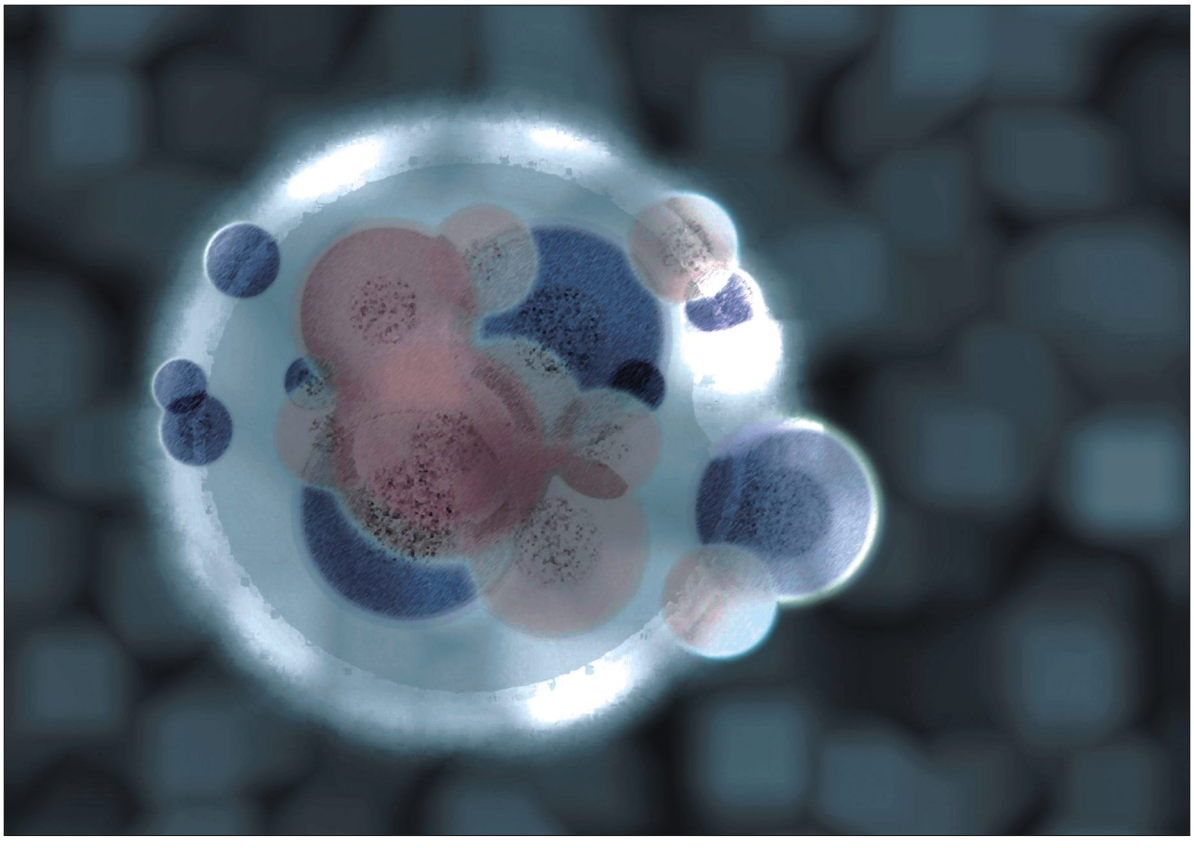

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Research Article
Applicability of Using Edible Algae (*Spirulina platensis*) to Prepare High Protein Quality Labenah

