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Enhancement the Chemical and Microbiological Properties of Milk Cream by Encapsulated Strawberry Leaves Extract

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Abstract

The ethanolic extract of strawberry leaves was estimated to total phenolic and flavonoid content as well as its antioxidant properties. The antimicrobial properties of the ethanolic leaves extract exhibited higher antimicrobial properties against eight reference strains of pathogens compared to the aqueous leaves extract. Encapsulated lyophilized strawberry leaves ethanolic (ELSLE) extract was added to milk cream at concentrations of 1, 1.5, and 2 g/100 g in an attempt to extend the shelf life of milk cream. Physicochemical, microbiological, total phenolic and flavonoid content analyses were performed during storage at 4±1°C for 28 days. ELSLE extract cream samples were higher in total solids, total phenolic and flavonoid content compared to the control. The use of ELSLE extract resulted in a decrease in L- values and an increase in b-values. All prepared cream samples showed a more or less intensive yellow colour shade (positive b-values). Treatments containing ELSLE extract had the lowest total bacterial count, yeast and mold, lipolytic, and proteolytic bacteria at the end of the storage period. All cream samples were sensory acceptable compared to the natural cream. Cream with ELSLE extract at a concentration of 2 g/100 g can be used as a natural antioxidant and antimicrobial to improve the oxidative stability and microbiological quality of milk cream.

Keywords: Antimicrobial; antioxidant; milk cream; total phenolic; total flavonoid

1. Introduction

Milk Cream is the fluid milk product rich in fat, in the form of an emulsion of fat-in-skimmed milk, obtained using either gravitational separation or centrifugal forces and enriched to varying degrees with milk fat. Creams can be whipped, acidified or not, and with or without additives. Cream is categorized according to its manufacture, use, and fat content [1]. Currently dairy cream is used in many forms and for many different purposes. Originally associated with desserts and fresh fruit, it is also used as an ingredient in sweet and savoury dishes, e.g., ice cream, soup, custard bases and cakes, and is the main ingredient used to make butter and butter oil [2].

Early [3] assumed that the high fat content of cream protects microbes during heating, necessitating a more severe heat treatment than that required for the pasteurization of milk. Most vegetative cells, including pathogens and yeasts and moulds, are inactivated by pasteurization, but some thermoduric bacteria, including spore- forming thermoduric Bacillus spp., survive pasteurization. Since that cream is still regarded as something of a luxury, it must have an excellent flavour, and as it is a high-fat product, any off-flavour of the milk lipids will be concentrated [4]. Expose the cream to oxygen and UV light cause oxidative degradation of lipids, producing off-flavours and rancidity and loss in quality value. Therefore, it must be protected to minimize chemical, organoleptic and bacteriological degradation by incorporation of additives having antioxidative and antimicrobial properties [5].

Food spoilage that results from the action of microorganisms is known as microbial spoilage. Additionally, it is the most typical source of foodborne illnesses. Perishable foods like cream are often attacked by different microorganisms [6].

Many food preservation systems such as heat treatments, refrigeration and preservatives are used to reduce the risk of food poisoning outbreaks. However,

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some of these techniques have often been associated with a negative impact on sensory characteristics and nutrient loss. The growing desire for chemical-free food has paved the way for the use of antimicrobials in the food sector [7]. Antimicrobials are a new technology that the food industry uses to increase the shelf life of food and address issues about quality and safety [8].
Food oxidation is a considerable challenge for the food preservation industry which not only decreases the sensory and nutritional quality of food products, but also their safety for the consumer due to the production of potentially toxic chemical compounds [9]. Antioxidants in lipid-containing foods both postpone the start of oxidation and slow its progression [10]. Synthetic antioxidants have been widely utilized in the past to retard lipid oxidation in food. Nevertheless, the uses of synthetic antioxidants have raised concerns about possible toxicity and food safety [11]. The Food industry is trying to decrease the chemical use of these synthetic compounds by replacing them with natural alternatives in order to respond to the concern of consumers that they are being exposed through their daily diet to potentially harmful chemical synthetic compounds. Therefore, employing natural antioxidants is becoming more and more popular as a way of controlling lipid oxidation and limiting its negative effects [12].
The goal behind the development of food preservation is the need to extend the shelf life of food like controlling microorganisms that spoil food or make it unsafe. Furthermore, much effort is being diverted toward the discovery of antioxidants from natural sources such as phenolic compounds to meet the growing desire for food products without synthetic additives [13].
The benefit optimization of food by-products is an enormous challenge, which are thought to be a significant cause of environmental contamination and should instead be used as a source of naturally occurring active ingredients [14]. The plant kingdom is the major source of bioactive compounds. These bioactive compounds present in plant extracts have similar antioxidant capacity when compared to synthetic ones so it can be used to control oxidation processes in different food matrices and avoid rancidity caused by oxidation. As far as natural antioxidants of plant origin are concerned, some of the most important groups are polyphenols, carotenoids and vitamins [15-17]. Virtually many phenolic compounds and plant phenolic extracts have successfully demonstrated the retarding effect on lipid oxidation in different foods.
Strawberry tree (*Arbutus unedo* L.) belongs to the *Ericaceae* family and is endemic to the Mediterranean area. Strawberry tree leaves extracts have a number of constituents such as tannins, flavonoids, phenolic, and iridoid glucosides exerting potent antioxidative and antimicrobial activity [18-20].
Encapsulation technology has been widely used in the food industry to protect bioactive compounds such as polyphenols, flavonoids, phytosterols, carotenoids and taurine against external factors and degradation. These techniques can coat bioactive core substances with wall materials, creating an effective barrier against environmental and chemical interactions [12]. Many polymers such as sodium alginate, Arabic gum, soy protein, chitosan, maltodextrin, cellulose and carboxymethylcellulose, pectin, maltodextrin, whey protein, galactomannan, modified starch, and sodium caseinate are used as wall materials to protect the core [14], [21-23]. It now demonstrates potential applications in obtaining functional ingredients incorporated into food and helps to prolong the release of bioactive compounds for longer times or under specific conditions, thereby increasing their stability [24].
As cream is prone to chemical and microbiological spoilage this study aimed to estimate the contents of bioactive components of the strawberry leaves to evaluate their antioxidant and antimicrobial characteristics to reduce microbial and oxidation activity in milk cream. Strawberry leaves were extracted and micro capsulated to apply as bio-preservatives in milk cream.

2. Materials and Methods

Bacillus cereus B-3711, *Listeria monocytogenes* 598, *Escherichia coli* ATCC 25922, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans* CAIM -22 were collected from dairy microbiological Lab., National Research Center. The pathogenic bacteria were activated in tryptone Soya broth. The broth culture was incubated at 35 °C until visible turbidity reached 0.5 “McFarland" standard solution. Fungi strains were activated in potato dextrose broth. The broth culture was incubated at 25 °C until visible turbidity reached 0.5 “McFarland" standard solution. Strawberry (*Fragaria orientalis*) leaves were obtained from a local market, Cairo, Egypt.

2.1 Strawberry leaves extracts

2.1.1 Aqueous extract: One hundred gram of dried strawberry leaves was suspended in 1000 ml of distilled hot water in a water bath (w/v) at 80°C ±1 for 30 min according to the method of Thorat et al. [25]. The extracts were cooled, filtered and the supernatant was collected and stored in an air tight bottle for further use [26].

2.1.2 Ethanolic extract: One hundred gram of dried strawberry leaves was mixed with 80% (v/v) ethanol and stirred mechanically (IKA RW 20 Digital Homogenizer) for 2 hrs. at room temperature, then allowed to stay in the refrigerator for 24 hrs. at 4±1˚C. The extracts were filtered and the supernatant was collected [27]. Then dried in a rotary evaporator (Stuart Rotary Evaporator Model RE300) at 40±1°C and completely dried by freeze drying (Snijders Scientific type 2040). The lyophilized extracts were stored at 4°C±1 for further use.

