



Enhancing the Nutritional Value and Chemical Composition of Functional Karish Cheese by Adding Microalgae Powder (*Chlorella vulgaris* and *Spirulina platensis*)

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DOI: 10.21608/AJAS.2024.295569.1367

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Abstract

Microalgae can enhance the nutritional value of conventional foods and hence to positively affect human health, due to their original chemical composition. The present study aimed to produce functional Karish cheese fortified with different types and ratios of microalgae powder (*Chlorella vulgaris* and *Spirulina platensis*) and evaluate the role of fortification with those algae on the quality of the product. Karish cheese was incorporated with *C. vulgaris* and *S. platensis* powder, separately at three levels 0.5, 1.0 and 1.5%. The evaluation of chemical composition, salt content, color parameters, antioxidant activity, total phenolic and total carotenoids content, texture profile and organoleptic properties of Karish cheese fortified with microalgae were determined. Addition of microalgae to Karish cheese showed an increasing ($P < 0.05$) in total solid content, protein, ash, acidity and enhanced the texture profile in the final product compared with control sample. Also, the results indicated highest content of antioxidant activity, total flavonoid and total phenolic among all treatments compared with control. Organoleptic preferred Karish cheese samples enriched with microalgae especially with 0.5 and 1.0 % level consequently. Based on these results microalgae powder enriched cheese may have a great benefit for the industry to improve the texture, shapes and the functional nutraceutical properties of the products.

Keywords: Antioxidant activity, *Chlorella vulgaris*, Karish cheese, *Spirulina platensis*.

Introduction

Recently, interest in functional foods has increased because of their positive impact on human health. The American Dietetic Association mentioned that functional foods include various types of foods like dietary supplemental and fortified foods. while the isolation of components that incorporated in different food products to improve health that cannot be obtained from normal foods were defined as nutraceuticals (Ross, 2000).

Microalgae could be used as functional ingredients as it is a good source of bioactive compounds so the attention of researcher with algae had increased. biological properties and chemical composition (antioxidants, minerals, good quality proteins, vitamins and balanced fatty acid profiles) can be applied in the production of new functional foods (Spolaore *et al.*, 2006). Microalgae are an enormous biological resource, representing one of the most promising sources for new food products (Pulz and Gross, 2004). Therefore, microalgae can be used to enhance the nutritional value of food products, due to their well-balanced chemical composition as well as a source of highly valuable molecules, such as polyunsaturated fatty acids, pigments, sterols, vitamins, hydrocolloids, and other biologically active compounds (Hadj *et al.*, 2013).

Chlorella and *spirulina* are two types of microalgae that are mostly used in food products, especially *spirulina* algae due to their high protein content *chlorella*, 51-58% and *spirulina*, 55.8-71%, respectively (Barka and Blecker 2016). The World Health Organization described algae *chlorella* and *spirulina* as among the best superfoods in the food field and they are among the most popular microalgae (Harun *et al.*, 2010). Meanwhile, according to Raya *et al.* (2016) stated that *C. vulgaris* is a good source of protein as well as Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA).

Spirulina has been widely used in several countries, it is considered generally recognized as safe (GRAS), without toxicological effects, and it is approved by the FDA (U.S.A.) and ANVISA (Navacchi *et al.*, 2012). *Spirulina* is considered a source of protein (60-70 g / 100 g) with high biological value, as it is rich in protein. A source of vitamins (Vitamin B₁₂ and Pro-Vitamin A) and minerals, such as iron, and high in its content of linolenic acid, the essential fatty acid that forms prostaglandins, there was an increasing the end for use as a food source throughout the world because it is safe for consumption by humans and animals (Habib *et al.*, 2008). On the other hand, *spirulina* contains components such as phenolic compounds, α -tocopherol phycocyanin, xanthophyll pigments and β -carotene and that are responsible for the antioxidant activities of these algae, as shown by several authors for in vivo and in vitro experiments (Patel *et al.*, 2006).

Chlorella vulgaris is a microscopic, green, single-celled algae that lives in freshwater, and has health benefits, such as stomach ulcers, anemia, wounds, constipation, high blood pressure, malnutrition in infants, diabetes, and helping disorders (Fradique *et al.*, 2010). It is also considered to be a rich source of antioxidants such as leucin, carotene, ascorbic acid and tocopherol and supplies large quantities of minerals and dietary fibers (Queiroz *et al.*, 2011). The biomass of *Chlorella vulgaris* also represents a valuable source of almost all vitamins A, B₁, B₂, B₆, B₁₂, C, E, nicotinic acid, folic acid, pantothenic acid, and biotin (Pulz and Gross, 2004). *Chlorella vulgaris* is also important as a source of natural pigments, namely carotenoids and can be used as a natural coloring agent (Fradique *et al.*, 2010).

Adding algae to soft cheese improved its properties, giving a significant effect on protein, water, fat, carotene, and texture (Agustinia *et al.*, 2016). Karish cheese is considered one of the most popular foods which eaten more than one time a day and is popularly known locally in Egyptian cities. It is one of the important foods in the consumer's diet (Abd-Ehamid, 2012). Karish cheese is one of the most popular types of soft cheese consumed in Egypt as it rich with proteins content and calcium as well as its low-fat content, and particularly its low price. This type of cheese is made from skimmed cows /buffalos' milk, or a mixture of them (Abu Donia, 2008). It is considered one of the cheapest and most famous soft cheeses in Egypt and Arabic countries. It has a soft texture and white, color slightly salty curds. It is rich in protein and calcium content, in addition to its low-fat content. Also rich in calcium and phosphorus. These elements are necessary for the formation of bones and teeth. It is also rich in sodium and potassium, which play an important role in the formation of body fluids and muscles (Francois *et al.*, 2004).