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Abstract

Background and Objective: The growing population number combined with limited food resources resulted in the great need for alternative protein sources and functional food. This study aimed to prepare functional labenah using edible green algae, *Spirulina platensis*, as a rich source of protein and enhancer of *Bifidobacterium* activity. **Materials and Methods:** This study was divided into two parts. The first one, was to study the effect of different concentrations of *Spirulina platensis* (1, 3 and 5 mg mL^{-1} pure media) on the activity of *Bifidobacterium*. The second was to study the properties of three different labenah samples; the first one used as control (T1) and contained 2% traditional yogurt starter culture. The second (T2) was prepared by adding 2% *Bifidobacterium* spp. The third treatment (T3) contained 5 mg mL^{-1} of *Spirulina platensis* powder plus 2% *Bifidobacterium* spp. All labenah samples stored at 5°C for 21 days and analyzed when fresh and after 7, 14 and 21 days for acidity percent; pH values and TVFAs contents. Color parameters and sensorial properties were also estimated during the same periods of storage. **Results:** Supplementation of *Spirulina platensis* markedly activated the growth of *Bifidobacterium* spp. While, slight differences were observed in the studied chemical parameters of the three different labenah samples. Obtained data revealed that the acidity percent of samples as well as TVFAs contents were slightly increased in treated samples rather than control and in stored samples rather than fresh. The pH values took an opposite trend. Noticeable and clear differences between control and treated samples were observed in color parameters and sensorial evaluation. Results also indicated that using of *Spirulina platensis* gave a clean and moderate acid taste with an acceptable color and favorite properties. **Conclusion:** Using 5 mg mL^{-1} of *Spirulina platensis* powder succeeded in preparing acceptable labenah samples and promote the growth of *Bifidobacterium*. So it is recommended to use this formulation to prepare labenah. Further studies should be done in food sector to prepare another algal dairy products using *Spirulina platensis* powder.

Key words: Algae, *Spirulina platensis*, functional labenah, *Bifidobacterium*

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

The global population growth combined with increasingly limited resources of arable land and fresh water resulted in urgent need for alternative protein sources and functional food[1]. Macro-algae (seaweed) and microalgae are examples of under-exploited "crops" that do not compete with traditional food crops[2]. Green algae are approximately 8000 species[3].

Seaweed and microalgae are considered a viable source of protein. Some species of seaweed and microalgae are known to contain protein levels similar to those of traditional protein sources, such as meat, milk and egg. Among the most edible micro algae is *Spirulina* spp.[4]. Spirulina is the best known genus of *Cyanobacteria* because of its unique nutritional properties[5,6]. Spirulina has been used for many years as food additive due its high nutritional value. The consumption of Spirulina is beneficial to health due to its specific chemical composition including important compounds like essential amino acids, vitamins, natural pigments and fatty acids, . In addition to high quality proteins, it contains high amounts of calcium, vitamin B_{12}, vitamin A, B_2, B_6, E, K and H, as well as essential minerals especially iron[7,8].

Yogurt, particularly probiotic yoghurts, contribute to health by providing natural nutrients and by enrichment of the intestinal biota with lactic acid bacteria or probiotic cultures. Limited number of studies recorded that *Spirulina platensis* powder promotes the growth of lactic acid bacteria in synthetic media[5] and milk[9] or yoghurt made with *Bifidobacterium animalis*[7].

This study was made to clarify the influence of *Spirulina platensis* powder on the activity *Bifidobacterium* spp; then preparing probiotic concentrated-yogurt (labenah) samples aiming to be accepted as functional food rich in protein. Another aim of this study was evaluating the properties of the resultant Labenah as a new dairy product which fortified with algae as food additive.

MATERIALS AND METHODS

Milk: Fresh raw cow milk (3.5% fat and 12% TS) were obtained from the herd of Faculty of Agriculture, Cairo University, Egypt.

Starter cultures: Yogurt starter culture containing *S. salivaricus* subsp. *thermophiles* and *L. delbrueckii* ssp. *bulgaricus* in a freeze dried direct vat setform were obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark.

Bifidobacterium spp. were obtained from Dairy Microbiology Lab., NRC, in Egypt.

***Spirulina platensis*:** Edible algae biomass in a powdered form; toxin-free; were obtained from Wal-Mart Stores, Inc., Bentonville, AR 72716; Walmart.com/ Spring Valley, USA.

The current study was carried out in National Research Centre, Dairy Lab. Giza , Egypt , in the period from march 2017 till February 2018.

Microbiological examination

Effect of *Spirulina platensis* on *Bifidobacterium* spp. activity: *Bifidobacterium* counts was carried out by adding 1 mL of *Bifidobacterium* spp. to tube containing 10 mL MRS broth medium supplemented with 3% lithium chloride and 2% sodium propionate. The plates were incubated at 37°C for 48 h. Counting colonies and then results were expressed as log cfu mL^{-1}.

