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EFFECT OF LONG STORAGE OF SINAPIS ALBA SEEDS AQUEOUS EXTRACT IN VIABILITY AGAINST SPOILED BACTERIA IN REFRIGERATED GROUND BEEF

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Abstract

White mustard seeds (Sinapis alba) have been extracted by hydroextraction since 2004 and stored in the refrigerator for 14 years until 2018. The antibacterial effect of the aqueous extract was tested by the well diffusion test against Gram-positive bacteria such as Bacillus cereus, Staphylococcus aureus and Micrococcus spp. The inhibition zones against Gram-negative bacteria such as Escherichia coli, Salmonella typhimurium, and Pseudomonas fluorescens were 23, 25 and 21 mm and 16, 20 and 15 mm, respectively. Ground beef was treated with 0, 5, 10 and 15 ml/100 g mustard extracts (ME0, ME5, ME10 and ME15) and refrigerated for two weeks. Bacteriological and sensory analyses were performed on frozen meat slices. The ME15 study showed the best reductions in total viable bacteria (TVC), coliforms, S. aureus, and psychrophiles. Compared to other treatment studies, the ME10 study was a super treatment that improved sensory attributes such as color, smell, and appearance within 14 days of refrigeration. The control experiment (ME0) was removed from the refrigerator after the seventh day of the experiment due to a high microbial load and poor color and odor.

Keywords: White mustard seeds, Aqueous extract, Antibacterial, Ground beef, Shelf-life.

تأثير الخزن الطويل للمستخلص المائي لبذور Sinapis alba في الفعالية الحيوية تجاه البكتريا المتلفة للحم البقر المفروم المبرد

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الخلاصة

أُجري استخلاص مائي لبذور الخردل الأبيض (*Sinapis alba*) منذ عام 2004 وخزنت في الثلاجة مدة 14 عامًا حتى عام 2018. أُختبر التأثير المضاد للمستخلص المائي عن طريق الانتشار بالحفر تجاه البكتريا الموجبة لصبغة كرام والسالبة لصبغة كرام مثل *Bacillus cereus*، *Staphylococcus aureus*، *Micrococcus spp.*، *Escherichia coli*، *Salmonella typhimurium* و *Pseudomonas fluorescens*. سجلت مناطق التثبيط 21، 25، 23،16، 20 و15 ملم على التوالي تجاه البكتريا الاختبارية. عُومل اللحم المفروم بنسب 0، 5، 10، 15 مل/ 100 غرام من المستخلص المائي للخردل (ME0، ME5، ME10، ME15) على التوالي وبُردت النماذج مدة أسبوعين. أُجريت الفحوص البكتريولوجية والحسية على شرائح اللحوم المبردة. أظهرت المعاملة ME15 أفضل تخفيض في العدد الكلي للبكتريا (TVC)، بكتريا القولون، العنقوديات الذهبية والبكتريا المقاومة للبرودة. بالمقارنة مع بقية المعاملات، سجلت المعاملة ME10 أفضل القيم في تحسين الخصائص الحسية مثل اللون والرائحة والمظهر خلال 14 يومًا من التبريد. استبعدت معاملة (ME0) من الثلاجة بعد اليوم السابع من الخزن بسبب ارتفاع الحمل الميكروبي ورداءة اللون والرائحة.

كلمات مفتاحية: بذور الخردل الأبيض، المستخلص المائي، الفعل المضاد للبكتريا، اللحم المفروم، العمر الخزني.

Introduction

Several alternatives use plant extracts and essential oils, which produce multiple secondary metabolites that are easily degradable, environmentally friendly, and non-toxic (36). Antibacterial activity against Gram-negative and Gram-positive bacteria suggests a broad role of the plant as a source of bioactive substances (2). Sinapis alba (white or yellow mustard) is a cruciferous vegetable widely eaten by humans. S. alba seeds have been used as a spice for over 5,000 years in ancient cultures such as the Romans, Egyptians, Sumerians and Chinese. (7). S. alba seeds are a rich source of bioactive substances such as glucosinolates and phenolic compounds. Sinalbin is an important glucosinolate found in yellow mustard. Upon mechanical injury, the plant's own myrosinase reacts with stored glucosinolates and hydrolyzes them into

bactericidal isothiocyanates (ITCs). Benzyl isothiocyanate is the main hydrolysis by-product of mustard (15). When ITC is used as a food preservative, ITC has relatively little impact on processed foods due to its high volatility, it is also considered a food additive due to its taste and antibacterial properties (12). The antimicrobial properties of mustard glucosinolate and its metabolite isothiocyanate have been shown to protect against a variety of foodborne pathogens, including Escherichia coli O157:H7, Listeria, and Salmonella spp. (8). As mustard powder can affect the physicochemical and sensory properties of processed poultry meat, antimicrobial films or coatings as a mode of transportation appear to be a more efficient method (23). Mustard powder is also used as a water retaining agent in meat products to facilitate the cutting of meat products (17). Meat is considered a good source of protein, lipids, vitamins and minerals (4).

