proceedings

MDPI

Proceedings

Incorporation of *Spirulina platensis* on Traditional Greek Soft Cheese with Respect to Its Nutritional and Sensory Perspectives †

Loulouda Bosnea [1,*], Antonia Terpou [1,2], Eleni Pappa [1], Efthymia Kondyli [1], Marios Mataragkas [1], Georgios Markou [3] and Georgios Katsaros [3]

[1] Hellenic Agricultural Organization DEMETER, Dairy Research Institute, Katsikas, 45221 Ioannina, Greece; email@gmail.com (A.T.); email@gmail.com (E.P.); email@gmail.com (E.K.); email@gmail.com (M.M.)
[2] Department of Agricultural Development, Agri-food, and Natural Resources Management, School of Agricultural Development, Nutrition & Sustainability, National and Kapodistrian University of Athens, Psachna, 34400 Evia, Greece
[3] Hellenic Agricultural Organization DEMETER, Institute of Technology of Agricultural Products, Leof. Sofokli Venizelou 1, 14123 Athens, Greece; email@gmail.com (G.M.); email@gmail.com (G.K.)
* Correspondence: louloudabosnea@gmail.com; Tel.: +30-26510-094781
† Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/.

Published: 10 November 2020

Abstract: *Spirulina platensis*, the most popular microalgae species known for its high protein content and bioactive compounds such as phycocyanin and allophycocyanin have been studied for cheese fortification. Incorporation of Spirulina in dairy products poses major sensorial challenges due to its characteristic odor and its insolubility in food formulation thus limiting consumer acceptance. The main objective was the production of a novel spread cheese fortified with Spirulina so powdered Spirulina was added at different concentrations (0.25, 0.5, and 1%), and the effect on physicochemical, microbiological, and sensory characteristics was assessed. Cheese samples were examined for pH, fat (Gerber-Van Gulik method), salt (Volhard method), protein (Kjeldahl), and moisture content by drying to constant weight at 102 ± 1 °C. Cheeses were, also, assessed organoleptically by five experienced panelists. Generally, the addition of *spirulina* slightly increased the protein content and affected the color of the cheeses. The cheeses achieved a good microbiological profile and were all characterized as acceptable for consumption by the panelists. However, the cheeses with 0.25 and 0.5% spirulina were mostly preferred by the evaluation panel due to the less intense characteristic odor and taste of spirulina. Concluding, it is possible to produce an acceptable spread cheese with the addition of spirulina without significant changes in the cheese production line.

Keywords: microalgae; *Spirulina platensis*; dairy; soft cheese; functional foods; sensory properties

1. Introduction

Milk and dairy products have an important role in human diet due to their nutritional benefits from proteins, mineral and vitamin. Especially in Greece, dairy products are consumed massively and are important part of the daily Greek diet. Recently, food scientists have tried the fortification of dairy products using natural products to ameliorate the overall dietary intake of foods with minimal effects [1]. Microalgae are known to be rich in proteins, amino acids, vitamins, and various minerals, as well as polysaccharides, sterols, and fatty acids. They have great potential to be used as fortification products in dairy as they contain varius macro- components (polysaccharides and sulphated polysaccharides as stabilizer) and micro-components (polyunsaturated fatty acids as bioactive compounds and pigments as a coloring agent) posing important functional characteristics [2]. *Spirulina platensis*, the most popular microalgae species known for its high protein content and bioactive compounds has been studied as

such potential natural resource for dairy products fortification [2]. Although the technological impacts of spirulina in fermented milks has been studied before, the effect on the nutritional and sensorial qualities of soft cheese has not been addressed. Also, incorporation of dried Spirulina in dairy products poses major sensorial challenges due to its characteristic odor and its insolubility in food formulation thus limiting consumer acceptance. The main objectives of the current study were to produce a novel functional soft cheese fortified with powdered Spirulina, to determine the concentration of *S. platensis* that can be added to the product and to evaluate its effects on the physicochemical, textural and sensorial characteristics of the produced cheese.

2. Materials and Methods

2.1. Production of Spirulina Enriched Fresh Soft Cheese

24 kg of ewe's milk (Fat: 5.43, Protein: 5.20, lactose: 4.61, Total Solids: 16.05) was collected after milking, cloth filtered to remove contaminants and external material and heated up to 85 °C and then cooled down to 35 °C where yogurt culture was added and divided into equal portions (6 kg each) in stainless steel buckets with a lid. In each bucket 1 mL Kg^{-1} of milk $CaCl_2$ (40% *w*/*v*) and liquid commercial rennin (1 mL Kg^{-1}) were added. Subsequently the samples were placed in a chamber at 20 °C for 24 h. After 24 h the curd was removed from each container and placed into perforated fabric called 'tsandila' for cheese whey removal. The cheese curd of each sample is left to drain into the fabric bags in a chamber at 12 °C for 48 h. Finally, the samples are removed from the fabric bags, weighed and placed in special storage containers and salt (type 2, 1 g kg^{-1} of cheese) and spirulina in powder form (0.25, 0.5, and 1 g kg^{-1}) were added.

