Evaluation of Microbiological Quality of Minced Beef Meat Coated with Chitosan Containing Garlic Esential Oil During Refrigerated Storage

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Abstract

The human population needs high requirements of protein which could get from red meat including mutton and beef. Beef meat is more inclined to spoilage because of high water content, suitable substrate for microbial growth. Beef meat can be preserved to enhance their shelf life by using naturally derived preservatives like chitosan and essential oils in forming edible coatings. In this study we used chitosan coating with garlic essential oil emulsion to inhibit bacterial growth in minced beef meat. We divided minced beef meat into three groups, one with chitosan coating supplemented with 0.5% garlic essential oil (G0.5) and second with chitosan coating supplemented with 1% garlic essential oil (G1) and third group with chitosan coating is control group (C). After microbiological analysis it was found that the initial total psychrophilic bacteria count was 1.91log CFU/g in beef meat which increased to 7.04 log CFU/g in C group while 5.99 log CFU/g and 5.24 log CFU/g in G0.5 and G1 respectively at the end of storage. Total mesophilic bacteria count was 2.27log CFU/g at the beginning of storage which increased to 7.33, 6.07 and 5.44 log CFU/g in C, G0.5 and G1 respectively at the end of the storage. Similarly Total Enterobacteriaceae count was found to be 1.59 log CFU/g at the beginning of the storage, which increased to 4.77, 3.78 and 3.26 log CFU/g in the C, G0.5 and G1 respectively. These results presented that G1 has less bacterial growth overall which proves that 1% garlic essential oil with chitosan coating could be good for decreasing bacterial growth in minced beef meat products for long time preservation.

Keywords: *Microbial spoilage, chitosan coating, essential oil, shelf life*

Introduction

Conferring the continually expanding human population, the nutritional issues resulting from animal protein deficiencies are extremely important to humans. Consequently, red meat species have a significant part in human nutrition as an animal-based diet (Mehta et al., 2015). Due to their characteristics (a favorable substrate for microbes, a suitable pH, high water activity, unsaturated fatty acid content, etc.), red meats have a limited shelf life, which makes storage and transit difficult (Olivera et al., 2013). Therefore, it must be consumed and processed quickly; otherwise, the items will be subject to chemical, microbiological, and sensory spoiling. Chemical and microbiological spoils are more of a problem than the degradation of the sensory qualities, even if spoiling impacts sensory qualities including color changes and putrefaction (Devatkal et al., 2014).

Packaging technology is frequently utilized to preserve quality, prolong shelf life, and maintain cleanliness, particularly in foods that are susceptible to oxidative and microbiological spoiling (Ahmad et al., 2012). Edible coating materials have been employed in food preservation in recent years (Aydin et al., 2017). Natural resources are used to create thin-layered, non-synthetic packaging materials that are produced on the surface of food products and ingested with the meal. These materials regulate the passage of solids, gases, and moisture. Applying edible films or coatings on food's outside or in between layers helps extend its shelf life and shield it from the elements (Işık et al., 2013). Edible coatings are becoming more popular in the food business because of their low cost, minimal technical requirements, and effective preservation (Beikzadeh et al., 2020; Duran & Kahve, 2016).

Meanwhile beef naturally spoils quickly the process is complex and involves a combination of microbiological, chemical, and physical changes. The main reasons for foodborne diseases and shorter shelf lives in beef are microbial contamination and lipid oxidation during processing and storage (Rao et al., 2008). People are becoming increasingly conscious of the harmful health effects associated with the use of chemical preservatives, such as potassium sorbate, sodium nitrate, and benzoic acid. To prevent the oxidation of lipids and proteins, researchers and the food industry concentrate on the consumption of naturally derived products, such as chitosan (Latou et al., 2014), bacteriocins (Lorenzo et al., 2018; Karimaei et al., 2022), and essential oils (EOs) (Simona et al., 2021; Soltan Dallal et al., 2024). The quality and shelf life of food items can be improved by applying active edible coatings (Raji et al., 2019).

Deacetylation of chitin yields chitosan, which is one of the most significant edible coatings utilized globally (Abdou et al., 2008; Kuzgun & İnanlı, 2013). Applying chitosan, a polysaccharide-based coating, to food's exterior helps regulate its morphological, physiochemical, and physiological alterations (Duran & Kahve, 2016). According to Coma et al. (2002), chitosan films have antibacterial and antioxidant properties and can regulate the permeability of oxygen and moisture in food. Chitosan's polycationic characteristics give it an antibacterial action. In fact, because it interacts to neutralize negatively charged molecules, it works well against germs. At pH 6.3, the amine group of the glucose monomer of chitosan interacts electrostatically with negatively charged microbial cell membranes through its pKa value. As a result, the microbial membranes become less intact, which causes the inner and outer membranes to leak cell components (Helander et al., 2001; Liu et al., 2004).

