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Sustainable Bio-Preservation of Concentrated Yogurt (Labneh) Using *Syzygium aromaticum* L.

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Abstract: Bio-preservation strategies for sustainable food and dairy products are some of the most in-demand techniques that expand shelf life and meet consumer requirements. The purpose of this study is to produce high quality, sensory-acceptable labneh cheese with a prolonged shelf life and sustainable preservation. Ethanolic extract of clove flower buds was applied during the manufacturing of labneh as a bio-preserving agent. The effect of the sprayed-clove extract on the chemical composition, microbiological composition, texture profile, antioxidant capabilities, aromatic compounds, and sensory properties of the labneh cheese were determined. Phytochemical profiling showed chaulmoogric acid, trans-cinnamic acid, propyl gallate, and sinapine as major constituents in clove extract. Antimicrobial inhibitory potential was estimated against both foodborne pathogens and food spoilage microorganisms. Clove extract showed a promising inhibitory effect against fungi recording 1 mg/mL maximally. Labneh samples with clove extract contained the highest records of antioxidant activity in addition to having no record of any fungal growth after 60 days. It is also distinguished by its eugenol, β-Caryophyllene, and acetyleugeno as aroma compound content. All spray-treated samples achieved the highest scores in sensory properties during the storage period. It can be concluded that clove extract, when sprayed on the surface of labneh cheese, produced an antifungal effect in a smart and economic way which boosted the shelf life, quality, enhanced nutritional value, and the antioxidant capacity of labneh cheese.

Keywords: bio-labneh; food preservation; antifungal effect; antimicrobial activity; sustainability

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1. Introduction

Fermented dairy products like yogurt and labneh are considered some of the most common products around the world. Labneh, a concentrated yogurt, is a commonly consumed dairy product in the Middle East, Lebanon, and the Balkan region [1]. It is obtained from yogurt after the removal of whey. Labneh cheese, a semisolid dairy product, has several characteristics including a creamy white color, a silky body, good spreadability, and a slightly pleasant acidic taste [2]. Labneh typically has a total solids content of around 23–25% and is manufactured from whole milk or recombined milk using different production methods, including the traditional cloth bag method and the UF technique. The

traditional method of producing labneh involves draining it in a cloth bag, then packaging and storing it in the refrigerator. The alternative method, known as the ultrafiltration process (UF), removes the whey or permeate to create a uniform cheese with a slightly sour taste [3].

Traditional labneh has a short shelf life even at low temperatures. Since it is not exposed to heat, the labneh produced using this method may have higher counts of lactic acid bacteria compared to labneh produced using other technique. As a result of a limited access to air during refrigerated storage, there is a risk of contamination by mold and yeast and, consequently, the quality and shelf life of the final product are negatively affected [4].

It was found that for yeast-free labneh products, a minimum concentration of sodium benzoate greater than 400 mg/kg is needed. Additionally, the ratio of potassium sorbate needed to inhibit various yeast species is also greater than 400 mg/kg. These requirements have led to the widespread use of benzoates and sorbates to control the growth of spoiled microorganisms, aiming to improve the poor quality and short shelf life of traditionally produced labneh. Furthermore, novel techniques for producing labneh cheese may reduce manual handling during the manufacturing process, which is considered a favorable trend for producing high-quality labneh with a longer shelf-life. This has resulted in the end product gaining superior microbiological quality [2].

To address the short shelf-life issues of fermented dairy products, various methods are typically employed, including pasteurization, refrigeration, and dehydration, which are easily survived by fungal spores [5]. The addition of bio-preservatives is the widely accepted way to sustainably prolong the shelf life of dairy products [6].

Essential oils, extracted from plant parts, have garnered increasing attention due to their safe and potent functional properties [7]. Steam distillation has been scientifically proven as the most effective method for extracting these oils. Known for their antimicrobial and antioxidant properties [2,8], essential oils can be utilized as food flavor agents, preservatives, and for pharmaceutical purposes [2]. Clove buds, scientifically known as *Syzygium aromaticum* (L.), belong to the largest genus of the Myrtaceae family, containing approximately 1200–1800 species. Clove has been traditionally used for its various health benefits, supported by many studies showcasing its antioxidant, antifungal, antiviral, antimicrobial, antidiabetic, anti-inflammatory, anti-thrombotic, anesthetic, analgesic, and insect repellent properties [9,10]. Clove is considered a rich source of phenolics such as flavonoids, cinnamic acid derivatives, hydroxyphenyl propene, and hydroxybenzoic acids [11]. Phenols and polypeptides in essential oils possess antimicrobial properties, with carvacrol from oregano and thyme and eugenol from cloves demonstrating themselves to be as effective the antimicrobial agents in various studies [12]. Clove oil is widely used in the food, medical, pharmaceutical, and cosmetic industries as a preservative agent [13]. Most essential oils are classified as GRAS (generally recognized as safe) and their antimicrobial activity can help extend the shelf life of various food products [8,14]. Pandey et al. [15] found that incorporating clove essential oil as an antimicrobial agent in cheese production extended the shelf life of the cheese product while maintaining high organoleptic properties and a low-cost.

The typical shelf life of cloth-bag labneh is approximately 14–21 days. Despite refrigeration, labneh has a short shelf life due to high levels of lactic acid and limited air access during its production, creating an ideal environment for yeast growth. At ambient temperatures (20–25 °C), labneh can spoil rapidly, with visible signs of yeast spoilage appearing with 7–14 days, even at 7 °C. Therefore, this study aims to extend the shelf life of labneh up to 8 weeks using biological preservation methods. The chemical properties, microbiological properties, textural profiles, and sensory properties of labneh were exam-

ined when the product was fresh and during storage, along with antioxidant activity and aroma compounds.