2.2. Physicochemical, Microbiological, and Sensory Analysis of Fresh Soft Cheese Enriched with Powered Spirulina

The milk for the cheesemaking was analyzed for physicochemical parameters, i.e., fat, protein, lactose, total solid by Milko-Scan, model 6000 (Foss Electric, Hillerød, Denmark). Milk pH was measured directly with a pH meter (Micro pH 2002; Crison, Barcelona, Spain). Microbiological evaluation was carried out by assessing the total viable counts (TVC) using the Bactoscan FC (Foss Electric, Hillerød, Denmark).

Cheese samples were examined for pH electrometrically (Micro pH 2002; Crison, Barcelona, Spain) and were analyzed for their fat according to the Gerber-Van Gulik method (Schneider, 1954), salt according to the modified Volhard method (Kosikowski, 1982) and moisture content by drying to constant weight at 102 ± 1 °C (International Dairy Federation, 1982).

On each sampling date, ten-gram portions of each cheese sample were added to 90 mL with sterilized Ringer solution ¼ strength and mixed with a stomacher (Bagmixer 400, Model VW, Interscience) for 120 s at room temperature. Subsequent dilutions were made in sterilised Ringer's solution ¼ strength. Viable counts for staphylococci, lactic acid bacteria, lactic cocci, moulds and yeasts, coliforms and enterobacteria were performed in duplicate. 1-mL or 0.1-mL samples of appropriate dilutions were poured or spread on total or selective agar plates for each species and according to instructions given by manufacturer. Unless otherwise stated, all media and supplements were purchased from Neogen Culture Media (Heywood, UK).

Coliform counts were enumerated on violet red bile agar after incubation at 30 °C for 24 h, and total Enterobacteriaceae were enumerated on violet red bile glucose agar after incubation at 37 °C for 24 h. Total mesophilic lactic acid bacteria (LAB) were enumerated on de Man, Rogosa, Sharpe (MRS) agar, incubated at 37 °C for 72 h under aerobic conditions. Mesophilic cocci were enumerated on M17 agar, incubated at 30 °C for 48–72 h. Total staphylococci were enumerated on Baird Parker agar base with egg yolk tellurite (BP), incubated at 37 °C for 48 h; yeasts and moulds were enumerated on rose Bengal chloramphenicol agar, incubated at 25 °C for 5 days.

A consumer acceptance test was conducted by 5 experts (25–55 years old) concerning attributes of dairy-sour taste, microalgae odor and taste, sweetness, bitterness, dairy flavor, crumbly texture, smoothness, color and overall acceptability. Assessors were non-smokers and they were familiar with the consumption of dairy and fermented milk products [3].

