Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 12, No. 2, 1-14, 2022
DOI: 10.30495/jfbt.2022.19331 https://dorl.net/dor/20.1001.1.22287086.2022.12.2.1.2

Characterization of a Traditional Egg-Free Crème Caramel Dessert Containing *Chlorella protothecoides*

F. Ziaziabari* [a], *V. Fadaei* [b]

[a] MSc Student of the Department of Food Science & Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
[b] Associate Professor of the Department of Food Science & Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

Received: 11 June 2020 Accepted: 10 June 2021

ABSTRACT: Crème caramel is sugar heated and turned into brown syrup to which milk and eggs are then added therefore each of its unit contains 200 kcal of energy for the consumer. *Chlorella protothecoides* is considered one of the commercially important microalgae due to its high nutritional potential and considerable energy content. This alga is used for the industrial production of lutein. Moreover, it contains high levels of fats, proteins, and fiber. It also has emulsifying properties for industrial applications. This research studied the effect of adding *Chlorella protothecoides* (0, 1, 2.5, and 4 gr/100 gr) on some properties of traditional crème caramel dessert. The results indicated that protein, calcium, phosphorous, and the oleic, linoleic, and linolenic fatty acids contents of the dessert increased when higher percentages of *Chlorella protothecoides* were added to its formulation ($p<0.05$). In general, the sample containing 4.0 gr/100 gr *Chlorella protothecoides* had higher overall acceptability score and titratable acidity, and more desirable nutritional properties compared to those containing lower percentages of this microalgae; it showed the maximum viscosity and protein content, the largest contents of carotenoid pigments, phosphorous, and calcium and the highest percentages of oleic, linoleic and linolenic fatty acids, and alpha-tocopherol.

Keywords: Chlorella protothecoides, Crème Caramel, Egg-Free, Nutritional Properties.

Introduction

Algae have received increasing attention for producing biologically active compounds exhibiting a wide range of activities such as antimicrobial (Guedesn *et al*., 2011; Abd El-Baky *et al*., 2009; Najdenski *et al*., 2013; Alves *et al*., 2016; Jun *et al*., 2015), antifungal (Najdenski *et al*., 2013), antioxidative (Abd El-Baky *et al*., 2009; Siriwardhana *et al*., 2008) and antitumor effects and hypotensive, hypoglycemic and hypolipidemic activities (Zhao *et al*., 2015). Microalgae are added as dietary supplements or natural edible dyes to pasta, snacks, or beverages; and they are also used in the production of functional edible oils that are rich in unsaturated essential fatty acids to be employed in food industry, especially for sea foods (Pulz and Gross, 2004).

Chlorella protothecoides is one of the commercially important microalgae species due to its high nutritional and energy potential that result from high

*Corresponding Author: vn.fadaei@gmail.com

photosynthetic productivity. It has great capability for industrial production of lutein and is a complete source of fat, protein, and fiber; and it also enjoys emulsifying properties. Its other applications are in the production of emulsified food products such as mayonnaise sauce and in bakery products such as cakes and cookies (Heredia-Arroyo *et al*., 2010).

Vegetable gums such as agar, carrageenan, and acacia gum are used as emulsifiers and stabilizers in the production of ordinary and low-calorie products in the frozen desserts industry (Joell and Amanda, 2006). Flavor and texture are of great importance in the production of desserts as Morais *et al*. (2014) reported that the most desirable sensory characteristics in milk chocolate desserts were their taste of chocolate milk, sweet flavor, and mouth-filling property. Consumption of large quantities of sugar causes various diseases, and that is why the dairy industry has reduced sugar contents in dairy products; hence, the quality stability of these products is of great importance (De Morais *et al*., 2015). Egg yolks have various applications in food formulations such as desserts, and no food ingredient can compete with their multi-functional properties (Li-Chan and Kim, 2008).