Several studies have found that mustard seeds reduce lipid oxidation and improve the sensory properties of fresh and processed meat products. Researchers report the inhibitory effect of mustard on spoilage and pathogenic microorganisms in meat products (24 and 35). Due to its strong microbial killing effect, some researchers tend to develop white mustard oil as a food preservative (25). The aim of this study was to determine the antimicrobial effect of aqueous extracts of S. alba seeds on food-borne bacteria during long-term storage and to study the bacteriological and sensory properties of ground beef under refrigerated conditions.

Materials and Methods

Aqueous extract: According to (1), hydroextraction of white flower seeds has been performed since 2004 with some modifications. To prepare the aqueous extract, infuse 100 g of mustard seed powder into 500 mL of distilled water to fully deplete (typically 24 h) in a shaking incubator at 40 °C. Then filter using muslin or Whatman No.1 filter paper; the filtrate is centrifuged at 3000 x g for 10 min (Beckman, England), the supernatant is concentrated by a rotary evaporator at 40 °C, and it is placed in a labeled sterile dark Bottles with screw caps keep for 14 years in the refrigerator.

Bacterial culture preparation: The three Gram-positive bacteria are *Staphylococcus aureus*, *Bacillus cereus*, and *Micrococcus* spp. Three Gram-negative bacteria, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas fluorescens*, were obtained from the Faculty of Agriculture, University of Basra. Mueller Hinton Broth (MHB) was used to activate the isolated bacteria at 37°C for 24 hours. Bacteria were maintained on Mueller-Hinton agar (MHA) and cultures were stored at 4 °C and passaged every 2 weeks. (25)

Antibacterial activity of *Sinapis alba* seeds aqueous extract: Mueller Hinton agar was used to study the antibacterial properties of aqueous extracts of *S. alba* seeds by well diffusion assay. Mueller Hinton agar medium is evenly streaked and marked according to the number of bacteria to be tested; use a sterile cup drill (6 mm) to drill holes in the medium. The bottom of each well was sealed with a drop of melted agar to prevent unwanted diffusion of the extract. Add 1 mL of the prepared extract

dropwise to each well and let the culture rest for 30 min before transferring to the incubator. Cultures were incubated at 37°C for 24–48 hours before final measurements. A control plate without added extract was also prepared for each test organism. Zone of inhibition measured with millimeter accuracy (10).

Preparation of raw ground beef: Fresh beef was obtained from a local butcher in Baghdad 24 hours after slaughter and stored at refrigerated temperature (4°C), with fat and connective tissue removed, then cut into 2 pieces with a sterile steel meat grinder (Philips, Japan). After this step, they were divided into four groups, including a control group (without mustard extract) and three groups treated with different concentrations of ME 5, 10 and 15 ml/100 g. Samples were refrigerated for two weeks (28).

Bacteriological and sensory analyses of ground beef: After 1, 4, 7 and 14 days of storage at refrigerator temperature, bacteriological and sensory parameters were examined. For bacteriological analysis of each bag of meat samples, 10 g were sterilely weighed into a stomacher bag and mixed with 90 mL of 0.1% peptone water for 2 min. Serial 10-fold dilutions of slurries were made in sterile peptone water for plating on selective agars, including standard plate count agar total viable and psychrophilic bacteria; incubation at 30°C for 48 h, (29), Violet Red Bile Glucose (VRBG) Agar (Coliforms; 48 hours at 37 °C) (16) and Mannitol Salts Agar (*Staphylococcus aureus*; C for 48 hours) (31). A jury of 12 judges aged 23 to 35 participated in the sensory evaluation of the smell, color and appearance of raw meat samples Hedonic use immediately after opening the package 9 on a scale of 1 dislike very much, 5 fair, and 9 very similar (20).

