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FUNCTIONAL CHARACTERISTICS OF BIOACTIVE PHYTOCHEMICALS IN *BETA VULGARIS L.* ROOT AND THEIR APPLICATION AS ENCAPSULATED ADDITIVES IN MEAT PRODUCTS

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ABSTRACT

Beetroot ethanolic extract contains active compounds and valuable elements such as phenols, carotenoids, alkaloids, tannin, flavonoids, and vitamins B_3, B_9, B_6, and C. Quality characteristics and microbiological activity, texture, and colour were examined in the storage process at refrigerated temperature (up to 9 days at 4±2 °C) of beef burger pads made directly and encapsulated in alginate beads of *Beta vulgaris subsp.* (BVE). Over time, the Encaps-SDW and SDW (Control Samples) total mesophile bacteria counts peaked at CFU 8.61±0.22 and 8.74±0.17 log CFU/g, respectively, during storage (9 days). The lowest values ($p < 0.05$) were shown in the Encaps-BVE and BVE samples, with 7.23±0.12 and 6.58±0.09 log CFU/g, respectively. However, the differences between all samples were significant ($p<0.05$), the BVE extract strongly inhibited *Enterobacterial* growth, with values on average two log units lower in BVE and Encaps-BVE than SDW and Encaps-SDW samples (control samples). Also, the addition of BVE extract kept the pH of beef minced nearly constant during storage; however, the pH value of control samples increased significantly ($p<0.05$). Furthermore, samples containing Encaps-BVE showed a more consistent trend in terms of texture and colour characteristics during the storage period than the other treatments, indicating the importance of using it as a natural preservative in meat product formulations to preserve quality standards and preservation.

1. Introduction

Consumers appear to be wary of chemical additives in these cases. Thus, attention is paid to discovering natural antimicrobials occurring to preserve food due to consumer knowledge and understanding of edible food products and an increasing concern around microbial resistance to traditional preservatives (Chouhan et al., 2017). However, some conventional or regulatory licensed antimicrobials have several limitations. In these cases, customers are likely to be wary of chemical additives. Growing market demand has led to high-quality, long-life storage foods and ready-to-eat foods that are only moderately reserved and retain a natural and fresh look as much as possible (Panchal, 2020). This has resulted in the quest for new anti-microbial compounds from natural sources. It is the potential to extract natural compounds and other natural products to contain bacteria in foods. Aromatic plants have considerable commercial value in foods, pharmaceutical industries, and cosmetics (Jain et al., 2019). Since ancient times, their use has taken place, and despite many of them being replaced by synthetic ones, demand for natural products is increasing. As an antimicrobial agent for food preservation, numerous extracts have emerged on the market in recent years. Scaling up antimicrobials' activity across the permitted regulatory substances is the primary

incentive to identify effective antimicrobials among natural compounds. Medicinal plant extracts are now emerging as alternatives to traditional natural preservatives to control the growth of foodborne pathogens and food spoilage bacteria, as they are generally healthy for humans and environmentally friendly. Microbe contamination, which impairs food quality and results in financial losses, is one of the most severe issues in the food sector processing. (Priyadarshani & Rath, 2012; Aneja et al., 2014; Flores & Toldrá, 2020; Fortunati et al., 2019; Ni et al., 2021).

Beta vulgaris L. subsp. vulgaris is a *Chenopodiaceae* (*Angiosperm*) family member and is commonly referred to as beets or garden beetroot. Beetroot varies in colour from yellow to purple red depending on the variety. Red beets are consumed by humans worldwide. Beetroot is a biannual herbaceous agricultural plant that is farmed for its edible roots and leaves. Salads, soups, jams, and juice are made with beetroots (Goldman & Navazio, 2003). Moreover, because the leaves are high in vitamins and antioxidants, they can be consumed raw or cooked as a spinach alternative. Due to betalains pigments in red beets, they have pharmaceutical and commercial applications, including food product coloring, medicinal formulations, cosmetics, and artwork (Neelwarne & Halagur, 2013; De-Ancos et al., 2015; Celli & Brooks, 2017; Kumar & Brooks, 2018; Miguel, 2018;). As a result, using effective antimicrobial agents found in fruits and vegetables to guard against microorganisms (bacteria and fungi) is a crucial strategy to solving this problem. Antibacterial, anti-inflammatory, antioxidant, antithrombotic, antiatherogenic, cardioprotective, antiallergenic, and vasodilatory effects have been found in red beets (Gliszczynska & Anna, 2013; De-Ancos et al., 2015).

One of the main aspects in enhancing study the chemical composition of red beetroots quality, prolonging shelf-life, maintaining consumer protection, product safety, and minimizing waste was the microbial control in minced beef. The primary purpose of this investigation was to improve the overall stability and quality of beef meat by using two different techniques to incorporate *Beta Vulgaris* L. root: direct processing and encapsulation in sodium alginate and encapsulation in sodium alginate. During refrigerated storage at 4±2 °C, the microbial characteristics, texture, colour, and pH of beef patties were investigated.

2. Materials and methods

2.1. Extraction Technique

The roots of *Beta vulgaris* subsp. *Vulgaris* var. Plano (sugar beet-red beetroot) was collected from the local market in Egypt and transported to the meat products laboratory in the Food Sci. and Tech. Dept., Faculty of Agri., Menoufia Univ. within 1-2 hours at room temperature into plastic boxes. The roots were manually peeled after being rinsed and cleansed with tap water, and then the roots were freeze-dried and ground into a soft powder (a crude extract) using an electric blender. The crud extract was dried in an oven at 35-40 °C for 24- 30 hr. The crud extract (500 mg) was separated for 24 hours using a shaker in 100 ml ethanol or distilled water, and then the solution was filtered and refrigerated at 4 °C until use.

2.1.1. Total Polyphenol

The Folin-Ciocalteu reagent assay was estimated to stain the extract's total phenolic content (Ozsoy et al., 2008). 0.4g dry sample obtained with 20 ml ethanol 80%, soaked in brawn bottle for 24 hours at room temperature, centrifuged for 5 minutes, volume adapted to 25 ml by ethanol 80%, filtered via Whatman no.1 filter paper, 10 ml of the solution evaporated to dryness, dissolved in 5 ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2 µm. Subsequently, the mixture was incubated for 30 minutes at room temperature (22 ºC ± 2), and the absorbance was measured with a spectrophotometer at 760 nm (School instrument, UV line 9400, EU). For the calibration curve, gallic acid was used as a standard material. Total phenolic content expressed as gallic acid equivalent. Both experiments were carried out in triplicate.

2.1.2. Total Flavonoids

The total flavonoids content was measured using the (Sakanaka et al., 2005) process. A 0.5 ml of extract was placed in a 10 ml volumetric flask. Distilled water was added to make an even volume of 5 ml, followed by 0.3 ml $NaNO_2$ (1:20). 5 min later, 3 ml $AlCl_3$ (1:10) were added. After 6 min, 2 ml of NaOH (4%) were added, and then by using distilled water, the total volume increased to 10 ml. The solution was mixed well again, and the absorbance was measured against a blank at 510 nm using a spectrophotometer (Schoot instrument, UV line 9400, EU). The findings were expressed as mg of sample quercetin/g. All measurements were collected in triplicate.

Condition of the instrument measuring total phenolic and flavonoid compounds: Agilent 1260 infinite HPLC Series (Agilent, USA) with Quaternary pump and a Kinetex XB-C18 (Phenomenex, USA) column 100 mm x 4.6 mm running at 35 °C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H_3PO_4 (v/v), (B) methanol, and (C) acetonitrile. The total volume injected was 20 µL. VWD detector calibrated at 284 nm for detection. The total volume injected was 20 µL to measuring total flavonoids compounds. UV detector tuned at 273 nm for detection and database management using claritychrom@ software. This methodology was modified from Mattila et al. (1989) and Goupy et al. (1999). For fractionating flavonoids and polyphenols.