Spirulina platensis suspension was used at three concentrations of 1, 3 and 5 mg mL^{-1} pure media in sterilized distilled water and added into MRS broth medium tubes containing the bacterial cultures. The tubes were then incubated at 37°C for 24 h and 48 h, respectively[10]. Control was also prepared without the addition of *Spirulina platensis.* Counting colonies was recorded to find the best concentration of the algae enhancing the bacterial growth.

Labenah samples preparation: Milk sample was heated at 85°C for 15 min, then cooled to 40°C and divided into three portions; to prepare three types of labenah: the first was control contained only 2% yogurt starter (*S. thermophilus+ L. bulgaricus*, 1:1) and named (T1). The second was probiotic sample contained the 2% yogurt starter in addition to 2% *Bifidobacterium* spp. and labeled as T2. The third treatment contained 2% yogurt starter, 2% *Bifidobacterium* spp. plus 5 mg/mL *Spirulina platensis* powder and labeled as T3. All cultured milks were manufactured according to Labenah manufacturer's instructions[11].

Chemical analytical methods: Fresh samples were checked for their gross chemical composition (TS, fat and protein) as well as acidity percentage of samples which were estimated during the 21 days of storage period as mentioned by AOAC[12]. The pH values were also measured during storage period using a digital laboratory pH-meter apparatus with glass electrode (Hanna instruments). Total volatile fatty acids values

(TVFAs) of samples were followed up during storage according to Kosikowski[13] and expressed as milliliter of 0.1 N Na OH/100 mg sample.

Color parameters estimation: The degree of samples-color was measured using Hunter colorimeter which was first standardized using a white tile (top of the scale) and a black tile (bottom of the scale). A specimen of the sample (flat layer) was placed at the specimen port. Tri-stimulus values of the color namely; L, A and B were measured.

Organoleptic properties: Labenah samples were evaluated for their sensory properties such as flavor; color and appearance; body and texture and all acceptability by a panel of trained Jude's of stuff members at Dairy Sciences Laboratory NRC, according to score card suggested by the authors. Each parameter ranked from 1-8.

RESULTS AND DISCUSSION

Microbiological examination: Data presented in Table 1 reflected the counts *Bifidobacterium* spp. (Log cfu mL^{-1}) as adding different ratios of *Spirulina platensis* powder in pure media.

A noticeable activity on *Bifidobacterium* spp. growth was observed due to addition of 5 mg mL^{-1} *Spirulina plantensis*. The *Bifidobacterium* counts were 8.85 (Log cfu mL^{-1}) after 24 h, reached 11.95 after 48 h of incubation, respectively. So it was more suitable for preparing probiotic products. Obtained results were in accordance with that obtained by Bensehaila *et al.*[14] and Kearney *et al.*[15].

Chemical composition of labenah samples: Results reported in Table 2 revealed that adding *Spirulina platensis* markedly elevated protein content of the prepared labenah (T3); where protein percent was 13.08 compared to 10.60% for control sample. No clear differences were noticed in fat content. It was well known that microalgae contain protein levels similar to those of traditional protein from animal and plant sources. *Spirulina platensis* algae was among the highest recorded protein content of any whole food[16]. It should be produced in a broad scale as an edible protein specially it grows in sea water and give higher protein yield/unit area compared to crops, such as soybean, plus legumes and wheat that needs fresh water[17].

Table 1: Count of *Bifidobacterium* (Log cfu mL^{-1}) added with *Spirulina platensis*

	Bifidobacterium spp.	
Concentration of *S. platensis*	24 h	48 h
0 (control)	7.30	8.60
1 mg mL^{-1}	7.50	8.70
3 mg mL^{-1}	7.90	9.85
5 mg mL^{-1}	8.85	11.95

Table 2: Gross chemical composition of different fresh Labenah samples

Gross composition (%)	T1	T2	T3
T.S	24.4	25.0	27.9
Fat	8.0	8.2	8.7
Protein	10.60	10.66	13.08

T1: Plain samples contained only 2% yogurt starters used as control, T2: Probiotic samples contained 2% *Bifidobacterium* spp.+Yogurt starter, T3: Algal samples contained 2% yogurt culture+2%, *Bifidobacterium* spp.+5 mg mL^{-1} *S. platensis* powder