Results and Discussion

Figure 1 shows the antibacterial susceptibility of aqueous extracts of white flower seeds to spoilage bacteria by the agar well diffusion method. The zone of inhibition was highest against *Staphylococcus aureus* (24 mm) and lowest against *Pseudomonas fluorescens* (15 mm). Aqueous extracts from S. alba seeds have been shown to be more effective against Gram-positive bacteria than Gram-negative bacteria. The differences in cell wall structure and composition between the two groups may be due to subtle differences between them. Gram-negative bacteria have an outer membrane that many small molecules cannot penetrate (5), which also showed the same result.

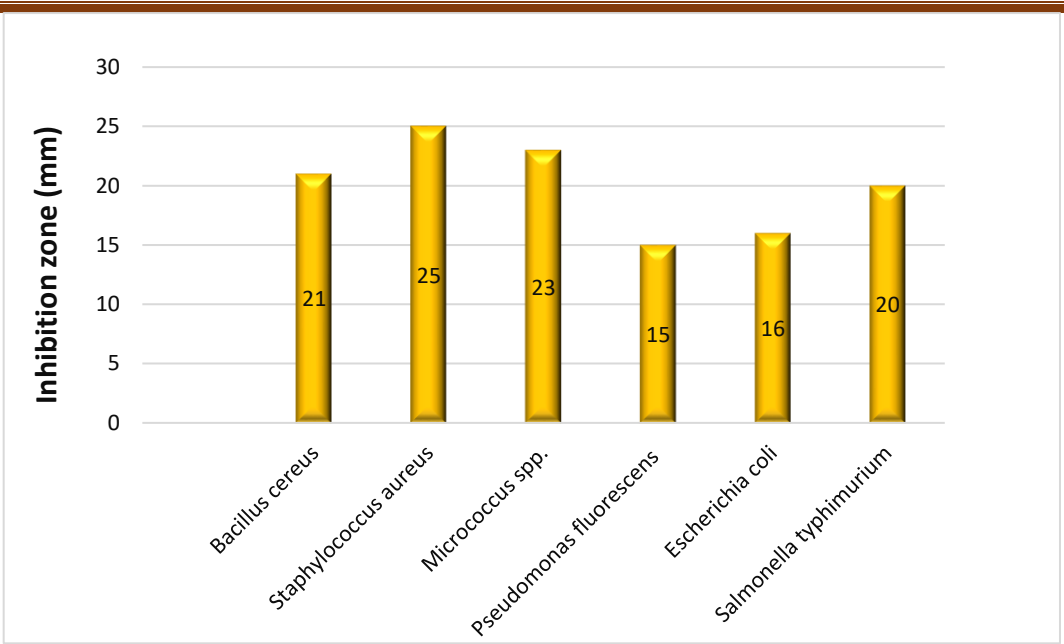

Figure 1 Antibacterial activity of *S. alba* seeds aqueous extract against spoiled bacteria.

Mustard is rich in glucosinolates, precursors of isothiocyanates (ITCs) (11). ITCs from mustard and other cruciferous vegetables have potent bactericidal activity against microorganisms (34). The inhibitory effect of aqueous extracts from plant leaves increased with increasing concentration (26). Both the essential oil and AITC have shown activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Bacillus subtilis*, *Shigella sonnei*, *Salmonella xylinum*, *Pseudomonas aeruginosa*, and fluorescent bacteria. Mustard essential oil acts on bacteria by lowering intracellular ATP concentration, lowering pH and increasing intracellular ATP concentration. This leads to damage to the bacterial cell membrane (13). The resulting inhibition of proton dynamics and membrane protein changes blocks cellular energy production and leads to bacterial death (19). (5) found that *S. nigra* and *S. alba* had the greatest inhibitory effect against *S. aureus* and *E. coli* (at a concentration of 20 mg/mL, approximately 22 mm and 20 mm inhibition zones respectively). This plant is one of the most important groups for inhibiting *Staphylococcus aureus* collagenase, which is considered important for healthy humans (27).

In Figure 2 the change in total mesophilicity of frozen ground beef treated with and without the aqueous extract of *S. alba* seeds is shown. The total viable count (TVC) of fresh ground beef was initially 3.92 log CFU/g. The initial TVC was consistent with that reported by (30), who stated that the mean TVC was 3-5 log CFU/g. Storage time had a significant effect on TVC values, which gradually increased during the study period. The control treatment (ME0) showed an increase in TVC over the upper limit of the ground beef microbiological standard during storage, and malodor was observed. Due to its flavoring and antimicrobial properties, AIT is also considered a food additive (5).