2. Materials and Methods

2.1. Materials

UF-skim buffalo retentate was obtained from the UF plant at the Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The chemical composition of UF-skim retentate showed total solids (TS) at 22.62, total protein (TP) at 17.12, fat at 0.5, lactose at 4.08, and ash at 1.08%. The pH value was 6.55. A mixed yogurt culture was obtained from Ch. Hansen's, Lab., in Hoersholm, Denmark. The starter culture was activated in 10% sterilized reconstituted skimmed milk at 35 °C for 24 h before use. A total of 9 strains of pathogens and food spoilage microbes; Gram-positive bacteria (*Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*), molds (*Penicillium verrocosum* and *Aspergillus flavus*), and yeast (*Saccharomyces cerevisiae*) were used in the study. Working cultures were kept at 4 °C on Tryptone Soy Agar (TSA) (Merk, Germany) or potato dextrose agar (PDA) (Biolife, Italy) slants and kept fresh through periodic subculturing. Clove flower buds extract was prepared from clove flower buds (*Syzygium aromaticum* L.) at the Dairy Department, National Research Centre, Dokki, Giza, Eygept

2.2. Methods

2.2.1. Ethanolic Clove Flower Buds' Extraction

An ethanolic extract was prepared according to [16] by shaking finely ground clove flower buds (*Syzygium aromaticum* L.) in 95% ethanol (1:10 *w*/*v*) at 150 rpm for an hour at room temperature. Mixtures were then filtered, and the supernatant was then dried in a fuming hood until a dry film was obtained.

2.2.2. LC-ESI-MS/MS Analysis of the Extract

The analysis was conducted at the chromatographic unit, NRC, Egypt, using the liquid chromatography with electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS).

Positive mode: As recommended by the system operator, the sample was separated using the Ascentis® C18 Column with dimensions of 2.1 × 150, 2.7 mm. Formic acid and formic acid in acetonitrile (0.1%:0.1%) were used as an eluent at a flow rate 0.3 mL/min. Samples were scanned at the range of 50–1000 Da, and compounds were identified using MS-DIAL (version 4.70), as followed by El-ssayad et al. [17].

2.2.3. Antimicrobial Evaluation of Ethanolic Clove Extract

Disk Diffusion Assay

The activity of the crude ethanolic clove extract against microorganisms was evaluated as recommended in the guidelines of the British Society [18]. Briefly, from a 0.5 "McFarland" culture, nutrient agar (N.A) were surface-swabbed for pathogenic bacteria, and potato dextrose agar (PDA) were surface-swabbed for yeast and molds (20 mL); they were then inoculated with a 250 µL spore suspension (1 × 105 cfu/mL) of fungal culture to obtain the semi-confluent growth. Within 15 min, disk papers with diameters of 6 mm and containing 10 µL of the extract were set on the inoculated agar. DMSO served as negative control, and Gentamycin (100 µg/mL) and Clotrimazole (1 mg/mL) were used as antibacterial or antifungal positive control, respectively. After incubation at 35 °C for 20 h for bacteria and 27 °C for 6 days for yeast and molds, the inhibition zone diameters (mm) were recorded. Tests were performed in triplicates.

Determination of Minimal Inhibitory Concentration (MIC)

Clove extract was tested for its MIC value using the agar dilution method following the EUCAST protocol [19]. To summarize, agar tubes were melted and then cooled in a water bath at 50 °C. The tubes were then supplemented with an accurately prepared dilution of the clove extract (6.0, 2.5, 1.0, 0.5, 0.25, 0.125, and 0.05 mg/mL), which was vortexed well and poured into sterile pre-labeled Petri dishes. Once the agar surface was completely dry at room temperature, 1 µL of a 10^7 CFU/mL microbial suspension was inoculated onto the plates. The plates were then incubated, and the MIC was determined from the lowest concentration of the extract which inhibited the organisms.

2.2.4. Manufacturing of Bio-labneh with Clove Extract

Bio-labneh was prepared using UF-retentate, following the method outlined by [20], with minor adjustments. UF-retentate from buffalo skim milk was heated to 85 °C for 15 s, then cooled and adjusted to 42 °C. Starter cultures of bio-labneh were added at a ratio of 2% to the UF-retentate. The mixture was poured into sterilized cups and incubated at 42 °C until complete coagulation. After fermentation, the cups were divided into two groups: one without surface spraying served as the control, while the other was sprayed with an ethanolic clove extract at the ratio of 15 mg/cup of bio-labneh to prevent the growth of spoilage fungi. All samples were then stored in the refrigerator at 7 °C for 8 weeks. Throughout storage, samples were periodically analyzed every 2 weeks for chemical composition and textural and antioxidant properties. Aroma compounds were also evaluated, and the levels of molds and yeasts (Log CFU/g) were monitored.

2.2.5. Microbiological Quality Analyses

Molds and yeasts were examined to indicate the microbiological quality of the bio-labneh using Potato Dextrose Agar. Serial 10-fold dilutions were prepared, and 1 mL/plate was transferred. After a 3-day incubation period at 30 °C, the fungal population were enumerated (CFU/gram) [21].

2.2.6. Chemical Composition of Bio-Labneh

Control and bio-labneh cheese were analyzed for total solids, fats, and total protein contents [22]. Readings of pH value for the fermented product were measured in different treatments (Hanna, Germany).

2.2.7. Antioxidant Activity of Bio-Labneh

Estimating the antioxidant potential, the free radical scavenging ability of labneh was investigated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay as mentioned by [23]. The following formula was used to determine DPPH scavenging activities:

$$\text{labneh antiradical activity (\%)} = [(A0 - A1)/A0] \times 100$$

A0 is the absorbance of the control (DPPH solution) and A1 is the absorbance of the sample.

2.2.8. Textural Profile of Bio-Labneh

The textural profile is crucial in determining the acceptance and quality of dairy products. Texture Profile Analysis (TPA) involves compressing a product twice to stimulate mouth conditions [24]. In this study, the bio-labneh samples were compressed in the double tester (W. Sussex, UK) at 5 different points on the surface. All of springiness (mm), gumminess (N), hardness (N), cohesiveness, and chewiness (mm) were determined based on the definition provided by [24].