The objectives of this study were to use *Spirulina platensis* and *Chlorella vulgaris* biomass as a new functional ingredient at different levels (0.5, 1 and 1.5%) in the preparation of imitation Karish cheeses.

Materials and Methods

Materials

Buffalos skim milk was obtained from milk production unit, Al-Azhar University (Assiut Branch), Egypt. Salt was purchased from the local market in Assiut governorate, Egypt. Commercial yoghurt culture (YC-X11 DIP 50u) containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Chr. Hansens Laboratiers, Denmark. Algae (*Spirulina platensis* and *Chlorella vulgaris*) in the form of freeze-dried powder were obtained from Algae Biotechnology Unit, National Research Centre, Dokki, Giza, Egypt.

Chemicals

All chemicals used in this study were of analytical grade supplied by different international chemicals companies such as BDH, Sigma and Difco chemical companies. Distilled water and Pyrex glassware were used throughout this study.

Methods

Cheese Preparing

Karish cheese was manufactured as previously described by Abou-Donia (2008). Buffalo's skim milk was heated to 85°C for 15 sec and cooled to 38-40°C. Active starters (1:1) of (*Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (3% w/w)) were added to milk and stirred well. The mixture was divided into seven portions, as following: The first portion was kept untreated (control). The second, third and fourth portions were fortified with 0.5%, 1% and 1.5% of *Chlorella vulgaris*, respectively. The fifth, sixth and seventh portions were fortified with 0.5%, 1% and 1.5% of *Spirulina platensis*, respectively and held until to coagulate. Sodium chloride was added as 0.5%, w/v of milk for all portions between layers

of cheese and left for whey draining into small cheese mold at room temperature for 4–5 h. The resultant samples were stored at $5^{\circ} \pm 1^{\circ} \text{C}$ and analyzed at zero time, and after 10, 20 and 30 days.

Chemical analysis

The total solid (TS), ash contents, total protein (TP), and developed titratable acidity (D.T.A) were determined in Karish samples according to (AOAC, 2012). The pH value was measured electronically in all samples using lad pH-meter with a glass electrode (Hanna model 8417 digital pH meter). The salt content determined by titration with AgNO_3 using the Mohr Method, Color was measured using a spectrophotometer (MOM, 100 D, Hungary) according to the instruction of Commission International using CIE Color coordinates system. The X, Y and Z values were converted to L a b values according to equation provided by the manufactures and as mentioned by (Francies 2005). Antioxidant activity (DPPH) was measured by the method of (Cervato *et al.*, 2000). The total phenol content of samples was determined according to the Folin–Ciocalteu method reported by Chan *et al.* (2007) and total flavonoid content was determined using aluminum chloride method as reported by Asha *et al.* (2010). Texture was determined according to the definition given by IDF (1991).

Sensory evaluation

The organoleptic properties were evaluated as given by El-Nawasany *et al.* (2015). The organoleptic properties included flavor (50 points), consistency (40 points) and general appearance (10 points) were evaluated by 10 panelists from the dairy department.

Statistical analysis

Data reported are the average of three measurements. Statistical analysis was carried out using ANOVA procedures followed by Duncan's Multiple Range Test with ($P < 0.05$) being considered statistically significant to compare between means. All procedures were in triplicate using Statistical Analysis System program (SAS, 2001).

Results and Discussion

Chemical composition of raw milk, and microalgae powder

Table (1) shows the chemical composition of raw milk, and microalgae powder *Spirulina platensis* (SPP) and *Chlorella vulgaris* (CVP). The results of the chemical composition of raw milk (before the sorting process) used for manufacturing of Karish cheese samples was determined as (14.88 % total solid, 0.76 ash%, 6.10 fat %, 4.01 protein %, 0.18 % acidity, 6.74 pH, 85.12 % moisture, 4.11 carbohydrate, and 8.78 % S.N.F). These results agree with Mesbah *et al.* (2022). Chemical composition of *Spirulina platensis* (TS, ash, fat, protein, acidity, pH, moisture, fiber, carbohydrate, and S.N.F) is in consistency with those obtained by Habib *et al.* (2008), Vijayarani *et al.* (2012), Dolly (2014), and Sharoba (2014). Chemical composition of *Chlorella vulgaris* (TS, ash, fat, protein, pH,

carbohydrate, and S.N.F) are similar to the results obtained by Widyaningrum and Prianto (2021) and Héctor *et al.* (2022).

Table 1. Chemical composition of raw milk, and microalgae powder used in manufacture of Karish cheese

Determination %	Materials		
	Milk	<i>Spirulina platensis</i>	<i>Chlorella vulgaris</i>
TS	14.88	94.00	94.14
Ash	0.76	7.99	9.50
Fat	6.10	7.00	12.00
Protein	4.01	61.92	51.46
Acidity	0.18	0.11	0.10
pH values	6.74	6.91	6.98
Moisture	85.12	6.00	5.86
Fiber	-	5.74	9.00
Carbohydrate	4.11	11.01	11.84
S.N.F.	8.78	87.00	82.14

TS: Total solids, S.N.F: Solids not fat

Phytochemicals of microalgae powder *Spirulina platensis* and *Chlorella vulgaris*

The data presented in Table (2) shows that there were significant differences in (DPPH (%), total phenolic content and total flavonoids among all treatments. *Spirulina platensis* and *Chlorella vulgaris* could be as source of free radical scavenging, total phenolic and total flavonoids content. The results are in good agreement with the results of with Bhavisha and Parula (2010), Deasy *et al.* (2019), and Basuny *et al.* (2023) who found that the algae riches with phenolic and other bioactive compounds.

