023)

2.1.2. Nutritional Composition of Chicken Meat

Poultry meat has a protein-rich component that is often regarded as high-quality foods. Poultry meat, which varies in composition based on the chicken species and feeds, cuts, and whether it is skinless, is often recognized as a nutritious and low-energy meat alternative. Chicken and turkey contain more than simply protein and minerals. In addition, it is consumed because of its very low fat and cholesterol values, especially skinless chicken meat. The protein content in meat and poultry ranges from 15% to 35%, depending on the product's other components. The Population Reference Intake (PRI), which is the amount needed to meet the nutritional needs of almost all healthy individuals

in the population, is calculated to be 0.83 grams of protein per kilogram of weight of the individual per day for adults based on data from the European Food Safety Authority (EFSA 2012). Table 2 shows the protein, lipid, and cholesterol levels of various raw and cooked poultry meat. The nutritional composition of chicken meat varies depending on the specific cutting and cooking method. Cooking raises the protein concentration in skinless chicken and turkey thighs by up to 60% by weight (Marangoni et al. 2015). While it is seen that the protein value is high in skinless whole, drumstick, and breast meats, the vice versa for lipid values. Cholesterol content increases when parts of the chicken are cooked.

Meat contains saturated fats; therefore, excessive consumption may relate to negative health results. In general, healthy people should aim to consume 25 to 35% of their total energy from fats. As a result, a person consuming 2,000 kcal per day receives at least 70 grams of these nutrients. Fat provides essential fatty acids like linoleic and alpha-linolenic acids when taken in sufficient quantities. It increases the feeling of fullness due to its effect on slowing gastric emptying and therefore reduces the bioavailability of carbohydrates. By enhancing the food's flavor, aroma, and texture, it also raises its quality (Marangoni et al. 2015).

Table 2. Protein, Lipid, and Cholesterol Content of Raw and Cooked Poultry Meat

	Chicken Protein Content(g/100g)	**Chicken Lipid Content (g/100g)**	**Chicken Cholesterol Content(mg/100g)**
Whole with skin, raw	18.6	15.1	75
Whole skinless, raw	21.4	3.1	70
Whole with skin, roasted	27.3	13.6	88
Whole skinless, roasted	28.9	7.4	89
Breast with skin, raw	20.9	9.3	64
Breast skinless, raw	22.5	2.6	73
Breast with skin, roasted	29.8	7.8	84
Breast skinless, roasted	31.0	3.6	85
Drumstick with skin, raw	18.1	9.2	92
Drumstick skinless, raw	19.4	3.7	89
Drumstick with skin, roasted	23.4	10.2	130
Drumstick skinless, roasted	24.2	5.7	130

Source: USDA National Nutrient Database for Standard Reference, release28(2015), (Bordoni and Danesi 2017).

The saturated to unsaturated fatty acid (SFA/UFA) ratio in poultry meat is typically 1:3, with breast meat having a smaller SFA/UFA ratio than other skinless cuts. Poultry meat's significant amount of long-chain n-3 poly-unsaturated fatty acids (n-3 LC-PUFA) is an important nutritional trait (Bordoni & Danesi, 2017). The calorie and macronutrient values of chicken meat are shown in Table 3. Based on the method of slaughter, poultry meat has varying amounts of fat. Because they are mostly present in the skin, fats are easily eliminated (Table 3). The leanest parts of chicken and turkey, including the breast and legs, have about 1% fat content, while cooked chicken wings with skin have about 17% fat content. Cooking can also boost fat content by eliminating water from meat or incorporating lipids included in seasonings used during preparation. However, chicken meat appears to contain less fat than other animal products (Marangoni et al. 2015).

Table 3. Calorie and Macronutrient Values in Chicken Breast and Thigh (Source: Joseph 2023)

Per 100g	Chicken Breast (meat only)	Chicken Breast (with skin)	Chicken Thigh (meat only)	Chicken Thigh (with skin)
Calories	110 kcal	172 kcal	119 kcal	211 kcal
Fat	1.2 g	9.2 g	3.9 g	15.3 g
Saturated	0.3 g	2.7 g	1.0 g	4.4 g
Monounsaturated	0.3 g	3.8 g	1.2 g	6.5 g
Polyunsaturated	0.3 g	2.0 g	1.0 g	3.4 g
Omega-3	40 mg	120 mg	100 mg	206 mg
Omega-6	170 mg	1740 mg	750 mg	3091 mg
Protein	23.1 g	20.8 g	19.7 g	17.3 g

Apart from being an excellent source of protein, chicken meat is also a very valuable food in terms of B vitamins (Table 4) and selenium minerals (Table 5). B complex vitamins, such as thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), vitamin B6, and vitamin B12, are abundant in chicken meat. It also contains folate, but it is a poor source of other B-group vitamins. It also contributes to the regular

functioning of the body by providing folate, which is important for DNA synthesis and cell growth, and vitamin B12, which is necessary for DNA synthesis. B-group vitamins help maintain healthy skin, maintain digestive and nervous system function, help produce red blood cells, and support the immune system. The natural and highly accessible B vitamins found in chicken meat make it a vital component of a balanced diet. Eating chicken makes it easy for the body to absorb these vitamins, which have beneficial impacts on energy metabolism, neurological system function, and skin health. Chicken is therefore a tasty and healthful component of a well-balanced diet plan.

Table 4. Content of Vitamins in Chicken Breast and Thigh (Source: Joseph 2023)

Per 100g raw (% RDI)	Chicken Breast (meat only)	Chicken Breast (with skin)	Chicken Thigh (meat only)	Chicken Thigh (with skin)
Vitamin B3	11.2 mg (56 %)	9.9 mg (50 %)	6.3 mg (32 %)	5.4 mg (27 %)
Vitamin B6	0.5 mg (27 %)	0.5 mg (26 %)	0.3 mg (17 %)	0.3 mg (13 %)
Vitamin B5	0.8 mg (8 %)	0.8 mg (8 %)	1.2 mg (12 %)	1.0 mg (10 %)
Vitamin B12	0.4 mcg (6 %)	0.3 mcg (6 %)	0.4 mcg (6 %)	0.3 mcg (5 %)
Vitamin B1	0.1 mg (5 %)	0.1 mg (4 %)	0.1 mg (5 %)	0.1 mg (4 %)
Vitamin B2	0.1 mg (5 %)	0.1 mg (5 %)	0.2 mg (11 %)	0.2 mg (9 %)
Folate	4.0 mcg (1 %)	4.0 mcg (1 %)	10 mcg (2 %)	8.0 mcg (2 %)
Vitamin E	0.1 mg (1 %)	0.3 mg (2 %)	0.3 mg (2 %)	0.3 mg (1 %)
Vitamin K	0.2 mcg (0 %)	0.0 mg (0 %)	2.9 mcg (4 %)	2.9 mcg (4 %)
Vitamin A	21.0 IU (0 %)	83 IU (2 %)	65 IU (1 %)	149 IU (3 %)

Selenium is an important mineral that prevents the toxic effects of heavy metals and cancer, acts as an antioxidant, supports immune function, and protects against oxidative damage (Joseph, 2023). Phosphorus, which ranks first after selenium, is followed by potassium, magnesium, zinc, iron, sodium, copper, calcium, and manganese, respectively, and is found in significant proportions in chicken meat (Table 5). The two

main dietary sources of zinc are poultry and red meat. Zinc is present in about 100 distinct enzymes and is a crucial trace element for humans. The amount of zinc in poultry varies by species and cut; skinless turkey thighs have 2,68 mg of zinc per 100 g, whereas chicken breasts have 0,67 mg (Bordoni and Danesi 2017).In general, chicken meat has a balanced mineral profile, with phosphorus and selenium at the top, and minerals like iron, zinc, and potassium that support bone health, energy production, and the immune system. Because of its low-fat content and ease of digestion, chicken meat has become a popular option for people looking to meet their mineral needs.

Table 5. Mineral Profile of Chicken Breast and Thigh (Source: Joseph 2023)

Per 100g raw (% RDI)	Chicken Breast (meat only)	Chicken Breast (with skin)	Chicken Thigh (meat only)	Chicken Thigh (with skin)
Selenium	17.8 mcg (25 %)	16.6 mcg (24 %)	13.5 mcg (19 %)	12.9 mcg (18 %)
Phosphorus	196 mg (20 %)	174 mg (17 %)	168 mg (17 %)	145 mg (14 %)
Potassium	255 mg (7 %)	220 mg (6 %)	231 mg (7 %)	192 mg (5 %)
Magnesium	28.0 mg (7 %)	25.0 mg (6 %)	24.0 mg (6 %)	20.0 mg (5 %)
Zinc	0.8 mg (5 %)	0.8 mg (5 %)	1.9 mg (13 %)	1.6 mg (11 %)
Iron	0.7 mg (4 %)	0.7 mg (4 %)	1.0 mg (6 %)	1.0 mg (6 %)
Sodium	65 mg (3 %)	63.0 mg (3 %)	86.0 mg (4 %)	76.0 mg (3 %)
Copper	Trace (2 %)	Trace (2 %)	0.1 mg (3 %)	0.1 mg (3 %)
Calcium	11.0 mg (1 %)	11.0 mg (1 %)	10.0 mg (1 %)	10.0 mg (1 %)
Manganese	Trace (1 %)	Trace (1 %)	Trace (1 %)	Trace (1 %)

2.1.3. Microbial Composition of Chicken Meat

Although poultry meat is preferred for its nutritional value, it is among the foods that spoil most quickly in the presence of pathogens and other microorganisms. According to studies, pathogenic organisms like *Salmonella, Campylobacter, S. aureus, E. coli*, and *Listeria* are frequently present in poultry meat. *Yersinia enterocolitica, Aeromonas*, and *Cl. perfringens* are additional microbes that could develop into significant diseases in poultry products. Nonetheless, it has been determined that the most common foodborne pathogens in the chicken sector are *Salmonella, Campylobacter jeuni*, and *Listeria monocytogenes* (Bhaisare et al. 2014). It has been observed that the pathogens causing food poisoning related to poultry meat are invasive species of Salmonella (*S.enteritidis, S. typhimurium, S.virchow*), *Campylobacter jejuni,* and *Campylobacter coli* (Yücel Baydur,2006). Saitama, Ono, and Yamamoto (1999) *C. jejuni*, which is reported to be the most important cause of enteritis and diarrhea in humans, was reported to be found in 45.8% of retail poultry meat in Japan and 3.7% of imported poultry meat.

2.1.3.1. Sources of Microorganisms and Contamination

Pathogenic organisms are not normally present on the surface of meat, but they can become contaminated by excrement or cross-contamination during the slaughter process. It is crucial to avoid cross-contamination that could happen during the stages of growing, slaughtering, and selling meat. Chicken meat is exposed to primary and secondary contamination during the production phase. The presence of microorganisms in the meat of animals while they are still alive indicates primary contamination. Environmental conditions as well as components such as equipment, personnel, and other animals can increase the microbial load in carcasses during the rearing process. Microorganisms usually enter the body through various ways and stay localized where they normally occur. However, animals under stress have a greater likelihood of microorganisms spreading through blood and lymph, which can harm their general health (Yücel 2006).

2.1.3.2. Spoilage and Shelf Life of Chicken Meat

Chicken meat's high protein and moisture content, as well as its abundance of minerals, vitamins, and growth factors, make it the ideal habitat for bacteria. Poultry meat has an approximate water activity (aw) of 0.98 to 0.99. Furthermore, the pH of the substance is appropriate for the development of bacteria (chicken breast = 5.7–5.9) (Bhaisare et al. 2014). Chicken meat spoils easily because it contains more than 70% moisture. Meat's color, texture, and flavor are all significantly impacted by the presence of water. It also reduces shelf life. Fat tissues hold less moisture, therefore as the animal grows bigger, the amount of water in its carcass reduces, and vice versa. Approximately 72% moisture content was found in younger, leaner animals (Ahmad, Imran, and Hussain 2018). This water ratio, which is necessary for the growth and survival of microorganisms, is an important situation to pay attention to in the process of chicken meat production to consumption.

One process that has a direct impact on meat quality is lipid oxidation. One of the nutrients that is most chemically unstable, lipids take part in oxidative reactions that are brought on by a multitude of factors. It significantly alters the color, texture, nutritional value, taste, and aroma of chicken meat, reducing its shelf life and degrading its quality. Eating chicken flesh may produce hazardous compounds and free radicals in addition to its detrimental sensory effects. These substances could lead to illnesses or have adverse health impacts. Malonaldehyde and 4-hydroxynonenal are two examples of the numerous primary and secondary lipid oxidation byproducts that appear to be possible carcinogens (Sabuncular, Akbulut, and Yaman 2021). The time it takes for lipid oxidation to occur in chicken meat depends on many factors. These include the presence of oxygen, the application of additives, vacuum, modified atmosphere, and active packaging; temperature- and time-dependent storage conditions; and processing conditions like cooking, milling, and irradiation. (Huang and Ahn 2019). According to a study on chicken meat kept at -7 °C, -12 °C, and -18 °C for six months, the peroxide value of the meat samples was significantly impacted by storage time, but there was not a noticeable differentiation between freezing temperatures. After 2 and 3 months of storage, peroxide levels in chicken leg and breast meat increased and decreased after 6 months of storage. It has been reported that the explanation for this fluctuation is that peroxide creation occurs at a quicker rate than peroxide decomposition into secondary oxidation products

during the first, second, and third months of storage (Soyer et al. 2010) Another study investigated the lipid fraction in uncooked and cooked chicken breast samples that were vacuum-stored and kept at 4°C for 0–6 days. Significant increases in TBA were observed in vacuum-stored samples only in cooked ones, while a milder increase was observed in aerobically packaged samples (Conchillo, Ansorena, and Astiasara´n 2003). These studies support the conclusion that lipid oxidation has multiple factors.

Total mesophilic bacteria, coliform bacteria, E. coli, S. aureus and psychrophilic bacteria found in the microbial ecology of chicken meat are considered spoilage microorganisms and bacteria that play an important role in hygiene criteria. Şahin et al. (2017) conducted an extensive study examining the microbiological quality of fresh packaged chicken meat samples offered for sale in terms of spoilage microorganisms such as total aerobic mesophilic bacteria (TAMB), Enterobacteriaceae, coliform bacteria, E. coli, S. aureus, Enterococcus spp., psychrophile bacteria, and yeast-mould. These indicator microorganisms and pathogens grow and spoil the chicken meat through many contamination sources.

2.2. Edible Coatings

There is a rising trend of consumers in developing countries seeking safe food products. To accommodate this need, food manufacturers are searching for novel strategies. Numerous foods have a restricted shelf life because of surface contamination. The food industry is working on edible coatings and films to fulfill the packaging function. Edible coatings offer a valuable solution to the industry for increasing their organoleptic properties by enriching them with organic acids, enzymes, natural extracts, and vitamins. As a result, their integration into biobased packaging is gaining importance. Food products' surfaces are covered with edible coatings. They create a barrier by preventing the transfer of gas and moisture from the atmosphere. They reduce the risk of food spoilage by restricting the respiration of cells and preventing microbial growth. In addition to hindering maturation, edible coatings reduce synthetic conventional packaging, reducing waste in the food industry (Nunes et al. 2023).

2.2.1. Types of Edible Coating Materials

Edible coatings are protective layers applied to food surfaces. These coatings are made of a combination of ecologically benign and natural biopolymers. These biopolymers' precise origins and functional characteristics might differ greatly. Biopolymer films from conventional agricultural commodities have become significantly more commercially viable in recent years. To preserve their clarity, these films are often hydrophilic, translucent, inexpensive, tasteless, and rich in phosphate esters(Andriani and Handayani 2023). Edible films and coatings made from natural ingredients such as proteins, polysaccharides, and lipids are critical to food quality and sustainability. These materials have many functional advantages, such as biodegradability and the prevention of harmful waste.

Proteins, composed of specific amino acid sequences, are the building blocks of biopolymers formed through deamination. During film formation, denaturation causes proteins to interact and form a resilient, interconnected, and viscoelastic film through hydrogen, ionic, or covalent bonds. Biopolymers are ideal for film and coating formulations due to their biological value, biodegradability, and exceptional film-forming properties(Matloob et al. 2023). Proteins can be extracted from both plant and animal sources and used to make edible coatings. Animal proteins include casein, whey, gelatin, and collagen, while plant-based proteins are found in multiple foods, such as soy protein, zein from corn, pea protein, gluten from wheat, and rice bran protein (Nunes et al., 2023). Protein-based edible coatings provide an excellent barrier against gases. These biopolymer-based coatings offer superior mechanical properties compared to those derived from lipids and carbohydrates (Priya, Thirunavookarasu, and Chidanand 2023).

One of the most popular biopolymers for making edible coatings and films is polysaccharide. Polysaccharide-based edible coatings are characterized by their hardness, high solubility, colorlessness, and flexibility, which are attributed to the linear structure of polysaccharides. These films can be used to extend the shelf life of a variety of goods, such as meat, vegetables, and fruits. Chitosan, alginate, starch, carrageenan, cellulose, pectin, and gums are commonly used polysaccharides for film formation. These types of coatings exhibit inert gas permeability and effective oxygen blocking due to their well-organized hydrogen-bonded structure, effectively blocking oxygen and moisture migration (Matloob et al. 2023). Although polysaccharides are hydrophilic, their poor

water vapor barriers may limit their integration into edible coatings in the food industry. They are frequently mixed with proteins, fats, or other polysaccharides to solve this problem (Priya, Thirunavookarasu, and Chidanand 2023).

Small hydrophobic molecules known as lipids are commonly used as ingredients in edible coatings to reduce the transfer of water vapor and moisture. Due to their limited mechanical strength, lipids are often combined with proteins or polysaccharides to form robust edible coatings. Lipid-based coatings improve food's appearance with a glossy, lustrous finish and provide superior moisture barrier qualities. Natural waxes, oils, resins, mineral oils, fatty acids, and petroleum-based waxes are used as common ingredients in lipid-based edible coatings (Priya, Thirunavookarasu, and Chidanand 2023). Because lipids have a low affinity for water, they reduce water loss when used as a food coating, improving its look and flavor. Hydrophobic compounds, such as some insoluble proteins, waxes, and resins, provide beneficial water absorption barrier properties to food. Water-soluble hydrocolloids (e.g., polysaccharides and proteins) often surpass lipids and other hydrophobic compounds in edible coatings due to their mechanical characteristics such as lengthening and tensile strength (Tavassoli-Kafrani, Shekarchizadeh, and Masoudpour-Behabadi 2016).

2.2.1.1. Coating Methods with Applications

Edible coatings are applied directly on the surface of food as the principal edible packaging. The choice of coating process varies for each food and is determined by the surface quality of the food and the function of the coating. The coating process includes covering materials on the food surface, adhering to it, and remaining there (Eser and Doğruer 2022). There are 4 types of coating models used in the food industry: dipping, spraying, sliding, and fluidized bed processing.

Dipping is the most popular edible coating procedure. The key advantage of this procedure is that it guarantees that the coating substance is fully and evenly coated on the food surface. The dipping process involves immersing the food sample in the coating solution, contacting it to generate a thin layer of edible coating, and lastly drying the coating material. In this approach, the product is dipped directly into aqueous solutions of coating formulations and then left to air dry, thus forming a thin layer on the product

surface (Tavassoli-Kafrani, Shekarchizadeh, and Masoudpour-Behabadi 2016). The immersion time in the dipping process varies from 3 to 5 minutes depending on the properties of the coating solutions such as viscosity, density, surface tension, etc. (Suhag et al. 2020). This interval has a direct impact on the coating's thickness and uniformity. While too lengthy immersion may cause the product to absorb too much moisture and cracks in the coating, too short immersion may cause the coating to fail to adhere enough (Priya, Thirunavookarasu, and Chidanand 2023). As a result, the optimal immersion time should be carefully determined based on the characteristics of the coating material and substance. In general, high-viscosity coating solutions are best applied by immersion. This procedure makes it simpler to get a homogenous coating since the whole surface of the product is in equal contact with the coating solution. The product's quality can be improved by adding chemicals like antimicrobials or antioxidants to the coating solution, which extends the product's shelf life and inhibits microbial development. Coating with the immersion technique, particularly on fresh items like fruits and vegetables, guarantees that the product retains its freshness by decreasing respiration and water loss(Priya, Thirunavookarasu, and Chidanand 2023). Furthermore, it protects the product's outside surface from damage, thus extending its shelf life.