2.2.9. Determination of Volatile Compounds of Bio-Labneh by GC-Mass Spectrometry

Samples were subjected to headspace solid phase microextraction (SPME) as performed by [25] with slight modifications. In detail, the extraction was carried out using a CAR/PDMS (carboxen/polydimethyl siloxane) fiber with 75-lm film thickness (Supelco, Bellefonte, PA, USA) for 15 min. The fiber was exposed to the sample headspace for 10 min at 50 °C. The highest extraction efficiency was achieved with 3 g of the sample in a headspace vial/15 min at 50 °C. After extraction, volatile compounds from the fiber were brought to the injector of the GC Agilent 8890/MS Agilent 5977B GC/MSD system. The isolated peaks were matched with the (National Institute of Standard and Technology, NIST) mass spectrum library.

Sensory Properties of Bio-Labneh

The sensory features of the samples were recorded when they were fresh and during the time of storage, as performed by [24]. The flavor was measure by 50 points, appearance by 10 points, and body and texture by 40 points, as well as the overall acceptability (sum of the previous scores); all scores were recorded. Twenty panelists (10 women and 10 men, 30–55 years old) from the Dairy Department, NRC, Giza, were involved in the measuring of the sensory properties.

Statistical Analysis

Data were analyzed using SAS Statistical Analysis Software Version 9.1.3. Probability equal ($p < 0.05$) was significant. Each assay was carried out in triplicates.

3. Results and Discussion

3.1. LC-ESI-MS/MS Profiling of Ethanolic Clove Extract

The ethanolic extract was injected into liquid chromatography/mass spectroscopy using positive scan mode to determine the compounds responsible for the reported antimicrobial action. The data are displayed in (Figure 1) and detailed in (Table 1). The results showed that the ethanolic extract contained fatty acids, phenolic acids, flavonoids, and alkaloids. These active compounds play important roles as antimicrobials and antioxidants [26]. Plentiful studies have shown that cloves possess considerable bioactive characteristics such as antioxidants, antinociceptives, anti-glycations, and antimicrobials, as offered in (Table 1). As exhibited in (Figure 1), the ethanolic extract of clove by LC-ESI-MS/MS analysis revealed the presence of nine major peaks at retention time 2.54, 7.54, 3.15, 4.48, 6.57, 7.54, 8.24, 19.53, and 26.77. Compounds such as hexadecenoic are considered precursors for fatty acids. In addition, the data showed the presence of chaulmoogric acid, propyl gallate, protocatechuic acid, quercetin, kaempferol, linoleic acid, trans-cinnamic acid, and sinapine, as they are considered biologically active substances.

Table 1. LC-ESI-MS/MS profiling of ethanolic Clove extract.

RT	Name	Area	Height	Nature	Activity	Reference
2.54655	Chaulmoogric Acid	23,252,350	868,137.5	Cyclopentenyl fatty acid	Antioxidant, Antimicrobial, anti-inflammatory	[27]
7.54475	Hexadecanedioic acid	3,556,171	153,683.8	Fatty acid		
3.155767	Propyl gallate	12,997,020	637,667.1	Phenolic acid derivative	Antioxidant	[28]
4.486117	Protocatechuic acid	1,147,789	53,354.38	Phenolic acid	Antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastroprotective, anticancer, anti-aging, neurological effective and immune-modulatory activities	[29]
6.5758	Quercetin	3,431,954	152,700.2	Flavonol		
7.54475	Kaempferol	2,636,916	114,283.4	Flavonol	Anti-inflammatory	[30]
8.249967	Linoleic acid	3,794,725	232,804.7	Fatty acid	antibacterial	[31]
19.53632	Trans-cinnamic acid	20,571,800	819,391.6	Aromatic carboxylic acid	Antioxidant, anti-inflammatory, anticancer, antimutagenic, anti-glycemic, neuroprotective, and antibacterial activities.	[32]
26.77547	Sinapine	11,317,340	651,271.3	Alkaloid		

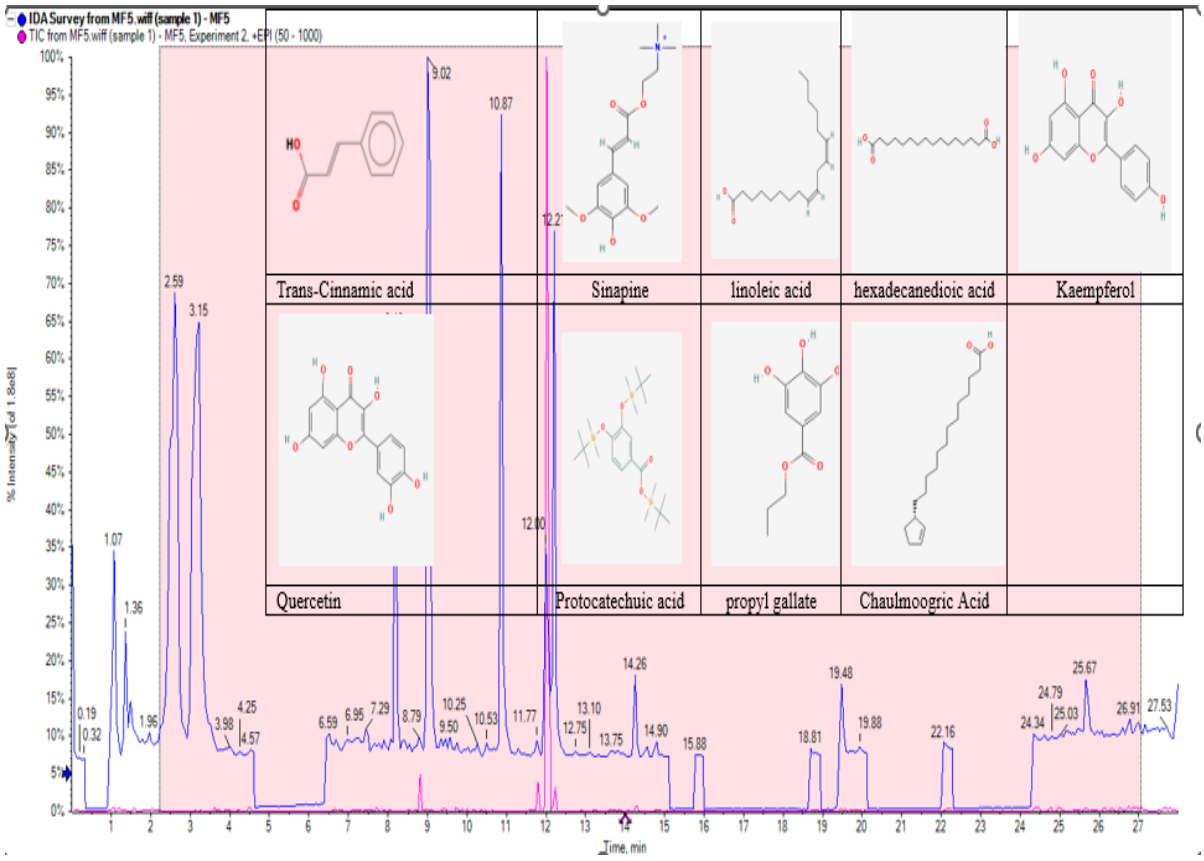

Figure 1. LC-MS chromatogram and spectra of clove flower buds' ethanolic extract.