2.2 Antibacterial activity of strawberry leaves extracts

The disk diffusion method is used to evaluate antimicrobial activity of the strawberry leaves aqueous and ethanolic extracts against pathogenic strains according to Mostafa et al. [28]. The strawberry leaves extracts (50 mg) were re- dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 mm). Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (as a basal layer) followed with 0.1 ml (approximately 10^9 cells ml^{-1}) of the tested microorganisms using a sterile swab. The plates were rested to 2 h at 37 ºC to allow the saturated with pathogenic strains. Sterile filter paper discs (6 mm in diameter) loaded with leaves extracts concentration of 0.5, 1.0, 1.5, and 2.0 mg were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 µg of Gentamycin was used as positive control. The plates were kept in the fridge at 5 ºC for 2 h. to permit plant extracts diffusion then incubated at 35 ºC for 24 h. After the incubation period the inhibition zones around each disc was measured in millimeters recorded and considered as indication for antibacterial activity of the extract.

2.3 Antifungal activity

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates. The inhibitory effect was determined using 0.5, 1.0, 1.5, and 2.0 mg of the leaves extracts was loaded on different sterile paper discs (whatman 6 mm) and placed on the surface of Czepak Dox media. The fungal species were compared to the antibiotic Fluconazole (25 µg), as a positive control. The plates were incubated at 30 °C for 5 days. The inhibition zone (mm) formed by the extracts against the particular test fungal strain determined as the antifungal activities of the extracts [29].

2.4 Determination of total phenolic compounds

Total phenolic content was determined using the Folin-Ciocalteu process [30]. The findings were expressed in mg of the equivalent of gallic acid (GAE)/g.

2.5 Determination of total flavonoids compounds

Total flavonoid content was determined by the aluminum chloride colorimetric assay according to Ordonez et al. [31]. 0.5mL of the extracts or standard solution of quercetin was mixed with 0.5mL of 20 g l^{-1} $AlCl_3$. After 1 hour at room temperature, the absorbance was measured at 420 nm. Total flavonoid content was expressed as mg quercetin equivalents (mgQE)/g dry weight.

2.6 Determination of antioxidant activity by 2, 2'-diphenyl 1- picrylhydrazyl (DPPH)

In terms of hydrogen donation or radical scavenging potential, the antioxidant role of the lyophilized strawberry leaves extracts was calculated using the stable DPPH technique. At room temperature, the reaction mixture containing 1 ml of lyophilized strawberry leaves extracts and 1 ml of DPPH (0.2 mm) at different concentrations (0.1, 0.3, 0.5, 0.7, and 1 mg/100ml). The mixtures were shaken vigorously and allowed to stand at room temperature in the dark for 60 min. Distilled water was used as a control instead of the extracts. The absorbance was measured at 515 nm by UV-visible spectrophotometer. The percent scavenging activity was calculated using the following formula:

The radical scavenging activity was expressed as a percentage of inhibition and was calculated using the following formula:

%DPPH = [Absorbance of Control – Absorbance of Sample / Absorbance of Control] x 100

The IC50 value was calculated from the graph plotted as the antioxidant concentration required to achieve 50% free radical scavenging activity [32].

2.7 Ferric reducing antioxidant power (FRAP)

A ferric reduction assay was used to determine the antioxidant activity. Lyophilized strawberry leaves extracts were dissolved in methanol to serial concentrations (0.1, 0.3, 0.5, 0.7, and 1 mg/100ml), and mixed in separate tubes with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide. The tubes were put at 50 °C for 20 minutes in a boiling water bath, cooled quickly, and mixed with 2.5 mL of 10% trichloroacetic acid and 0.5 mL of 0.1% ferric chloride. The sum of iron (II)-ferricyanide complex formed was calculated by measuring at 700 nm after 10 minutes the formation of Perl's Prussian blue. Increased absorption of the reaction mixtures suggests increased power reduction [33].

2.8 Encapsulation of the lyophilized strawberry leaves extracts

Encapsulation of lyophilized strawberry leaves extract conducted as previously described by Badr *et al*. [14]. Maltodextrin (MD) at a ratio of 10% dissolved in distilled water (50ºC/1hr) to prepare the first solution; the soy protein (SP, 80% purity) was completely dissolved in water (60ºC/30 min) at a ratio of 8% as the second solution of coating material. The two solutions were mixed at 2 MD: 1 SP; then, Arabic gum (1% AG) had been added before stirred (30 min.) till completely dissolved. After that, 1 g of the targeted lyophilized extract (at 10% wt /vol) was added and dissolved gently using stirrer till the solution completely homogenous. The microcapsule emulsions were formed using an Ultra-Turrax homogenizer T18 basic (IKA, Wilmington, USA) at an operating speed of 15,000 rpm for 10 min.

2.9 Estimated encapsulation efficiency (EE)

Encapsulation efficiency is expressed as the total phenolic content (as %) that is entrapped in the matrix of wall material after freeze-drying as compared to that present in the initial system. It has been determined by adapting the method and equations presented by other authors Gonzalez-Ortega et al. [34] on the microencapsulated products by precisely weighing an amount of powder (20 mg) on microcentrifuge tubes to which 1.4 ml absolute ethanol was added. Powder suspension was immediately vortexed (10 s) and centrifuged at 12000g for 2 min (Eppendorf Centrifuge 5425 R). The supernatant ethanol fraction was collected and the pellet was dissolved in 1.4 ml of water by vortexing. Both fractions were analyzed for phenolic compounds content. Phenolic compounds in the ethanol fraction were considered to be surface/non-ncapsulated fraction, while phenolics in the water fraction correspond to the encapsulated fraction.
The encapsulation efficiency (EE) for total phenolic content and flavonoid was calculated according to Eq. (1):

$$EE\ (\%) = \frac{\text{Encapsulated phenolics (mg/g) powder}}{\text{Encapsulated phenolics (mg/g) powder} + \text{Surface phenolics (mg/g) powder}} \times 100 \qquad \text{Eq. (1)}$$

2.10 Transmission electron microscopy (TEM)

The morphology and size of the prepared encapsulated extract were studied using a JEOL JEM-1400 transmission electron microscope (TEM) with acceleration voltage of about 80 kV. A small drop of the dispersion of polymer encapsulated ethanolic strawberry leaves EESL extract onto a Lacey carbon film-coated copper grid and allowed to dry initially in air then by applying high vacuum [35].

2.11 Preparation of milk cream samples

Fresh buffalo's milk cream was obtained from dairy manufacturing unit at the Faculty of Agriculture, Cairo University, Giza, Egypt. Fresh cream was prepared using a cream separator was heated up to (80 ° C / 10 min) followed by cooling down to 40 °C. After that the cream was divided into four portions. The first portion was kept as a control (cream without any additives) and cooled down to 4±1°C immediately. Encapsulate lyophilized strawberry leaves ethanolic ELSLE.
extract was added at the ratio of 1.0, 1.5 and 2.0% (w/w) to second, third and fourth portion and mixed well to prepare (T1), (T2) and (T3) cream with ELSLE extract respectively. Three replicates were done for each treatment. All cream treatments were packaged into plastic container (100 g) and stored in refrigerator at 4±1°C for 28 day (Fig. 1). The samples were analyzed at fresh, 7,14,21 and 28 days of cold storage

2.12 Physicochemical analysis

Cream samples were analyzed for their fat content (Soxhlet method), protein content (micro-Kjeldahl method), ash, total solids, and total carbohydrate, according to the methodology established by the Association of Official Analytical Chemists, AOAC [36]. The pH values were measured by a digital laboratory Jenway 3510 pH meter, UK.

2.13 Apparent viscosity

The viscosity of the fresh and at the end of the storage period of cream samples were measured using a Brookfield digital
viscometer (DV1 Digital Viscometer, AMETEK Inc., USA). The shear rates at 10 rpm.

2.14 Colorimetric analysis

The color was evaluated using a chromameter (Konica Minolta CR- 400, Minolta Camera, Co., Osaka, Japan) fitted with an 8 mm measuring head and a D65 illuminant at three separate sample points. The chromameter was calibrated using a standard white plate provided by the manufacturer. In the L*, a*, b* color space, color modifications were quantified.