2.1.3. Water-soluble vitamins (WSV)

The WSV was evaluated by HPLC analysis following sample extraction as suggested by Albalá-Hurtado et al. (1997). A sample of dried beets (0.2 g) was weighed to a centrifuge tube together with 15 mL deionized water. Centrifuge at 4000 rpm for 5 minutes after 15 minutes of extraction, then transfer quantitatively to a 25 mL volumetric flask adding more water to the mark: before treatment, filter over a 0.2µm nylon membrane.

Condition of the instrument measuring Water-soluble vitamins: Agilent 1260 infinite HPLC Series (Agilent, USA) with Quaternary pump and a Kinetex XB-C18 (Phenomenex, USA) column 100 mm x 4.6 mm running at 35 °C. A double linear elution gradient using (A) 25 mM NaH_2PO_4 at pH = 2.5 and (B) methanol is used to separate the samples. The total volume injected was 20 µL. Ascorbic acids are detected at 254 nm, while vitamins B6, B3, B9, and B12 are detected at 220 nm (Mattila et al., 1989).

2.1.4. Total Tannin

Folin-Denis's reagent is used to determine the tannin concentration in red beetroots, as suggested by (Saxena et al., 2016). A spectrophotometer (Schoot instrument, UV line 9400, EU) was used to determine the absorbance at 700 nm.

2.1.5. Total Alkaloid

The alkaloids were determined using Adham's technique (Adham et al., 1998). The % alkaloid was defined as follows:

$$\% \text{ Total Alkaloid} = \frac{\text{Weight of remain}}{\text{Weight of Sample}} \; x\; 100$$

2.1.6. Total Anthocyanins

In methanol containing 1% HCl (v/v), fresh beetroot was homogenized and then filtrated. A spectrophotometer (Schoot instrument, UV line 9400, EU) was used to read the filtration at 530 and 657 nm, as Mancinelli et al. (2006) suggested.

2.1.7. Total carotenoid

Total carotenoids of beetroot were obtained using a mixture of acetone: hexane (1:1 v/v), as Jeyanthi et al. (2014) presented. And to use a spectrophotometer (Schoot instrument, UV line 9400, EU), the absorbance of carotenoids was determined at 630 nm.

2.2. Preparation of roots of *Beta vulgaris* in Berger:

Red-colored extract (dry crud extract) was diluted in an equivalent volume of (1:3) sterile distilled water (SDW) was used to add directly or encapsulated to minced beef.

2.2.1. Encapsulation of BVE

Encapsulation of *Beta vulgaris* subsp. extract (BVE) in sodium alginate was administered as designed by (Ribeiro & Veloso, 2020). In summary, according to the

method described above, a defined volume with diluted BVE was blended with sodium alginate 0.5 % (w/v) and allowed the solution to dissolve homogeneously. Once the BVE-alginate solution was homogeneous, the weight was recorded, and the solution was injected into a calcium chloride solution of 1.5% (w/v) using a syringe (0.80 mm × 25 mm). The beads were cleaned and filtered using sterile Whatman® class I paper, then allowed to settle for 20 minutes in the air before being weighed to ensure any BVE during the pelleting process, and there is a loss. According to (Aguirre & Santagapita, 2016), the projected pill size, which might include SDW or BVE, was estimated by scanning digital images of beads (taken with a brother MFC-7360N scanner) the free license program ImageJ1. The standard deviation is an expression of the size of the beads with an average diameter of the ferret that agrees with the total distance (mm) between any two or more point's places and the bead limits.

2.2.2. Beef Burger Patty Preparation

Beef mince from two different batches (3 kg x 2 kg) was purchased at a local market in Shebin El-Kom (Egypt), brought to the meat products lab in a transportable refrigerator at 4 ± 2 °C (within 30min), and rapidly utilized for further meat combinations. To achieve a homogeneous mixture, each lot (1 kg) of minced beef was mixed in a bowl with 0.8 % (w/w) of NaCl for 3 minutes, which had been categorized into four 250g experimental groups; each module was added with sterile distilled water (SDW 5%, v/w), encapsulated sterile distilled water (Encaps-SDW 5%, v/w), *Beta vulgaris* subsp. extract (BVE 5%, v/w), encapsulated *Beta vulgaris* subsp. extract (Encaps-BVE 5%, v/w). The 5% (v/w) concentration of *Beta vulgaris subsp*. the extract was chosen based on previous research on beef slices (Aminzare et al., 2015); Parafiti et al., 2018). Several concentrations of *Beta vulgaris* extract were tested to see which one type of most inhibited microbial growth while refrigerated storage. Burger samples were made with a burger patty builder to achieve a comparable weight and size (about 50 g, with a diameter ranging of 5 cm and a thickness of 2 cm) and labeled as shown in Table 1. All samples were mobilized in an aerobic environment on a food tray with a sealed plastic polyethylene membrane and kept at 4±2 °C in a refrigerator. Prepared burger samples were used to add SDW, each encapsulated with or without sodium alginate, to ground beef as controls. Each sample from every batch was investigated for pH values, microbial parameters, colour characteristics (CIE *a*, L*, and b**), and profile of texture properties after 0, 3, 6, and 9 days of storage period at 4±2 °C.

Table 1. The study's beef burger formulation.

Treatment	Ingredients in burger patties
SDW	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) sterile distilled water
Encaps-SDW	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) of sterile distilled water encapsulated in alginate beads
BVE	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) encapsulated *Beta vulgaris* subsp. extract
Encaps-BVE	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) *Beta vulgaris* subsp. extract encapsulated in alginate beads

2.3. Microbiological Analysis

The antibacterial activity of potential microbial evaluation of the *Beta vulgaris subsp*. extract (BVE) added to the preparation of pies burgers, both coated and non-coated in sodium alginate granules, was predestined by observing the increase of the microbial population after 0, 3, 6, and 9 days of storage at 4±2°C. In summary, a part of every sample (10 g) was aseptically placed in a stomacher filter package containing 90 ml of sterile Ringer's solution, homogenized for 5 minutes, then serially diluted afterward. To observe the growth of total *Pseudomonas*, total mesophilic bacteria, and *Enterobacteriaceae*, suitable dilutions were plated in Petri dishes plate Count Agar with

Violet Red Bile Glucose Agar, cycloheximide 0.1% solution, and *Pseudomonas* Agar Base, supplemented with *Pseudomonas* CFC selective agar supplement (SR-0103). At 35 or 25 °C, the dishes were incubated for 24–48 hours (*Pseudomonas spp*. count). Microorganisms were calculated using three replicates, with the mean represented as log CFU/g of burger ± standard deviation.

2.4. pH Values

The pH values of samples were measured by using a digital pH meter (Model 3510, Jenway Technology, Italy). With the help of two buffer solutions of pH 4 and 7, the pH meter electrode was calibrated. In a Cyclo-Mixer, 15 g of finely powdered sample was mixed in 50 ml of distilled water in a test tube (CM- Model 3000 USA). It was obtained using Whitman filter paper No. 1 as a filter. The pH meter's electrode was immersed in the filtrate, and the sample pH was measured. All values were calculated as the average of three replicates ± standard deviation (Khandil *et al.,* 2020).

2.5. Instrumental colour

The beef samples patty was pressed on a flat surface. The Hunter (*a*, b*,* and *L**) scale colour difference was determined using a scale colour spectrophotometer (machine colors Tristimulus) and a CIE Lab colorimeter (Hunter, Lab Scan XE - Reston, VA, USA) in reflection mode, with the colorimeters held over the samples at room temperature at 25°C (Tatli et al., 2020; Muge et al., 2020).

