J. of Food and Dairy Sci., Mansoura Univ., Vol. 16 (7):119 - 127, 2025

Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Comparative Study on the Impact of *Spirulina*, *Chlorella*, and *Nannochloropsis* on Custard Quality Attributes

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Article Information
Received 1/ 6 /2025
Accepted 2/7 /2025

ABSTRACT

Microalgae are considered a rich and complete nutritional source due to their high content of proteins, carbohydrates, fats, vitamins, minerals, fibers, and bioactive compounds with various health benefits.The aim of this study is fortification of custard with powder of *Spirulina, Chlorella*, and *Nannochloropsis* and study the effect of addition of them to custard at 0.5, 1, 1.5,and 2%(W/W) on chemical, color,rheological and sensory properties.The results showed that, when powder of *Spirulina, Chlorella*, and *Nannochloropsis* was added to custard samples, the amount of protein, fat, fiber and total energy value in functional custard samples increased compared with control. As the amount of *Spirulina,Chlorella*, and *Nannochloropsis* powder increased, the moisture content decreased. L* of custard samples fortified with *spirulina* powder was lower than those of custard samples fortified with powder of *Chlorella* and *Nannochloropsis*.The results from this study showed that the addition of *spirulina*, *Chlorella* and *Nannochloropsis* powder to custard samples reduced the lightness, redness and yellowness values. All treated custard samples showed higher viscosity and hardness than control. The bioactive compounds content in the studied samples increased with increasing microalgae powder. Custard samples fortified with 0.5, 1, 1.5, and 2% powder of *Nannochloropsis* receiving the highest scores in sensory evaluation.The findings of the study suggest that *spirulina* powder can be incorporated into custard formulations at levels up to 0.5%,whereas *Nannochloropsis* and *Chlorella* powders can be included at levels up to 2%, in order to enhance the functional value of the product while maintaining acceptable sensory properties as evaluated by the panelists.

Keywords: *Spirulina, Chlorella, Nannochloropsis, Chemical, Rheological, Sensory Properties and Custard.*

INTRODUCTION

In recent decades, there has been a significant rise in interest toward foods rich in protein, dietary fiber, and antioxidants, due to their well-established health benefits (Tyagi *et al*., 2007). This growing awareness has contributed to the development of a large market for nutritious products and ingredients that are high in fiber and antioxidants. Custard is a popular food product consumed globally and is typically made from ingredients such as milk, sugar, colorants, and may also be fortified with essential vitamins and minerals. In many developing tropical countries, custard is commonly used as a cereal-based breakfast or as a weaning food, especially for children (Ihekoronye & Ngoddy, 1985). However, as traditional custard is mainly carbohydrate-based, there is a clear need to enhance its nutritional value by incorporating protein-rich and micronutrient-dense ingredients (Okoye *et al.,* 2008).

In recent years, significant efforts have been directed toward enhancing the nutritional quality and functional properties of custard by enriching its composition with proteins, minerals, and dietary fiber. Additionally, modifications aim to improve the viability and efficacy of probiotics within the final product. This has resulted in intense market competition to cater for consumer demand for natural, health-promoting, and functional food products. The growing public awareness of nutrition has contributed to a heightened interest in foods abundant in antioxidants, which play a crucial role in the prevention of various cancers and cardiovascular diseases. Among these bioactive compounds, phenolic substances are recognized as potent natural antioxidants. Consequently, the consumption of foods rich in phenolics is considered beneficial for boosting the body's antioxidant defenses (Sudha *et al*., 2007).

For centuries, microalgae have been part of the traditional diet in various Asian countries and are widely regarded as nutritious due to their high levels of vitamins, minerals, proteins, fibers, and other biologically active compounds (Barba, 2017; Niccolai *et al*., 2019 and Abdel-Moatamed *et al*., 2025). Research indicates that the protein quality in microalgae is superior to that found in many plant-based sources (Mendes *et al*., 2007). In recent years, the global microalgae sector has experienced remarkable growth, with microalgae increasingly being used in nutraceuticals and as functional ingredients in both food and animal feed (Batista *et al*., 2017). Because of their rich content of minerals, polyunsaturated fatty acids (PUFAs), antioxidants, pigments, and amino acids, microalgae are considered sustainable and valuable ingredients for the production of healthy, clean-label food products (Cabrol *et al*., 2023).

Chlorella species are among the most common types of green microalgae. Various meta-analyses have shown that *Chlorella* supplementation can significantly improve health markers such as total cholesterol, low-density lipoprotein (LDL) cholesterol, systolic and diastolic blood pressure, and fasting blood glucose levels. These health benefits are attributed to the synergistic effects of the numerous nutrients and antioxidants found in *Chlorella* (Bito *et al*., 2020).

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DOI: 10.21608/jfds.2025.390314.1203

Moreover, *Chlorella vulgaris* may also promote muscle regeneration, which could be an effective strategy to prevent sarcopenia, especially in individuals with dysphagia (Azlan *et al*., 2019; Abdel-Moatamed *et al*., 2025).

Spirulina (*Arthrospira platensis*) is a type of blue-green algae that grows naturally in warm, alkaline freshwater environments. It is cultivated worldwide and consumed as both a dietary supplement and a whole food, available in tablet, flake, and powder forms. *Spirulina* offers a wide range of health benefits, including immune system enhancement, cholesterol reduction, cardiovascular support, diabetes management, wound healing, digestive improvement, and relief from depression and anxiety. Additionally, it serves as a natural detoxifying agent by oxygenating the blood and helping to eliminate toxins and other harmful substances from the body. Research has also highlighted its therapeutic potential, including anti-cancer properties (Mao *et al*., 2005), lipid-lowering effects (Narmadha *et al*., 2012), and protective roles against diabetes and obesity (Anitha & Chandralekh, 2010).