In the spraying method, the liquid solution is sprayed onto the food. The spray coating method involves spraying the coating solution onto the ground food matrix with an atomizer and high pressure. In this way, a thin film layer of micrometer and nanometer dimensions is formed on the surface. Surface tension, spray temperature, and solution viscosity all have an impact on coating efficiency (Suhag et al. 2020). As a result, the atomizer nozzle is filled with a coating solution that has the right rheological characteristics. The coating is allowed to dry for a specific amount of time after spraying. Drying parameters including temperature, humidity, and duration have a direct impact on coating quality (Priya, Thirunavookarasu, and Chidanand 2023).

The slide method is a versatile coating technique suitable for a variety of food and confectionery products. This method allows multiple round or oval-shaped products to be coated simultaneously. A large pan holds the food while it rotates. The pan is rotated to spray the coating solution onto the food. The amount of solution sprayed determines the final coating thickness. Air circulation facilitates evaporation of the solvent and drying of the coating(Campos, Gerschenson, and Flores 2011; Eser, and Doğruer 2022).

One popular approach for applying a coating layer on small, lower-density foods (like wheat or nuts) is the fluidized bed coating process (Eser, and Doğruer 2022). The

solution of coating or suspension is sprayed over the top layer of the fluidized powder with several nozzles, forming a shell-like structure. Fluidized bed processes can be applied in 3 different ways: rotary fluidized bed, bottom spray, and top spray. In the food industry, top spraying is preferred over others because the traditional top spraying method gives more effective results. Powder agglomeration facilitates the coating material's solubility and dispersion in fluidized bed coating. Before being sprinkled over the meal, a liquid binder is added by heat treatment to improve material durability. Particle adhesion, aggregation, and drying are the outcomes of this process. Both batch and continuous operations can use fluidized bed coating (Suhag et al. 2020).

2.2.1.2. Effect of Chitosan on Meat Products

Chitosan is one of the edible coatings that have attracted great attention in the food industry due to its biodegradability, non-toxicity, physicochemical properties, and antifungal and antimicrobial activities (Priya, Thirunavookarasu, and Chidanand 2023). Chitosan coatings increase the stability of food by delaying oxidative deterioration and microbial growth by acting as an excellent oxygen barrier. Chitosan contains hypolipidemic and hypoglycemia antioxidant properties, which inhibit the microbial degradation of coated foods (Abdel-Naeem, Zayed, and Mansour 2021). There are many studies on the application of chitosan to meat and meat products, fruits and vegetables, and various foods. Edible coatings are used in meat products to prevent loss, control microbial contamination, and prevent oxidation and undesirable color formation. Many edible biopolymers are used in meat products. Chitosan is used in these types of products because of its good film-formation properties. Table 6 summarizes the publications that have been examined for shelf life, sensory analysis, lipid oxidation, and protein degradation by combining chitosan as a coating material with some plant extracts and essential oils. According to the studies, all of them, except one, were applied using the dipping method. It has been stated that chitosan applied to meat and meat products slows down microbial growth, increases antioxidant activity, contributes to texture and nutritional value, and provides no off-flavor or odor.

Table 6. Studies on the integration of chitosan coating with ingredients including essential oils and other plant-based extracts for meat and meat products

Type of hydrocolloid	Application Method	Applied Product	Results	Reference
Chitosan + duck oil	Dipping (2 min)	Chicken meat	DFC2 (2% chitosan and duck fat) inhibited the development of microbes, lipid oxidation, and protein degradation. Increases the raw chicken meat's shelf life by 15 days.	Shin et al. 2022
Chitosan + rosemary essential oil	Dipping	Rabbit meat	CH (1% chitosan) and REO (0.2% rosemary (Salvia rosmarinus) essential oil) remained acceptable until the 12th day, resulting in considerably lower meat rejection scores in these treated groups than in untreated meat.	El Bayomi, Shata, and Mahmoud 2023
Carrageenan/chitosan/allyl isothiocyanate/mustard extract	Dipping (20 seconds)	Chicken breasts	Inhibition of *C. jejuni*, lactic acid bacteria, and aerobic bacteria growth	Olaimat, Fang, and Holley 2014
Chitosan coatings (0, 1, 2, and 3%)	Dipping (30 seconds)	Harbin red sausage	Inhibiting the pH decline, stabilizing the L* value and water migration, and slowing down the growth of aerobic and lactic acid bacteria are the primary results that an edible coating film containing 2% chitosan helps to improve the storage stability of Harbin red sausage at room temperature.	Dong et al. 2020
Chitosan + lauric arginate (LAE)	Dipping	Chicken drumsticks	Chitosan coating improved the microbiological load, oxidative stability, and sensory properties of chicken drumsticks. This effect was greatly enhanced when combined with LAE.	Abdel-Naeem, Zayed, and Mansour 2021
Chitosan (1.0%, 1.5%, and 2.0%)	Dipping	Chicken fillets	It was determined that chitosan-coated samples, particularly 2% chitosan-coated samples, had an acceptable sensory score and the lowest bacterial count even after 12 days in cold storage.	Eldaly et al. 2018

Table 6. (cont.) Studies on the integration of chitosan coating with ingredients including essential oils and other plant-based extracts for meat and meat products

Chitosan + oregano essential oil	Dipping (1.5 min)	Chicken fillet	Using chitosan and oregano essential oil together under MAP circumstances can suppress the formation of microbial spoilage flora, slow lipid oxidation, retain lightness, and improve the sensory quality of fresh chicken meat.	Petrou et al. 2012
Chitosan with *M. piperita* and *P. amboinicus*	Dipping (5 min)	Chicken breast fillets	As compared to treated samples, chitosan with *P. ambonicus* and *M. piperita* efficiently reduced and regulated pH, exudate loss, lipid oxidation, and microbial load in chicken during storage.	Mariam Johnson et al. 2021
Chitosan + *Elettaria Cardamomum*	Dipping (1 min)	Chicken drumsticks	The shelf-life of the chicken samples was extended by two to three days by coating them with chitosan, and it was extended until the sixteenth day of the trial by adding 1% essential oil of green cardamom.	Khorshidi, Mehdizadeh and Ghorbani 2021
Chitosan + oregano essential oil	Direct coating (edible film)	Chicken fillet	Chitosan film with 2% oregano oil had the best effect on chicken fillet shelf life following 12 days of storage in the refrigerator.	Karimnezhad et al. 2019
Chitosan +Lemon + oregano essential oils	Dipping (1 min)	Broiler breast meat	Up to the ninth day, the treatment groups had significantly lower levels of lactic acid bacteria, Enterobacteriaceae, mold, and yeast than the control group. Overall, the chicken meat with 1% lemon essential oil and chitosan had the best acceptability rate.	Sharafati 2017

2.2.1.3. Chitosan Safety Profile

Chitosan is a natural polymer obtained from deacetylated chitin and is used as an edible film in foods due to its antimicrobial and antifungal properties. In the present day, it is widely utilized to preserve and enhance the high-quality aspects of products. However, since chitosan application comes into direct contact with food, it needs to be approved for safety before commercial use. Therefore, all compounds used in edible coatings must pass stringent safety testing to guarantee that they do not endanger

consumers' health and must be designated "Generally Recognized as Safe" (GRAS) by the American Food and Drug Administration (FDA) for product safety and public acceptability (Matloob et al. 2023). Additionally, compounds like essential oils and plant extracts that are natural should be used carefully and frequently inspected because they may occasionally trigger reactions that are allergic. Edible coatings, as confirmed by research on ecologically friendly packaging, assist in decreasing waste by prolonging food shelf life(Priya, Thirunavookarasu, and Chidanand 2023). There are worldwide standards for the manufacturing and application of these coatings, and coatings that meet these requirements are more commonly utilized in the food industry. Also, when applying edible coatings to food, Goods Manufacturing Practices (GMP) should be followed. The materials used in forming edible coatings should be non-toxic with proper hygienic processing practices; from this point of view, chitosan is suitable for food applications.

The FDA and EFSA (European Food Safety Authority) have classified them into three groups for a thorough knowledge of the nature of chemicals present in food sectors. Food coating materials (FCM) are compounds included in coating formulations such as nanoparticles, antibacterial, anti-browning, antioxidants, and antifungals. Food contact materials (FCA) are the end products of packaging, such as coatings or films, whereas food contact materials (FCS) are the chemicals utilized in their creation (Paidari et al. 2021). Food makers must follow Codex Alimentarius standards when manufacturing packaging materials (Priya, Thirunavookarasu, and Chidanand 2023).

2.3. Bioactive Compounds

Bioactive compounds are substances that are found in the natural world, are part of the chain of food production, and have been shown to have an impact on human health. They can be essential or non-essential (e.g., vitamins or polyphenols). They may contain a variety of components, such as probiotics, plant extracts, vitamins, and minerals (Biesalski et al. 2009). In general, people use these goods to protect themselves from illnesses and lead healthy lives. In recent years have seen a sharp rise in interest in bioactive compounds, particularly as chronic illnesses and health consciousness have grown. These components may have properties such as antioxidant, anti-inflammatory, and anti-cancer. In addition, they have positive effects such as strengthening the immune

system, improving brain functions, reducing inflammation, and protecting heart health. Bioactive components are found in a wide range and are obtained from different food sources. Polyphenols, carotenoids, glucosinolates, and omega-3 fatty acids are naturally found in fruits vegetables, and fish products. Glutathione, taurine, anserine, and other bioactive substances are also present in poultry meat, and proper feeding can raise the amount of most of these substances (Fairoze 2021). In addition, bioactive compounds, in addition to providing good health protection, can have dangerous and even fatal effects. The amount of dose taken by the consumer is of great importance in this regard. There are limits specified in the regulations for this. The manufacturer must produce food consisting of bioactive components in accordance with these limits.

Essential oils and plant extracts are utilized as natural food preservatives to drastically lower spoilage. Essential oils are a combination of volatile components that are hydrophobic in nature. They permeate the fatty tissue of the cell membrane, increasing the production of cellular components and eventually eliminating bacteria (Naseri et al. 2020). These natural components create an effective barrier against various microorganisms found in foods. These extracts can contribute to food safety by extending the shelf life of perishable products, especially meat and meat products, fresh fruits and vegetables. For example, oils derived from sage, thyme, rosemary, and oregano effectively suppress gram-positive bacterial activity. The thymol carvacrol, p-cymene, and terpinene, all of which are found in Thymus species, have been used for this purpose since they include phenolic substances or various hydrophobic constituents (Tohidi, Rahimmalek, and Arzani 2017).

The use of bioactive ingredients in combination with edible coatings is a very promising area in the food industry. Adding essential oils directly to food can result in drastic flavor changes and changes to the food's appearance, these drawbacks are eliminated when the essential oil is used in conjunction with edible films (Nunes et al. 2023). Recently, functional foods with antimicrobial and antioxidant properties have been created by incorporating essential oils into other coatings such as polysaccharide-based coatings, which enhance the sensory palatability of food. However, the edible coating must be specially created to fulfill the requirements of the food product since including bioactive ingredients in the coating material may cause the coating to lose its essential functional qualities (Nunes et al. 2023).

Natural antimicrobial agents of plant-derived (essential oils, plant extracts), animal-origin (enzyme, protein), and microbial-origin (yeast, lactic acid bacteria,

microbial products) are used in edible coatings for food. Many studies in the literature show that this natural method is safer and more effective than chemical preservatives. In the study conducted on meat and fish, it was reported that samples coated with chitosan together with essential oils had a longer shelf life than samples coated with pure chitosan or uncoated (Yuan, Chen, and Li 2016). Karam et al. (2019), who examined the effects of carvacrol, thymol, and vacuum packaging on marinated chicken in terms of antimicrobial properties, reported that the shelf life of marinated chicken was extended by 6 days, and it was sensorially more appreciated than normal chicken meat. It was obtained as a result of the study conducted by integrating tomato plant extract into chitosan-based edible coatings that contributed to the shelf life and microbiological safety of chicken while maintaining some quality criteria at refrigeration temperature (Ruíz-Cruz et al. 2019). In the study conducted by Alvarez, Ponce, and Moreira (2013), it was reported that the antimicrobial effect of chitosan and bioactive compounds in essential oils inhibited the growth of mesophilic and psychotropic bacteria in broccoli, obtaining positive outcomes in their sensory properties and also synergistically controlled the survival of *E. coli* and *L. monocytogenes.*

2.3.1. Antimicrobial and Antioxidant Properties of Allicin

For many years, a significant portion of the food industry has used chemical preservatives to prevent food spoilage and contamination by microorganisms. However, due to consumer demand for natural products and the adverse health effects of chemical preservatives, safer and more natural alternatives are being sought. Plant-based antimicrobials, which can extend food shelf life by inhibiting microbial growth and lipid peroxidation, have recently gained importance(Salehi et al. 2019). The effects and application techniques of natural chemicals derived from plants, spices, and essential oils in food systems are the subject of much study in this field. Allicin is among these antimicrobials.

Allicin, a sulfur-containing molecule, is the primary cause of garlic's intense odor and flavor. One clove of fresh garlic has 4–5 mg of allicin, which is identifiable by its distinct smell (Horev-Azaria et al. 2009). Allicin, found in many Allium species, including wild garlic, field garlic, elephant garlic, and white garlic, is one of the most

prevalent organosulfur compounds produced by these plants. Initially, absent in intact garlic cloves, allicin is formed upon crushing or maceration. The precursor, alliin, a stable and odorless amino acid, reacts with the enzyme alliinase to produce allicin, pyruvate, and ammonia. This reaction occurs when the compartments containing alliin and alliinase are disturbed and come into interaction (Salehi et al. 2019). Allicin's antibacterial capabilities are mostly owing to its suppression impact on specific thiol-containing enzymes, while its antioxidant characteristics are explained by its suppression of hydroxyl and superoxide radicals (Rahman 2007).

In addition to antioxidant and antibacterial effects, allicin has been demonstrated to be anti-inflammatory, antihypertensive, antifungal, and anticancer (Marchese et al. 2016). Allicin is high in vitamins A, C, and E, as well as beta-carotene and other essential minerals, which can improve the nutritional value and organoleptic quality of beef and chicken (Balogun, Sobande, and Oyeyinka 2023). The most effective plant species for treating bacterial illnesses including headaches, fever, bites, intestinal worms, cholera, and dysentery has been allicin since ancient times. Its antiviral, antifungal, antibacterial, immunostimulating, antioxidant, and cholesterol-lowering effects have all been validated by recent laboratory studies (Dziri et al. 2012).

The impact of allicin on intestinal bacteria was investigated in vitro, and it was shown that garlic effectively inhibited the development of some bacteria(Rahman 2007). According to Sharma et al. (1977), *Allium sativum* water extracts have antibacterial effects since they strongly suppress the formation of a gram (-) and gram (+) flora in chickens' digestive systems. According to another study, a variety of microorganisms including multidrug-resistant types with bactericidal or bacteriostatic properties, were inhibited in their growth by extract from Allium sativum (Magryś, Olender, and Tchórzewska 2021).

As a result of the study on the antimicrobial antioxidant activities of pink garlic, it was found that the antibacterial effect was on the bacterial species *Enterococcus feacium* and the extract obtained from the garlic stem had the greatest effect in inactivating *Bacillus subtilis*, *Enterococcus feacium, Escherichia coli* and *Staphylococcus aureus* (Dziri et al. 2012). Furthermore, allicin has significant antioxidant properties and inhibits several diseases, including viruses, bacteria, fungi, and protozoa. *Aspergillus niger, Escherichia coli, Staphylococcus, Mycobacterium tuberculosis, Bacillus, Proteus, Streptococcus, Clostridium, Salmonella, Penicillium notatum*, and

Saccharomyces cerevisiae are just a few of the many bacteria and fungi that it is very efficient against (Wang et al. 2018).

CHAPTER 3

MATERIALS & METHODS

3.1. Experimental Design

The study was designed to examine the shelf life studies, and chemical, microbiological, and sensory analyses of chicken drumsticks by dipping them in chitosan solution and adding allicin. Two different sample groups were studied. Firstly, three different sample groups were studied: the control group, 1%, and 2% chitosan-coated chicken meat on days 0, 2, 4, 6, 8, and 12. Secondly, four different combinations of allicin with chitosan which are K1A1(1% Chitosan + 10% Allicin), K1A2(1% Chitosan + 20% Allicin), K2A1(2% Chitosan + 10% Allicin), K2A2(2% Chitosan + 20% Allicin) and the control group were studied on days 0, 2, 4, 6, 8, 9 and 10.

3.2. Preparation and Application of Chitosan Coating Solution and Allicin Solution to Chicken Drumsticks

Chitosan edible coating solutions were prepared according to a study reported in the literature (Johnson et al. 2021). 100 ml of 1% acetic acid (Merck, Darmstadt, Germany) was used to dissolve 2% chitosan (90–95% deacetylation degree). 1% glycerol (Difco, Becton, Dickinson, and Company Sparks, USA) was added to the solution to give its plasticizing effect.1% chitosan solution was prepared similarly, and the solutions were dissolved using a magnetic stirrer for 24 hours until they became homogeneous.

Allicin solutions were prepared at 10% and 20% with pure water. With the dipping method, chicken drumsticks were first immersed in the allicin solutions for 3 minutes and then allowed to dry. After that again with the dipping method, chicken drumsticks were immersed in chitosan solution for 3 minutes and then allowed to dry.

3.3. Chemical Analyses

3.3.1. pH Value Determination

The AOAC Official Method's protocols were used to calculate pH. In a stomacher device, 50 mL of distilled water was combined with 10 g of chicken sample. A standardized pH probe meter (Mettler Toledo, Turkey) was used to measure the pH readings.

3.3.2. Chemical Composition Analyses

Chemical composition analysis is an analytical method that determines the types and quantities of elements, compounds, or chemicals found in a sample. In this study, chemical composition analysis includes moisture value, protein values, total ash values, and total fat value.

3.3.2.1. Moisture Analyses

The moisture was calculated using the procedures provided by the AOAC 950.46 (Soderberg, n.d.). Chicken samples are homogenized with the help of a homogenizer. The moisture-weighing container is cleaned and dried in the oven. After cooling to room temperature, the dried container is weighed in a desiccator. 2 grams of the sample is weighed with 1 mg sensitivity. The weighed sample is placed in a moisture-weighing container. It is kept in the oven at 130 °C for 4 hours until a constant weight is reached. The container is taken out of the oven at the end of the time and weighed when it's completely cooled.

3.3.2.2. Protein Analyses

The protein content was calculated using the procedures provided by the AOAC 981.10 with the Kjeldahl method (German 2021a). 0.5 grams of chicken samples are properly weighed and placed in tubes. Kjeldahl catalysts are introduced into the glass tube. 15 mL of 98% sulfuric acid is added to the tube. The tubes are placed in the nitrogen-protein determination device's wet combustion unit, which has been warmed to 420 °C, together with the carrying case. They are held at this temperature for 120 minutes. The tubes are hung on the device's hanger until the gases in the tubes have been entirely extracted. After the tubes have completely cooled, the distillation step begins. 30 mL of 4% boric acid is introduced to the 250 mL Erlenmeyer flask. The Erlenmeyer flask is put within the distillation unit. The device automatically takes pure water and a 40% NaOH solution. The distillation process continues for a further 5 minutes. The Erlenmeyer flask is removed from the distillation equipment. An acid titration with 0.1 N HCL is done. The process continues until the color of green in the Erlenmeyer flask becomes pinkish.

3.3.2.3. Total Ash Analyses

The AOAC 920.153 techniques were used to determine the total ash content (Latimer 2023). Firstly, porcelain crucibles were weighed. 2 grams of the chicken samples were weighed. The ash furnace is filled with it and heated to 600 °C. It is kept in the ash furnace for approximately 4 hours. After that, the ash furnace is closed. Following their cooling in the desiccator to room temperature, the crucibles exiting the ash furnace are weighed again.

3.3.2.4. Total Fat Analyses

The total fat was calculated using the procedures provided by the AOAC 960.39 (German 2021b). Chicken samples are homogenized. Approximately 1 to 1.5 grams of

the sample is weighed on filter paper and dried in the oven for approximately 30-45 minutes. The oil paper is placed in the cartridges and then in the extraction device. 100 ml of petroleum gasoline is added to each of the weighed aluminum oil containers. The device is turned on. After the extraction process is completed, the aluminum oil containers are removed from the device and kept in the oven at 130 °C for 30-45 minutes.

3.4. Thiobarbituric Acid Reactive Substance (TBARS) Analysis

With few changes, the extraction technique outlined by Selani et al. (2011) was used to calculate the TBARS values. 20% acetic acid solution was mixed with 0.72 grams of TBA (2-Thiobarbituric acid) (Merck, Darmstadt, Germany) and dissolved in a water bath for 1 hour to make it homogeneous and stored in the dark. 200 grams of TCA (Trichloroacetic acid) (Merck, Darmstadt, Germany) was homogeneous with 1 L of pure water. 5 grams of chicken samples were weighed, and 50 ml of TCA and 50 ml of pure water were mixed with a homogenizer. The resulting solution was passed through filter paper. 5 ml of the resulting solution and TBA solution were taken and transferred to test tubes. After leaving it in the water bath for 35 minutes at 80 °C, the absorbance value was measured at 538 nm in a UV spectrophotometer. TBARS values of samples are calculated by multiplying the measured absorbance value with the conversion factor which is 7.8.

