ISSN: 2347-467X, Vol. 08, No. (2) 2020, Pg. 531-540

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Effect of *Arthrospira* Microalga Fortification on Physicochemical Properties of Yogurt

WANIDA PAN-UTAI[1]*, JARIYA ATKONGHAN[2], TIPAKORN ONSAMARK[3] and WACHIRAYA IMTHALAY[2]

[1]Institute of Food Research and Product Development, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.
[2]Division of Fermentation Technology, Faculty of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
[3]Division of Food Science and Technology, Faculty of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Abstract

Arthrospira (Spirulina) platensis, a blue-green microalga, is a rich source of organic nutrients. Microalgae are used as food supplements with enhanced nutritional and functional values. Consumption of fermented milk products such as yogurt has recently increased. Both oven-dried and fresh *Arthrospira* biomass supplemented into yogurt at different concentrations of 0.1, 0.3, and 0.5% (w/v) and 1, 5, and 10% (v/v), respectively improved physicochemical properties. *Arthrospira* supplemented in yogurt fermentation gave faster decrease in pH value with green color index of finished yogurt. Oven drying is a convenient method; however, fresh biomass gave similar nutritional value with higher C-phycocyanin content. During storage, pH, titratable acidity, and color of yogurt showed negligible variations. Results indicated that *Arthrospira* increased both nutritional composition and functional properties of yogurt as a natural food ingredient.

Article History

Received: 13 April 2020
Accepted: 18 June 2020

Keywords

Arthrospira,
Food Ingredient,
Nutritional Composition,
Spirulina,
Yogurt.

Introduction

Expanding public health knowledge, with a preference for natural ingredients and colorants, has led to an increase in consumption of functional health-promoting foods.[1] A balanced and healthy nutritional diet contains vitamins, minerals, and polyunsaturated fatty acids (PUFAs).[2] Microalgae are a rich natural source of bioactive compounds for a healthy diet. They contain proteins, carbohydrates, lipids, minerals, vitamins, essential amino acids, PUFAs, carotenoids, enzymes, and fiber.[3] The microalga *Arthrospira* or *Spirulina* is produced

CONTACT Wanida Pan-utai ifrwdp@ku.ac.th Institute of Food Research and Product Development, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.

Doi: http://dx.doi.org/10.12944/CRNFSJ.8.2.19

commercially all over the world and has been used in human food for thousands of years.[4] *Arthrospira* is a micro-filamentous cyanobacterium with generally recognized as safe (GRAS) status as a functional food by the US Food and Drug Administration.[5] Previous data relating to the safety and side effects of *Arthrospira* were mainly concerned about human clinical potential and application.[6] *Arthrospira* has potential health benefits including carbohydrate, protein, essential amino acids, essential fatty acids, minerals, vitamins, and pigments.[7] Moreover, the lack of cellulosic cell walls allows easy digestion of *Arthrospira* biomass as suitable and acceptable for human consumption.[8]

Fermented milk products or yogurt are preferred foods for probiotics[9] as live microorganisms of bacteria or yeast that provide their host with health benefits.[10] They promote gastrointestinal health benefits through various intestinal functions and electrolyte balance, nutrient absorption, transit time, and management of digestive issues. Probiotics improve nutrient digestibility and metabolic utilization by generating volatile fatty acids that indirectly modify gut microsystems.[11,12] *Lactobacillus* and *Bifidobacterium* are the main genera used in probiotic products.[10]

Market trends for natural sources in food and beverages indicate the potential of utilizing microalgae to satisfy demand. Various species of microalga including *Arthrospira, Chlorella, Dunaliella,* and *Haematococcus* have been developed and incorporated in food products.[4] *Arthrospira* farms produce food supplements but studies are lacking in food product ingredients. Yogurt is a commercially popular fermented food in Asian regions and has potential for supplementation as an alternative natural source of functional food. Therefore, the main objective of this study was to supplement yogurt with *Arthrospira* microalgae rich in bioactive compounds as healthy food. Nutrient composition, functional and physicochemical properties, and significant parameters of *Arthrospira* supplemented yogurt products were evaluated during 4 weeks of storage.

Materials and Methods

Arthrospira Preparation

Arthrospira (Spirulina) platensis IFRPD 1182 was provided by the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Bangkok, Thailand and maintained in Zarrouk medium. The condition of *A. platensis* cultivation followed Pan-utai *et al.,*[13] *Arthrospira* was cultured in a glass photobioreactor which was incubated in chamber equipment with temperature controlled at 30°C, light intensity at 15 Klux and light and dark cycles at 16 and 8 hours respectively. Carbon dioxide mixed with air at 2% was used as a continuous bubble feed with flow rate at 0.67 vvm. Cells were grown to log phase and used as inoculum at 10% in an open raceway pond system containing 200 liters of working volume. The culture was well mixed using a paddle wheel at 15 rpm. Cells were grown to exponential phase around 1 at an optical density of 560 nm, harvested by filtration through a nylon cloth and washed with tap water to completely remove residual culture medium. Fresh *Arthrospira* biomass was mixed with sterilized water at a ratio of 1:1 (w/w) using homogenization at 11,000 rpm for 3 min (Ultra Turrax, T25 basic, IKA, USA) and filtered through 0.45 µm nylon membrane to remove residual cells. Dried *Arthrospira* biomass was obtained by drying at 55°C for 6 hours or until less than 5% moisture content. Dried biomass samples were then milled to 0.5 mm particle size for use in yogurt as supplemented dried *Arthrospira* biomass.