Crème caramel is sugar that has been heated and turned into brown syrup to which milk and eggs are then added. Many studies have been conducted so far on replacing sugar and fats in various desserts (Specter and Setser, 1994; René *et al*., 2003; Lethuaut *et al*., 2003; Joell and Amanda, 2006; Gonza´ lez-Toma *et al*., 2008; Bayarri *et al*., 2010; Morais *et al*., 2014). Some researchers have referred to replacement of eggs in desserts in their studies (Li-Chan and Kim, 2008; García *et al*., 2015). Raymundo *et al*. (2005) studied the properties of the microalgae *Chlorella vulgaris* as a fat mimic and emulsifier in emulsions. No report, however, has been published on the application of *Chlorella protothecoides* in food industry although food safety of whole algal flour (WAF) obtained from dried and powdered *Chlorella protothecoides* has been confirmed in rats and rodents (Szabo *et al*., 2012). It must be mentioned that replacement of eggs with *Chlorella protothecoides* in traditional crème caramel and improved nutritional value of ready-to-eat produced crème caramel desserts was studied for the first time in this research. Therefore, this research studied the nutritional effects of adding *Chlorella protothecoides*, as a substitute for eggs, on some of the properties of traditional crème caramel with lower sugar.

Materials and Methods

- *Additives*

Chlorella protothecoides powder was bought from Roquette Company, France, and transferred to the processing place in Iran under a dry, cool, and dark condition. carrageenan, milk, sugar, vanilla, and eggs were purchased from Milad Fakhr Iranian Co., Damdaran Co., Ghazvin Sugar Factory, Shahsavand Zarin Co. and Telavang Co., Iran, respectively.

- *Method of producing traditional crème caramel samples containing various percentages of Chlorella protothecoides*

As shown in Table 1, milk, vanilla, and sugar were mixed and heated to 95˚C, and *Chlorella protothecoides* was added to the mixture together with carrageenan, then poured into molds and placed in an oven at 180˚C for 25 minutes to be cooked; caramel syrup was then added. The dessert was packaged in 100-gram polyethylene bags and stored at refrigerator temperature

Table 1. Treatments used in the research

Ingredients* Treatments	Sugar	Carrageenan	Alga	Eggs	Caramel Syrup	Vanilla	Milk (with 1.5%Fat)
TD1	14.5	0	0	26.5	10	0.5	48.5
TD2	14	0.1	4	15.9	10	0.5	58.5
TD3	13.25	0.25	2.5	---	10	0.5	73.5
TD4	11.65	0.35	4	---	10	0.5	73.5

* gr/100 gr

(4˚C). It must be mentioned that the packages were almost impermeable to oxygen and light.

It should be noted that formulation of the treatments and percentages of the added *Chlorella protothecoides* were selected according to the results of sensory properties of pre-tested samples (unpublished data).

- *Chlorella protothecoides and crème caramel samples analysis*

The AACC 46-12.01 and the AACC 58-18.01 methods (AACC, 2011) were employed to measure the protein content and characterize the fatty acids profile in *Chlorella protothecoides* and crème caramel samples, respectively.

The AACC 80-68.01, AACC 30-16.01, AACC-86-06.01 methods (AACC, 2011) were conducted to measure the total sugar, total fat, and vitamin E, respectively. The Iranian National Standards No. 9266, 5533, 7149, 13579 and 10699 (Anon, 2007b; Anon, 2001; Anon, 2003a; Anon, 2010; Anon, 2007a) were used to measure minerals, water soluble vitamins (B1, B2, B3 and B5), vitamins A and D, and amino acids profile, respectively in *Chlorella protothecoides*. The Iranian National Standards No. 18544 and 2442 (Anon, 2024; Anon, 1995) were implemented for the qualitative study of *Chlorella protothecoides* flavor and color, respectively.

In the crème caramel samples, the Iranian National Standards No. 7123 and 2852 (Anon, 2003b; Anon, 2004) were used to determine beta-carotene and titratable acidity, respectively.