3.2. Antimicrobial Activity of Clove Extract

3.2.1. Disk Diffusion Assay

An experiment was conducted to qualitatively estimate the antimicrobial potential of ethanol-extracted clove flower through disk diffusion assay. The results, presented as inhibition diameter (mm) in (Table 2), showed that ethanol-extracted clove bud flowers had little impact on Escherichia coli, Staphylococcus aureus, Saccharomyces cerviceae, Listeria monocytogenes, Salmonella enterica, and Bacillus cereus, with inhibition zones ranging from 6.17 to 7.33 mm. Sofia et al. [33] and Pandey et al. [15] outlined that the antibacterial effect of clove was resisted by many types of microorganisms. Sofia et al. [33] clarified that clove with a concentration of 3% was the most inhibiting for Escherichia coli, Bacillus cereus, and Staphylococcus aureus, where the diameter of inhibition ranged from 16.0 to 19.3 mm using the disk diffusion technique, while the zone of inhibition for the same strains ranged from 23.3 to 25.6 mm using the well method. However, ethanol-extracted clove flower could not inhibit Pseudomonas aeruginosa. The ethanol-extracted clove flower showed maximum inhibition for Penicillium verrocosum and Aspergillus flavus (20.33 mm) with zones of inhibition larger than positive control (Table 2). The same observation was mentioned by [34] when studying the effect of clove-nano emulsion on some fungi. The results showed that A. flavus was the most sensitive to clove-nano emulsion.

It is clear that the diversity of microbial strains is an important factor in the antimicrobial effect of ethanol-extracted clove flower. Most spices have more of an antibacterial effect on Gram-positive bacteria than on Gram-negative ones. The difference in the composition of the cell wall of Gram-positive and Gram-negative bacteria also plays a part in the antibacterial effect. Phytochemical compounds, such as cyclopentenyl fatty acid, phenolic acid, flavonol, aromatic carboxylic acid, and alkaloid, available in ethanolic extract of clove are responsible for the antibacterial and antifungal activity observed. Many studies have

shown that clove can destroy cell walls and membranes of microorganisms, inhibiting the normal synthesis of DNA and proteins [35]. Eugenol, a major component of clove, can also inhibit the production of amylase and protease in *B. cereus* [36]. Pandey et al. [15] observed that clove extract with a 1% ratio acts as a potent antimicrobial agent with broad spectrum activity. This oil also exhibited antibacterial effects against *E. coli* O157:H7 due to the presence of eugenol, which is considered the main ingredient responsible for its antimicrobial properties.

Table 2. Qualitative estimation for antimicrobial potential of clove extract.

Test Organisms	Inhibition Diameter (mm) *	
	Ethanolic Extract	Positive Control
Bacillus cereus	7.33 ± 0.34^{F}	16.50 ± 0.00^{C}
Staphylococcus aureus	6.33 ± 0.34^{HI}	16.50 ± 0.00^{C}
Listeria monocytogenes	6.83 ± 0.17^{FGH}	15.0 ± 0.00^{D}
Escherichia coli	6.17 ± 0.17^{I}	18.83 ± 0.17^{B}
Salmonella enterica	7.0 ± 0.00^{FG}	20.0 ± 0.00^{A}
Pseudomonas aeruginosa	ND	ND
Penicillium verrocosum	20.33 ± 0.34^{A}	12.0 ± 0.00^{E}
Aspergillus flavus	20.33 ± 0.34^{A}	17.0 ± 0.00^{C}
Saccharomyces cerviceae	6.67 ± 0.34^{GHI}	12.0 ± 0.00^{E}

* Including disk diameter of 6 mm. ND: not detected Data expressed as mean ± SE; means that do not share a letter within the same column are significantly different.

3.2.2. Minimal Inhibitory Concentration

Although the antimicrobial effect was not obtained by the disk diffusion method on the bacteria under study (Table 2), the use of minimal inhibitory concentration (MIC) values gave satisfactory results. MIC values were determined using the poisoned plate technique and are presented in (Table 3) as (mg/mL). MIC values ranged from 1 to 6.5 mg/mL for bacterial strains. *Bacillus cereus* showed more sensitivity among the bacteria studied. This result is consistent with the [37] study, which showed that the components of cloves, especially eugenol, have an effect on the production of some enzymes important for bacterial growth. Clove ethanol extract showed the lowest inhibition for *Pseudomonas aeruginosa* (6.5 mg/mL). Hoque et al., 34, reported that clove ethanol extract showed the lowest inhibition for *P. aeruginosa* PA 01(5.5 mg/mL). However, fungal strains showed the highest inhibition of 0.125 mg/mL, 0.25 mg/mL, and 1 mg/mL for *Aspergillus flavus*, *Penicillium verrocosum*, and *Saccharomyces cerviceae*, respectively. These results align with the findings reported by [15,38].

Table 3. Estimated values of minimal inhibitory concentration for clove extract.

Indicator Strain	MIC Value (mg/mL)
Bacillus cereus	1.00 ± 0.00^{C}
Staphylococcus aureus	2.50 ± 0.00^{B}
Listeria monocytogenes	2.50 ± 0.00^{B}
Escherichia coli	2.50 ± 0.00^{B}
Salmonella enterica	2.50 ± 0.00^{B}
Pseudomonas aeruginosa	6.50 ± 0.00^{A}
Penicillium verrocosum	0.25 ± 0.00^{D}
Aspergillus flavus	0.125 ± 0.00^{E}
Saccharomyces cerevisiae	1.00 ± 0.00^{C}

Data expressed as mean ± SE; means that do not share a letter are significantly different.

