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Research Article
Functional Flavoured Mandarin Fermented Goat Milk with Algae Oil Nanoemulsion

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Abstract

Background and Objective: Fermented dairy products are healthy products, especially when fortified with the source of omega 3. This study aimed to prepare nanoemulsions for Algae oil as a rich source of omega 3 and 6 using whey protein isolate (WPI) and sodium caseinate (NaCas) and incorporated in flavoured fermented goat milk and evaluate it. **Materials and Methods:** Using WPI: NaCas were mixed in ratios 10:0, 8:2, 6:4, 4:6, 2:8 and 0:10 to prepared nanoemulsions with 10% algae oil and characterized for size, zeta potential and stability. Preparation of flavoured fermented goat milk and evaluate the effect of algae oil addition on the nutritional value and physicochemical and sensory characteristics. **Results:** The emulsions with only WPI had a significantly smallest droplet size 77.74 nm, while the size is increased with increased NaCas ratio in emulsions, with only NaCas is 89.03 nm. The results of this study showed that algae oil is characterized by its high content of unsaturated fatty acids (omega-3 fatty acids). While goat milk fat was rich in saturated fatty acids. The addition of algae oil at different ratios to the flavoured fermented goat milk enhanced the growth of starter culture and increases the percentage of unsaturated fatty acids and therefore decrease the percentage of saturated fatty acids. The addition of mandarin juice and peels to fermented goat milk has improved the aroma and reduced goaty flavour in all samples. Also, the addition of nano-emulsion algae oil is a good way for developing flavoured fermented goat milk. **Conclusion:** It could be concluded that using Algae oil nanoemulsion in flavoured fermented goat milk for acceptable quality and high antioxidant activity.

Key words: Fermented goat milk, chlorella algae oil, lactic acid bacteria, whey protein isolate, sodium caseinate, nano-emulsion

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microalgae are one of the promising sources of functional food ingredients[1]. Which are considered an important natural source of bioactive molecules, such as n3 polyunsaturated fatty acids (n3 PUFA) in particular DHA. DHA has been recognized as a source of anti-inflammatory specialized pro-resolving mediators, such as protectins, resolvins and maresins[2]. The use of algae oil, which is the primary source of PUFAs, presents the advantage of the absence of pollutants and the lack of unpleasant odor[3].

Fermented dairy products are widely accepted health products and are included as essential components of the daily diet. The yogurt is a suitable food matrix to successfully incorporate bioactive ingredients and the high bioaccessibility of a given food component is rendered available for intestinal absorption after digestion of lipid nutrients and bioactive compounds. Moreover, yoghurt was demonstrated as the best form to rapid providing absorption of all lipids, inclusive Ω 3 PUFA 4[4,5]. The use of goat milk as a probiotic carrier has fast increased over the last decade[6]. Goat milk can be suited for fermented milk production, mainly because of its higher nutritional value compared to cow milk as its much richer in lactose-derived oligosaccharides (lactulose, lactitol, lactobionic acid and galactooligosaccharides), which are beneficial to humans as a result of their prebiotic and anti-infective features. The use of specific types of milk with appropriate nutritional properties, such as goat milk, in combination with bacterial strains that have probiotic properties, represents one valuable method of manufacturing novel dairy functional foods[7,8] and promising strategy for enhancing the nutritional quality and therapeutic potential of goat milk[9,10].

However, oils rich in omega-3 are more sensitive to oxidation, including the loss of nutritional value, production of oxidation products like peroxides and makeup of ketones and aldehydes compounds with bad odor and taste, these reducing consumer acceptance[11]. For these reasons, there is a need to develop effective strategies to overcome this challenge. One of the most promising methods of maintaining bioactive components from oxidation is using encapsulation techniques[12].

The nanostructures' trend has been paid attention, because of its ability to improve solubility and bioavailability for bioactive ingredients without compromising other food properties[13]. Whey protein isolate and sodium caseinate (milk proteins) have excellent emulsifier properties in food production and are mostly used as food ingredients[14-16]. It is used to include monoglycerides in matrices of food to the enhancement of physico-chemical properties and stability of the protein-stabilized emulsion[17] since the lipid oxidation stability is very important as a quality parameter of the food product.

Citrus fruits and mandarins in particular are appreciated and consumed by billions of people worldwide because of their unique, delightful and attractive flavour, which derives from a blend of sweet, sour, fruity, fresh and earthy notes[18]. Mandarins an especially rich source of bioactive carotenoid compounds and contain a unique carotenoids composition, governed by the radical-scavenging pigment, β-cryptoxanthin, which was repeatedly proven to reduce symptoms of diabetes, obesity, oxidative stress and carcinogenesis in humans and rats[19,20].

This study aimed to apply algae oil as a rich source of omega 3 and 6 in whey protein isolate and sodium caseinate nanoemulsions to enhance stability and acceptability of nano-encapsulated algae oil to Fortify Flavoured Fermented Goat Milk (FFGM), comparing with control. Physicochemical, microbiological, viscosity, oxidative interactions, microstructural and sensorial analyses for the fresh products and after 21 days of cold storage at 5±1°C were conducted to evaluate the potential changes.

