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Effect of Edible Garden Cress Mucilage (*Lepidium sativum*) Coatings Containing Chitosan and Black Pepper Oil on Quality and Preservation of Chilled Beef Burger

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ABSTRACT

This study explores the potential of edible coatings to extend the shelf life of beef burgers, focusing on health and market demands. The research evaluated the effectiveness of garden cress mucilage (GSM) seed extract as an edible coating, with or without black pepper oil (BPO) and chitosan (CH), in preserving beef burgers stored at 4±1°C for 12th days. Five treatments were tested: an uncoated control, GSM alone, GSM with BPO, GSM with CH, and GSM with both CH and BPO. The coated burgers were stored in PET bags and their physical, chemical and microbiological characteristics were assessed, including cook loss, moisture content, texture, pH, lipid oxidation, microbial counts and sensory qualities, over the storage period. The results showed that GSM coatings significantly improved the quality of the chilled beef burgers, delaying cook loss, maintaining texture and reducing lipid oxidation, especially in the GSM+CH+BPO combination. Microbial counts were lower in all coated samples compared to the control and the GSM coatings maintained high sensory quality, with no off-tastes or color changes. The control group had the most variability in quality, while the GSM+CH+BPO coated samples had the most consistent quality. Overall, the study highlights the potential of garden cress mucilage, particularly when combined with chitosan and black pepper oil, as a natural alternative to enhance the shelf life and safety of refrigerated beef burgers.

1. Introduction

With the escalating global consumption of meat products, concerns about sustainability, fat content, and shelf life are growing. Traditional methods of preserving meat often involve artificial additives that can pose health and environmental risks. Natural polysaccharides, such as seed mucilage, offer a promising alternative due to their functional properties like emulsification, film formation, and water retention. This study investigates the potential of using seed mucilage in meat processing and preservation. Its water-holding and emulsifying capabilities could help reduce fat content, while its antibacterial and film-forming properties might enhance shelf life by inhibiting microbial growth and oxidation. However, the long-term stability of these hydrophilic polymers is essential for maintaining product quality. Additionally, while seed mucilage-based coatings can provide unique flavors, their strong taste and natural additive nature might present challenges for consumer acceptance (Xueqin et al., 2024).

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Beef, particularly ground beef products, contains high levels of protein, fats and moisture, making it highly susceptible to microbial growth. The beef industry is grappling with growing concerns about sustainability, texture and fat content. As consumers seek healthier options without sacrificing flavor or texture, demand for low- and reduced-fat beef products is on the rise. This trend reflects a broader shift in dietary preferences toward lower fat intake while maintaining taste (Safdar et al., 2022). Meat can undergo lipid oxidation, which can deteriorate its sensory qualities. This process leads to the formation of chemicals such as acids, alcohols, ketones, hydrocarbons and aldehydes resulting in off-flavors and odors (Reda et al., 2016). Red meat is prone to lipid oxidation, which can cause a reduction in color. Ground beef is a crucial component of meat products especially hamburgers. The grinding process damages the muscle membrane increasing the surface area and promoting lipid oxidation and microbial growth in stored meat products (Hawashin et al., 2016). The potential sources of contamination include the animal's health before slaughter as well as consumer handling, transportation and marketing practices. Microorganisms such as *Pseudomonas spp*. and *Enterobacteriaceae* can cause spoilage (Martins et al., 2012). Contamination may be due to the presence of psychrotrophic and pathogenic species including *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Campylobacter jejuni* and *Yersinia enterocolitica*. Enteropathogenic bacteria like *E. coli* and *Salmonella ssp*. are significant concerns (Comi et al., 2015). In recent years, there has been a growing trend toward using natural additives instead of synthetic ones in the meat industry. This shift is driven by consumer concerns about the potential negative effects of chemical additives such as their ability to inhibit lipid oxidation and microbial growth (Martins et al., 2012).

Plastic packaging has seen a substantial increase in usage in recent years. A current trend in the food sector is to replace petro-based plastic materials with compostable and natural alternatives. The application of edible films or bio-films as a substitute for petrochemical polymers is a compelling perspective offering an alternative to nondegradable materials. This has led to a recent surge in the use of edible coatings and films due to concerns about environmental hazards as well as the cost and availability of petroleum (Janjarasskul & Krochta 2010). Foodborne pathogens and oxidation are significant factors that impact qualitative characteristics during processing and storage (Wu et al., 2019). Edible coatings have recently gained significant attention as innovative food packaging solutions that enhance quality and improve shelf-life by reducing physical, chemical and biological deterioration (Ojagh et al., 2010). The use of cress seed mucilage *(Lepidium sativum L*.) as a pharmaceutical ingredient has become increasingly common due to the growing demand for natural components. The seeds contain significant quantities of protein, fat, calcium, iron and have high nutritional value. They have been recognized as galactagogues, anticarcinogens, antidiabetics, antiasthmatics and antidiarrheals. The recent discovery of the promising, intriguing and valuable properties of cress seed has highlighted its potential for creating beneficial pharmacological dosage forms. *Lepidium sativum Linn*. is a rapidly growing annual plant that contains mucilage and is also edible. The various components of the plant, including the roots, leaves and seeds have been used to treat numerous human ailments (Kamlesh et al., 2022).*Lepidium sativum*, also known as garden cress or pepper cress, is a member of the Brassicaceae family (Mohite et al., 2012). The garden cress plant consists of seeds, leaves and branches each containing distinct phytochemicals such as flavonoids, glycosides, alkaloids and polyketides. Additionally, these components also contain vitamins, minerals, proteins, lipids and carbohydrates. Garden cress primarily contains oleic acid (23%) and linolenic acid (33%), which are both essential fatty acids. The main sterol is β-sitosterol (50%) followed by tocopherol at a level of 1.5-1.9g/kg, with α-tocopherol being the predominant form (Chatoui et al., 2020). Garden cress has gained recognition for its diverse medicinal and herbal properties due to the presence of phytochem-