Yogurt Production

Yogurt preparation was based on pasteurized fresh cow's milk (CP-Meiji Co., Ltd., Thailand) as raw material. The milk formulation was composed of sugar at 7% (w/v) and gelatin as thickener at 0.5% (w/v). *Arthrospira* microalgal were supplemented with oven-dried *Arthrospira* biomass at 0.1, 0.3, and 0.5% (w/v) and fresh *Arthrospira* at 1, 5, and 10% (v/v). Fresh *Arthrospira* content was examined on a dry weight basis and expressed as around 6.9% DW. Thus, concentrations of fresh *Arthrospira* supplemented into yogurt at 1, 5 and 10% (v/v) in terms of dry basis were expressed as 0.07, 0.35, and 0.69% (w/v), respectively. Milk formulation without *Arthrospira* supplement was used as the control. Milk formulations were heated for 20 min at 85°C and then cooled to 40°C. A commercial starter culture YC-X11 (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) was mixed according to the manufacturer's instructions (Chr. Hansen, Denmark) and added to the samples. After mixing well and transferring into plastic cups, all samples were incubated at 43°C for 4 hours. The pH and acidity were determined every hour during fermentation. After fermentation, the yogurt samples were cooled and kept at 4°C. All samples were analyzed for pH, titratable acidity, water holding capacity, nutritional composition, C-phycocyanin content, and color after fermentation. In addition, pH, titratable acidity, and color were analyzed during storage at 1, 2, 3, and 4 weeks.

Physicochemical Measurement

All samples were determined for pH and acidity during yogurt fermentation and storage. The pH was measured using a pH meter (Schott, Lab850, Germany). The percentage of titratable acidity (TA) of the yogurt samples was analyzed using 0.1 N NaOH solution using phenolphthalein as the indicator.[14] Acidity was calculated as a percentage of lactic acid produced. The factor is 90.08 (g/mol) which is the molecular weight of lactate. The pH and acidity were measured at different stages of sample preparation including during fermentation, finished products and during storage.

Water holding capacity (WHC) of the different yogurt samples was determined according to the method of Barkallah *et al.,*[15] WHC was analyzed by centrifuging 10 g of each sample at 4,500 x g for 20 min at 4°C (Sorvall RC6 Plus, Thermo Scientific, Germany) and calculated according to the following equation:

$$WHC\ (\%) = (1 - W1/W2) \times 100$$

where W1 is the weight of supernatant after centrifugation and W2 is the weight of yogurt.

Nutritional Composition

Proximate composition of the yogurt samples was analyzed using the AOAC standard method.[16] Dry weights of the samples were analyzed by oven-drying at 105°C overnight to constant weight. Ash content was determined by ignition of the dried samples in an electric furnace at 550°C. Protein content was analyzed by the Kjeldahl method using a nitrogen conversion factor of 6.25. Total lipid contents of the yogurt samples were determined using the ether extraction method (AOAC Official Method 989.05). Carbohydrate content was calculated by difference as the sum of moisture, protein, lipid, and ash content in 100 g dry weight.

C-phycocyanin Content

For determination of C-phycocyanin content, 1 ml of each samples was centrifuged at 5,000 rpm for 10 min. The pellet was dissolved and then sonicated in 1 ml of distilled water for 30 min. The supernatant was collected by centrifugation at 10,000 rpm for 5 min and measured for optical density at 615 and 652 nm (SP-80001, UV/vis Spectrophotometer, Metertech, Taiwan). C-phycocyanin content was calculated using the following equation.[17]

$$\text{C-phycocyanin (mg/mL)} = (OD_{615} - 0.474OD_{652})/5.34$$

Color Evaluation

Color parameters were determined using a Datacolor Spectraflash Spectrophotometer (SF 600 plus, Datacolor International, USA). The instrument was standardized using a standard black trap and white and green plates. Color measurements were expressed in terms of 3 values: lightness (L*) from 0 (black) to 100 (white) with chromaticity parameters, a* as green color (-) to red (+) and b* as blue (-) to yellow (+).

Statistical Analysis

All experiments were analyzed in triplicate. Data from the parameters were subjected to analysis of variance (ANOVA) using SPSS 12.0 (SPSS, Inc., USA). Multiple comparisons in each parameter were conducted using Duncan's multiple range test (DMRT) with significance set at 0.05 ($P<0.05$).