2.6. Instrumental texture

The texture characteristics of the burger samples were determined using Mercadante et al. (2010) methodologies with slight modifications. The burger samples were first taken from the casings and sliced into 2.5 cm length pieces before being compressed twice with a Texture Analyzer (Texture Analyzer TA-HD plus, 750 kg. (7.5 kN), England). Textural characteristics were evaluated at room temperature with the following processing parameters: 5.0 mm/s crosshead speed, 99.0 g surface sensing force, and 30.0 g threshold using a diameter cylindrical aluminum probe (5cm×4cm) 1*s* time gap between the first and second compressions. The Texture Professional version 1.0 program collected and analyzed the data (Stable Micro Systems, Surrey, England). The force-time curves were used to determine each sample's hardness, gumminess, springiness, cohesiveness, and chewiness (Bourne, 1978; Muge et al., 2020). The results were based on the average and standard deviation (±) of three replicates with one burger for each measurement.

2.7. Statistical Analysis

All experimental data were represented as mean values with ± standard deviation. Data from various tests were examined separately and compared using a one-way analysis of variance (ANOVA). Based on (Artimage & Berry, 1987; Kowalczewski & Andreani, 2015) (Laleg et al., 2019) protocols with minor adjustments, the significance of differences between the means was compared using Fisher's test ($p<0.05$). The data is displayed as mean SD (+). According to Fisher's least significant difference test ($p<0.05$), results followed by different letters within the same storage duration (0, 3, 6, and 9 days) are significantly different in each row.

3.Results and discussions

3.1. Red beet root's chemical components.

Which is shown in Table 2, total phenolics (130.44±0.11 mg/g DW), total flavonoids (1.22±0.251 mg/g DW), total tannin (4.89±0.114 mg/g DW), total anthocyanins (59.89±0.082 g/100g FW), total alkaloid (2.54 ±0.018 g/100g DW), and carotenoids (1.55±0.221 mg/100g FW) are present in the ethanolic extract.

Table 2. The Quantitative chemical composition, Phenolic and Flavonoid compounds, and Water-soluble Vitamins of *Beta vulgaris* root

Components	Composition
Total phenolic (mg Gallic acid /g DW)	130.44±0.11
Total flavonoid (mg Quercetin /g DW)	1.22±0.251
Total tannin (mg Tannic acid /g DW)	4.89±0.114
Total alkaloid (g/100g DW)	2.54± 0.018
Total anthocyanin (µg/100g FW)	59.89±0.082
Carotenoids (mg/100g FW)	1.55±0.221
Phenolic compounds (Conc. mg/100g DW)	
Gallic acid	10.22±0.02
Catechol	6.88±0.11
p-Comuaric acid	0.58±0.14
Ferulic acid	0.88±0.08
o-Coumaric acid	1.47±0.24
Cinnamic acid	0.74±0.43
Flavonoid compounds (Conc. mg/100g DW)	
Myricetin	20.22±0.11
Naringenin	19.42±0.24
Kaempferol	2.98±0.47
Apigenin	2.78±0.42
Vitamins contents (Water-soluble Vit.: mg/100g DW)	
Ascorbic acid (Vit. C)	25.87±0.17
Niacin (Vit. B3)	1.47±0.10
Pyridoxine (Vit. B6)	5.879±0.08
Folic acid (Vit. B9)	2.59±0.31
Note: **Values are mean ± SD of three replicate analyses**	

Soluble vitamins are identified using HPLC. The ethanolic extract of red beetroot includes vitamin C (25.87±0.17 mg/100g DW), vitamin B3 (1.47±0.10 mg/100g DW), vitamin B6 (5.879±0.08 mg/100g DW), and vitamin B9 (2.60 mg/100g DW, according to the data in Table 1. Phenolic compounds are separated by using HPLC). Table 1 shows that catechol (6.88±0.11 mg/100g DW), ferulic acid (0.88±0.08 mg/100g DW), gallic acid (10.22±0.02 mg/100g DW), *p*-Comuaric acid (0.58±0.14 mg/100g), and o-Cinnamic acid (0.74±0.43 mg/100g) are all present in the ethanolic extract of red beetroot. Furthermore, the ethanolic extract of red beetroot includes a variety of flavonoids, including naringenin (19.42±0.24 mg/100g DW), myricetin (20.22±0.11 mg/100g DW), apigenin (2.78±0.42 mg/100g DW), and kaempferol (2.98±0.47 mg/100g DW).

3.2. pH Determination

The pH values are presented in Fig. 1 correspond to burger patties containing BVE, either encapsulated or/not encapsulated in sodium alginate, and to Encaps-SDW or SDW samples control stored at 4±2°C for 9 days. The samples Encaps-SDW, SDW, BVE, and Encaps-BVE, had comparable beginning pH values of 5.97±0.13, 5.94±0.11, 6.03±0.11, and 5.98±0.14 for Encaps-SDW, SDW, BVE, and Encaps-BVE extract, respectively, right after treatment (0 times). As compared to the SDW (control samples), the BVE sample had a significantly ($p < 0.05$) higher pH value of 6.03±0.11. While the pH of the Encaps-SDW and SDW control samples remained stable (approximately at 5.96) after three days of

storage, the pH of the Encaps-BVE and BVE samples significantly decreased ($p<0.05$), reaching 5.31±0.15 and 5.49±0.11, respectively. The pH value in the SDW sample increased significantly ($p<0.05$) after six days of storage (Figure1), reaching 6.61±0.15. This was nearly constant (6.03±0.12) in Encaps- SDW, whereas sample BVE had the lowest values of 5.17±0.09, substantially ($p<0.05$), confirmed by Encaps-BVE at 5.13±0.05. After 9 days of storage, the variation in pH values was significantly greater. Indeed, the pH of BVE and Encaps-BVE samples was 5.16±0.12 and 5.54±0.10, respectively. In contrast, the pH of SDW and Encaps-SDW samples was significantly higher at 7.11±0.14 and 6.89±0.05, respectively.

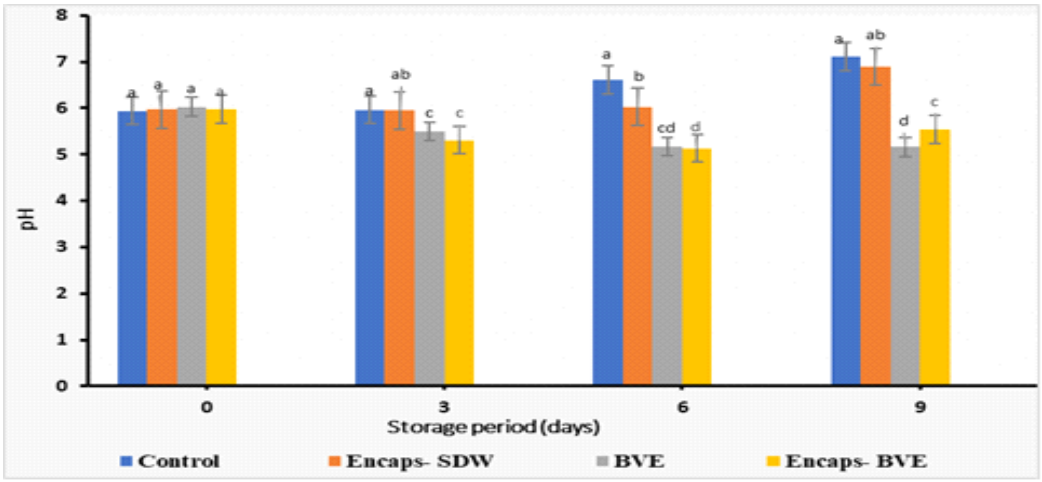

Figure 1. pH values of beef burger patties incorporating *Beta vulgaris* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