MATERIALS AND METHODS

Study area: The study was carried out at the Dairy Department, Food industries and Nutrition Division, National Research Centre, [1]Dairy Research and Technology Department and Fats and Oils Research Department Food Technology Research Institute, Agricultural Research Centre from March, 2021 to August, 2021.

Materials: Goat Milk was obtained from a goat farm at Brajil, Giza, Egypt. The gross composition of raw goat's milk was: 10.68±0.21% total solids, 2.90±0.02% total protein, 3.15±0.05% fat, 4.10±0.19% lactose, 0.71±0.03% ash, 0.18±0.01%, titratable acidity and 6.7±0.02 pH. Whey protein isolate (WPI, Pipro™, comprised mainly of β-lactoglobulin and α-lactalbumin, was obtained from Davisco Foods International, Le Sueur, USA. Sodium caseinate (NaCas, ALANATE™ 180, protein content 92.7%) was provided by Fonterra Ltd., New Zealand (NZ). Ethanol, methanol, ethyl acetate and acetonitrile were purchased from Himedia, India. All chemicals and reagents from different suppliers were of analytical grade.

Starter, namely: ABT-2 containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium*

bifidium, was supplied by the Chr-Hansen company (Horsholm, Denmark). The freeze-dried bacterial starter was activated separately in sterilized (121°C/10 min) skimmed cow's milk (0.1% fat and 10% SNF) using 0.02% (w/v) inoculums. The activated cultures were used for the inoculation of the goat milk. The Mandarin was obtained from the local market in Giza, Egypt which was used to obtain juice and the peel was used after drying by air-dried.

Fatty acid composition: For the determination of the fatty acid composition of the algae oil, fatty acid methyl esters were prepared according to AOAC[21]. Determination of fatty acids composition was carried out according to Hamed *et al.*[22].

Emulsion preparation: Whey protein isolate and sodium caseinate each of both 10% was dissolved in Milli-Q water by gentle magnetic stirring at 55°C for 1 hr after which the solutions were left standing at room temperature for 12 hrs to ensure complete dispersion. The resulting mixture was adjusted to pH 6.7 using NaOH (1 M). The wall materials, WPI : NaCas were mixed in ratios 10:0, 8:2, 6:4, 4:6, 2:8 and 0:10 by gentle magnetic stirring for 1 hr, to give T_1, T_2, T_3, T_4, T_5 and T_6 respectively. Algae oil was then added to the wall material 10 %, at ratio 1:10 was then blended with the above aqueous solution using an Ultra-Turrax (Ingenieurbüro CAT, M. Zipperer Gmbh, Germany) at 22.000 rpm for 1 min. Fine nano-emulsions were produced by ultra-sonication at 160 W power, 20 kHz frequency, with 50% pulse (Sonic Vibra Cell USA).

Droplet size and zeta potential measurement: Nano-emulsion was characterized using a Zetasizer NICOMP 380 ZLS Dynamic Light Scattering (DLS) instrument (PSS, Santa Barbara, CA, USA), using the 632 nm line of a He Ne laser as the incident light with angle 90° and Zeta potential with external angle 18.9°. To describe the mean globule size diameter and globule distribution in solution reported as polydispersity index (PDI) with the globule electrical charge (ζ-potential).

Transmission electron microscopy: Samples of nano-emulsion, algae oil were prepared for Transmission Electron Microscopy (TEM). The samples were diluted (1:100 v/v) with deionized water. A drop of the diluted suspension was placed on the format-coated electron microscopy grid, left for 1 min and then a drop of phosphotungstic acid solution (2% at pH 7.2) was added. The grid was air-dried and examined by TEM using a JEOL JEM-1400 plus TEM with an accelerating voltage of 100 kV at a magnification of 200,000 x[23].

Milk fat extraction and analysis: Lipids was extracted from the goat milk and fermented goat milk samples with chloroform and methanol as described by Herzallah *et al.*[24].

Chemical and physical properties of oils: Free fatty acids content (%) oleic acid, peroxide value (meq O_2 kg^{-1} oil), Anisidine value (meq kg^{-1} oil), iodine number and saponification value were carried out following the analytical methods described by AOAC[21].

Nano-encapsulated algae oil flavoured fermented goat milk preparation: The goat milk was heated at 85°C for 30 min, followed by cooling to 42°C, inoculated with 3% (v/v) of ABT-2 activated culture (10^5-10^6 CFU mL^{-1}) and incubated at 42±1°C for 3 hrs until a firm curd was obtained. Then, the curd was refrigerated at 4°C overnight before being blended with 13% of mandarin juice, 2% peel mandarin powder, 5% sucrose. Then divided into four portions, The first portion was kept as a control (GC), while three portions were fortified with 10, 20, 30% nanoemulsion, which contains 1, 2 and 3% algae oil respectively, to prepare GTA, GTB and GTC. The prepared nano-encapsulated algae oil flavoured fermented goat milk was immediately stored in a refrigerator at 5±1°C and their chemical, microbiological, rheological and sensory properties were evaluated during storage.