Results and Discussion

Arthrospira platensis microalga was used as the ingredient supplemented in yogurt and investigated as a functional food. All parameters including physicochemical composition, nutritional composition, C-phycocyanin content, and color index value changes during fermentation were determined. Changes in pH, acidity, and color indexes of yogurt products were also assessed during 4 weeks of storage. Yogurt without *Arthrospira* supplementation was used as the control.

Physicochemical Measurement

Arthrospira platensis was prepared as oven-dried biomass and fresh biomass for supplementation in yogurt. The pH values during yogurt fermentation for 4 hours at 43°C and storage at 4°C for 4 weeks are shown in Table 1. The pH values decreased with increasing time during the fermentation process in all experiments. Initial pH values of different conditions were approximated at 6 and decreased to around 4 after 4 hours of fermentation, whereas pH did not fluctuate under storage for 4 weeks. Milk fermentation was inoculated from thermophilic bacteria consisting of a mixture of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* as gelation form. Milk formulation involved heating at high temperature. This caused the denaturation of whey proteins. Gelation, as caseins reach their isoelectric point, results in denatured whey proteins interacting with casein on the surface of casein micelles from around pH 6.6 to 4.6 during yogurt fermentation.[18] Moreover, variations in fermentation time were due to differences in the ability of lactic acid bacteria to grow and ferment in milk.[19] The pH values among various sample treatments were slightly lower than the control during 2-4 hours of fermentation. Similar results from previous reports of A. *platensis* addition in fermented milk showed larger decrease in pH[20]. High protein content of *Arthrospira* microalgae promoted the growth of probiotics in milk.[21] Therefore, both oven-dried and fresh *Arthrospira* supplemented in yogurt gave pH values lower than the control and supported the growth of lactic acid bacteria. Valuable proteins and carbohydrates from *Arthrospira* increased nitrogen and carbon sources in milk formulation, and also increased solid content that supported growth of probiotics during fermentation. These properties were attributed to different yogurt buffering capacities. Yogurt supplemented with *Arthrospira* presented lower buffering capacity during fermentation. Moreover, increase in *Arthrospira* supported growth rates and viability of lactic acid bacteria.[10]

Table 1: The pH values during yogurt fermentation for 4 hours at 43 °C and 4 weeks of storage at 4°C.

Time	Control	Oven-dried *Arthrospira* (%w/v)			Fresh *Arthrospira* (%v/v)		
		0.1	0.3	0.5	1	5	10
Fermentation (h)							
0	6.18 ± 0.00^{abcA}	6.17 ± 0.01^{bcA}	6.21 ± 0.02^{aA}	6.20 ± 0.01^{abA}	6.17 ± 0.00^{bcA}	6.17 ± 0.00^{bcA}	6.16 ± 0.01^{cA}
1	5.97 ± 0.01^{aB}	5.96 ± 0.00^{aB}	5.95 ± 0.02^{abB}	5.92 ± 0.01^{bB}	5.72 ± 0.02^{eB}	5.84 ± 0.02^{cB}	5.79 ± 0.01^{dB}
2	5.35 ± 0.04^{aC}	5.31 ± 0.02^{aC}	5.20 ± 0.01^{bC}	5.19 ± 0.02^{bC}	5.09 ± 0.06^{cC}	5.18 ± 0.01^{bC}	5.10 ± 0.02^{cC}
3	4.75 ± 0.02^{aD}	4.67 ± 0.06^{bcD}	4.70 ± 0.01^{abD}	4.71 ± 0.03^{abD}	4.60 ± 0.01^{dD}	4.66 ± 0.03^{bcdD}	4.61 ± 0.02^{cdD}
4	4.39 ± 0.01^{aE}	4.28 ± 0.02^{cE}	4.18 ± 0.01^{eE}	4.21 ± 0.00^{dE}	4.37 ± 0.01^{aE}	4.32 ± 0.02^{bE}	4.31 ± 0.00^{bE}
Storage (weeks)							
1	4.13 ± 0.01^{abcF}	4.08 ± 0.00^{bcF}	4.06 ± 0.01^{cF}	4.11 ± 0.02^{abcF}	4.15 ± 0.03^{abF}	4.19 ± 0.07^{aF}	4.11 ± 0.05^{abcF}
2	4.08 ± 0.02^{aG}	4.00 ± 0.00^{bG}	3.99 ± 0.01^{bG}	4.06 ± 0.00^{aF}	4.08 ± 0.02^{aG}	4.00 ± 0.03^{bH}	3.96 ± 0.02^{bH}
3	4.08 ± 0.02^{abG}	3.93 ± 0.03^{dG}	4.04 ± 0.04^{abcFG}	4.01 ± 0.02^{cG}	4.09 ± 0.04^{aFG}	4.10 ± 0.02^{aG}	4.02 ± 0.00^{bcG}
4	4.13 ± 0.00^{aF}	4.00 ± 0.01^{bG}	4.01 ± 0.05^{bFG}	4.01 ± 0.04^{bG}	4.09 ± 0.01^{aFG}	4.13 ± 0.02^{aFG}	4.08 ± 0.00^{aF}

Values are averages of triplicates ± standard deviation. Mean values with different superscript letters indicate significant differences.

a, b,… Means in the same row with different letters are significantly different (P<0.05).