Viscosity of the samples was measured using a Brookfield viscometer (RV-DVII, USA) (De Wijk *et al*., 2003).

A panel of 50 untrained assessors evaluated the sensory properties (color, flavor, aroma, texture, and overall acceptability) of the samples based on a 5-point hedonic scale 24 hours after production. Since the final evaluation index is the overall acceptability, only its related results were reported in this research.

- *Statistical analysis*

The statistical analysis was performed using a completely randomized design and one-way ANOVA; and comparison of the means was carried out using Duncan's test at the significance level of 5% to determine the significant differences between various treatments using SPSS 18. The experiment was conducted with 4 treatments and there were 3 replications for each one.

Results and Discussion

- *Total protein measured 24 hours after sample production*

The analysis and characteristics of *Chlorella protothecoides*in Table 2. are presented Total protein declined from TD1 to TD3 ($p<0.01$) due to the reduction in the number or omission of eggs from the control to TD3 (Table 3). It must be

Table 2. Chlorella protothecoides characteristics used in producing traditional low-calorie Cream Caramel dessert samples containingmicroalga

Characteristics	Results
pH	6.7
Protein(gr/100gr)	14.2٠
Total Fat(gr/100gr)	54.3٠
Total Sugar (gr/100gr)	7
Calcium (mgr/100gr)	140
Phosphorus (mgr/100gr)	406
Iron (mgr/100gr)	0.47
Arsenic (mgr/100gr)	<0.2٠
Mercury (mgr/100gr)	<0.02
Cadmium (mgr/100gr)	<0.1٠
Lead (mgr/100gr)	<0.2٠
Vitamin A (IU/100gr)	11.5٠
Vitamin E (mgr/100gr)	78
Vitamin D (IU/100gr)	400
Vitamin B_1 (mgr/100gr)	3.5٠
Vitamin B_2 (mgr/100gr)	9
Vitamin B_3 (mgr/100gr)	30
Vitamin B_5 (mgr/100gr)	1
Myristic Fatty Acid (gr/100gr of total fatty acid)	0.96
Palmitic Fatty Acid (gr/100gr of total fatty acid)	16.5٠
Stearic Fatty Acid (gr/100gr of total fatty acid)	2.5٠
Oleic Fatty Acid (gr/100gr of total fatty acid)	60.31
Linoleic Fatty Acid (gr/100gr of total fatty acid)	8.31
Linolenic Fatty Acid (gr/100gr of total fatty acid)	1.68
Aspartic Amino acid (mg/100gr)	0.54
Glutamic Amino acid (mg/100gr)	1.26
AsparagineAmino acid (mg/100gr)	1.21
Serine Amino acid (mg/100gr)	0.99
Threonine Amino acid (mg/100gr)	0.53
Arginine Amino acid (mg/100gr)	0.51
Alanine Amino acid (mg/100gr)	0.69
Cysteine Amino acid (mg/100gr)	0.22
TyrosineAmino acid (mg/100gr)	0.40
IsoleucineAminoacid (mg/100gr)	1.11
Phenol alanineAmino acid (mg/100gr)	1.51
TyrosineAmino acid (mg/100gr)	2.11
TryptophanAmino acid (mg/100gr)	1.3
Color	Dark yellow
Taste	Taste Floury
Total Solid (gr/100gr)	97.2

*Numbers are an average of three replications.

mentioned that eggs contain about 13% protein (Belitz *et al.*, 2009). The addition of algae, at 2.5%, to TD3 was able to compensate this reduction to some extent but the protein content was still lower compared to TD1 (0 % algae). Comparison of TD3 and TD4 revealed that the protein content of the dessert increased by 1.76% when the *Chlorella protothecoides* content raised from 2.5 to 4% because this alga contains about 14.2% protein (Table 2). These results conform to those obtained by Mamatha *et al.* (2007) who showed that the protein content of a snack (called *Pakoda* in India) increased when it was enriched by marine algae

Enteromorpha compressa, and to those observed by Danesi *et al*. (2010) who used Spirulina algae as a protein supplement in bakery products, and to the results found by Prabhasankar *et al*. (2009) who observed that protein content of pasta increased when it was enriched with *Wakame* (a brown alga).