Acidity percent, pH values and total volatile fatty acids of labenah samples: Data presented in Fig. 1 reflected the acidity percent and pH values as well as total volatile fatty acids (TVFAs) in labenah samples during 21 days of cold storage. It could be observed that the acidity percentage of samples was slightly increased in treated samples T2, T3 rather than control T1 and in stored ones rather than fresh. The pH values took an opposite trend. The pronounced effect was cleared in algal-supplemented samples (T3). TVFAs contents showed slight increase in treated samples rather than control and in stored ones rather than fresh samples. The present study showed enhanced culture growth in the samples fortified with *Spirulina plantensis*. This finding was in coincidence with Bensehaila *et al.*[14] who found enhanced

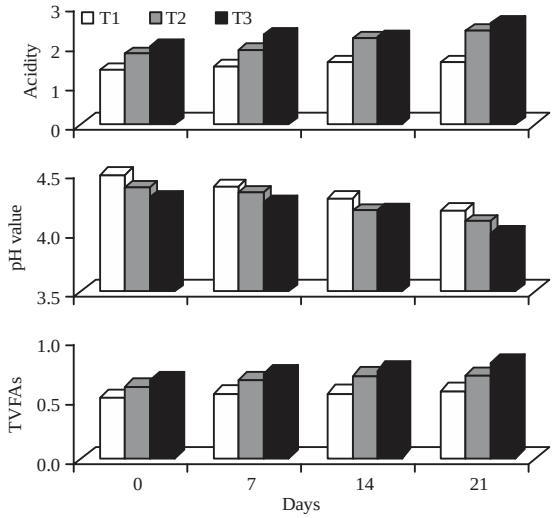

Fig. 1: Acidity %; pH values and TVFAs contents of Labenah samples during storage period, T1: Plain samples contained only 2% yoghurt starters used as control, T2: Probiotic samples contained 2% *Bifidobacterium* spp.+ yogurt starter, T3: Algal sample contained 2% yogurt culture+2% *Bifidobacterium* spp.+5 mg mL^{-1} *S. platensis* powder. TVFAs: Total volatile fatty acids expressed as mL 1.0 N Na OH/100 mg sample

Bifidobacterium breve growth with addition of *Spirulina plantensis* in milk. In the same time; Varga *et al*.[9] found that the growth rate, acid production and viability of *Bifidobacterium strains* were beneficially influenced by adding dried *Spirulina platensis* during manufacture and refrigerated storage of cultured milks. This may be explained in the light of the need of the probiotic bacteria to the nutrients provided by *Sprulina platensis* where, it contains high concentrations of vitamins, mineral, amino acids[15]. The present study indicated also that the acidity percent was slightly increased in treated samples (T2, T3) rather than control (T1) and in stored one rather than fresh. Several authors stated that *Sperulina platensis* promoted the growth of *S. thermophilus* in treated milk compared to control samples at the end of the refrigerated storage[5,7,9].

The results of pH values were in coincidence with previous studies that reported the decrease in pH values of yoghurt samples fortified by *Sperulina platensis* powder and this decrease continued by samples-storage. This was referred to the ability of *Sperulina platensis* to enhance the growth of lactic acid bacteria[18,9]. Microalgae area hugely diverse group containing approximately 200,000 species. Several of these species are currently exploited for a variety of biotechnological purposes, including fatty acids, biofuel production, alginates and waste water treatment[6].

Current study showed also an elevated TVFAs content in samples fortified by *Sperulina platensis* rather than control samples. This was referred to the exposure of algae to high oxidative and free-radical stresses[19] and enhancement of culture growth[7,9]. This had led to the evolution of natural protective systems, such as volatile fatty acids which can supply health benefits when eaten by consumers[20]. Significant body of evidence suggests that TVFAs have a beneficial role in appetite, digestion, colon disorders and energy homeostasis[21].

Color properties: Data presented in Table 3, reflected the tri-stimulus values of the samples color (L, A, B). It could be observed that the samples fortified with 5 mg mL^{-1} *S. platensis* powder (T3) mainly had a slight green color where the (A) values were higher than other samples. So; the values of (A) stimulus was higher in T3 than other samples .This finding may be referred to the green color of Spirulina itself. The other two stimulus (l or b) had no clear differences.