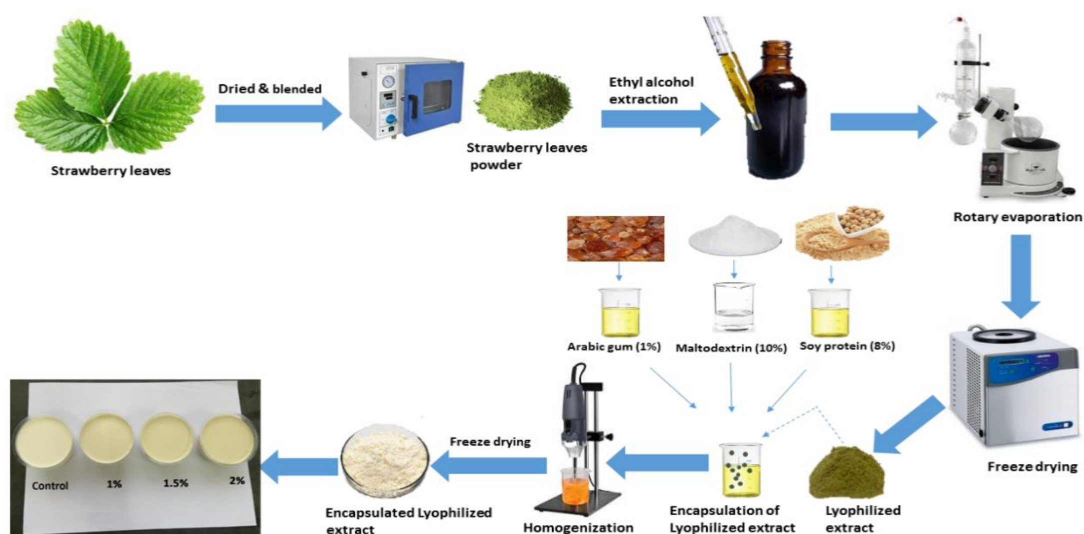

Figure 1. Preparation of cream with encapsulated lyophilized strawberry leaves ethanolic extract

Where L: luminosity (relative darkness or lightness) a value of L* = 100 represents pure white and L* = 0 represents pure
black; a* is chromaticity coordinate (+a*: red; -a*: green) and the b* is chromaticity coordinate (+ b*: yellow; -b*: blue). The hue angle (h°) when a<0 and b>0 was determined as h° = tan-1 (b/a). Yellowness index (YI) =142.6 b*/ L*. The analyses were performed in triplicate [37].

2.15 Microbiological examinations

Ten grams of cream was diluted with 90 mL of sterilized peptone water (Merck, 0.1%) and was thoroughly stirred in a vortex device for 60 s at room temperature. Serial 10-fold dilutions were made in 0.1% peptone water solution before plating on Petri dish .The microbiological analysis of cream samples were done using plate count agar (Oxoid) incubated at 37 °C for 48 h for total bacteria as a method recorded by Laird et al. [38] ; yeast peptone dextrose agar (Oxoid) enriched with chloramphenicol (0.1 g/L, Oxoid) plates incubated for 72 h at 30°C for yeasts and molds according to the IDF [39]; violet red bile agar (Oxoid) incubated for 24 h at 37°C for total coliform bacteria as in Hitchins et al. [40]; the total proteolytic bacterial count was determined using plate count agar (Merck) supplemented with 4 % skimmed milk powder and incubated at 37°C for 48 h. Proteolytic colonies were identified as colonies surrounded by a clear halo in an otherwise opaque medium ; and the total lipolytic bacterial count was determined using tributyrin agar (Oxoid) and incubated at 37°C for 48 h. Lipolytic colonies were identified as colonies surrounded by a clear halo in an otherwise turbid culture medium [41] .The cell concentrations were expressed as the logarithm of colony-forming units per gram of sample (log cfu g^{-1}).

2.16 Sensory properties

Samples of cream were analyzed by 9 panelists in the scope of sensory analyses according to the scoring card described by Bodyfelt et al. [42].

2.17 Analysis of fatty acids

2.17.1 Preparation of fatty acids methyl esters

Fatty acids methyl esters were prepared from total lipid by using rapid method according to the method of ISO 12966-2 [43]. However, fatty acid methyl esters were formed by trans-esterification with methanolic potassium hydroxide as an intermediate stage before saponification occurs. Approximately 0.1 g of the oil was placed in 5- ml screw- top test tube and isooctane (2ml) were added to the tube then the tube was shaken. Methanolic potassium hydroxide solution (0.1 ml, 2 N) was put on the cap fitted with a polytetrafluoroethylene (PTFE) joint, tighten the cap, and shaken strenuously for 30 seconds. The tube was left to stratify until the upper solution became clear and the upper layer containing the methyl ester was decanted. The isooctane solution is suitable for injection into the gas chromatograph.
flow rate 1.5 ml/min, splitting ratio of 1:5. The injector temperature was 250º C and that of flame ionization detector (FID) was 280º C.

The temperature setting was as follows:150 º C to 210º C; rising at 5 º C/min, and then held at 210 º C for 25 min. Peaks were identified by comparing the retention times obtained with stander methyl esters.

2.18 Data analysis

Data were statistically analyzed using Co-STAT software, V.6.13 (CoHort software, Berkeley, CA 94701). Mean comparison was done using Duncan's multiple ranges test (DMRT) at 5% probability test [44].

3. Results and Discussion

3.1 Total phenolic and flavonoid of strawberry leaves extract

The primary objectives of this investigation were to determine the level of total phenolic and specific groups of phenolic compounds (flavonoids), as these chemicals appear to be predominantly responsible for antioxidant and antimicrobial activity Table 1. Total phenolic content (mg/g) of ethanolic (Et OH 80%) strawberry leaves extract was 227.25 mg GAE/g. According to data from the literature, the finding of this investigation demonstrated that ethanol was a suitable solvent for the extraction of phenolics [45]. The level of total phenolic in this study is higher than the total phenol levels determined in strawberry leaves from Croatia, Turkey, Portugal and Algeria which was (96.96 , 197.16 , 170.3 , 179.6 mg GAE/g), respectively [46-49]. However, this is lower than previously reported in Portuguese samples (329 mg GAE/g) in acetonic extracts [50]. Table 1 also demonstrates the flavonoid concentration expressed in quercetin equivalents. Strawberry leaves ethanolic extract also contains high amount of flavonoids 186.51mg QE/g compared to previous studies. From published data, the flavonoid content of strawberry leaves seems quite variable. In Moroccan ethanolic extract samples, Amezouar et al. [51] reported 54 mg QE/g ; while Guendouze-Bouchefa et al. [46] recorded 21.4 mg QE/g in Algerian methanolic extract samples.

The reasons for such differences in both the concentration of total phenolic and flavonoid compounds can lead to sample preparation, geographical, climate conditions or time of sample collection [52,53].

3.2 Antioxidant activity of strawberry leaves extract

Antioxidant activity determined by FRAP and DPPH showed a better antioxidative effect of ethanolic strawberry leaves extract (Table 1). For DPPH test the results were expressed in IC50 value, the concentration of extract required to inhibition 50% of the DPPH radicals, where lower IC50 indicates higher antioxidant activity. The IC50 of strawberry leaves extract is 0.171 mg/100ml. Ethanolic strawberry leaves extract showed better activity to scavenge the DPPH radical compared with the values from published data [46,47,50].

Ferric reducing assay employed to determine reducing potential of ethanolic strawberry leaves extract. As the FRAP value measures the reduction of the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) by donor electrons in the sample [54]. In this, it was observed that the absorbance of the reaction mixture at 700 nm increased as the content of ethanolic strawberry leaves extract increased, indicating that the extract has a reducing potential.