3.3. Microbiological Analysis

Figures 2, 3 and 4 show the microbiological counts on burger patties with or without *Beta vulgaris* extract, each encapsulated or/not encapsulated in sodium alginate, after 9-days of storage (4±2 °C). In furthermore, vitamins A, B, and C, as well as folic acid, can help with motor function. On plate count agar, the initial estimate of Total Mesophilic Bacteria has been 4.8 log CFU/g throughout all treatment (BVE and SDW). Despite this, it immediately rose after three days of storage, exceeding the European regulation's limit of 7.14 to 7.32 log CFU/g (5×10^6 CFU/g) for total aerobic bacterial counts (Regulation EC-2073, 2005). Although Total Mesophilic Bacteria count increased during six days of storage in Encaps-SDW and control samples SDW, it stayed relatively constant in Encaps- BVE and BVE samples. Total Mesophilic Bacteria count achieved peak values of 8.61±0.22 and 8.74±0.17 log CFU/g in Encaps-SDW and SDW (control samples), respectively, at the end of storage (9 days). Encaps-BVE and BVE samples, on the other hand, had the lowest values ($p<0.05$) at 7.23±0.12 and 6.58±0.09 log CFU/g, respectively, even though the Encaps-BVE was inside the microbiological limit (Fig. 2). The count of *Enterobacteriaceae* on burger patties processed with Encaps-SDW, Encaps-BVE, and BVE samples (Fig. 3), were not statistically different ($p>0.05$) from the SDW sample (5.41±0.13 log CFU/g) initially after procedures (0 days). The BVE and Encaps-BVE samples had the lowest enterobacteria counts ($p<0.05$) after 3, 6, and 9 days of storage when evaluated to Encaps-SDW and control samples SDW. Although there were some differences between samples, the BVE strongly inhibited enterobacterial growth, with values averaged 2 log units lesser in measurements of Encaps-BVE and BVE (6.45±0.15 and 6.01±0.09 log CFU/g, respectively) than SDW

and Encaps-SDW samples at the end of storage (9 days).

Figure 4, *Pseudomonas* spp. counts began at 4.12±0.11 log CFU/g and grew in all tests performed after 9-days of storage, although significant differences probably depend on treatments and experimental conditions. During 6 and 9 days, all samples containing BVE had considerably lower values ($p<0.05$) than SDW and Encaps-SDW, which had the highest values of 8.77±0.13 and 8.12±0.11 log CFU/g, respectively. Immediate addition of BVE (6.44±0.16 log CFU/g) produced the most significant review ($p<0.05$) at the end of storage (9 days), followed by encapsulated SDW (8.12±0.05 log CFU/g) and encapsulated BVE (7.55±0.09 log CFU/g).

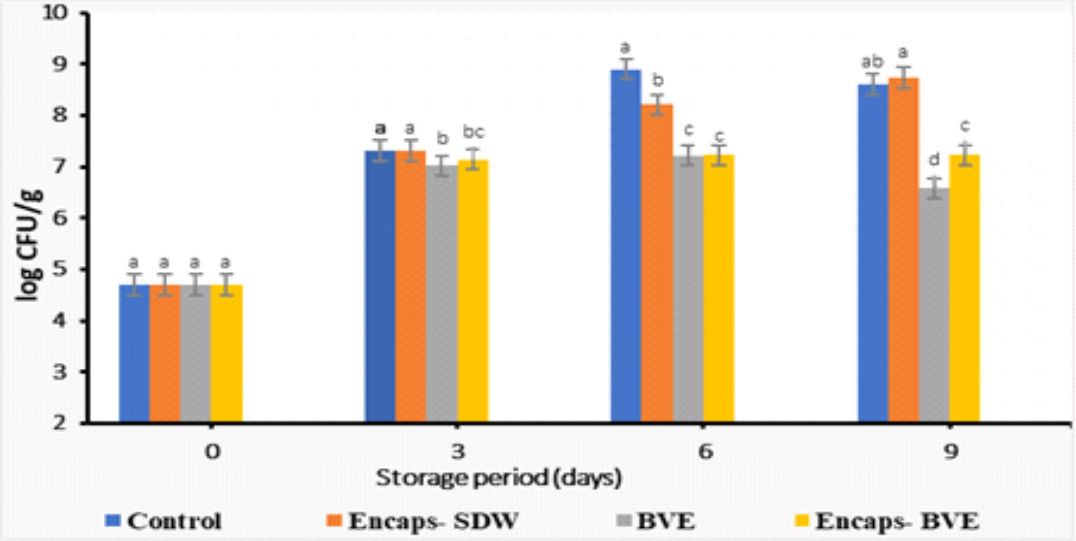

Figure 2. Total mesophilic bacteria (TMB) of beef burger patties incorporating *Beta vulgaris L.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

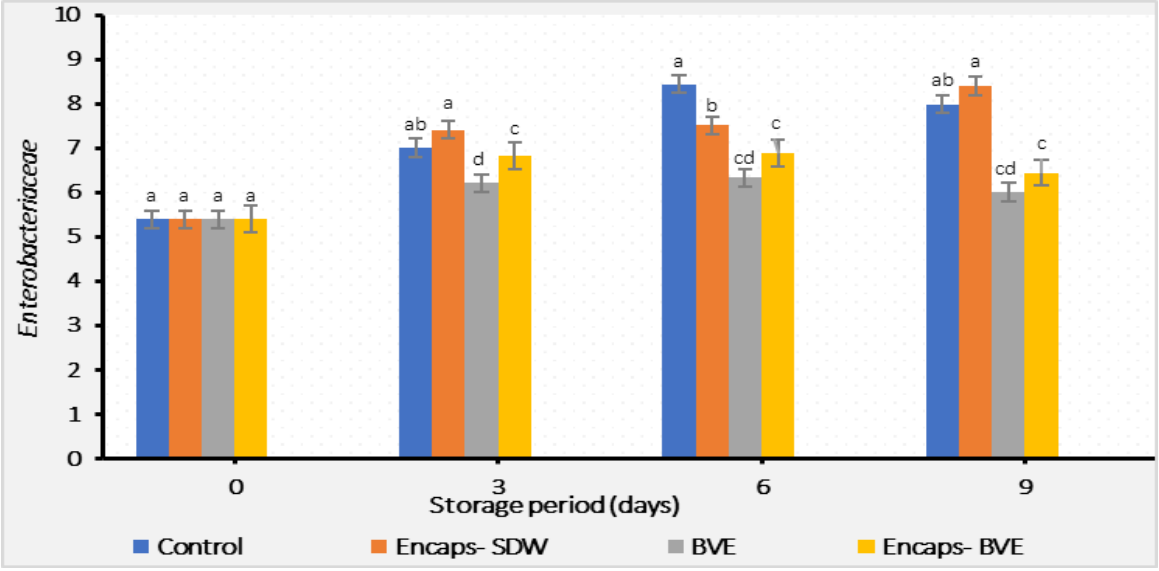

Figure 3. Growth of *Enterobacteriaceae* of beef burger patties incorporating *Beta vulgaris L.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