Viscosity: The apparent viscosity was measured in fresh products using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden) attached to a workstation loaded with V88 viscometer programming software. The viscometer probe, system C30, was placed in the nano-encapsulated fermented goat milk sample cup and measurements of viscosity were carried out at 20±2°C in the up mode at shear rates ranging from 19-578 sec^{-1} [13].

Chemical analysis: Total solids, total nitrogen, ash, pH, titratable acidity (%), lactose content, Total carbohydrates content and specific gravity were determined according to procedures described by the AOAC[21].

Microbiological analysis: Enumeration of *Streptococcus thermophilus* was carried out using a modified M-17 medium and that of *Lactobacillus bulgaricus* was done on a modified MRS medium. The plates were incubated at 37°C for 48 hrs. Pour plate techniques using a plate count agar medium incubated at 32°C for 48 hrs were used for the total bacterial count. The colonies were counted according to Marshall[25]. The identification of purified colonies was carried out according to Barrow and Feltham[26].

Sensory evaluation: Drinks were organoleptically scored when fresh by 9 panelists of the experienced staff members of Dairy Research and Technology Department, Food Technology Research Institute, ARC as follows: Flavour 50 points, appearance 25 points, color 25 points and overall acceptability 100 points[13].

Statistical analysis: The data presented as mean values±standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's test. The differences were considered significant at ($p \leq 0.05$). We used IBM SPSS Statistics 23 software program for statistical analyses [IBM Corp[27] IBM SPSS Statistics for Windows, Version 23.0. IBM Corp, Armonk, NY] with $p \leq 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Fatty acid composition: The identification of fatty acids of goat milk fat and microalgae oil using Gas-Liquid Chromatography apparatus were given in Table 1. From the obtained results, it could be noticed that algae oil is featuring as having a high content of unsaturated fatty acids (omega-3 fatty acids). Where, the major fatty acids in microalgae oil were omega-3 fatty acids EPA, DHA and DPA which represented 24.00, 35.70 and 5.37% of the total fatty acids, respectively. These results agree with[28,29], who reported that algae oil is a good natural source of long-chain n-3 fatty acids. On the other hand, goat milk fat was rich in saturated fatty acids. Where, palmitic, myristic and stearic acid were the most abundant saturated fatty acids in goat milk fat.

Regarding the results of the same table, it can be noted that goat milk fat does not contain omega fatty acids (C20:5 n3 EPA, C22:6 n3 DHA and C22:5 n3 DPA). While algae oil is characterized by containing a high percentage of these fatty acids that have health and nutritional benefits for the consumer, so goat milk has been fortified with these fatty acids using algae oil in the manufacture of flavoured fermented goat milk.

Preparation algae oil nanoemulsion: The parameters of dynamic laser scattering for droplet size, polydispersity index and surface charge ζ (zeta potential) of nanoemulsions prepared from WPI, NaCas and mix with different ratios was illustrated in Table 2. The emulsions with only WPI had a significantly smallest droplet size (77.74 nm) while the size is increased with increased NaCas ratio in emulsions, with only NaCas 89.03 nm. The small droplets size of NaCas is owing to colloidal texture, which has a high ability to bind oil and form smaller particles compared to whey proteins[14,30].

Table 1: Fatty acids composition of goat milk fat and algae oils

Fatty acids	Microalgae oil	Goat milk fat
C16:0	9.07	24.64
C16:1	1.57	1.11
C18:0	2.80	9.52
C18:1	8.28	21.87
C18:2	5.37	2.41
C18:3	7.84	0.99
C20:5 n3 EPA	24.00	-
C22:6 n3 DHA	35.70	-
C22:5 n3 DPA	5.37	-
TSFA	7.06	57.76
TUSFA	88.13	31.14

*Means with the same letter are not significantly different, GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Table 2: Particle size (nm), zeta potential and particle dispersity index (PDI) of algae oil nanoemulsion

Samples	Particle size (nm)	PDI	Zeta potential
T_1	77.74±4.4	0.217	-48.40±7.23
T_2	78.86±5.3	0.238	-44.80±5.57
T_3	79.93±4.9	0.318	-43.80±4.60
T_4	82.83±4.8	0.353	-41.70±5.03
T_5	83.09±5.2	0.382	-37.80±5.03
T_6	89.03±6.7	0.393	-36.50±6.20

*Means with the same letter are not significantly different, T_1: 10% WPI, T_2: 8WPI: 2 NaCas, T_3: 6WPI: 4 NaCas, T_4: 4WPI: 6 NaCas, T_5: 2WPI : 8 NaCas, T_6: 10% NaCas