The Food and Drug Administration (FDA) has designated essential oils (EOs) derived from natural sources as safe (GRAS), meaning they are generally safe for consumer consumption (Jackson-Davis et al., 2023). The fruits, buds, flowers, leaves, seeds, and roots of many plants can all provide these essential oils. As for their antibacterial qualities, EOs may be a suitable substitute for antibiotics (Calo et al., 2015; Karimaei et al., 2024). Thus, the increasing desire for natural food additives has led to a rise in the usage of EOs as potential alternative antimicrobials (Wi´nska et al., 2019). These secondary metabolites prevent food pathogens and spoilage bacteria from growing (de Silva et al., 2021). However, essential oils' stability during processing and storage is diminished by their hydrophobic and volatile characteristics as well as their susceptibility to light and oxygen. Furthermore, effective application levels are limited in direct applications due to the potential for essential oils to promote organoleptic degradation in meals. Thus, another method is to combine essential oils with edible coatings or films. Edible coatings become more stable and effective when emulsions made with essential oils are added (Ucak & Afreen, 2022; Renur et al., 2016; Ebadi et al., 2019; Ucak et al., 2021; Ucak et al., 2019). As a result, the primary goal of this research is to make an emulsion with garlic essential oil and then cover minced beef flesh with the resulting chitosan coating. It is said to prevent the growth of microorganisms in beef meat when it is being stored in the refrigerator.

Materials and Methods

Materials

Minced beef meat Garlic essential oil provided from local market in Niğde region.

Method

Preparation of chitosan coatings and application to minced beef meat samples

Chitosan solution was prepared as the prescribed method (Ojagh et al., 2010). Chitosan (Chitosan from shrimp shells, ≥75% -deacetylated chitin) used by mixing and stirring 1g of 2% chitosan (w/v) in 100ml of 1% acetic acid (v/v), aqueous solution at room temperature for 3 hours. To provide the solution plasticity, glycerol (0.75 ml/g) and 75% (w/w) chitosan were then added. The mixture was then stirred with a magnetic stirrer for 10 minutes at 95°C. Emulsions were made using Tween 80 at the same rate as 0.5% and 1% concentration of garlic essential oil, as established in early studies. The ultrathorax homogenizer was used to homogenize the emulsions for three minutes at 70°C and 13.500 rpm. After cooling to 55°C, the emulsion was left for some time to allow the gas bubbles to go.

Three groups were control group (C) without coating, chitosan coated groups supplemented with 0.5% (G0.5) and 1% (G1) concenteration of garlic essential oil were prepared. Every sample was shaped into small balls of 10g. After 30 seconds of soaking in the previously made chitosan solutions, the minced beef meat was left to stand for two minutes. They were then given another 30-second soak in the solution and given another 2-minute drying period (Ojagh et al., 2010). Every sample was wrapped in stretch film and placed on styrofoam plates. The control group consisted of one set of minced beef meat that was not covered with chitosan solution. Every group was stored on styrofoam plates and wrapped in stretch film. After every two days, quality analyses were performed on all samples which were kept at 4ºC±1 in a refrigerator.

Microbiological analysis

To perform microbiological investigations, a beef meatball sample of 10g was homogenized in 90ml of ringer solution. One ringer tablet was diluted in 500 milliliters of distilled water to create the ringer solution, which was then autoclaved, and nine milliliters were added to tubes to create serial dilutions. The smear culture technique was used for the counts of total mesophilic and total psychrophilic bacteria into Plate Count Agar media. The plates were incubated for two days at 30°C and for one week at 8–10°C correspondingly (Anonymous, 1998). Dilutions were inoculated into Violet Red Bile Agar (VRBA) medium using the bulk sowing method, and the samples were cultured for 24 to 48 hours at 37°C to determine the number of Enterobacteriaceae (Anonymous, 1998).

Statistical analysis

The data was compared using the Duncan multiple comparison test (one-way Anova at $P<0.05$ significance level) and statistical analyses were conducted using SPSS software (Statistical Analysis System, Cary, NC, USA).

Results and Discussion

Bacterial growth is one of the main causes of spoilage of meat and meat products. The effect of chitosan coating enriched with garlic essential oil emulsion on the total psychrophilic bacteria count in the minced beef meat is presented in figure 1. Significant differences ($P<0.05$) were observed between control group and chitosan coating treated groups with different concenterations of essential oil emulsions during the storage time. The initial total psychrophilic bacteria count was determined to be 1.91log CFU/g in beef meat. During the storage period, this value increased in all samples as control group has 7.04 log CFU/g while G0.5 and G1 have 5.99 log CFU/g and 5.24 log CFU/g respectively at the 10^{th} day of storage. It shows that control group has the highest bacterial growth while G1 group of chitosan coating with 1% garlic essential oil emulsion has strong antibacterial effect.