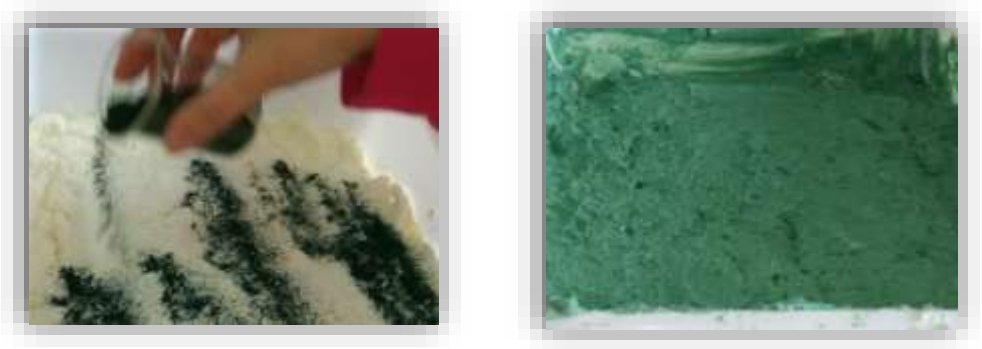
3. Results & Discussion

3.1. Cheese Production and Physicochemical Analysis

Galotyri is a fresh traditional Greek soft cheese. It is prepared using full fat sheep milk either raw or pasteurized. In the present study ewe's milk was applied without pasteurization to maintain its aromatic and nutritional characteristics as the initial samples were submitted to cheese making instantly after milk collection. Milk was collected immediately after milking, filtered and heat treated at 85 °C, then was cooled down to 35 °C and yogurt culture was added and divided into equal portions (6 kg each). The yield of milk in cheese is estimated at 40% as from the 6kg of ewe's milk we receive 2.4 kg ± 0.4 of cheese. In each bucket 1 mL/Kg of cheese of $CaCl_2$ (40% *w*/*v*) and liquid rennin were added. In Figure 1 are illustrated the stages of production until the final cheese curd is retrieved (Figure 1c). To produce galotyri with incorporated spirulina the samples are removed from the fabric bags, weighed and placed in special storage containers (Figure 1c), salted (type 2, 1 g kg^{-1} of cheese) and mixed with powder spirulina (0.25, 0.5, and 1 g kg^{-1}) (Figure 2).

(**a**) (**b**) (**c**)

Figure 1. Manufacture stages of galotyri fresh cheese. (**a**) Samples removed from their initial containers and placed into perforated fabric called 'tsandila' targeting cheese whey removal; (**b**) The cheese curd of each sample is left to drain into fabric bags in a chamber at 12 °C for 48 h; (**c**) Cheese curd retrieved after drainage.



(**a**) (**b**)

Figure 2. (**a**) Addition of powder spirulina in fresh cheese; (**b**) Fresh cheese type-Galotyri with incorporated S*pirulina platensis*.

The chemical composition of galotyri was determined during storage at 4 °C after two days of production (Table 1). Products of microalgae origin have been widely used as a high-protein supplements especially in human nutrition as well as for nutraceutical purposes. In the present study a slight rase in protein content was observed as the content of incorporated spirulina was incorporated in higher amounts. This result was mostly expected as *Spirulina platensis* is one of the most nutritious microalgae known containing a wide capacity of essential amino acids and protein content of up to 70% of its dry biomass [4].

Table 1. Chemical composition of fresh cheese so named 'galotyri' determined 2 days after the addition of salt and powered spirulina.

(%)	Control	1% spirulina	0.5% spirulina	0.25% spirulina
Moisture	69.60 ± 0.16	68.70 ± 0.34	69.52 ± 0.15	69.67 ±0.30
Fat	11.54±0.00	12.55±0.35	12.05 ±0.35	11.75±0.35
Protein	11.02±0.22	12.10 ± 0.35	11.55±0.11	11.22±0.40
NaCl	0.98±0.01	1.09±0.02	1.05±0.02	1.02 ±0.05

3.2. Cheese Microbiological Profile

Microorganisms in dairy products impose a significant impact on physicochemical and organoleptic characteristics of the final product. Likewise, microbial stability is of crucial role for maintenance of cheese products original character [1]. In the present study, the different concentrations (0.25, 0.5, 1%) of incorporated spirulina did not affect the final microbiological profile of produced cheeses (Table 2). The usual storage days of microbiological accepted fresh cheeses such as galotyri is up to 30 days after production. Likewise, in the present study, we can observe the increase in population of possible spoilage microorganism such as yeast and moulds after 60 days of storage in all cheese samples.

Table 2. Microbiological profile of galotyri during cold storage at 4 °C for 60 days.

Cheese Sample	Days of Storage	Mesophilic Lactobacilli	Mesophilic Lactococci	Staphylococci	Total Coliforms	Total Enterobacteria	Molds and Yeasts
Control	7	7.81	9.72	2.91	1.90	1.78	0.00
1% spirulina		8.80	9.34	0.00	1.60	2.08	0.00
0.5% spirulina		8.07	9.56	1.85	2.26	2.40	0.00
0.25% spirulina		7.90	9.62	0.00	2.40	2.45	0.00
Control	14	9.18	5.72	0.00	0.00	0.00	3.21
1% spirulina		9.20	5.34	0.00	0.00	0.00	3.00
0.5% spirulina		9.67	5.56	0.00	1.85	0.00	1.85
0.25% spirulina		8.54	5.62	1.00	1.48	2.00	1.48
Control	21	5.00	7.75	0.00	0.00	0.00	4.21
1% spirulina		5.66	7.89	0.00	0.00	0.00	2.98
0.5% spirulina		5.00	7.99	0.00	0.00	0.00	2.98
0.25% spirulina		5.70	7.92	0.00	0.00	0.00	2.86
Control	30	4.88	8.94	1.70	0.00	0.00	5.26
1% spirulina		5.44	9.02	0.00	1.90	1.70	4.31
0.5% spirulina		4.79	9.04	0.00	0.00	0.00	4.26
0.25% spirulina		4.77	8.77	0.00	0.00	0.00	4.16
Control	45	4.12	8.26	0.00	0.00	0.00	5.35
1% spirulina		5.60	8.90	0.00	0.00	0.00	5.66
0.5% spirulina		4.42	9.30	0.00	0.00	0.00	5.29
0.25% spirulina		4.78	8.70	0.00	0.00	0.00	5.06
Control	60	4.60	8.51	1.30	0.00	0.00	6.46
1% spirulina		6.30	8.04	0.00	0.00	0.00	5.48
0.5% spirulina		6.10	8.28	0.00	0.00	0.00	5.52
0.25% spirulina		5.34	8.26	0.00	0.00	0.00	5.27