phytochemicals (Baregma & Goyal, 2019). Garden cress mucilage, a water-soluble hydrocolloid, can be used as a raw material to produce edible films and coatings. This is due to its mechanical, optical, barrier and physical properties which are crucial for food packaging applications (Jouk et al., 2013). The seeds of *L. sativum* are brown in color and have an oval shape. When immersed in water, they readily absorb water and form a viscous and flavorless substance. The seeds are rich in mucilaginous compounds and have been found to possess high molecular weight gum (Karazhiyan et al., 2011). Previous studies (Salehi 2019) investigated the biodegradable and edible properties of the GSM coating, as well as the characterization of the seeds according to their physical, microstructural, mechanical, and thermal features. The application of garden cress seed gum containing 10% carvacrol resulted in a significant enhancement in the microbiological, chemical and sensory quality of shrimp samples. Furthermore, the shelf-life of the samples improved when stored under cold conditions. This finding was reported by Karamkhani et al. (2018). Edible films and coatings offer significant advantages such as biocompatibility, eco-friendliness (resulting in a 66% reduction in total packaging waste), affordability and exceptional barrier properties against gases, lipids and aromas. In addition, edible coatings can serve as a medium for incorporating natural bioactive components and food additives, such as vitamins, antioxidants and antibacterial substances (El-Mogy 2020 & Rizzo and Muratore 2020). A novel active edible coating, combining Satureja hortensis essential oil and *Lepidium sativum* seed mucilage, was developed and applied to lamb meat. Stored at 4°C for eighteen days, the coated lamb meat exhibited enhanced quality and extended shelf life compared to uncoated samples (Mina et al., 2024). Chitosan, a naturally produced polymer, is non-toxic, biodegradable and economically viable. These characteristics make it suitable for many uses in the food industry, where it can be used as an alternative to non -biodegradable polymers for quality assurance purposes (Dos Santos et al., 2020). Chitosan is a naturally produced carbohydrate biopolymer that contains many functional groups, such as primary -OH, secondary -OH and NH (Li & Yu 2001 & Abbasi et al., 2009). Numerous investigators (Amadio et al., 2011) have found that plant essential oils (EOs) had the capacity to preserve food from pathogenic and spoilage bacteria as well as oxidation. However, the type of essential oils required to produce efficient antibacterial and antioxidant activity plays an important role in assessing sensory acceptance. This is because strong odors from the essential oils can be absorbed into food products (Chouliara et al., 2007). Hence, future research should prioritize the integration of essential oils into edible coatings as an additional method in food packaging. Chitosan a naturally occurring polymer is non-toxic, biodegradable and economically viable. These characteristics make it useful in many applications in the food industry, where it can serve as a replacement for non-biodegradable polymers to ensure quality (Dos Santos et al., 2020). Several researchers Amadio et al. (2011) have discovered that plant essential oils (EOs) possess the ability to preserve food from both harmful bacteria and spoilage, as well as from oxidation. However, the specific type of essential oils needed to have significant antioxidant activity and antibacterial properties is crucial in evaluating sensory properties. The absorption of strong scents from essential oils into food products is the reason for this phenomenon (Chouliara et al., 2007). Therefore, it is recommended that future studies prioritize the incorporation of essential oils into edible coatings as an additional technique in food processing packaging. Therefore, the aim of this research was to investigate the ability of the edible coating from garden cress seed mucilage *(Lepidium sativum),* enhanced with or without the addition of chitosan and essential black pepper oil, to improve the shelf life, microbial load and chemical properties of chilled beef burgers through cold storage at 4±1°C for 12th days.

2. Materials and Methods

Materials

Golden grass seed (*Lepidium sativum*) was purchased from the local market in Kafr El-Sheikh, Egypt. Chitosan was purchased from the Mifad

Company, Badr City, Egypt. Black pepper oil was purchased from the Gretco Essential Oils Company, Giza Governorate, Egypt.

Fresh meat samples were obtained from a local market in Kafr El-Sheikh City, Egypt, directly after slaughter. All chemicals used were of analytical grade and were obtained from Sigma – Aldrich Elgomhoria Company.

Methods

Extraction of Garden Seeds Mucilage (GSM)

The GSM coatings were manufactured using the process suggested by Karazhiyan et al., (2011), with certain modifications.

Approximately 100 grams of cress seeds were first sifted to exclude immature and damaged seeds. Following this, they were cleaned by soaking them in three times their weight of ethanol for 15 minutes, while being constantly agitated. After removing the ethanol residue by drying the seeds in an oven at 70°C, the seeds were subsequently soaked in distilled water (with a ratio of 30 parts distilled water to 1 part seed by volume/weight) at 40°C for a duration of 8 hours. The seed-water mixture was gradually stirred during the soaking period.

After that, a blender (Blender, Moulinex 400W, Model: LM2420, French) was used to roughly mix the seeds in 1 L of distilled water at room temperature for 15 minutes, keeping a continuous stirring speed of 1500 rpm. The gum was separated from the swelled seeds through a filtration process using cheese cloth.

Burger manufacture

Minced meat was purchased from a commercial producer. The beef burgers were produced using a traditional recipe consisting of 85% ground beef (with a fat percentage of 20%), 7.5% onion, 1.5% salt, 1% seasonings, 5% soybean and 5% water. The ingredients were thoroughly mixed in a bowl mixer (Tefal, QA400, France) for a duration of 5 minutes, serving as a standard.

Beef burgers weighing approximately 30±2 grams were created. Each meatball had an approximate width of 5 cm and a thickness of 1 cm.

Preparing the coatings solution and coated samples

Firstly, the seed mucilage extract was pasteurized at a temperature of 90°C for 1 minute, followed by the addition of 0.5% glycerol.

Next, we assembled the various treatments as follows:

- Control: Uncoated burger
- GSM: Coating with garden seed mucilage extract
- GSM+CH: Coating with garden seed mucilage extract and 0.25% chitosan
- GSM+BPO: Coating with garden seed mucilage extract and 1% black pepper oil
- GSM+CH+BPO: Coating with garden seed mucilage extract, 0.25% chitosan and 1% black pepper oil

The chitosan solution was made by dissolving it in distilled water containing 1% (v/v) acetic acid and stirring it for 30 minutes until complete dissolution at room temperature using a magnetic stirrer. Then, the black pepper oil was added and stirring was continued for another 5 minutes. For three minutes, the beef burgers were completely soaked in each coating; after that, sieves were used to remove excess liquid. Following the application of the treatments, the burgers were placed in PET trays (polyethylene terephthalate) and covered. These packages were then stored in a refrigerator at a temperature of 4±1°C for a duration of 12th days.

Microbiological analyses

The following tests were used to investigate the microbial count of the beef burgers during storage time:

- Number of viable bacteria
- Number of psychrotrophic bacteria
- Number of Escherichia coli bacteria
- Number of mold and yeast on potato dextrose agar at a temperature of 27°C for a duration of 72 hours

A 45 ml aliquot of sterile peptone water was added to the sample, using aseptic techniques. The combination underwent homogenization at a speed of 2000 rpm using a homogenizer (Electro+ plus,

Model SHG-307) for approximately 2 minutes to achieve a dilution of 1/10.

The user conducted a serial dilution using sterile peptone water at dilution ratios of 1/100, 1/1000, and 1/10000. The total number of bacteria was determined using Plate Count Agar (PCA) medium, which was incubated at a temperature of 30°C for 48 hours. To enumerate the psychrophilic bacteria, the same medium (Shahbazi, et al., 2016*)* was used but incubated at a temperature of 7°C for 10th days.

The presence of *E. coli* was determined using MacConkey agar (MAC) following the method described by (ISO 16654).

Physical and chemical analysis

The chemical and texture analyses were performed in triplicate on the day of processing (considered as day 0), as well as on days 3, 6, 9, and 12 of storage. While the physical analyses were carried out at zero time.