Table 1. Phenolic compounds, antioxidant and antibacterial activity of ethanolic ((Et OH 80%)) strawberry leaves extract

				Antioxidant activity	
Sample	Total phenolic compounds (mgGAE/g)	Total flavonoid (mg QE/g)	Concentrations	DPPH radical scavenging activity (%)	The absorbance of ferric reducing Power
Ethanolic extract solution	227.25	186.51	0.1 mg/100ml	39.90^{i}	0.804^{f}
			0.3 mg/100ml	57.50^{g}	0.884^{e}
			0.5 mg/100ml	78.79^{d}	1.061^{c}
			0.7 mg/100ml	86.75^{c}	1.380^{b}
			1 mg/100ml	94.64^{a}	1.932^{a}
			IC50 (mg/100ml)	0.171^{k}	-

*Means with small superscript letters are significantly different at $p \leq 0.05$

FRAP—Ferring reducing antioxidative power; DPPH—2,2-diphenyl-1-picryl-hydrazyl-hydrate cation; IC50—Substrate concentration to produce 50% reduction of the DPPH

3.3 Antimicrobial activity

The aqueous and ethanolic extract of strawberry leaves were tested together for comparative purposes for studied the antimicrobial activity against foodborne pathogens that were *Pseudomonas aeruginosa*, *E. coli*, *B.cereus, L. monocytogenes*, *Salmonlla typhi*, *Staph aureus* , and yeast and mold which are *A. niger* and *Candida albicans* using the disk diffusion assay (Table2). The inhibition zones for the tested agents (aqueous and ethanolic leaves extracts) were measured. Table 2 has demonstrated that the strawberry leaves extract has antimicrobial properties against the tested organisms. This was shown by the production of inhibition zones. Results showed that the highest antimicrobial effect was found against *B.cereus* (11 and 16mm) and *L. monocytogenes* (10.5 and 15 mm), followed by *Staph. aureus* (9 and 14 mm) and *S.typhi* (8 and 10.2 mm) for both the aqueous and alcoholic extracts, respectively.

These values may be associated with increased extracellular β-glucosidase activity, which is secreted by *B.cereus* and *L.monocytogenes*. The antimicrobial effect of arbutin present in leaves strongly depends on the extracellular activity of β-glucosidase, an enzyme responsible for converting arbutin to free hydroquinone [55] . The minimum inhibitory concentration of arbutin is reported ranges from 0.4 to 0.8% depending on the microorganism [56]. In summary, it has been proposed that there is a direct correlation between the level of enzymatic activity of the microorganism and the antibacterial action of arbutin. Aqueous and ethanolic leaves extracts showed a weak inhibition effect for *P. aeruginosa, E. coli* and *S.typhi* which was in accordance with studies by other authors for *A. unedo* leaf extracts [57-61]. *A.niger* had higher antimicrobial properties for either the aqueous or alcoholic extract (10 and 13).

Table 2. Antimicrobial activity of aqueous and ethanolic extract solutions (EtOH 80%) of strawberry leaves on pathogenic strains

Microorganism	Antibiotic	A	Aqueous extract solution				Ethanolic extract solution (80%)			
			0.5	1.00	1.5	2.00	0.5	1.00	1.5	2.00
			mg/ml				mg/ml			
			Diameter of inhibition zone, mm*				Diameter of inhibition zone, mm*			
Pseudomonas aeruginosa ATCC 27853	G	18.0	-	-	6.0	7.0	6.0	7.0	7.8	9.0
Escherichia coli ATCC	G	19.0	4.0	6.0	6.5	7.0	6.3	7.5	8.0	9.5
Salmonlla typhi	G	18.6	4.0	6.0	7.0	8.0	7.0	8.0	9.0	10.2
Bacillus cereus B-3711	G	16.5	7.00	8.0	9.0	11.0	8.0	13.0	15.0	16.0
Listeria monocytogenes 598	G	18.0	5.0	6.0	7.5	10.5	7.6	10.0	12.0	15.0
Staphylococcus aureus 25922	G	20.4	5.0	7.0	7.4	9.0	8.0	9.2	10.1	14.0
Aspergillus niger	FN	23.0	6.5	7.0	8.4	10.0	7.0	7.5	10.0	13.0
Candida albicans CAIM -22	FN	15.0	-	-	3.0	5.5	-	4.0	5.0	6.5

A = G Gentamycin (5 µg), FN Fluconazole (25 µg), (–) no effect. Results are expressed as mean (n = 3).

This study showed weak inhibitory effect of strawberry leaves extracts for *Candida albicans* (Table 2) which is in correlation with other studies [57, 58, 60]. Results in the same table showed also that this inhibition effects varied among of concentrations of leaves extracts in addition to the method of extraction. In general, the antimicrobial activity increased with increasing the concentration of the extract, whether for the aqueous or alcoholic extract of strawberry leaves. Which was consistent with Rodriguez *et al* [62], who reported that the phenolic compound effect on microbial growth according to their constitution and concentration.

Due to the higher antioxidant capability and antimicrobial activity of ethanolic extract of strawberry leaves compared to the aqueous extract [47]. Lyophilized strawberry leaves ethanolic ELSLE extract was encapsulated and used in the ratio of (0,1, 1.5, and 2 g /100 g) to prepare milk cream samples.

3.4 Morphology

Porosity, strength, and entrapment of functional food components are all impacted by the structural properties of the freeze-dried matrices, which are predominantly produced during freezing [63]. The sublimation of the ice produced a porous hard shell in all experimental formulations following freeze-drying, resulting in a structure made of a glassy matrix with air cells whose size and shape depend on the makeup of the original system and the processing parameters used.

In Fig. 2, TEM micrographs of freeze-dried powders alone (control) (Fig. 2a) or ELSLE extract (encapsulation efficiency 85.72%) (Fig. 2b), images obtained with a transmission microscope suggest that ELSLE extract have a spherical or slightly

oblong in shape with a size <100 nm. The use of homogenization treatment of strawberry leaves extract with capsule materials reduces the average size of ELSLE extract and it could be hypothesized that a fraction of the ELSLE extract dispersed in the carrier solution separated at a nanoscale level during the spray drying process, without any evidence of structural collapse/ aggregation of strawberry leaves extracts at a nanoscale level [64, 65].

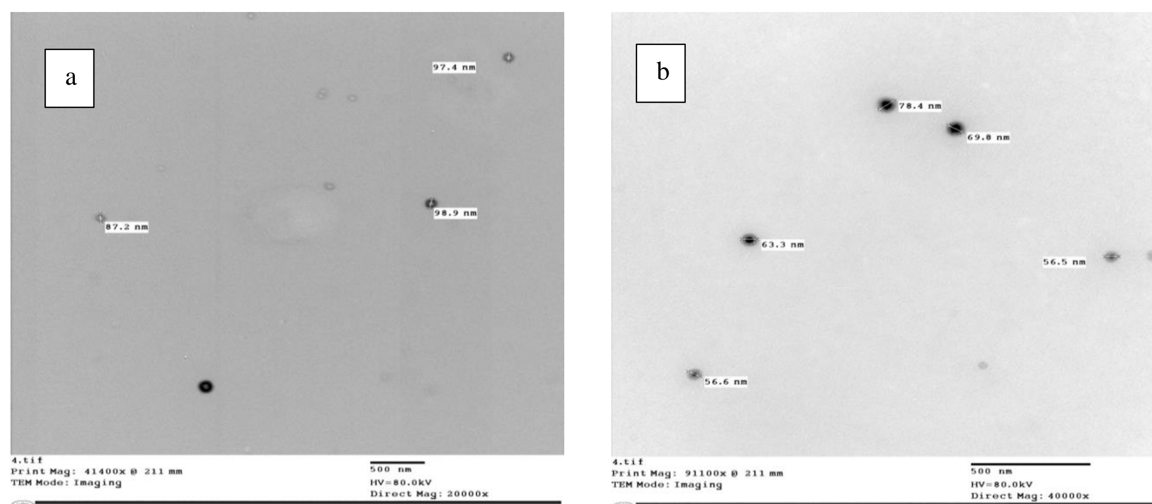

Figure 2. Micrographs of control and microencapsulated strawberry leaves powders: (a) control, (b) ethanolic extract 80%

3.5 Cream compositional analysis

The cream composition with or without the addition of encapsulated lyophilized strawberry leaves ethanolic ELSLE extract during storage for 28 days at 4 ±1ºC are shown in Table 3. Moisture is an important factor in food quality, preservation, and resistance to degradation. The dry matter that remains after moisture evaporated is commonly referred to as total solids [66]. As expected, the addition of ELSLE extract increase the total solid contents of creams, The data show that the moisture ranged from 50.63 to 53.95% (wt/wt) in fresh samples. It could be observed from these results that the moisture content in ELSLE extract cream were lower than that of control cream this could be attributed to the elevated level of solids not fat in ELSLE cream treatments. A slight decrease in moisture toward the end of the storage for all treatments was noted. These findings are in agreement with the literature [67,68]. Fat is a major component to determine total solids and influence the characteristics of cream. The fat ratio of the fresh cream samples with different samples ranged from 37.50 to 37.72% (wt/wt). The fat content decreased as the level of ELSLE increased. The fat contents decline with prolonging of the refrigerated storage period these results might be due the lipolytic activity of natural lactic acid bacteria. These findings are in agreement with the literature [68].