The polydispersity index showed for all emulsions below 0.393, which indicated the small droplet size and narrow range distribution. The monomodal size distribution is due to the sufficient ultrasonication at 160 W power, 20 kHz frequency. The result obtained from droplet size and polydispersity index of nanoemulsions by WPI, NaCas and a mix of different ratios showed that NaCas had the utmost capability to make smaller, more uniform oil droplets compared to WPI. The ease of discussing the results ζ of nanoemulsion produced by WPI, NaCas and mix of different ratios refers to the negative charge of all emulsions samples. Also, an increase in surface charge refers to the increase in the negativity of the zeta potential. The zeta-potential explain changes in the stability of oil droplets in the medium around caused by changes in shell material WPI: NaCas. The nanoemulsions prepared by WPI shown the lowest zeta-potential, while the highest zeta-potential has appeared in NaCas emulsions. Also, showed an increase in zeta-potential with the increased ratio of NaCas in the emulsion shell. This finding agreed with the report by Loi *et al.*[31].

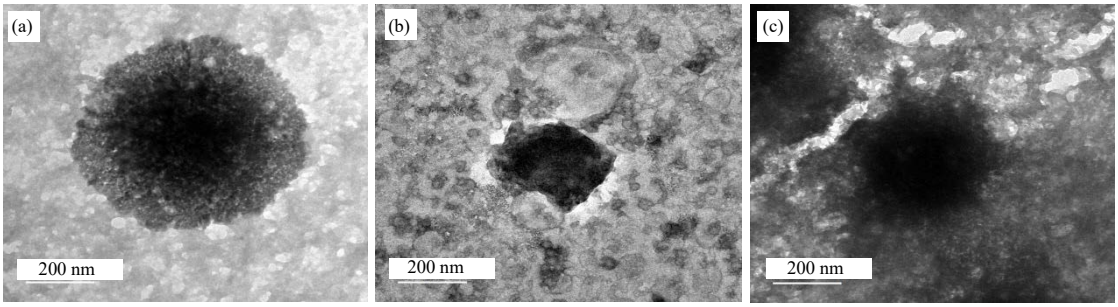

Fig. 1(a-c): Transmission electron micrographs of algae oil nanoemulsions prepared with (a) WPI, (b) WPI60:40 NaCas and (c) NaCas

Table 3: Physicochemical analysis of fermented goat milk

Treatments	Moisture (%)	Total carbohydrates (%)	Fat (%)	Protein (%)	Ash (%)
GC	82.25^{a}	10.13^{d}	3.32^{d}	3.20^{b}	0.76^{c}
GTA	82.02^{b}	10.43^{c}	4.02^{c}	3.45^{b}	0.78^{bc}
GTB	79.32^{c}	10.73^{b}	4.82^{b}	3.72^{a}	0.81^{ab}
GTC	78.33^{d}	10.93^{a}	5.65^{a}	3.85^{a}	0.84^{a}

*Means with the same letter are not significantly different ($p<0.05$), GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk + 1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Table 4: Changes in the pH and titratable acidity as citric acid or lactic acid (g/100 mL) of fermented goat milk during storage at 4±1°C

	Storage period (days) at 4±1°C							
	0		7		14		21	
Treatments	pH	TA	pH	TA	pH	TA	pH	TA
GC	4.52^{a}	0.89^{a}	4.50^{b}	0.89^{a}	4.44^{c}	0.92^{a}	4.28^{d}	0.94^{a}
GTA	4.54^{a}	0.88^{a}	4.51^{b}	0.89^{a}	4.46^{bc}	0.91^{a}	4.41^{c}	0.93^{a}
GTB	4.58^{a}	0.87^{a}	4.53^{b}	0.88^{a}	4.51^{ab}	0.89^{a}	4.49^{b}	0.92^{a}
GTC	4.61^{a}	0.86^{a}	4.57^{a}	0.87^{a}	4.54^{a}	0.89^{a}	4.52^{a}	0.89^{a}

*Means with the same letter are not significantly different, GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Morphology of nanoemulsion: After prepared the formulations of algae oil nanoemulsion was applied the morphology of nanoemulsion using Transmission Electron Microscopy (TEM). The result of TEM was done to emphasize the size of particles and inside assert the complete encapsulation of algae oil within WPI, NaCas and a combination of ratios as shell materials (Fig. 1). The Sample's apparent particle size diameter is nearly 90 nm, this was consistent with the particle size of the dynamic laser scattering. The morphology of emulsions particles was spherical with slight variation. Mohammed *et al.*[32], reported that the image of the transmission electron microscope for Nigella sativa oil emulsion had a globular form with a size of nearly 100 nm. Furthermore, the morphological of NaCas emulsions manifested dark and the environment circumference is bright indicating a spindle-shaped asymmetrical shape. This may be due to the collapse of the globule when the nano-emulsion is deposited on the TEM copper grid coated with a Holly carbon film[33].

Characterization of fermented goat milk: The fermented goat milk samples were determined with some physio-chemical, microbiological and rheological properties and sensory evaluation. Three replicates were done for every treatment and the obtained data were statistically analyzed.