Table 2. Phytochemicals of microalgae powder *S. platensis* and *C. vulgaris*

Constituents	Values of microalgae	
	<i>Spirulina platensis</i>	<i>Chlorella vulgaris</i>
DPPH (%)	467.8 ^A	116.4 ^B
Total phenols (mg GAE/100g)	844.20 ^A	677.67 ^B
Total flavonoids (mg/100g)	681.10 ^A	230.80 ^B

DPPH: (Radical scavenging capacity) 2,2-diphenyl-1-picryl-hydrazyl

A, B, C, D: Means with the same letter for the same row are not significantly different.

The chemical composition of functional Karish cheese fortified with *Chlorella vulgaris* and *Spirulina platensis* powders

Total solid

The obtained data in Table (3) indicated that functional Karish cheese containing *Chlorella vulgaris* and *Spirulina platensis* had significant differences of total solids content between treatments than that of control treatment. The increase in total solids content in functional Karish cheese containing algae powder compared with the control sample may be due to the higher total solids content of algae powder used in functional Karish cheese manufacturing. These results agree with the result of Mohamed *et al.* (2013) who found that there were significant

differences between cheese sample analogue enhanced with 3% *C. vulgaris* in total solids compared with the control.

On the other hand, there were no significant differences between T1c and T1s in fresh samples and after 10 days of storage. Also, there were no significant differences in total solids content among treatments than that of control treatment after 20 and 30 days of storage.

Total solids content slightly increased in all functional Karish cheese manufacture with different types and ratio of algae powder as the refrigerated storage period progressed up to 30 days. This increase in total solids during storage period may be due to the water evaporation during the storage period. Our results agreed with those results obtained by Qureshi *et al.* (2012) who indicated that the total solid content in yoghurt samples were increased with the passage of storage time. The increase in solid content is due to the decrease in moisture content.

Table 3. Total solid content of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Treatments	CSP (Day)			
	Fresh	10	20	30
C	23.42 ^{Bc}	24.74 ^{Ab}	25.23 ^{Aa}	25.59 ^{Aa}
T1c	23.79 ^{ABc}	25.00 ^{Ab}	25.41 ^{Aa}	25.73 ^{Aa}
T2c	24.17 ^{Ab}	25.20 ^{Aa}	25.59 ^{Aa}	25.79 ^{Aa}
T3c	24.55 ^{Ab}	25.46 ^{Aa}	25.75 ^{Aa}	25.95 ^{Aa}
T1s	23.78 ^{ABc}	24.98 ^{Ab}	25.39 ^{Aa}	25.71 ^{Aa}
T2s	24.15 ^{Ab}	25.18 ^{Aa}	25.56 ^{Aa}	25.81 ^{Aa}
T3s	24.52 ^{Ab}	25.42 ^{Aa}	25.71 ^{Aa}	25.90 ^{Aa}

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris*, respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis*, respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different.

a, b, c, d: Means with the same letter for the same row are not significantly different

Total Protein

According to Beheshtipour *et al.* (2013), *Spirulina platensis* is rich in protein content (60–70% of its dry weight). The data in Table (4) showed that there are significant differences ($P < 0.05$) in total protein among all treatments. At the same time, by increasing of added algae there was an increase in protein content in the Karish cheeses because of the high protein content in *C. vulgaris* and *S. platensis*. The highest protein content in Karish cheese was 1.5% *S. platensis* and 1.5% *C. vulgaris*. These data are in good agreement with those of Lupatini *et al.* (2017). Other studies reported that the protein content in yogurt increased significantly ($P < 0.05$) due to the addition of *Spirulina* Mesbah *et al.* (2022). The total protein in all treatments increased steadily during storage period at 5 °C which could be related to the decrease in moisture content. A similar observation was reported by Hendy *et al.* (2023).

Table 4. Total protein content of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Treatments	CSP (Day)			
	Fresh	10	20	30
C	16.33 ^{Aa}	16.39 ^{Aa}	16.67 ^{Aa}	16.84 ^{Aa}
T1c	16.48 ^{Bd}	16.55 ^{Bc}	16.81 ^{Bb}	16.97 ^{Ba}
T2c	16.65 ^{Ad}	16.73 ^{Ac}	17.00 ^{Ab}	17.17 ^{Aa}
T3c	16.80 ^{Cd}	16.89 ^{Cc}	17.18 ^{Cb}	17.37 ^{Ca}
T1s	16.52 ^{Ad}	16.59 ^{Ac}	16.85 ^{Ab}	17.02 ^{Aa}
T2s	16.70 ^{Bd}	16.77 ^{Bc}	17.05 ^{Bb}	17.23 ^{Ba}
T3s	16.90 ^{Cd}	16.98 ^{Cc}	17.27 ^{Cb}	17.44 ^{Ca}

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris* respectively.T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis* respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different.

a, b, c, d: Means with the same letter for the same row are not significantly different

Ash content

The data in Table (5) showed that functional Karish cheese containing *C. vulgaris* and *S. platensis* had significant differences in ash content between treatments than that in control sample. On the other hand, the data showed that functional Karish cheese containing algae powder exhibited significant higher ash content during storage period than that in control sample (without algae). These data agree with Agustinia *et al.* (2016) and Darwish (2017) who found that increasing in the addition of *Spirulina* powder led to significant difference ($P < 0.05$) in ash content of Karish cheese.