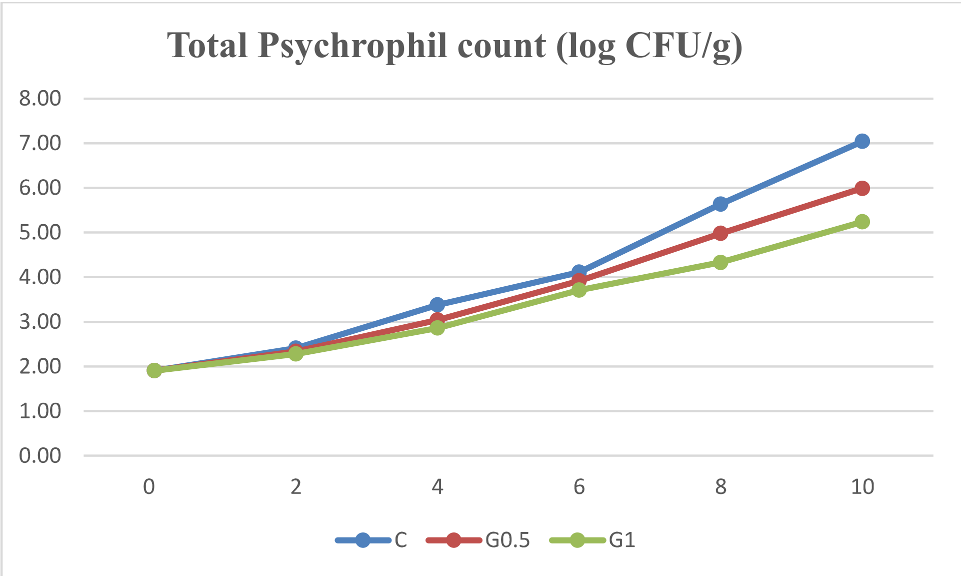

Figure 1. Changes in total psychrophilic bacteria count of minced beef meat during storage.

Total mesophilic bacteria count was 2.27log CFU/g in the minced beef meat at the beginning of storage. This number increased in all groups and control groups have the highest value of bacterial growth as 7.33 log CFU/g while G0.5 and G1 have 6.07 log CFU/g and 5.44 log CFU/g respectively at the end of the storage. The effect of chitosan coating enriched with garlic essential oil emulsion on the total mesophilic bacteria count in the minced beef meat is presented in figure 2.

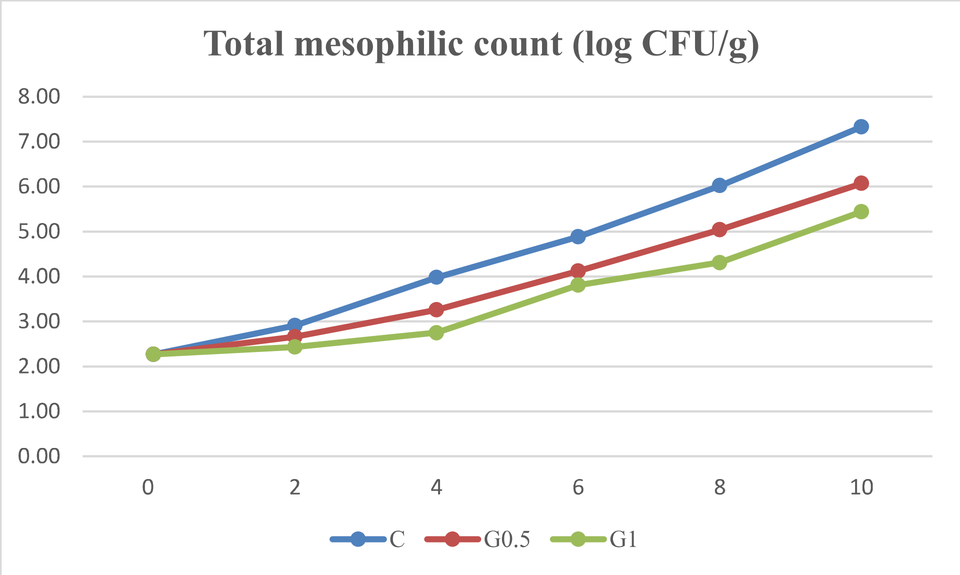

Figure 2. Changes in total mesophilic bacteria count of minced beef meat during storage.

