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FUNCTIONAL RICOTTA CHEESE WITH *DUNALIELLA SALINA* ALGAE EMULSION

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ABSTRACT

Microalgae are a remarkable source of essential biomolecules with several uses that may be exploited for commercial applications. The objective of the present work was to prepare a novel Ricotta cheese supplemented with *Dunaliella salina* algae emulsion formula containing mint essential oil (mint EO) as a functional food ingredient. Total phenolic compounds (TP), total flavonoids (TF), DPPH antioxidant capacity, and identification of phenolic and flavonoids compounds of algae were determined. Moreover, the chemical, physical, rheological, microbiological, organoleptic characteristics, and chemical score of the resulted Ricotta cheese were investigated. The results showed that TP, and TF contents in *D. salina* algae crude extract are 8.48 (mg GAE/g DW), and 5.93 (mg QE/g DW), respectively. Also, *D. salina* has high levels of phenolic acids (e.g., gallic acid, chlorogenic acid, caffeic acid, and rosmarinic acid); as well as high flavonoid content (e.g., catechin, naringenin, quercetin, rutin, and hesperetin). The DPPH procedure showed that *D. salina* algae antioxidant activity is 42.19±0.39 mg/ml as IC_{50} value. The amino acids profile revealed that *D. salina* algae contains 17 amino acids with the highest value of threonine, as well as total amino acid (TAA) is 949.69, which contains 424.2 essential amino acids (EAA) with a 0.447 TAA/EAA ratio. However, *D. salina* algae/Mint EO emulsion was prepared using a non-ionic surfactant (Tween 80). Ricotta cheese supplemented with an algae emulsion formula 0.25%(I), 0.5%(II), and 1.00%(III) showed gradual adequate free radical scavenging ability as the algae level increased. The incorporation of *D. salina* algae emulsion into Ricotta cheese processing led to an increase in their protein, fat, and mineral levels compared to control cheese. Also, all color attributes were decreased for algae Ricotta cheese in comparison to the control sample. Texture profile of cheese showed that algae Ricotta cheese had lower hardness, gumminess, and chewiness than control cheese with opposite trends for other textural parameters; while no significant ($p \leq 0.05$) changes in adhesiveness, cohesiveness and springiness parameters were recorded after 21 days of storage at 5 °C. The total bacterial counts were significantly ($p \leq 0.05$) lower in the algae cheese samples than the control. The yeast and mold count in control cheese appeared after 14 days, whereas it didn't appear in II and III algae Ricotta cheese until day 21 of storage. However, the addition of an algae emulsion formula improved all sensorial attributes of Ricotta cheese during storage at 5 °C for 21 days, especially at the level of 0.5% (II), and the same level of algae emulsion led to an increase in most amino acid content, and the chemical score of amino acids (e.g., methionine + cysteine, histidine, and isoleucine) compared to control cheese.

1.Introduction

The global consumption of cheese is increasing continuously and is predicted to rise by ~13.5% between 2016 and 2025 (OECD/FAO, 2016). However, the cheese industry produces approximately ~145 million tons of whey annually which represents an environmental issue (Ganju and Gogate, 2017). Recent research has focused on remanufacturing cheese whey, a well-known unconventional protein source, into different food products to address the societal dilemma of food and protein scarcity to sustain human population growth. Ricotta cheese, which is primarily made from cheese whey, is a creamy, soft, un-ripened cheese with a high moisture content. It also has important nutritional, health, and functional features. Traditionally prepared by heat-induced coagulation of whey protein and adding acetic, citric, or lactic acid to the heated whey to reach a pH of ~5. The flavor of fresh ricotta cheese is nutty, mild, and pleasant flavor (Camerini *et al*., 2016; Rubel *et al*., 2019; Nzekoue *et al.*, 2021; Hesarinejad *et al*., 2021). The whey proteins in Ricotta cheese have a high biological value because they contain a large amount content of sulfur-containing amino acids (Smithers, 2008). Fresh ricotta is used extensively around the world due to its nutritional advantages. The increasing awareness among consumers regarding the significance of diet for health and overall well-being has led food scientists and cheese manufacturers to focus on enhancing the quality of their current products or creating novel and innovative ones. (Lamichhane *et al*., 2018). Many natural components, such as dietary fiber, natural antioxidants, vitamins, etc., can be used to boost Ricotta cheese into a functional product. (Siyar *et al*., 2022). Consumers are often looking for dairy products with new flavors in addition to their high nutritional value. Many consumers continued to enjoy exploring new flavors and combinations with recognizable and approachable formats and flavors during the COVID-19 pandemic, such as key lime-flavored yoghurt or barbecue flavored unripe firm cheese. (Falcão *et al*., 2023). The addition of essential oils with their characteristic flavor and microalgae with their potent health benefits to a traditional product like cheese conforms to this trend. Microalgae are a remarkable source of essential biomolecules with several uses that may be exploited for commercial applications. Thus, the use of microalgal biomass resources presents a resource-efficient and ecological way to utilize algal metabolism to synthesize and accumulate a wide range of molecules of industrial interest and high value-added products including proteins, lipids, vitamins, carotenoids, pigments, and other bioactive compounds (Becker, 2007; De Jesus Raposo *et al*., 2013; Sui and Vlaeminck, 2019).

Dunaliella salina (Dunaliellaceae), one of the few species of green microalga that may naturally flourish in very salty media. It synthesizes extreme amounts of carotenoids, especially β-carotene and zeaxanthin beside lutein and astaxanthin (El-Baz *et al*., 2020a; El-Baz and Ali, 2023). In addition to carotenoids, *D. salina* produce remarkable amounts of polyphenolic compounds, including caffeic acid, vanillic acid, gallic acid, coumarin, and vanillin (Simon *et al*., 2015). Different carotenoids, in particular the lipid-soluble orange pigment β-carotene, are utilized as coloring constituents in food and feed thanks to their strong antioxidant and free radical scavenging efficiency (Cakmak *et al*., 2014). *D. salina* had remarkable anti-inflammatory, hepatoprotective, and antioxidant therapeutic benefits (El-Baz *et al*., 2020a) and counteractive agent in liver fibrosis (El-Baz *et al*., 2019, 2020b). Different epidemiological findings advised that daily consumption of enough amounts of natural antioxidants like phenols and carotenoids could potentially lower the likelihood of cardiovascular complications and inhibit the growth of different cancer cells (Sheu *et al*., 2008; Singh *et al.*, 2016; Gaafar *et al*., 2020). Nonetheless, the US Food and Drug Administration (FDA) has designated Dunaliella as a form of microalgae with Generally Regarded as Safe (GRAS) classification. It is primarily utilized in various applications, producing proteins with excellent quality and digestibility. It is primarily utilized in various uses, producing proteins with

excellent quality and digestibility (Amaya *et al*., 2014).

According to our hypothesis, using cheese whey to make Ricotta cheese will assist to reduce the environmental problem of excessive cheese whey waste, while incorporating new functional microalgae *Dunaliella salina* into such cheese will satisfy customer desire for highly nutritive and therapeutic dairy product. As a result, the main goal of this research is to develop a novel functional Ricotta cheese enriched with *Dunaliella salina* microalgae emulsion formula which includes mint essential oil (mint EO). The chemical composition and antioxidant characteristics of Ricotta cheese and *Dunaliella salina* microalgae were determined. Additionally, the physical, rheological, microbiological, organoleptic characteristics, and chemical score of the developed Ricotta cheese were examined.

2.Materials and methods

2.1. Materials

The *D. salina* pulverized biomass utilized in this investigation was secured from the National Research Center in Cairo, Egypt by the Algal Biotechnology Group at the New Central Laboratories Network. Mint essential oil (Mint EO) was obtained from the production and marketing of medicinal plants and their extracts unit, National Research Centre, Egypt. Sweet whey was got from the dairy plant at the Faculty of Agriculture, Cairo University, Giza, Egypt. Commercial fine grade salt (Sodium-chloride, NaCl) was obtained from the Saudi-Egyptian company for salt and minerals (SECOASALT), 10th of Ramadan, Egypt. Citric acid was obtained from SRL-INDIA. Gallic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Tween 80, standard amino acids, and Folin-Ciocalteau phenol reagent were purchased from Sigma Aldrich (St. Louis, Missouri, USA). All other chemicals were used in analytical grade.