3.3. Bio-labneh and Its Properties

3.3.1. Microbiological Quality of Manufactured Bio-labneh Cheese

At the end of the study, an in situ application study was conducted to evaluate the preservation efficiency of the extract. The results of microbiological quality were displayed in (Figure 2). During the shelf-life period of refrigerated bio-labneh, the most common concern, the mold and yeast populations, were enumerated and the count was recorded as Log CFU/g. Prior to storage (zero time), both the control sample and the spray-treated sample recorded 1 Log CFU/g. As time passed, the control sample showed an increase in fungal count, reaching 8.04 Log CFU/g at 56 days of cold storage. In contrast, no growth was revealed in the spray-treated sample after 5 days until the end of the 56 days in the refrigerator, achieving significant reduction in fungal count than the control under the same conditions ($p < 0.05$). Similar data were observed by [39] who detected no growth of mold and yeast in labneh containing essential oils throughout a storage period of 21 days. El-Awady et al. [40] showed a significant ($p < 0.05$) effect on mold and yeast counts in yogurt fortified with clove extract nano-formulations at ratios of 0.0125, 0.025, and 0.05% in up to 15 days of cold storage. On the other hand, Rifky et al. [41] studied the effects of various essential oils (EOs) such as cinnamon, garlic, caraway, and clove oils on yogurt production. They found that mold and coliform counts were not detected in any treatments for up to 30 days of storage, while garlic extract had the highest impact after 40 days of cold storage.

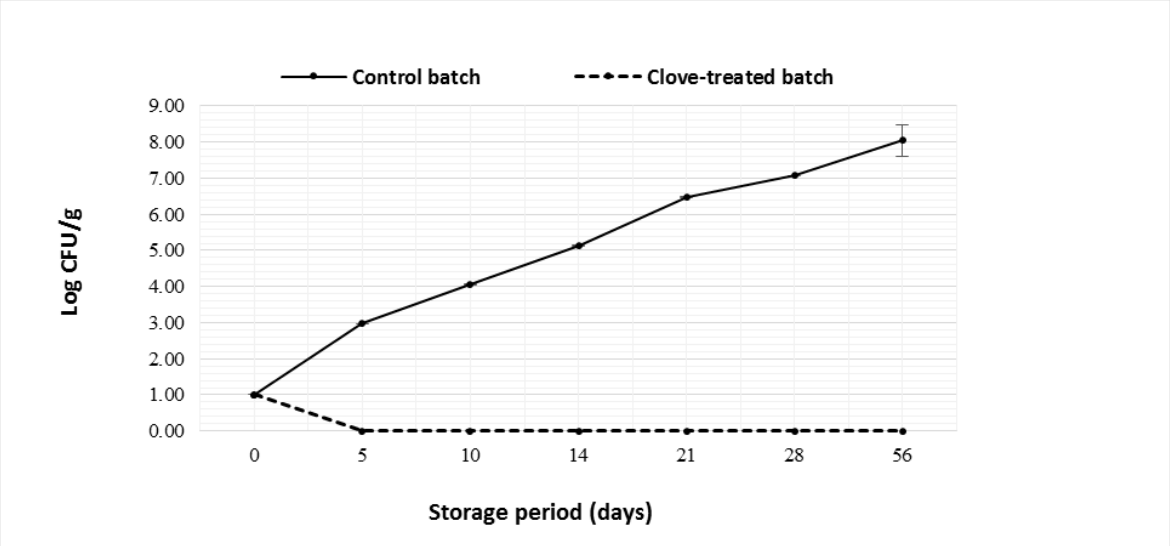

Figure 2. Investigation of microbiological quality for spray-treated labneh cheese compared to control one.

3.3.2. Chemical Composition of Bio-Labneh

Bio-labneh samples were analyzed for their composition when fresh and during an eight-week storage period, as detailed in (Table 4). Overall, the total solids content of the bio-labneh samples increased during the cold storage period, likely due to moisture loss. Initially, there were no significant differences ($p \leq 0.05$) between the control and the treated samples. The highest total solids contents were recorded at the end of the trial, with values of 26.74% for the control and 27.15% for the treated samples. Interestingly, the bio-preservative extract did not impact the total solids content, whether in fresh samples or during cold storage, aligning with findings from [2,41].

Similarly, there were no significant differences ($p \leq 0.05$) in fat content between the samples. Regarding total protein (TP%), as the storage period increased, TP content also

slightly increased according to the increase in total solids. The increase had no significant differences ($p \leq 0.05$) in both control and treated samples. Thabet et al. [2] noted that labneh containing 0.8% of cumin and cinnamon oils had higher protein content than untreated samples.

Table 4. Chemical composition of bio-labneh when fresh and during storage period/weeks.

Parameters	Samples	Storage Period/Weeks				
		Fresh	2	4	6	8
TS %	C	25.27 ^{Ab} ± 0.03	25.29 ^{Ab} ± 0.04	25.98 ^{Ba} ± 0.02	26.43 ^{Aa} ± 0.03	26.74 ^{Aa} ± 0.55
	T	25.42 ^{Ab} ± 0.47	25.85 ^{Ab} ± 0.04	26.20 ^{Aab} ± 0.03	26.63 ^{Aab} ± 0.03	27.15 ^{Aa} ± 5.75
Fat %	C	0.75 ^{Ac} ± 0.25	0.79 ^{Ac} ± 0.28	0.85 ^{Ab} ± 0.38	0.89 ^{Ab} ± 0.20	0.91 ^{Aa} ± 0.20
	T	0.76 ^{Ac} ± 0.50	0.75 ^{Ac} ± 0.25	0.84 ^{Ac} ± 0.38	0.85 ^{Ab} ± 0.18	0.89 ^{Aa} ± 0.20
TP %	C	11.68 ^{Aa} ± 0.19	11.95 ^{aa} ± 0.05	12.75 ^{Aa} ± 0.29	13.01 ^{Aa} ± 0.13	13.17 ^{Aa} ± 0.17
	T	11.735 ^{Aa} ± 0.11	11.97 ^{Aa} ± 0.04	12.45 ^{Aa} ± 0.28	12.90 ^{Aa} ± 0.12	13.24 ^{Aa} ± 0.14
pH	C	4.81 ^{Bb} ± 0.01	4. 85 ^{Aa} ± 0.01	4.56 ^{Ac} ± 0.02	4.44 ^{Ad} ± 0.01	4.35 ^{Be} ± 0.02
	T	4.86 ^{Aa} ± 0.005	4.71 ^{Bb} ± 0.01	4.58 ^{Ac} ± 0.03	4.48 ^{Ad} ± 0.02	4.40 ^{Ad} ± 0.02