Among the various recommended microalgal strains, species belonging to the genus *Nannochloropsis* should be prioritized due to their notably high levels of polyunsaturated fatty acids, antioxidants, carotenoids, polyphenols, and essential vitamins, as well as their compatibility with intensive cultivation systems (Zanella & Vianello, 2020). The green microalga *Nannochloropsis* is widely recognized for its potential in biochemical, nutraceutical, and pharmaceutical applications (Shah & Abdullah,2018). This species is particularly abundant in proteins and polyunsaturated fatty acids, along with antioxidant pigments that significantly contribute to the suppression of free radical overproduction by enhancing the antioxidant defense mechanism (Selvendran, 2013). Furthermore, *Nannochloropsis* has been reported to contain substantial amounts of sterols, which may play a role in promoting anti-inflammatory and anticancer effects (Sanjeewa *et al*., 2016).

The enrichment of custard with microalgae has attracted significant scientific interest due to the high protein, dietary fiber, and antioxidants content of these organisms (Ampofo & Abbey, 2022 and Abdel-Moatamed *et al*., 2025). As noted by Khanra *et al*., (2018), approximately 50% of the global protein and peptide supply is currently derived from terrestrial plants; however, by 2054, this may be partially replaced by alternative protein sources such as microalgae and insects. In line with this trend, peptides from microalgae such as *Chlorella*, *Nannochloropsis*, Tetraselmis, and *Spirulina* have been increasingly studied for their potential applications (Levasseur *et al*., 2020).

The present study aimed to fortify custard with powder of *Spirulina*, *Chlorella*, and *Nannochloropsis* and to evaluate the impact of the incorporation of *Spirulina*, *Chlorella*, and *Nannochloropsis* powder on the chemical, color, rheological and sensory characteristics of such functional custard.

MATERIALS AND METHODS

Custard powder

Custard powder was produced by Dreem Mashreq Foods, located in New Borg El-Arab, Alexandria, Egypt. It was procured from a local retail outlet in Damanhour City, Beheira Governorate, Egypt.

Microalgae Source

Three dried microalgae species *Spirulina platensis*, *Chlorella vulgaris*, and *Nannochloropsis salina* were obtained from the Algal Biotechnology Unit at the National Research Centre, Dokki, Egypt. Chemical composition of the microalgal powder in Table (1).

Buffalo's Milk Source

Buffalo's milk used in this study was collected from Damnhour Agriculture Secondary School in Damnhour, Egypt. The milk had the following composition: 7.6% fat, protein 4.20%, lactose 5%, ash 0.75% and 9.88% solids-not-fat (SNF).

Experimental procedure

Buffalo's milk (7.6% fat and 9.88 % SNF) was heated at 72±1 °C/ 15 sec and cooled to 4±1 °C. Divided into 13 parts, each part 400ml milk (Figure 1); Addition to each part 25g custard valnia +40g sugar and T1: with 0.5% *Spirulina platensis* powder ; T2: with 1% *Spirulina platensis* powder; T3: with 1.5% *Spirulina platensis* powder; T4: with 2% *Spirulina platensis* powder ;T5: with 0.5% *Chlorella vulgari* powder; T6: with 1% *Chlorella vulgari* powder; T7: with 1.5% *Chlorella vulgari* powder; T8: with 2% *Chlorella vulgari* powder; T9: with 0.5% *Nannochloropsis salina* powder; T10: with 1% *Nannochloropsis salina* powder; T11: with 1.5% *Nannochloropsis salina* powder; T12: with 2% *Nannochloropsis salina* powder.

Chemical Analyses

The protein, moisture, fat, fiber and ash contents of each of the custard samples were analyzed in triplicates according to the methods of AOAC (2003). The carbohydrate was estimated by difference (Cort *et al*., 1986). The total energy value of the custard samples was calculated based on the proximate composition, using the method reported by Passmore and Eastwood (1986).

Color Assessment

Color measurements were performed according to the Hunter Associates Laboratory protocol (2012), using tristimulus values (X, Y, and Z) obtained via a Hunter Lab spectrophotometer (model MOM-D-100, Hungary). Each sample was analyzed in at least three replicates. The X, Y, and Z values were converted to Hunter L*, a*, and b* values using the manufacturer's formula. Chroma (C) was computed using the equation: $C = \sqrt{(a^2 + b^2)}$, while the hue angle was calculated as arctangent (b/a), adjusted by adding 180°.

Water holding capacity (WHC) measurement

The water holding capacity after storage at 4 °C for five days was assessed using the method described by Spada *et al*. (2015).

Total Phenolic Content (TPC)

Total phenolic content in the examined samples was evaluated using a modified Folin–Ciocalteu method, as previously outlined by Gajula *et al*. (2009).

Chlorophyll A and B Estimation

Chlorophyll A and B were determined spectrophotometrically at a wavelength of 665 and 649 nm. Calculations were performed using the formulas reported by Seely *et al*. (1972).