2.2.Methods

2.2.1.Preparation of D. salina extract

Two grams of pulverized *D. salina* were sonicated (5 sec. on, 50 sec. off, for 30 min) in 40 ml of a water/methanol/formic acid (28:70:2, v/v/v) mixture (Martini *et al*., 2019), using a Prop Ultrasonic (Vibra Cell (VCX750), USA) with 750 Watts power and 20 KHz frequency. The mixture was centrifuged (5000 xg, 20 min, 4 °C). The resultant supernatant was completed to 50 ml with the same solvent solution.

2.2.2.Total phenolic content (TP) assessment

Using the Folin Ciocalteu reagent test and our previously reported procedures, the TP of the crude extract was assessed (Ali *et al*., 2018). In a 25 ml volumetric flask, the extract (1 ml), distilled water (9 ml), and Folin reagent (1 ml) were mixed well. Then 7 % Na2CO3 (10 ml) was added to the mixture after 5 min. The mixture was completed to 25 ml with distilled water, mixed well, and incubated at room temperature for 90 min. A spectrophotometer (Unicum UV 300) was used to measure the absorbance at 750 nm against the blank (all reagents without the sample). The sample's TP was reported as mg Gallic acid equivalents (GAE)/g DW. Every sample was assessed three times.

2.2.3. Total flavonoid content (TF) assessment

The aluminum chloride method was used for the determination of total flavonoid (TF) content in the crude extract following our former published methods (Ali *et al*., 2018). In a volumetric flask, the extract (1 ml), distilled water (4 ml), and 5 % NaNO2 (0.3 ml) were mixed well. After 5 min, 10 % AlCl3 (0.3 ml) was added. Following an additional six minutes, 2 milliliters of 1M NaOH were added, and then the mixture was completed with distilled water to a final volume of 10 ml. A spectrophotometer (Unicum UV 300) was used to measure the absorbance at 510 nm against the blank (all reagents without the sample). The sample's TF was reported as mg quercetin equivalents (QE)/g DW. Every sample was assessed three times.

2.2.4. Identifying phenolic ingredients

For fifteen minutes, a 10 mg sample of *D. salina* extract was vortexed in 2 mL of HPLC spectral grade methanol. A 0.2µm Millipore membrane filter was used to filter this extract, and its volume was set at 2 mL, and 5 µl of it was injected into an HPLC (Agilent Technologies 1260 series, Germany) with an auto-sampling injector. To separate phenolic

ingredients, C8 column (ZORBAX Eclipse Plus, 4.6 mm x 250 mm i.d., 5 µm) was utilized. The temperature in the column was kept constant at 40°C. At a flow rate of 0.9 ml/min, the mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The following mobile phases were programmed in a linear gradient: 0 min (82% A); 0-1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). At 280 nm, the multi-wavelength detector was observed (Goupy *et al.*, 1999). The relative retention durations of phenolic compounds were compared to those of the reference mixture chromatogram. The peak area measurements were used to compute the concentration of each phenolic component (µg/g DW).

2.2.5. Determination of antioxidant activity

DPPH• radical scavenging test

After preparing the DPPH• (0.1 mM) in methyl alcohol, 1 ml of this solution was added to 100 mg/ml of Ricotta cheese or 2 ml of *D. salina* extract at concentrations ranging from 1 to 7 mg/ml. After giving the mixture a good shake, it was left to remain at room temperature in the dark for half an hour. The absorbance was then measured at 515 nm (Gaafar *et al.*, 2020). The control sample comprised all reagents except the extract. Using the following formula, the ability to scavenge the DPPH• radical was determined:

DPPH• scavenging effect (%) = (Ac- As / Ac) × 100

Where Ac is the absorbance of the control sample and As is the absorbance of the tested sample. The results were represented as IC50 (the extract's concentration (in mg/ml) that scavenges 50% of the DPPH• radical).

2.2.6. Amino acids profile

The amino acids profile of *D. salina* algae powder and Ricotta cheese samples were determined using HPLC-Pico-Tag method according to Cohen *et al.*, (1989). The Pico-Tag method was developed commercially by Waters Associates which was an integrated technique for amino acids analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamyl (PTC) derivatives, which were detected by their UV absorbance. The Liquid chromatography apparatus was equipped with 600 E Multisolvent Delivery System and the following gradient of Pico-Tag solvent A and B (Waters Eluent A and B) at 38 °C, flow rate 1 ml/min. Twenty microliter of sample was injected and loaded on amino acids column Pico-Tag amino acids (150 x 3.9 mm, stainless steel) using linear gradient elution. Detection of the PTC derivatives is by ultraviolet absorption measurements using a fixed wavelength 254 nm (2489 UV/Vis Detector).

2.2.7. Preparation of algae emulsion formula

The algae emulsion formula was prepared by mixing 1.5% *D. salina* algae powder, 1.5% Mint EO, and 0.1% Tween 80 with distilled water using high speed homogenizer (Ingenieurbüro CAT, M. Zipperer GmbH) for 15 min at 25000 rpm/min. The mixture was sonicated using ultrasonicator (ULTRASONIC Get 750) micro tip probe of 400 watt with amplitude of 40% for 30 min. During sonication, the temperature of algae emulsion formula was maintained at 25°C by cooling in an ice bath. The resulted emulsion was stored in sterile containers at 5 °C until further analysis.

2.2.8. Characterization of algae emulsion formula

The algae emulsion formula was assessed by Transmission Electron Microscopy (TEM, JEM-1230, JEOL) to evaluate the structure and surface morphology of the prepared emulsion. Also, the particle size of the resulted algae emulsion was performed and expressed as intensity particle size distribution and average particle size at 20 °C using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK) using dynamic light scattering technique.

2.2.9. Preparation of Ricotta cheese

Sweet whey collected after the production of Mozzarella cheese from cow milk, was heated up to 85 – 90°C, thereafter, citric acid (25 ml/L) was added followed by the addition of 1 % NaCl to the mixture and a gentle stirring was provided. Following coagulation, the cheese curd was formed and left in the whey for 10-15 min and

then scooped in a mold lined with muslin filter to separate the whey and allowed to attain complete drainage in 24 hr. The curd was divided into four parts. The first part was considered as a control Ricotta cheese without any additives. *D. salina* algae/Mint EO emulsion was added at the ratio of 0.25, 0.5 and 1.0% (w/w) to second, third, and fourth portion and mixed well to prepare (I), (II), and (III) Ricotta cheese with algae emulsion formula, respectively. Three replicates were done for each treatment. All Ricotta cheeses were packaged in a plastic container (100 g) and stored in refrigerator at 5±1°C for 21 day. The samples were analyzed fresh and after 7, 14, and 21 days of cold storage.

2.2.10. Cheese chemical analysis

Moisture, fat, total protein, ash, and titratable acidity were analyzed according to the method described in AOAC (2012). The pH value of cheese was measured electrometrically using Lab. pH meter with a plastic electrode, JENWAY 3510 digital pH meter. Minerals content was estimated as described by Hankinson (1975) using atomic absorption spectrophotometer No. 3300 (PerkinElmer, US instrument Division Norwalk, CT, USA).

2.2.11. Cheese texture profile analysis

Texture profile analysis was determined using the (TMSPro testing machine) equipped with (250 lbf) load cell and linked to a computer programmed with ProTM texture analysis software. Texture of cheese samples was evaluated in triplicate for each batch of cheese at room temperature (±2ºC). Cheese samples were analyzed through a storage period; they were measured in a 25-ml cup, and two-bite compression experiments with a flat cylinder probe (25 mm diameter) were applied to each sample. All texture profile analysis (TPA) measurements are performed with two cycles of uniaxial compression tests which generated a plot of force (g) vs. time (s). Compression and release of the samples and the force exerted on the probe was automatically registered. The data obtained from the force decompression curve was used to calculate the maximum and residual force, while the data got from the TPA curve was used for the calculation of textural parameters. The parameters simulating involved hardness (the peak force through the first compression cycle), adhesiveness (the negative force area of the first compression cycle), cohesiveness (the ratio of the positive force area through the second compression cycle to that during the first compression cycle) and springiness (the length to which the sample recovers in height during the time that terminates between the end of the first compression cycle and the start of the second compression cycle).

2.2.12. Color development of cheese

Color parameters of Ricotta cheese during storage for 21 days were performed using Hunter colorimeter (model D2s A-2, Hunter Assoc. Lab., Virginia, USA) tri-stimulus values of the color namely l*, a* and b* as described by Hunter and Harold (1987).