Data expressed as means ± standard deviation; capital letters are the difference between samples, small letters are the difference between periods, TS: total solids; TP: total proteins.

The pH values were similar between the two samples, indicating that the bio-preservative extract did not affect the acidity of the product. There was a slight reduction in pH values over the storage period, with the samples at the end of the 8-week period showing the lowest values. This decrease in pH values may be attributed to the activity of starter cultures, in line with observations by [42].

3.3.3. Antioxidant Activity of Bio-Labneh

Several studies have clarified that clove oil or extract is a powerful antioxidant agent [14]. Natural antioxidant agents have been used in many dairy products to slow down damage factors, such as lipid oxidation and hydrolysis, and to minimize nutritional losses, prevent the release of free radicals, and provide numerous health benefits. The antioxidant power of clove extract owes to the existence of phenolic compounds, which are considered the most effective factor in antioxidant activity [14,43]. Several experiments using clove buds, either in water or ethanolic extract, have shown powerful minimizing potential, metal chelating, free radical scavenging, and superoxide anion radical scavenging activities at different concentrations (60, 40, and 20 µg/mL) [10].

The antioxidant activity of the bio-labneh samples was tested when fresh and during storage periods, as shown in (Figure 3). A significance ($p \leq 0.05$) was reported in the difference observed between treated and untreated samples at all times. Over time, the antioxidant activity of all samples slightly increased. The sprayed-clove extract provided the treated samples with the highest antioxidant properties compared to the control. The high antioxidant activity of sprayed-clove samples is attributed to their unique compounds, such as chaulmoogric acid, propyl gallate, protocatechuic acid, quercetin, trans-cinnamic acid, and eugenol, which were identified through LC-MS and GC-MS analysis, as mentioned in the aroma compounds and microbiological analysis section. These findings align with the results reported by [44], who emphasized that clove extract has the strongest antioxidant activity compared to cinnamon stick, oregano, pomegranate peel, grape seed, black cumin, and black pepper. Additionally, Fouad et al. [45] noted that the antioxidant activity of bio-labneh with different microcapsule treatments significantly increased during cold storage.

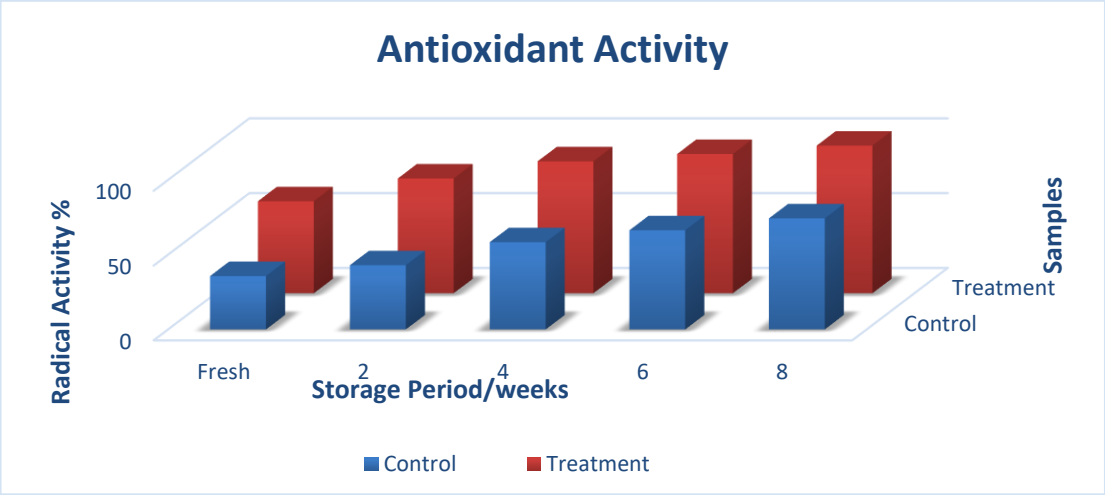

Figure 3. Antioxidant activity (%) of bio-labneh when fresh and during storage period/weeks.

3.3.4. Texture Profile Analysis of Bio-Labneh

The rheological behavior of labneh cheese is significantly influenced by processing techniques and chemical composition [4]. In a study on bio-labneh, changes in texture parameters were observed both when the samples were fresh and during storage periods, as shown in (Table 5). Hardness is a prevalent evaluated parameter when determining yogurt and labneh texture. Significant differences ($p \leq 0.05$) in hardness values were noticed between the control and treated samples. As the storage period increased, the hardness values were raised in both the control and treated samples. The use of clove extract in the treated samples resulted in a smoother texture contrasted to the control during the initial 15 days of storage, potentially due to the preservation of starter cultures by the clove extract. Exopolysaccharides produced by *S. thermophilus*, a major species, contributed to fast acidification and may have influenced the texture changes observed [46]. Over time, the hardness values significantly ($p \leq 0.05$) increased in each of the control and treated samples. Changes in hardness can be attributed mainly to the changes in the total solid's contents of the labneh as a close relation was apparent between hardness and total solids. El-Sayed et al. [47] reported an increase in the hardness of labneh cheese during storage in agreement with the present results. Cohesiveness is defined as the strength of internal bonds making up the body of the product. Significant differences ($p \leq 0.05$) were noticed in cohesiveness and springiness values between the control and treated sample. Also, during the first 4 weeks of storage, there were significant differences ($p \leq 0.05$) in both the control and treated samples. Gumminess is defined as the force needed to break down a food to be swallowable [48]. Results showed that there were significant differences ($p \leq 0.05$) in gumminess between the fresh controls and treated samples. The treated samples had the lowest values of gumminess compared to the control. The values of the control samples increased during the storage period, in contrast with the treated samples, whose gumminess values increased in the first three weeks and then decreased in the last two weeks. This decrease in treated gumminess values may be due to the cohesiveness values in the last two weeks. The calculation method of gumminess is dependent on cohesiveness, (gumminess (N) = hardness $\times$ cohesiveness). A similar trend was observed with chewiness (work needed to masticate a solid food to a state ready for swallowing) values in the control and treated samples due to its relationship with gumminess. Chewiness (mm) = gumminess $\times$ springiness. These results were in accordance with 20. As mentioned by [4], UF-retentate presented excellent texture compared to the traditional method. They also suggested that products with high protein ratios have greater gel strength.