3.5. Microbiological Analyses

All microbiological analyses except Pseudomonas were performed using the Tempo (Biomerieux, Türkiye) device. It is performed on the Tempo device, one of the leading devices in the industry, in determining a wide range of bacteria such as total aerobic mesophilic bacteria count, E. coli count, Total Coliform count, EMS Coliform Count, Staphylococcus count, Enterobacteriaceae family count, yeast & mold count and lactic acid bacteria count. All tests provide results within 72 hours at the latest.

3.5.1. Total Viable Count

As mentioned in Section 3.4, total plate counts were made with the Tempo device. 10 mL/g sample is diluted using Tryptone salt broth (Peptone water) with a 1/10 dilution setting. Take a medium bottle from the Tempo AC Kit and add 3 ml of sterile pure water into the bottle with the help of a dispenser. 1.0 mL of the 1:10 sample suspension was taken and transferred to a glass bottle containing medium to which 3.0 mL sterile distilled water was added and mixed in a vortex mixer for 10 seconds. (Paulsen et al., 2008). The cards were then incubated for 22-28 hours at 30°C for total organisms. At the end of the incubation, the system determined the number of microorganisms by reading the cards placed in the device. The system performs calculations using statistical methods and gives results in colony-forming units/g (CFU/g) (Owen, Willis & Lamph, 2010).

3.5.2. Yeast & Mold Count

In yeast and mold count same procedure is in Section 3.4.1. was done until the incubation. Another material that was different from the Total plate count was the kit used, the Tempo YM Kit. The incubation time and temperature for yeast and mold were set to 72-76 hours at 25°C, and the results were again given as CFU/g (Yahyaoğlu,2019).

3.5.3. *Enterobacteriaceae* Count

In Enterobacteriaceae count same procedure in Section 3.4.1. was done until the incubation. Another material that was different from the Total plate count was the kit used, the Tempo EB Kit. The incubation time and temperature for *Enterobacteriaceae* were set to 22-27 hours at 35°C, and the results were again given as CFU/g (Yahyaoğlu,2019).

3.5.4 *Pseudomonas* Count

For the Pseudomonas analysis, 90 ml of 0.1% peptone water was added to a stomacher bag containing 10 g of material, and the mixture was homogenized for one minute. Appropriate decimal dilutions were prepared and inoculated on a cetrimide agar medium (Biomerieux, Türkiye). Petri dishes, on which 1 ml sample was taken and inoculated, were incubated at 37 °C for 48 hours.

3.6. Color Measurements

Instrumental color values were measured on the surface of chicken drumsticks for each chicken immediately after opening the package. The instrumental color (CIELAB, lightness, L*; redness, a*; yellowness, b*) of chicken drumsticks was measured using a calorimeter (Lovibond, Türkiye).

3.7. Sensorial Evaluation

Sensory analyses were conducted with 30 panelists in standard-size tasting booths at 20–22°C. Sensory testing was performed separately for 1% chitosan, 2% chitosan, and control groups, and separately for K1A1, K1A2, K2A1, K2A2, and control groups. The samples were coded with independent 3-digit numbers and prepared for the panelists to taste. Before and after tasting, the panelists were told to rinse their mouths with water to get rid of any tastes that could have lingered from the sample they had just tasted. The internal temperature of the chicken drumsticks reached 72°C after 24 minutes of baking at 220°C. Samples were served independently on plates encoded with three-digit random numbers. Thirty regular consumers rated chicken drumsticks' flavor, color, texture, and odor using a 5-point hedonic scale (1 = highly dislike, 5 = extremely like) (Passos et al., 2022).

3.8. Statistical Analysis

Excel was used to compute the averages and standard deviations of all the data that was collected. Analysis of variance (ANOVA) with general linear fit model and also one-way ANOVA was used to analyze the outputs using Minitab (Version 18 Statistical Software). Variables are compared using the Tukey test ($\alpha = 005$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Chemical Analyses

Chemical properties of chicken drumsticks coated with chitosan and allicin combinations, no chitosan coating, and only chitosan was characterized by pH, moisture, fat, ash, TBARS, and protein analysis.

4.1.1. pH Value

pH is one of the important quality parameters for raw meat and meat products.The pH value is an important characteristic that influences the quality, microbiological stability, and shelf life of food goods. For chicken flesh, pH is critical in affecting both physical and chemical qualities. The pH of fresh chicken drumstick meat typically ranges between 62 and 6.5. This range influences the sensory qualities of meat, including flavor, texture, and color. pH control was performed on chicken drumsticks coated with combinations of chitosan and allicin, without chitosan coating, and coated with chitosan only, throughout their shelf life. Figure 3 compares pH values between sample groups coated with chitosan at different concentrations and those without coating during the 0. ,2. ,4. ,6. ,8. and 12th days of cold storage. pH values of the control samples were higher than the samples coated with chitosan. The pH scale of the control group fluctuated between 6.51 and 6.55. The pH values of the samples coated with 1% chitosan were found to be higher than the samples coated with 2% chitosan, except for the 2nd, 6th and 12th days. It is thought that this fluctuation in the results obtained in pH values during the shelf life is due to the buffering properties of meat and meat products.

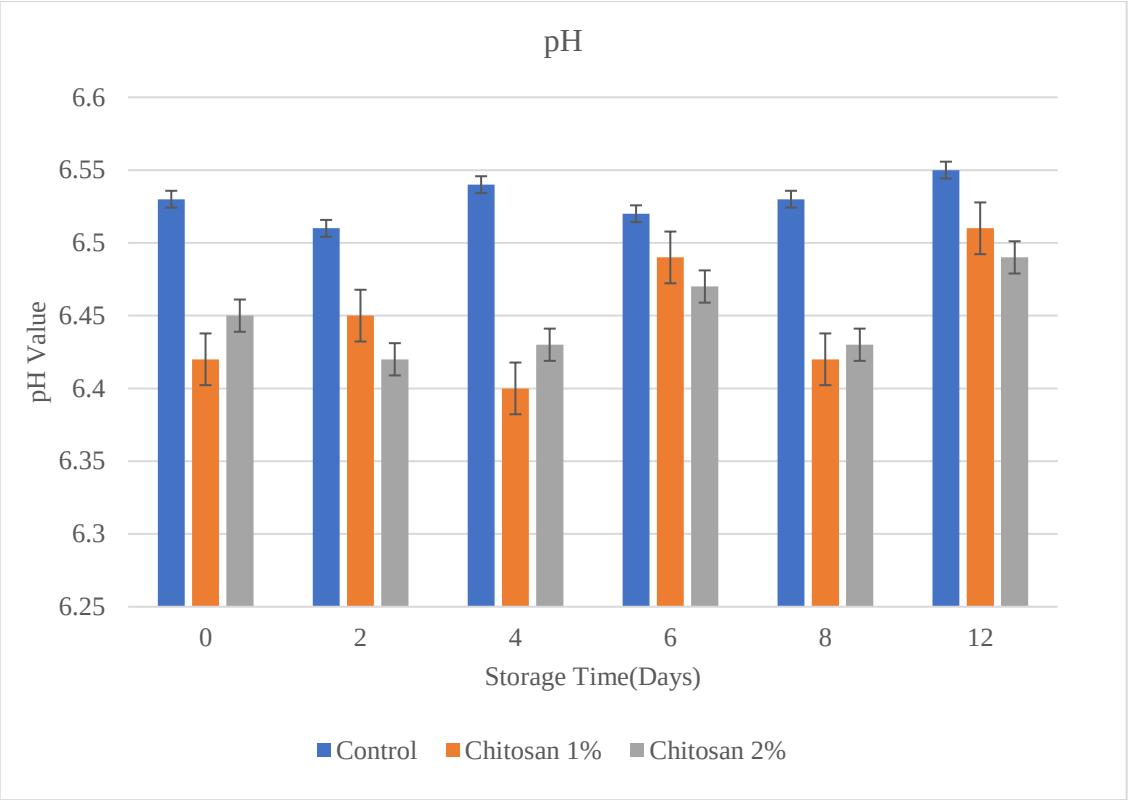

Figure 3. pH values of chicken drumsticks with different chitosan solutions

In Figure 4, the pH values of chitosan and allicin combinations and the control group during cold storage on days 0, 2, 4, 6, 8, 9, and 10 are given. Again, the control group gave results in a higher pH range than the other samples. On day 4, a significant pH decrease occurred from 6.48 to 6.23 in the K1A1 treatment. On day 10 of the shelf life, K1A1 gave the lowest pH value as 6.35, while the control and K1A2 samples gave the highest pH value as 6.55. When the results are examined in general, there are fluctuations in pH values throughout the shelf life of the samples. One reason for these fluctuations may be due to the antioxidant and antibacterial activity of allicin and chitosan. At the same time, chitosan, which adheres to the surface of chicken drumsticks, tries to balance the pH, and it can be said that it changes depending on the relationship that bacteria form with the chitosan coating. The higher pH is connected with the generation of nitrogenated basic chemicals such as ammonia and amines produced by the degradation of proteins under the action of proteolytic enzymes such as lipase or protease released by microorganisms, endogenous and microbial enzymes (Manju et al. 2007; Triki et al. 2018).

In the study investigating the effect of the combination of extracts and essential oils of the Elettaria Cardamomum plant with chitosan coatings on the shelf life of chicken drumsticks, a smaller decrease in pH was observed on the 12th day in the application containing chitosan coating compared to the sample groups applied with other combinations (Khorshidi, Mehdizadeh, and Ghorbani 2021). In addition, the fluctuations and increases in pH values during the shelf life in this study show that similar results were obtained with the study conducted. It has been reported that there is a decrease and increase in pH values during the shelf life of chitosan in combination with thyme essential oil obtained from chicken breast (Zheng et al. 2023), chitosan films formulated with nano-liposomal garlic essential oil obtained from chicken breast fillets (Kamkar et al., 2021), and chitosan-gelatin coating containing nano-encapsulated tarragon essential oil obtained from pork slices (Zhang et al., 2020).

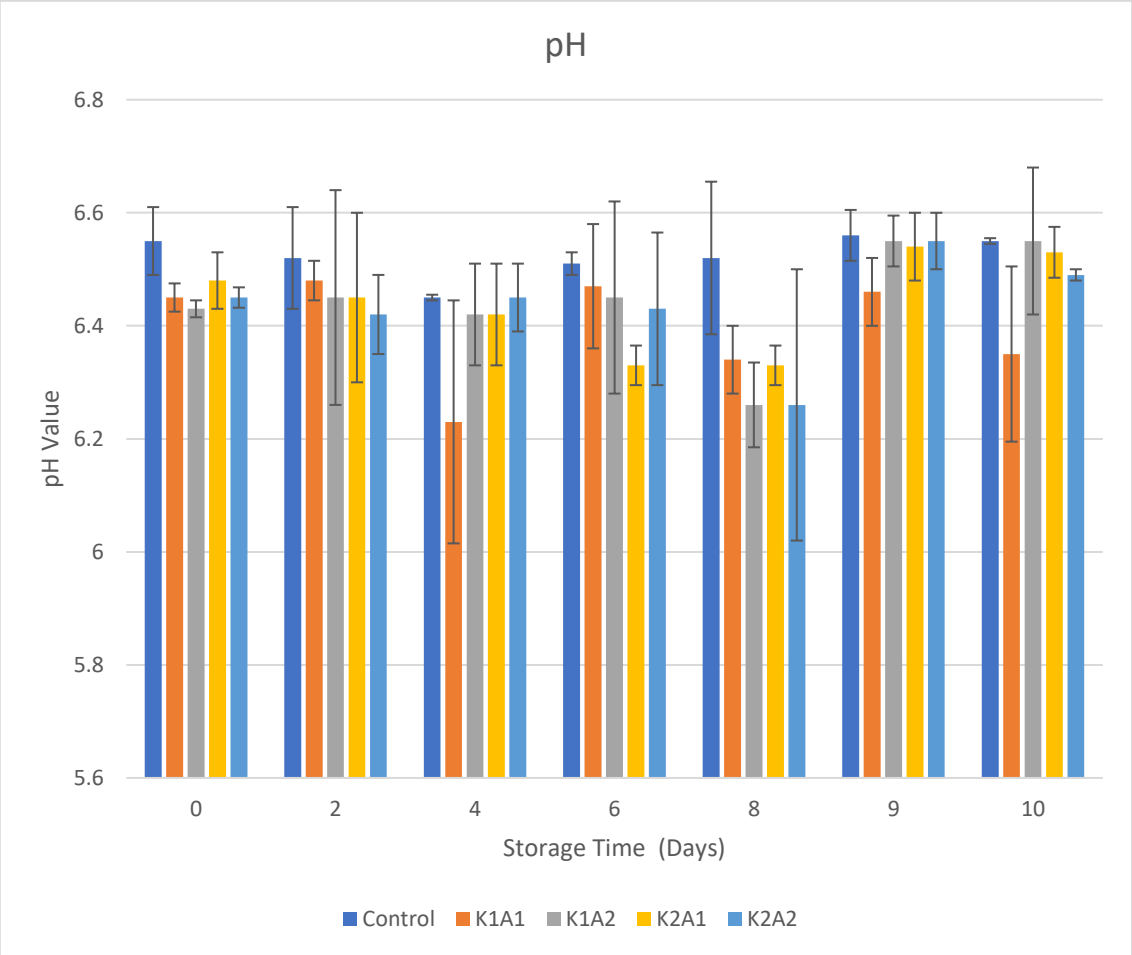

Figure 4. pH results of chicken drumsticks with a combination of chitosan solutions and allicin

4.1.2. Chemical Composition Analyses

The quality of meat depends on changes in its chemical components. The chemical components of meat vary depending on differences such as animal type, age, breed, gender, feed, and body weight, and the quality of meat also changes accordingly (Elsharawy, Ahmad, and Abdelrahman 2018). Chemical composition analyses include moisture, total fat, total protein and total ash.

The overall quality of meat and poultry products is significantly impacted by the amount of moisture they contain. Moisture levels directly influence the texture, flavor, and shelf life of these products. Excessive moisture can lead to spoilage, while insufficient moisture results in a dry and tough product. Chemical composition of chicken drumsticks with different chitosan solutions was given in Table 7. When the moisture results were compared, the value of the sample coated with 1% chitosan was lower than the others, but there was still no significant difference between them. The chemical composition of chicken drumsticks containing the combination of chitosan solutions and allicin is given in Table 8. While the K1A2 and control groups gave similar values in terms of moisture values, no significant difference was observed among the remaining chitosan-allicin combinations.

An essential criterion for meat quality categorization and labeling is protein analysis. One of the most significant nutritional components of chicken meat is protein, which serves as the fundamental building block of muscular development. The high protein content of chicken meat supplies the amino acids required for immune system enhancement, muscular growth, and tissue repair. Results in Table 7 showed that when the protein values in chicken drumsticks were compared, it was observed that the 1% chitosan sample gave the highest value. No significant difference was observed between the other samples. The protein values of the combination of chitosan solutions and allicin were found to be around 18% and no significant difference was observed between the values. Considering this result, it can be concluded that chitosan and allicin did not have a negative or positive effect on the protein value of chicken meat. While the lowest value belongs to sample K1A1, the highest value belongs to sample K2A2.

Table 7. Chemical composition of chicken drumsticks with different chitosan solutions(%)[a]

Treatments	Moisture(%)	Fat(%)	Protein (%)	Ash(%)
Control	77.05±0.007^{A}	4.18±0.071^{A}	17.28±0.042^{B}	0.96±0.014^{A}
1% Chitosan	76.41±0.012^{B}	4.08±0.000^{A}	18.19±0.021^{A}	0.85±0.021^{B}
2% Chitosan	77.26± 0.071^{A}	3.58±0.056^{B}	17.16±0.078^{B}	0.86±0.014^{B}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. Each chemical analysis is grouped within itself.

Another essential ingredient that affects meat's texture and energy content is fat. Meat's taste, softness, and juiciness are all impacted by its fat level. However, the kind of fat (monounsaturated, saturated, and polyunsaturated fatty acids) also matter for healthy living. In the total fat analysis results performed in this study, it was found that the sample coated with 2% chitosan gave the lowest value, but there was no significant difference between the other two samples (Table 7). While the K1A2 and K2A2 sample groups gave similar values in fat values, differences were observed among themselves in the remaining chitosan-allicin combinations (Table 8). It was discovered that the K2A1 sample group had the lowest fat level (5.36%), whereas the control group had the highest fat content (6.20%).

The residue left over after the burning of inorganic (mineral) materials in meat is called ash. Ash analysis reveals information on the mineral content of meat. Minerals like calcium, phosphorus, and iron are essential for biological activities including bone health, blood generation, and energy production. High ash content implies that the meat is mineral-rich. In the ash analysis results performed in this study, it was found that the highest value was in the control sample, however, the other two samples did not differ significantly (Table 7). According to Table 8., in ash values, K2A1 and K2A2 sample groups gave similar values, and higher results were obtained compared to other samples. It was observed that keeping the chitosan ratio high had a good effect on chicken meat in terms of retaining minerals.

Table 8. Chemical composition of chicken drumsticks with combination of chitosan solutions and allicin (%)[a]

Treatments	Moisture(%)	Fat(%)	Protein (%)	Ash(%)
Control	72.93±0.137^{C}	6.20±0.070^{A}	18.41±0.488^{A}	0.86±0.011^{B}
K1A1	74.14±0.142^{AB}	5.69±0.078^{B}	18.28±0.246^{A}	0.85±0.015^{B}
K1A2	73.93±0.109^{B}	5.59±0.088^{B}	18.55±0.070^{A}	0.84±0.011^{B}
K2A1	74.92±0.568^{A}	5.36±0.206^{B}	18.38±0.073^{A}	0.94±0.021^{A}
K2A2	74.69±0.340^{AB}	5.53±0.178^{B}	18.81±0.157^{A}	0.92±0.010^{A}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. Each chemical analysis is grouped within itself. C= Control, K1A1= 1% chitosan+10% Allicin, K1A2= 1% chitosan+20% Allicin, K2A1= 2% chitosan+10% Allicin, K2A2= 2% chitosan+20% Allicin

In a study on the addition of 0.5% and 1% green tea extract to chicken patties, moisture values were found to be around 71%, fat values were around 7.8-7.9, ash values were between 1.12 and 1.65, and protein values were around 16% (Passos et al. 2022). It is seen that the chemical composition results of the study are different from each other, but this is normal since the gender of the chicken, the feed it is fed, and production conditions may differ.

4.1.3. Thiobarbituric Acid Reactive Substance (TBARS) Analysis

Lipid oxidation reduces meat's sensory, functional, and nutritional qualities. Malondialdehyde is the second and most major byproduct of lipid oxidation, and it indicates the degree of oxidation and rancidity. The amount of malondialdehyde in meat and the level of lipid oxidation are frequently estimated using the TBARS value (Khorshidi, Mehdizadeh, and Ghorbani 2021). Figure 5 shows the findings of studies done on chicken drumstick samples about the quantity of TBA value when they were stored at 4 °C in a refrigerator. Analysis was conducted on days 0, 3,5, 7, 10, and 17 at 4 °C storage conditions. The TBA amount in the control sample kept at 4 °C for 17 days showed an increasing trend except for the 3rd day. There was no significant increase or

decrease in all sample groups between the 7th and 10th days. During the shelf-life analysis of 1% chitosan coated chicken drumsticks, the TBA value was always found to be lower than the other sample groups. It was concluded that the most effective rate in retardation lipid oxidation was 1% chitosan; the TBA value on the 0th day of the study (0.041±0.008) reached a significant amount (1.43±0.022) on the 17th day of the study.

Up until day 17, there was an increase-decrease trend between all treatments; the K1A2 sample group had the largest TBA quantity (0.98±0.004) on day 0, while the 1% chitosan group had the lowest amount (0.041±0.008). It is thought that the formation of secondary oxidation by-products that do not react with the TBA reagent, the Maillard reaction between malondialdehyde and proteins, and the breakdown of malondialdehyde and other TBARS forms by chemical-based and microbial-based sources that cause the chicken to degenerate are the causes of the decrease in TBA level between the third and fifth days of storage. This decrease was similar to the results reported by Chouliara et al. (2008) study on the combined effect of irradiation and modified atmosphere packaging on chicken meat breast and the study conducted by Khorshidi, Mehdizadeh, and Ghorbani (2021), who examined the effect of *Elettaria Cardamomum* and chitosan-coated chicken drumsticks on the shelf life. These fluctuations in TBA values have been observed in many studies in the literature (Matiacevich, Acevedo, and López 2015; Passos et al. 2022; Aşik and Candoğan 2014).