(22) reported an average total of 8.86 and 5.63 log CFU/g for mesophilic bacteria and Enterobacteriaceae, respectively. In general, a count of 7 log CFU/g is considered the approximate point at which meat is no longer acceptable (14 and 21).

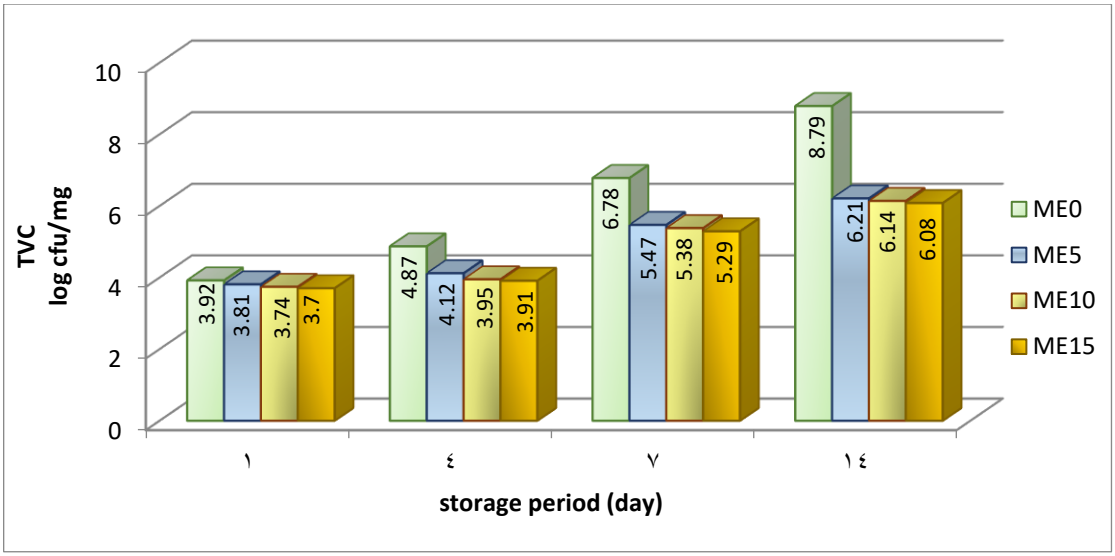

Figure 2 Total viable count (TVC) of ground beef treated with mustard extract during refrigerated storage.

Figures 3, 4 and 5 show the changes in coliform, psychrophilic and *S. aureus* populations in ground beef treated with or without aqueous mustard extract during cold storage. (ME0) showed a gradual increase in the number of coliforms, psychrophiles, and *Staphylococcus aureus* over the study period, above the upper limit of the microbiological standard for ground beef, and a foul odor was observed. Ground beef samples treated with mustard extract reduced the growth rate of test microorganisms during storage. The ME15 study showed the best reductions in total viable bacteria (TVC), *E. coli*, *S. aureus* and psychrophiles. Several studies have shown that coliforms and *E. coli* are present in ground beef. Coliforms are indicator organisms because Enterobacteriaceae are of intestinal origin. Indicator organisms can be used to reflect the microbiological quality of food in relation to product shelf life or product safety against foodborne pathogens. Microbiological indicators are more commonly used to assess food safety and hygiene than quality. (30). It is well known that the main causes of spoilage in meat products are lipid oxidation and microbial spoilage. However, the use of antioxidants and antimicrobials provides an effective way to prevent the spoilage of meat products. Today, interest is focused on plant extracts as a source of phenolic antioxidant, antibacterial, antiviral, and anti-inflammatory effects (3).