Table 5. Rheological properties of bio-labneh when fresh and during storage period/weeks.

Parameters	Samples	Storage Period/Weeks				
		Fresh	2	4	6	8
Hardness (N)	C	$11.5^{Ad} \pm 0.15$	$14.30^{Ac} \pm 0.30$	$17.86^{Ab} \pm 0.11$	$19.11^{Ba} \pm 0.18$	$19.27^{Ba} \pm 0.19$
	T	$7.88^{Bd} \pm 0.04$	$12.00^{Bc} \pm 0.15$	$17.55^{Ab} \pm 0.18$	$20.22^{Aa} \pm 0.16$	$21.05^{Aa} \pm 0.31$
Cohesiveness (B/A area)	C	$0.613^{Ba} \pm 0.01$	$0.617^{Ba} \pm 0.01$	$0.522^{Bc} \pm 0.0$	$0.569^{Ab} \pm 0.01$	$0.576^{Ab} \pm 0.01$
	T	$0.704^{Ab} \pm 0.01$	$0.624^{Ac} \pm 0.01$	$0.770^{Aa} \pm 0.01$	$0.328^{Bd} \pm 0.00$	$0.352^{Bd} \pm 0.03$
Springiness (mm)	C	$0.769^{Bb} \pm 0.01$	$0.780^{Aa} \pm 0.01$	$0.698^{Bc} \pm 0.01$	$0.714^{Ac} \pm 0.01$	$0.721^{Ac} \pm 0.01$
	T	$0.860^{Aa} \pm 0.01$	$0.730^{Bc} \pm 0.01$	$0.743^{Ab} \pm 0.01$	$0.538^{Bd} \pm 0.01$	$0.535^{Bd} \pm 0.01$
Gumminess (N)	C	$7.050^{Bd} \pm 0.19$	$8.820^{Ac} \pm 0.23$	$9.320^{Bb} \pm 0.29$	$10.87^{Aa} \pm 0.27$	$10.973^{Aa} \pm 0.12$
	T	$5.547^{Ad} \pm 0.03$	$7.49^{Bb} \pm 0.16$	$13.51^{Aa} \pm 0.27$	$6.960^{Bc} \pm 0.13$	$6.85^{Bc} \pm 0.21$
Chewiness (N/m)	C	$5.421^{Ac} \pm 0.18$	$6.88^{Ab} \pm 0.32$	$6.50^{Bb} \pm 0.25$	$7.761^{Aa} \pm 0.24$	$7.966^{Aa} \pm 0.47$
	T	$4.77^{Bc} \pm 0.08$	$5.47^{Bb} \pm 0.15$	$10.04^{Aa} \pm 0.24$	$3.744^{Bd} \pm 0.14$	$3.70^{Bd} \pm 0.10$

Data expressed as means ± standard deviation; capital letters are the difference between samples (in column), small letters are the difference between periods (in row).

3.3.5. Aroma Compounds

Volatile aroma compounds in fermented dairy products, such as yogurt or labneh cheese, are mainly produced through lactic acid fermentation. These compounds provide a unique flavor to the fermented product. The aroma compounds of bio-labneh cheese are listed in (Table 6). There were significant variations in aroma compounds between the two samples, whether fresh or after a storage period. More than 30 aroma compounds were identified through GC–MS analysis for both the control and treated samples. The most abundant compounds in the fresh control sample were benzene (15.44), dimethyl silanedio (16.29), methylbenzene (5.96), 3-Hydroxy-2-butanone (5.81), 3-Heptanone (2.93), styrene (6.66), cyclopentyl 4-ethylbenzoate (9.29), benzaldehyde (11.02), heptanal (2.84), 1,6-Dihydrocarveol (4.14) and 2,7-Dimethyl-1-octanol (1.04). Similarly, the fresh treated sample contained benzene (2.87), dimethyl silanedio (9.82), styrene (9.3), cyclopentyl 4-ethylbenzoate (7.6), 1,6-Dihydrocarveol (2.72), eugenol (11.58), copaene (3.4), β-caryophyllene (56.02), α-humulene (5.6), and aroma ndendrene (1.25) as the most prominent compounds. Eugenol and β-caryophyllene were the most abundant compounds in the treated sample, resembling the clove extract [11]. Eugenol is known for its antioxidant, antimicrobial and antifungal properties. Throughout the experiment, some compounds disappeared while others emerged, such as toluene, methyltartronic acid, ethyl benzene, palmitic acid, and methyl N-hydroxy benzene carboxi-midoate.

Table 6. Aroma compounds of bio-labneh when fresh and during storage period/weeks.