When the TBA results of the K2A2 sample group are examined, a general increase is always observed after day 0 compared to the results of the other sample groups. The reason for this may be the prooxidant activity of allicin in the chitosan coating, causing possible changes in its permeability. These changes may increase oxygen transmission from the edible coating as a result of the intermolecular interactions of the produced structural matrix and increase lipid oxidation (Aşik and Candoğan 2014). Both peanut shell and pink pepper residue extracts were found to be as efficient as butylated hydroxytoluene in maintaining the oxidative stability of chicken products in terms of peroxide value and TBARS substances when compared to the control in the analysis of chicken products with chitosan films containing natural antioxidants(Serrano-León et al. 2018) .

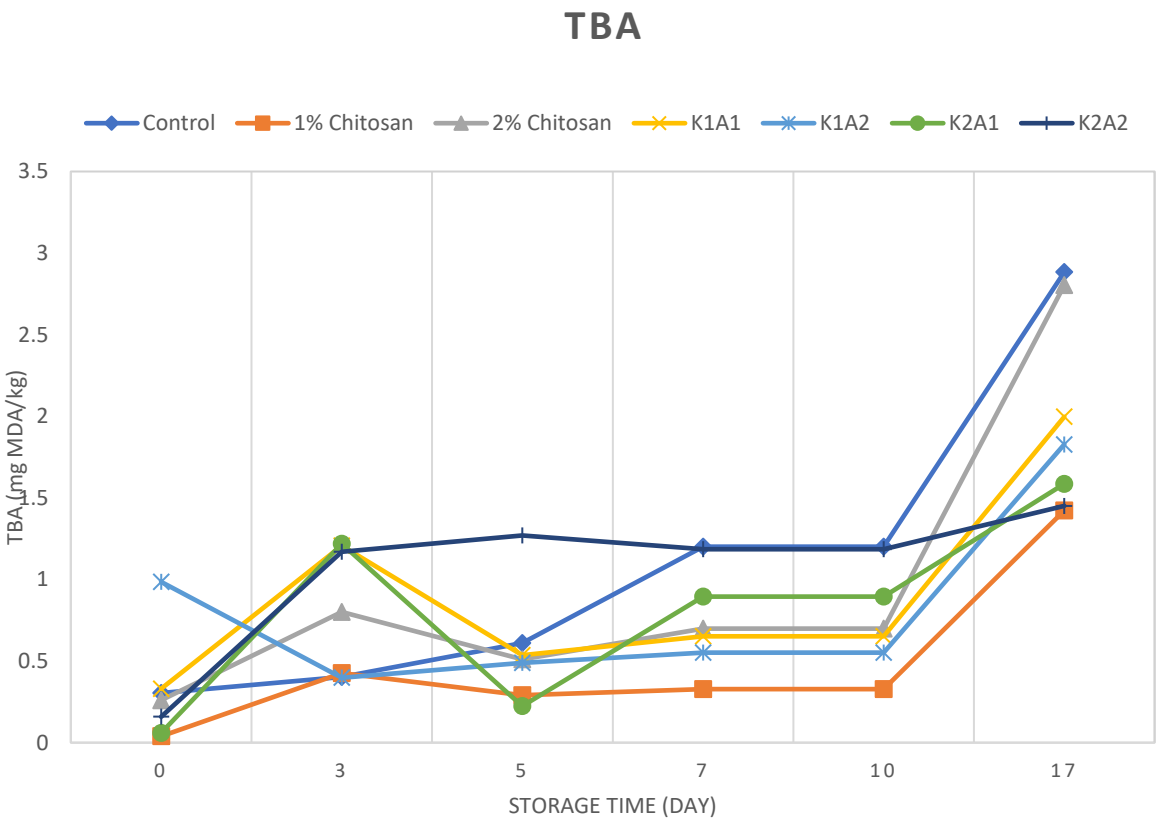

Figure 5. TBA analysis results of chitosan coated, chitosan+allisin and no-coated chicken drumsticks

4.2. Microbiological Analyses Results

The microbiological quality of meat is very important for consumer health. However, it can be exposed to various microbiological contamination risks during production, processing and storage processes. Cases of food poisoning and death due to meat consumption are seen worldwide (Elsharawy, Ahmad, and Abdelrahman 2018). In chicken meat, carelessness during cold storage from production to market shelves also becomes risky and affects the shelf life of chicken meat. In this study, total viable, mold&yeast, *Pseudomonas*, and *Enterobacteriacea* counts were made in sample groups containing chitosan concentrations in chicken drumsticks and different combinations with allicin added to these concentrations during the shelf life of refrigerated storage. Microbiologic analysis was performed at day 0, 2, 4, 6, 8, and 12 for different chitosan

solutions and at days 0, 2, 4, 6, 8, 9, and 10 for combination of chitosan solutions and allicin.

4.2.1. Total Viable Count

Results of the total viable bacteria growth of the chicken drumsticks with different chitosan solutions are presented in Figure 6. Since there was deterioration in the control sample, it was not included in the analysis on the 12th day. The initial value of total viable count (day 0) for fresh chicken meat was found to be 4.6 log CFU/gr, indicating that the meat was of reasonably good quality. It is seen that 2% chitosan results give the best results in the first 6 days compared to the other two groups. On the 8th day, there was an unexpected decrease in 1% chitosan compared to the previous analysis day. It is seen that there is no significant difference between 1% and 2% chitosan on the shelf life of 12th day. The total viable bacteria normally observed on day 0 in chicken drumsticks started to give similar results in chitosan coated samples from the 6th day of shelf life. It has been proven that chitosan coating delays microbial growth by acting as a moisture barrier. This finding is reinforced by the fact that chitosan has antibacterial properties by adhering to the charge of the negative bacterial cell wall and breaking the cell membrane (Pabast et al. 2018). Chicken breast fillets coated with chitosan or chitosan combined with oregano essential oil effectively inhibited microbiological growth on the surface of chilled chicken breasts during 12 days, supported by total viable count results (Zheng et al. 2023) A study of rainbow trout coated with chitosan stated that for 16 days, total aerobic plate counts were reduced by 1.64 logs compared to the control at the end of storage (Ojagh et al. 2010).These researches shows the positive effects of chitosan on meat and meat products. Some results in the literature varied slightly due to chitosan's propensity to chelate metals in the presence of bacteria, which prevents the transfer of critical nutrients and limits bacterial development. pH, targeted bacteria, exposure duration, degree of acetylation, and chitosan's positive charge all have an impact on its antimicrobial efficacy (Nunes et al. 2023).

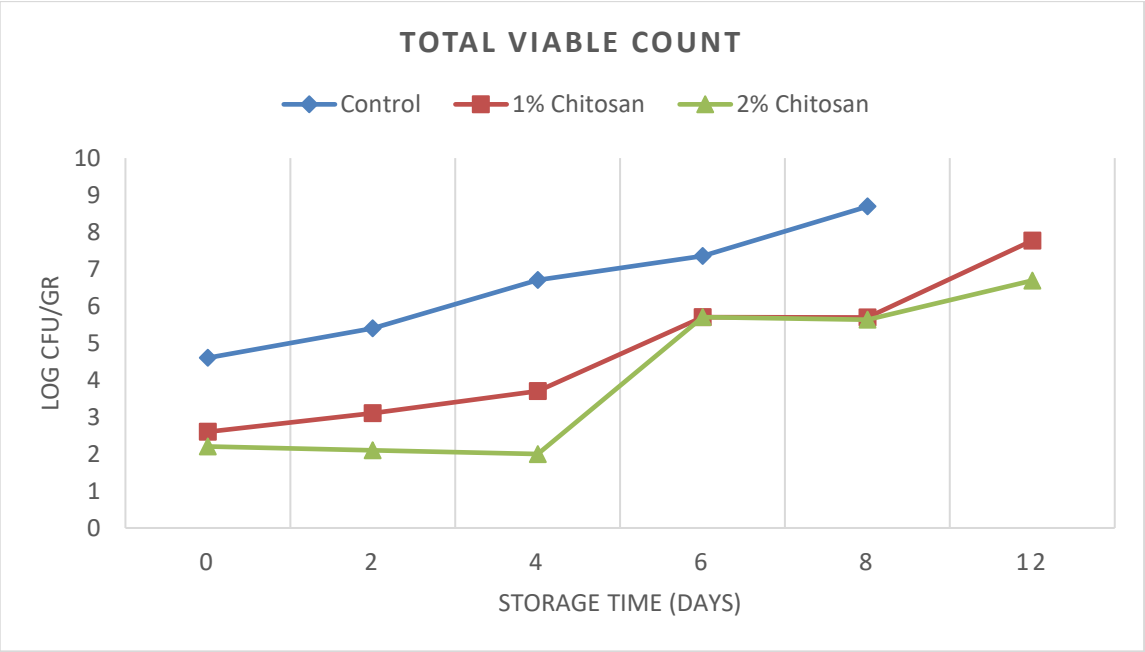

Figure 6. Total viable bacteria count results of chicken drumsticks with different chitosan solutions

The results of total viable bacteria count of chicken drumsticks with the combination of chitosan solutions and allicin are given in Figure 7. After 10 days of storage, the highest microbial growth occurred in the control sample. From the 6th to the 8th day, the logarithmic increase in the K2A2 and K1A1 groups proportionally increased, and a logarithmic decrease occurred in the other treatment groups proportionally. On the 6th, K1A1-treated chicken drumsticks had the lowest total viable bacteria count (5.43 log CFU/gr). However, on the last day of storage, the K1A1 and control groups had the highest microbial growth and there was no significant difference between the other treatment groups. It was determined that chitosan coating and allicin could successfully limit microbiological development in chicken drumsticks for two additional days. Other formulations than the K1A1 sample can be evaluated in chicken drumstick coatings in the future.

When the effects of the treatment of chicken products with a solution containing the combination of chitosan and natural antioxidants obtained from some plant extracts on total aerobic living populations were examined; it was concluded that the chicken samples coated with chitosan with pink pepper residue extract and peanut shell extract showed the lowest microbial growth at the end of the 7th day. Chitosan active films containing residue extracts have been shown to retain the quality of chicken products

because of their antioxidant and antibacterial capabilities (Serrano-León et al. 2018). In the study investigating the antimicrobial properties of onion and garlic extracts in beef and chicken samples, it was proven that onion and garlic extract significantly reduced the total viable population, showing lower numbers than control meat samples in each storage period (Balogun, Sobande, and Oyeyinka 2023). The lower total viable count observed in chicken drumstick samples treated with allicin and chitosan may be due to the antimicrobial effects of garlic extracts, which can be attributed to their active principles such as diallyl sulfide and diallyl disulfide (Maria Nuutila et al. 2003). Karam et.al (2019) reported that the use of active essential oils ingredients, in combination with vacuum packaging, reduced the growth of spoilage microbiota by approximately 2.9-3.1 log CFU/gr, extending the duration of marinated chicken by more than 6 days according to total viable count data.

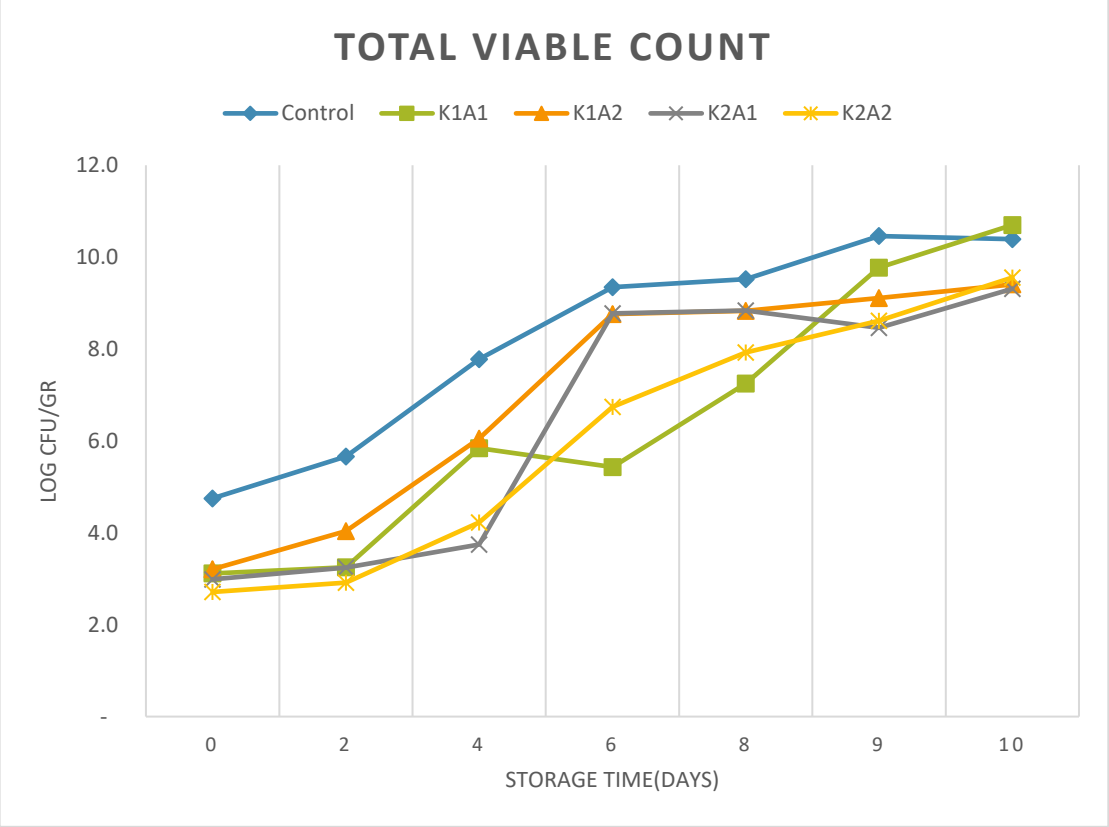

Figure 7. Total viable bacteria count results of chicken drumsticks with combination of chitosan solutions and allicin

4.2.2.Yeast & Mold Count

Yeast and mold count results of chicken drumsticks with different chitosan solutions (Figure 8), in all treatments, there was an increase from the beginning to the last day of storage. No significant difference was observed between the two chitosan coated samples on the 2nd day of storage. On the 8th day, there was an approximately 3 log decrease in both chitosan-coated samples compared to the control sample, and the results were found to be 6.16 log CFU/gr, 3.35 log CFU/gr, 3.45 log CFU/gr, respectively. On the 12th and final day of storage, there was no significant change between the two chitosan-coated chicken samples. Chitosan's antibacterial activity varies depending on the kind of microbe; it works best against bacteria, but it can also work against certain molds and yeast (Yuan, Chen, and Li 2016b). It is interpreted that this approximately 3 log decrease observed in the analysis results is due to this activity of chitosan.

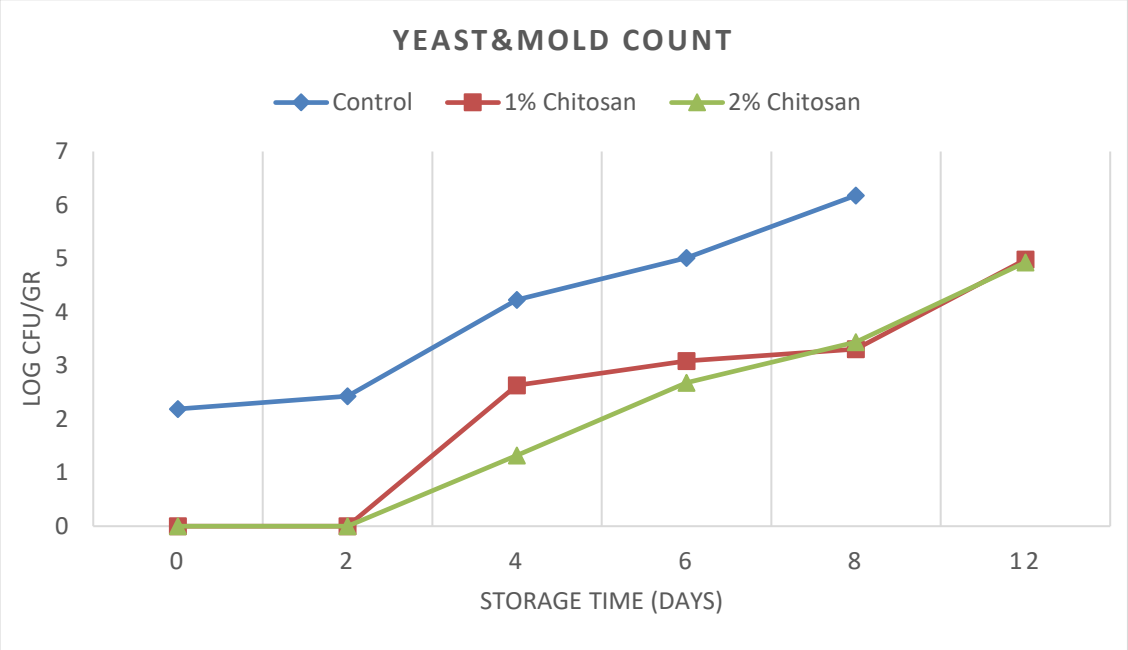

Figure 8. Yeast and mold count results of chicken drumsticks with different chitosan solutions

According to the yeast&mold count results of chicken drumsticks with combination of chitosan solutions and allicin (Figure 9), there was a fluctuation in K2A1 and K2A2 treatments from day 0 to day 6 of storage. Microbial growth was observed in

the results in a linear manner from day 6 to day. According to the data obtained on day 9, all treatments were similar (around 6.6-6.8 log CFU/gr) to each other and no significant differences were found. On the last day of cold storage, K2A1 treatment showed less microbial growth than other sample groups and was successful in inhibiting yeasts & molds. The lower total viable, yeast and mold count results at the beginning of the shelf life are an indication that hygienic conditions were well provided during the production and storage of chicken drumsticks, and as expected, they increased during the shelf life.

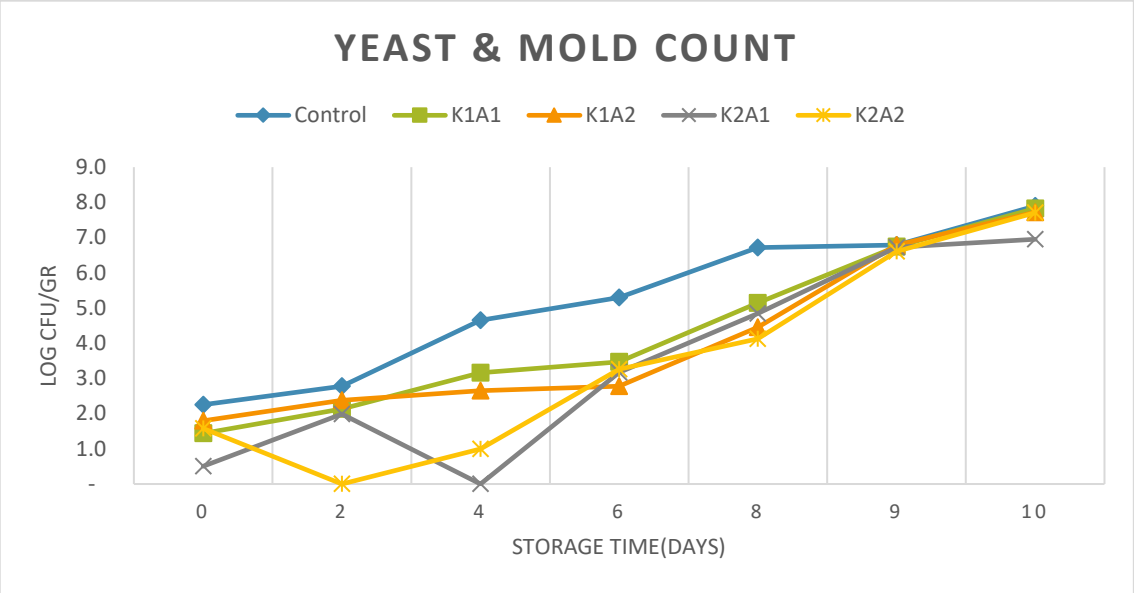

Figure 9. Yeast and mold count results of chicken drumsticks with combination of chitosan solutions and allicin

4.2.3. *Enterobacteriaceae* Count

Gram-negative bacteria such as Enterobacteriaceae are seen as a sign of hygiene. (Chouliara et al. 2008). Figure 10 shows the Enterobacteriaceae count results of chicken drumsticks with different chitosan solutions. The control group was the one with the highest enteric bacterial growth throughout the entire storage period. When the samples coated with chitosan solution were compared, no growth was observed in the 2% chitosan sample until the 8th day, while an increased microbial growth was observed in the 1% chitosan sample until the 6th day and interestingly, the enteric bacterial count

decreased by approximately 1 log on the 8th day. No significant difference was found between the results of two chitosan coated samples on the 8th day of storage. In the study examining the effect of the combination of chitosan and lauric alginate on the number of Enterobacteriaceae in chicken drumsticks, the Enterobacteriaceae count results in all sample groups were lower than the control sample throughout the entire shelf life (Abdel-Naeem, Zayed, and Mansour 2021).