The total number of Enterobacteriaceae is accepted as an indicator of hygiene in fish. Total Enterobacteriaceae count of the minced beef meat was found to be 1.59 log CFU/g at the beginning of the storage. At the end of the storage this value reached 4.77 log CFU/g in the control group while G0.5 and G1 reached 3.78 log CFU/g and 3.26 log CFU/g respectively. The effect of chitosan coating enriched with garlic essential oil emulsion on the total Enterobacteriaceae count in the minced beef meat is presented in figure 3.

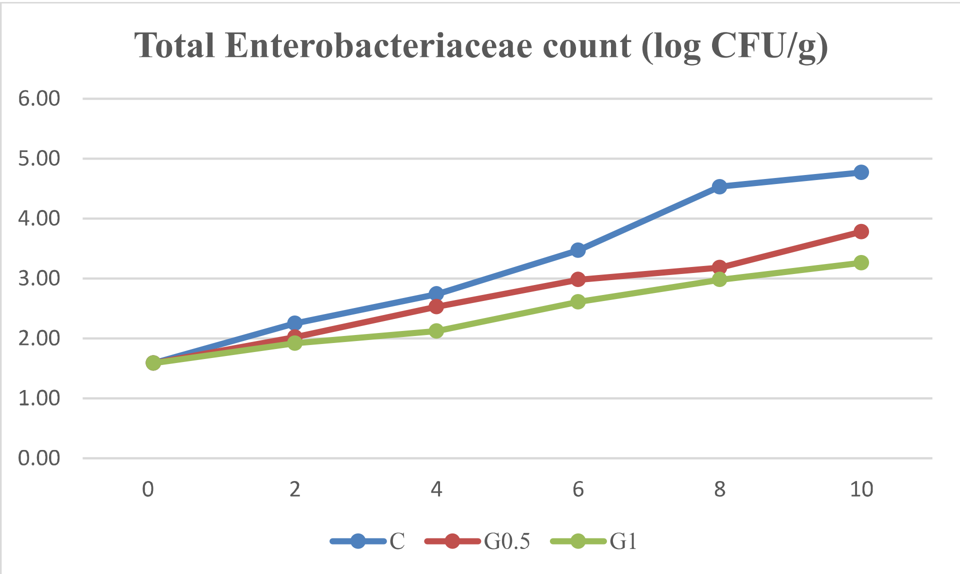

Figure 3. Changes in total Enterobacteriaceae count of minced beef meat during storage.

It was found that the G1 group with chitosan coating combined with 1% garlic essential oil showed strong antimicrobial effect. So, chitosan coating with garlic essential oil emulsion can be used to extend the shelf life of minced beef meat.

Antimicrobial properties of rosemary have been shown in studies using meatballs (Fernández-López et al., 2005), boiling beef (Ahn et al., 2007), lamb meat packed in a modified environment (Camo et al., 2008), and pig sausage (Pandit & Shelef, 1994). The compounds rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, and isorosmanol work together to suppress the growth of microbes. These substances interact with the cell membrane, change nutrition, fatty acid synthesis, electron transport, genetic material, and cause cellular components to leak (Nieto et al., 2018).

Our findings are consistent with a different study that found that minced beef samples coated with a chitosan–kombucha tea coating exhibited a delay in microbial development throughout storage when compared to samples that were not coated (Ashrafi et al., 2018). Like the current findings, Hasani-Javanmardi et al. showed that a mixture of safflower oil and cumin essential oil reduced the amount of lactic acid bacteria, mesophilic and psychrophilic bacteria, and Enterobacteriaceae in samples of lamb meat (Hasani-Javanmardi et al., 2021). According to Langroodi et al. (2021), turkey breast flesh coated with chitosan and grape seed extract exhibited antibacterial activity against Enterobacteriaceae. According to other research, chitosan–cinnamon essential oil prevented the development of Enterobacteriaceae in beef patties both during storage and while refrigerated (Ghaderi-Ghahfarokhi et al., 2017). Georgantelis et al. (2007b) also found that samples containing chitosan and rosemary had the lowest microbial counts in pork sausages, suggesting a potential synergistic impact. Through MICs ranging from 0.01% to 1%, chitosan's antibacterial efficacy against several food spoilages and pathogenic microorganisms is well-established (Sagoo et al., 2002).

Conclusion

The study's results suggest that adding an emulsion of garlic essential oil to the chitosan coating can prevent microbiological development in beef meat for ten days while it is stored in the refrigerator. Therefore, to improve the microbiological quality of minced beef meat, garlic essential oil might be suggested as a natural antibacterial ingredient with chitosan coating.

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