Determination of cooking loss (CL)

Cooking loss of beef burger was calculated as described by Erdogdu et al. (2007) following the formula:

Cooking Loss (CL %) = (Mi - Mf) / Mi × 100

Where: Mi = Initial mass of the raw burger in gram, Mf = Final mass of the cooked burger in grams

Determination of cooking yield

Cooking yield was determined and calculated according to Murphy et al. (1975):

Cook yields = (Weight of cooked meat ball)/ (Weight of raw meat ball) ×100

Determination of Beef burger shrinkage

Beef burger shrinkage is the difference between the raw and cooked areas of the beef burger sample, expressed as a percentage of the raw area according to (Darweash and Moghazy, 1998*)* following the equation:

Beef meatball shrinkage % = (Fresh burger area - cooked burger area) / (Fresh burger area) × 100.

Moisture content

The moisture contents were carried out following the method described by (AOAC, 2000).

pH measurement

pH values were determined using the method described by Yetim et al. (2011).

Approximately 5 grams of the sample was blended with 50 mL of deionized distilled water. The mixture was then filtered, and pH was measured using a digital pH meter (Hanna Instruments, Milano, Italy).

Texture analysis

The texture measurement of beef burgers was evaluated using a texture analyzer as outlined by Zhang et al. (2021). By following this procedure: Compression ratio of 50%,Trigger force of 2N, Test speed of 1 mm/s and 50mm diameter aluminum cylindrical probe. The maximum force needed to compress the samples was reported as the hardness (N).

Determination of Lipid Oxidation

The investigation of lipid oxidation was conducted using the evaluation of thiobarbituric acid (TBA) and peroxide value (PV). The quantity of thiobarbituric acid (TBA) in a 4g sample of burger was determined by homogenizing it with a 20mL solution of trichloroacetic acid (20% w/v) and subsequently subjecting it to centrifugation at 3000g for 10 minutes. The 2 mL supernatant was mixed with 2 mL of a solution containing 0.1% thiobarbituric acid in double distilled water. The mixture was then heated in a water bath at a temperature of 100° C for a duration of 30 minutes and thereafter cooled to room temperature. Consequently, TBARS were extracted under refrigerated conditions. The spectrophotometer (PEAK INSTRUMENTS C-7200) was used to measure the absorbance of each extract at a wavelength of 520 nm. The compound 1,1,3,3-tetraethoxypropane was utilized to establish the standard curve for the TBARS assay. The TBARS values were expressed as milligrams of malonaldehyde per kilogram of beef meatball, as published by Zhang et al. (2016). Furthermore, the peroxide value (PV) was subjected to lipid extraction using a chloroform-methanol solution. Next, the lipid phase was infused with a 1% starch solution, distilled water, and saturated potassium iodide to liberate iodine. Zanganeh et al. (2021) utilized a 0.01 N solution of thiosulfate to titrate the liberated iodine. The findings were expressed as milliequivalents of oxygen per kilogram of lipids (meq O_2/kg).

Sensory properties

The burger samples were investigated for texture, color, overall acceptance, and odor by 25 panelists. The nine-point scale was as follows: 9 = very good and 1 = refused (Kang et al., 2007)

Statistical Analysis

The data were analyzed in triplicate using SPSS software (version 18.0) using the one-way ANOVA method. The difference between the data means was determined by Duncan's test at ($P < 0.05$).

3. Results and Discussion

Microbial analysis for beef burger samples

Total viable count (TVC)

Figure 1 showed the variations in Total Viable Count (TVC) of control and coated burger samples over the period of cold storage. Every sample showed a significant increase in total viable count (TVC) during storage, with the coated samples displaying significantly ($P<0.05$) lower TVC compared to the uncoated sample. The TVC of the control sample ranged from 2.00 to 7.675 (log CFU/g) on the 9th day. The findings showed that the beef burger samples treated with GSM+CH and GSM+CH+BPO exhibited a significantly reduced total viable count (TVC) compared to both the control group and other treated samples for the entire storage duration. Nevertheless, the rise in TVC was less pronounced in the GSM+BPO coated burger compared to the beef burger coated only with GSM. The total viable count in the uncoated burger rose to 7.55 log CFU/g and 7.97 log CFU/g on the 6th and 9th days of storage, respectively. The International Commission of Microbiological Specification for Foods (ICMSF) has set a maximum permissible TVC level of 7.0 log CFU/g or 107 CFU/g for fresh beef (ICMSF 1986). The control beef burger displayed an appropriate quantity of TVC (4.842 log CFU/g) on the 3rd day, which is clearly observable. Due to the prolonged storage duration, the Total Viable Count (TVC) of the uncoated beef burger exceeded the permissible threshold, suggesting a microbiological shelf-life of three days. Thus, TVC results showed that the beef burgers were coating can be stored for a period of 12 th before it expires. Based on the latest research, using a food coating that contains BPO and CH successfully increased the period in which beef patties can be stored without bacterial development. These findings indicate that the coating effectively released BPO and CH in a regulated manner, and the testing validated the antibacterial characteristics of these compounds. The likely cause for this is the antibacterial characteristics of the pepper, along with the Chitosan and oxygen barrier functions of the edible coatings. Our results are consistent with previous studies conducted by Alizadeh et al. (2017a). In addition, more research has found comparable findings about the antibacterial characteristics and ability to prolong the shelf-life of beef products by the utilization of essential oils *(*Liana et al., 2018 and Takma & Korel 2019).

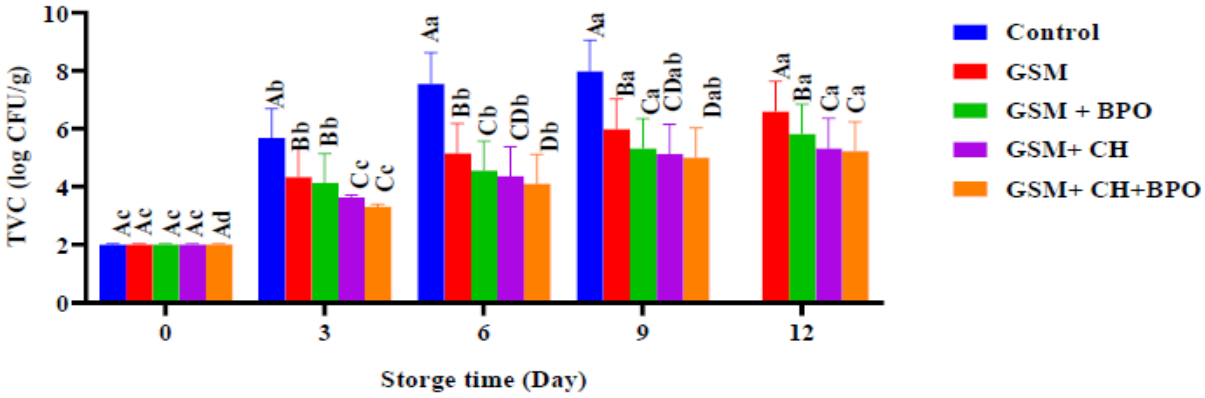

Figure 1. Effect of different treatments on Total viable count (TVC) during cold storage of burger samples