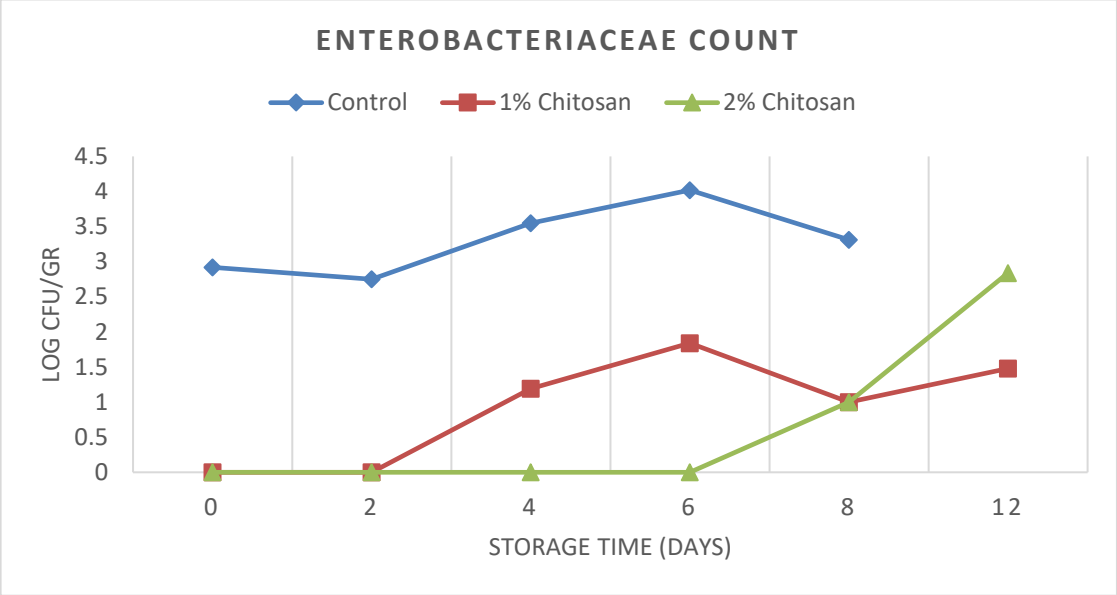

Figure 10. *Enterobacteriaceae* count results of chicken drumsticks with different chitosan solutions

According to the Enterobacteriaceae count results of chicken drumsticks with combination of chitosan solutions and allicin, the control treatment gave the highest microbial growth results throughout storage (Figure 11). No significant enteric bacterial growth was observed between K1A1 and K1A2 treatments on the 4th day of storage, with results of 1.54 and 1.76 log CFU/g, respectively. Also, no growth was observed in K2A1 and K2A2 treatments. On the 9th day of storage, there was no important difference in between the samples treated with K1A1 and K2A2 and between the other three applications. On the 10th and last day of storage, enteric bacterial counts of chicken drumstick samples treated with allicin, and chitosan were 1 log lower than those in the control group. In a study conducted for 16 days of storage, a decrease of approximately 3 log CFU/gr was observed in the Enterobacteriaceae counts in the samples combined with

chitosan, chitosan and extract, chitosan and essential oil, and approximately 3.1 log CFU/gr in the samples combined with chitosan+essential oil+extract compared to the control group (Khorshidi, Mehdizadeh, and Ghorbani 2021). Regarding Enterobacteriaceae in the literature, bacterial counts were lower at the first day of storage and on the 3rd day but were observed to increase from the 7th day onwards, decreasing by 1 log CFU/gr compared to the control sample of chicken patties with 1% green tea extract added (Passos et al. 2022). In this study, edible coating was not applied, and it was seen that this affected the microbiological results and spoilage in chicken meat.

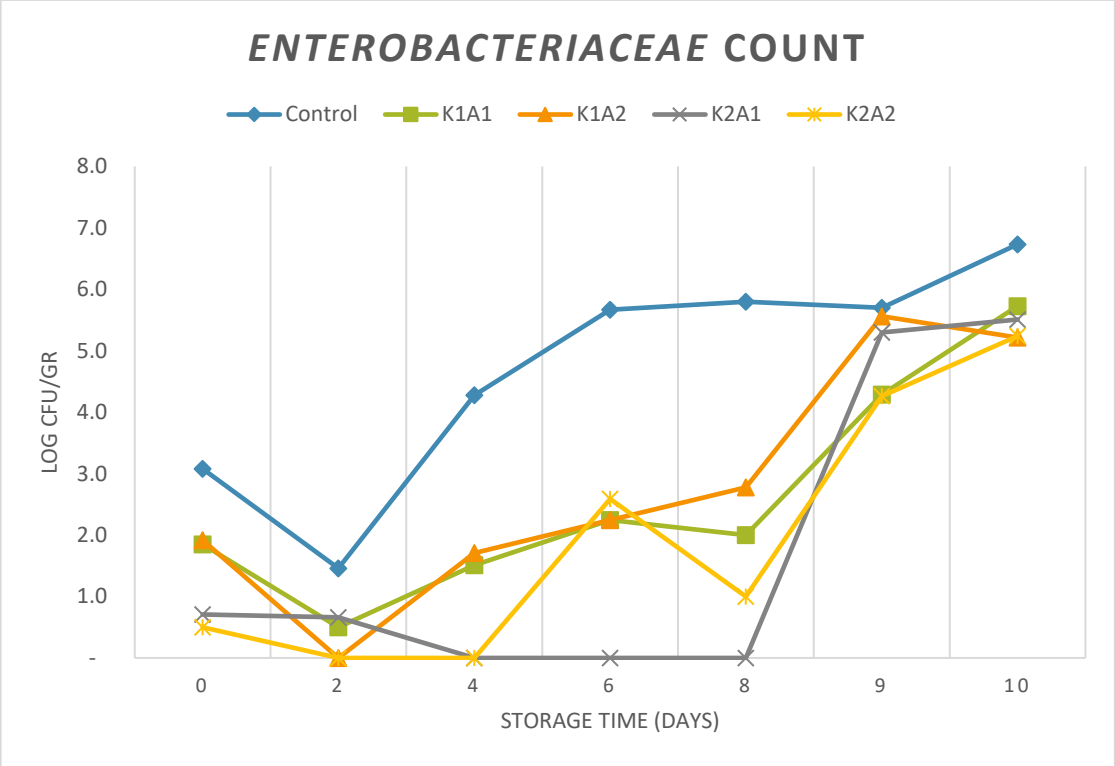

Figure 11. *Enterobacteriaceae* count results of chicken drumsticks with combination of chitosan solutions and allicin

4.2.4. *Pseudomonas* Count

Considered as one of the principal spoilage germs in meat, Pseudomonas spp. are gram negative bacteria that causes the development of highly unpleasant putrid flavours (Chouliara et al. 2008). The result of Pseudomonas counts of chicken drumsticks with

different chitosan solutions are given in Figure 12, and as in other microbiological analyses, a linearly increasing graph was obtained in the control group. While 2.53 log CFU/gr growth was obtained on day 0, 4.65 log CFU/gr growth was obtained on day 8. When the other two treatment groups were compared, it was understood from the results that there was no growth in chicken drumsticks treated with 2% chitosan until the 6th day of storage, while there was no growth in chicken drumsticks treated with 1% chitosan until the 4th day of shelf life. On the 8th day of storage, there was a decrease in the Pseudomonas count in both samples, and when the last day results were examined, it was concluded that 2% chitosan was better at inhibiting pseudomonas than other treatments.

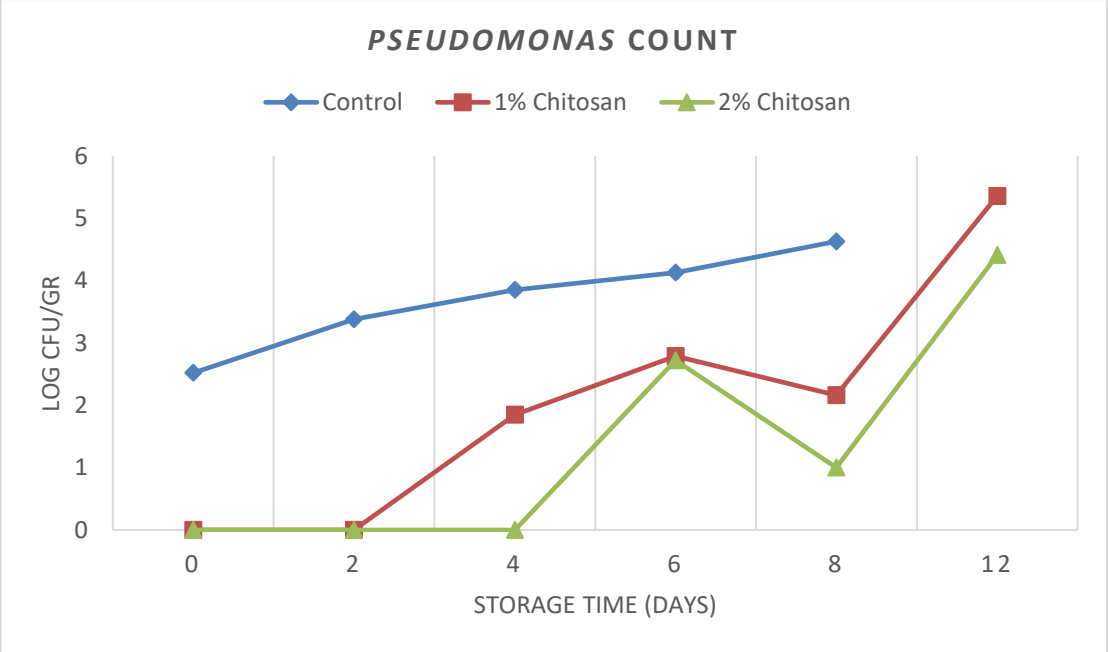

Figure 12. *Pseudomonas* count results of chicken drumsticks with different chitosan solutions

In the Pseudomonas count results of the treatment groups containing combinations of chitosan and allicin (Figure 13), the highest values were given including the 8th day of storage. However, on the 9th day, K1A1 and K1A2 treated samples gave similar results to the control sample, while on the 10th day, only the K1A1 sample gave similar results. According to these results, it was observed that the effect of the K1A1 treatment in terms of Pseudomonas reduction decreased towards the last days of storage. A Pseudomonas growth result that increased and decreased from the beginning to the end of storage was obtained from the K2A1 sample. The most stable growth curve was obtained from the

K2A2 treatment, while there was no growth until the 6th day, and then with a linear increase. Successful results were in the K2A1 and K2A2 treatments in terms of inhibiting Pseudomonas. On the 9th day, there was approximately 2 log decrease in Pseudomonas growth results in K2A2 treatment compared to the control group, and 1.5 log decrease on the 10th day. These results are in line with earlier studies that discovered that chicken meat processed using chitosan and various essential oils contained fewer Pseudomonas species. A study on chitosan coating of chicken breast meat combined with propolis extract and Zataria multiflora boiss oil concluded that Chitosan 1%-Zataria 1% applications were most beneficial in inhibiting Pseudomonas growth in chicken meat applications, resulting in a 2.81 log cycle reduction compared to the control sampletreatment (Mehdizadeh and Mojaddar Langroodi 2019). In another study, where chitosan coating was combined with Zataria multiflora boiss essential oil and chicken meat was immersed in different concentrations of pomegranate juice and monitored throughout the shelf life, it was concluded that the Pseudomonas spp. counts in the first days of shelf life were 0.8, 1.76, 2.65 and 2.91 log CFU/g lower than the corresponding counts in control samples for other treatments, respectively (Bazargani-Gilani, Aliakbarlu and Tajik 2015).

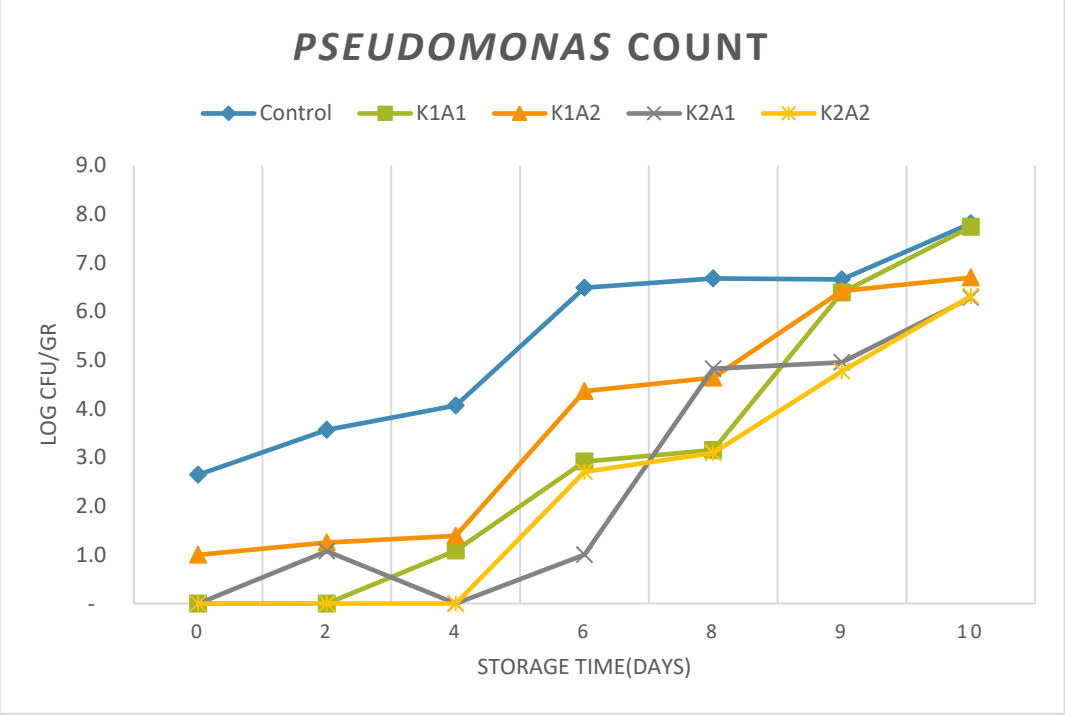

Figure 13. *Pseudomonas* count results of chicken drumsticks with combination of chitosan solutions and allicin

4.3. Color Measurements Results

Color is a significant quality indicator for food goods. The color of the product immediately reflects its outward look and influences its acceptance level among consumers (Petković et al. 2021). The color and opacity of the coatings impact the look and acceptance of food products. The opacity of coatings is another topic of interest, as higher opacity might be associated with better light barrier properties (Zhang et al. 2021).

Since chitosan is a colorless and odorless material and will not cause any color change in chicken meat, color analysis was examined only in sample groups containing combinations of chitosan and allicin during 10 days of storage at 4 °C. Color analysis results (Table 5) show that in the 4 different combination of chicken drumsticks of samples , chitosan and allicin tries to neutralize the pH value due to its acidic feature, and accordingly, fluctuations in L *, a *, and b * values are likely to have occurred. The L* value, which indicates the degree of lightness of the product, was measured in the control sample between 63.21 - 51.90 throughout the shelf life. Samples treated with K1A2 (52.16-58.65) and K2A2 (54.47-59.98) gave lower results compared to the others. When the L* value results are examined in general, fluctuating results were obtained between the storage days. The a* value, which indicates the degree of redness, gave different results between the days in all samples. The average lowest value range was 1.68-4.61 for the control group, while the average highest value range was 2.56-6.61 for the K2A1 group. The b* value, which indicates the degree of yellowness of the product, gave different results between the days in all samples. While the control group gave positive results between 0.21-4.23, treatments containing combinations of chitosan and allicin gave (-) and (+) results. The average lowest value range in the storage days belonged to the K1A1 group with -0.33 and +3.10, while the average highest value range belonged to the K2A2 group with -0.21 and +4.76 among those other than the control group.

The decrease in a* values between storage days in chicken meat is most likely due to the increased tendency of oxymyoglobin to oxidize to gray-brown metmyoglobin (MetMb) when the meat is stored in cold conditions (Zheng et al. 2023). Since free radicals can denature myoglobin particles or oxidizing ferrous atoms, changing the color of meat, it may also be connected to lipid oxidation (Serrano-León et al. 2018). In the literature, similar results were obtained with the study conducted by Munekata et al. (2015) during the storage of chicken meatballs containing peanut shell extract and

Munekata et al. (2016) during the storage of lamb meatballs, and Serrano-León et al. (2018) with pink pepper residue and peanut shell extracts on the shelf life of chicken meat, obtaining decreasing values in the a* value. Passos et al. (2022), who examined the color values of chicken patties using green tea extract as a natural preservative, observed changes in L and a* values, while no significant difference was found in b* values. Zheng et al. (2023) found generally similar results in L, a*, b* values between days in chicken breast fillets treated with chitosan coating or combination with oregano essential oil during 12 days of storage at refrigeration temperature. The difference between the two studies can be interpreted as the fact that Zheng et al. (2023) worked on chicken breasts. Since the chicken drumsticks have skin, the points measured in color cannot always be the same, so it is normal for the differences between the values to be high.

4.4. Sensorial Evaluation Results

Sensory analysis is essential for creating new products, refining existing products, and enhancing customer satisfaction. It determines how consumers perceive products, whether they are liked or not, and whether they will remain on the shelves for a long time when they enter the market. It also assesses sensory impacts on shelf life, packaging materials, and manufacturing processes. The sensory qualities of treated chicken drumsticks were presented to the panelists to be evaluated in terms of appearance, color, odor and texture. Changes in the scores of sensory factors that have been presented in Table 6. In the flavor and texture results, chicken drumsticks coated with chitosan were liked more than the control group. 2% chitosan and the control group gave similar results in the odor evaluation, while 1% chitosan gave the lowest result. With an evaluation score of 3.87, 2% chitosan made a difference compared to the other treatments in color result. The coating substance shouldn't impart any flavor, odor, or dark color to the food. Therefore, the sensory qualities should remain unchanged (Priya, Thirunavookarasu, and Chidanand 2023). The fact that no such comments were made by the panelists as a result of the sensory analysis supports the use of chitosan in chicken drumsticks.