Aroma Compounds (Area%)	Storage Period/Weeks					
	Fresh		4 Weeks		8 Weeks	
	C	T	C	T	C	T
Benzene	15.44	2.87	4.76	18.61	4.64	4.59
2,3-Pentanedione	3.21			3.34		
Dimethylsilanedio	16.29	9.82		20.27		0.49
Methylbenzene	5.96		2.43			
2-Hexanone	2.13		1.42			
3-Heptanone	2.93				1.47	
Styrene	6.66	9.3	10.79	7.31	4.99	15.32
Styrene	3.86	4.28	3.24	3.86		
5-Methyl-3-hexanol	1.43			1.3		
Cyclopentyl 4-ethylbenzoate	9.29	7.6				
Benzaldehyde	11.02				1.76	0.74
Heptanal	2.84		2.12	1.41	2.57	1.26
p-Cymene	0.75		1.82	3.74	2.95	5.13
1,6-Dihydrocarveol	4.14	2.72	1.77	2.72	2.28	5.15

Table 6. *Cont.*

Aroma Compounds (Area%)	Storage Period/Weeks					
	Fresh		4 Weeks		8 Weeks	
	C	T	C	T	C	T
γ-Terpinene	0.62		0.76	1.41	1.36	2.19
2-Propyl-1-pentanol	0.95	0.95				14.26
2-Nonanone	0.93					0.91
α-Cubebene		0.89				
Eugenol		11.58		2.65		
Copaene		3.4				
β-Caryophyllene		36.02		3.8		
Aromandendrene		1.25				
α-Humulene		5.6		0.35		
Acetyleugenol		1.74		0.42		
δ-Cadinene		1.98				
Toluene			13.04		10.84	18.72
Methyl tartronic acid					20.94	
Ethyl Benzene			26.05		11.63	0.93
Palmitic acid			1.47		5.15	1.5
Methyl N-hydroxy benzene carboximidoate			9.36	14.02		5.5

Dan et al. [49] highlighted that benzaldehyde, a volatile compound, contributes a unique flavor to fermented products, resembling bitter almonds at low levels and maraschino cherries at higher levels. This compound is commonly found in dairy products. Additionally, heptanal provides a "green, sweet" flavor, enhancing the taste of yogurt and labneh products. They found low levels of heptanal (0.21 to 0.6%) in S. thermophilus-fermented milk after 14 days of storage, whereas our data showed a ratio of 2.84% for heptanal.

On the other hand, 2,3-butanedione, derived from pyruvate, through lactose and citrate metabolism, imparts a "buttery, creamy, vanilla" flavor to fermented products, as described by [49].

3.3.6. Sensory Attributes of Bio-Labneh

The critical method for determining the quality and shelf life of any product is examining its sensory characteristics. Control and spray-treated samples had been evaluated for sensory parameters, as presented in (Figure 4). There were no significant ($p \leq 0.05$) differences between the two samples at the fresh time in all parameters. By that time, the significant ($p \leq 0.05$) differences clearly appeared between the control and treated samples. All spray-treated samples gained the highest scores in flavor, appearance, body, and texture during the storage period compared to the control ones. The best scores were at 2, 4, and 6 weeks, they then started to slightly decrease at the end. The sprayed extract gave the product a refreshing taste even with its small amount.

In the same vein, both studies in 4 found that labneh which contained 0.2 ppm thyme, marjoram, or sage essential oil had gained the highest scores of flavor and it was more acceptable compared to the control. There was a gradual decrease in all parameters at the end of storage period. Zaky et al. [50] were on the same track when they fortified salt-free labneh with dill and caraway essential oils. Many studies deduced that labneh, which is made from milk, contained 4% fat and 14% TS, with yogurt starter cultures considered a good sensory property in labneh [4].

Thus, it could be concluded that the sprayed-clove extract can be used for extending the shelf life of labneh cheese and improving its quality parameters.

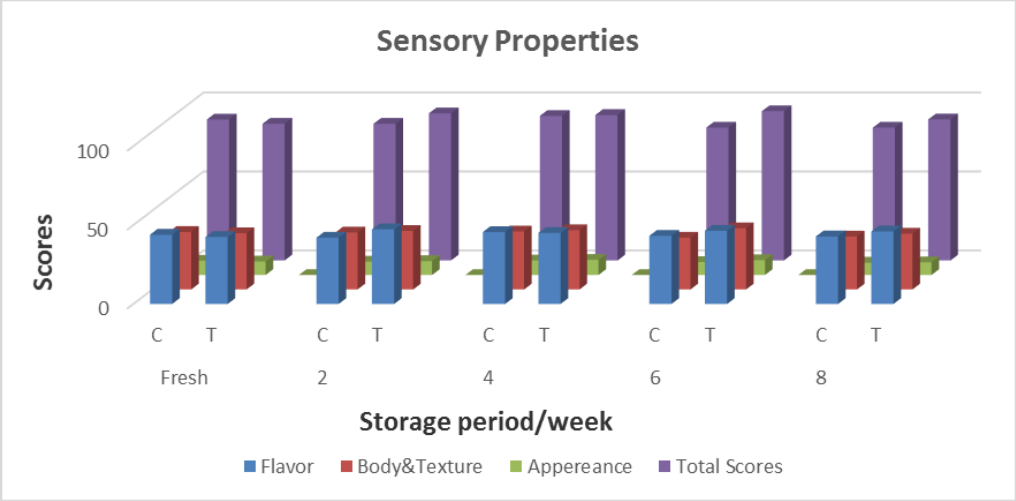

Figure 4. Sensory properties of bio-labneh when fresh and during storage period/weeks.

4. Conclusions

This research aimed to expand and sustain the shelf life of labneh cheese using a bio-preservation method involving clove extract sprayed on the surface at a specific ratio. Clove extract has a positive antifungal activity against *Aspergillus flavus*, *Penicillium verrocosum*, and *Saccharomyces cerviceae*. According to LC-Mass, the clove extract contained some active components, such as chaulmoogric acid, trans-cinnamic acid, propyl gallate, sinapine, linoleic acid, quercetin, and kaempferol, which have antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastro-protective, anticancer, anti-aging, neurological effective, and immune-modulatory activities. Thus, the sprayed-clove extract grants the treated samples the highest antioxidant properties compared to the control. The addition of clove extract had no negative impact on the structure of bio-labneh cheese. The most quantitative compounds in the sprayed sample were eugenol and β-caryophyllene, which are among the most plentiful substances in clove extract. Overall, the incorporation of clove extract improved the microbial, antioxidant, rheological, and sensory properties, as well as the aroma compounds of the labneh, enhancing its quality and longevity.

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