- ***Calcium measured 24 hours after sample production***

As can be seen in Table 3, calcium content significantly increased from TD1 to TD4 ($p<0.01$). This could be attributed to the increase in the percentage of alga used and the increased percentage of milk used in the formulation of the crème caramel dessert. Calcium content of *Chlorella protothecoides* is approximately 140 mg/100g (Table 2) while that of milk is about 120 mg/100g (Walstra, 2006). Therefore, it is expected that calcium content will increase when the algae and milk form higher percentages of the dessert formulation. In recent experiments, Gutierrez-Salmean *et al*. (2015) stated that Spirulina alga is rich in minerals (calcium, phosphorous, magnesium, iron, sodium, potassium, zinc, copper, manganese, and chromium), therefore, it can be used for enrichment of foods as it would increase the quantities of the mentioned minerals in them; Mamatha *et al*. (2007) increased the contents of iron and calcium in a kind of snack in India called Pakora by using the marine alga *Enteromorpha compressa*.

- ***Phosphorous measured 24 hours after producing the samples***

Table 3 indicates that phosphorous content decreased significantly from TD1 to TD4. This could be attributed to the reduction in the number of eggs in TD2 and their omission in TD4 because phosphorous constitutes 0.58-0.98 percent of the egg yolks weight (Belitz *et al*., 2009). The phosphorous content of TD4 was significantly higher than that of TD3. This was related to the increase in the percentage of algae used on one hand and to the increase in the percentage of milk used in the formulation of the dessert on the other hand. It must be mentioned that these algae are rich in phosphorous and their phosphorous content is 406 mg/100g (Table 2), while cow's milk has a phosphorous content of 96.3 mg/100g (Walstra, 2006).

- ***Beta-carotene measured at 24-hour intervals for three days***

Table 4 shows that there was a significant reduction in color from TD1 to TD2 ($p<0.01$). This could be attributed to the reduced number of eggs in TD2 as egg yolk contains 3.89 ppm carotenoids, especially the lutein pigment which is one of the factors causing the yellow color in egg yolk (Belitz *et al*., 2009). In egg yolks, which have the average weight of 17-19 grams, the lutein and zeaxanthin contents are 292±117 µg and 213±85 µg, respectively (Abdel-Aal *et al*., 2013). Moreover, a significant increase in the color of crème caramel dessert was observed from TD3 to TD4, which could be due to the increase in *Chlorella protothecoides* content in the samples. These algae are one of the important sources of carotenoids pigments, especially beta carotene and lutein (Wei *et al*., 2008); therefore, the yellow color could be observed in TD3 and TD4 although they did not contain any eggs. Fradique *et al*. (2010) enriched pasta using different quantities of microalgae (*Chlorella vulgaris* and *Spirulina maxima*) and observed that the color of the pasta which contained microalgae remained relatively stable after it was cooked. Gouveia *et al.* (2007) reported that the green color of cookies containing 1%

Table 3. Average values of protein (gr per 100 gr), calcium, phosphorus (mg per 100 gr) and overall acceptability in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* 24 hours after production*

Treatment Parameters	TD1	TD2	TD3	TD4	Standard Deviation
Protein	7.77 [a]	5.13 [b]	2.66 [c]	4.42 [d]	2.12
Calcium	84.64 [a]	89.33 [b]	93.83 [c]	96.03 [d]	5.052
Phosphorus	136.33 [a]	110.35 [b]	80.92 [c]	87.02 [d]	25.34

*In each row and column, different superscript letters denote significant differences between samples (p<0.05).