Table 9. Color parameters of chicken drumsticks coated with chitosan and allicin combination.[a]

Color Parameters	Treatment	Storage Period (Days)						
		0	2	4	6	8	9	10
L*	Control	63.21±4.88^{AB}	55.1 2±1.91^{A-C}	51.90±2.73^{BC}	55.85±3.12^{A-C}	54.11±2.01^{BC}	52.72±2.02^{BC}	52.87±0.29^{BC}
	K1A1	58.45±1.36^{A-C}	61.63±0.92^{A-C}	52.82±5.15^{BC}	57.74±3.58^{A-C}	53.49±0.76^{BC}	54.09±3.01^{BC}	52.96±0.53^{BC}
	K1A2	56.62±2.62^{A-C}	58.65±2.71^{A-C}	58.35±5.40^{A-C}	52.16±0.65^{BC}	54.19±1.39^{BC}	52.87±1.30^{BC}	53.76±1.04^{BC}
	K2A1	65.74±7.26^{A}	60.42±2.16^{A-C}	58.29±1.92^{A-C}	54.72±0.41^{A-C}	52.12±1.94^{BC}	51.62±0.24^{C}	53.76±1.04^{BC}
	K2A2	58.05±2.77^{A-C}	59.98±1.63^{A-C}	55.89±2.30^{A-C}	56.41±6.36^{A-C}	58.41±2.43^{A-C}	54.47±0.36^{A-C}	55.42±0.84^{A-C}
a*	Control	3.21±0.83^{A-C}	4.19±1.32^{A-C}	3.90±1.53^{A-C}	1.73±1.34^{BC}	4.61±1.96^{A-C}	1.68±0.60^{BC}	2.84±0.86^{A-C}
	K1A1	1.98±0.97^{A-C}	5.70±0.38^{A-C}	5.94±3.06^{A-C}	4.30±1.32^{A-C}	4.66±1.37^{A-C}	4.88±2.23^{A-C}	2.07±0.46^{A-C}
	K1A2	1.06±0.65^{C}	4.45±1.05^{A-C}	3.22±1.49^{A-C}	4.09±0.99^{A-C}	5.04±1.90^{A}	3.11±0.44^{A-C}	2.45±0.44^{A-C}
	K2A1	3.77±1.39^{A-C}	3.91±0.46^{A-C}	5.34±1.43^{A-C}	2.56±1.79^{A-C}	6.61±2.54^{AB}	2.75±0.56^{A-C}	5.19±0.61^{A-C}
	K2A2	3.34±0.78^{A-C}	3.22±0.36^{A-C}	4.96±2.04^{A-C}	4.51±0.32^{A-C}	5.33±1.41^{A-C}	0.76±0.13^{C}	4.91±0.28^{A-C}
b*	Control	0.57±2.63^{AB}	0.21±1.36^{AB}	3.28±0.53^{AB}	1.46±0.43^{A-C}	1.96±1.91^{AB}	4.03±0.43^{AB}	1.83±0.79^{AB}
	K1A1	-1.21±0.47^{AB}	3.10±0.80^{AB}	2.65±0.50^{AB}	1.74±0.85^{AB}	-0.33±1.25^{AB}	0.90±2.71^{AB}	1.32±0.65^{AB}
	K1A2	-0.43±1.53^{AB}	3.52±4.02^{AB}	1.98±3.16^{AB}	1.12±1.45^{AB}	4.46±1.97^{A}	0.31±0.11^{AB}	-1.22±0.64^{AB}
	K2A1	-0.48±1.24^{AB}	-0.09±0.44^{AB}	0.94±2.92^{AB}	3.20±2.50^{AB}	1.87±3.28^{AB}	1.39±0.51^{AB}	-0.92±1.04^{AB}
	K2A2	1.18±0.75^{AB}	3.29±0.60^{AB}	4.76±1.73^{AB}	3.58±1.91^{AB}	2.93±2.01^{AB}	-0.21±1.07^{AB}	-1.67±0.42^{BAB}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. Each color parameter is grouped within itself. .C= Control, K1A1= 1% chitosan+10% Allicin, K1A2= 1% chitosan+20% Allicin, K2A1= 2% chitosan+10% Allicin, K2A2= 2% chitosan+20% Allicin. (L*: lightness, a*: redness, and b*: yellowness)

Table 10. Sensory analyses result of chicken drumsticks with different chitosan solutions[a]

Treatments	Flavor	Texture	Odor	Color
Control	3.2 ± 0.91^{A}	3.27 ± 0.93^{A}	3.53 ± 0.96^{A}	3.53 ± 0.50^{A}
1% Chitosan	3.67 ± 0.70^{A}	3.6 ± 0.71^{A}	3.33 ± 0.60^{A}	3.4 ± 0.71^{A}
2% Chitosan	3.4 ± 0.75^{A}	3.73 ± 0.85^{A}	3.6 ± 0.80^{A}	3.87 ± 0.72^{A}

[a] Means ± standard deviation. Means that do not share a letter are significantly different.

According to the sensory analysis results of chicken drumsticks coated with chitosan and allicin combination (Table 7), all the samples treated with chitosan coating and allicin were liked more in the flavor results with the comparison of the control. K1A2 and K2A2 sample groups also gave the closest and highest results to each other. K1A2 sample gave the highest values according to color and odor comparison of the other treatments. The panelists commented that the slightly yellowish redness that appeared after cooking in the K1A2 treatment group was appreciated in the analysis results. Due to the low chitosan concentration and high allicin ratio, allicin had a positive effect on the color during cooking. This positive response is a significant finding for the study because customers' acceptance of meat products is mostly influenced by their color. The textural evaluation of the K1A1 sample was found to be at the highest value.

Table 11. Sensory analyses results of chicken drumsticks coated with chitosan and allicin combination. [a]

Treatments	Flavor	Texture	Odor	Color
Control	2.92 ± 0.81^{A}	3.20 ± 0.98^{A}	3.37 ± 1.02^{A}	3.50 ± 0.81^{A}
K1A1	3.2 ± 0.91^{A}	3.30 ± 1.07^{A}	3.40 ± 0.80^{A}	3.60 ± 0.76^{A}
K1A2	3.23 ± 0.96^{A}	3.07 ± 1.09^{A}	3.63 ± 1.02^{A}	3.70 ± 0.82^{A}
K2A1	3.03 ± 1.11^{A}	3.03 ± 1.20^{A}	3.27 ± 1.12^{A}	3.57 ± 1.15^{A}
K2A2	3.20 ± 1.33^{A}	3.17 ± 1.37^{A}	3.17 ± 1.27^{A}	3.33 ± 1.22^{A}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. C= Control, K1A1= 1% chitosan+10% Allicin, K1A2= 1% chitosan+20% Allicin, K2A1= 2% chitosan+10% Allicin, K2A2= 2% chitosan+20% Allicin

The overall score given to all treatments, which was evaluated out of 5 points in total, as the total evaluation of all these features, was plotted as radar as overall acceptance (Figure 14).While chicken drumsticks coated with 2% chitosan gave the highest score of 3.65 which is an acceptable score, the lowest scores were given by sample groups K2A1 and K2A2 with 3.21 and 3.22, respectively. When the results were evaluated generally, higher approval scores were obtained in samples with lower chitosan and allicin concentrations.

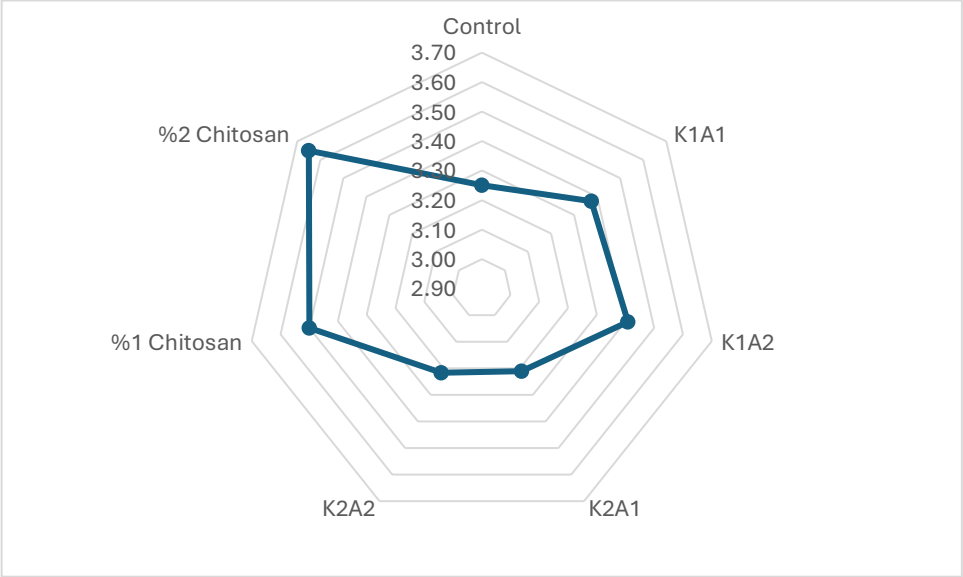

Figure 14. Overall acceptance of treated chicken drumsticks in sensory profile results

CHAPTER 5

CONCLUSION

In this study, instead of using chemical preservatives such as nitrites, nitrates, organic acids, and hydrogen peroxides, to enhance the quality and microbiological safety features of white meat products such as chicken, the shelf life of chicken drumsticks was investigated using chitosan and allicin. In the findings of the study, which included the effect of the combination of chitosan and allicin and chitosan alone, it was found that these natural antimicrobial agents significantly reduced the total viable bacterial count, Pseudomonas and Enterobacteriaceae load in chicken drumsticks. Especially in the last two days of storage (9th-10th), the K2A1 and K2A2 sample groups caused a significant decrease in the total viable count in chicken drumsticks. Chitosan is known to attach to bacterial cell walls, increasing permeability and causing bacterial death. Allicin is a substance that has broad-spectrum antibacterial properties. The combination of these two substances inhibited the microbiological deterioration of chicken drumsticks more efficiently. Thiobarbituric acid reactive substance (TBARS) values were investigated in this study as an indicator of lipid peroxidation. It was determined that chitosan and allicin coating significantly slowed down the enhance in TBARS values with the comparison of control sample group. 1% chitosan was the most successful rate at preventing lipid oxidation, while K1A2 treatment was the second most effective group. This finding shows that chitosan and allicin contribute to the longer preservation of sensory properties of the product by delaying the oxidative degradation of lipids in chicken drumsticks. As a result of chemical composition analyses such as moisture, fat, protein and ash, it was not observed that allicin and chitosan caused significant differences in chicken drumsticks. There was a continuous instability in the pH values of chicken drumsticks during the storage period. This is due to the buffering properties of chicken meat. Sensory analyses by panelists were performed by considering parameters such as odor, color, texture, taste and of chicken meat. While some panelists stated that the yellowish redness and crispiness after cooking in chitosan and allicin-coated samples contributed to the flavor, some stated that they did not like the slight aroma given by allicin. Sensory analysis results showed that the coating did not cause any negative changes in the

product's flavor, aroma and texture in sample groups where the concentration was low. The most appreciated treatment in terms of overall acceptability was found to be 2% chitosan.

As a result, the combination of chitosan and allicin may be employed as a preservative agent and is a potential natural strategy for extending the shelf life of easily perishable products like chicken meat. In particular, the development of these combinations designed for the specific needs of consumers in line with personalized nutrition trends will add vitality to the sector. However, the efficiency of this approach must be explored in various meat varieties and storage circumstances. Furthermore, it is advised that future research examines matters like cost analysis and customer approval of the chitosan and allicin combination.

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APPENDICES

APPENDIX A. RESULTS OF CHEMICAL ANALYSIS

One-way ANOVA: Moisture versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	0.78703	0.393517	55.56	0.004
Error	3	0.02125	0.007083		
Total	5	0.80828			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0841625	97.37%	95.62%	89.48%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	2	76.4100	0.1273	(76.2206, 76.5994)
%2 Chitosan	2	77.2600	0.0707	(77.0706, 77.4494)
Control	2	77.0550	0.0071	(76.8656, 77.2444)

Pooled StDev = 0.0841625

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%2 Chitosan	2	77.2600	A
Control	2	77.0550	A
%1 Chitosan	2	76.4100	B

Means that do not share a letter are significantly different.

Figure A1. Moisture analysis results (statistically) of chicken drumsticks with different chitosan solutions

One-way ANOVA: Fat versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	0.413333	0.206667	75.61	0.003
Error	3	0.008200	0.002733		
Total	5	0.421533			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0522813	98.05%	96.76%	92.22%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	2	4.080	0.000	(3.962, 4.198)
%2 Chitosan	2	3.5800	0.0566	(3.4623, 3.6977)
Control	2	4.1800	0.0707	(4.0623, 4.2977)

Pooled StDev = 0.0522813

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
Control	2	4.1800	A
%1 Chitosan	2	4.080	A
%2 Chitosan	2	3.5800	B

Means that do not share a letter are significantly different.

Figure A2. Fat analysis results (statistically) of chicken drumsticks with different chitosan solutions

One-way ANOVA: Protein versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	1.27423	0.637117	230.28	0.001
Error	3	0.00830	0.002767		
Total	5	1.28253			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0525991	99.35%	98.92%	97.41%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	2	18.1950	0.0212	(18.0766, 18.3134)
%2 Chitosan	2	17.1650	0.0778	(17.0466, 17.2834)
Control	2	17.2800	0.0424	(17.1616, 17.3984)

Pooled StDev = 0.0525991

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%1 Chitosan	2	18.1950	A
Control	2	17.2800	B
%2 Chitosan	2	17.1650	B

Means that do not share a letter are significantly different.

Figure A3. Protein analysis results (statistically) of chicken drumsticks with different chitosan solutions

One-way ANOVA: Ash versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	0.015633	0.007817	27.59	0.012
Error	3	0.000850	0.000283		
Total	5	0.016483			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0168325	94.84%	91.41%	79.37%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	2	0.8450	0.0212	(0.8071, 0.8829)
%2 Chitosan	2	0.8600	0.0141	(0.8221, 0.8979)
Control	2	0.9600	0.0141	(0.9221, 0.9979)

Pooled StDev = 0.0168325

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
Control	2	0.9600	A
%2 Chitosan	2	0.8600	B
%1 Chitosan	2	0.8450	B

Means that do not share a letter are significantly different.

Figure A4. Ash analysis results (statistically) of chicken drumsticks with different chitosan solutions

One-way ANOVA: Moisture versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	7.2450	1.81124	18.50	0.000
Error	10	0.9791	0.09791		
Total	14	8.2241			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.312911	88.09%	83.33%	73.21%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	3	72.9300	0.1375	(72.5275, 73.3325)
K1A1	3	74.1400	0.1418	(73.7375, 74.5425)
K1A2	3	73.9267	0.1097	(73.5241, 74.3292)
K2A1	3	74.920	0.568	(74.517, 75.323)
K2A2	3	74.687	0.340	(74.284, 75.089)

Pooled StDev = 0.312911

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping	
K2A1	3	74.920	A	
K2A2	3	74.687	A	B
K1A1	3	74.1400	A	B
K1A2	3	73.9267		B

Figure A5. Moisture analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

One-way ANOVA: Fat versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	1.2008	0.30021	16.18	0.000
Error	10	0.1855	0.01855		
Total	14	1.3864			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.136211	86.62%	81.26%	69.89%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	3	6.2000	0.0700	(6.0248, 6.3752)
K1A1	3	5.6900	0.0781	(5.5148, 5.8652)
K1A2	3	5.5900	0.0889	(5.4148, 5.7652)
K2A1	3	5.363	0.206	(5.188, 5.539)
K2A2	3	5.533	0.178	(5.358, 5.709)

Pooled StDev = 0.136211

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
Control	3	6.2000	A
K1A1	3	5.6900	B
K1A2	3	5.5900	B
K2A2	3	5.533	B

Figure A6. Fat analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

One-way ANOVA: Protein versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	0.5134	0.12834	1.92	0.183
Error	10	0.6680	0.06680		
Total	14	1.1814			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.258457	43.46%	20.84%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	3	18.407	0.488	(18.074, 18.739)
K1A1	3	18.277	0.246	(17.944, 18.609)
K1A2	3	18.5500	0.0700	(18.2175, 18.8825)
K2A1	3	18.3767	0.0737	(18.0442, 18.7091)
K2A2	3	18.8100	0.1572	(18.4775, 19.1425)

Pooled StDev = 0.258457

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
K2A2	3	18.8100	A
K1A2	3	18.5500	A
Control	3	18.407	A
K2A1	3	18.3767	A

Figure A7. Protein analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

One-way ANOVA: Ash versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	0.025027	0.006257	30.27	0.000
Error	10	0.002067	0.000207		
Total	14	0.027093			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0143759	92.37%	89.32%	82.84%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	3	0.85667	0.01155	(0.83817, 0.87516)
K1A1	3	0.84667	0.01528	(0.82817, 0.86516)
K1A2	3	0.83667	0.01155	(0.81817, 0.85516)
K2A1	3	0.9367	0.0208	(0.9182, 0.9552)
K2A2	3	0.92000	0.01000	(0.90151, 0.93849)

Pooled StDev = 0.0143759

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping	
K2A1	3	0.9367	A	
K2A2	3	0.92000	A	
Control	3	0.85667		B
K1A1	3	0.84667		B

Figure A8. Ash analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

APPENDIX B. RESULTS OF TBARS ANALYSIS

Table B. TBARS analysis results of chicken drumsticks with combination of chitosan solutions and allicin during shelf-life [a]

TREATMENT	STORAGE TIME (DAY)					
	0	**3**	**5**	**7**	**10**	**17**
Control	$0.31\pm0.002^{P-U}$	$0.40\pm0.012^{N-U}$	$0.63\pm0.073^{K-Q}$	$1.61\pm0.502^{B-E}$	$1.22\pm0.163^{F--I}$	2.89 ± 0.014^{A}
1% Chitosan	0.041 ± 0.008^{U}	$0.42\pm0.006^{N-T}$	$0.29\pm0.015^{P--U}$	$0.34\pm0.043^{O-U}$	$0.33\pm0.045^{N-U}$	$1.43\pm0.022^{E-G}$
2% Chitosan	$0.27\pm0.004^{Q-U}$	$0.84\pm0.056^{J-M}$	$0.51\pm0.007^{M-S}$	$0.71\pm0.016^{K-N}$	$0.72\pm0.042^{K-N}$	2.83 ± 0.049^{A}
K1A1	$0.35\pm0.0036^{N-U}$	$1.24\pm0.041^{E-I}$	$0.53\pm0.004^{L-R}$	$0.65\pm0.033^{K-P}$	$0.67\pm0.043^{K-O}$	1.95 ± 0.067^{B}
K1A2	$0.98\pm0.004^{H-K}$	$0.40\pm0.024^{N-U}$	$0.49\pm0.0014^{M-S}$	$0.56\pm0.016^{L-R}$	$0.55\pm0.0023^{L-R}$	$1.82\pm0.015^{B-D}$
K2A1	$0.063\pm0.006^{T-U}$	$1.21\pm0.014^{G-I}$	$0.23\pm0.016^{R-U}$	$0.89\pm0.039^{I-L}$	$0.89\pm0.004^{I--L}$	$1.58\pm0.008^{C-F}$
K2A2	$0.163\pm0.029^{S-U}$	$1.17\pm0.025^{G-J}$	$1.27\pm0.031^{E-H}$	$1.18\pm0.001^{G-J}$	1.18 ± 0.030^{BC}	$1.46\pm0.015^{D-G}$

[a] Means ± standard deviation. Means that do not share a letter are significantly different.

General Linear Model: TBARS versus Storage Time, Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	6	0, 3, 5, 7, 10, 17
Sample Groups	Fixed	7	%1 Chitosan, %2 Chitosan, Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	5	23.3745	4.67491	623.85	0.000
Sample Groups	6	4.2599	0.70999	94.75	0.000
Storage Time*Sample Groups	30	10.1145	0.33715	44.99	0.000
Error	42	0.3147	0.00749		
Total	83	38.0637			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0865661	99.17%	98.37%	96.69%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.90626	0.00945	95.95	0.000	
Storage Time					
0	-0.5949	0.0211	-28.17	0.000	1.67
3	-0.0935	0.0211	-4.43	0.000	1.67
5	-0.3401	0.0211	-16.10	0.000	1.67
7	-0.0558	0.0211	-2.64	0.012	1.67
10	-0.0057	0.0211	-0.27	0.789	1.67
Sample Groups					
%1 Chitosan	-0.4228	0.0231	-18.27	0.000	1.71
%2 Chitosan	0.0746	0.0231	3.22	0.002	1.71
Control	0.2692	0.0231	11.63	0.000	1.71

Figure B. TBARS analysis results (statistically) of chicken drumsticks with all treated sample groups

Comparisons for TBARS

Tukey Pairwise Comparisons: Storage Time*Sample Groups

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping
17 Control	2	2.89000	A
17 %2 Chitosan	2	2.83500	A
17 K1A1	2	1.94750	B
10 K2A2	2	1.87450	B C
17 K1A2	2	1.82300	B C D
7 Control	2	1.60500	B C D E
17 K2A1	2	1.58000	C D E F
17 K2A2	2	1.46100	D E F G
17 %1 Chitosan	2	1.43750	E F G
5 K2A2	2	1.26685	E F G H
3 K1A1	2	1.24100	E F G H I
10 Control	2	1.22500	F G H I
3 K2A1	2	1.21000	G H I
7 K2A2	2	1.18605	G H I J
3 K2A2	2	1.17200	G H I J
0 K1A2	2	0.97850	H I J K
7 K2A1	2	0.89545	I J K L
10 K2A1	2	0.89050	I J K L
3 %2 Chitosan	2	0.84000	J K L M
10 %2 Chitosan	2	0.72000	K L M N
7 %2 Chitosan	2	0.70900	K L M N
10 K1A1	2	0.66950	K L M N O
7 K1A1	2	0.65150	K L M N O P
5 Control	2	0.62850	K L M N O P Q
10 K1A2	2	0.56795	L M N O P Q R
7 K1A2	2	0.56165	L M N O P Q R
5 K1A1	2	0.53340	L M N O P Q R
5 %2 Chitosan	2	0.50950	M N O P Q R S
5 K1A2	2	0.48800	M N O P Q R S
3 %1 Chitosan	2	0.42200	N O P Q R S T
3 K1A2	2	0.40300	N O P Q R S T U
3 Control	2	0.40100	N O P Q R S T U
0 K1A1	2	0.35900	N O P Q R S T U
10 %1 Chitosan	2	0.35650	N O P Q R S T U
7 %1 Chitosan	2	0.34450	O P Q R S T U
0 Control	2	0.30300	P Q R S T U
5 %1 Chitosan	2	0.29950	P Q R S T U
0 %2 Chitosan	2	0.27150	Q R S T U
5 K2A1	2	0.23750	R S T U
0 K2A2	2	0.16325	S T U
0 K2A1	2	0.06330	T U
0 %1 Chitosan	2	0.04100	U

Means that do not share a letter are significantly different.