Table 5. Ash content of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Treatments	CSP (Day)			
	Fresh	10	20	30
C	1.88 ^{Bc}	1.95 ^{Bb}	2.02 ^{Bb}	2.11 ^{Bb}
T1c	1.92 ^{ABc}	1.99 ^{Ab}	2.06 ^{ABb}	2.15 ^{ABa}
T2c	1.96 ^{Ad}	2.04 ^{Ac}	2.12 ^{Ab}	2.22 ^{Aa}
T3c	1.99 ^{Ad}	2.07 ^{Ac}	2.15 ^{Ab}	2.25 ^{Aa}
T1s	1.91 ^{ABd}	1.98 ^{ABc}	2.05 ^{ABb}	2.14 ^{ABa}
T2s	1.94 ^{Ad}	2.02 ^{Ac}	2.10 ^{Ab}	2.20 ^{Aa}
T3s	1.98 ^{Ad}	2.06 ^{Ac}	2.14 ^{Ab}	2.24 ^{Aa}

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris* respectively.T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis* respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different.

a, b, c, d: Means with the same letter for the same row are not significantly different

Salt content

The data in Table (6) revealed that functional Karish cheese containing *C. vulgaris* and *S. platensis* had no significant differences in salt content between treatments than that of control sample. While there were slight differences between

samples during storage compared with the control. There was low salt content in control and other treatments Karish cheese due to low percentage of salt used and the partially loses during the whey syneresis step. During the storage period the salt content increased slightly due to decreasing in cheese moisture content. The same trend was found by Hendy *et al.* (2023).

Table 6. Salt content of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Treatments	CSP (Day)			
	Fresh	10	20	30
C	0.50 ^{Ac}	0.56 ^{Ab}	0.62 ^{Aab}	0.70 ^{Aa}
T1c	0.49 ^{Ac}	0.56 ^{Ab}	0.61 ^{Aab}	0.69 ^{Aa}
T2c	0.49 ^{Ac}	0.54 ^{Ab}	0.60 ^{Aab}	0.69 ^{Aa}
T3c	0.50 ^{Ac}	0.54 ^{Ab}	0.59 ^{Aab}	0.68 ^{Aa}
T1s	0.49 ^{Ac}	0.56 ^{Ab}	0.61 ^{Ab}	0.69 ^{Aa}
T2s	0.50 ^{Ac}	0.55 ^{Ab}	0.60 ^{Ab}	0.68 ^{Aa}
T3s	0.50 ^{Ab}	0.54 ^{Ab}	0.60 ^{Ab}	0.68 ^{Aa}

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris* respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis* respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different.

a, b, c, d: Means with the same letter for the same row are not significantly different

The titratable acidity and pH value

The titratable acidity and pH value of functional Karish cheese is summarized in Table (7). There was an increase ($p < 0.05$) in acidity as expressed in % lactic acid by the addition of *Chlorella* and *Spirulina* compared to the control, this may be the *Chlorella* and *Spirulina* stimulated the growth of bacteria. These results agree with those reported by Akalin *et al.* (2009) and Darwish (2017). Bhowmik *et al.* (2009) recorded that the growth of lactic acid bacteria was enhanced by *S. platensis*. Antimicrobial effect of *S. platensis* on antifungal and pathogenic bacteria cause increase in the growth of lactic acid bacteria (Usharani *et al.*, 2015). In addition, the acidity of control and functional Karish cheese treatments increased significantly during the storage period ($P < 0.05$), this may be due to the activity of lactic acid bacteria.

Also, there were significant differences ($p < 0.05$) in pH values between treatments compared with control sample. The pH values of functional Karish cheese were found to gradually decrease with increasing algae Powder concentration and storage period at refrigerator temperature up to 30 days. This may be due to the stimulant effect of algae Powder on the starter or lactic acid bacteria

The data indicated that during the storage period there were slight pH values compared with the control. The acidity showed a slight increase, and pH decreased in all samples including control. During storage of cheese, the remaining lactose was converted into lactic acid by lactic acid bacteria, causing the pH to decrease. This result is agreed with Awad *et al.* (2012) who reported that during storage the

decreasing in pH values may be due to the hydrolysis happening in lactose and converting into lactic acid.

Table 7. The titratable acidity and pH value of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Treatments	Acidity (%)				pH value				
	Fresh	CSP (Day)				Fresh	CSP (Day)		
		10	20	30	10		20	30	
C	0.94 ^{Ac}	1.00 ^{Bc}	1.16 ^{Bb}	1.22 ^{ca}	4.47 ^{Aa}	4.38 ^{Aa}	4.16 ^{Ab}	4.12 ^{Ab}	
T1c	0.95 ^{Ad}	1.05 ^{ABc}	1.19 ^{ABb}	1.46 ^{Ba}	4.44 ^{Aa}	4.37 ^{Aa}	4.13 ^{Ab}	3.97 ^{Ab}	
T2c	0.97 ^{Ad}	1.11 ^{Ac}	1.24 ^{Ab}	1.64 ^{ABa}	4.40 ^{Aa}	4.28 ^{Aab}	4.10 ^{Ab}	3.88 ^{ABc}	
T3c	1.00 ^{Ad}	1.19 ^{Ac}	1.32 ^{Ab}	1.77 ^{Aa}	4.38 ^{Aa}	4.13 ^{Aab}	4.05 ^{Ab}	3.69 ^{Bc}	
T1s	0.96 ^{Ad}	1.06 ^{ABc}	1.21 ^{ABb}	1.47 ^{Ba}	4.43 ^{Aa}	4.35 ^{Aa}	4.12 ^{Ab}	3.96 ^{Ab}	
T2s	0.98 ^{Ad}	1.13 ^{Ac}	1.24 ^{Ab}	1.66 ^{ABa}	4.38 ^{Aa}	4.26 ^{Aab}	4.09 ^{Ab}	3.88 ^{ABc}	
T3s	1.02 ^{Ad}	1.21 ^{Ac}	1.39 ^{Ab}	1.79 ^{Aa}	4.35 ^{Aa}	4.10 ^{Ab}	4.02 ^{Ac}	3.66 ^{Bd}	