2.2.13. Cheese microbiological analysis

The total bacterial count was enumerated using plate count agar (Oxoid), incubated at 37 °C for 48 h as a method recorded by Laird *et al*., (2004). Coliform was counted using violet red bile agar (VRBA) at pH 7.0-7.2, 37˚C for 48 hrs as described in Hitchins (1992). Yeast and mold were detected according to IDF (1990).

2.2.14. Organoleptic evaluation of cheese

Sensorial attributes of Ricotta cheese for 21 days of storage were evaluated as described by Pappas *et al*., (1996). Cheese samples were evaluated by means of regular panelists from the staff of the Dairy Sciences and Technology Research Department (Food Technology Research Institute, Giza, Egypt), with a maximum score of 50 points for flavor, body and texture (40 points) and cheese appearance (10 points).

2.2.15. Chemical score of cheese

The chemical score, protein efficiency ratio and biological value of Ricotta cheese samples were calculated based on their amino acid content according to Bhanu *et al*., (1991), as follows:

Chemical score = (mg of amino acid in 1g test protein/ mg of amino acid in 1g reference protein) × 100

2.2.16. Statistical analysis

The results average values were analyzed by SAS software (SAS Institute, Cary, North

Carolina, USA) using ANOVA procedure for analysis of variance. The results were expressed as mean ± standard error and the differences between means were tested for significance using Duncan's multiple range at $p \leq 0.05$.

3.Results and discussion

3.1. Algae phenolic and flavonoid contents

The result showed that the total phenolic and total flavonoid contents of *D. salina* crude extract are 8.48±0.11 (mg GAE/g DW) and 5.93±0.40 (mg QE/g DW), respectively (Table 1). The total phenolics of the *D. salina* extract in the current research is consistent with the content (8.78±1.49 mg GAE g^{-1} DW) of *D. salina* aqueous extract and higher than the content (1.30±0.37 mg GAE g^{-1} DW) of *D. salina* methanol extract as reported by Andriopoulos *et al.*, (2022). Other studies reported various total phenolic contents in different *D. salina* extracts, including 4.672 mg GAE g^{-1} methanol extract (Widowati *et al.*, 2017), 53.27±7.7 mg GAE g^{-1} hexane extract and 56.45±4.50 mg GAE g^{-1} dichloromethane extract (Cakmak *et al.*, 2014), and 19.3±0.70 mg GAE g^{-1} ethanol extract (Maadane *et al.*, 2015).

Table 1 shows that the *D. salina* crude extract contains high concentrations (µg/g DW) of phenolic acids, including gallic acid (47.82), chlorogenic acid (46.84), caffeic acid (25.05), and rosmarinic acid (12.42), in addition to high flavonoid concentrations (µg/g DW), catechin (22.58), naringenin (14.88), quercetin (14.25), rutin (11.10), and hesperetin (9.80). Our findings are in accord with Simon *et al.*, (2015), who identified many phenolic components in the HPLC-MS/MS chemical profile of *D. salina* ethyl acetate extract, including caffeic acid, gallic acid, vanillic acid, coumarin, and vanillin. The concentration of total phenolic content and phenolic individuals in *D. salina* microalgae varies in this study compared to other studies because of differences in algal growth conditions, extraction solvent selection, and condition of extraction processes (Khawli *et al.*, 2021).

Table 1. Phenolic and flavonoid compounds profile of *D. salina* microalgae powder.

Compounds	***D. salina* algae content (µg/g DW)**
Phenolic compounds	
Gallic acid	47.82
Chlorogenic acid	46.84
Caffeic acid	25.05
Rosmarinic acid	12.42
Cinnamic acid	8.21
Ferulic acid	3.95
Syringic acid	3.06
Flavonoids	
Catechin	22.58
Naringenin	14.88
Quercetin	14.25
Rutin	11.10
Hesperetin	9.80
Kaempferol	7.06
Daidzein	6.05
Other phenolics	
Vanillin	4.33
Methyl gallate	1.94
Total phenolic contents (TP)	8.48±0.11 (mg GAE/g DW)
Total flavonoid contents (TF)	5.93±0.40 (mg QE/g DW)

3.2.Antioxidant activity of algae

The antioxidant efficiency of *D. salina* algal crude extract increased with concentration using the DPPH technique (Fig. 1a), with adequate IC_{50} value of 42.19±0.39 mg/ml. The powerful free radical scavenging capacity of *D. salina* extract is strongly linked to its quantities of various phenolic acids and flavonoids, these findings are in accord with former conclusions that demonstrated the high antioxidant ability of *D. salina* different extracts and attributed it to their contents of phenolic elements (Cakmak *et al*., 2014; Maadane *et al*., 2015; Widowati *et al*., 2017; Andriopoulos *et al*., 2022). The antioxidant effects of *D. salina* microalgae in this research could also be attributed to its high quantities of carotenoids, the powerful natural antioxidant pigments, in recent study (El-Baz and Ali, 2023) found that *D. salina* microalgae contain total carotenoids (0.0039-1.39 mg/g DW), β-carotene (0.0037-0.0565 mg/g DW), lutein (0.0004-0.1596 mg/g DW), zeaxanthin (0.0002-0.0341 mg/g DW), and astaxanthin (0.0003-0.0231 mg/g DW) depending on the extraction solvent. The free radical scavenging ability of the Ricotta cheese significantly ($p \leq 0.05$) enhanced as the quantities of the supplemented algae emulsion formula (*D. salina* algae/Mint EO) increased, revealing DPPH scavenging effects of 12.21, 43.36, and 43.99 % for I (0.25%), II (0.5%), and III (1%), respectively, compared to 5.71% of the control cheese (Fig. 1b). The numerous identified phenolic compounds in *D. salina* microalgae, including rosmarinic acid, gallic acid, caffeic acid, chlorogenic acid, rutin, catechin, naringenin, hesperetin, and quercetin are principally in charge of the elevated antioxidant capacity of Ricotta cheese fortified with algae emulsion formula (*D. salina* algae/Mint EO) compared to the control cheese in this study. These outcomes align with those of Falco *et al.*, (2023), who showed that adding *Chlorella vulgaris* microalga increased the antioxidant capacity of cream and quark cheese significantly in contrast to the cheese used as a control. Tohamy *et al*., (2018) revealed that spreadable cheese treated with *C. vulgaris* had stronger antioxidant activity than control cheese. The current findings suggested *D. salina* microalgae as a natural, safe, and potent source of antioxidants to lessen oxidative cellular damage and stop oxidative deterioration in manufactured dairy products like Ricotta cheese. Antioxidants, such as phenolic compounds and carotenoids found in *D. salina* microalgae, work through a variety of mechanisms to prevent chain initiation, chelate transition metal ion catalysts, degrade peroxidases, stop further hydrogen absorption, and act as radical scavengers (Valko *et al*., 2006; Andriopoulos *et al*., 2022).

3.3. Algae amino acids profile

The amino acids analysis of *D. salina* microalgae found that it contained 949.69 mg of total amino acids (TAA) and 424.2 mg of essential amino acids (EAA), with a ratio of 0.447 between TAA and EAA. The amino acid profile of *D. salina* microalgae contains 17 essential amino acids (Fig. 2), that are important for human growth and health including threonine (117.98 mg/g protein), lysine (112.88 mg/g protein), histidine (60.06 mg/g protein), valine (56.96 mg/g protein), phenylalanine (53.17 mg/g protein), methionine (40.16 mg/g protein), leucine (19.93 mg/g protein), and isoleucine (18.64 mg/g protein). Similar findings were recorded for the amino acids profile of *Chlorella vulgaris* microalgae (Mohamed *et al.*, 2013).