Compared to fat and moisture content, protein and carbohydrate contents had a relatively smaller range. Protein content ranged from 3.65 to 3.85% (wt/wt). Protein contents were significantly decreased with prolonging of cooled storage ($P < 0.05$). Total carbohydrate content ranged from 4.43 to 4.89% (wt/wt) and significantly decreased ($P < 0.05$) with prolonging of the cooled storage period (Table 3). However, high carbohydrate or protein content was observed in ELSLE samples compared to the control.

Ash content ranged from 0.39 to 0.41% (wt/wt) for a storage period ($P > 0.05$). The apparent increase in concentrations of ash may be due to moisture loss of all samples during the storage period.

The pH and titratable acidity values were varied depending on the storage period and the treatment. All cream treatments occurred a gradual decline toward the end of the storage period. Higher pH values were observed in ELSLE extract samples ranged from (6.85-6.89) this may be due to the tendency of the strawberry leaves extract to be alkaline. There was significant difference observed ($p<0.05$) in pH for all samples in all stages of the experiment (Table 3). The initial pH decrease may be due to the normal lactic acid bacteria leading to the degradation of lactose to lactic acid and associated with the formation of carbonic acid resulting from the dissolution of CO_2 in water. These findings are in agreement with those reported by Rodarte et al. and Mieželienė et al. [69,70]

Table 3. Effect of encapsulated lyophilized strawberry leaves ethanolic extract on the chemical composition of cream during storage at 4 ±1℃ for 28 days

Treatment	Storage period (day)	% (wt/wt)					Acidity %	pH
		Moisture	Fat	Protein	Total Carbohydrate	Ash		
C	1	53.95^{a}	37.72^{a}	3.65^{ad}	4.43^{cde}	0.39^{a}	0.17^{de}	6.68^{af}
	7	53.80^{ab}	37.54^{abc}	3.60^{be}	4.41^{cde}	0.40^{a}	0.19^{be}	6.58^{dg}
	14	53.55^{ab}	37.46^{abc}	3.45d^{e}	4.39^{de}	0.40^{a}	0.21^{bc}	6.46^{fi}
	21	53.26^{b}	37.34^{abc}	3.24^{f}	4.17^{ef}	0.42^{a}	0.27^{a}	6.32^{ij}
	28	52.00^{cf}	37.25^{bc}	3.01^{g}	3.95^{f}	0.43^{a}	0.30^{a}	6.15^{j}
T1	1	52.44^{c}	37.70^{a}	3.77^{abc}	4.59^{ad}	0.40^{a}	0.17^{de}	6.85^{ab}
	7	52.32^{cd}	37.65^{ab}	3.71^{abc}	4.56^{ad}	0.41^{a}	0.18^{cde}	6.75^{ae}
	14	52.16^{cde}	37.63^{ab}	3.68a^{bc}	4.53^{ae}	0.41^{a}	0.20^{bcd}	6.62^{cg}
	21	51.80^{cg}	37.59^{abc}	3.57^{cde}	4.50^{be}	0.42^{a}	0.21^{bc}	6.44^{ghi}
	28	51.52^{eh}	37.38^{abc}	3.41^{ef}	4.47^{be}	0.44^{a}	0.22^{b}	6.35^{hi}
T2	1	51.70d^{h}	37.65^{ab}	3.83^{a}	4.83^{ab}	0.41^{a}	0.16^{e}	6.86^{a}
	7	51.54^{eh}	37.60^{abc}	3.80^{ab}	4.67^{ad}	0.41^{a}	0.18^{cde}	6.78^{ad}
	14	51.36^{fgh}	37.55^{abc}	3.74^{abc}	4.62^{ad}	0.42^{a}	0.19^{be}	6.64^{bg}
	21	51.14^{ghi}	37.50^{abc}	3.65^{ad}	4.61^{ad}	0.43^{a}	0.20^{bcd}	6.52^{fi}
	28	50.99^{hij}	37.41^{abc}	3.60^{be}	4.57^{ad}	0.44^{a}	0.21^{bc}	6.47^{fi}
T3	1	50.63^{ijk}	37.50^{abc}	3.85^{a}	4.89^{a}	0.41^{a}	0.16^{e}	6.89^{a}
	7	50.56^{ijk}	37.20^{c}	3.82^{ab}	4.79^{abc}	0.41^{a}	0.17^{de}	6.82^{abc}
	14	50.40^{jk}	36.80^{d}	3.73^{abc}	4.76^{ad}	0.42^{a}	0.18^{cde}	6.68^{af}
	21	50.24^{k}	36.20^{e}	3.65^{ad}	4.72^{ad}	0.44^{a}	0.20^{bcd}	6.55^{eh}
	28	50.19^{k}	36.19^{e}	3.61^{be}	4.69^{ad}	0.45^{a}	0.21^{bc}	6.43^{ghi}

*Means with small superscript letters are significantly different at $p \leq 0.05$

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

As storage period progressed, acidity slightly increased in all treatments. The control sample (C) had the highest content of acidity. Titratable acidity ranged from 0.16 to 0.17% and increased significantly ($P < 0.05$) during the storage period reaching 0.21 to 0.30% on the 28 th day. The increase in the acidity can be related to an increase in lactic acid bacteria and their conversion of lactose to lactic acid. These results are in agreement with the literature [68,70,71]. The titration acidity values of T3 samples were lower than those of T1 and the control group. However, there was no significant ($P > 0.05$) differences between T2 and T3 treatments at the end of storage period. The acidity of the cream should not exceed 0.225% as far as the percentage of lactic acid is concerned [71]. The acidity of the ELSLE cream samples remained below the limits during the storage period. On the other hand, the acidity value of the control samples on the 28th day was higher than the upper limit of acidity determined for the cream.

3.6 Color parameters of cream samples

The color of a food product is one of the most important factors influencing consumer acceptance and it is very important parameter because excessively dark color could be regarded by consumers as sign of lower quality indicator. The light, process temperature, oxygen capacity, or pH can change the color pigments of foods [72]. Additionally, spoilage by microorganism especially by yeast and molds can cause to surface discoloration [73]. The colorimetric analysis of cream samples is shown in (Table 4). The evaluated color parameters included lightness (L-value), redness (a- value), yellowness (b-value), chroma (C-value), hue (H-value), yellowness index (YI) and total color index (ΔE value). The lightness (L-value) of the tested samples were in the range of 68.22 to 84.3 in fresh samples and 58.09 to 70.86 at the end of storage. Use of encapsulated strawberry leaves in preparation of cream resulted in decreased L- value ($P < 0.05$), simply as a result of the presence of the phenolic fraction of strawberry leaves. The redness (a-value) of the prepared cream samples showed negative sign indicating the greenish color shade of cream. All cream samples showed a fade greenish color shade (a-values from - 0.39 to -2.28). The increase in b* value that occur during storage attributed to the non-enzymatic degradation (Maillard reaction) [74]. All prepared cream samples showed more or less intensive yellow color shade (positive b-values). The b* values of the samples became increasingly yellow with time.