APPENDIX C. RESULTS OF MICROBIOLOGICAL ANALYSIS

Table C1. Bacterial counts (Log_{10} CFU/g) of chicken drumsticks coated with chitosan. [a]

Bacterial Counts(CFU/g)	Treatment	Storage Time (Day)					
		0	**2**	**4**	**6**	**8**	**12**
Total Viable Count	Control	4.66±0.028^{E}	5.4±0.014^{D}	6.78±0.014^{C}	7.35±0.056^{BC}	8.41±0.410^{A}	-
	1% Chitosan	2.61±0.028^{GH}	3.11±0.156^{FG}	3.73±0.028^{F}	5.74±0.042^{D}	5.68±0.014^{D}	7.75±0.049^{AB}
	2% Chitosan	2.25±0.056^{H}	2.09±0.007^{H}	2.04±0.092^{H}	5.37±0.530^{D}	5.66±0.042^{D}	6.79±0.134^{C}
Yeast & Mold	Control	2.22±0.056^{H}	2.43±0.014^{G}	4.23±0.035^{C}	5.02±0.021^{B}	6.16±0.056^{A}	-
	1% Chitosan	*	*	2.67±0.028^{F}	3.08±0.014^{E}	3.35±0.049^{D}	4.99±0.014^{B}
	2% Chitosan	*	*	1.33±0.028^{I}	2.67±0.035^{F}	3.45±0.035^{D}	4.93±0.028^{B}
Pseudomonas	Control	2.53±0.028^{H}	3.39±0.014^{F}	3.87±0.021^{E}	4.13±0.028^{D}	4.65±0.049^{B}	-
	1% Chitosan	*	*	1.86±0.014^{J}	2.83±0.038^{G}	2.13±0.028^{I}	5.36±0.028^{A}
	2% Chitosan	*	*	*	2.73±0.035^{G}	1.01±0.049^{K}	4.43±0.035^{C}
Enterobacteriaceae	Control	2.93±0.043^{D}	2.80±0.141^{D}	3.55±0.056^{B}	4.05±0.049^{A}	3.34±0.056^{C}	-
	1% Chitosan	*	*	1.19±0.014^{G}	1.85±0.049^{E}	1.01±0.014^{G}	1.47±0.021^{F}
	2% Chitosan	*	*	*	*	1.02±0.035^{G}	2.85±0.056^{D}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. Each microbiological analysis is grouped within itself. * No microbial growth

(-) The sample in the control group was not analyzed because it was spoiled.

Table C2. Bacterial counts (Log_{10} CFU/g) of chicken drumsticks coated with chitosan and allicin combination.[a]

Bacterial Counts(CFU/g)	Treatment	Storage Time (Day)						
		0	2	4	6	8	9	10
Total Viable Count	Control	4.75 ± 0.021^{T}	5.66 ± 0.028^{R}	7.77 ± 0.008^{M}	9.35 ± 0.007^{EF}	9.51 ± 0.007^{D}	10.45 ± 0.0005^{B}	10.39 ± 0.005^{B}
	K1A1	3.12 ± 0.006^{Y}	3.24 ± 0.015^{X}	5.85 ± 0.016^{Q}	5.43 ± 0.001^{S}	7.30 ± 0.072^{N}	9.77 ± 0.005^{C}	10.71 ± 0.007^{A}
	K1A2	3.21 ± 0.002^{X}	4.05 ± 0.011^{V}	6.06 ± 0.019^{P}	8.76 ± 0.003^{I}	8.85 ± 0.026^{H}	9.13 ± 0.031^{G}	9.41 ± 0.011^{E}
	K2A1	2.97 ± 0.014^{Z}	3.25 ± 0.013^{X}	3.77 ± 0.031^{W}	8.78 ± 0.010^{HI}	8.84 ± 0.001^{HI}	8.46 ± 0.003^{K}	9.32 ± 0.005^{F}
	K2A2	2.72 ± 0.008^{AA}	2.92 ± 0.003^{Z}	4.24 ± 0.020^{U}	6.76 ± 0.028^{O}	7.94 ± 0.028^{L}	8.62 ± 0.006^{J}	9.57 ± 0.015^{D}
Yeast & Mold	Control	2.27 ± 0.015^{LM}	2.78 ± 0.012^{J}	4.66 ± 0.009^{EF}	5.29 ± 0.002^{D}	6.74 ± 0.028^{BC}	6.78 ± 0.007^{BC}	7.89 ± 0.021^{A}
	K1A1	1.43 ± 0.005^{P}	2.13 ± 0.015^{MN}	3.25 ± 0.011^{HI}	3.53 ± 0.042^{H}	5.18 ± 0.021^{D}	6.78 ± 0.005^{BC}	7.84 ± 0.021^{A}
	K1A2	1.85 ± 0.005^{NO}	2.46 ± 0.011^{KL}	2.72 ± 0.014^{JK}	2.85 ± 0.007^{J}	4.46 ± 0.035^{F}	6.86 ± 0.016^{BC}	7.78 ± 0.027^{A}
	K2A1	0.55 ± 0.005^{R}	2.07 ± 0.106^{MN}	*	3.24 ± 0.024^{I}	4.87 ± 0.014^{E}	6.72 ± 0.042^{BC}	7 ± 0^{B}
	K2A2	1.63 ± 0.085^{OP}	*	0.95 ± 0.071^{Q}	3.35 ± 0.021^{HI}	4.05 ± 0.212^{G}	6.68 ± 0.017^{C}	7.7 ± 0.283^{A}
Pseudomonas	Control	2.65 ± 0.077^{K}	3.54 ± 0.070^{I}	4.01 ± 0.127^{H}	6.45 ± 0.106^{CD}	6.66 ± 0.026^{BC}	6.65 ± 0.018^{BC}	7.83 ± 0.042^{A}
	K1A1	*	*	1.15 ± 0.053^{LM}	2.93 ± 0.049^{JK}	3.23 ± 0.078^{J}	6.67 ± 0.313^{BC}	7.73 ± 0.042^{A}
	K1A2	1 ± 0.071^{M}	1.3 ± 0.071^{LM}	1.45 ± 0.035^{L}	4.45 ± 0.042^{G}	4.67 ± 0.028^{FG}	6.47 ± 0.003^{CD}	6.78 ± 0.019^{B}
	K2A1	*	1.11 ± 0.014^{M}	*	1.05 ± 0.078^{M}	4.86 ± 0.014^{EF}	5.05 ± 0.071^{E}	6.34 ± 0.028^{D}
	K2A2	*	*	*	2.73 ± 0.028^{K}	3.13 ± 0.028^{J}	4.85 ± 0.064^{EF}	6.33 ± 0.064^{D}
Enterobacteriaceae	Control	3.07 ± 0.029^{G}	1.47 ± 0.027^{L}	4.26 ± 0.035^{F}	$5.57\pm0.177^{B-E}$	5.82 ± 0.099^{B}	5.69 ± 0.014^{BC}	6.75 ± 0.035^{A}
	K1A1	$1.84\pm0.028^{J-L}$	0.54 ± 0.028^{N}	1.54 ± 0.071^{L}	2.23 ± 0.005^{IJ}	2.05 ± 0.064^{JK}	4.36 ± 0.021^{F}	5.73 ± 0.028^{BC}
	K1A2	1.94 ± 0.028^{JK}	*	1.76 ± 0.042^{KL}	2.5 ± 0.354^{HI}	2.88 ± 0.049^{GH}	$5.64\pm0.009^{B-D}$	5.26 ± 0.042^{DE}
	K2A1	0.77 ± 0.035^{MN}	0.76 ± 0.014^{MN}	*	*	*	$5.35\pm0.042^{C-E}$	$5.53\pm0.004^{B-E}$
	K2A2	0.54 ± 0.035^{N}	*	*	2.59 ± 0.105^{HI}	1.07 ± 0.311^{M}	4.35 ± 0.049^{F}	5.23 ± 0.035^{E}

[a] Means ± standard deviation. Means that do not share a letter are significantly different. * No microbial growth. Each microbiological analysis is grouped within itself.

C= Control, K1A1= 1% chitosan+10% Allicin, K1A2= 1% chitosan+20% Allicin, K2A1= 2% chitosan+10% Allicin, K2A2= 2% chitosan+20% Allicin

General Linear Model: Total Viable Count versus Storage & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	6	0, 2, 4, 6, 8, 12
Sample Groups	Fixed	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	11.714	5.8572	204.46	0.000
Storage Time	5	57.758	11.5517	403.24	0.000
Storage Time*Sample Groups	10	115.469	11.5469	403.07	0.000
Error	18	0.516	0.0286		
Total	35	185.457			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.169255	99.72%	99.46%	98.89%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.7469	0.0282	168.28	0.000	
Sample Groups					
%1 Chitosan	0.0239	0.0399	0.60	0.557	1.33
%2 Chitosan	-0.7103	0.0399	-17.80	0.000	1.33
Storage Time					
0	-1.5736	0.0631	-24.95	0.000	1.67
2	-1.2119	0.0631	-19.21	0.000	1.67
4	-0.5619	0.0631	-8.91	0.000	1.67
6	1.4081	0.0631	22.32	0.000	1.67
8	1.8364	0.0631	29.11	0.000	1.67
Storage Time*Sample Groups					
0 %1 Chitosan	-0.5872	0.0892	-6.58	0.000	2.22

Figure C1. Total viable count results (statistically) of chicken drumsticks with different chitosan solutions

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping							
8 Control	2	8.410	A							
12 %1 Chitosan	2	7.755	A	B						
6 Control	2	7.350		B	C					
12 %2 Chitosan	2	6.795			C					
4 Control	2	6.780			C					
6 %1 Chitosan	2	5.740				D				
8 %1 Chitosan	2	5.680				D				
8 %2 Chitosan	2	5.660				D				
2 Control	2	5.400				D				
6 %2 Chitosan	2	5.375				D				
0 Control	2	4.660					E			
4 %1 Chitosan	2	3.730						F		
2 %1 Chitosan	2	3.110						F	G	
0 %1 Chitosan	2	2.610							G	H
0 %2 Chitosan	2	2.250								H
2 %2 Chitosan	2	2.095								H
4 %2 Chitosan	2	2.045								H
12 Control	2	-0.000									I

Means that do not share a letter are significantly different.

General Linear Model: Yeast&Mold versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	6	0, 2, 4, 6, 8, 12
Sample Groups	Fixed	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	10.874	5.4369	6097.40	0.000
Storage Time	5	66.699	13.3397	14960.47	0.000
Storage Time*Sample Groups	10	61.349	6.1349	6880.26	0.000
Error	18	0.016	0.0009		
Total	35	138.937			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0298608	99.99%	99.98%	99.95%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.58528	0.00498	519.47	0.000	
Sample Groups					
%1 Chitosan	-0.23778	0.00704	-33.78	0.000	1.33
%2 Chitosan	-0.52194	0.00704	-74.16	0.000	1.33
Storage Time					
0	-1.8453	0.0111	-165.82	0.000	1.67
2	-1.7753	0.0111	-159.53	0.000	1.67
4	0.1597	0.0111	14.35	0.000	1.67
6	1.0081	0.0111	90.58	0.000	1.67
8	1.7314	0.0111	155.58	0.000	1.67
Storage Time*Sample Groups					
0 %1 Chitosan	-0.5022	0.0157	-31.91	0.000	2.22

Figure C2. Yeast & mold count results (statistically) of chicken drumsticks with different chitosan solutions

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping
8 Control	2	6.160	A
6 Control	2	5.025	B
12 %1 Chitosan	2	4.990	B
12 %2 Chitosan	2	4.930	B
4 Control	2	4.235	C
8 %2 Chitosan	2	3.445	D
8 %1 Chitosan	2	3.345	D
6 %1 Chitosan	2	3.080	E
6 %2 Chitosan	2	2.675	F
4 %1 Chitosan	2	2.670	F
2 Control	2	2.430	G
0 Control	2	2.220	H
4 %2 Chitosan	2	1.330	I
0 %1 Chitosan	2	0.000	J
12 Control	2	-0.000	J
2 %2 Chitosan	2	-0.000	J
0 %2 Chitosan	2	-0.000	J
2 %1 Chitosan	2	-0.000	J

Means that do not share a letter are significantly different.

General Linear Model: Enterobacter versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	6	0, 2, 4, 6, 8, 12
Sample Groups	Fixed	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	32.2568	16.1284	7560.19	0.000
Storage Time	5	5.3497	1.0699	501.54	0.000
Storage Time*Sample Groups	10	34.4194	3.4419	1613.41	0.000
Error	18	0.0384	0.0021		
Total	35	72.0643			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0461880	99.95%	99.90%	99.79%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.44833	0.00770	188.14	0.000	
Sample Groups					
%1 Chitosan	-0.5267	0.0109	-48.38	0.000	1.33
%2 Chitosan	-0.8025	0.0109	-73.71	0.000	1.33
Storage Time					
0	-0.4717	0.0172	-27.40	0.000	1.67
2	-0.5150	0.0172	-29.92	0.000	1.67
4	0.1317	0.0172	7.65	0.000	1.67
6	0.5183	0.0172	30.11	0.000	1.67
8	0.3433	0.0172	19.95	0.000	1.67
Storage Time*Sample Groups					
0 %1 Chitosan	-0.4500	0.0243	-18.49	0.000	2.22

Figure C3. *Enterobacteriaceae* results (statistically) of chicken drumsticks with different chitosan solutions

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping							
6 Control	2	4.045	A							
4 Control	2	3.550		B						
8 Control	2	3.340			C					
0 Control	2	2.930				D				
12 %2 Chitosan	2	2.850				D				
2 Control	2	2.800				D				
6 %1 Chitosan	2	1.855					E			
12 %1 Chitosan	2	1.475						F		
4 %1 Chitosan	2	1.190							G	
8 %2 Chitosan	2	1.025							G	
8 %1 Chitosan	2	1.010							G	
12 Control	2	0.000								H
0 %1 Chitosan	2	0.000								H
4 %2 Chitosan	2	0.000								H
6 %2 Chitosan	2	-0.000								H
0 %2 Chitosan	2	-0.000								H
2 %1 Chitosan	2	-0.000								H
2 %2 Chitosan	2	-0.000								H

Means that do not share a letter are significantly different.

General Linear Model: Pseudomonas versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	6	0, 2, 4, 6, 8, 12
Sample Groups	Fixed	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	18.359	9.17935	12746.63	0.000
Storage Time	5	32.400	6.48008	8998.37	0.000
Storage Time*Sample Groups	10	69.596	6.95962	9664.27	0.000
Error	18	0.013	0.00072		
Total	35	120.368			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0268354	99.99%	99.98%	99.96%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.16181	0.00447	483.35	0.000	
Sample Groups					
%1 Chitosan	-0.13222	0.00633	-20.90	0.000	1.33
%2 Chitosan	-0.80097	0.00633	-126.63	0.000	1.33
Storage Time					
0	-1.3185	0.0100	-131.83	0.000	1.67
2	-1.0318	0.0100	-103.17	0.000	1.67
4	-0.2501	0.0100	-25.01	0.000	1.67
6	1.0657	0.0100	106.56	0.000	1.67
8	0.4349	0.0100	43.48	0.000	1.67
Storage Time*Sample Groups					
0 %1 Chitosan	-0.7111	0.0141	-50.28	0.000	2.22

Figure C4. *Pseudomonas* results (statistically) of chicken drumsticks with different chitosan solutions

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping
12 %1 Chitosan	2	5.3600	A
8 Control	2	4.6450	B
12 %2 Chitosan	2	4.4250	C
6 Control	2	4.1300	D
4 Control	2	3.8750	E
2 Control	2	3.3900	F
6 %1 Chitosan	2	2.8275	G
6 %2 Chitosan	2	2.7250	G
0 Control	2	2.5300	H
8 %1 Chitosan	2	2.1300	I
4 %1 Chitosan	2	1.8600	J
8 %2 Chitosan	2	1.0150	K
12 Control	2	0.0000	L
0 %1 Chitosan	2	0.0000	L
0 %2 Chitosan	2	0.0000	L
4 %2 Chitosan	2	0.0000	L
2 %2 Chitosan	2	-0.0000	L
2 %1 Chitosan	2	-0.0000	L

Means that do not share a letter are significantly different.

General Linear Model: Total Viable Count versus Storage &Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	418.240	69.7067	179862.31	0.000
Sample Groups	4	40.333	10.0832	26017.47	0.000
Storage Time*Sample Groups	24	31.811	1.3255	3420.08	0.000
Error	35	0.014	0.0004		
Total	69	490.398			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0196864	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.88488	0.00235	2926.03	0.000	
Storage Time					
0	-3.52891	0.00576	-612.28	0.000	1.71
2	-3.06231	0.00576	-531.32	0.000	1.71
4	-1.34385	0.00576	-233.16	0.000	1.71
6	0.93316	0.00576	161.91	0.000	1.71
8	1.60411	0.00576	278.32	0.000	1.71
9	2.40305	0.00576	416.94	0.000	1.71
Sample Groups					
control	1.38877	0.00471	295.11	0.000	1.60
K1A1	-0.39729	0.00471	-84.42	0.000	1.60
K1A2	0.18314	0.00471	38.92	0.000	1.60

Figure C5. Total viable count results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping
10 K1A1	2	10.7050	A
9 control	2	10.4586	B
10 control	2	10.3962	B
9 K1A1	2	9.7701	C
10 K2A2	2	9.5670	D
8 control	2	9.5153	D
10 K1A2	2	9.4147	E
6 control	2	9.3547	E F
10 K2A1	2	9.3153	F
9 K1A2	2	9.1309	G
8 K1A2	2	8.8472	H
8 K2A1	2	8.8391	H I
6 K2A1	2	8.7857	H I
6 K1A2	2	8.7626	I
9 K2A2	2	8.6195	J
9 K2A1	2	8.4606	K
8 K2A2	2	7.9448	L
4 control	2	7.7757	M
8 K1A1	2	7.2985	N
6 K2A2	2	6.7573	O
4 K1A2	2	6.0663	P
4 K1A1	2	5.8534	Q
2 control	2	5.6600	R
6 K1A1	2	5.4298	S
0 control	2	4.7550	T
4 K2A2	2	4.2418	U
2 K1A2	2	4.0452	V
4 K2A1	2	3.7680	W
2 K2A1	2	3.2553	
2 K1A1	2	3.2362	
0 K1A2	2	3.2092	
0 K1A1	2	3.1202	
0 K2A1	2	2.9779	
2 K2A2	2	2.9161	
0 K2A2	2	2.7176	

Storage Time*Sample Groups			
10 K1A1			
9 control			
10 control			
9 K1A1			
10 K2A2			
8 control			
10 K1A2			
6 control			
10 K2A1			
9 K1A2			
8 K1A2			
8 K2A1			
6 K2A1			
6 K1A2			
9 K2A2			
9 K2A1			
8 K2A2			
4 control			
8 K1A1			
6 K2A2			
4 K1A2			
4 K1A1			
2 control			
6 K1A1			
0 control			
4 K2A2			
2 K1A2			
4 K2A1			
2 K2A1	X		
2 K1A1	X		
0 K1A2	X		
0 K1A1		Y	
0 K2A1			Z
2 K2A2			Z
0 K2A2			AA

Means that do not share a letter are significantly different.