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris* respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis* respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different.

a, b, c, d: Means with the same letter for the same row in each value are not significantly different

Color evaluation of functional Karish cheese

The color of the products is important factor for consumer's perceptions as it effects on characteristic and quality of food products (Ghaeniand and Roomiani 2016) especially dairy products (Dönmez *et al.*, 2017). Color of Karish cheese fortified by algae powder samples are shown in Table 8. In the first storage period it was L* values, indicating brightness ranged from 44.86 to 53.10 and 34.68 to 47.73 for Karish cheese fortified *C. vulgaris* and *S. platensis*, respectively. The highest L* values was recorded in control sample which decreased significantly by increasing amounts of algae Karish cheese containing 1.5% *C. vulgaris* and *S. platensis* both separately was greenish this may be due to the green color of algae which change the color of Karish cheese (Table 8). These results agree with Ki jeon (2006) and Darwish (2017) who studied the effect of addition *Spirulina* and *Chlorella* on cheese color. *S. platensis* microalgae concentration causes whiteness reduction that affected color (Mazinani *et al.*, 2016). The a* values, indicating redness ranged from -15.99 to -14.26 and -16.35 to -15.68 in Karish cheese fortified with *C. vulgaris* and *S. platensis*, respectively. The control and fortified Karish cheese by algae showed negative a* values which decreased by increasing amounts algae powder. The b* value that indicating yellowness, ranged from 13.95 to 8.68 and 13.84 to 13.19 in Karish cheese fortified with *Chlorella* and *Spirulina*, respectively. The control Karish cheese presented the lowest b values (4.01) and rising in concentration of algae lead to the value raised. As the concentration of *Spirulina* powder increased, the yogurt drink's greenness increased, which reflected the negative a* value. This may be related to carotene and Chlorophyll Habib *et al.* (2008), Vijayarani *et al.* (2012), Dolly (2014) and Sharoba (2014).

Cold storage of Karish cheese samples at $5\pm 1^\circ\text{C}$ for 30 days increased the brightness of the cheese samples since L values were increased from average of 53.10 to the level of 55.95. of the cheese samples fortified with 0.5 algae powder *Chlorella vulgaris*. Control yoghurt sample showed the highest increase in brightness 81.27 compared with all treatment.

The Hue – value of the control Karish cheese sample was 165.74° , while that of the treatment samples fortified with *C. vulgaris* and *S. platensis* samples was in the range of 143.34° to 134.14° and 141.59° to 131.72° , respectively, being less green than the control samples.

Storage of Karish cheese samples for 30 days caused further reduction in green colour of all samples (142.29° , 138.83° , 133.12° , 140.55° , 135.39° and 130.68° respectively), while the change in color type of Control treatment from 165.74° to 165.60° was slightly and could be neglected.

Overall, the present study showed that addition microalgae powder (*C. vulgaris* and *S. platensis*) to Karish cheese samples decrease the a^* and L^* value and increased in b^* value by increasing amount of algae.

Table 8. Color parameters of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at $5\pm 1^\circ\text{C}$ for 30 days

CSP (Day)	Treatments	Color parameters					
		L^*	a^*	b^*	I	Chroma	Hue
Fresh	C	80.68	-11.73	4.01	82.29	16.23	165.74
	T1c	53.10	-14.26	8.68	53.94	9.49	143.34
	T2c	51.77	-15.58	12.03	53.18	12.17	139.88
	T3c	44.86	-15.99	13.95	47.21	14.72	134.14
	T1s	47.73	-15.68	13.19	49.67	12.77	141.59
	T2s	42.27	-15.87	13.50	44.43	14.69	136.42
	T3s	34.68	-16.35	13.84	38.37	16.43	131.72
30 days	C	81.27	-11.87	3.87	82.87	15.74	165.60
	T1c	55.95	-14.83	6.60	58.69	17.76	142.29
	T2c	54.20	-15.75	6.69	56.91	17.36	138.83
	T3c	47.71	-15.97	7.19	50.76	17.32	133.12
	T1s	50.40	-15.86	7.79	53.38	17.59	140.55
	T2s	45.41	-15.96	8.58	48.61	17.32	135.39
	T3s	38.68	-16.66	9.00	42.05	16.50	130.68

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris*, respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis*, respectively.

Evaluation of antioxidant activity, total phenolic and total flavonoid content of functional Karish cheese.

The phenolic and flavonoid compounds show extensive benefits to human health with various beneficial pharmacological effects like acting as antioxidants (López *et al.*, 2011). The obtained data in Table 9 showed that there are significant differences in TFC, TPC and scavenging activity among all treatments. Karish

cheese fortified with different types of microalgae powder (*C. vulgaris* and *S. platensis*) has antioxidant activity higher than control. This may be due to the high antioxidants activity in algae added to Karish Cheese. These results agree with (Darwish 2017) who revealed that significantly increasing ($P < 0.05$) of antioxidant in Karish cheese fortified by *S. platensis* due to increasing antioxidant activity of algae.