Table 4. Average values of Color (mg per 100 gr In terms of beta-carotene) in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* during 3 days at intervals of 24 hours*

Day Treatments	1	2	3
TD1	0.51[a]	0.49[a]	0.51[a]
TD2	0.42[b]	0.41[b]	0.41[b]
TD3	0.46[c]	0.45[c]	0.46[c]
TD4	0.60[d]	0.60[d]	0.59[d]
Standard Deviation	0.079	0.082	0.078

* In each row and column, different superscript letters denote significant differences between samples (p<0.05).

Chlorella vulgaris exhibited maximum stability during storage. Moreover, Powell *et al*. (1961) stated that addition of *Chlorella vencedesmus* to ginger bread, chocolate cake, and cookies changed the color of the products.

In none of the produced crème caramel samples was any change in color intensity observed during storage because the pigments in *Chlorella* protothecoides are of the carotenoids type and form complexes with proteins that, in general, are highly resistant. In addition, during the 3-day storage period the samples were kept in packages impermeable to oxygen, which is the most destructive factor among those destroying these pigments (Belitz *et al*., 2009). Results of the present research conform to those of Gouveia *et al*. (2007) and of Fradique *et al*. (2010) who found the color stability in *Chlorella vulgaris* and *Spirulina maxima* containing cookies and macaroni, respectively, during storage.

- ***Profile of fatty acids measured at 24-hour intervals for 3 days:***

The contents of myristic and myristoleic fatty acids (Tables 5 and 6) increased significantly from TD1 to TD4 (p<0.01). This could be attributed to the increased percentage of the dessert formulation formed by the algae on one hand and the increased percentage of milk used in the formulation on the other hand. It must be mentioned that myristic acid in *Chlorella protothecoides* forms 0.96 percent of the total fatty acids (Table 2), while the corresponding figure for cow's milk is 9.5 percent of the total fatty acids (Tamime, 2009). No tangible changes occurred in the contents of these fatty acids during storage because myristic acid has no covalent bonds and is resistant to oxidizing agents, oxygen and light. Since the quantity of vitamin E in the samples increased due to the presence of *Chlorella protothecoides*, which has antioxidant properties, myristoleic acid was protected against oxidation (Belitz *et al*., 2009).

As shown in Table 5, contents of palmitic and stearic fatty acids decreased significantly from TD1 to TD4 (p<0.01). This could be attributed to the reduced number of eggs from TD1 to TD2 and the

Table 5. Average values of myristic, palmitic and stearic fatty acids (gr per 100 gr) in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* during 3 days at intervals of 24 hours*

Day	**1**			**2**			**3**		
Treatments	myristic	palmitic	stearic	myristic	palmitic	stearic	myristic	palmitic	stearic
TD1	0.051[a]	1.143[a]	0.496[a]	0.051[a]	1.143[a]	0.496[a]	0.053[a]	1.145[a]	0.493[a]
TD2	0.095[b]	1.033[b]	0.301[b]	0.097[b]	1.032[b]	0.301[b]	0.096[b]	1.033[b]	0.301[b]
TD3	0.136[c]	0.969[c]	0.185[c]	0.136[c]	0.968[c]	0.185[c]	0.135[c]	0.970[c]	0.182[c]
TD4	0.155[d]	0.886[d]	0.213[d]	0.155[d]	0.886[d]	0.211[d]	0.156[d]	0.885[d]	0.213[d]
Standard deviation	0.045	0.108	0.140	0.046	0.108	0.140	0.045	0.108	0.140

[*] In each row and column, different superscript letters denote significant differences between samples(p<0.05)

Table 6. Average values of myristoleic, oleic, linoleic and linolenic fatty acids (gr per 100 gr) in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* during 3 days at intervals of 24 hours*