Physicochemical analysis of fermented goat milk was determined and the results were recorded in Table 3. The data shows that there were significant differences in moisture content (%), total carbohydrates (%) and fat (%) of GC and all other treatments the highest significant moisture content was recorded in GC (82.25%) and the least moisture content was in GTC (78.33%). Total carbohydrates, Fat, protein and ash content were high in GTC (10.93, 5.65, 3.85 and 0.84%, respectively). While, the least total carbohydrates, fat, protein and ash content were in GC (10.13, 3.32, 3.20 and 0.76%, respectively).

The treatments were examined for their pH and acidity (%) during the storage period Table 4. Titratable acidity (TA %) slightly increased whereas pH values decreased as the

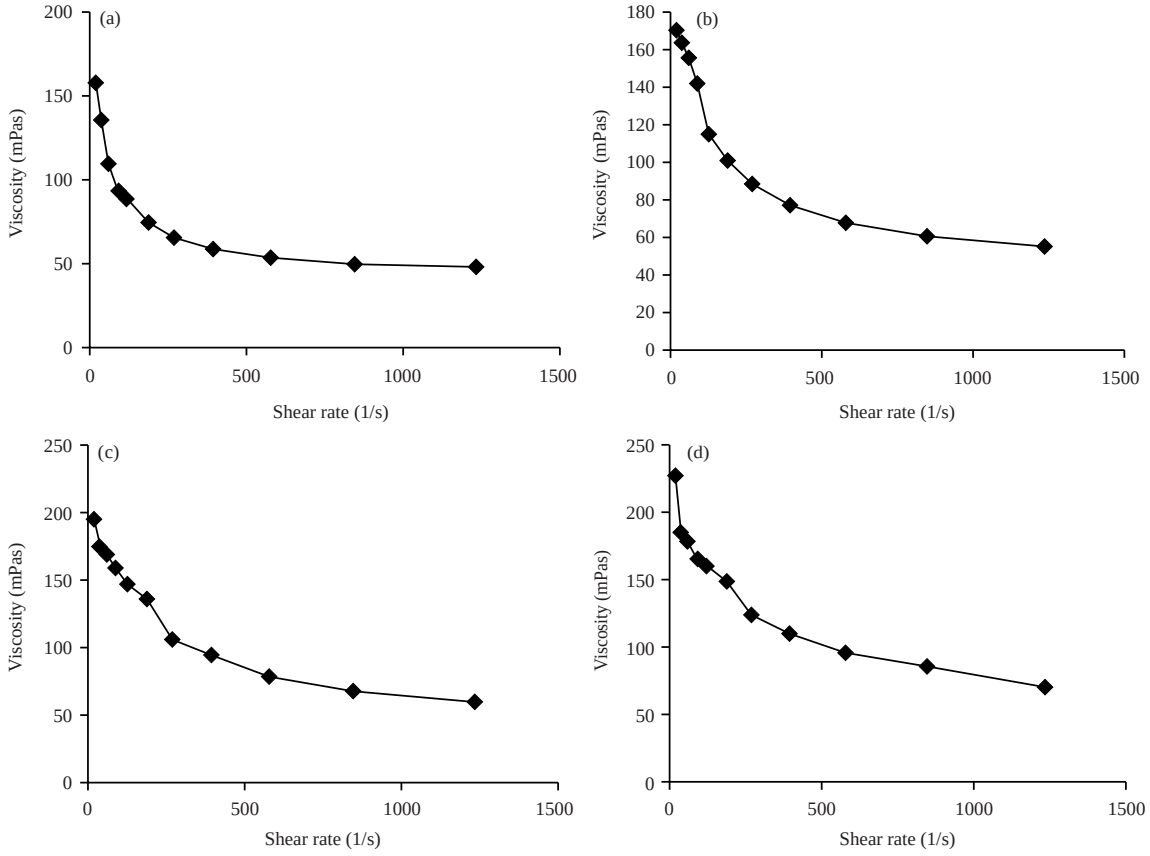

Fig. 2(a-d): Viscosity of flavoured fermented goat milk, (a) Control (GC), (b) 1% algae oil (GTA), (c) 2% algae oil (GTB) and (d) 3% algae oil (GTC)

storage period progressed in all treatments due to lactose hydrolysis during the storage by Lactic Acid Bacteria (LAB) and lower pH with a subsequent increase in organic acid production (acid lactic) this result agrees with[34,35]. The pH and acidity of treatments were in the range (4.52-4.61), (0.86-0.89 at zero time and after 21 days of storage were (4.28-4.52), (0.89-0.94), respectively.

Viscosity: The apparent viscosity of fermented goat milk fortified with nanoemulsion of Algae oil was illustrated in Fig. 2. Fermented goat milk displays a complex, shear-thinning, time-subject ow behavior and fermented goat milk viscosity is subsequently very important for processing, handling, process design, product development and quality control aspects[36]. The viscosity of fermented goat milk (control) was the lowest of the samples compared with fermented goat milk fortified with algae oil nanoemulsion and the viscosity increased with the increasing addition of Algae oil nano-emulsion. Darwish *et al.*[13] found the yogurt enrichment with nanoparticles recorded the highest values of apparent viscosity comparing to control.