Table 4. Effect of encapsulated lyophilized strawberry leaves ethanolic extract on color of cream during storage at 4±1°C for 28 days

Treatment	Storage period (day)	Parameters%					
		L*	a*	b*	YI	c*	H
C	1	84.3[a]	-1.90[b]	9.03[j]	15.30[h]	9.05[h]	101.05[a]
	7	74.10[b]	- 1.94[b]	9.23[j]	17.79[g]	9.43[gh]	101.64[a]
	14	72.39[c]	- 2.36[a]	9.95[ij]	19.64[fg]	10.14[fgh]	102.34[a]
	21	71.56[d]	- 2.15[ab]	10.00[ij]	19.97[f]	10.37[fgh]	102.25[a]
	28	70.86[d]	- 2.28[ab]	10.11[hij]	20.38[f]	10.48[eh]	102.55[a]
T1	1	73.58[b]	- 0.58[def]	10.23[gj]	19.86[f]	11.04[eh]	95.84[b]
	7	67.13[f]	- 0.85[cde]	10.78[fi]	22.94[e]	10.16[fgh]	95.90[b]
	14	66.08[g]	-1.01[cd]	11.37[dh]	24.58[de]	11.38[dg]	95.18[bc]
	21	64.53[h]	-1.09[c]	11.20[ei]	25.42[de]	11.54[cg]	93.18[cde]
	28	62.92[i]	-1.19[c]	11.71[def]	26.59[d]	11.72[cf]	92.99[de]
T2	1	71.07[d]	- 0.61[def]	11.46[dh]	23.04[e]	11.50[cg]	95.71[b]
	7	63.23[i]	- 0.91[cd]	11.56[dg]	26.12[d]	11.62[cf]	95.64[b]
	14	61.21[j]	- 1.14[c]	12.32[cde]	28.75[c]	12.33[bf]	94.04[be]
	21	60.27[k]	- 1.17[c]	13.24[bc]	33.18[b]	13.43[ad]	93.97[be]
	28	59.68k[l]	- 1.20[c]	14.36[ab]	34.37[b]	14.39[ab]	92.93[de]
T3	1	68.22[e]	- 0.39[f]	11.79[def]	24.69[de]	11.85[cf]	95.18[bc]
	7	63.15[i]	- 0.46[ef]	12.58[cd]	28.46[c]	12.63[be]	94.54[bcd]
	14	59.58k[l]	- 0.89[cd]	13.57[abc]	32.54[b]	13.58[abc]	92.43[def]
	21	59.14[l]	- 0.97[cd]	14.15[ab]	34.27[b]	14.10[ab]	90.58[f]
	28	58.09[m]	- 1.00[cd]	14.76[a]	36.30[a]	14.78[a]	91.96[ef]

* Means with small superscript letters are significantly different at $p \leq 0.05$

a: Redness; **b:** Yellowness; **L:** Lightness; **H:** Hue ; **C:** Chroma; **(YI):** Yellowness index

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

Encapsulated lyophilized strawberry leaves ethanolic extract (ELSLE extract) ratio also appeared to play a role in the color of the powders. The most intensive yellow color shade was recorded for cream with higher concentration of ELSLE extract resulted in a more intense color as shown by higher values of the chromatic indices (11.79 to 14.76), higher b* and YI values and lower L* values than control. This indicates a higher retention of strawberry leaves pigment such as polyphenolic compounds in encapsules matrices. It has been demonstrated that saccharides with α-(1,4) glycoside link generates a polar surfaces, increasing their ability to bind a polar molecules [75]. Thus, since maltodextrin has α-(1,4) link and a higher molecular weight, it is expected to interact and bind a higher fraction of such a polar pigment. The trend of color parameters detected in this study is in agreement with what were detected by Perna et al . and Sert et al. [76,77].

The hue (H-values) represent the color type on a scale from 0 ˚to 360 ˚ with 0˚, 360˚ (pure red), 90˚(pure yellow),180˚(pure green) and 270˚(pure purple). All control samples showed a yellow-greenish color type in the range of (101.50 to 102.55) while ELSLE extract cream samples tended to be in the pure yellow color type with Hue-values between 91.96˚ to 95.84˚ along storage period. The chroma (C-values) are indicator for the intensity (saturation) of the color. The color intensity (C-values) of ELSLE extract cream were higher (11. 4 to 14.78) than those of control cream sample (9.50 to 10.48) indicating that ELSLE extract gave the cream more saturated color and this may be because of its higher phenolic content.

3.7 Viscosity

Viscosity is an important physical property for describing mouth feel of dairy products. Consumer generally judges the quality of cream by the manner of its flow from a container, deeming the thickness of the cream a measure of its fat content [78-80]. Data presented in Table 5 show that C sample (control) recorded the lowest values of viscosity either when fresh or at the end of storage period as it recorded 3600 cP when fresh and increased to reach 3650 cP after 28 days, while the other treatments recorded higher values in descending order as follows T3 > T2 > T1. The use of ELSLE extract resulted in cream formulations with a higher viscosity than cream without any additives. Since viscosity of cream was directly associated with total solids, which might explain the greater viscosity of T3.

Table 5. Viscosity (cP) determination of cream with encapsulated strawberry leaves extract samples at 10 rpm at room temperature 25 ± 2 ° C

Sample	Storage period	
	Fresh	After 28 days of storage
Control	3600^{d}	3650^{d}
T1	3800^{c}	3870^{c}
T2	4200^{b}	4260^{b}
T3	4400^{a}	4480^{a}

*Means with small superscript letters are significantly different at $p \leq 0.05$
C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

3.8 Microbiological analysis
The standard specification for cream and prepared creams ES: 780-1/ 2014 recorded general microbiological properties for all types of cream is free of pathogenic bacteria and its toxins; coliform bacteria must not exceed 10 cfu/g for pasteurized cream only; free of *E. coli* ; molds and yeasts spores must not exceed 20 cfu/g for pasteurized cream only. The changes in the number of total bacterial count TBC, yeast and molds Y&M , lipolytic bacteria LP , proteolytic bacteria PB and coliform bacteria of cream samples during the storage period at 4±1˚C (log cfu ml^{-1}) are shown in Table 6. The TBC count increased gradually all over the storage period. The highest values were recorded with control and the lowest was recorded with T3 , resulting in significant differences. Yeasts and molds were not detected at the beginning of the storage period in all treatments. A gradual increase ($P < 0.05$) was observed in yeast and mold counts after zero time in the control sample. However, it appeared by the end of the storage period in a very low count in treatments to which strawberry leaves extract was added. According to reports, concentrated creams made using conventional procedures on the market typically contain 3.32 log cfu g-1 of yeast and mold [81].

Table 6. Microbiological analysis of cream with different concentration of lyophilized encapsulated strawberry leaves ethanolic extract during storage at 4±1˚C for 28 days (Log cfu ml^{-1})

Sample	Storage period (day)	TBC*	Y&M	Lipolytic Bacteria	Proteolytic bacteria	Coliform bacteria
C	1	3.08^{g}	N.D	N.D	N.D	N.D
	7	3.18^{fg}	1.30^{i}	1.90^{h}	N.D	N.D
	14	3.41^{f}	4.11^{c}	2.04^{h}	N.D	N.D
	21	5.43^{b}	4.41^{b}	4.85^{b}	4.18^{c}	N.D
	28	6.04^{a}	5.34 ^{a}	5.44^{a}	5.21^{a}	N.D
T1	1	2.04^{i}	N.D	N.D	N.D	N.D
	7	2.18^{i}	N.D	N.D	N.D	N.D
	14	3.30^{fg}	3.00^{f}	N.D	N.D	N.D
	21	4.36^{de}	3.70^{d}	3.20f^{g}	4.08^{cd}	N.D
	28	5.23^{b}	4.52^{b}	4.30^{c}	4.85^{b}	N.D
T2	1	1.95^{ij}	N.D	N.D	N.D	N.D
	7	2.08^{i}	N.D	N.D	N.D	N.D
	14	3.23f^{g}	N.D	N.D	N.D	N.D
	21	4.30^{e}	2.78^{g}	3.34^{f}	3.85^{e}	N.D
	28	4.83^{c}	3.16^{e}	3.84^{d}	4.02^{d}	N.D
T3	1	1.78^{j}	N.D	N.D	N.D	N.D
	7	1.95^{ij}	N.D	N.D	N.D	N.D
	14	2.70^{h}	N.D	N.D	N.D	N.D
	21	4.18^{e}	2.30^{h}	3.15^{g}	3.34^{g}	N.D
	28	4.54^{d}	2.95^{f}	3.52^{e}	3.72^{f}	N.D

*Means with small superscript letters are significantly different at $p \leq 0.05$,
TBC: Total bacterial count; Y&M: Yeast and mold; ND: Not detected
C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream

with 2% ELSLE extract

The count of total proteolytic and lipolytic bacteria (Table 6) was not detectable at zero time in all samples. In general, the counts of total proteolytic and lipolytic bacteria in the control sample were higher than those in the other treatments after 28 days of cold storage. Coliform bacteria were not detected during storage in all cream treatments. This may be due to the adequate heat treatment of cream during manufacture and high sanitation conditions during making and storage of cream.