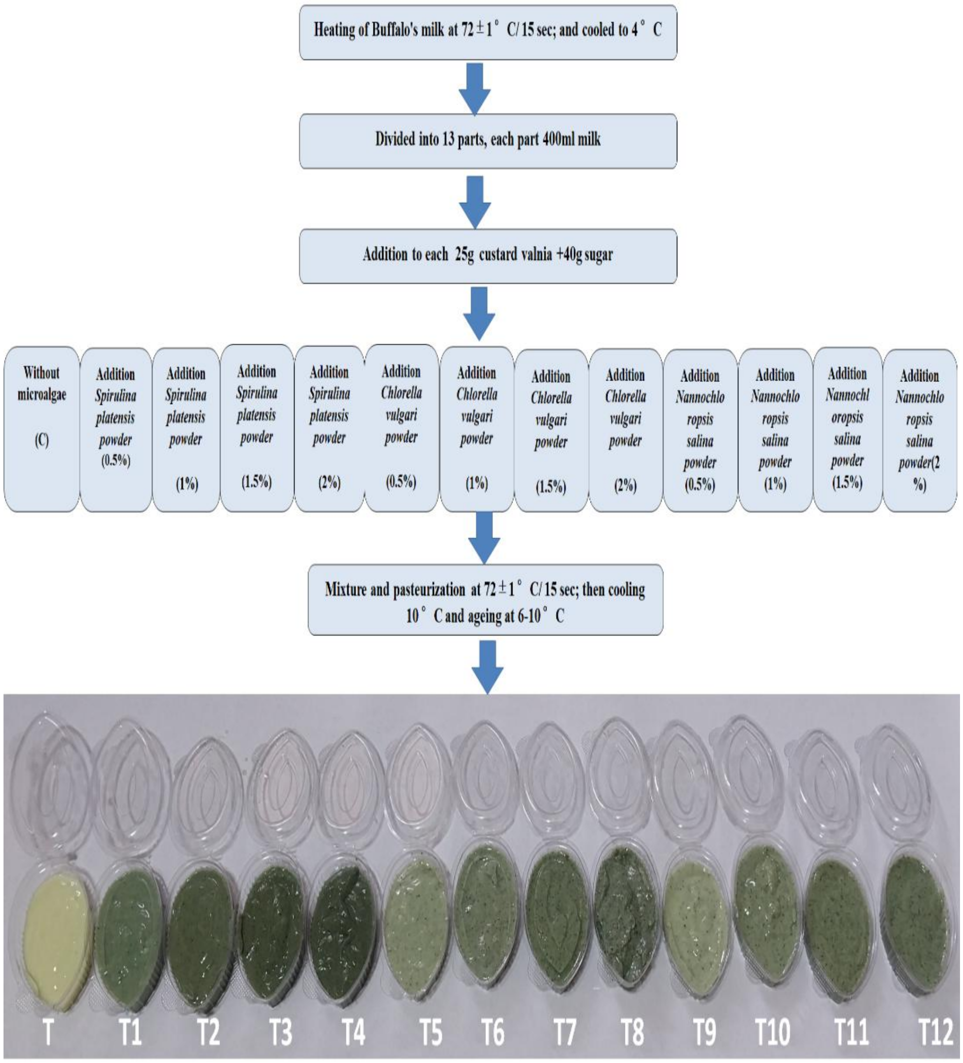

Fig. (1): Flow chart for the production of Custard Fortified with Powder of *Spirulina*, *Chlorella*, and *Nannochloropsis*. T: without microalgae; T1: with 0.5% *Spirulina platensis* powder ; T2: with 1% *Spirulina platensis* powder ; T3: with 1.5% *Spirulina platensis* powder ; T4: with 2% *Spirulina platensis* powder ;T5: with 0.5% *Chlorella vulgari* powder ; T6: with 1% *Chlorella vulgari* powder ; T7: with 1.5% *Chlorella vulgari* powder ; T8: with 2% *Chlorella vulgari* powder ; T9: with 0.5% *Nannochloropsis salina* powder ; T10: with 1% *Nannochloropsis salina* powder ; T11: with 1.5% *Nannochloropsis salina* powder ; T12: with 2% *Nannochloropsis salina* powder .

Assessment of Total Carotenoids

Carotenoid content was quantified using a spectrophotometric method at 450 nm, following the official procedures described by AOAC (1995). β-carotene was employed as the standard to generate the calibration curve.

Texture Measurement

After 24 hours of refrigeration at 6-10°C, the textural attributes of the custard samples were measured using a Texture Analyzer (CNS-Farnell, Borehamwood, Hertfordshire, UK). Measurements were conducted following the procedure described by Szczesniak *et al.* (1963).

Sensory Evaluation:

Sensory characteristics of both commercial and experimental custard formulations were evaluated by a trained panel of ten faculty members from the Department of Food and Dairy Science and Technology. A 9-point hedonic scale was applied to assess attributes such as color, aroma, mouthfeel, taste, consistency, and overall acceptability (Iwe, 2002). On the scale, 1 indicated strong dislike, 5 was neutral, and 9 represented strong liking. Data were statistically analyzed using analysis of variance at a 5% significance level.

Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS, 2000) software package. Analysis of variance (ANOVA) procedures were used to assess differences, and Duncan's multiple range test was applied to determine significant differences between means.

RESULTS AND DISCUSSION

Chemical characteristics of studied custard

Table (2) illustrates the chemical composition of functional custard samples enriched with different concentrations of *Spirulina, Chlorella*, and *Nannochloropsis* powder. The moisture content of these samples ranged between 76.75% and 78.83%, which is comparable to the findings reported by Zarroug *et al.* (2022). It was observed that custard samples fortified with 2% microalgae powder exhibited lower moisture levels than the control samples.

Moreover, a significant reduction ($P < 0.05$) in moisture content occurred as the powder concentration increased from 0.5g to 2g per 100g of custard. These results align with the observations of Agustini *et al*. (2016), who reported that increasing the amount of *Spirulina platensis* led to a decrease in water content in soft cheese.

Regarding protein and fat contents, the functional custard samples contained between 3.62% and 4.80% protein, and 6.53% to 6.93% fat. Both protein and fat levels showed a significant increase with higher additions of microalgae powder, likely due to the rich protein content of *Spirulina, Chlorella*, and *Nannochloropsis* powders, thereby improving the nutritional value of the custard. These findings are consistent with previous studies, including Sahin (2019), who found that incorporating 2% dried *Spirulina* into homemade chocolates and biscuits enhanced protein content. Similarly, Agustinia *et al*. (2016) demonstrated a significant increase in protein content in ice cream after the addition of *Spirulina* algae. Additional research has confirmed that using dry microalgae powder as a food supplement can elevate protein levels in products like cookies (De Marco *et al*., 2014 and Batista *et al*., 2017). Abdel-Moatamed *et al*. (2024) demonstrated that adding *Chlorella vulgaris* powder to burger samples resulted in an increase in their protein content. Furthermore, the protein concentration rose proportionally with higher amounts of *Chlorella vulgaris* powder.