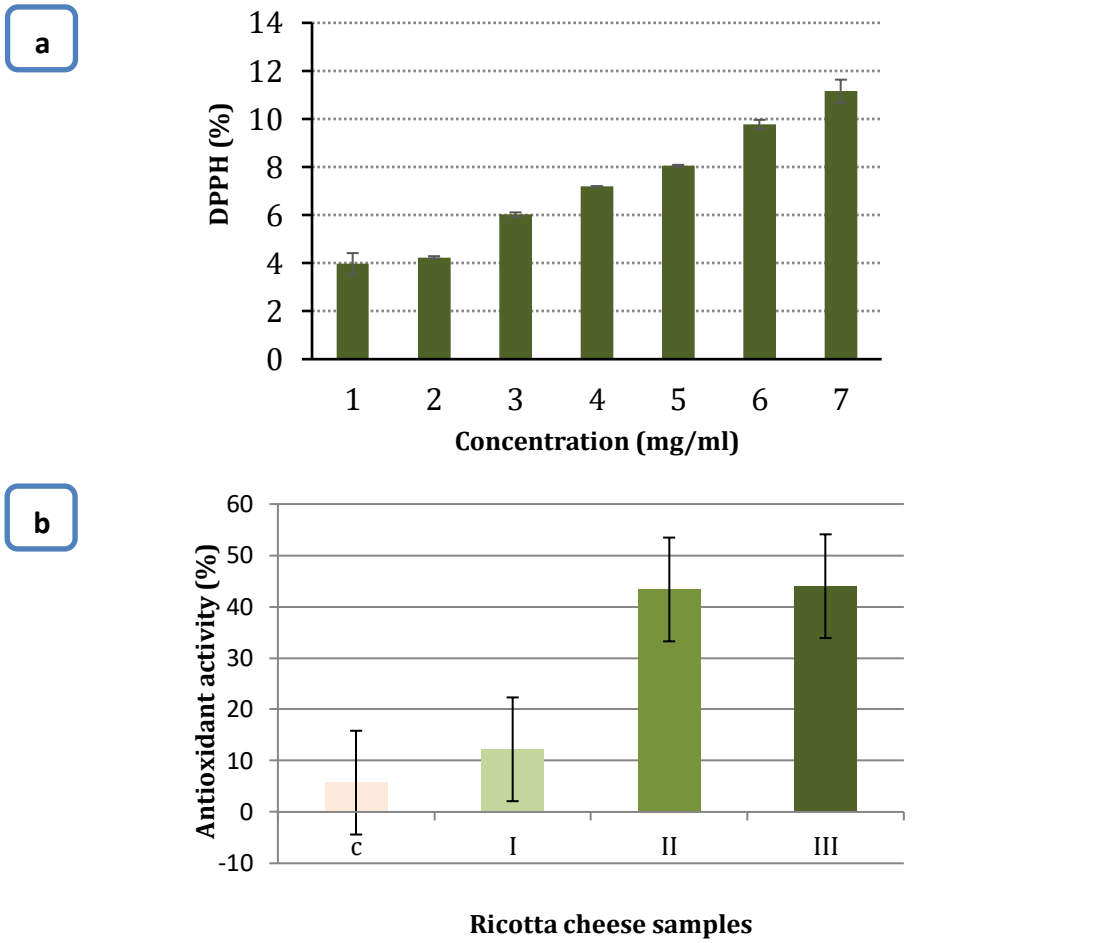


Figure 1. Antioxidant activity of *D. salina* algae and Ricotta cheese.
(a) DPPH• radical scavenging assay of *D. salina* algae crude extract, **(b)** Antioxidant activity of Ricotta cheese.
C: Ricotta cheese without algae emulsion, **I**: Ricotta cheese supplemented with 0.25% algae emulsion, **II**: Ricotta cheese supplemented with 0.5% algae emulsion, **III**: Ricotta cheese supplemented with 1% algae emulsion.

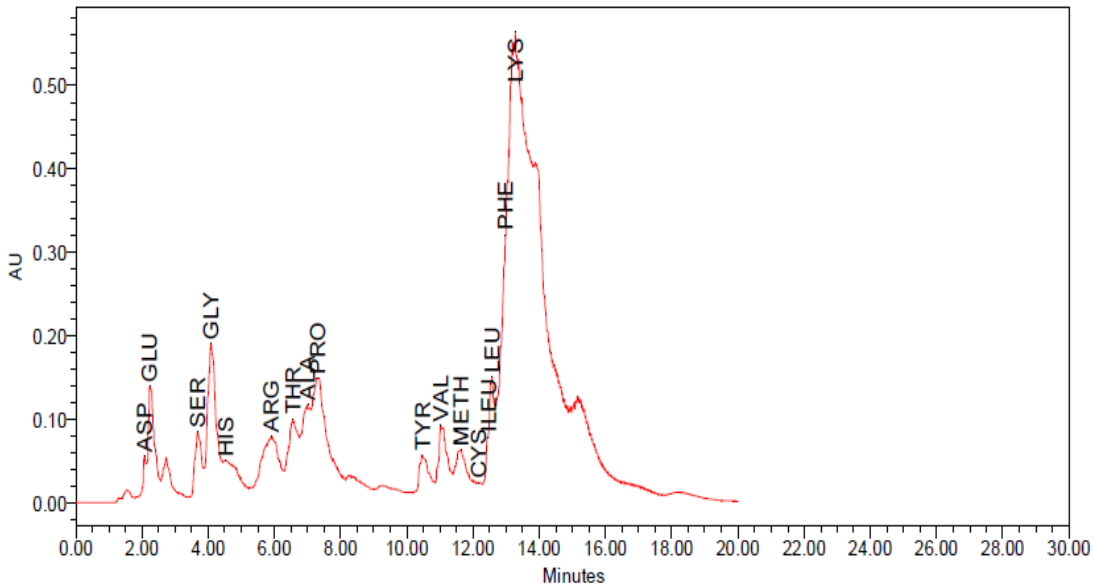


Figure 2. Amino acids profile of *D. salina* algae powder.

3.4. Characteristics of algae emulsion

The algae emulsion contains *D. salina* algae powder, and mint essential oil was prepared with non-ionic surfactant (Tween 80) using ultrasonication technique. The morphology of algae emulsion formula using TEM shows that it was mostly sphere-shaped, or oval in shape and very smooth surface as shown in Fig. 3 (A1, A2) at low and high magnifications. Also, the dynamic light scattering (DLS) result of particle size analyzer of the prepared algae emulsion is shown in Fig. 3 (B) which displays the average diameter is about 116 nm which similar with the average diameter of algae/cinnamon oil/epirubicin (117.2 nm) (Alkhatib *et al.*, 2020). In particular, the molecular geometry of a surfactant is known to play a major role in determining the formation and stability of emulsions and nanoemulsions (Israelachvili, 2011). It means the presence of double bonds in the nonpolar chains of Tween 80 as non-ionic surfactants improves the formation of *D. salina* algae/mint EO emulsion. Similar effects in other studies which have been reported that the surfactant type also had an appreciable effect on the droplet diameter mean, which Tween 80 as a food-grade nonionic surfactants had the smallest droplets (Wang *et al.*, 2009; Chang *et al.*, 2013).

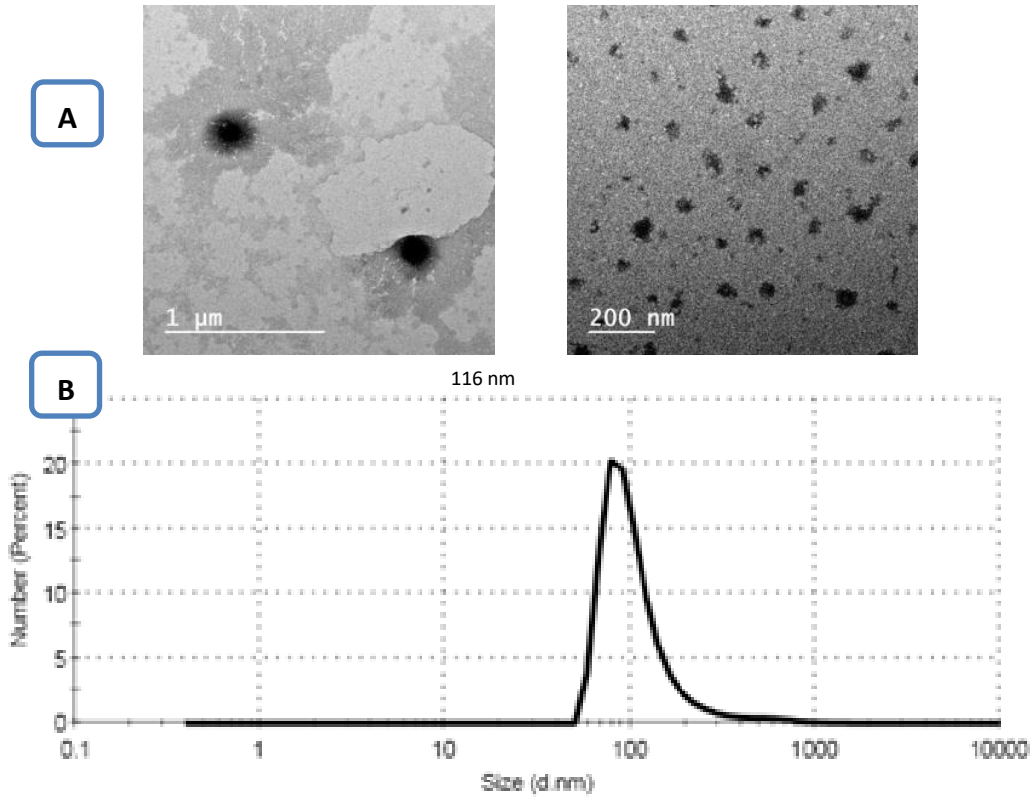


Figure 3. TEM images different magnifications (A), mean diameter and relative particle size (B) of the prepared algae emulsion formula.