Table 9. Antioxidant activity, total phenolic and total flavonoid content of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5 ± 1 °C for 30 days

Samples	Storage Period	Mean (%) \pm SE		
		DPPH* inhibition (%)	TPC (mg GAE/g)	TFC (mg QE /g)
Control	Fresh	14.01 ^{Ea}	0.054 ^{Ea}	0.021 ^{Ea}
	10 days	11.83 ^{Eb}	0.048 ^{Eb}	0.018 ^{Eb}
	20 days	7.62 ^{Ec}	0.042 ^{Ec}	0.014 ^{Ec}
	30 days	4.59 ^{Ed}	0.033 ^{Ed}	0.007 ^{Ed}
T1c	Fresh	24.05 ^{Da}	0.070 ^{Da}	0.033 ^{Da}
	10 days	20.49 ^{Db}	0.065 ^{Db}	0.030 ^{Db}
	20 days	14.90 ^{Dc}	0.057 ^{Dc}	0.026 ^{Dc}
	30 days	7.11 ^{Dd}	0.047 ^{Dd}	0.019 ^{Dd}
T2c	Fresh	34.09 ^{Ca}	0.081 ^{Ca}	0.041 ^{Ca}
	10 days	29.64 ^{Cb}	0.075 ^{Cb}	0.037 ^{Cb}
	20 days	22.78 ^{Cc}	0.067 ^{Cc}	0.031 ^{Cc}
	30 days	13.22 ^{Cd}	0.057 ^{Cd}	0.022 ^{Cd}
T3c	Fresh	45.23 ^{Ba}	0.090 ^{Ba}	0.050 ^{Ba}
	10 days	40.31 ^{Bb}	0.084 ^{Bb}	0.046 ^{Bb}
	20 days	32.16 ^{Bc}	0.076 ^{Bc}	0.040 ^{Bc}
	30 days	22.44 ^{Bd}	0.066 ^{Bd}	0.031 ^{Bd}
T1s	Fresh	35.92 ^{Ca}	0.082 ^{Ca}	0.042 ^{Ca}
	10 days	30.48 ^{Cb}	0.077 ^{Cab}	0.039 ^{Cab}
	20 days	23.07 ^{Cc}	0.070 ^{Cb}	0.035 ^{Cb}
	30 days	12.78 ^{Cd}	0.061 ^{Cc}	0.028 ^{Cc}
T2s	Fresh	46.96 ^{Ba}	0.093 ^{Ba}	0.051 ^{Ba}
	10 days	41.09 ^{Bb}	0.087 ^{Bab}	0.047 ^{Bab}
	20 days	33.77 ^{Bc}	0.079 ^{Bb}	0.041 ^{Bb}
	30 days	24.95 ^{Bd}	0.069 ^{Bc}	0.032 ^{Bc}
T3s	Fresh	58.13 ^{Aa}	0.104 ^{Aa}	0.060 ^{Aa}
	10 days	52.29 ^{Ab}	0.098 ^{Aab}	0.056 ^{Aa}
	20 days	44.59 ^{Ac}	0.090 ^{Ab}	0.050 ^{Ab}
	30 days	33.88 ^{Ad}	0.081 ^{Ac}	0.041 ^{Ac}

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris*, respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis*, respectively.

DPPH: Radical scavenging capacity 2,2-diphenyl-1-picryl-hydrazyl

TPC: Total phenolic content, TFC: Total flavonoid content

A, B, C, D: Means with the same letter for the same column are not significantly different between treatment.

a, b, c, d: Means with the same letter for the same column are not significantly different between storage period in the same treatment

The highest content of DPPH, total phenols and total flavonoid were in Karish cheese fortified with *Spirulina platensis* followed by Karish cheese fortified with *Chlorella vulgaris* compared with control. The differences between all treatments related to parameters may be due to the differences in DPPH, TPC and TFC contents of added algae. A similar observation was reported by Swelem (2015).

The DPPH radical scavenging activity of control Karish cheese was 14.01% increased gradually as added algae was increased 0.5, 1.0 and 1.5% reached of 24.05%, 34.09% and 45.23 % respectively with *C. vulgaris*, and 35.92%, 46.96% and 58.13 % with *S. platensis*, respectively. The increase in chlorophyll content may be the reason for the removal of free radicals (Ismaiel *et al.*, 2016).

On the other hand, there was a significant decrease in antioxidant activity of all samples during storage period up to 30 days is possible that most of the degradation of total phenols resulted from oxidation reactions. The results are in harmony with those obtained by Davinder (2012) who found that there was a significant decrease in antioxidant activity and total phenol content of all ice cream samples during storage period up to 14 days, The decrease in antioxidant activity might be due to loss of bioactive compounds i.e. total phenols during storage. There was a significant ($p < 0.01$) effect of treatment and storage on reduction in total phenols. The loss of total phenols could be due to oxidation of phenols with extension in storage time.

Texture profile analysis of functional Karish cheese

The texture profile analyses of Karish cheese fortified by *C. vulgaris* and *S. platensis* are observed in Table 10. these values differ significantly ($p < 0.05$) among all Karish cheese which fortified by algae powder. Hardness cohesiveness, adhesiveness, gumminess and chewiness values recorded increased with increasing *C. vulgaris* and *S. platensis* to Karish cheese, but springiness values recorded decreased. Hardness increased from 4.33 N in control Karish cheese to 4.82 N, 4.87 N in Karish cheese containing 1.5% *C. vulgaris* and *S. platensis*, separately at fresh samples. The hardness increased in Karish cheese with increasing of algae due to raise of dry matter and retention of water (Mazinani *et al.*, 2016). This results in agreement with Darwish (2017) who clarified that the texture profile was significantly difference on Karish cheese as added microalgae 0.5%1.0 and 1.5% *Spirulina platensis*.

In all samples of the texture profile increased during storage periods except springiness values decreased that might be because of the proteolysis during storage, bringing about smoother and finer texture cheese (Molander *et al.*, 1990). In the present study texture analysis showed that Karish cheeses fortified by *C. vulgaris* and *S. platensis* at 0.5%1.0 and 1.5% improved the development of texture characteristics.