The T2 and T3 treatments were the most effective for the inhibition of bacterial count and yeast and mold ($P < 0.05$). This is probably because of an inhibitory effect of strawberry leaves extract on the spoilage bacteria growth. These results are in agreement with the literature [47,57,58,60,82, 83] , who reported that the strawberry leaves arbutin and its metabolite hydroquinone has an antimicrobial activity .

3.9 Sensory evaluation

The Scores of sensory evaluation (flavor, body and texture, appearance and color, and overall acceptability) for cream containing ELSTLE extract at the levels of 1.0, 1.5 and 2% during cold storage (fresh ,7, 14, 21 and 28 days) were presented in Fig. 3. The data in this figure reveal that the flavor of the cream (Fig.3 a) was affected by adding ELSLE extract. There were significant differences ($P \leq 0.05$) in flavor scores for all treatments when fresh and during cold storage. The control treatment gained the highest score either when fresh or throughout the interval storage periods. Also, the data in same figure indicated that cream containing 1% ELSLE extract was the most acceptable and did not differ from the control. While the addition level over 1 % ELSLE extract resulted in a product with slightly darker color. However, scores decreased slightly in all treatments as storage period advanced. At the beginning of storage, significant ($P \leq 0.05$) differences in appearance and color (Fig.3b) scores were observed. Total acceptability scores are presented as mean flavor, body and texture, and appearance and color ($P \leq 0.05$) during storage period. The 28 th day T3 samples were recorded the lowest score ($P \leq 0.05$) of total acceptability (Fig.3d). The C sample followed by T1 and T2 samples recorded relatively higher scores.

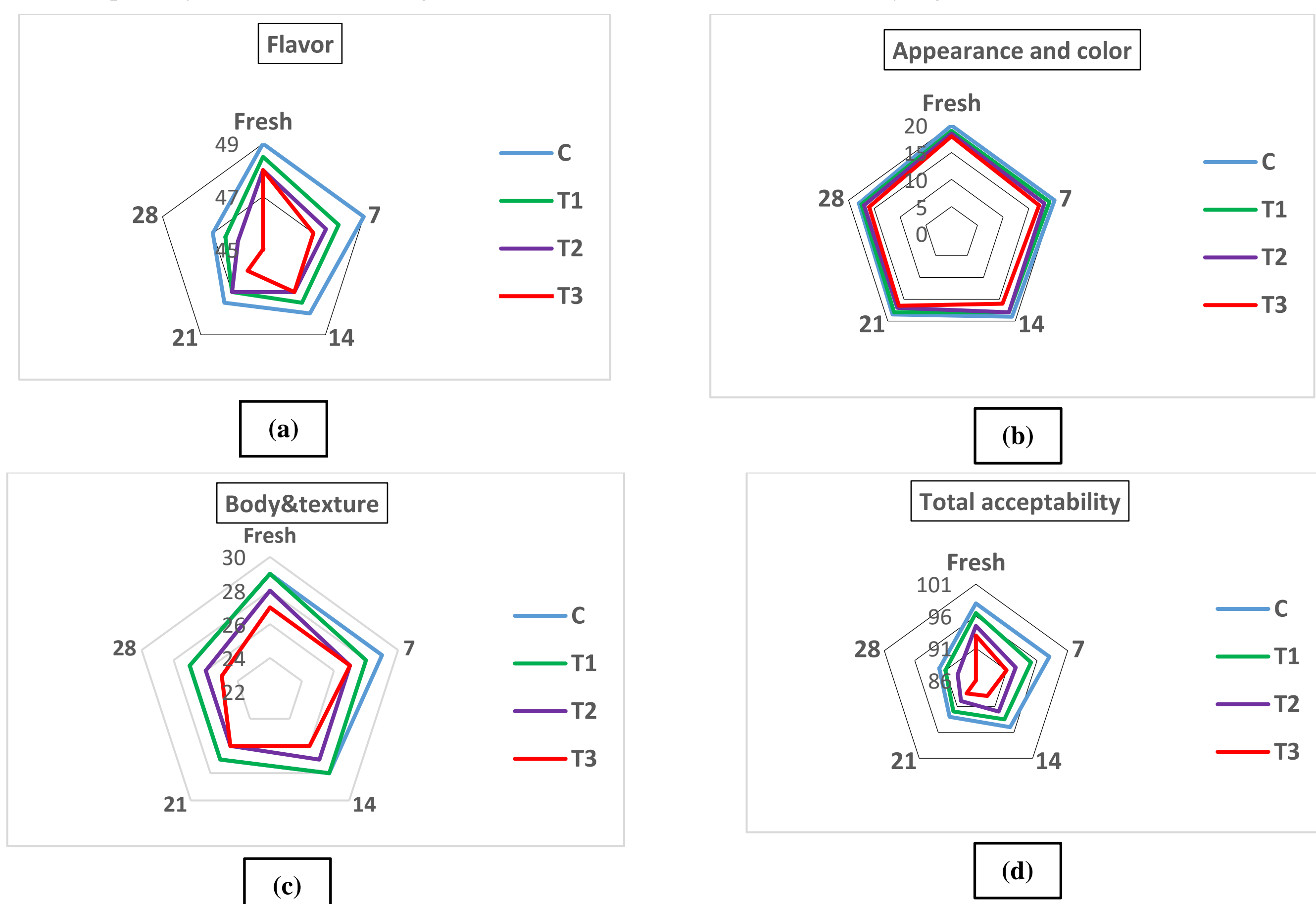

Figure 3. Organoleptic properties of cream with different levels of lyophilized encapsulated strawberry leaves ethanolic extract during storage at 4±1° C for 28 days

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

3.10 Saponification value and peroxide value

The changes in saponification value (SV), Peroxide value (PV) of cream containing different concentrations of ELSLE extract were taken as indices for oxidation stability during storage period (28 days) at 4± 1 ºC were estimated. One of the primary issues limiting the quality and acceptability of dairy products is lipid oxidation. This process results in color changes, the development of unfavorable aromas and flavors, as well as the creation of potentially toxic compounds [9].

Peroxide is a primary product of oxidation. Lipid reacts with oxygen which is contained in the product resulting into a rancid flavor, therefore the peroxide value (PV) is an indicator of freshness as viewed through the assessment of the primary oxidation of hydroxyl groups of unsaturated fats in oils by molecular oxygen into hydroperoxides and peroxides [83-85]. The data in Table 7 demonstrates that the addition of ELSLE extract led to a decrease in PV, compared with PV of control, but it was more remarkable in cream containing higher level of addition (2%) of ELSLE extract.

The peroxide values of all samples decreased on the 28 th day of storage. This can be a result of the unstable nature of peroxide which turns easily into a secondary oxidized products [86,87]. There was significant difference between the samples during storage ($p < 0.05$).