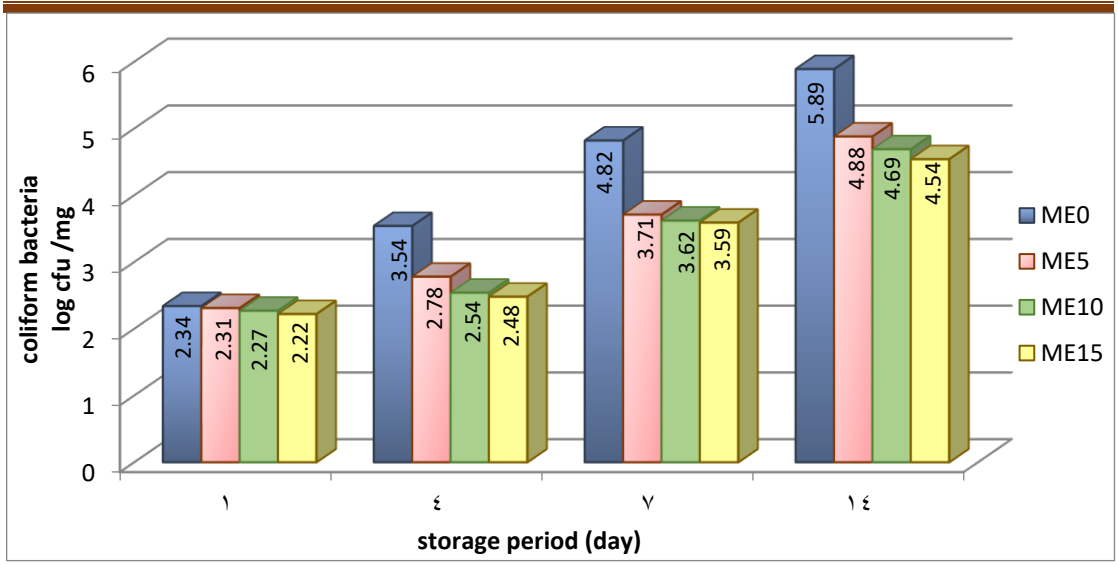

Figure 3 Coliform count of ground beef treated with mustard extract during refrigerated storage.

Pseudomonas was reported by (33). Isolated from ground beef at a concentration of 4.66 log CFU/g. Addition of mustard extract reduces the growth rate of mesophilic and psychrophilic bacteria over the expected storage period compared to the control.

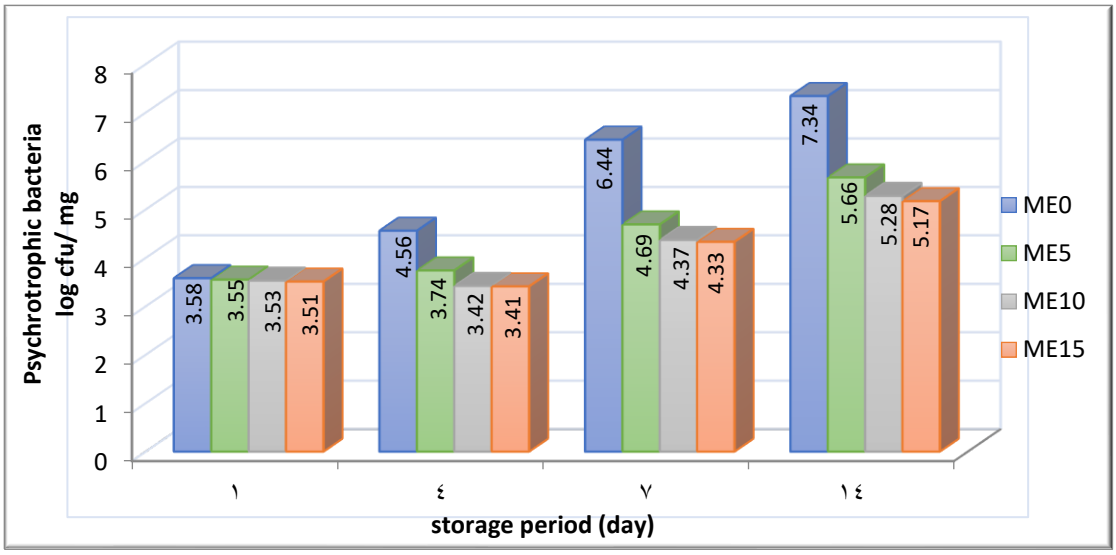

Figure 4 psychrotrophic count of ground beef treated with ml/100 g of mustard extract during refrigerated storage.

(9) found that in ground beef samples at 4.43-8.30, 2.54-7.65 and 2.81-6.56 log CFU/g TVC, total coliforms, *Staphylococcus aureus* were identified. Controlling lipid oxidation and microbial growth using antioxidants and antimicrobial compounds can help maintain meat quality. Currently, manufacturers and consumers primarily prefer herbal antibacterial and antioxidant compounds (6). Several studies have concluded that mustard seed reduces lipid oxidation and improves the sensory properties of fresh and processed meat products. have reported inhibitory effects (24 and 35).