A, B,… Means in the same column with different letters are significantly different (P<0.05).

Titratable acidity values during yogurt fermentation and storage are shown in Table 2. Increased acidity of yogurt during fermentation was observed in all experiments. *Arthrospira* addition increased acidity faster than the control and was related to the higher buffering capacity of media containing *Arthrospira*.[10]The finished yogurt product supplemented with 0.5% oven-dried *Arthrospira* gave the highest acidity of 0.78%. Acidity of finished products with oven-dried *Arthrospira* supplemented in yogurt increased at higher concentrations of oven-dried *Arthrospira*, whereas fresh *Arthrospira* showed no significant differences with concentration. The control showed the lowest acidity. Moreover, results also indicated that pH decreased and titratable acidity increased during yogurt fermentation with thermophilic bacteria over 4 hours. Lactic acid production from lactose fermentation led to decreasing pH and increasing titratable acidity as a result of microorganism metabolism.[22] Titratable acidity also slightly increased during the first week of storage due to the decrease in temperature from fermentation to storage. As mentioned, lactic acid fermentation continuing growth and produced lactic acid from active cell viability until becoming stable at storage temperature. Lactic acid bacteria (LAB) instigate three significant biochemical conversions of milk constituents consisting of carbohydrate to lactic acid or other metabolite substances, casein hydrolysis to peptides and breakdown of free amino acids and milk fat to free fatty acids. The role in milk fermentation by lactic acid bacteria produces the final product as lactic acid. Moreover, organic acids provide the flavor and aroma of yogurt as well as acting as a preservative.[22,23]

Table 2: Titratable acidity (%) during yogurt fermentation for 4 hours at 43°C and 4 weeks of storage at 4°C.

Time	Control	Oven-dried *Arthrospira* (%w/v)			Fresh *Arthrospira* (%v/v)		
		0.1	0.3	0.5	1	5	10
Fermentation (h)							
0	0.19 ± 0.04^{aF}	0.17 ± 0.01^{aF}	0.19 ± 0.01^{aE}	0.20 ± 0.00^{aE}	0.18 ± 0.00^{aF}	0.17 ± 0.01^{aG}	0.17 ± 0.01^{aH}
1	0.19 ± 0.01^{cF}	0.20 ± 0.00^{cF}	0.23 ± 0.01^{abE}	0.24 ± 0.01^{aE}	0.21 ± 0.01^{bcF}	0.20 ± 0.00^{cG}	0.21 ± 0.00^{bcG}
2	0.30 ± 0.01^{bE}	0.30 ± 0.00^{bE}	0.36 ± 0.02^{aD}	0.39 ± 0.01^{aD}	0.41 ± 0.04^{aE}	0.36 ± 0.03^{aF}	0.37 ± 0.01^{aF}
3	0.50 ± 0.00^{bcD}	0.51 ± 0.01^{bcD}	0.56 ± 0.02^{aC}	0.59 ± 0.02^{aC}	0.46 ± 0.02^{cD}	0.51 ± 0.04^{bE}	0.49 ± 0.00^{bcE}
4	0.55 ± 0.01^{eC}	0.65 ± 0.00^{cC}	0.76 ± 0.02^{bB}	0.78 ± 0.01^{aB}	0.61 ± 0.01^{dC}	0.61 ± 0.01^{dD}	0.61 ± 0.01^{dD}
Storage (weeks)							
1	0.70 ± 0.03^{cdB}	0.75 ± 0.01^{bB}	0.84 ± 0.01^{aA}	0.86 ± 0.01^{aA}	0.72 ± 0.00^{bcB}	0.68 ± 0.01^{dC}	0.68 ± 0.00^{dC}
2	0.81 ± 0.03^{abcA}	0.83 ± 0.03^{abcA}	0.87 ± 0.01^{abA}	0.90 ± 0.08^{aA}	0.78 ± 0.01^{cA}	0.82 ± 0.01^{bcA}	0.80 ± 0.01^{bcA}
3	0.74 ± 0.03^{cB}	0.80 ± 0.01^{bA}	0.84 ± 0.03^{abA}	0.86 ± 0.01^{aA}	0.74 ± 0.01^{cAB}	0.73 ± 0.02^{cB}	0.74 ± 0.00^{cB}
4	0.73 ± 0.00^{cB}	0.81 ± 0.02^{bA}	0.87 ± 0.01^{aA}	0.90 ± 0.02^{aA}	0.76 ± 0.00^{cA}	0.72 ± 0.00^{cB}	0.72 ± 0.04^{cB}

Values are averages of triplicates ± standard deviation. Mean values with different superscript letters indicate significant differences.

a, b,… Means in the same row with different letters are significantly different ($P<0.05$).