3.5. Chemical changes of algae Ricotta cheese

The results obtained in this study for moisture, protein, fat, and ash, are shown in Table 2. The moisture content of Ricotta cheese ranged from 73.29 to 75.11%. Consequently, Ricotta cheese is categorized as a high moisture cheese as its moisture content exceeds 55% (Mangione *et al.*, 2023). Treatments III showed the lowest moisture value and differed significantly ($p \leq 0.05$) from control. Throughout the storage period, all cheese samples showed a decrease in moisture, most likely as a result of moisture evaporation from the cheese surfaces

(Pérez-Soto *et al.*, 2021). The current results of moisture content are consistent with those reported by Niro *et al.*, (2013), and Miele *et al.*, (2021).

The protein content of Ricotta cheese ranged from 17.59 to19.04%. The incorporation of the algae emulsion into the Ricotta cheese formulation causes significant changes ($p \leq 0.05$) in the protein content of cheeses. The fat content of Ricotta cheese ranged from (0.40-0.85%) for control and III treatments, respectively. The ash content significantly different ($p \leq 0.05$) between treatments; ash content of 1.78% was reported for Ricotta with 1% algae emulsion. However, the moisture loss of all samples during the storage period is most likely the cause of the apparent gradual increase in fat, protein, and ash content in the cheeses.

3.6. Acidity and pH changes of algae Ricotta cheese

The pH and titratable acidity (TA) values of the Ricotta cheeses that contained different concentrations of algae emulsion during storage period are shown in Table 2. The titratable acidity and pH values of the cheese samples were in the ranges of 0.47–1.06% and 5.02–5.39, respectively. The pH of cheese samples decreased and TA significantly increased ($p \leq 0.05$) in all samples throughout the storage period. According to Dermiki *et al.*, (2008), the production of organic acid, mainly by lactic acid producing bacteria, increased during storage, which is likely what caused the increase in TA. The slight increase in the total number of bacteria in the cheese samples from algae emulsion in the first three weeks could explain the constant TA values.

Table 2. Chemical changes of algae Ricotta cheese during storage at 5 °C for 21 days.

Treatment	Storage period (Day)	% (wt/wt)				Titratable acidity (%)	pH
		Moisture	Protein	Fat	Ash		
Control	**Fresh**	75.11±0.03^{aA}	17.59±0.10^{iA}	0.40±0.01^{dA}	0.70±0.02^{gB}	0.53±0.02^{dD}	5.39±0.05^{ghA}
	7	74.93±0.46^{abAB}	17.61±0.04^{hiA}	0.40±0.01^{dA}	0.75±0.02^{fgAB}	0.64±0.02^{cC}	5.29±0.01^{hiAB}
	14	74.60±0.16^{abcAB}	17.87±0.09^{ghiA}	0.43±0.02^{dA}	0.80±0.02^{fgAB}	0.84±0.02^{hiB}	5.23±0.07^{iB}
	21	74.32±0.04^{bcdeB}	17.95±0.03^{fghA}	0.44±0.01^{dA}	0.87±0.04^{fA}	1.05±0.02^{aA}	5.02±0.01^{jC}
I	**Fresh**	74.57±0.17^{abcA}	18.08±0.14^{efgA}	0.60±0.01^{cA}	1.56±0.01^{eC}	0.35±0.02^{hiBC}	5.60±0.08^{cdeA}
	7	74.49±0.56^{abcdA}	18.11±0.19^{efgA}	0.62±0.01^{cA}	1.58±0.02^{deBC}	0.39±0.02^{ghB}	5.52±0.08^{efAB}
	14	74.35±0.08^{bcdeA}	18.25±0.17^{efA}	0.64±0.02^{cA}	1.61±0.08^{cdeABC}	0.46±0.02^{bA}	5.49±0.01^{efgAB}
	21	74.01±0.03^{cdefA}	18.33±0.06^{eA}	0.65±0.02^{cA}	1.73±0.03^{bcA}	0.47±0.01^{efA}	5.42±0.02^{fgB}
II	**Fresh**	73.99±0.27^{cdefA}	18.69±0.03^{dA}	0.72±0.02^{bA}	1.71±0.02^{bcdA}	0.34±0.02^{ghB}	5.76±0.03^{bA}
	7	73.85±0.07^{defgA}	18.73±0.10^{dA}	0.74±0.01^{bA}	1.74±0.02^{bcA}	0.37±0.03^{fghAB}	5.67±0.01^{bcAB}
	14	73.70±0.11^{efghA}	18.79±0.03^{cdA}	0.74±0.01^{bA}	1.79±0.06^{abA}	0.38±0.01^{fghAB}	5.58±0.03^{cdBC}
	21	73.51±0.07^{fghiA}	18.90±0.04^{bcdA}	0.76±0.01^{bA}	1.85±0.03^{abA}	0.42±0.02^{efA}	5.50±0.02^{defC}
III	**Fresh**	73.29±0.10^{ghiA}	19.04±0.10^{abcdA}	0.85±0.02^{aA}	1.78±0.02^{abA}	0.32±0.02^{iC}	5.88±0.05^{aA}
	7	73.24±0.08^{ghiA}	19.12±0.19^{abcA}	0.86±0.01^{aA}	1.80±0.12^{abA}	0.35±0.03^{hiBC}	5.78±0.02^{abA}
	14	73.10±0.09^{hiA}	19.19±0.11^{abA}	0.87±0.01^{aA}	1.85±0.06^{abA}	0.39±0.02^{ghAB}	5.61±0.01^{cdeBC}
	21	72.96±0.02^{iA}	19.32±0.18^{aA}	0.89±0.04^{aA}	1.92±0.04^{abA}	0.42±0.02^{fgA}	5.59±0.03^{deC}

All parameters are represented as mean of triplicates ± standard error. Different lowercase letters in the same row differ significantly between treatments at $p \leq 0.05$. Different capital letters in the same column differ significantly at $p \leq 0.05$ over the storage period. **Control:** Ricotta cheese without algae emulsion, **I:** Ricotta cheese supplemented with 0.25% algae emulsion, **II:** Ricotta cheese supplemented with 0.5% algae emulsion, **III:** Ricotta cheese supplemented with 1% algae emulsion.

3.7. Minerals content of algae Ricotta cheese

Data presented in Table 3 showed that the content of minerals (mg/100 g) in Ricotta cheese increased in linear proportion to the concentration of *D. salina* microalgae that was incorporated in the algal Ricotta cheese. Along with having a high protein content, algal emulsion ricotta cheese (1%), had the greatest micronutrient concentration, with 398, 143.63, 118.75, 22.35, and 0.85 mg/100g of calcium, phosphor, potassium, magnesium, and iron, respectively. These findings are consistent with those of Osman *et al*. (2011), Michalak and Chojnacka (2014), and Oluwamukomi and Adeyemi (2015). In the current investigation, fortification of *D. salina* microalgae in the ricotta cheese resulted in ricotta cheese enriched with appropriate concentrations of minerals including magnesium, zinc, copper, and iron, which are necessary for immune support (Weyh *et al*., 2022). Because dairy products are often low in iron (Gaucheron, 2000), the fortified ricotta cheese used in this study with *D. salina* microalgae had a high iron content, which is a significant advantage for consumers, particularly those who consume vegetarian or plant-based diets. Hence, in accordance with Regulation (EU) No. 1169/2011, *D. salina* microalgae-enriched Ricotta cheese can use the nutritional demand of 'source of iron'. Falcão *et al*., (2023) also revealed that the addition of 4 % of *C. vulgaris* into cream cheese can use the nutritional demand of a source of iron compared to control cheese.

Table 3. Minerals content of algae Ricotta cheese.