Psychrotrophic count (PTC)

Both the control and coated beef burgers exhibited a significant increase in PTC as time progressed. In addition, all samples that were coated exhibited a much lower PTC in comparison to the uncoated samples, as shown in Figure 2. The initial psychrotrophic total count (PTC) of the control beef burger was 2.20 logCFU/g. Following a period of 9 th in a cold storage environment, the PTC had risen to 9.92 logCFU/g. After 12 th of storage, the coated beef burger samples, particularly the ones coated with GSM+BPO+CH, exhibited a decreased rate of psychrotrophic bacteria with a value of 4.20 logCFU/g. This was lower than what was observed in the other coated and uncoated beef burger samples. This suggests that the edible coating successfully limited the movement of oxygen (serving as a barrier against oxygen) and prevented the growth of these bacteria, which are important psychrotrophic microorganisms. Figure 2 clearly demonstrates that PTCs were the primary microorganisms accountable for the degradation of the meat samples. The degradation of fresh beef held in aerobic-cold storage settings is mostly due to the growth and metabolic processes of psychrotrophic bacteria. Under certain conditions, these bacteria can break down glucose and amino acids (Zinoviadou et al., 2009, 2010). According to our research, found that using edible coatings produced from polysaccharides and plant extracts can significantly improve the safety against microorganisms and prolong the freshness of fresh meat. These findings were published in the papers by (Alizadeh et al., 2017a & 2017b and Alizadeh & Imani 2018a and 2018b).

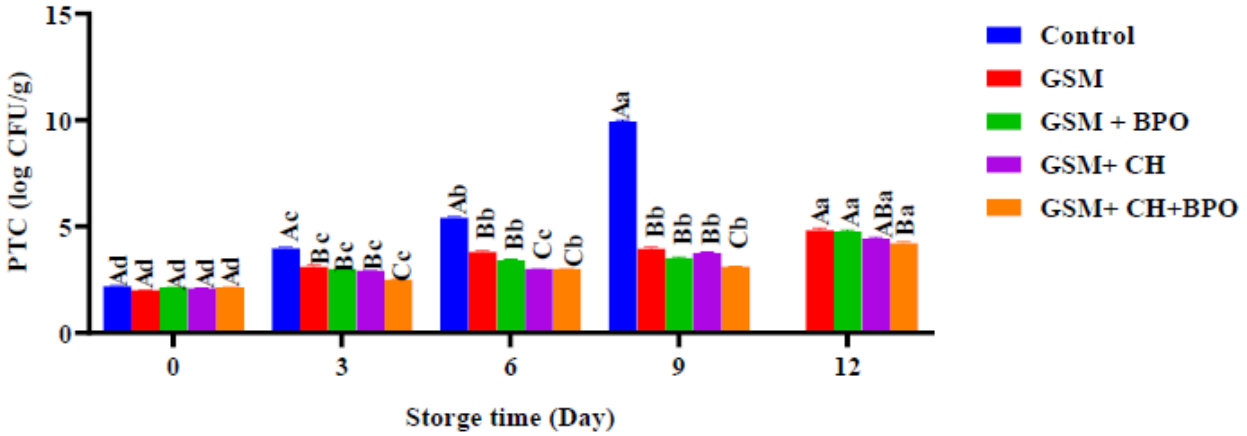

Figure 2. Effect of different treatments on Psychrotrophic count (PTC) during cold storage of burger samples

Escherichia coli counts

The data presented in Figure 3 show the changes in *E. coli* counts for both coated and uncoated burgers over periods of refrigerated storage. Throughout the storage time, the *E. coli* count of all samples exhibited a significant increase. The samples that were coated exhibited a significantly reduced *E. coli* count in contrast to the samples that were not coated. The uncoated beef burgers experienced the most significant increase, with a rise of 4.90 log CFU/g within the 9th day of cold storage. On the 12th day of storage, the GSM+CH+BPO and GSM+CH coated samples showed only a slight increase of 1.37 and 1.56 logCFU/g, respectively. These results align with the microbial standards outlined in National Food Safety Authority Decision No. 1/2021, which establishes technical regulations for microbial criteria in fresh meat, poultry, comminuted, and mechanically separated meat (NFSA Decision No. 1/2021). The samples that were coated indicate a significant antibacterial effect, which improves the microbiological safety and prolongs the shelf life of the beef burger samples. The main factor behind this is the oil's antibacterial activity and the ability of edible coating as an oxygen barrier. This shows a remarkable ability of edible coatings

to enhance the microbiological and sustainability safety of beef meat and its products. Emiroglu et al., (2010) have documented the capacity of coatings containing EOs to inhibit the increase of coliform bacteria in meat products. According to our research, previous studies conducted by (Alizadeh et al., 2017a and Eshghinezad et al., 2018) have also shown that EO and EO-loaded edible coatings have the ability to reduce the increase of *S. aureus* and *E. coli* in meat.

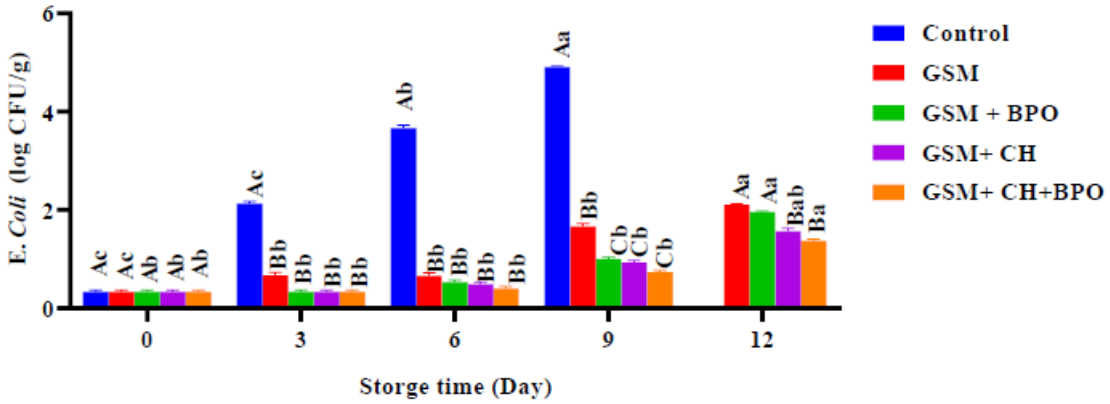

Figure 3. Effect of different treatments on *Escherichia coli* (EC) (logCFU/g) during cold storage of burger samples

Mold and yeast (fungi) count

Both the control and coated beef burgers showed a significant rise in the count of mold and yeast (fungi) as time progressed. Nevertheless, the samples that had been coated exhibited a much-reduced growth compared to the samples that were not coated, as seen in Figure 4. The initial fungus count in the uncoated beef burger samples was 1.33 (log CFU/g) within the 1st day of storage time, which increased to 4.20 logCFU/g on the 9th day of storage. Because fungal species are aerobic and have an ability to develop on the surface of burgers, all GSM coatings acted as an oxygen barrier. Consequently, the increase of fungi on the burgers was markedly reduced in coated samples compared to those uncoated. The results are consistent with the studies conducted by (Alizadeh & Imani 2018 and Kiarsi et al., 2020), where they created edible coatings using essential oils to improve the shelf-life of beef. In addition, the edible coatings containing EOs-loaded seed mucilage successfully prevented the increase and growth of different microorganisms,