The gross energy values of custard samples fortified with 2% powder from *Spirulina, Chlorella,* and *Nannochloropsis* were 122.84, 123.33, and 124.04 kcal per 100 g, respectively, which were higher than those of the control sample without microalgae. This increase in energy is likely due to the higher levels of protein, fat, and carbohydrates present in these microalgae species. These results are consistent with those reported by Batista *et al*. (2017), who observed a rise in energy content in custard samples as the concentration of microalgae powder increased. Similar findings were also reported by Onacik-Gur *et al*. (2018).

Additionally, the functional custard samples showed ash and fiber contents ranging from 0.66 to 0.91% and 0.024 to 0.104%, respectively. A slight increase in both ash and fiber content was observed with increasing levels of microalgae powder. These chemical composition findings agree with the results obtained by Agustinia *et al*. (2016) and Abdel-Moatamed *et al*. (2024), who noted that the ash content in burger samples increased with higher percentages of *Chlorella vulgaris* powder. This is attributed to the mineral richness of *Chlorella vulgaris*, which elevates the ash content when added to meat products. Similarly, Cabrol *et al*. (2023) found that incorporating honey and 3% white *Chlorella* significantly increased ash levels content in frankfurter samples compared to the control.

Table1. Chemical Composition of the Microalgal Powder Used in the Experiments.

Natural component	*Spirulina platensis*	*Chlorella vulgari*	*Nannochloropsis salina*
Protein (%)	61.72	59.31	54.82
Fat (%)	8.79	16.11	20.21
Carbohydrates (%)	20.30	11.30	15.37
Fiber (%)	4.71	5.23	3.68
Ash (%)	4.45	7.1	5.7
Total Phenolic (mg/g DW)	92.56	81.27	80.72
Chlorophyll A (mg/g DW)	9.80	7.32	6.93
Chlorophyll B (mg/g DW)	0.43	0.22	0.12
Total Carotenoids (mg/g DW)	7.61	5.92	5.18

Table 2. Chemical Composition in Functional Custard Fortified with a Powder of *Spirulina*, *Chlorella*, and *Nannochloropsis.*

Components Treatments	(W/W) %	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Kcal/100g
Control	0	78.83 ± 0.93^{a}	3.62 ± 0.12^{f}	6.53 ± 0.23^{b}	0.64 ± 0.07^{i}	0.000 ± 0.05^{i}	114.77 ± 1.03^{e}
Spirulina platensis	0.5	78.34 ± 1.03^{ab}	3.92 ± 0.15^{e}	6.57 ± 0.24^{b}	0.66 ± 0.04^{h}	0.024 ± 0.03^{gh}	115.75 ± 1.12^{e}
	1	77.87 ± 1.10^{ab}	4.23 ± 0.11^{d}	6.61 ± 0.22^{ab}	0.68 ± 0.09^{g}	0.047 ± 0.04^{ef}	118.77 ± 1.04^{bcde}
	1.5	77.35 ± 0.95^{ab}	4.55 ± 0.13^{bc}	6.66 ± 0.20^{ab}	0.70 ± 0.05^{f}	0.071 ± 0.02^{cd}	120.86 ± 1.13^{abcd}
	2	77.88 ± 1.04^{ab}	4.84 ± 0.15^{a}	6.70 ± 0.20^{ab}	0.72 ± 0.05^{e}	0.094 ± 0.05^{ab}	122.84 ± 1.10^{ab}
Chlorella vulgari	0.5	78.58 ± 1.01^{a}	3.68 ± 0.13^{f}	6.61 ± 0.30^{ab}	0.67 ± 0.09^{gh}	0.026 ± 0.07^{gh}	116.01 ± 1.05^{de}
	1	77.86 ± 0.89^{ab}	4.21 ± 0.12^{d}	6.69 ± 0.31^{ab}	0.70 ± 0.07^{f}	0.052 ± 0.05^{ef}	119.09 ± 1.03^{abcde}
	1.5	77.36 ± 1.07^{ab}	4.51 ± 0.19^{c}	6.71 ± 0.28^{ab}	0.74 ± 0.08^{d}	0.078 ± 0.03^{bc}	121.21 ± 1.05^{abc}
	2	76.88 ± 1.03^{b}	4.80 ± 0.15^{a}	$6.75.93\pm0.28^{ab}$	0.78 ± 0.08^{c}	0.104 ± 0.06^{a}	123.33 ± 1.06^{ab}
Nannochloropsis salina	0.5	78.21 ± 1.07^{ab}	3.89 ± 0.80^{e}	6.63 ± 0.27^{ab}	0.71 ± 0.02^{ef}	0.018 ± 0.05^{h}	117.11 ± 1.12^{cde}
	1	77.77 ± 1.11^{ab}	4.17 ± 0.27^{d}	6.72 ± 0.25^{ab}	0.77 ± 0.05^{c}	0.036 ± 0.07^{fg}	119.45 ± 1.13^{abcde}
	1.5	77.27 ± 1.04^{ab}	4.42 ± 0.30^{c}	6.83 ± 0.29^{ab}	0.85 ± 0.07^{b}	0.055 ± 0.04^{de}	121.71 ± 1.10^{a}
	2	76.75 ± 0.93^{b}	4.70 ± 0.16^{ab}	6.93 ± 0.19^{a}	0.91 ± 0.05^{a}	0.073 ± 0.07^{c}	124.04 ± 1.15^{a}

***Means of triplicates ±SD. Means followed by the same superscript at the column are not significantly different at *P*<0.05.**

Water Holding Capacity (WHC)

Water holding capacity (WHC) of custards fortified with 0.5%, 1%, 1.5%, and 2% powder of *Spirulina, Chlorella*, and *Nannochloropsis* ranged from 92% to 100% (Fig. 2). This improvement is likely due to the incorporation of microalgal fibers, which are known for their ability to retain water. The high WHC values observed suggest that *Spirulina, Chlorella*, and *Nannochloropsis* could serve as effective natural ingredients in food products aimed at enhancing texture and viscosity. This behavior can be attributed to the excellent water-binding properties of the microalgal fibers, as previously reported by Huber *et al*. (2016); Roohinejad *et al*. (2016); and Akinwale *et al*. (2017).