General Linear Model: Yeast&Mold versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	353.279	58.8799	12737.10	0.000
Sample Groups	4	28.182	7.0455	1524.12	0.000
Storage Time*Sample Groups	24	29.017	1.2090	261.55	0.000
Error	35	0.162	0.0046		
Total	69	410.641			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0679905	99.96%	99.92%	99.84%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.12520	0.00813	507.63	0.000	
Storage Time					
0	-2.5760	0.0199	-129.41	0.000	1.71
2	-2.2347	0.0199	-112.27	0.000	1.71
4	-1.8088	0.0199	-90.87	0.000	1.71
6	-0.4709	0.0199	-23.66	0.000	1.71
8	0.9348	0.0199	46.96	0.000	1.71
9	2.6389	0.0199	132.57	0.000	1.71
Sample Groups					
control	1.0795	0.0163	66.42	0.000	1.60
K1A1	0.1796	0.0163	11.05	0.000	1.60
K1A2	0.0176	0.0163	1.08	0.287	1.60

Figure C6. Yeast & mold count results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping
10 control	2	7.89500	A
10 K1A1	2	7.83500	A
10 K1A2	2	7.77950	A
10 K2A2	2	7.70000	A
10 K2A1	2	7.00000	B
9 K1A2	2	6.86150	B C
9 control	2	6.78500	B C
9 K1A1	2	6.77600	B C
8 control	2	6.74000	B C
9 K2A1	2	6.72000	B C
9 K2A2	2	6.67800	C
6 control	2	5.29850	D
8 K1A1	2	5.17500	D
8 K2A1	2	4.87000	E
4 control	2	4.65950	E F
8 K1A2	2	4.46500	F
8 K2A2	2	4.05000	G
6 K1A1	2	3.53000	H
6 K2A2	2	3.34500	H I
4 K1A1	2	3.25250	H I
6 K2A1	2	3.24300	I
6 K1A2	2	2.85500	J
2 control	2	2.78600	J
4 K1A2	2	2.72000	J K
2 K1A2	2	2.46250	K L
0 control	2	2.26905	L M
2 K1A1	2	2.12900	M N
2 K2A1	2	2.07500	M N
0 K1A2	2	1.85600	N O
0 K2A2	2	1.63000	O P
0 K1A1	2	1.43600	P
4 K2A2	2	0.95000	Q
0 K2A1	2	0.55500	R
2 K2A2	2	0.00000	S
4 K2A1	2	-0.00000	S

Means that do not share a letter are significantly different.

General Linear Model: Enterobacter versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	219.225	36.5374	4134.03	0.000
Sample Groups	4	73.599	18.3998	2081.85	0.000
Storage Time*Sample Groups	24	39.561	1.6484	186.51	0.000
Error	35	0.309	0.0088		
Total	69	332.694			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0940117	99.91%	99.82%	99.63%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.7748	0.0112	246.94	0.000	
Storage Time					
0	-1.1429	0.0275	-41.52	0.000	1.71
2	-2.2214	0.0275	-80.71	0.000	1.71
4	-1.2618	0.0275	-45.84	0.000	1.71
6	-0.1957	0.0275	-7.11	0.000	1.71
8	-0.4108	0.0275	-14.92	0.000	1.71
9	2.3058	0.0275	83.78	0.000	1.71
Sample Groups					
control	1.8896	0.0225	84.08	0.000	1.60
K1A1	-0.1626	0.0225	-7.24	0.000	1.60
K1A2	0.0806	0.0225	3.59	0.001	1.60

Figure C7. *Enterobacteriaceae* results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping													
10 control	2	6.75500	A													
8 control	2	5.82000		B												
10 K1A1	2	5.73000		B	C											
9 control	2	5.69000		B	C											
9 K1A2	2	5.64300		B	C	D										
6 control	2	5.57450		B	C	D	E									
10 K2A1	2	5.52750		B	C	D	E									
9 K2A1	2	5.35000			C	D	E									
10 K1A2	2	5.26000				D	E									
10 K2A2	2	5.23500					E									
9 K1A1	2	4.36500						F								
9 K2A2	2	4.35500						F								
4 control	2	4.26500						F								
0 control	2	3.07950							G							
8 K1A2	2	2.88500							G	H						
6 K2A2	2	2.59600								H	I					
6 K1A2	2	2.50000								H	I					
6 K1A1	2	2.22500									I	J				
8 K1A1	2	2.04500										J	K			
0 K1A2	2	1.94000										J	K			
0 K1A1	2	1.84000										J	K	L		
4 K1A2	2	1.76000											K	L		
4 K1A1	2	1.54000												L		
2 control	2	1.46685												L		
8 K2A2	2	1.07000													M	
0 K2A1	2	0.76500													M	N
2 K2A1	2	0.76000													M	N
2 K1A1	2	0.54000														N
0 K2A2	2	0.53500														N
4 K2A2	2	0.00000														O
2 K2A2	2	-0.00000														O
8 K2A1	2	-0.00000														O
6 K2A1	2	-0.00000														O
2 K1A2	2	-0.00000														O
4 K2A1	2	-0.00000														O

Means that do not share a letter are significantly different.

General Linear Model: Pseudomonas versus Storage Time & Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	369.913	61.6522	11456.61	0.000
Sample Groups	4	79.791	19.9477	3706.81	0.000
Storage Time*Sample Groups	24	29.786	1.2411	230.62	0.000
Error	35	0.188	0.0054		
Total	69	479.678			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0733578	99.96%	99.92%	99.84%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.45871	0.00877	394.47	0.000	
Storage Time					
0	-2.7297	0.0215	-127.10	0.000	1.71
2	-2.2687	0.0215	-105.63	0.000	1.71
4	-2.1372	0.0215	-99.51	0.000	1.71
6	0.0603	0.0215	2.81	0.008	1.71
8	1.0501	0.0215	48.89	0.000	1.71
9	2.4802	0.0215	115.48	0.000	1.71
Sample Groups					
control	1.9387	0.0175	110.56	0.000	1.60
K1A1	-0.3585	0.0175	-20.44	0.000	1.60
K1A2	0.2736	0.0175	15.61	0.000	1.60

Figure C8. *Pseudomonas* results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping													
10 control	2	7.83000	A													
10 K1A1	2	7.73000	A													
10 K1A2	2	6.78400		B												
9 K1A1	2	6.66900		B	C											
8 control	2	6.65885		B	C											
9 control	2	6.65300		B	C											
9 K1A2	2	6.47750			C	D										
6 control	2	6.44500			C	D										
10 K2A1	2	6.34000				D										
10 K2A2	2	6.33500				D										
9 K2A1	2	5.05000					E									
8 K2A1	2	4.86000					E	F								
9 K2A2	2	4.84500					E	F								
8 K1A2	2	4.67000						F	G							
6 K1A2	2	4.45000							G							
4 control	2	4.01000								H						
2 control	2	3.54000									I					
8 K1A1	2	3.22500										J				
8 K2A2	2	3.13000										J				
6 K1A1	2	2.92500										J	K			
6 K2A2	2	2.73000											K			
0 control	2	2.64500											K			
4 K1A2	2	1.44500												L		
2 K1A2	2	1.30000												L	M	
4 K1A1	2	1.15250												L	M	
2 K2A1	2	1.11000													M	
6 K2A1	2	1.04500													M	
0 K1A2	2	1.00000													M	
4 K2A2	2	0.00000														N
2 K2A2	2	-0.00000														N
0 K2A2	2	-0.00000														N
2 K1A1	2	-0.00000														N
0 K2A1	2	-0.00000														N
4 K2A1	2	-0.00000														N
0 K1A1	2	-0.00000														N

Means that do not share a letter are significantly different.

APPENDIX D. RESULTS OF COLOR MEASUREMENT

General Linear Model: L versus Storage Time, Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	684.47	114.08	9.33	0.000
Sample Groups	4	57.40	14.35	1.17	0.330
Storage Time*Sample Groups	24	453.97	18.92	1.55	0.081
Error	70	855.70	12.22		
Total	104	2051.55			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.49633	58.29%	38.03%	6.15%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	55.967	0.341	164.03	0.000	
Storage Time					
0	4.446	0.836	5.32	0.000	1.71
2	3.192	0.836	3.82	0.000	1.71
4	-0.518	0.836	-0.62	0.538	1.71
6	-0.592	0.836	-0.71	0.481	1.71
8	-1.502	0.836	-1.80	0.077	1.71
9	-2.812	0.836	-3.36	0.001	1.71
Sample Groups					
Control	-0.855	0.682	-1.25	0.214	1.60
K1A1	-0.086	0.682	-0.13	0.900	1.60
K1A2	-0.738	0.682	-1.08	0.283	1.60
K2A1	0.701	0.682	1.03	0.308	1.60
Storage Time*Sample Groups					

Figure D1. Color measurement results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for L value

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping		
0 K2A1	3	65.7433	A		
0 Control	3	63.2067	A	B	
2 K1A1	3	61.6300	A	B	C
2 K2A1	3	60.4167	A	B	C
2 K2A2	3	59.9800	A	B	C
2 K1A2	3	58.6467	A	B	C
0 K1A1	3	58.4467	A	B	C
8 K2A2	3	58.4067	A	B	C
4 K1A2	3	58.3500	A	B	C
4 K2A1	3	58.2867	A	B	C
0 K2A2	3	58.0467	A	B	C
6 K1A1	3	57.7367	A	B	C
0 K1A2	3	56.6200	A	B	C
6 K2A2	3	56.4067	A	B	C
4 K2A2	3	55.8933	A	B	C
6 Control	3	55.8533	A	B	C
10 K2A2	3	55.4167	A	B	C
2 Control	3	55.1200	A	B	C
6 K2A1	3	54.7233	A	B	C
9 K2A2	3	54.4733	A	B	C
8 K1A2	3	54.1933		B	C
8 Control	3	54.1133		B	C
9 K1A1	3	54.0900		B	C
10 K2A1	3	53.7633		B	C
10 K1A2	3	53.7633		B	C
8 K1A1	3	53.4900		B	C
10 K1A1	3	52.9567		B	C
9 K1A2	3	52.8700		B	C
10 Control	3	52.8667		B	C
4 K1A1	3	52.8167		B	C
9 Control	3	52.7233		B	C
6 K1A2	3	52.1567		B	C
8 K2A1	3	52.1200		B	C
4 Control	3	51.9000		B	C
9 K2A1	3	51.6200			C

Means that do not share a letter are significantly different.

General Linear Model: a versus Storage Time, Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Time	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Time	6	108.87	18.145	6.93	0.000
Sample Groups	4	18.08	4.519	1.73	0.154
Storage Time*Sample Groups	24	108.98	4.541	1.73	0.039
Error	70	183.23	2.618		
Total	104	419.16			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.61788	56.29%	35.05%	1.65%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.837	0.158	24.30	0.000	
Storage Time					
0	-1.165	0.387	-3.01	0.004	1.71
2	0.459	0.387	1.19	0.239	1.71
4	0.835	0.387	2.16	0.034	1.71
6	-0.397	0.387	-1.03	0.308	1.71
8	1.811	0.387	4.68	0.000	1.71
9	-1.200	0.387	-3.10	0.003	1.71
Sample Groups					
Control	-0.672	0.316	-2.13	0.037	1.60
K1A1	0.384	0.316	1.22	0.228	1.60
K1A2	-0.205	0.316	-0.65	0.519	1.60
K2A1	0.469	0.316	1.48	0.142	1.60
Storage Time*Sample Groups					

Figure D2. Color measurement results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for a* value

Tukey Pairwise Comparisons: Storage Time*Sample Groups

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping		
8 K1A2	3	7.03667	A		
8 K2A1	3	6.60667	A	B	
4 K1A1	3	5.94000	A	B	C
2 K1A1	3	5.70333	A	B	C
4 K2A1	3	5.34000	A	B	C
8 K2A2	3	5.32667	A	B	C
10 K2A1	3	5.19000	A	B	C
4 K2A2	3	4.96000	A	B	C
10 K2A2	3	4.91333	A	B	C
9 K1A1	3	4.88333	A	B	C
8 K1A1	3	4.66000	A	B	C
8 Control	3	4.60667	A	B	C
6 K2A2	3	4.51333	A	B	C
2 K1A2	3	4.45333	A	B	C
6 K1A1	3	4.30333	A	B	C
2 Control	3	4.19333	A	B	C
6 K1A2	3	4.08667	A	B	C
2 K2A1	3	3.91333	A	B	C
4 Control	3	3.89667	A	B	C
0 K2A1	3	3.77333	A	B	C
0 K2A2	3	3.33667	A	B	C
4 K1A2	3	3.22000	A	B	C
2 K2A2	3	3.21667	A	B	C
0 Control	3	3.20667	A	B	C
9 K1A2	3	3.11333	A	B	C
10 Control	3	2.84333	A	B	C
9 K2A1	3	2.75000	A	B	C
6 K2A1	3	2.56333	A	B	C
10 K1A2	3	2.45000	A	B	C
10 K1A1	3	2.07333	A	B	C
0 K1A1	3	1.98000	A	B	C
6 Control	3	1.73000		B	C
9 Control	3	1.67667		B	C
0 K1A2	3	1.06333			C
9 K2A2	3	0.76000			C

Means that do not share a letter are significantly different.

General Linear Model: b versus Storage Tıme, Sample Groups

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Storage Tıme	Fixed	7	0, 2, 4, 6, 8, 9, 10
Sample Groups	Fixed	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage Tıme	6	123.35	20.559	4.74	0.000
Sample Groups	4	19.64	4.909	1.13	0.349
Storage Tıme*Sample Groups	24	184.29	7.679	1.77	0.034
Error	70	303.93	4.342		
Total	104	631.22			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.08372	51.85%	28.46%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.487	0.203	7.31	0.000	
Storage Tıme					
0	-1.560	0.498	-3.13	0.003	1.71
2	0.522	0.498	1.05	0.298	1.71
4	1.234	0.498	2.48	0.016	1.71
6	0.736	0.498	1.48	0.144	1.71
8	0.891	0.498	1.79	0.078	1.71
9	-0.205	0.498	-0.41	0.682	1.71
Sample Groups					
Control	0.420	0.407	1.03	0.305	1.60
K1A1	-0.318	0.407	-0.78	0.437	1.60
K1A2	0.047	0.407	0.12	0.908	1.60

Figure D3. Color measurement results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for b* value

Grouping Information Using the Tukey Method and 95% Confidence

Storage Time*Sample Groups	N	Mean	Grouping	
8 K1A2	3	5.46333	A	
4 K2A2	3	4.76333	A	B
9 Control	3	4.02667	A	B
6 K2A2	3	3.58000	A	B
2 K1A2	3	3.52000	A	B
2 K2A2	3	3.29333	A	B
4 Control	3	3.28000	A	B
6 K2A1	3	3.20333	A	B
2 K1A1	3	3.10333	A	B
8 K2A2	3	2.92667	A	B
4 K1A1	3	2.64667	A	B
4 K1A2	3	1.97667	A	B
8 Control	3	1.95667	A	B
8 K2A1	3	1.86667	A	B
10 Control	3	1.83333	A	B
6 K1A1	3	1.74333	A	B
6 Control	3	1.46333	A	B
9 K2A1	3	1.38667	A	B
10 K1A1	3	1.32333	A	B
0 K2A2	3	1.18000	A	B
6 K1A2	3	1.12333	A	B
4 K2A1	3	0.93667	A	B
9 K1A1	3	0.90333	A	B
0 Control	3	0.57333	A	B
9 K1A2	3	0.30667	A	B
2 Control	3	0.21333	A	B
2 K2A1	3	-0.08667	A	B
9 K2A2	3	-0.21333	A	B
8 K1A1	3	-0.32667	A	B
0 K1A2	3	-0.43333	A	B
0 K2A1	3	-0.47667	A	B
10 K2A1	3	-0.92000	A	B
0 K1A1	3	-1.21000	A	B
10 K1A2	3	-1.22000	A	B
10 K2A2	3	-1.67333		B

Means that do not share a letter are significantly different.

APPENDIX E. RESULTS OF SENSORY ANALYSIS

One-way ANOVA: Flavor versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	1.644	0.8222	0.88	0.423
Error	42	39.333	0.9365		
Total	44	40.978			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.967733	4.01%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	15	3.667	0.724	(3.162, 4.171)
%2 Chitosan	15	3.400	1.183	(2.896, 3.904)
Control	15	3.200	0.941	(2.696, 3.704)

Pooled StDev = 0.967733

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%1 Chitosan	15	3.667	A
%2 Chitosan	15	3.400	A
Control	15	3.200	A

Means that do not share a letter are significantly different.

Figure E1. Sensory analysis results (statistically) of chicken drumsticks with different chitosan solutions for flavor

One-way ANOVA: Texture versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	1.733	0.8667	1.16	0.324
Error	42	31.467	0.7492		
Total	44	33.200			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.865567	5.22%	0.71%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	15	3.600	0.737	(3.149, 4.051)
%2 Chitosan	15	3.733	0.884	(3.282, 4.184)
Control	15	3.267	0.961	(2.816, 3.718)

Pooled StDev = 0.865567

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%2 Chitosan	15	3.733	A
%1 Chitosan	15	3.600	A
Control	15	3.267	A

Means that do not share a letter are significantly different.

Figure E2. Sensory analysis results (statistically) of chicken drumsticks with different chitosan solutions for texture

One-way ANOVA: Odor versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	0.5778	0.2889	0.42	0.658
Error	42	28.6667	0.6825		
Total	44	29.2444			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.826160	1.98%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	15	3.333	0.617	(2.903, 3.764)
%2 Chitosan	15	3.600	0.828	(3.170, 4.030)
Control	15	3.533	0.990	(3.103, 3.964)

Pooled StDev = 0.826160

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%2 Chitosan	15	3.600	A
Control	15	3.533	A
%1 Chitosan	15	3.333	A

Means that do not share a letter are significantly different.

Figure E3. Sensory analysis results (statistically) of chicken drumsticks with different chitosan solutions for odor

One-way ANOVA: Color versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	3	%1 Chitosan, %2 Chitosan, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	2	1.733	0.8667	1.91	0.161
Error	42	19.067	0.4540		
Total	44	20.800			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.673772	8.33%	3.97%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
%1 Chitosan	15	3.400	0.737	(3.049, 3.751)
%2 Chitosan	15	3.867	0.743	(3.516, 4.218)
Control	15	3.533	0.516	(3.182, 3.884)

Pooled StDev = 0.673772

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
%2 Chitosan	15	3.867	A
Control	15	3.533	A
%1 Chitosan	15	3.400	A

Means that do not share a letter are significantly different.

Figure E4. Sensory analysis results (statistically) of chicken drumsticks with different chitosan solutions for color

One-way ANOVA: Flavor versus Sample Groups

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	2.040	0.5100	0.46	0.767
Error	145	161.800	1.1159		
Total	149	163.840			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.05634	1.25%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	30	2.933	0.828	(2.552, 3.315)
K1A1	30	3.200	0.925	(2.819, 3.581)
K1A2	30	3.233	0.971	(2.852, 3.615)
K2A1	30	3.033	1.129	(2.652, 3.415)
K2A2	30	3.200	1.349	(2.819, 3.581)

Pooled StDev = 1.05634

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
K1A2	30	3.233	A
K2A2	30	3.200	A
K1A1	30	3.200	A
K2A1	30	3.033	A

Figure E5. Sensory analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for flavor

One-way ANOVA: Texture versus Sample Groups Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	1.373	0.3433	0.25	0.907
Error	145	196.100	1.3524		
Total	149	197.473			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.16293	0.70%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	30	3.200	0.997	(2.780, 3.620)
K1A1	30	3.300	1.088	(2.880, 3.720)
K1A2	30	3.067	1.112	(2.647, 3.486)
K2A1	30	3.033	1.217	(2.614, 3.453)
K2A2	30	3.167	1.367	(2.747, 3.586)

Pooled StDev = 1.16293

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
K1A1	30	3.300	A
Control	30	3.200	A
K2A2	30	3.167	A
K1A2	30	3.067	A
K2A1	30	3.033	A

Figure E6. Sensory analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for texture

One-way ANOVA: Odor versus Sample GroupsMethod

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	3.667	0.9167	0.80	0.530
Error	145	167.167	1.1529		
Total	149	170.833			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.07372	2.15%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	30	3.367	1.033	(2.979, 3.754)
K1A1	30	3.400	0.814	(3.013, 3.787)
K1A2	30	3.633	1.033	(3.246, 4.021)
K2A1	30	3.267	1.143	(2.879, 3.654)
K2A2	30	3.167	1.289	(2.779, 3.554)

Pooled StDev = 1.07372

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
K1A2	30	3.633	A
K1A1	30	3.400	A
Control	30	3.367	A
K2A1	30	3.267	A
K2A2	30	3.167	A

Figure E7. Sensory analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for odor

One-way ANOVA: Color versus Sample Groups Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample Groups	5	Control, K1A1, K1A2, K2A1, K2A2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Groups	4	2.227	0.5567	0.57	0.683
Error	145	141.033	0.9726		
Total	149	143.260			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.986227	1.55%	0.00%	0.00%

Means

Sample Groups	N	Mean	StDev	95% CI
Control	30	3.500	0.820	(3.144, 3.856)
K1A1	30	3.600	0.770	(3.244, 3.956)
K1A2	30	3.700	0.837	(3.344, 4.056)
K2A1	30	3.567	1.165	(3.211, 3.923)
K2A2	30	3.333	1.241	(2.977, 3.689)

Pooled StDev = 0.986227

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Groups	N	Mean	Grouping
K1A2	30	3.700	A
K1A1	30	3.600	A
K2A1	30	3.567	A
Control	30	3.500	A
K2A2	30	3.333	A

Figure E8. Sensory analysis results (statistically) of chicken drumsticks with combination of chitosan solutions and allicin for color