Mineral	Control	I	II	III
Ca	121±0.58^{d}	225±0.42^{c}	297±0.72^{b}	398±0.78^{a}
Na	415.38±0.06^{d}	442.86±0.61^{c}	495.04±0.03^{b}	569.69±0.04^{a}
K	114±0.29^{d}	115.04±0.07^{c}	116.38±0.12^{b}	118.75±0.03^{a}
Zn	0.030±0.003^{b}	0.032±0.001^{b}	0.035±0.002^{ab}	0.040±0.002^{a}
Fe	0.14±0.01^{d}	0.30±0.04^{c}	0.58±0.02^{b}	0.85±0.02^{a}
Cu	0.001±0.02^{a}	0.0013±0.01^{b}	0.0015±0.0001^{d}	0.061±0.01^{c}
Mn	0.025±0.003^{c}	0.050±0.01^{bc}	0.085±0.003^{b}	0.250±0.03^{a}
P	142.11±0.31^{c}	142.49±0.06^{bc}	142.87±0.06^{b}	143.63±0.07^{a}
Mg	13.02±0.06^{c}	21.08±0.04^{b}	21.96±0.26^{a}	22.35±0.12^{a}

All parameters are represented as mean of triplicates ± standard error. Different lowercase letters in the same row differ significantly between treatments at $p \leq 0.05$. Different capital letters in the same column differ significantly at $p \leq 0.05$ over the storage period. **Control**: Ricotta cheese without algae emulsion, **I**: Ricotta cheese supplemented with 0.25% algae emulsion, **II**: Ricotta cheese supplemented with 0.5% algae emulsion, **III**: Ricotta cheese supplemented with 1% algae emulsion.

3.8. Color development of algae Ricotta cheese

The enrichment of Ricotta cheese with *D. salina* algae emulsion led to innovative green color which mainly due to the presence of varied pigments in the microalga biomass including chlorophyll and carotenoids (El-Baz and Ali, 2023). The addition of algae emulsion formula during Ricotta cheese processing showed significant ($p \leq 0.05$) darkening of the cheese treatments, where it can be seen reduction in all color tonalities (Fig. 4). It could be mainly due the color of *D. salina* algae is green while the control Ricotta cheese is creamy color. The

obtained results are in the line with Falcão *et al.*, (2023) who demonstrated that the incorporation of *C. vulgaris* microalga results in a significant ($p \leq 0.05$) decrease in the L* color attributes for both cream and quark cheese samples compared to control cheese.

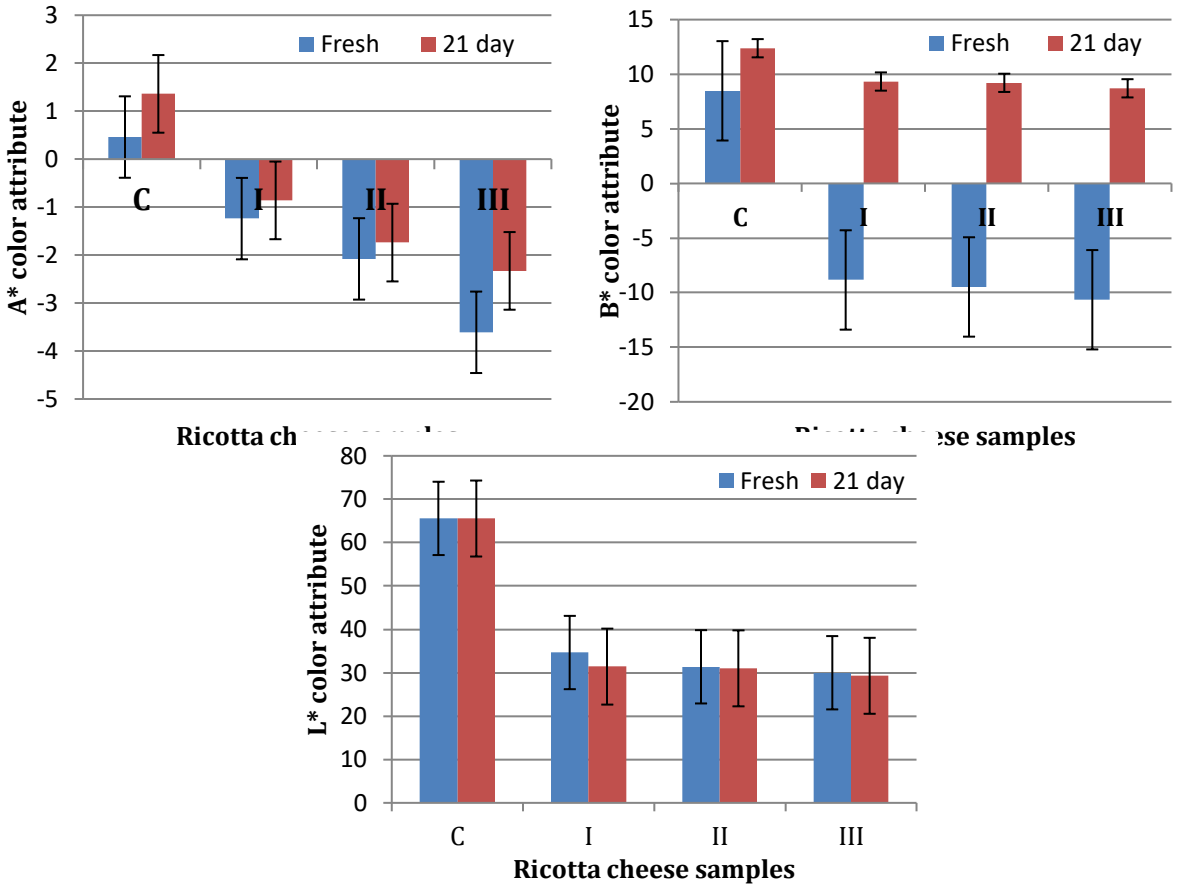


Figure 4. Color development of algae Ricotta cheese during storage at 5 °C for 21 days.
l* value represents darkness from black (0) to white (100); **a*** value represents color ranging from red (+) to green (−); **b*** value represents yellow (+) to blue (-).
C: Ricotta cheese without algae emulsion, **I**: Ricotta cheese supplemented with 0.25% algae emulsion, **II**: Ricotta cheese supplemented with 0.5% algae emulsion, **III**: Ricotta cheese supplemented with 1% algae emulsion.

3.9. Texture profile analysis of algae Ricotta cheese

The texture profile of Ricotta cheese is characterized as a viscoelastic food (Fox *et al.*, 2000) not pasty and friable with fragile characteristics (Tunick *et al.*, 2012). Texture profile analysis (TPA) analysis of Ricotta cheese at different ratios of algae emulsion was carried out on fresh samples and after 21 days of storage at 5 °C (Table 4). Hardness is the force required to attain a specific deformation. Hardness values of fresh samples ranged from 10.3 to 16.8 N. It could be seen that the control cheese sample had significantly higher hardness than the other varieties that contained different percentages of algae emulsion. This result agrees with Bryant *et al.*, (1995); Araque *et al.*, (2017); Azarashkan *et al.*, (2022) who reported that fat content affected cheese firmness significantly. As the cold storage period progressed, the hardness values of the cheeses significantly increased at the end of storage ($p \leq 0.05$) a similar finding was reported by Araque *et al.*, (2017). Adhesiveness is the work required to overcome attraction between food and other surfaces. Because the fat content affected the adhesiveness, therefore significant change was observed in the adhesiveness of algae-based Ricotta cheese

treatments. Algae-based Ricotta cheese samples showed greater adhesiveness than the control sample at the end of the cold storage period. Cohesiveness is the mechanical textural attribute relating to the degree to which a substance can be deformed before it breaks. The cohesiveness value without the addition of algae emulsion was 0.45. The lower value of cohesiveness in Ricotta cheese can be attributed to the low fat/protein ratio (Pizzillo *et al*., 2005). The addition of algae emulsion increasing cheese cohesiveness which means that the force exerted by the internal cheese bonds was stronger. The ability of the deformed cheese to gradually return to its original position following the removal of force is measured by its springiness. The higher values of springiness of II and III treatments show that the deformability of Ricotta cheese remained unchanged (Ferrandini *et al*., 2011). No significant differences in springiness ($p \leq 0.05$) observed in cheese samples; but only among control and III treatments. There was a slight decrease in springiness, which could be related to the proteolytic breakdown of the protein matrix. Chewiness is a secondary cheese textural characteristic. and derivative of hardness which means the overall work required for the double compression. Zheng *et al.*, (2016) found that chewiness values were highest for low-fat cheese samples and lowest for high-fat or high-moisture cheese samples. Additionally, it was noted that restructured Ricotta cheese made with mozzarella cheese whey including a combination of gelatin and guar gum had a higher chewiness value (Hesarinejad *et al*., 2021). In our study, the value of chewiness decreased with increasing the percentage of algae emulsion (4.75mj). There were significant differences ($p \leq 0.05$) in gumminess between the cheese treatments. The control recorded the highest value of guminness (9.07 N). At the end of the storage period, this secondary attribute increased and exhibited similar behavior with primary attribute of hardness. Our TPA results are in agreement with literature (Tunick *et al*., 2012; Hesarinejad *et al*., 2021) who described that Ricotta cheese texture is very soft consistency, compressible, and not too cohesive. In terms of textural parameters, adhesiveness, cohesiveness, and springiness, which are essential for preserving product acceptance over its shelf life, there were no significant changes observed after 21 days of refrigerated storage and the texture profile was generally stable.