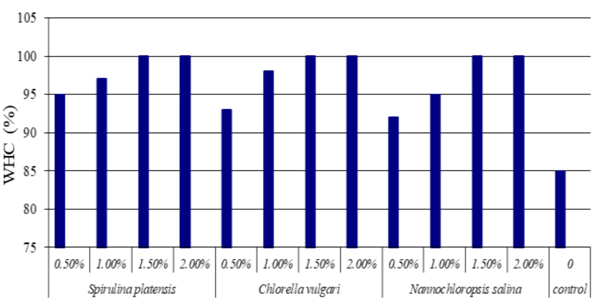

Fig. (2). Water holding capacity(WHC) of functional custard samples fortified with microaglae.

Bioactive Compounds

With respect to bioactive compounds such as carotenoids, the control custard exhibited the lowest concentration, showing a significant difference compared to the fortified samples (Figure 3). The total carotenoid content increased with the addition of dried *Spirulina*, ranging from 26.23 µg/g in the control to a maximum of 150.52 µg/g in the custard fortified with 2.0% *Spirulina*. This is due to the rich carotenoid content of *Spirulina, Chlorella*, and *Nannochloropsis* powder. These results are in agreement with previous studies by Barakat *et al*. (2016) and Sharma and Dunkwal (2012), who reported that β-carotene levels in biscuits enriched with 10% dried *Spirulina* reached approximately 349.75 µg/100g.

In addition, total phenolic content (Fig. 3) significantly increased with higher levels of *Spirulina*, reaching its value in the custard fortified with 2% *Spirulina* (1847.21 µg/g). These results are in agreement with those reported by Barakat *et al*. (2016) and Rodríguez De Marco *et al*. (2014), who demonstrated that pasta enriched with *Spirulina* showed higher phenolic content and antioxidant activity compared to the control, suggesting its potential role in improving the nutritional value of food products.

The main biochemical differences observed in the studied custard samples were due to the presence of chlorophyll-A (Figure 4), a prominent compound found in microalgae. In the control custard (without microalgae), chlorophyll-A and chlorophyll-B were absent.

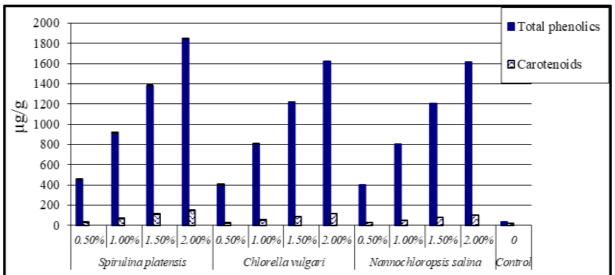

Fig. (3). Total phenolics and carotenoids of functional custard samples fortified with microaglae.

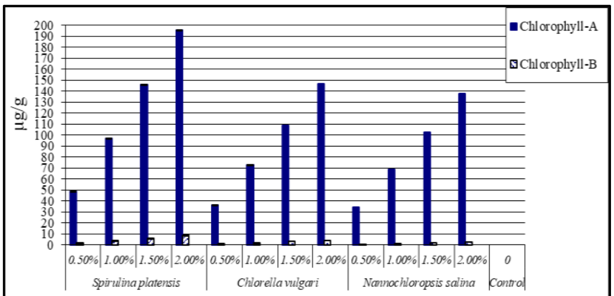

Fig. (4). ChlorophyII-A and ChlorophyII-B of functional custard samples fortified with microaglae.

However, its concentration increased progressively with the addition of dried microalgae, reaching a maximum of 195.86 and 8.56 µg/g , respectively in samples fortified with 2% dried *Spirulina*. This trend is consistent with the findings of Barakat *et al*. (2016).

Viscosity of studied custard:

As presented in Table (3), viscosity values of the functional custard samples varied from 9407 to 9489 centipoise (C.p). When compared to the control sample (8854 C.p), custard formulations enriched with *Spirulina, Chlorella*, and *Nannochloropsis* powders at concentrations of 0.5, 1.0, 1.5, and 2.0 g/100 g exhibited significantly higher viscosity values ($P < 0.05$). The viscosity increased proportionally with increasing concentration of added microalgal powders. The highest viscosity was recorded in custard fortified with 2% *Spirulina* (9489 C.p), followed by the sample containing 2% *Nannochloropsis* (9487 C.p). This increase may be attributed to the higher solid content of dried *Spirulina* compared to *Nannochloropsis*. Hanou *et al*. (2016) suggested that the enhanced interactions between the components of *Spirulina* such as proteins, carbohydrates, and lipids play a major role in increasing viscosity. The viscosity results observed in this study are consistent with those reported by Bchir *et al.* (2019).

Table 3. The Viscosity and Textural Parameters of Functional Custard Samples Fortified with Powder of *Spirulina*, *Chlorella*, and *Nannochloropsis*.