Day	**1**				**2**				**3**			
Treatments	myristolec	Oleic	linoleic	linolenc	myristoleic	oleic	linoleic	linolenic	myristoleic	oleic	linoleic	linolenic
TD1	0.005[a]	1.238[a]	0.255[a]	0.005[a]	0.003[a]	1.283[a]	0.254[a]	0.003[ab]	0.003[a]	1.281[a]	0.253[a]	0.002[abc]
TD2	0.007[b]	1.341[b]	0.299[b]	0.006[b]	0.006[b]	1.340[b]	0.299[b]	0.005[bc]	0.006[b]	1.341[b]	0.299[b]	0.003[bcd]
TD3	0.011[c]	1.420[c]	0.324[c]	0.008[c]	0.012[c]	1.419[c]	0.323[c]	0.007[cd]	0.013[c]	1.420[c]	0.321[c]	0.005[cde]
TD4	0.014[d]	1.855[d]	0.497[d]	0.013[d]	0.013[d]	1.854[d]	0.495[d]	0.012[dc]	0.015[d]	1.854[d]	0.493[d]	0.011[dce]
Standard Deviation	0.004	0.259	0.105	0.003	0.004	0.259	0.105	0.003	0.005	0.259	0.106	0.003

[*] In each row and column, different superscript letters denote significant differences between samples(p<0.05).

omission of eggs from T2 to T4. Palmitic and stearic fatty acids form 25.96 and 9.6% of the total fatty acids in eggs, respectively (Celebi and Macit, 2008). Although eggs were completely replaced by *Chlorella protothecoides* in TD3 and TD4, yet the quantities of these fatty acids in this alga was not high enough to compensate for the lack of eggs. Palmitic and stearic fatty acids in *Chlorella protothecoides* form 16.5 and 2.5% of the total fatty acids, respectively (Table 2), while in cow's milk they form 26.3 and 14.6% of the total fatty acids, respectively (Tamime, 2009). During the storage, no tangible reduction happened in the amounts of these two fatty acids because they do not have covalent bonds and, therefore, are resistant to oxidizing agents (Belitz *et al*., 2009).

Oleic acid content significantly increased from TD1 to TD4 ($p<0.01$) probably due to the higher content of the alga in the samples (Table 6). In *Chlorella protothecoides*, oleic acid constitutes 60.31% of the total fatty acids (Table 2). Although there were eggs in TD1 and TD2, yet oleic acid accounts for 38.68% of the total fatty acid in eggs (Celebi and Macit, 2008), which is lower compared to *Chlorella protothecoides*. Moreover, oleic acid makes up 29.8% o the total fatty acids in milk (Tamime, 2009). During the storage of the samples, no tangible reduction was observed in the quantity of oleic acid as the content of vitamin E increased due to the presence of *Chlorella protothecoides*. This vitamin shows antioxidant properties which protects this fatty acid against oxidation (Belitz *et al*., 2009).

As shown in Table 6, the contents of linoleic and linolenic acids increased significantly from TD1 to TD4 ($p<0.01$). The reasons for this increase could be the higher content of the alga in the samples on the one hand and the increase in milk content of the samples on the other hand. As shown in Table 2, linoleic and linolenic acids constitute 8.31 and 1.68% of the total fatty acids in *Chlorella protothecoides*, respectively, while the corresponding figures for milk are 2.4 and 0.8% of the total fatty acids, respectively (Tamime, 2009). During the storage of the samples, the quantity of linolenic acid declined. The instability of this fatty acid in the oxidation process is due to the presence of the three covalent bonds in its structure. Furthermore, this fatty acid can enter the process of flavor reversion (which reduces its content) even in the presence of slight quantities of oxygen, and cause the development of an undesirable flavor in dairy products (Damodaran *et al*., 2007). TD4 had a high content of *Chlorella protothecoides* (about 4%). However, less change was observed in linoleinc acid contents in TD1 and TD2 due to the fact that they contained eggs and high levels of carotenoid pigments, that have antioxidant properties (Belitz *et al*., 2009).