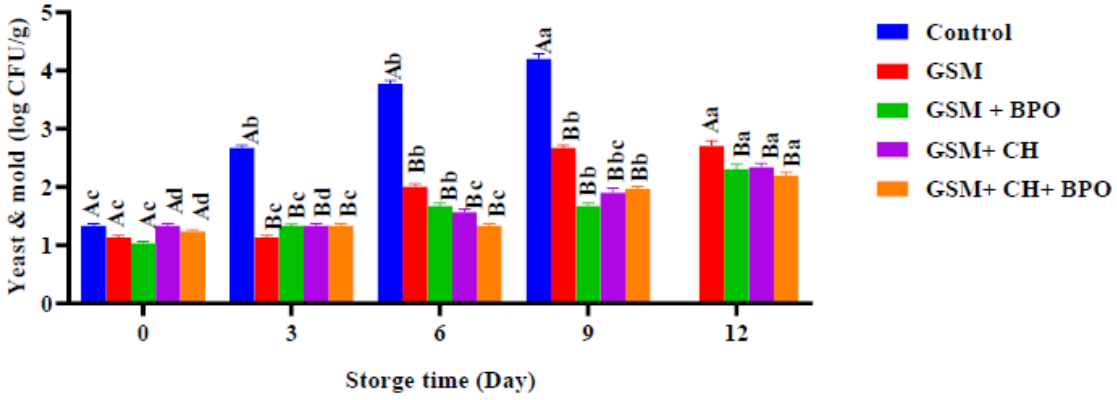

Figure 4. Effect of different treatments on Yeast and Molds (logCFU/g) during cold storage of burger samples

Physical and chemical analyses of beef burger samples

Cook Loos (CL), Cook yield (CY) and shrinkage (SH) of beef burger samples in Zero time

The statistical analysis of Table 1 showed that the different GSM coatings had a significant effect ($p<0.05$) on the cooking loss, cook yield and shrinkage of beef burgers. The treatment results showed a statistically significant decrease ($p<0.05$) in cooking loss and shrinkage of beef burgers compared to the control samples. The cook yield of the samples represents a significantly greater increase in coated samples compared to the control samples. The samples coated with GSM+CH and GSM+CH+BPO displayed significantly reduced cook loss and shrinkage values compared with the other coated samples. The control sample exhibited a significantly higher cook loss value of approximately 36.017%, whereas the samples coated with GSM+CH and GSM+CH+BPO displayed lower cook loss values of around 26.093% and 27.000%, respectively. The control sample had a cook yield value of around 60.983%, which was significantly ($p<0.05$) lower compared to the coated samples treated with GSM+BPO and GSM+CH+BPO. These coated samples had cook yield values of 73.000% and 73.907%, respectively. The decrease in cook loss seen in the GSM edible coating can be related to the coating's ability to act as a barrier to water and its capacity to form a gel, as proposed by Ruan et al., (2019) and Behrouzian et al., (2014).

Table 1. Effect of different GSM coatings on cook loos (CL), cook yield (CY) and shrinkage (SH) % in beef burger at zero time

Storage time / Samples	CL	CY	SH %
Control	36.017±2.78 A	60.983±4.11 C	25.653±2.10 A
GSM	30.773±2.64 B	65.927±4.22 B	16.693±2.11 B
GSM+BPO	30.750±2.58 B	66.250±4.12 B	17.080±1.98 B
GSM+CH	26.093±2.31 C	73.000±4.56 A	12.443 ±2.00 C
GSM+CH+ BPO	27.000±2.33 C	73.907±4.68 A	12.841±1.85 C

Means of treatments having the same case letter (s) (capital letters within a column are not significantly different ($p < 0.05$). Control: (uncoated burger); GSM: Garden seed mucilage extract only as edible coatings; GSM+BPO:Garden seed mucilage extract+1% black pepper oil; GSM+CH:Garden seed mucilage extract+0.25%chitosan and GSM+CH+BPO:Garden seed mucilage extract+0.25% chitosan+1% black pepper oil.

Moisture Content

The moisture content of all samples exhibited a significant decrease with storage time, as shown in Figure 5. The edible coating effectively saved the beef burgers from experiencing weight loss over a period of time. The beef burgers that were coated exhibited a markedly greater level of retained moisture in comparison to the untreated sample. The moisture content in the control samples varied between 64.23% and 47.06% on the 9th day. Comparatively, the moisture content of the GSM, GSM+BPO, GSM+CH, and GSM+CH+BPO samples ranged from 63.52% to 56.35%, 64.77% to 57.44%, 65.02% to 58.98%, and 64.96% to 60.51%, respectively, at the end of the storage period.

The burgers that were coated, particularly those with GSM+CH+BPO, demonstrated higher water content in comparison to uncoated burgers. This suggests that the reduction in weight of the beef burgers was successfully inhibited. The edible coating's high moisture retention capability can be ascribed to its low permeability to water vapors and its capacity to act as a physical barrier (Saffari et al., 2023). In addition, our study discovered that meat samples covered with a bioactive-laden edible coating showed very little emissions. Furthermore, Alexandre et al., (2020) showed that the coating successfully inhibited water losses in the meat during storage.

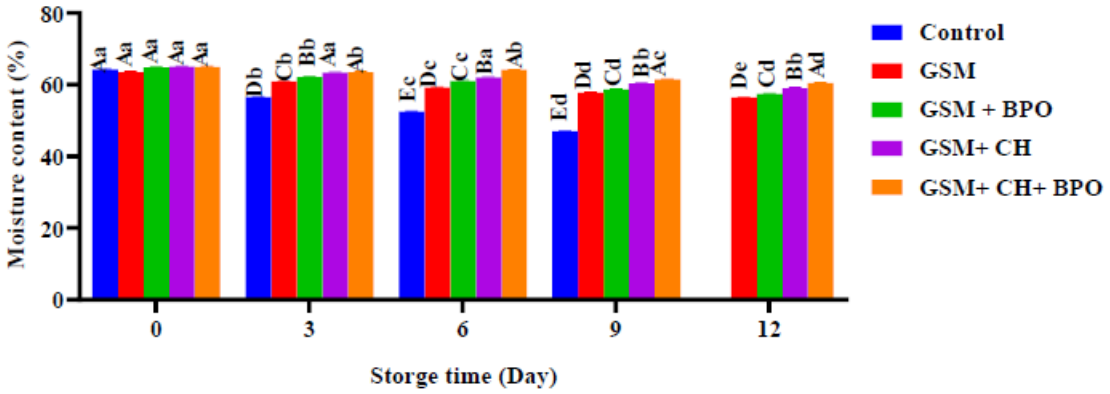

Figure 5. Effect of different treatments on Moisture content (%) during cold storage of burger samples

pH changes

The change in pH level of beef burger samples through the storage period is shown in Figure 6.