A, B,… Means in the same column with different letters are significantly different ($P<0.05$).

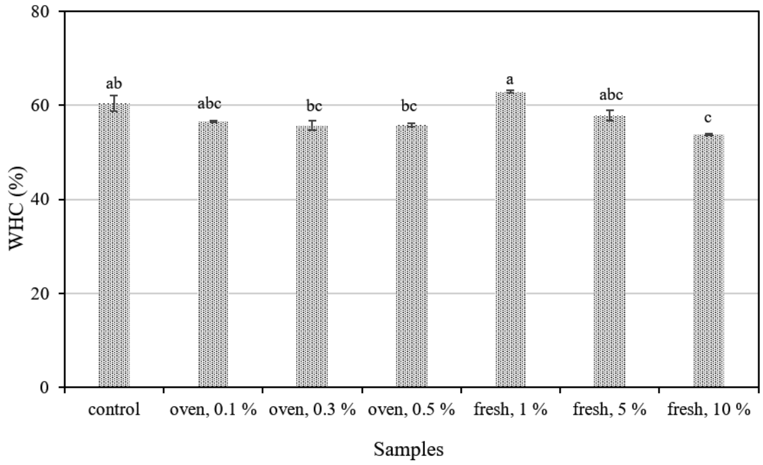

Fig. 1: Water holding capacity of different supplemented concentrations of oven-dried and fresh *Arthrospira* and the control

Water holding capacity in fermented milk like yogurt is mainly determined by the microstructure of the protein network. Water holding capacity indicates the ability of proteins to prevent water releases from their three-dimensional structure. Furthermore, whey protein is vented on the surface of the product if the water-binding capacity is not sufficient.[24] Results showed that water holding capacity in all experiments ranged at 53-62% (Figure 1). Fresh *Arthrospira* supplemented in yogurt displayed the highest water holding capacity. However, the values of water holding capacity showed little difference among various conditions. Yogurt products supplemented with *Arthrospira* showed the ability to retain water after fermentation.

Nutritional Composition

Nutritional compositions of yogurt supplemented with various concentrations of oven-dried and fresh *Arthrospira* and the control treatment as moisture content, protein, fat, ash, and carbohydrate are shown in Table 3. All treatments had similar nutritional compositions compared to the control. Although *Arthrospira* has high protein content, yogurt products cannot be added with large amounts of oven-dried *Arthrospira* powder because this imparts an unsuitable flavor. Previous experiments showed that all organoleptic properties of yogurt with high microalgal powder concentration at 0.75 and 1% gave lower sensory acceptability scores. Unacceptable flavor at higher *Arthrospira* powder concentration results from the oxidation of lipids and minerals that act as pro-oxidant molecules and produce metallic off-flavors.[25]

Arthrospira platensis is considered as a food source of natural molecules with functional composition and nutritional health benefits.[26] *Arthrospira* is a dried food, easy to store and transport with long shelf life.[27] Moreover, oven drying of *Arthrospira* is a conventional low-cost process that also affects protein denaturation in cell structure and composition.[13] However, fresh *Arthrospira* biomass has higher nutritional value compared with the dried biomass. Oven-dried *Arthrospira* biomass showed reduced protein content of 35% with respect to the fresh biomass.[28] Therefore, fresh *Arthrospira* supplemented into yogurt treatments gave similar nutritional composition with oven-dried *Arthrospira*. Yogurt products supplemented with fresh *Arthrospira* produced an off-flavor and smell compared with oven-dried biomass. Furthermore, *Arthrospira* has high iron content. Iron bio-availability has been trialed in both rats and humans without side effects. Thus, yogurt supplemented with *Arthrospira* has proven nutritional beneficial iron content enrichment.[29]

Table 3: Nutritional composition of control, oven-dried, and fresh *Arthrospira* supplemented in yogurt after production.