In agreement with results of the present research regarding the contents of fatty acids in dessert samples enriched with alga, Gouveia *et al*. (2008a) showed that oleic, palmitic and linoleic acids were the dominant fatty acids in cookie samples enriched with *Spirulina platensis* and the quantity of γ-linolenic acid significantly increased when the microalgae formed a higher percentage in the formulation of the cookies. Gouveia *et al*. (2008b) confirmed the presence of omega-3 fatty acids in biscuit samples enriched with the microalga *Isochrysis galbana.* Vahmani *et al*. (2013) reported that microalga supplementation improved fatty acid composition of cows' milk. Prabhasankar *et al*. (2009) showed that cookies containing *Spirulina platensis* had greater nutritional value compared to the control because of the increase in the content of γ-linolenic acid.

Chen *et al*. (2007), Tichivangana and Morrissey (1985) and Arab-Tehrany *et al*. (2012) reported that during storage, the content of fatty acids with one or more covalent bonds exhibited the maximum reduction compared to the saturated fatty acids in rainbow trout muscle, muscle systems and fish oil, respectively. These results conform, in general, to those of the preset research about changes in the dessert samples during their storage. Gomez-Estaca *et al*. (2017) also reported that after storage for 120 days at room temperature, no reduction occurred in the content of the dominant fatty acids of shrimp extract which were mainly omega-3 and palmitic fatty acids.

- ***Titratable acidity measured at 24-hour intervals for 3 days:***

Titratable acidity (Table 7) increased slightly from TD1 to TD4. This increase might be attributed to the compounds present in the dessert such as proteins, vitamins B, A and K, and gamma-linolenic acid that stimulate acid production by lactic acid bacteria (Ásványi-Molnár *et al*., 2009). Moreover, eggs, especially egg yolks, are very acidic because they contain large amounts of free fatty acids, phospholipids and lecithin. Therefore, lower acidity was expected in TD2 because it contained fewer eggs. However, there was no reduction in acidity due to the 1% algae ingredient and, as a matter of fact, acidity increased. The microalgae species *Chlorella protothecoides* contains a high percentage of the amino acid lysine (2.11%) and its pH is about 6.7 (Table 2). The α-amino group of lysine takes part in the maillard reaction and its α-carboxylic acid group remains free which can increase acidity. Akalin *et al*. (2009) stated that use of spirulina to enrich yoghurt enhanced *Lactobacillus bulgaricus* viability thus increased the acidity of the sample. Ásványi- Molnár *et al*. (2009) reported a significant increase in acidity of the products during fermentation by lactic acid in the presence of spirulina powder. However, Beheshtipour *et al*. (2013) concluded that spirulina stabilized pH of food products by playing a buffering role.

There was a slight increase in acidity during storage time that could be attributed to the activity of lactic acid bacteria (Ásványi-Molnár *et al*., 2009). Some researchers have pointed to the buffering role of *Spirulina platensis* resulting from the proteins, peptides and amino acids it contains and reported that the descending trend in pH of dairy products containing microalgae during their storage time was a minor one compared to the control (Varga *et al*., 2012; Guldas and Irikh, 2020).

- *Viscosity measured at 24-hour intervals for 3 days:*

The apparent viscosity of the samples (Table 8) exhibits a substantial decrease from TD1, which contains eggs, to TD3, which does not contain any eggs (p<0.05). This decline can be attributed to the fact that TD3 was egg-free. Eggs contain proteins such as ovomucin that increases the viscosity. In addition, the presence of ovalbumin, which has a very high gelling capacity, in eggs also enhances viscosity in desserts. There is a considerable increase in viscosity from TD3 to TD4, which is associated with the increase in the content of *Chlorella protothecoides* microalgae from 2.5% to 4%. The dry matter of this microalgae species is 97.2% (Table 2) and its addition to desserts increases their dry matter contents. Consequently, the viscosity of egg-free crème caramel containing *Chlorella protothecoides* increases. It must be mentioned that TD4 contained the largest amount of milk among the treatments and milk, due to its protein and fat content, contributes to the increase in viscosity (Walstra *et al*., 2006).