Table 10. Texture Profile Analysis of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

CSP (Day)	T	Parameters					
		Hardness N	Adhesiveness (mj)	Cohesiveness (B/A area) (Ratio)	Springiness mm	Gumminess N	Chewiness N/m
Fresh	C	4.33 ^{Bb}	0.42 ^{Ba}	0.271 ^{Bb}	3.35 ^{Aa}	1.173 ^{Bb}	3.930 ^{Bb}
	T1c	4.43 ^{Bb}	0.45 ^{Ba}	0.313 ^{Ab}	3.29 ^{Aa}	1.366 ^{Ab}	4.460 ^{Ab}
	T2c	4.66 ^{ABb}	0.47 ^{ABb}	0.315 ^{Ab}	3.25 ^{Aa}	1.448 ^{Ab}	4.571 ^{Ab}
	T3c	4.87 ^{Ab}	0.50 ^{Aa}	0.317 ^{Ab}	3.22 ^{Aa}	1.524 ^{Ab}	4.772 ^{Ab}
	T1s	4.41 ^{Bb}	0.49 ^{Ba}	0.322 ^{Ab}	3.37 ^{Aa}	1.389 ^{Ab}	4.584 ^{Ab}
	T2s	4.63 ^{AB}	0.54 ^{ABa}	0.327 ^{Ab}	3.33 ^{Aa}	1.468 ^{Ab}	4.786 ^{Ab}
	T3s	4.82 ^{Ab}	0.59 ^{Aa}	0.329 ^{Ab}	3.28 ^{Aa}	1.537 ^{Ab}	4.967 ^{Ab}
30 days	C	5.03 ^{Aa}	0.44 ^{Ba}	0.450 ^{Aa}	2.75 ^{Ab}	2.364 ^{Ba}	6.201 ^{Aa}
	T1c	5.14 ^{Aa}	0.48 ^{Ba}	0.555 ^{Aa}	2.59 ^{Ab}	2.424 ^{Aa}	6.420 ^{Aa}
	T2c	5.16 ^{Aa}	0.50 ^{ABa}	0.561 ^{Aa}	2.56 ^{Ab}	2.474 ^{Aa}	6.556 ^{Aa}
	T3c	5.19 ^{Aa}	0.53 ^{Aa}	0.569 ^{Aa}	2.53 ^{Ab}	2.534 ^{Aa}	6.787 ^{Aa}
	T1s	5.03 ^{Aa}	0.53 ^{Ba}	0.571 ^{Aa}	2.69 ^{Ab}	2.443 ^{Aa}	6.532 ^{Aa}
	T2s	5.05 ^{Aa}	0.58 ^{ABa}	0.589 ^{Aa}	2.65 ^{Ab}	2.498 ^{Aa}	6.699 ^{Aa}
	T3s	5.09 ^{Aa}	0.63 ^{Aa}	0.498 ^{Aa}	2.61 ^{Bb}	2.563 ^{Aa}	6.878 ^{Aa}

T: Treatments

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c, Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris*, respectively.T1s, T2s, T3s, Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis*, respectively.

A, B, C, D: Means with the same letter for the same column are not significantly different between treatment.

a, b, c, d: Means with the same letter for the same column are not significantly different between storage period in the same treatment

Sensory evaluation

Organoleptic properties of functional Karish cheese.

The results in Table (11) showed that there was significant difference ($P < 0.05$) among all Karish cheese samples fortified with algae. Karish cheese manufacture by adding 0.5% algae recorded the highest score compared with the other concentration compared with control in appearance. Increasing microalgae by more than 1.5% causes darken of Karish cheese and the primary experiment was rejected by consumers. The most acceptable flavor sample was control treatment then sample cheese fortified with 0.5%. 1.0% and 1.5%, respectively of *S. platensis* and *C. vulgaris*.

The most acceptable consistency sample was Karish cheese fortified by *C. vulgaris* and *S. platensis* %1.5 then 1.0% and 0.5%, respectively. These results agree with Mazinani *et al.* (2016) who found that the highest degree of consistency was in the Karish cheese fortified with 1.5% *S. Platensis*, due to the increase in dry matter by algae and water retention.

Table 11. Sensory evaluation (%) of functional Karish cheese fortified with *C. vulgaris* and *S. platensis* during storage at 5±1 °C for 30 days

Property	CSP (Day)	Treatments						
		Control	T1c	T2c	T3c	T1s	T2s	T3s
Appearance (10 points)	1	9.50	8.90	8.00	7.00	9.00	8.20	7.20
	10	9.00	8.30	7.50	6.50	8.60	7.80	6.60
	20	8.50	8.00	7.00	6.00	8.10	7.10	6.10
	30	7.50	7.00	6.50	5.50	7.40	6.60	5.70
Consistency (40 points)	1	37.10	38.20	38.10	38.80	37.00	37.50	38.00
	10	36.00	37.10	37.20	37.90	36.00	36.50	37.50
	20	35.10	36.20	36.20	37.00	35.00	35.50	36.00
	30	32.20	33.40	33.10	34.00	32.00	32.50	33.10
Flavor (50 points)	1	48.50	45.40	44.20	41.00	46.50	43.50	40.10
	10	47.00	42.20	41.30	38.20	43.50	40.00	37.50
	20	45.50	40.00	38.10	34.90	40.00	37.00	34.00
	30	42.50	37.10	34.20	31.00	38.50	33.00	30.10
Total score (100 points)	1	95.10 ^{Aa}	92.50 ^{ABa}	90.30 ^{Ba}	86.80 ^{Ca}	92.50 ^{ABa}	89.20 ^{Ba}	85.30 ^{Ca}
	10	92.00 ^{Aab}	87.60 ^{Bb}	86.00 ^{Cb}	82.60 ^{Db}	88.10 ^{Bb}	84.30 ^{Cb}	81.60 ^{Db}
	20	89.00 ^{Ab}	84.20 ^{Bc}	81.30 ^{Cc}	77.90 ^{Dc}	83.10 ^{Bc}	79.60 ^{Cc}	76.10 ^{Dc}
	30	82.00 ^{Ac}	77.50 ^{Bd}	73.80 ^{Cd}	70.50 ^{Dd}	77.90 ^{Bd}	72.10 ^{Cd}	68.90 ^{Dd}

CSP: Cold storage period

C: Karish cheese without any microalgae powder (Control treatment).