Composition (g/100 g dry weight)	Control	Oven-dried *Arthrospira* (%w/v)			Fresh *Arthrospira* (%v/v)		
		0.1	0.3	0.5	1	5	10
Moisture	81.57 ± 0.04	82.11 ± 0.57	82.22 ± 1.14	81.20 ± 0.24	81.84 ± 0.07	82.53 ± 0.03	83.24 ± 0.01
Protein	18.43 ± 0.04	17.89 ± 0.57	17.78 ± 0.14	18.80 ± 0.24	18.16 ± 0.07	17.47 ± 0.03	16.76 ± 0.01
Fat	15.54 ± 0.76	17.14 ± 1.54	17.47 ± 1.21	18.65 ± 0.20	16.91 ± 0.42	16.79 ± 0.06	16.08 ± 0.09
Ash	5.02 ± 0.53	3.61 ± 0.07	4.53 ± 0.54	4.59 ± 0.60	3.62 ± 0.11	4.00 ± 0.86	3.80 ± 0.68
Carbohydrate	0.66 ± 0.01	0.63 ± 0.04	0.66 ± 0.01	0.68 ± 0.02	0.67 ± 0.00	0.63 ± 0.01	0.60 ± 0.01

Color Evaluation

Yogurt with *Arthrospira* supplemented treatments achieved different colors of final product as shown in Figures 2-4. Color shade of yogurt supplemented with *Arthrospira* changed from green to blue depending on the added concentration. The color of dairy products is important for consumer acceptance.[30.] Values of L*, a*, and b* indexes recorded lightness, green to red, and blue to yellow respectively. All treatments showed negligible variations after storage for 4 weeks. Yogurt with fresh *Arthrospira* samples showed increased whiteness when compared with oven-dried biomass supplementation, whereas higher quantity of oven-dried biomass gave more greenness than fresh *Arthrospira* supplement. Moreover, yogurt samples with fresh *Arthrospira* had higher blue color indexes as fresh *Arthrospira* gave more C-phycocyanin.

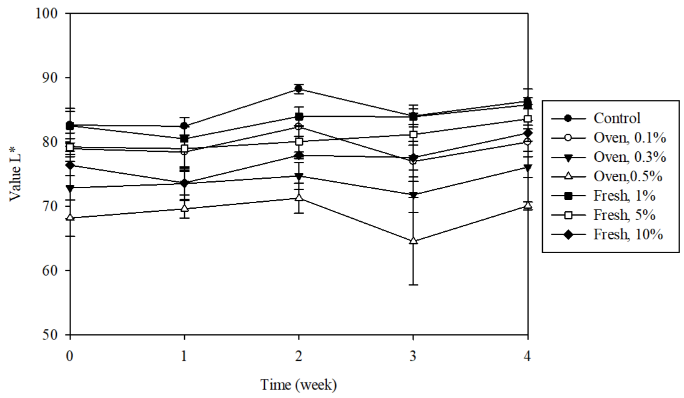

Fig. 2: The L* index values of different oven-dried and fresh *Arthrospira* concentrations in supplemented and control experiments.

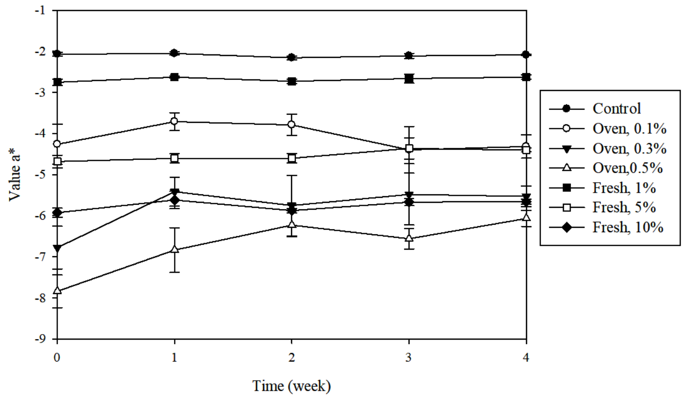

Fig. 3: Value a* index of different oven-dried and fresh *Arthrospira* concentrations in supplemented and control experiments.

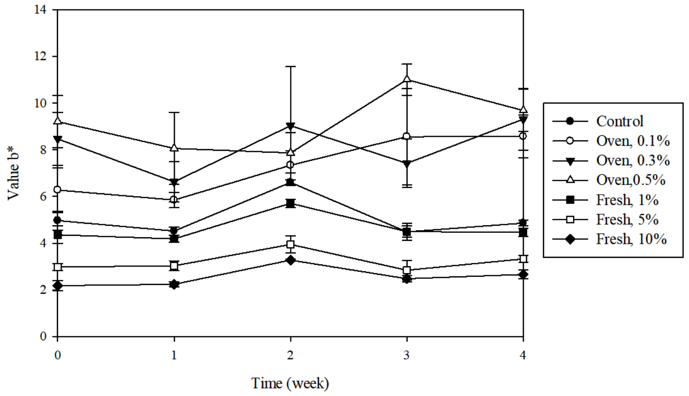

Fig. 4: The b* index values of different oven-dried and fresh *Arthrospira* concentrations in supplemented and control experiments.