Parameters Treatments	(W/W) %	Viscosity (C.p)	Hardness (N)	Adhesiveness (J)	Cohesiveness
Control	0	8854±8.13 ^{f}	235±2.35 ^{g}	-62.4±1.05^{a}	1297±9.34^{g}
Spirulina platensis	0.5	9437±6.13 ^{bcde}	254±2.86 ^{f}	-70.7±1.52^{d}	1545±7.57^{f}
	1	9440±9.13 ^{bcde}	265±1.97 ^{e}	-71.5±1.13 ^{de}	1561±8.62^{f}
	1.5	9452±7.13 ^{bc}	280±2.58 ^{bc}	-72.6±0.97 ^{e}	1702±7.78^{b}
	2	9489 ±8.13 ^{a}	284±2.67 ^{a}	-80.3±1.34 ^{h}	1687±9.93^{abc}
Chlorella vulgari	0.5	9407±7.13 ^{e}	275±2.81^{j}	-78.7±1.17 ^{gh}	1678±8.72^{c}
	1	9415±9.13 ^{de}	275±1.39 ^{cd}	-67.2±1.35^{c}	1684±6.35^{c}
	1.5	9421±8.13 ^{cde}	263±1.81 ^{e}	-65.4±1.45^{b}	1656±8.73^{d}
	2	9468 ±9.13 ^{ab}	298±2.58^{b}	-82.4±0.98 ^{i}	1845±7.99^{a}
Nannochloropsis salina	0.5	9425±5.13 ^{cde}	264±2.35 ^{e}	-65.8±1.24^{bc}	1632±8.37^{e}
	1	9443±9.13 ^{bcd}	275±1.99 cd ^{i}	-71.5±1.24 ^{de}	1690±9.88^{bc}
	1.5	9449±6.13 ^{bc}	274±2.91 ^{cd}	-77.6±1.80 ^{g}	1689±9.33^{bc}
	2	9487 ±9.13 ^{a}	267±1.01 ^{de}	-75.4±2.05 ^{f}	1625±8.13^{e}

***Means of triplicates ±SD. Means followed by the same superscript at the same column are not significantly different at P<0.05.**

Rheological analysis of studied custard:

The textural attributes of the control custard and those enriched with varying concentrations of *Spirulina, Chlorella*, and *Nannochloropsis* are summarized in Table (3). Measurements were conducted after 24 hours of storage at 6-10°C. Adhesiveness and cohesiveness of the custard samples ranged from -82.4 to -62.4 J and 1297 to 1845, respectively. Hardness values were notably higher in samples enriched with 2% (w/w) of the microalgal powders, reaching 284, 298, and 267 N for *Spirulina, Chlorella*, and *Nannochloropsis*, respectively. A significant increase in hardness was observed with higher levels of microalgae incorporation. This may be attributed to the elevated dry matter and reduced moisture content in the enriched custards. The addition of microalgal powder influenced the formation of the gel matrix, leading to enhanced curd strength. These findings align with those of Bchir *et al*. (2019) and Malik *et al*. (2013), and are further supported by Abd El-Baky *et al*. (2015).

Color values for different custard samples

Table (4) shows color parameters of tested custard samples. As seen, all samples including control showed negative assigned redness (a*) indicating greenish color shade of the products. Except of custard sample fortified with 0.5% powder of *Chlorella*, all tested custard preparation showed close greenish a*-value in the range of - 11.53 to - 14.83. Except of custard sample fortified with 0.5% powder of *Chlorella* and control samples all custard preparation showed a hidden yellow color component (b*). Yellowness (b*) ranging between 28.13 to 33.61 for custard sample fortified with 2% powder of *Chlorella* and custard sample fortified with 0.5% powder of *Nannochloropsis* and 24.93 to 28.36 for custard sample fortified with 2 and 0.5% powder of *Spirulina*, respectively. The yellowness (b*) of the *spirulina*, *Chlorella* and *Nannochloropsis* fortified custard samples was higher than that of the control. This result agrees with Minh (2014) and Ismail *et al*. (2023).

Correspondingly, the color saturation (Chroma values) showed high level with C-values in the range 30.73 to 35.58 for custard samples fortified with 2% powder of *Chlorella* and *Nannochloropsis*, respectively, while the custard samples fortified with powder of *Spirulina* was relatively less saturated with C-values in the range of 28.27 to 31.16. The control sample showed the highest lightness and brightness appearance with L* of 90.42. Incorporation of the green color ingredient at low concentration in custard samples fortified with powder of *Chlorella* and *Nannochloropsis* substantially decreased the lightness (L*) to the level of 72.12 to 72.71. However, increasing the level of substitution to 1, 1.5 and 2.0 produced custard samples with less lightness, where L* moved towards darker level and reached values of 58.29 to 60.34 for custard samples fortified with1.5% powder of *Chlorella* and *Nannochloropsis*, respectively. These findings are consistent with those of Golmakani *et al*. (2019) ,who reported a decrease in lightness with the addition of *spirulina* to acidified feta-type cheese. Also, Ismail *et al*. (2023), observed that, the L* values of the fortified Ricotta cheese samples decreased with increasing concentrations of *spirulina*. The same trend was assured by the obtained Hue-angle values on the color circuit where the color type changed from yellow–greenish type with values of 109.80° and 110.85° to more greenish component and H-values of 113.75° and 116.21° for custard samples fortified with 0.5, 2% powder of *Chlorella* and *Nannochloropsis*, respectively. All microalgae powder fortified custard samples were greener than the control ones, which may be due to the presence of the blue-green pigments chlorophyll and phycocyanin in *spirulina*, *Chlorella* and *Nannochloropsis*. This result agrees with Minh (2014) and Ismail *et al.* (2023).

Lightness level of the custard samples fortified with powder of *Spirulina* was lower than those of custard samples fortified with powder of *Chlorella* and *Nannochloropsis*, with L-value of only 63.36 for custard samples fortified with 0.5% powder of *Spirulina* and 46.00 for the custard samples fortified with 2% powder of *Spirulina*. This means that incorporation of powder of *Spirulina* produced duller custard samples than did custard samples fortified with powder of *Chlorella* and *Nannochloropsis*. The corresponding Hue-values recorded angel-level of 114.46° to 118.12° with more greenish intensity. These results agree with Al-Soudy *et al*. (2024), who found that, the Hue – value of the control Karish cheese sample was higher than the treatment samples fortified with *Chlorella vulgaris* and *Spirulina platensis* samples, being less green than the control samples. In all tested samples, the differences in color parameters between 1.5 to 2.0 substitution was not remarkable, so that both concentrations showed almost the same effect on color parameters. The results from this study showed that the addition of *spirulina*, *Chlorella* and *Nannochloropsis* to custard samples reduced the lightness, redness and yellowness values. These findings are in agreement with previous research on the impact of microalgal pigments on cheese color (Mazinani, *et al*., 2016; Darwish *et al*., 2017 and Ismail *et al*., 2023).