Table 7. Average values of titratable acidity i(%) n the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* during 3 days at intervals of 24 hours*

Day Treatments	1	2	3
TD1	0.05[a]	0.04[a]	0.24[a]
TD2	0.07[a]	0.07[a]	0.08[a]
TD3	0.11[a]	0.11[a]	0.12[a]
TD4	0.14[a]	0.15[a]	0.15[a]
Standard Deviation	0.04	0.04	0.06

* In each row and column, different superscript letters denote significant differences between samples (p<0.05).

Table 8. Average values of viscosity (Pa.s) in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* during 3 days at intervals of 24 hours*

Day Treatments	1	2	3
TD1	88.66[a]	89.33[a]	90.33[ab]
TD2	86.33[b]	86.33[b]	87.33[bc]
TD3	85.33[c]	84.66[c]	86.33[cd]
TD4	87.66[d]	88.33[d]	89.33[dc]
Standard Deviation	1.465	2.081	1.825

* In each row and column, different superscript letters denote significant differences between samples (p<0.05)

During the storage time, the viscosity of the dessert samples increased ($p<0.05$). On the second and third days of storage, the viscosity values of TD1, TD2, TD3 and TD4 were 89.33, 86.33, 84.66, 88.33 and 90.33, 87.33, 86.33, and 89.33 (Pa s), respectively. Therefore, the viscosity of the product was significantly higher on the third day of storage on compared to the second. The reason for this increase might be the decrease in moisture due to its absorption by the microalgae, resulting from their high dry matter content (Sung *et al*., 2004).

- *Overall acceptability measured 24 hours after producing the samples:*

The results of overall acceptability (Table 9) indicate that there were no significant differences between the samples ($p>0.05$). The reason for this is that the carotenoid in *Chlorella protothecoides* is similar to lutein, the dominant pigment in eggs, that is used in the common crème caramel formulations. The caramelized sugar is the main ingredient giving crème caramel its aroma and taste and this microalga species has no dominant taste or aroma (Table 2). As for texture, since the microalgae absorb water (Sung *et al*., 2004) and create suitable consistency as the common dessert formulations do, no considerable differences were observed. Consequently, TD4 had a slightly higher overall acceptability score than the other samples.

One of the disadvantages of using microalgae in food products is their undesirable sensory properties. Many researchers have suggested that small quantities of microalgae be used to improve overall yoghurt acceptability (Guldas and Irkin, 2010; Akalin *et al*., 2009). Studies by Ásványi-Molnár *et al*. (2009) on low-fat yoghurt indicated that the highest sensory score was received by the yoghurt sample containing 3% spirulina powder together with a mixture of 1.5% of a kiwi fruit, strawberry and 10% sugar. Beheshtipour *et al*. (2012) suggested addition of fruit purees and oligosaccharides to mask the flavor of *Spirulina platensis*. It was reported that microalgae content of higher than 0.5% in the yoghurt led to consumer perception of sandiness influenced by the presence of agglomerates in the fermented milk.

Table 9. Average values of overall acceptability in the traditional low-calorie cream caramel dessert samples containing *Chlorella protothecoides* 24 hours after production

Day Treatments	1
TD1	4.18±0.84[a]
TD2	4.36± 0.76[a]
TD3	4.4± 0.60[a]
TD4	4.52± 0.60[a]

* In each column, the same superscript letter doesn't denote any significant differences between samples ($p>0.05$)

Conclusion

This research studied the effect of adding *Chlorella protothecoides* (0, 1, 2.5, and 4 gr/100 gr) on some properties of traditional crème caramel dessert. The low-calorie traditional crème caramel sample containing 4% *Chlorella protothecoides* alga had the maximum protein, viscosity, carotenoid pigments, vitamins D and E, phosphorous and calcium contents and the minimum sugar content and the highest percentages of oleic, linoleic and linolenic fatty acids. This sample had a slightly higher overall acceptability score than the other samples.

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