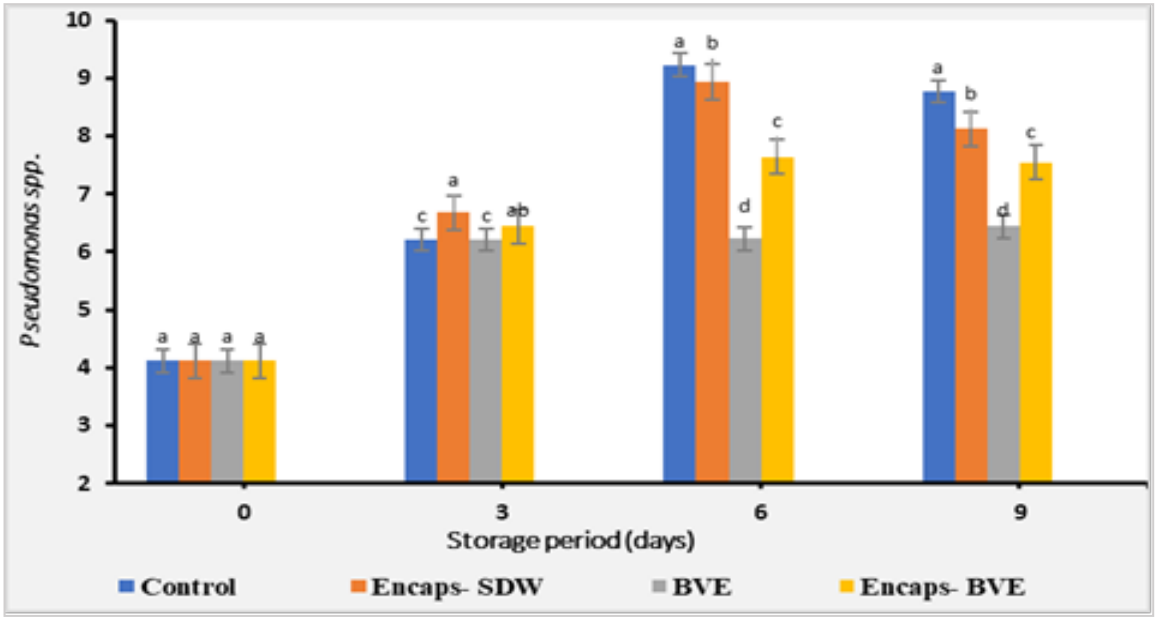

Figure 4. Growth of *Pseudomonas* spp. of beef burger patties incorporating Beta vulgaris L. root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C

3.4. Estimation of Color Characteristics

The addition of BVE, including direct and encapsulated, significantly ($p<0.05$) affected the initial *L*, a*,* and *b** characteristics of beef patties compared to the comparable controls of SDW and Encaps-SDW. While BVE was directly added to minced beef, *L** revealed the significantly lowest value ($p<0.05$) of 41.21±0.23, followed by Encaps-BVE and Encaps-SDW samples (42.18±0.14 and 46.25±0.11, respectively). After six days of storage, the lightness levels of all different treatments increased (Fig. 5). After 9 days, they were 53.87±0.12, 54.13±0.11, 46.15±0.18, and 46.01±0.17, respectively, for Encaps-SDW, SDW, BVE, and Encaps-BVE. As illustrated in Figure 6, Encaps-BVE and BVE beef samples showed significantly higher ($p<0.05$) *a** values than Encaps-SDW and SDW samples control (13.57±0.12 and 13.45±0.11, respectively). During 9 days of storage, Encaps-SDW and SDW control samples had *a** values at 9.15±0.17 and 7.99±0.14, respectively, whereas Encaps-BVE and BVE samples had *a** values of 15.33±0.12 and 15.47±0.14, respectively. Furthermore, the sample Encaps-BVE had the lowest *a** value variations over the storage period. Compared to sample Encaps-BVE, sample BVE showed an unusual trend, declining *a** value of nearly twice after 9 days. The *b** values observed in Encaps-BVE and BVE beef samples were significantly affected ($p<0.05$), with initial levels of 15.24±0.13 and 15.82±0.14, respectively, when compared to Encaps-SDW and SDW samples, which had values of 13.34±0.24 and 11.08±0.18, respectively (Fig. 7). After 3 days of storage, the SDW and Encaps-SDW were significantly lower (7.32±0.15 and 7.99±0.12, respectively); around the same time, samples of BVE and Encaps-BVE were less likely (11.20±0.21 and 10.54±0.09) to have a relationship with previously identified antimicrobial effect (Figures 5, 6, and 7). In comparison to BVE, Encaps-BVE had no significant impact ($p>0.05$) on the beginning of colour parameters; when particularly in contrast to Encaps-SDW and SDW, samples BVE and Encaps-BVE had one of the most constant trends up to 9 days of storage.