Microbiological properties: The survival of the LAB strains analysis during the fermented goat milk storage was summarized in Table 5. Lactic acid bacterial counts gradually increased significantly by increasing the storage period in all treatments until 7 days of the storage followed by a gradual decrease until the end of the storage. Microbial growth during cold storage was affected by formulation. Whereas microbial growth increased gradually throughout storage in GC and other treatments, a different pattern was observed in formulations containing nanoemulsion algae oil. Significant increases in *B. bifidum* and *Str. thermophilus* levels in treatments were only observed after 7 days, with no significant changes with increasing storage time. *Lb. acidophilus*, *B. bifidum* and *Str. thermophilus* viability decreased markedly; however, the cell count was still above the minimal count (10^6 cfu/mL after 21-day storage). *S. thermophilus* presented significantly higher viability in GTB and GTC treatments compared to its viability in the other treatments after 21 days.

Algae oil had a positive effect on *Lb. acidophilus*, *B. bifidum* and *Str. thermophilus* survival during storage

probably due to the presence of amino acids and other compounds in algae oil. Also, sulfur amino acid release during heat treatment of whey could lower the redox potential leading to a beneficial effect on *Lb. acidophilus*, *B. bifidum*, and *Str. thermophilus* survival. The obtained results agreed with these reported by Akalin *et al*., 43.

In this experiment, the highest microbial counts after 21 days were recorded in the GTB and GTC. Current finding also illustrated that the algae oil nanoemulsion flavuored fermented goat milk can serve as a vehicle for delivering lactic acid bacterial (LAB) cells to the consumers since cells maintained high viability after 21 days of storage period.

As a result of high hygienic conditions during manufacturing and storage in addition to high total carbohydrates content. Also, LAB addition resulted in increased lactose hydrolysis during the storage of fermented milk, with a subsequent increase in organic acid production (lactic acid) and lower pH and creating unfavorable conditions for the development of other microorganisms which can provide protective effects against foodborne pathogens. Total plate count and molds and yeasts were low ranging ($<10^3$) and (<10), respectively. Coliforms were not detected in all treatments when fresh and throughout the storage period Table 6.

Sensory properties: Probiotic cultures have been utilized to enhance the sensory acceptance of goat fermented milk[6]. In particular, specific probiotic bacteria release volatile compounds as a result of their metabolism, which are known to play a major role in the aroma profile and sensory aspects of the final products. Additionally, fermentation with certain bacterial strains was reported as an effective method to reduce caprylic, capric and caproic acids and inhibit the production of goaty flavour [40]. The results of sensory evaluation of flavoured fermented goat milk were shown in Table 7. All fermented goat milk samples presented satisfactory acceptability these might be due to the fermentation processing compounds besides the addition of mandarin juice with peels, which offers a pattern of potential for improving the flavour of goat milk-based products.

Fermented goat milk GC was more acceptable than GTB and GTC treatments. GTA obtained the highest score among all treatments.

Effect of processing fermented goat milk fortified with algae oil nanoemulsion on fatty acid: The influence of adding different amounts of free algae oil 1, 2 and 3% and algae oil nanoemulsions 10, 20, 30 mL which containing 1, 2 and 3% algae oil to flavoured fermented goat milk on some chemical characteristics of fat extracted from all samples of flavoured fermented goat milk were shown in Table 8. The chemical properties of fat in different flavoured fermented goat milk treatments were evaluated at the beginning of the experiment and after 3 weeks.

The obtained data revealed that the values of acidity, Peroxide Value (PV), saponification value and unsaponifiable matter of milk fat extracted from fermented goat milk samples

Table 5: Viability (log CFU mL^{-1}) of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidium* in fermented goat milk after the fermentation process and during storage at 5±1°C

	Storage period (days)			
Treatments	0	7	14	21
Lactobacillus acidophilus				
GC	7.0000^{a}	7.9100^{a}	7.5900^{a}	7.4000^{a}
GTA	7.1800^{ab}	8.4300^{b}	8.1200^{b}	7.4100^{a}
GTB	7.3500^{bc}	8.4800^{b}	8.1800^{b}	7.5200^{a}
GTC	7.5900^{c}	8.5100^{b}	8.2000^{b}	7.7700^{a}
Bifidobacterium bifidum				
GC	6.000^{a}	6.9500^{a}	7.1300^{a}	7.1100^{a}
GTA	6.1000^{ab}	7.4000^{b}	7.3200^{ab}	7.1200^{a}
GTB	6.2600^{ab}	7.8800^{c}	7.5300^{b}	7.1800^{a}
GTC	6.4600^{b}	8.8300^{d}	8.4000^{c}	7.3000^{a}
Streptococcus thermophilus				
GC	8.1000^{a}	8.8100^{a}	8.6700^{a}	8.6500^{a}
GTA	8.2300^{a}	9.4800^{b}	9.4300^{b}	8.7500^{a}
GTB	8.5000^{b}	9.7200^{c}	9.6400^{bc}	9.4900^{b}
GTC	8.6800^{b}	9.9900^{d}	9.7600^{c}	9.6200^{b}