3.3. Sensory Evaluation: Consumers Acceptance Test

Spirulina enriched fresh cheeses were produced by the addition of powder spirulina in different concentrations (Figure 2a). Spirulina as most microalgae is known to pose an undesirable odor. As matter of fact scientific researcher have investigated methods targeting to reduce the algae-related odor of Spirulina using activated charcoal absorption, heating, lysozyme enzymatic hydrolysis, β-

cyclodextrin inclusion, fermentation, and solvent extraction. Fermentation and ethanol extraction have been proved as the most effective methods for the reduction of algae-like odor of spirulina [5]. Likewise, in the present study algae-like odor was detected in cheese samples but in the intense of powder spirulina. Specifically, the cheeses with incorporated 0.25 and 0.5% spirulina were mostly preferred by the panelists as they posed a less intense odor and taste of spirulina. In general, all cheese samples received high scores of preferences and were characterized as acceptable for consumption by the expert's evaluation panel.

4. Conclusions

Microalgae biomass is considered a sustainable source of proteins that can meet the growing global demand for these biomolecules. Spirulina is one of the most nutritious microalgae known and can reach protein content of up to 70% of the dry biomass. Spirulina poses great potential to be used as functional fortification ingredient in dairy. Likewise, in the present study the protein fortification, microbiological stability and consumers acceptance were verified in the production of a novel fresh cheese containing powder spirulina providing a nutritional product will in parallel the original recipe and character of traditional galotyri cheese were preserved.

Author Contributions: Conceptualization, L.B. and G.M.; methodology, L.B., G.M.; software, M.M.; validation, G.K., A.T. and L.B.; formal analysis, E.P., E.K.; investigation, L.B., G.M. & A.T.; resources, L.B.; data curation, E.P. and A.T.; writing—original draft preparation, A.T.; writing—review and editing, L.B.; visualization, L.B., G.M. & A.T.; supervision, L.B.; project administration, L.B.; funding acquisition, L.B.". All authors have read and agreed to the published version of the manuscript.

Funding: This research (MIS number: 5040303, project title: Innovative cheese products with microalgae) was funded by the action 'Support of research, development and innovation projects- RIS3 Agri-foods' and was co-financed by the European Union (European Regional Development Fund) and Greece, under the "Operational Program Western Greece 2014–2020" of the National Strategic Reference Framework.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Terpou, A.; Papadaki, A.; Lappa, I.K.; Kachrimanidou, V.; Bosnea, L.A.; Kopsahelis, N. Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved Viability and Delivery of Enhanced Beneficial Value. *Nutrients* **2019**, *11*, 1591.
2. Durmaz, Y.; Kilicli, M.; Toker, O.S.; Konar, N.; Palabiyik, I.; Tamtürk, F. Using spray-dried microalgae in ice cream formulation as a natural colorant: Effect on physicochemical and functional properties. *Algal Res.* **2020**, *47*, 101811, doi:10.1016/j.algal.2020.101811.
3. Terpou, A.; Papadaki, A.; Bosnea, L.; Kanellaki, M.; Kopsahelis, N. Novel frozen yogurt production fortified with sea buckthorn berries and probiotics. *LWT* **2019**, *105*, 242–249, doi:10.1016/j.lwt.2019.02.024.
4. da Fontoura Prates, D.; Duarte, J.H.; Vendruscolo, R.G.; Wagner, R.; Ballus, C.A.; da Silva Oliveira, W.; Godoy, H.T.; Barcia, M.T.; de Morais, M.G.; Radmann, E.M.; et al. Role of light emitting diode (LED) wavelengths on increase of protein productivity and free amino acid profile of Spirulina sp. cultures. *Bioresour. Technol.* **2020**, *306*, 123184, doi:10.1016/j.biortech.2020.123184.
5. Bao, J.; Zhang, X.; Zheng, J.-H.; Ren, D.-F.; Lu, J. Mixed fermentation of Spirulina platensis with Lactobacillus plantarum and Bacillus subtilis by random-centroid optimization. *Food Chem.* **2018**, *264*, 64–72, doi:10.1016/j.foodchem.2018.05.027.