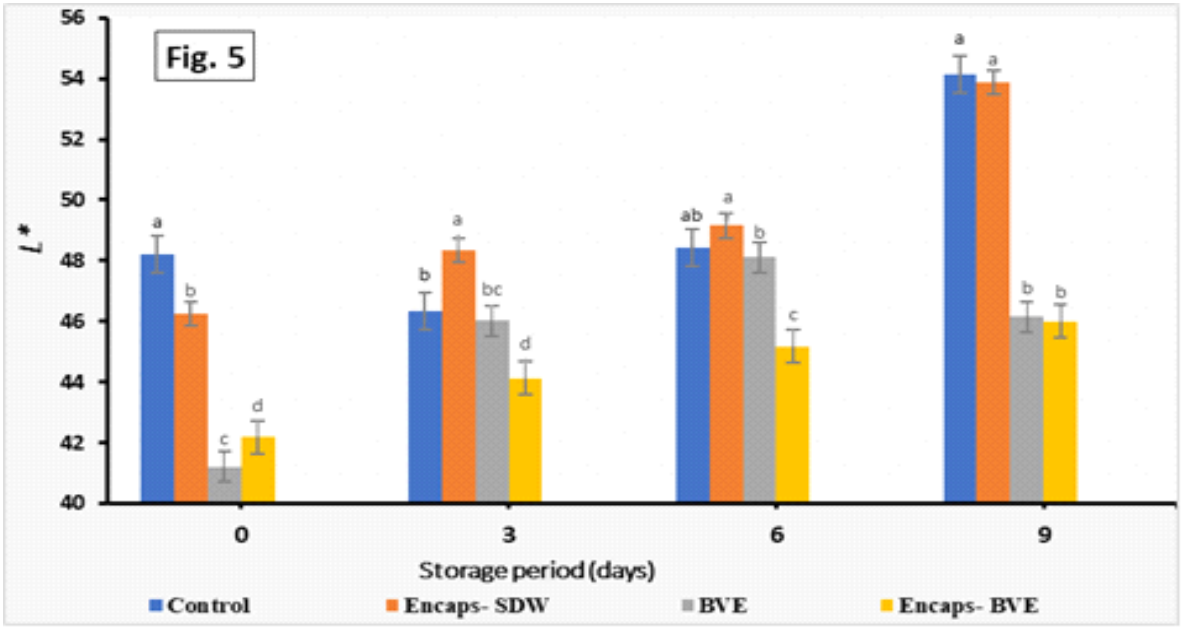

Figure 5. Color values L* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

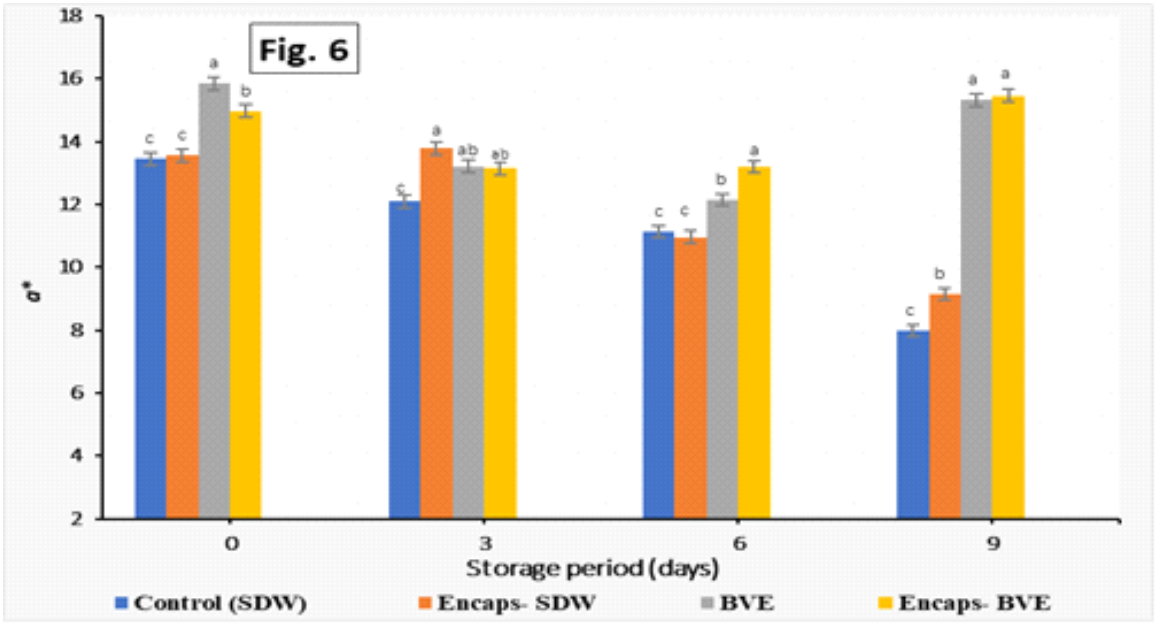

Figure 6. Color values a* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

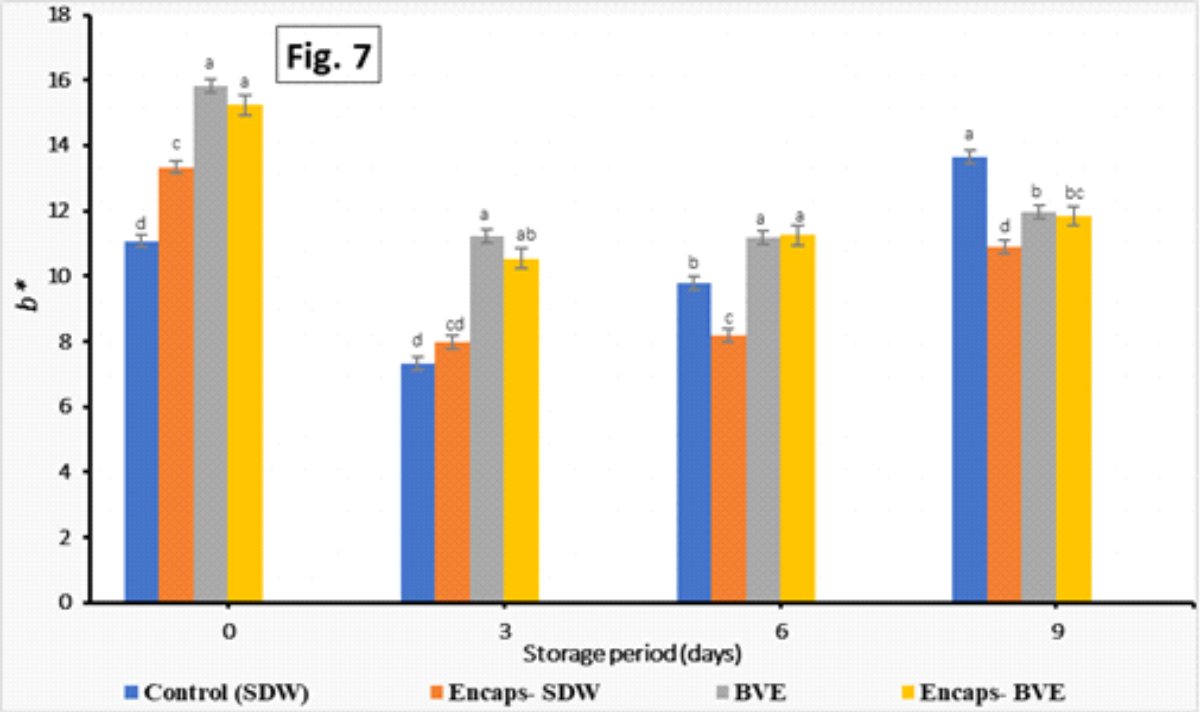

Figure 7. Color values b* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

3.5. Texture profile

According to the storage period and considered parameters, the addition of BVE and Encaps-BVE considerably altered the textural characteristics of beef patties during storage (springiness, hardness, and cohesiveness) shown in Table 8, 9, and 10. Shortly after treatment (time 0), it was hardness comparable amongst treatments ($p>0.05$). After three and six days of storage at 4 ±2 °C, all except one of the samples, the SDW control samples, had increased hardness values. After nine days of storage (Fig. 8), samples of BVE extract had the highest levels ($p<0.05$), followed by Encaps-BVE extract; there was no significant difference ($p>0.05$) between the Encaps-SDW and SDW samples, which had the lowest hardness values. This impact was less apparent in sample Encaps-BVE extract due to the encapsulation of BVE extract. In addition to SDW and Encaps- SDW samples, cohesiveness and springiness improved in BVE and Encaps-BVE extract samples with storage period, achieving significantly affected ($p<0.05$) a higher value during 3, 6, and 9 days (Fig. 9 and 10).

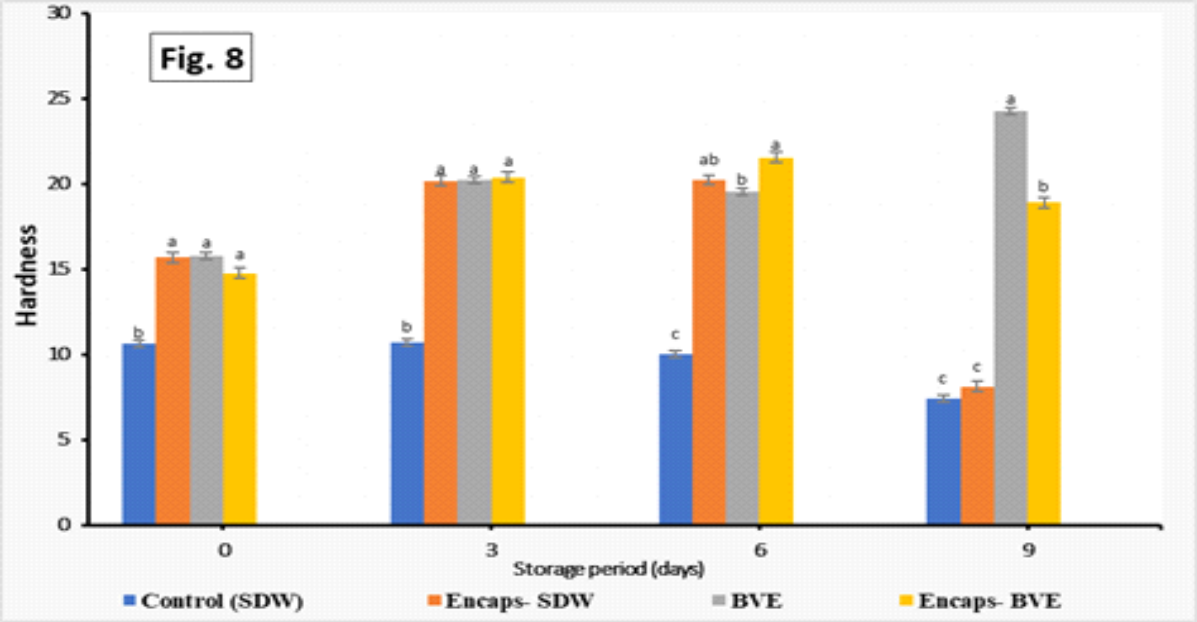

Figure 8. Hardness of beef burger patties incorporating *Beta vulgaris l.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