As the storage time progressed, both the control and coated beef burgers had a significant increase in pH value. There was a significant difference between the control and the coated samples, but the coated samples showed no significant differences during the storage period. Beef burger samples which were coated with the GSM+CH+BPO showed little change in pH value. The reduction in pH value can be attributed to the coating's capacity to impede the permeability of carbon dioxide, leading to the buildup of carbon dioxide in the meat and a subsequent decline in pH. Consequently, the presence of acidic conditions can lead to a decrease in the microbial population in the meat (Alizadeh et al., 2017a).

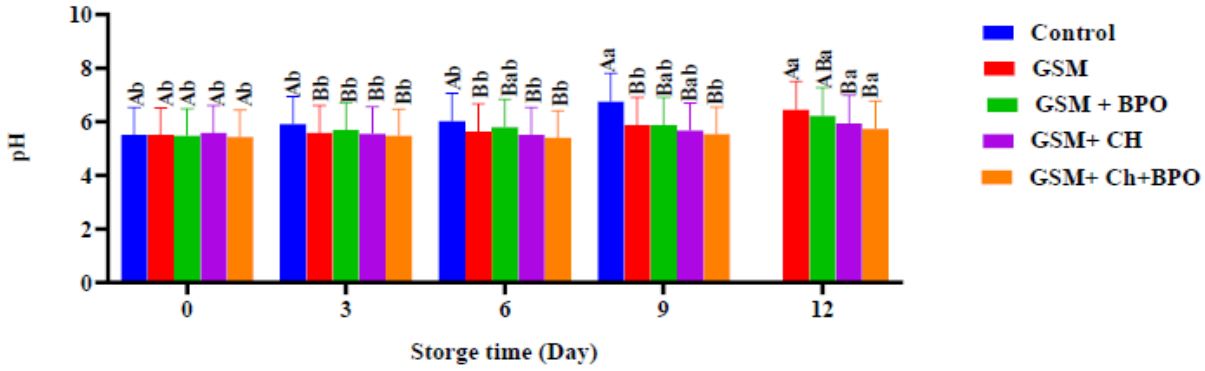

Figure 6. Effect of different treatments on PH value during cold storage of burger samples

Texture analysis

Texture is an important factor for meat products that has a substantial impact on its overall quality and the degree of approval from consumers (Krzywdzinska et al., 2016). Figure 7 displayed the hardness readings of all burgers that were maintained at 4°C. The decrease in hardness of beef burgers was clearly observed as the storage period progressed. Nevertheless, the rate at which hardness decreased was significantly slower in the coated burgers in comparison to the control. After being stored for 9th days, the uncoated beef burger (control) sample exhibited a hardness value of 51.61N. On the end of storage time (12th day), the samples coated with GSM+CH+BPO had a hardness value of 58.27N. Throughout the storage period, the hardness values of the GSM+CH, GSM+BPO, and GSM coated burgers were lower than those of the GSM+CH+BPO coated samples.

The observed decrease in hardness in the beef burgers can be related to the degradation of beef burgers resulting from the activity of both bacterial microorganisms and enzymes (Heydari et al., 2020; Alizadeh et al., 2021; and Saffari et al., 2023).

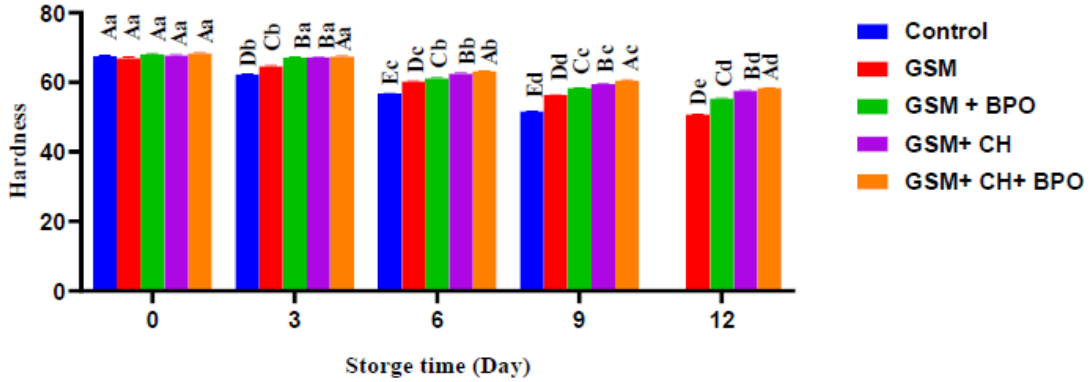

Figure 7. Effect of different treatments on Hardness (N) during cold storage of burger samples

Changes of Thiobarbituric acid (TBA)

The thiobarbituric acid value is a quantitative evidence of secondary oxidation products, specifically malondialdehyde (MDA), which is the main compound formed via lipid oxidation (Nogueira et al., 2019). Alizadeh & Imani (2018 a,b) state that the allowed permissible limit of TBA level in beef is 1.0 mg MDA/kg. According to the Egyptian Organization for Standards and Quality (2005) and Tarladgis et al., (1960), a rancid odor in meat is detected when the TBA level is within the range of 0.5 to 1.0 MDA/kg. Based on the results shown in Figure 8, the TBA value of all samples exhibited substantial growth over the refrigeration period. Nevertheless, the coated samples exhibited much lower TBA (<1.0MDA/kg) in comparison to the uncoated beef burger. The TBA for the control reached the highest value of 1.98 MDA/kg within the 9th day of storage. The samples treated with GSM+BPO and GSM+CH+BPO showed significantly reduced TBA values. The data suggested that BPO can effectively inhibit oxidation of lipids in meat, mostly due to its phenolic components, which possess remarkable antioxidative capabilities. According to Kim et al., (2002), an edible coating was effectively utilized to prolong the oxidation process of beef for a duration of 12th days while stored in a refrigerator. The delay is most likely due to the antioxidant qualities of BPO, as well as the capacity of the edible coatings GSM and CH to minimize exposure to both light and oxygen (Alizadeh and Imani 2018a and 2018b, and Barzegar et al., 2020).