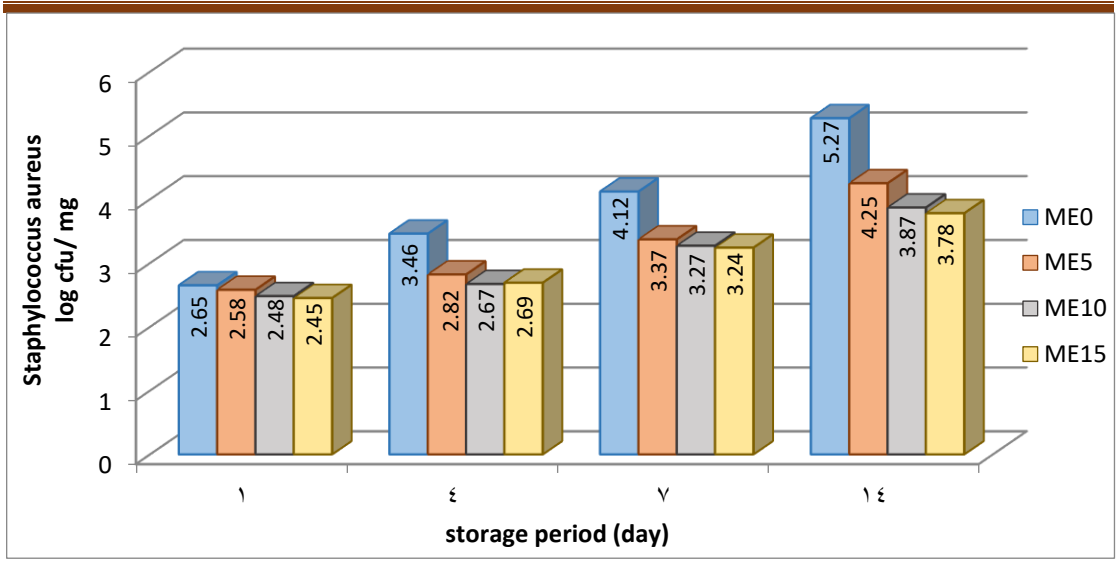

Figure 5 *Staphylococcus aureus* counts during cold storage of ground beef treated with mustard extract in ml/100 g.

Table 1 shows the effect of aqueous mustard extract on the organoleptic properties (color, odor and appearance) of ground beef during storage under refrigerated conditions. Acceptance of these attributes is found to be consistent with favorable microbial growth outcomes. According to the results of this study, the ME10 study had no adverse effects on organoleptic properties during storage compared to control samples.

Table 1 Sensory attributes (color, odor and appearance) of ground beef treated with mustard extract during storage at refrigerated condition.

	Color				Odor				Appearance			
	Storage period/day				Storage period/day				Storage period/day			
	1	4	7	14	1	4	7	14	1	4	7	14
ME0	9	6	3.7	0	9	5.8	3.6	0	9	5.7	4	0
ME5	9	8	7.2	5.1	9	8	6.8	5.3	9	7.6	7	6
ME10	8.5	8.2	7.6	7.1	8.8	8.1	7.5	7.2	8.7	8.5	8.1	7.3
ME15	8	7.4	6.8	5.8	8.2	7.3	6.4	5.3	8	7.1	6.5	5.4

During the entire 14-day storage period, ground beef that had been treated with mustard extract (10 ml/100 g) had excellent smell, color, and appearance. Due to increased microbial growth, untreated ground beef samples were less acceptable on the seventh day of the research period. Since ancient times, herbal spices have been used as food seasonings, preservatives, and even as folk medicines. In addition to giving food a unique flavor, several herbs and spices can protect food from going bad by acting as an antioxidant, bacteriostatic, or bactericidal agent (5). (32) concluded that white mustard bran and flour could be a very interesting strategy as they can delay microbial spoilage and extend the shelf life of baked goods.

According to several studies, mustard seed lowers lipid oxidation and enhances the sensory qualities of both fresh and processed beef products. Mustard seeds have been found to suppress the growth of harmful microbes and the deterioration of meat products (24 and 35). Natural antioxidants found in medicinal plants, such as phenolic acids, flavonoids, and tannins, are well-known to exist. (18). Because

processed poultry meat's physicochemical and sensory qualities can be impacted by mustard powder, antimicrobial films or coatings seem to be a more effective way to transport the substance (23).

Conclusion: White mustard aqueous extract had a strong antimicrobial activity. although it can be stored in refrigerator for many years. The action of aqueous extract against pathogenic gram positive and gram negative was done despite not fully understood. The ability of watery extract to preserve minced beef meat was applied with 14 days under refrigeration with maintenance quality properties.

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