Table 4. Texture profile changes of algae Ricotta cheese during storage at 5 °C for 21 days.

Parameter	Storage period (day)	Ricotta cheese treatments			
		Control	I	II	III
Hardness (N)	**Fresh**	16.80 ± 0.04^{bB}	14.30 ± 0.06^{dB}	11.70 ± 0.11^{fB}	10.30 ± 0.13^{hB}
	21	17.20 ± 0.02^{aA}	16.10 ± 0.15^{cA}	13.20 ± 0.10^{eA}	11.28 ± 0.09^{gA}
Adhesiveness (mj)	**Fresh**	0.80 ± 0.01^{fA}	0.87 ± 0.02^{efB}	1.15 ± 0.04^{dB}	1.51 ± 0.02^{bB}
	21	0.83 ± 0.006^{efA}	0.94 ± 0.06^{eA}	1.34 ± 0.07^{cA}	1.84 ± 0.03^{aA}
Cohesiveness (Ratio)	**Fresh**	0.54 ± 0.03^{dA}	0.61 ± 0.02^{cdA}	0.69 ± 0.02^{abcA}	0.71 ± 0.04^{abA}
	21	0.59 ± 0.03^{dA}	0.68 ± 0.03^{bcA}	0.74 ± 0.02^{abA}	0.77 ± 0.03^{aA}
Springiness (mm)	**Fresh**	0.65 ± 0.03^{bA}	0.65 ± 0.03^{bA}	0.83 ± 0.03^{abA}	0.86 ± 0.07^{aA}
	21	0.66 ± 0.03^{bA}	0.66 ± 0.03^{bA}	0.85 ± 0.08^{aA}	0.89 ± 0.05^{aA}
Gumminess (N)	**Fresh**	9.07 ± 0.09^{cB}	8.72 ± 0.02^{cB}	8.07 ± 0.22^{dB}	7.31 ± 0.06^{eB}
	21	10.15 ± 0.04^{bA}	10.95 ± 0.36^{aA}	9.77 ± 0.32^{bA}	8.69 ± 0.15^{eA}
Chewiness (mj)	**Fresh**	7.78 ± 0.09^{bB}	7.24 ± 0.14^{cB}	6.05 ± 0.02^{dB}	4.75 ± 0.12^{eB}
	21	9.03 ± 0.21^{aA}	9.31 ± 0.17^{aA}	7.43 ± 0.12^{bcA}	5.74 ± 0.06^{dA}

All parameters are represented as mean of triplicates ± standard error. Different lowercase letters in the same row differ significantly between treatments at $p \leq 0.05$. Different capital letters in the same column differ significantly at $p \leq 0.05$ over the storage period. **Control**: Ricotta cheese without algae emulsion, **I**: Ricotta cheese supplemented with 0.25% algae emulsion, **II**: Ricotta cheese supplemented with 0.5% algae emulsion, **III**: Ricotta cheese supplemented with 1% algae emulsion.

3.10. Organoleptic attributes of algae Ricotta cheese

In 2020, green colored food varieties a major pattern as consumers looked for items that reconnected them with nature which such variety is frequently connected with nutritional and healthy products (Smail, 2021; Falcão *et al*., 2023). In the present investigation, the addition of algae emulsion formula during Ricotta cheese processing improved all sensorial attributes of cheese including flavor, appearance and texture during the storage period for 21 days at 5 °C. The Ricotta cheese with algae emulsion formula (0.5 %) had the highest flavor (Fig. 5A), and appearance (Fig. 5B) scores among other treatments; while at the higher level of algae emulsion such sensorial attributes were decreased. The mint essential oil content of algae emulsion improved the acceptability of cheese flavor and appearance especially for both *D. salina* algae color is green and its taste could be unaccepted, while the higher level of algae emulsion increased the green color level in the resulted cheese which decreased their overall acceptability. Also, Fig. 5C shows that the body and texture of Ricotta cheese was increased with the algae emulsion increased. Falcão *et al*., (2023) also reported a promising sensory testing result in cream cheese supplemented with *C. vulgaris* compared to control cheese.

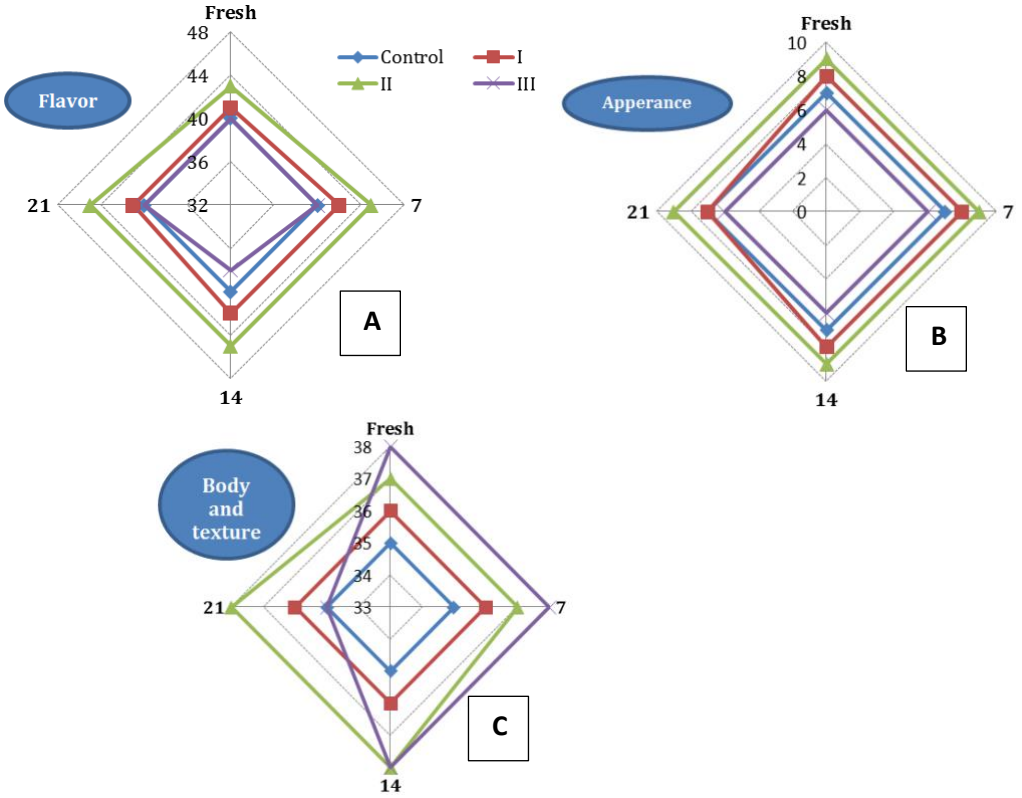


Figure 5. Organoleptic attributes change of algae Ricotta cheese during storage at 5 °C for 21 days. **Control**: Ricotta cheese without algae emulsion, **I**: Ricotta cheese supplemented with 0.25% algae emulsion, **II**: Ricotta cheese supplemented with 0.5% algae emulsion, **III**: Ricotta cheese supplemented with 1% algae emulsion.