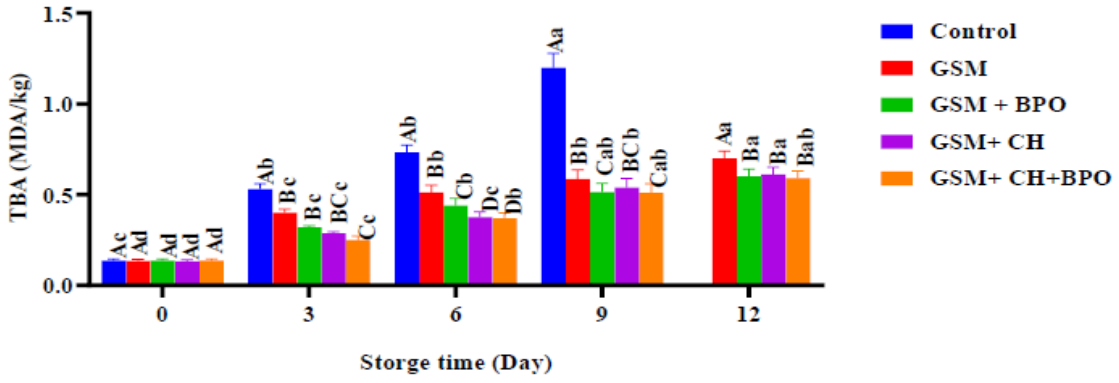

Figure 8. Effect of different treatments on Thiobarbituric acid (TBA) during cold storage of burger samples

Changes of Peroxide value (PV)

The peroxide value (PV) is usually carried out to quantify the formation of primary products of lipid oxidation, namely odorless hydroperoxides (Saengsorn and Jimtaisong 2017). Thus, peroxide value for both coated and uncoated burgers significantly increased over time in storage ($p<0.05$) (Fig. 9). Clearly, the PV values experienced a significant rise during the refrigeration storage time. The beef sample that was not coated experienced the highest increase in peroxide value (PV) during storage, reaching 10.44 meq oxygen/kg within the 9th day. The coated samples showed increasing PV values over time, with the samples coated with GSM+CH+BPO, GSM+BPO, GSM+CH, and GSM displaying oxygen levels of around 6.19, 6.23, 6.77, and 7.00 meq oxygen/kg, respectively. As the duration of storage extended from 1 to 12th days, the (PV) value likewise rose. The approved allowable threshold for beef is 7 meq oxygen/kg (Alizadeh et al., 2020). Consequently, while the uncoated beef burger (control) surpassed the permissible limit within the 6th day and was expected to have a shelf life of only 3th days under refrigerated conditions, the beef burgers coated with GSM+BPO and GSM+CH+BPO exhibited a PV lower than the stipulated limit, suggesting that their shelf life is 12th days. In accordance with our results, the application of coatings containing EOs effectively decreased the oxidation of beef lipids. The oxygen barrier qualities of the edible coating can be attributed to this, as shown by (Vital et al., 2016, Alizadeh & Imani 2018b and Alizadeh et al., 2017a).

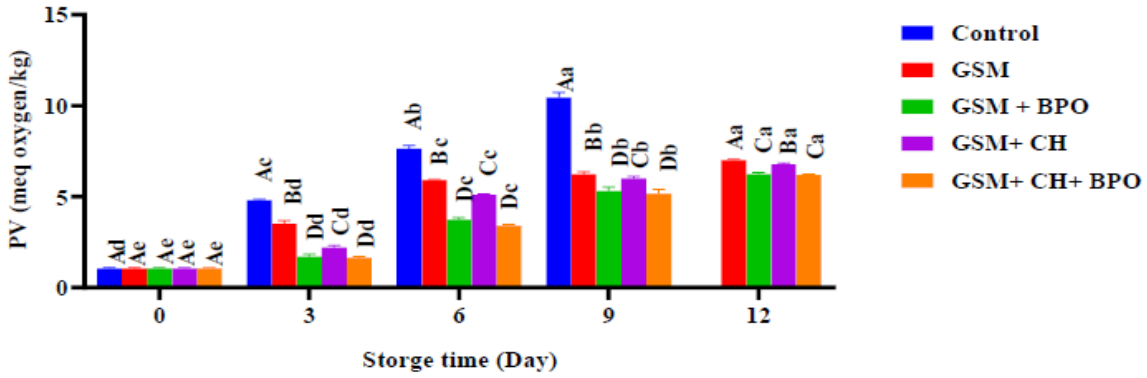

Figure 9. Effect of different treatments on Peroxide Value (PV) during cold storage of burger samples

Sensory properties

Over time, beef burger observed a decrease in its odor, color, texture, and overall acceptability (Fig.10a,b,c&d). Barzegar et al., (2020) state that beef burger samples are deemed suitable for human ingestion only if they achieve high ratings (>4) in terms of their sensory characteristics. In this particular situation, the control sample was deemed unsatisfactory after being stored for a duration of 3th days. Conversely, the samples that were coated remained in excellent condition during the whole storage period. Following a storage period of 3 days, the control samples were deemed unsatisfactory in terms of odor, color, texture and overall acceptability. During all of the storage period, the beef burgers that had been coated appeared to be satisfactory. The burgers that were coated with GSM+CH+BPO received notably higher sensory scores compared to the control and other coated samples. Figure 10d the GSM, GSM+BPO, GSM+CH and GSM+CH+BPO samples achieved ratings of approximately 4.11, 5.39, 5.89 and 6.67 respectively, during the whole storage period, showing their overall acceptance. The results of sensory evaluation appear to be strongly associated with the findings of the chemical and microbiological investigations. Consistent with our findings, the application of a consumable layer enriched with essential oil significantly extends the period of beef's freshness until the end of refrigerated preservation (Hassan et al., 2000).

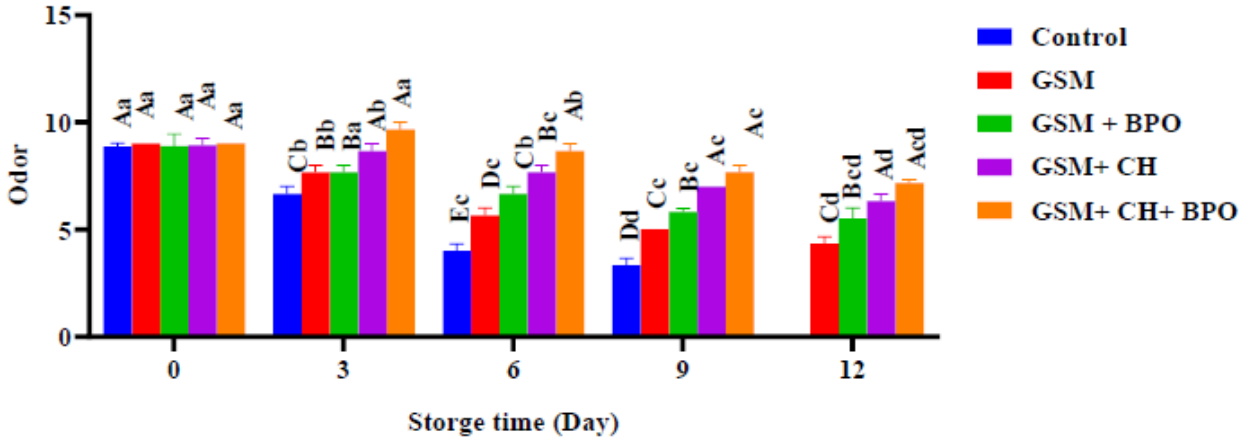

Figure 10a. Effect of different treatments on odor during cold storage of burger samples