C-phycocyanin Content

Arthrospira, a blue-green alga that contains blue pigment as C-phycocyanin (C-PC), is mostly consumed as a natural edible and functional brilliant blue colorant. The alga has excellent antioxidant, anticancer and anti-inflammatory properties. C-phycocyanin (C-PC) is found in cyanobacteria as a blue water-soluble light-harvesting protein-pigment complex.[17] It is normally used as a food colorant ingredient and also as a pharmaceutical reagent with antioxidant and anti-inflammatory.[31] Yogurt products were added with C-PC content under different treatments in the range 0.2-16.5 mg/g (Figure 5). All experiments showed that C-PC content increased with increasing *Arthrospira* addition. Oven-dried *Arthrospira* biomass gave lower C-PC content compared with fresh biomass caused by denaturation at high temperature. Higher C-PC content showed greater antioxidant activity. Our results concurred with Papalia *et al.,*[28] who found that fresh *Arthrospira* gave higher antioxidant activity than frozen and oven-dried *Arthrospira* biomass. Yogurt supplemented with fresh *Arthrospira* achieved high nutritional and antioxidant activity as a functional product.

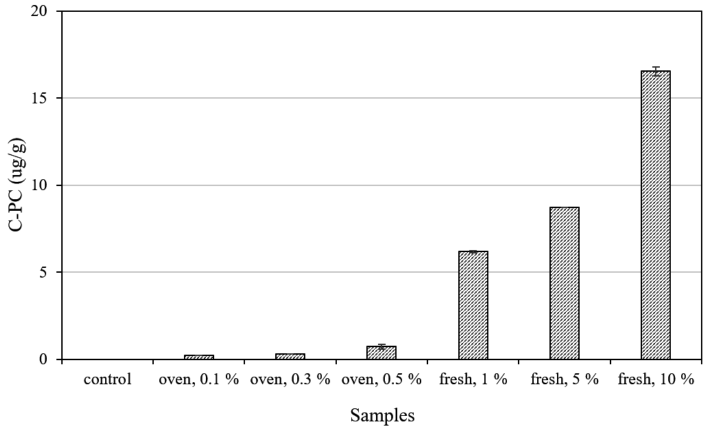

Fig. 5: C-phycocyanin content of different oven-dried and fresh *Arthrospira* concentrations in supplemented and control experiments.

Conclusions

Arthrospira platensis has long been used as a food ingredient with important nutritional and functional properties. Milk fermented with *Arthrospira* addition can be used to develop new healthy yogurt products. Our results showed that yogurt supplemented with *Arthrospira* improved nutritional and functional properties, depending on type of preparation and concentration. It is difficult to assess the optimal condition for *Arthrospira* addition to yogurt. Fresh *Arthrospira* supplementation remains largely understudied; however, addition of fresh *Arthrospira* into yogurt improved nutritional and functional properties of the final product.

Acknowledgements

Thanks are due to the Institute of Food Research and Product Development (IFRPD), Kasetsart University for supplying the research instruments. Partial aspect of this research is protected by petty patent number 1903000763 under the Kasetsart University license.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Vukasovic T. Chapter 20 - Functional foods in line with young consumers: challenges in the marketplace in Slovenia. In: Bagchi D, Nair S, eds. *Developing New Functional Food and*