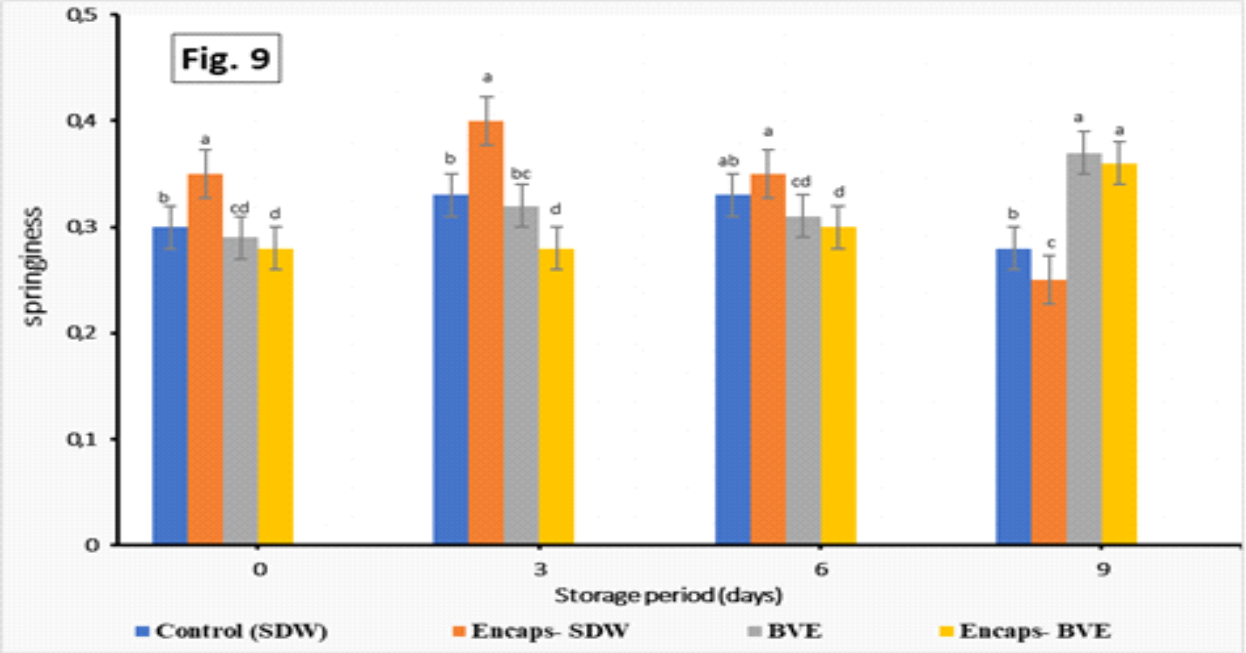

Figure 9. Springiness of beef burger patties incorporating *Beta vulgaris l.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

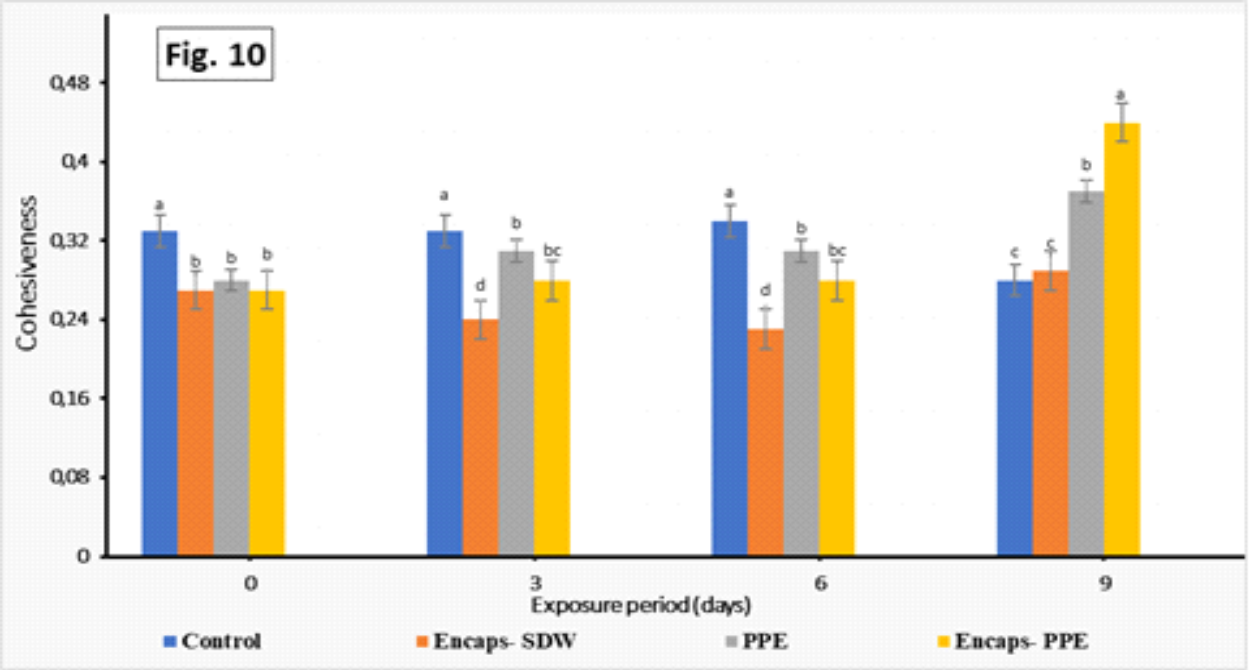

Figure 10. Cohesiveness of beef burger patties incorporating *Beta vulgaris l*. root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

3.6. Discussion

The existence of bioactive compounds in red beetroot has influenced its pharmacological and physiological properties. Polyphenols, flavonoids, alkaloids, folic acid, tannins, ascorbic acid, and reducing sugars were identified as the primary ingredients of the red beetroot extract in earlier investigations (Azmir et al., 2013; Castro-Enríquez *et al.,* 2020; Rashmi & Negi, 2020). Flavonoids, for example, have been demonstrated to have antibacterial, antineoplastic, antiviral, anti-allergic, and antioxidant, and antimicrobial properties (Sameeh et al., 2016; Karak, 2019; López-Lázaro, 2009;), acting as scavenging free radicals and metal chelators. Alkaloids have biological activities used in food industries, recreational drugs, and medicinal (Gülçin, 2011; El-Beltagi et al., 2018; Akanni et al., 2019). Tannins have been shown to protect carbohydrates and proteins against microbial breakdown in meat products (Jakobek, 2015; Bunglavan & Dutta, 2013; Allam & Dolgonova, 2017[b]).

Furthermore, carotenoids protect against cancer, inflammatory processes, cardiovascular disease, and age-related dystrophy muscular (Ahmed et al., 2014) and act as an antibacterial and antioxidant (Allam & Dolgonova, 2017[a]; El-Beltagi *et al.,* 2018; Kandil et al., 2020). Odoh and Okoro (2012) observed that beetroot contains considerable amounts of vitamins, particularly Vit. C (4.36 mg/100 g), and their findings are identical to ours. Red beetroot has a high content of ascorbic acid, according to the investigation. This vitamin is essential for human food and nutrition, especially tissue maintenance and growth, neurotransmitter synthesis, hormone production, and immune responses. These findings are comparable to those of Vuli et. al., (2010) who found vanillic, ferulic, caffeic, *p*-hydroxybenzoic, and protocatechuic acids in beetroot. Pyo *et. al.*, (2004) reported similar findings, identifying the following compounds: myricetin (2.2 mg/100 g FW), catechin (6.7 mg/100 g FW), quercetin (7.5 mg/100 g FW), and kaempferol (9.2 mg/100 g FW).