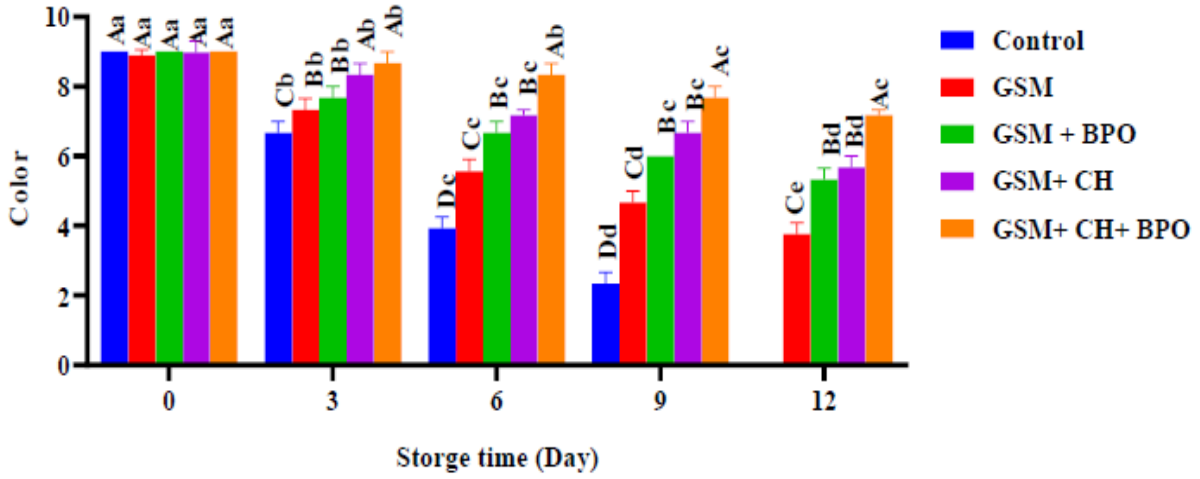

Figure 10b. Effect of different treatments on color during cold storage of burger samples

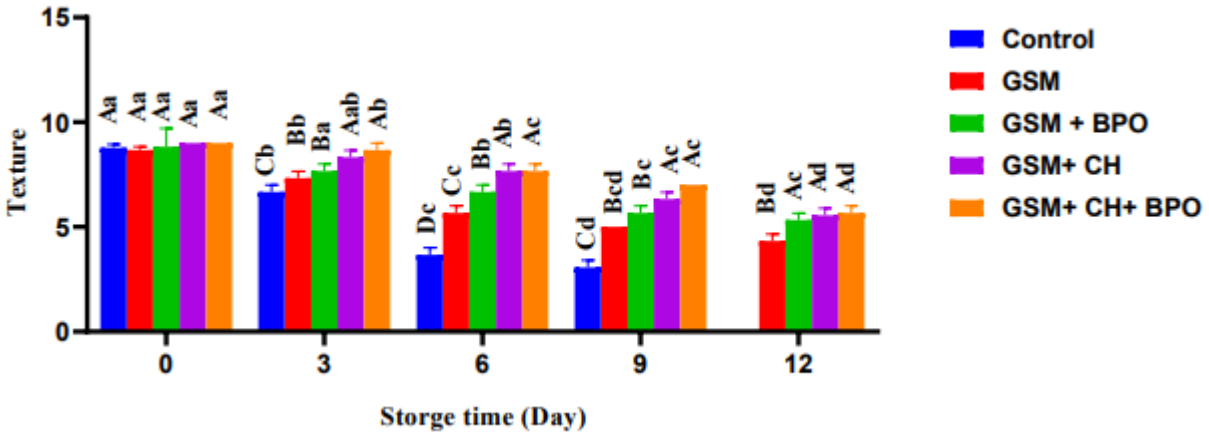

Figure 10c. Effect of different treatments on texture during cold storage of burger samples

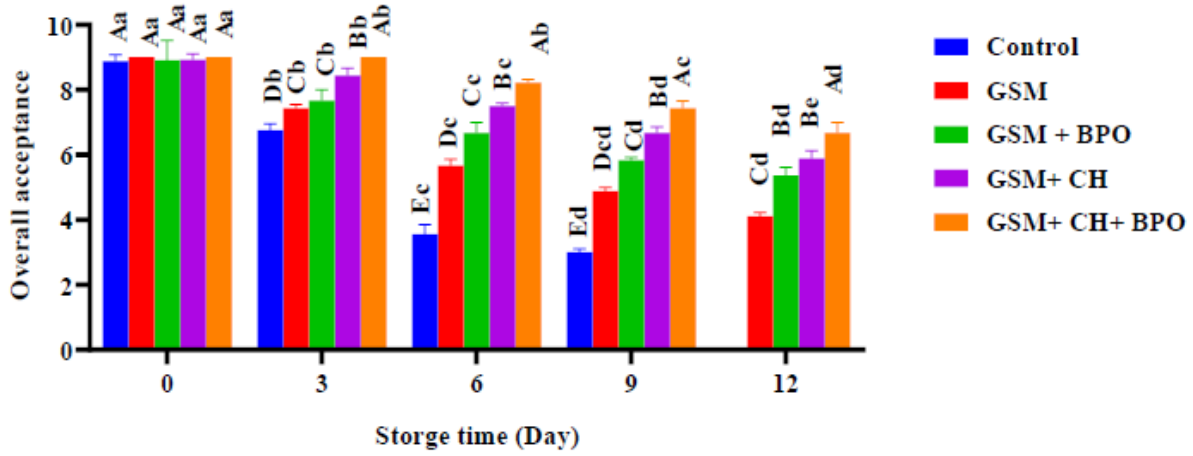

Figure 10d. Effect of different treatments on overall acceptability during cold storage of burger samples

Visually, the coatings adhered uniformly to the beef burger samples, presenting a natural, bright appearance. Their effect on quality maintenance during storage is also evident. The appearance of the samples can be seen in Figure 11.

Storage (Days) / Coatings	0	3	6	9	12
Control (uncoated)					
GSM					
GSM+BPO					
GSM+CH					
GSM+CH+BPO					

Figure 11. Images of beef burger samples (control and coated) during storage period

4. Conclusion

Novel edible coating was developed by blending black pepper essential oil with a natural hydrocolloid chitosan and garden seed mucilage. This coating showed promise for many uses in food packaging. The edible coatings significantly increased the shelf life of beef burgers by effectively avoiding microbial deterioration and reducing lipid oxidation. The use of a GSM coating with 1% black pepper essential oil and 0.25% chitosan enhanced the beef burgers, prolonging its shelf-life and quality when refrigerated. The incorporation of black pepper essential oil and chitosan-enriched garden seed mucilage as active packaging materials can improve the qualitative characteristics and microbiological safety of beef burgers and other perishable food products. Overall, the results suggest that the developed edible coating is a promising alternative to traditional plastic packaging for preserving the quality and safety of beef burgers. Furthermore, it is important to explore future commercial applications of seed mucilage in meat processing and preservation, carefully considering the safety and economic factors associated with its use.

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