T1c, T2c, T3c: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris*, respectively.

T1s, T2s, T3s: Karish cheese fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis*, respectively.

Means in the same column with different small letters are significantly different ($p < 0.05$).

Means in the same row with different capital letters are significantly different ($p < 0.05$).

During the storage period, significant differences ($P < 0.05$) were observed between fresh and stored cheeses, after 30 days of storage there was evaluation a decrease in the sensory for all tested samples. This may be due to the development of acidity or the production of microbial metabolism that slightly affects the sensory and rheological properties of the product.

The total score for control was 95.10 points and Karish cheese fortified by microalgae powder samples T1c, T2c, T3c, T1s, T2s, and T3s were 92.50, 90.30, 86.80, 92.50, 89.20, and 85.30 respectively in fresh samples. Cheese samples containing algae powder had significantly higher total score in T1c and T1s compared with other cheese samples compared with control. The total sensory scores, reflecting the overall sensory evaluation of the products confirmed that, after Karish cheese sample containing algae powder 0.5% of gained the highest score as good as the control followed by Karish cheese sample containing algae powder 1.0% and 1.5%.

Generally, all cheese samples had good organoleptic properties, but cheeses fortified with 0.5% and 1.0% algae powder were the most preferred, and cheese samples with good functional properties were fortified with 1.5% algae.

Finally, the foregoing results led to the conclusion that, the use of 0.5%, 1.0% and 1.5% from microalgae powder of *C. vulgaris* and *S. platensis* led to improve the functional and nutraceutical properties of Karish cheese.

Conclusion

Based on our results, microalgae powder (*Chlorella vulgaris* and *Spirulina platensis*) with different concentrations could be used in the production of functional Karish cheese to increase nutraceutical value of the cheese as it rich with antioxidant. Our results showed that addition of algae powder 0.5, 1% and 1.5% to Karish cheese have significant effect of protein, total solids content and antioxidant activity compared with control sample. Furthermore, texture profile show differences ($P < 0.05$) among Karish cheese fortified with microalgae powder compared with the control sample. The panelists preferred Karish cheese samples enriched with microalgae (especially 0.5 and 1.0 % level), Consequently, enriched cheese by algae may have a great potential for the cheese industry to develop the shapes, texture, and functional products.

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تعزيز القيمة الغذائية والتركيبة الكيميائية للجبن القريش الوظيفي عن طريق إضافة مسحوق الطحالب الدقيقة (*Chlorella vulgaris* and *Spirulina platensis*)

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الملخص

تعتبر الطحالب الدقيقة أحد المصادر التي يمكن أن تعزز المحتوى الغذائي للأغذية التقليدية حيث إنها تؤثر بشكل إيجابي على صحة الإنسان، وذلك بسبب تركيبها الكيميائي الأصلي. تهدف الدراسة الحالية إلى إنتاج جبن قريش وظيفي مدعم بأنواع ونسب مختلفة من مسحوق الطحالب الدقيقة (*Spirulina Platensis* and *Chlorella vulgaris*) وتقييم مدى تأثير تلك الإضافة على خصائص المنتج. تم تدعيم الجبن القريش بـ *Spirulina Platensis* و *Chlorella vulgaris* بشكل منفصل في شكل مسحوق على ثلاثة مستويات: 0.5، 1.0، 1.5% وتم أخذ عينة جبن خالية من الطحالب الدقيقة كمجموعة كنترول. تم تقييم الخواص الحسية، التركيب الكيميائي، نسبة الملح، درجات اللون، النشاط المضاد للأكسدة، محتوى الفينول الكلي والكاروتينات الكلية والتقييم الريولوجي للجبن القريش المدعم بالطحالب الدقيقة. أوضحت النتائج أن إضافة الطحالب الدقيقة للجبن القريش إلى وجود اختلافات معنوية بالزيادة ($P < 0.05$) في الجوامد الصلبة الكلية والبروتين والرماد والحموضة وتحسين المظهر العام للجبن القريش المدعم بالطحالب الدقيقة (*Spirulina* و *Chlorella vulgaris* و *Platensis*) عند مقارنتها مع عينة الكنترول. أظهر الجبن القريش المدعم بالطحالب الدقيقة أعلى محتوى من النشاط المضاد للأكسدة، والفينول الكلي والكاروتينات الكلية بين جميع المعاملات. حصلت عينات الجبن القريش المدعم بالطحالب الدقيقة (خاصة عند مستوى 0.5 و 1.0%) على اعلي درجات للتقييم الحسي بعد الكنترول وبناءً على هذه النتائج، قد يكون للجبن المدعم بمسحوق من الطحالب الدقيقة إمكانية لاستخدامها في صناعة الجبن وذلك لتحسين الشكل والقوام والملحس وكذلك تحسين الخصائص الغذائية الوظيفية للمنتجات الغذائية.