Quality characteristics and Microbiological activity, texture, and colour were examined in the storage process at refrigerated temperature (up to 9 days at 4±2 °C) of beef burger pads made directly and encapsulated in alginate beads of BVE extract. Microbial results showed

that both immediately added and or/not encapsulated BVE extract was conservative, which reduced significantly ($p<0.05$) *Enterobacteriaceae*, *Pseudomonas* ssp. counts and total mesophilic bacteria, compared to samples addicted with control samples SDW or SDW encapsulated. The commonly added BVE extract to burger formulations, on the other hand, appeared to be more capable of limiting the growth of approximated microbial populations of organisms, especially after 6 days; justifications for this must be attributed to the reality that almost all of the additional extracts interacted instantly with microorganisms and bacterial cells, decreasing their feasibility and effectiveness, or even with meat tissue and so limiting its degradation, resulting in the formation of smaller molecules that microorganisms can consume (Hassan et al., 2018). Parafati *et al*. (2019) previously published similar results on sliced beef, demonstrating the potential of BVE to decrease microbial activity while significantly at 4 °C of storage.

Furthermore, Kharrat et al. (2018) found that using BVE as a natural additive and preservative increases the microbial stability of salami, owing to the high content of betalains, phenolic, and flavonoids compounds in BVE extract. Various alternative strategies and preservation techniques were investigated because burgers are hazardous food from a microbiological or quality standpoint. Compared to control burger samples, Özvural *et al*. (2016) found that using encapsulated extract of green tea on burger pie preparation significantly decreased the coliform, total Mesophilic Bacteria, mold, and yeast count. According to observations from the last day of storage (6-8 days), the impacts of various preparations of chitosan and chitosan/sodium tripolyphosphate composite alternatives which include β carotene as edible coatings and preservatives in hamburgers on bacteriological activity, oxidative, and quality characteristics, features were recently investigated by the same authors (Özvural et al., 2016; Özvural and Huang, 2018; Parafati et al., 2019; Hemmatkhah et al., 2020; Chaudhary et al., 2020). The findings demonstrate that using the solution as a functional ingredient and edible coating was improved and much more successful microbiological characteristics and lipid oxidation. Chemical agents extracted from animals, plants, bacteria, and their metabolites that avoid the decomposition of different food items are natural preservatives. They work by inhibiting the growth of microbial, oxidation, and particular food enzymatic reactions. A preservative is a natural or synthetic derived chemical compound that protects finished products from decomposing due to microbial growth or other undesired chemical changes. They are applied to different foods to prevent them from spoiling, discoloration, or infection by microorganisms, and they improve their texture, colour, taste, and nutrition (Adham et al., 1998; Meyer et al., 2002; Kabak et al., 2006; Mei et al., 2019).

After storage, the pH of Encaps-SDW and SDW (control samples) increased (Biswas et al., 2004), most commonly due to the development of metabolites generated or primary microbial metabolites from protein beef deamination. The application of BVE extract had no discernible impact ($p>0.05$) on the pH of beef at the beginning of the storage period studied, so excluding that, it had observed effectiveness on microbial development. But even so, samples were treated with BVE extract (including both encapsulated and bulk) had the lowest of pH levels throughout the storage, implying an antibacterial activity of the bioactive extract components over time, a preventive role of meat tissue, and/or heterofermentative fungi and bacteria producing organic acids from BVE sugars (Sánchez et al., 2003; Del-Río et al., 2007; Hemmatkhah et al., 2020). When BVE and Encaps-BVE were compared, the latter had a relatively increased pH value because the extract was encapsulated in alginate beads. These findings agree with (Campolo et al., 2018; Parafati et al., 2019; Marrone et al., 2021;), who found that varying doses of BVE extract significantly impacted the pH of preserved beef treated with the extract. In terms of colour characteristics, control samples

showed a considerable decrease in red colour (*a** parameter) during storage, most likely because of microbial degradation and the resulting rise in pH, often associated with a shift in colour toward green. Meanwhile, the *a** values of treated BVE extract samples, whether encapsulated or not, illustrated comparative stability and suggested that the extract has provided a preventive action against myoglobin oxidation, as previously characterized by Parafati *et al*. (2019). While there were non-significant changes ($p>0.05$) in *a** values between samples containing encapsulated and non-encapsulated BVE, samples having encapsulated BVE showed a more consistent trend, except at four days. That according to (Campolo *et al*., 2018; Hemmatkhah et al., 2020). most packaged and raw minced beef samples demonstrated a significant decrease ($p<0.05$) in the *a** parameter during the first day and throughout storage.

Additionally, the researchers also concluded that both *a** and *b** characteristics, owing to metmyoglobin synthesis, cause a decrease in the *b** value. The Encaps-SDW and SDW samples demonstrate a more severe reduction in the *b** values over storage, likely due to aerobic microorganisms consuming oxygen, resulting in lower oxymyoglobin contributing significantly to the yellow colour formation (Gülçin, 2011; Hemmatkhah et al., 2020). In this instance, the BVE extract encapsulated resulted in reduced *b** parameter changes over the storage period, leading to colour preservation. The addition of BVE extract had a significant impact on hardness at the storage period (8 days), with the highest levels in samples treated or/not treated with BVE extract encapsulated, most certainly associated with the extract's carbohydrate content (Özvural et al., 2016; Parafati et al., 2019). Similarly, because once compared with untreated samples, springiness qualities in BVE and encapsulated BVE extracted samples increased significantly; the providing additional soluble proteins and carbohydrates in the extract may have improved the texture of meat products, resulting in structural resistance higher elasticity after the first component of beef product. Lastly, the cohesiveness characteristic demonstrated the most significant values of meat samples containing encapsulated extract, probably due to alginate's gelling characteristic.

4. Conclusions

One of the main aspects in enhancing quality, prolonging shelf-life, maintaining consumer protection, product safety, and minimizing waste was the microbial control in minced beef. This study follows a trend of identifying the various chemical compounds in red beetroot and evaluating their preventative role in extending food products' shelf life, including meat and its byproducts. Microbiological studies indicate that the addition of BVE and Encaps-BVE to burger preparation improved significantly ($P<0.05$) compared to control samples to total mesophilic bacteria, *Pseudomonas* spp. count, and *Enterobacteriaceae*. Moreover, results revealed that during the storage period, the addition of BVE kept beef minced almost constant in pH (average: 5.2-5.5), while control samples increased significantly ($p<0.05$) in pH values, possibly due to bacterial activity and degradation of protein caused by the production of amines, ammonia, and other essential substances.

Furthermore, after 9 days of the storage period, the sample containing Encaps-BVE demonstrated a steadier trend in terms of colour and texture characteristics than the other treatments. Confident that BVE is an effective technique of microorganisms' growth while storage, further research will be conducted to study the impact of beetroot extract, encapsulated or/not, on the cooked and grilled product's technological features as its sensory characteristics and acceptability. The research results have confirmed the feasibility of using BVE extract, whether encapsulated or/not in alginate beads. In contrast, a natural additive to meat product compositions maintains overall quality characteristics comparable to those of other extracts while also containing a significant inhibitor of antimicrobial agents,

indicating that it could be used as a multifunctional value-added component.

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