Sensory properties: Figure 2 reflected the organoleptic properties of all fresh and stored samples. It could be observed that T3 sample; which fortified with 2% *Bifidobacterium* spp. and 5% *Spirulina platensis*; gained the high scores for flavor

Table 3: Changes in color parameters of Labenah samples during storage period

Parameters	Storage period (day)	T1	T2	T3
L	Fresh	88.16	88.21	79.75
	7	87.11	87.48	78.40
	14	86.90	87.03	78.62
	21	86.74	86.90	77.45
A	Fresh	-1.41	-1.39	-7.90
	7	-1.46	-1.44	-8.22
	14	-1.52	-1.50	-8.85
	21	-1.67	-1.64	-9.90
B	Fresh	23.50	22.90	35.90
	7	24.30	23.85	36.88
	14	25.60	25.45	37.11
	21	26.03	25.80	37.90

T1: Plain sample contained only 2% yogurt starters used as control, T2: Probiotic sample contained 2% *Bifidobacterium* spp.+Yogurt starter, T3: Algal sample contained 2% yogurt culture+2% *Bifidobacterium* spp.+5 mg mL^{-1} *S. platensis* powder, L: Darkness from black (0) to white (100), A: Color ranging from red (+) to green (-), B: Yellow (+) to blue (-)

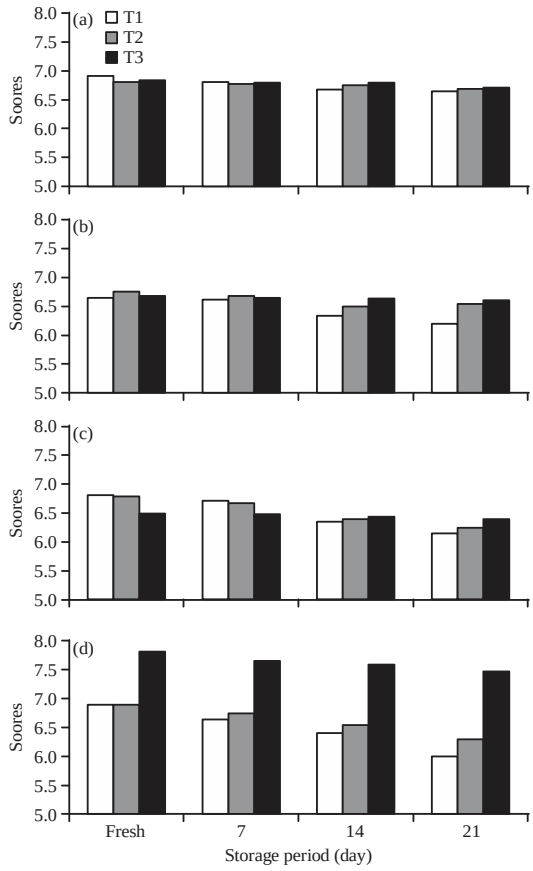

Fig. 2(a-d): Sensory evaluation of Labenah samples during storage period. T1: Plain sample contained only 2% yogurt starters used as control, T2: Probiotic sample contained 2% *Bifidobacterium* spp., plus yogurt starter, T3: Algal sample contained 2% yogurt culture+2% *Bifidobacterium* spp.+5 mg mL^{-1} *Sperulina platensis* powder, (a) Body and texture, (b) Flavor, (c) Appearance and color and (d) All acceptability

and all acceptability. It was also noticed that using of *Spirulina platensis* gave a clean and slightly acidic taste with an acceptable body and texture and favorite properties. No clear differences were observed in body and texture properties between other samples. The acceptability of all samples was decreased at 21 days of storage.

CONCLUSION

It could be concluded that fortification of labenah sample with 5 mg mL^{-1} *Sperulina platensis* powder enhanced the activity of the *Bifidobacterium* spp. and promoted their growth to prepared probiotic-functional product .In addition, this study was succeeded in preparing healthy dairy product rich in high protein quality.

This research recommend that it could be used *Bifidobacterium* spp. to prepare probiotic product. It could be recommended also that, it was beneficial for dairy sector to use the *Spirulina platensis* to rise the nutritive values of the different dairy products. It will help also the researchers to uncover the critical areas that many researchers were not able to explore.

Further studies should be also make on using *sperulina* spp. or other algae spp. in different dairy products.

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