*Means with the same letter are not significantly different, GC: Flavoured fermented goat milk; GTA: Flavoured fermented goat milk +1% algae oil; GTB: Flavoured fermented goat milk + 2% algae oil, GTC: Flavoured fermented goat milk + 3% algae oil"

Table 6: Microbiological analysis (CFU mL^{-1}) of fermented goat milk during storage at 5±1°C

		Storage period (days)				
Treatments	Microbiological test	0	7	14	21	28
GC	Yeast and mold	<10	<10	<10	<10	<10
	Coliform group	<10	<10	<10	<10	<10
GTA	Yeast and mold	<10	<10	<10	<10	<10
	Coliform group	ND	ND	ND	ND	ND
GTB	Yeast and mold	<10	<10	<10	<10	<10
	Coliform group	ND	ND	ND	ND	ND
GTC	Yeast and mold	<10	<10	<10	<10	<10
	Coliform group	<10	<10	<10	<10	<10

ND: Not detected, GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Table 7: Sensory evaluation of fermented goat milk

Treatments	Flavour 50	Appearance 25	Color 25	Over all acceptability 100
GC	47.5^{a}	24^{a}	22.5^{a}	94^{a}
GTA	48^{a}	23.5^{a}	23^{a}	94.5^{a}
GTB	46^{b}	23^{a}	23.5^{a}	92.5^{b}
GTC	45^{b}	23^{a}	24^{a}	92^{b}

*Means with the same letter are not significantly different, GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Table 8: Chemical composition of milk fat extracted from flavoured fermented goat milk during storage at 4±1°C

Treatments	Chemical characteristics				
	FFA (as oleic acid %)	PV (meq O_2 kg^{-1} oil)	Anv (meq kg^{-1} oil)	Iodine value (gI2/100 g oil)	Saponification value (mg KOH g^{-1}) fat
Zero time					
GC	0.85	4.20	1.5	23.25	130.319
GTA	0.88	4.36	1.65	33.33	139.885
GTB	0.97	4.42	1.70	34.43	149.981
GTC	1.02	4.54	1.76	35.66	150.763
After 21 days					
GC	0.98	4.54	2.25	23.32	130.35
GTA	1.01	4.65	2.5	33.35	139.98
GTB	1.25	4.82	2.62	34.62	150.35
GTC	1.54	4.92	2.70	35.85	151.23

GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

Table 9: Effect of addition algae oil on the saturated fatty acids of nano-encapsulated flavoured fermented goat milk

Fatty acids (g 100 g^{-1})	Goat milk fat	GC	Algae oil	GTA	GTB	GTC
C4:0 butyric	2.3	1.98	-	1.35	1.21	1.02
C6:0 caproic	2.8	2.20	-	1.32	1.25	1.21
C8:0 caprylic	2.92	2.25	-	2.15	2.10	1.58
C10:0 capric	9.27	9.26	-	8.22	6.20	5.12
C12:0 lauric	4.65	4.00	-	3.90	2.98	2.97
C14:0 myristic	10.05	9.15	-	6.12	6.10	6.09
C15:0	0.78	0.74	-	0.65	0.70	0.74
C16:0 palmitic	24.64	26.83	9.07	23.45	22.25	21.20
C18:0 stearic	9.52	10.92	2.8	8.98	8.28	6.35
C20:0 arachidic	0.10	0.24	-	0.24	0.23	0.23
TSFA	57.76	67.57	11.87	56.38	51.3	46.51

GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

were inside the range reported by[41] for fermented milk products which limited that the levels of acidity, peroxide value and saponification number mustn't exceed than 0.2% as oleic acid, 10 meq O_2 kg^{-1} oil and 189-195 mg KOH g^{-1} oil, respectively.

The anisidine value is used to measure the level of aldehydes, principally 2-alkenals and 2,4-alkadienals, formed in oils as a result of the oxidation process[42].

Conversely, after 4 weeks, a very slight increase occurred as a result of peroxide value and anisidine value for flavoured fermented goat milk fat extract. These results are in harmony with those reported by Xing Huimin *et al.*[43], who found no significant increase in peroxide levels and AnV during storage of the algae oil-supplemented milk samples for 4 weeks. Also, Naohiro and Shun[44] reported that the peroxide number (PV) of the nanoemulsion fish oil in yoghurt recorded a value of less than 5 meq O_2 kg^{-1} oil until 5 weeks later. Due to the low level of consumption of seafood in the human diet, which is a rich source of omega-3 fatty acids and milk, is relatively poor in these essential fatty acids. The effect of adding algae oil as a cheap source and rich in omega fatty acids to flavoured fermented goat milk on the fatty acid composition was studied to produce fermented goat milk rich in omega fatty acids and the obtained results were given in Table 9 and 10.