3.11. Amino acids content and chemical score of algae Ricotta cheese

Data of Table 5 showed that most of amino acids, including glutamic acid, serine, glycine, histidine, arginine, proline, methionine, cysteine, isoleucine, and phenylalnine, were increased in the Ricotta cheese when *D. salina* algae emulsion formula was added at a level of 0.5% in comparison of untreated cheese. On the other hand, control Ricotta cheese and algal

cheese had comparable TAA, EAA, and EAA/TAA ratio. Fig. 6 demonstrates that the chemical score of various amino acids, including methionine + cysteine, histidine, and isoleucine, increased when algal emulsion formula was added to Ricotta cheese compared to control Ricotta cheese. The fortified Ricotta cheese used in this study with *D. salina* microalgae had high contents of essential amino acids, which are vital to human nutrition and health. Different amino acids have a variety of vital roles in several functional processes, including cell signaling, gene expression, DNA synthesis, immunological response, and food intake and metabolism, in addition to serving as the building blocks for proteins (Manjarín *et al*., 2020; Ryan *et al*., 2021).

Table 5. Amino acids content of algae Ricotta cheese.

Amino acids (mg/g protein)	Ricotta cheese treatments	
	Control	**Best algae cheese (II)**
Aspartic acid	80.02	79.98
Glutamic acid	180.45	184.71
Serine	49.63	57.32
Glycine	19.09	22.83
Histidine	25.79	33.09
Arginine	34.76	41.51
Threonine	119.24	112.75
Alanine	66.18	46.89
Proline	89.61	115.23
Tyrosine	55.28	49.03
Valine	55.61	46.04
Methionine	30.64	31.5
Cysteine	2.86	3.8
Isoleucine	24.95	32.62
Leucine	38.39	36.2
Phenylalnine	12.95	17.78
Lysine	65.00	37.75
Total amino acids (TAA)	950.45	949.03
Total essential amino acids (EAA)	288.09	276.49
EAA/TAA ratio	0.303	0.291

II: Ricotta cheese supplemented with 0.5% algae emulsion formula.

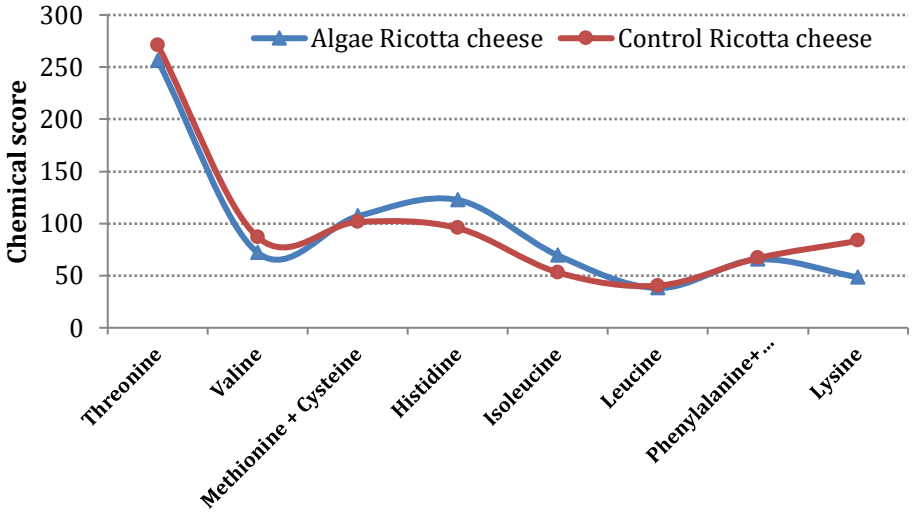
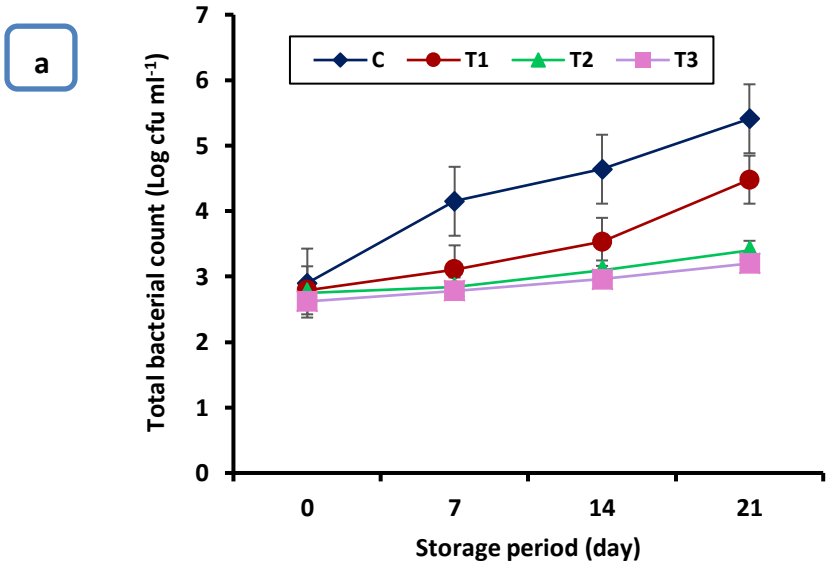


Figure 6. Chemical score of algae Ricotta cheese.
Algae Ricotta cheese: Ricotta cheese supplemented with 0.5% algae emulsion formula.

3.12. Microbiological analysis of algae Ricotta cheese

A slight increase in total bacterial count (TBC) during storage period in algae Ricotta samples was recorded. The TBC was significantly lower ($p \leq 0.05$) in algae Ricotta cheese samples than in control sample (Fig. 7a). The yeast and mold counts showed a different trend in control sample than II and III algae Ricotta samples cheese (Fig. 7b). In the control sample, it appeared after 14 days of storage, whereas in the algae Ricotta cheese samples II and III it appeared only on the 21st day of storage period. There were significant differences between all treatments at the end of the storage period ($p \leq 0.05$).

Coliform bacteria were not detected in all samples either fresh or during the refrigerated storage period. This may be due to the high sanitation conditions during manufacturing and cold storage.



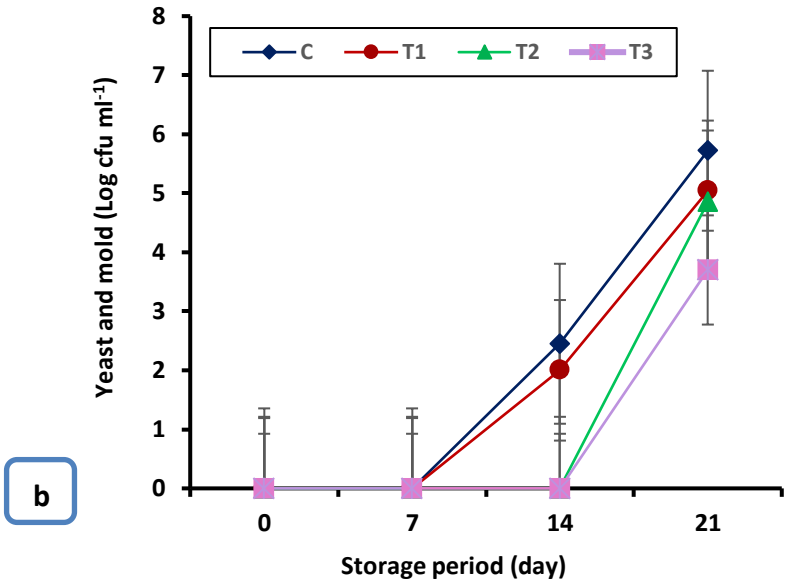


Figure 7. Microbiological changes of algae Ricotta cheese during storage at 5 °C for 21 days. **(a)** Total bacterial count (TBC), **(b)** yeast and mold count.
C: Ricotta cheese without algae emulsion, **T1**: Ricotta cheese supplemented with 0.25% algae emulsion, **T2**: Ricotta cheese supplemented with 0.5% algae emulsion, **T3**: Ricotta cheese supplemented with 1% algae emulsion.

4.Conclusion

Adequate total phenolics, total flavonoids, and phenolics components, particularly gallic acid, chlorogenic acid, caffeic acid, rosmarinic acid, catechin, naringenin, quercetin, rutin, and hesperetin, are present in *D. salina* microalgae, which have the potent ability to scavenge free radicals. *D. salina* microalgae contain significant amounts of several amino acids, including threonine, lysine, histidine, valine, and phenylalanine, which are necessary for human growth and health. Therefore, the incorporation of *D. salina* algae/mint essential oil emulsion into Ricotta cheese processing led to improve the final characteristics of such cheese including more antioxidant activity, protein content, mineral profile, and good sensorial acceptability. Hence, *D. salina* microalga could be utilized as a promising functional ingredient of plant origin for the improvement of conventional dairy items, resulting in inventive and more sustainable products.

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