Table 4. Color Parameters of Functional Custard Samples Fortified with Powder of *Spirulina*, *Chlorella*, and *Nannochloropsis.*

Parameters	Color parameters					
Treatments	**(W/W)%**	**Lightness (L*)**	**Redness (a*)**	**Yellowness(b*)**	**Saturation Chroma (c)**	**Color type Hue (H°)**
Control	0	90.42±2.45 a	- 13.20±0.59 cde	21.27±1.23 g	25.03±1.46 g	121.80±3.13 a
Spirulina platensis	0.5	63.36±1.45 d	- 12.90±0.65 cde	28.36±1.45 de	31.16±.136de	114.46±3.62 cd
	1	53.30±3.14 g	- 13.54±0.48 e	27.33±1.24 e	30.50±1.98 e	116.36±2.24 bc
	1.5	48.58±2.25 h	- 11.53±0.78 b	27.53±1.26 e	29.58±1.65 ef	112.72±3.41 def
	2	46.00±2.78 i	- 13.32±0.69 de	24.93±1.24 f	28.27±1.78 f	118.12±2.36 b
Chlorella vulgari	0.5	72.12±4.47 b	- 5.89±0.69a	16.36±1.36 h	17.39±1.45 h	109.80±3.45 f
	1	64.73±2.46 d	- 12.55±0.48 cd	30.90±1.37 bc	33.35±1.62 c	112.10±3.84 def
	1.5	58.29±3.47 f	- 12.94±0.78 cde	29.86±1.62 cd	32.54±1.78 cd	113.43±2.74 cde
	2	58.33±2.45 f	- 12.38±0.98 c	28.13±1.52 e	30.73±1.36 e	113.75±2.46 cde
Nannochloropsis salina	0.5	72.71±2.36 b	- 12.80±0.45 cde	33.61±1.46 a	35.96±1.72 a	110.85±2.58 ef
	1	66.64±4.58 c	- 14.66±0.98 f	31.89±1.46 b	35.10±1.65 ab	114.69±2.85 cd
	1.5	60.34±3.45 e	- 14.49±0.78 f	31.98±1.26 ab	35.11±1.45 ab	114.38±2.95 cd
	2	58.40±2.45 f	- 14.83±0.68 f	30.13±1.34 c	33.58±1.35 bc	116.21±2.85 bc

***Means of triplicates ±SD. Means followed by the same superscript at the same column are not significantly different at *P*<0.05.**

Sensory analysis of the studied custard samples

The sensory evaluation results of custard samples fortified with powder of *Spirulina, Chlorella*, and *Nannochloropsis* are presented in Table (5). After one day, the panelists awarded high scores to the custard samples fortified with 0.5%, 1%, 1.5%, and 2% *Nannochloropsis* powder. In contrast, samples fortified with 1.5% and 2% *Spirulina* powder received lower scores for color, likely due to the formation of a darker hue. Significant differences were observed in the color attributes among custard samples fortified with the three types of microalgal powder. Panelists showed a clear preference for the taste of custard samples containing *Nannochloropsis* at 0.5%, 1%, 1.5%, and 2%, awarding scores of 8.89, 8.80, 8.75, and 8.70, respectively. Aroma scores of functional custard on day one ranged from 8.42 to 8.94.

The mouthfeel and consistency varied significantly among the custard samples fortified with *Chlorella* and *Nannochloropsis*. Mouthfeel scores ranged from 5.93 to 8.82 across all treatments. Custard samples fortified with *Spirulina* powder at 0.5%, 1%, 1.5%, and 2% received the lowest scores in this category. Although increasing *Spirulina* levels initially improved the mouthfeel, sensory scores decreased when its concentration surpassed 0.5%, likely due to the emergence of earthy and musty off-flavors typical of *Spirulina* powder. These findings align with those of Khemiri *et al*. (2022), who reported that custard samples fortified with 0.2% *Chlorella* spp. achieved the highest sensory acceptance.

Samples containing *Nannochloropsis* powder consistently received the highest scores in sensory evaluation. Overall acceptability scores ranged from 6.09 to 8.90, with the lowest ratings recorded for samples fortified with *Spirulina*. These results are consistent with the findings of Ismail *et al*. (2023), who reported favorable sensory outcomes for samples containing 0.2% and 1% *Chlorella* spp., while the 1.5% mixture was the least accepted due to its pronounced fishy odor and aftertaste.

Furthermore, Abdel-Moatamed *et al*. (2025) found that cake samples treated with *Chlorella vulgaris* at concentrations of 0.5%, 1%, and 2% had significantly lower sensory scores than the control. In contrast, Al-Soudy *et al*. (2024) reported that adding 0.5%, 1.0%, and 1.5% of *Chlorella vulgaris* and *Spirulina platensis* powders improved the functional and nutraceutical qualities of Karish cheese. These findings are consistent with those reported by Kim *et al*. (2012).

Table 5. Sensory Properties of Functional Custard Fortified with Powder of *Spirulina*, *Chlorella*, and *Nannochloropsis* after one day at 6-10o C.