Nutraceutical Products. San Diego: Academic Press; 2017:391-405.
2. Sathasivam R, Radhakrishnan R, Hashem A, Abd_Allah EF. Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences.* 2019;26(4):709-722. DOI: 10.1016/j.sjbs.2017.11.003
3. Barkia I, Saari N, Manning SR. Microalgae for high-value products towards human health and nutrition. *Marine drugs.* 2019;17(5):304. DOI: 10.3390/md17050304
4. Koyande AK, Chew KW, Rambabu K, Tao Y, Chu D-T, Show P-L. Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness.* 2019;8(1):16-24. DOI: 10.1016/j.fshw.2019.03.001
5. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. Spirulina in clinical practice: evidence-based human applications. Evidence-based complementary and alternative medicine : eCAM. 2011;2011:531053-531053.
6. Wan D, Wu Q, Kuca K. Chapter 42 - *Spirulina.* In: Gupta RC, ed. Nutraceuticals. Boston: Academic Press; 2016:569-583.
7. Kent M, Welladsen HM, Mangott A, Li Y. Nutritional evaluation of Australian microalgae as potential human health supplements. *PLOS One*. 2015;10(2):e0118985.
8. Kose A, Ozen MO, Elibol M, Oncel SS. Investigation of in vitro digestibility of dietary microalga *Chlorella vulgaris* and cyanobacterium *Spirulina platensis* as a nutritional supplement. *3 Biotech.* 2017;7(3):170-170. DOI: 10.1007/s13205-017-0832-4
9. Patel P, Jethani H, Radha C, *et al.,* Development of a carotenoid enriched probiotic yogurt from fresh biomass of *Spirulina* and its characterization. *Journal of Food Science and Technology.* 2019;56(8):3721-3731. DOI: 10.1007/s13197-019-03844-0
10. Beheshtipour H, Mortazavian AM, Mohammadi R, Sohrabvandi S, Khosravi-Darani K. Supplementation of *Spirulina platensis* and *Chlorella vulgaris* Algae into Probiotic Fermented Milks. *Comprehensive Reviews in Food Science and Food Safety.* 2013;12(2):144-154. DOI: 10.1111/1541-4337.12004
11. Vieco-Saiz N, Belguesmia Y, Raspoet R, et al. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology.* 2019;10:57-57. DOI: 10.3389/fmicb.2019.00057
12. Freitas M. Chapter 24 - The benefits of yogurt, cultures, and fermentation. In: Floch MH, Ringel Y, Allan Walker W, eds. *The Microbiota in Gastrointestinal Pathophysiology.* Boston: Academic Press; 2017:209-223.
13. Pan-utai W, Kahapana W, Iamtham S. Extraction of C-phycocyanin from *Arthrospira* (*Spirulina*) and its thermal stability with citric acid. *Journal of Applied Phycology.* 2018;30(1):231-242. DOI: 10.1007/s10811-017-1155-x
14. Dabija A, Codin GG, Ropciuc S, Gatlan A-M, Rusu L. Assessment of the antioxidant activity and quality attributes of yogurt enhanced with wild herbs extracts. *Journal of Food Quality*. 2018;2018:12. DOI: 10.1155/2018/5329386
15. Barkallah M, Dammak M, Louati I, *et al.,* Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. *LWT.* 2017;84:323-330. DOI: 10.1016/j.lwt.2017.05.071
16. AOAC. (Association of Official Analytical Chemistry). Official methods of analysis of the Association of Analytical Chemists International. 2005.
17. Pan-utai W, Iamtham S. Extraction, purification and antioxidant activity of phycobiliprotein from *Arthrospira platensis*. *Process Biochemistry.* 2019;82:189-198. DOI: 10.1016/j.procbio.2019.04.014
18. Lee WJ, Lucey JA. Structure and physical properties of yogurt gels: effect of inoculation rate and incubation temperature. *Journal of Dairy Science.* 2004;87(10):3153-3164. DOI: 10.3168/jds.S0022-0302(04)73450-5
19. Mani-López E, Palou E, López-Malo A. Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. *Journal of Dairy Science.* 2014;97(5):2578-2590. DOI: 10.3168/jds.2013-7551

20. Beheshtipour H, Mortazavian AM, Mohammadi R, Sohrabvandi S, Khosravi-Darani K. Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks. 2013;12(2):144-154. DOI: 10.1111/1541-4337.12004
21. de Caire GZ, Parada JL, Zaccaro MC, de Cano MMS. Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. *World Journal of Microbiology and Biotechnology.* 2000;16(6):563-565.
22. Barros RF, Cutrim CS, Costa MPd, Conte Junior CA, Cortez MAS. Lactose hydrolysis and organic acids production in yogurt prepared with different onset temperatures of enzymatic action and fermentation. *Ciência Animal Brasileira.* 2019;20.
23. Chen C, Zhao S, Hao G, Yu H, Tian H, Zhao G. Role of lactic acid bacteria on the yogurt flavour: A review. *International Journal of Food Properties*. 2017;20(sup1):S316-S330. DOI: 10.1080/10942912.2017.1295988
24. Mortensen G, Andersen U, Nielsen JH, Andersen HJ. 24 - Chemical deterioration and physical instability of dairy products. In: Skibsted LH, Risbo J, Andersen ML, eds. *Chemical Deterioration and Physical Instability of Food and Beverages.* Woodhead Publishing; 2010:726-762.
25. Shimamatsu H. Mass production of *Spirulina*, an edible microalga. *Hydrobiologia*. 2004;512(1):39-44.
26. Seghiri R, Kharbach M, Essamri A. Functional composition, nutritional properties, and biological activities of moroccan *Spirulina microalga*. *Journal of Food Quality.* 2019;2019:11. DOI: 10.1155/2019/3707219
27. de Farias Neves F, Demarco M, Tribuzi G. Drying and quality of microalgal powders for human alimentation. In:2019.
28. Papalia T, Sidari R, Panuccio MR. Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass. Molecules (Basel, Switzerland). 2019;24(15):2810. DOI: 10.3390/molecules24152810
29. Malik P, Kempanna C, Paul A. Quality characteristics of ice cream enriched with *Spirulina* powder. *International Journal of Food and Nutritional Sciences.* 2013;Volume 2:44-50.
30. Dönmez Ö, Mogol BA, Gökmen V. Syneresis and rheological behaviors of set yogurt containing green tea and green coffee powders. *Journal of Dairy Science.* 2017;100(2):901-907. DOI: 10.3168/jds.2016-1126
31. Kumar D, Dhar DW, Pabbi S, Kumar N, Walia S. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). *Indian Journal of Plant Physiology.* 2014;19(2):184-188. DOI: 10.1007/s40502-014-0094-7