The saponification value SV of fats and oils is one of the most common quality indices. A high SV indicates triacylglycerols with shorter fatty acyl chains. Consequently, SV becomes an easy approach to assess fatty acids' chain length of specific fats/oils [88]. The saponification values of cream containing different concentrations of ELSLE extract were lower than in control sample. These results reflected the impact of ELSLE extract, as natural antioxidants in retarding of cream oxidation. This fact indicated that antioxidant activities of ELSLE extract to the presence of phenolic compounds. These findings are in agreement with the literature [82, 89-93].

Table 7. Saponification value and peroxide value of cream with different concentration of lyophilized encapsulated strawberry leaves ethanolic extract during storage at 4±1° C for 28 days

Treatment	Storage period									
	1st day		7th day		14th day		21th day		28th day	
	Saponification value (mg $KOH.gm^{-1}$)	Peroxide value (meq $O2.kg^{-1}$)	Saponification value (mg $KOH.gm^{-1}$)	Peroxide value (meq $O2.kg^{-1}$)	Saponification value (mg $KOH.gm^{-1}$)	Peroxide value (meq $O2.kg^{-1}$)	Saponification value (mg $KOH.gm^{-1}$)	Peroxide value (meq $O2.kg^{-1}$)	Saponification value (mg $KOH.gm^{-1}$)	Peroxide value (meq $O2.kg^{-1}$)
C	216.18^{g}	2.95^{i}	217.59^{e}	3.61^{g}	219.33^{c}	5.54^{a}	221.20^{b}	5.12^{b}	223.18^{a}	4.82^{c}
T1	215.23^{h}	2.73^{jk}	216.36^{g}	2.98^{i}	218.51^{d}	4.48^{d}	219.73^{c}	4.17^{e}	221.06^{b}	3.84^{f}
T2	214.42^{i}	2.56^{kl}	215.05^{h}	2.57^{kl}	215.93^{g}	3.33^{h}	216.94^{f}	2.90^{ij}	218.34^{d}	2.98^{i}
T3	211.99^{l}	2.41^{l}	212.31^{kl}	2.16^{m}	212.74^{k}	2.97^{i}	213.33^{j}	2.54^{kl}	214.03^{i}	2.02^{m}

*Means with small superscript letters are significantly different at $p \leq 0.05$

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

3.11 Fatty acid composition

Fatty acid composition of cream treatments was determined and the obtained data was recorded as in Table 8. Dib et al. [57] identified the 12 fatty acids in strawberry leaves extracts and recorded that the fatty acid fractions of the leaves contained 38.5% palmitic acid followed by lauric acid (5.2%). The main unsaturated fatty acids were oleic acid (10.6%), linolenic acid (9.3%) and linoleic acid (5.5%). Through this study, it could be explained the reason for the increase in the proportions of these fatty acids in treatments containing strawberry leaves extract compared to the control. strawberry leaves extract had a high saturated fatty acid content, of which palmitic acid was the predominant compound (up to 33%), followed by stearic acid (11.25%). The main unsaturated fatty acid was oleic acid (25.74). However, the predominate polyunsaturated fatty acids were linoleic acid (1.22%) followed by octadecatetraenoic acid (0.83) for cream without any additives.

Table 8. Fatty acid composition of fresh cream with different concentration of lyophilized encapsulated strawberry leaves ethanolic extract for 28 days

Fatty acids %	Control	1	2	3
Butyric (C4:0)	2.59^{a}	2.21^{b}	1.70^{c}	0.73^{d}
Caproic (C6:0)	1.57^{a}	1.52^{a}	1.27^{b}	1.24^{b}
Caprylic (C8:0)	0.82^{a}	0.72^{a}	0.73^{a}	0.71^{a}

Capric (C10:0)	1.54[a]	1.48[a]	1.48[a]	1.40[a]
Lauric (C12:0)	2.00[a]	2.02[a]	2.03[a]	2.05[a]
Myristic (C14:0)	9.84[b]	9.87[b]	10.02[b]	10.90[a]
Pentadecanoic acid (C15:0)	0.77[a]	0.81[a]	0.83[a]	0.84[a]
Palmitic acid (C16:0)	32.52[b]	33.05[ab]	33.11[ab]	33.33[a]
Heptadecanoic acid (C17:0)	0.99[a]	0.97[a]	0.97[a]	0.92[a]
Stearic (C18:0)	10.82[a]	10.91[a]	11.13[a]	11.15[a]
Arachidic (C20:0)	0.29[a]	0.29[a]	0.30[a]	0.32[a]
Behenic (C22:0)	0.10[a]	0.11[a]	0.11[a]	0.12[a]
(C15:1)	0.50[a]	0.49[a]	0.48[a]	0.46[a]
Myristoleic (C14:1)	1.37[a]	1.36[a]	1.35[a]	1.32[a]
Palmitoleic (C16:1)	2.51[a]	2.50[a]	2.50[a]	2.48[a]
Margaric acid (C17:1)	0.43[a]	0.47[a]	0.47[a]	0.48[a]
Oleic (C18:1)	24.78[c]	25.04[bc]	25.32[b]	25.74[a]
Linoleic (C18:2)	1.22[a]	1.23[a]	1.34[a]	1.39[a]
Linolenic (C18:3)	0.27[a]	0.27[a]	0.28[a]	0.29[a]
Octadecatetraenoic acid (C18:4)	0.83[a]	0.83[a]	0.82[a]	0.78[a]
Unknown	2.78[b]	3.23[a]	3.26[a]	3.29[a]
*Total SFA	62.86[b]	63.96[a]	63.68[a]	63.71[a]
**Total USFA	31.91[c]	32.19[c]	32.56[b]	32.94[a]

*Means with small superscript letters are significantly different at $p \leq 0.05$

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

3.12 Total Phenolic and flavonoid content

The effectiveness of antioxidants in protecting foods against oxidative deterioration is very dependent on complex phenomena determined by the relative physical states of the lipid substrates, the conditions of oxidation, the methods used to follow oxidation and the stages of oxidation [94]. The content of total phenolic and flavonoid were determined in fresh samples and at the end of storage period (Table 9). A higher amount of total phenolic and flavonoid content was found in the cream with ELSL extract which decreased slightly at 28 th day of storage.

Table 9. Total Phenolic and flavonoid content (mg/g) of cream with lyophilized encapsulated strawberry leaves ethanolic extract

Treatment	Storage period			
	1st day		28 th day	
	Phenolic mg/g	Flavonoids mg/g	Phenolic mg/g	Flavonoids mg/g
C	0.63[f]	0.98[g]	0.60[f]	0.96[g]
T1	1.71[cd]	1.29[e]	1.51[e]	1.18[f]
T2	1.80[c]	1.67[b]	1.62[de]	1.47[d]
T3	2.79[a]	1.91[a]	2.49[b]	1.60[c]

*Results were expressed as means ±SD (standard division; n=3; p=0.05)

C: Cream without any additives; T2 :Cream with 1.5% ELSLE extract; T1: Cream with 1% ELSLE extract; T3: Cream with 2% ELSLE extract

4. Conclusion

The effect of encapsulated lyophilized strawberry leaves ethanolic ELSLE extract on shelf life extension and quality attributes of milk cream treatments was studied for 28 days at 4 ±1° C. Three different concentration of ELSLE extract were investigated (1, 1.5, 2 g/100g cream) which represented (T1, T2,T3) treatments in addition to one cream sample without any additives (control). Color analysis recorded significant differences between all treatments, intensive yellow color shade was recorded for cream with higher concentration of ELSLE extract and gave the cream more saturated color. The highest viscosity cream formulation was recorded for T3 treatment. Microbiological analysis revealed that T3 followed by T2 were the most effective treatments inhibiting the growth of bacterial count and yeasts and molds. T1 and T2 recorded relatively higher sensory evaluation scores. Lower saponification values of cream containing different concentrations of ELSLE extract. Milk cream with ELSLE extract contained more total phenolic and flavonoid content than control. ELSLE extract could be used till to 2 g/ 100 g cream to improve the microbiological safety and oxidative stability of milk cream and prolong the shelf life till 28 days at 4 ±1° C.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

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