Parameters Treatments	(W/W)%	Color (9)	Aroma (9)	Mouth feel (9)	Taste (9)	Consistency (9)	Overall acceptability (9)
Control	0	9.00 ± 0.62^{a}	9.00 ± 0.77^{a}	9.00 ± 0.96^{a}	9.00 ± 0.76^{a}	9.00 ± 0.96^{a}	9.00 ± 0.48^{a}
Spirulina platensis	0.5	8.62 ± 0.92^{a}	8.65 ± 0.68^{abcd}	7.21 ± 0.77^{d}	7.31 ± 0.99^{c}	7.81 ± 0.89^{c}	7.51 ± 0.79^{d}
	1	8.33 ± 0.89^{cd}	8.60 ± 0.88^{abcd}	6.92 ± 0.58^{de}	7.00 ± 0.58^{c}	7.63 ± 0.47^{c}	7.30 ± 0.89^{de}
	1.5	7.65 ± 0.75^{e}	8.51 ± 0.66^{abcd}	6.63 ± 0.98^{e}	6.71 ± 0.76^{d}	7.30 ± 0.92^{cd}	6.89 ± 0.55^{e}
	2	7.02 ± 0.16^{f}	8.42 ± 0.54^{bcd}	5.93 ± 0.48^{f}	6.00 ± 0.82^{e}	7.00 ± 0.73^{d}	6.09 ± 0.44^{f}
Chlorella vulgari	0.5	8.81 ± 0.38^{abc}	8.80 ± 0.78^{abc}	8.82 ± 0.78^{ab}	8.60 ± 0.98^{ab}	8.86 ± 0.75^{ab}	8.80 ± 0.58^{ab}
	1	8.62 ± 0.95^{abc}	8.86 ± 0.96^{ab}	8.59 ± 0.86^{abc}	8.41 ± 0.48^{ab}	8.62 ± 0.94^{ab}	8.68 ± 0.84^{abc}
	1.5	8.45 ± 0.75^{bcd}	8.32 ± 0.78^{cd}	8.33 ± 0.72^{bc}	8.25 ± 0.79^{b}	8.51 ± 0.79^{ab}	8.37 ± 0.84^{bc}
	2	8.11 ± 0.99^{de}	8.27 ± 0.85^{d}	8.29 ± 0.94^{c}	8.19 ± 0.36^{b}	8.40 ± 0.82^{b}	8.20 ± 0.79^{c}
Nannochloropsis salina	0.5	8.95 ± 0.52^{ab}	8.94 ± 0.96^{a}	9.39 ± 0.48^{bc}	8.89 ± 0.79^{a}	8.96 ± 0.58^{a}	8.90 ± 0.84^{a}
	1	8.83 ± 0.78^{abc}	8.91 ± 0.78^{ab}	8.82 ± 0.98^{ab}	8.80 ± 0.58^{ab}	8.83 ± 0.86^{ab}	8.82 ± 0.86^{ab}
	1.5	8.79 ± 0.69^{abc}	8.85 ± 0.98^{ab}	8.37 ± 0.38^{bc}	8.75 ± 0.78^{ab}	8.76 ± 0.45^{ab}	8.71 ± 0.48^{ab}
	2	8.63 ± 0.47^{abc}	8.72 ± 0.48^{abcd}	8.69 ± 0.79^{abc}	8.70 ± 0.35^{ab}	8.70 ± 0.65^{ab}	8.63 ± 0.85^{abc}

***Means of triplicates ±SD. Means followed by the same superscript at the same column are not significantly different at P<0.05.**

CONCLUSION

In conclusion, *Spirulina platensis* powder can be effectively incorporated into custard formulations at levels up to 0.5%, while *Nannochloropsis salina* and *Chlorella vulgaris* powders can be included at levels up to 2%, enhancing the functional value of the custard without adversely affecting its quality attributes or sensory properties.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the valuable cooperation provided by Prof. Dr. Samh Aly Awad, Professor of Dairy Microbiology & Technology, Faculty of Agriculture, Alexandria University; Prof. Dr. Yehia Abd El-Razik Heikal; and Prof. Dr. Mohamed Mustafa, Department of Food Science, Faculty of Agriculture, Ain Shams University, Egypt. Special thanks are extended to them for their significant contributions and assistance.

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دراسة مقارنة لتأثير السبيرولينا والكلوريلا والنانوكلوروبسيس على جودة الكاسترد

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الملخص

تعد الطحالب الدقيقة مصدرا غذائيا غنيًا ومتكاملًا، لما تحتويه من نسب مرتفعة من البروتينات والكربوهيدرات والدهون، بالإضافة إلى الفيتامينات والمعادن والألياف والمركبات الحيوية النشطة ذات الفوائد الصحية المتعددة. هدفت الدراسة الى تدعيم الكاسترد بمسحوق الـ *Spirulina* ، *Chlorella* و *Nannochloropsis* ، وتأثيرإضافتهم الى الكاسترد بتركيزات ٠,٥ ، ١ ، ١,٥ و ٢ %(وزن / وزن) على الخصائص الكيميائية ، واللونية ، والرويولوجية والحسية. أوضحت النتائج أنه عند إضافة مسحوق الـ *Spirulina* ، *Chlorella* و *Nannochloropsis* إلى عينات الكاسترد، زادت كمية البروتين والدهون والألياف والقيمة الكلية للطاقة في عينات الكاسترد الوظيفي. كانت قيمة سطوع اللون *L لعينات الكاسترد المدعمة بالـ *Spirulina* أقل من عينات الكاسترد المدعمة بمسحوق الـ *Chlorella* و *Nannochloropsis* ، وكذلك أظهرت نتائج هذه الدراسة أن إضافة مسحوق الـ *Spirulina* ، *Chlorella* و *Nannochloropsis* إلى عينات الكاسترد أدت إلى تقليل قيم سطوع اللون ودرجتى الاحمرار والاصفرار. وقد أظهرت العينات التي تحتوي على مسحوق الـ *Spirulina* ، *Chlorella* و *Nannochloropsis* لزوجة وصلابة أعلى من الكنترول . ازداد محتوى المركبات النشطة بيولوجيًا مع زيادة نسبة الإضافة من مسحوق الطحالب. و حصلت عينات الكاسترد المدعمة بـ ٠,٥ ، ١، ١,٥، و٢٪ بمسحوق الـ *Nannochloropsis* على أعلى القيم من سطوع اللون *L . تشير نتائج الدراسة إلى أن مسحوق السبيرولينا يمكن إدماجه في تركيبة الكاسترد بنسبة لا تتجاوز ٠,٥٪، بينما يمكن إدماج كلٍ من مسحوقي النانوكلوروبسيس والكلوريلا بنسبة تصل إلى ٢٪، وذلك بهدف تعزيز القيمة الوظيفية للمنتج دون التأثير السلبي على قبوله الحسي من قِبل المستهلكين.