From the obtained data in Table 9 and 10 it could be noticed that palmitic, myristic and stearic acid were the most abundant saturated fatty acids in goat milk fat. Regarding the effect of the fermentation process on the fatty acid composition of goat milk fat, there are decreases in the amount of butyric, caproic, caprylic, capric, lauric and myristic acids, while there are increases in palmitic, stearic and arachidic acids in fermented goat milk fat compared with fresh goat milk fat. Also, there was an increase in total unsaturated fatty acids especially oleic acid and Conjugated Linoleic Acid (CLA) content during fermentation process. Abbas *et al.*[45], who reported that the fermentation process increased Conjugated Linoleic Acid (CLA) content of goat milk from 3.09 mg g^{-1} fat in fresh milk to 3.26 mg g^{-1} fat in fermented milk.

The impact of the addition of algae oil at different ratios 1, 2 and 3% on saturated fatty acids of fermented goat milk and the data were presented in Table 9. Data showed that with increasing addition of algae oil from 1-3% there is a decrease in total saturated fatty acids from 56.38-46.51%

Table 10: Effect of addition algae oil on the unsaturated fatty acids of flavoured fermented goat milk

Fatty acids (g 100 g^{-1})	Goat milk fat	Algae oil	GTA	GTB	GTC
C15:1 pentadecenoic	1.22	-	1.20	1.15	1.15
C16:1 palmitoleic	1.11	1.57	1.28	1.30	1.32
C18:1 cis-9c, oleic C18:1n9c	21.87	8.28	23.96	23.75	22.15
C18:1 n-9t, oleic	2.6		2.41	2.40	2.25
C18:2 cis-9, cis-12, Linoleic C18:2n6c	2.41	5.37	3.67	2.82	2.88
C18:2 cis-9, t-11, CLA	0.62	0.59	0.88	0.86	0.85
C18:3 n-3 cis-9,cis-12,cis-15, α-Linolenic	0.99	7.84	1.02	1.85	2.09
C18:3 n-6 gamma Linolenic	0.32		0.32	0.30	0.30
C20:5 n3 EPA	-	24.00	2.4	4.8	7.02
C22:6 n3 DHA	-	35.70	4.57	7.14	10.71
C22:5 n3 DPA	-	5.37	0.537	1.074	1.611
TUFA	31.14	88.13	42.247	48.518	52.331
PUFA	4.34	78.28	13.397	18.844	25.461
MUFA	26.8	9.85	28.85	28.6	26.87
*Atherogenicity index	2.2315	0.226	1.2268	1.0229	0.927

*Calculated according to Ulbricht and Southgate (1991): Atherogenicity index: (C12:0+4×C14:0+C16:0)/∑ of total unsaturated fatty acids. GC: Flavoured fermented goat milk, GTA: Flavoured fermented goat milk+1% algae oil, GTB: Flavoured fermented goat milk+2% algae oil, GTC: Flavoured fermented goat milk+3% algae oil

compared with the control sample (fermented milk without addition algae oil). Estrada *et al.*[46], studied the fatty acid composition of strawberry yogurt supplemented with nano-encapsulated fish oil. They noticed that the addition of fish oil led to an increase palmitic, stearic and oleic acids and a small increment in linoleic acid and a decrease in linolenic acid.

From the results in Table 10, it could be noticed that total unsaturated fatty acids of flavoured fermented goat milk samples achieved a higher increase by increasing the amount of algae oil addition, especially polyunsaturated fatty acids (omega-3 fatty acids EPA, DHA and DPA). One of the most important results observes by using algae oil at the ratio of 3% in this study was the greater increase inn-3 PUFAs (EPA, DHA and DPA were 7.02, 10.71 and 1.61%, respectively) compared to the control sample (0%). On the contrary, increasing the percentage of algae oil in the fermented goat milk samples led to a decrease in the Atherogenicity index (AI) values. This has a positive and protective effect on cardiovascular disease for consumers of fermented goat milk enriched with algae oil.

CONCLUSION

The WPI and NaCas had been able to bind and emulsification of algae oil with good polydispersity and the lowest surface charge. Also, concluded that the algae oil-enriched fermented goat milk is a stable and acceptable quality during 21 days of storage. Increased in total unsaturated fatty acids of flavoured fermented goat milk samples with addition algae oil nanoemulsion, especially polyunsaturated fatty acids (omega-3 fatty acids EPA, DHA and DPA) and decrease in the Atherogenicity Index (AI) values. These effects are very beneficial from the point of view of human nutrition for the prevention of cardiovascular disease for fermented goat milk consumers.

SIGNIFICANCE STATEMENT

This study discovers the algae oil can be emulsified with NaCas and WPI. Also, the first time using algae oil nanoemulsion as the main source of omega-3 fatty acids EPA, DHA and DPA in flavoured fermented goat milk beverage, with stability and good functional properties during manufacture conditions and storage period. Thus it could be concluded that flavoured fermented goat milk containing algae oil nanoemulsion was sensory acceptable quality.

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