

Influence of Incorporated *Arthrospira* (Spirulina) *platensis* on the Growth of Microflora and Physicochemical Properties of Feta-Type Cheese as Functional Food [†]

Antonia Terpou ^{1,2,*}, Loulouda Bosnea ¹, Marios Mataragkas ¹ and Giorgos Markou ³

¹ Dairy Research Institute, Hellenic Agricultural Organization DEMETER, Katsikas, 45221 Ioannina, Greece; louloudabosnea@gmail.com (L.B.); mmaster@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, 34400 Psachna, Evia, Greece

³ Institute of Technology of Agricultural Products, Hellenic Agricultural Organization DEMETER, 14123 Athens, Greece; gmarkou@itap.com.gr

* Correspondence: aterpou@upatras.gr

[†] Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/.

Abstract: The prevalence of functional foods has increased within the industrial production era. In this context, microalgae (including cyanobacteria) have become an innovative and promising resource of nutritional supplements as they are commercially cultivated to produce valuable compounds, including protein, pigments, lipids, essential amino acids, monounsaturated and polyunsaturated fatty acids, carotenoids, steroids, and vitamins, among others. Specifically, *Arthrospira* (Spirulina) *platensis*, which has been recognized as safe (GRAS) for human consumption, provides important properties, such as anticancer and antihypertensive activity, immune system enhancement, high content of antioxidants, vitamins and trace elements, and it belongs to the category of superfoods. Concerning the above, the main target of the current study was to produce a novel functional feta-type cheese with incorporated Spirulina and study its effect on microflora and physicochemical properties of produced cheeses. Feta cheese was prepared with pasteurized sheep milk. The effects of supplemented Spirulina (0.25%) were studied during manufacture and storage (4 °C) of feta-type cheese for 60 days and compared with commercial feta cheese. Growth and viability of *Lactobacilli* and *Lactococci* were reported more often in cheese samples containing Spirulina and, as a result, the starter culture (lactic acid bacterial culture) used in feta cheese production is not disturbed, and can be even enhanced, by the presence of microalgae. Specifically, Spirulina showed a positive effect on the growth of *Lactobacilli*, while *Lactococci* viability was detected enhanced in cheese sample with incorporated Spirulina. In addition, no pathogenic microorganisms were detected after the 30th day of production. Finally, incorporation of Spirulina significantly affected the color and mouthfeel of produced cheeses, while higher moisture content was detected as the content of Spirulina increased. To conclude, Spirulina has great industrialization potential as an additive in dairy products, and especially ones produced by *Lactobacilli* as starter cultures, while enhancing products nutritional value in parallel.

Keywords: *Arthrospira* (Spirulina) *platensis*; feta cheese; functional foods; sensory properties; nutritional value

Citation: Terpou, A.; Bosnea, L.; Mataragkas, M.; Markou, G. Influence of Incorporated *Arthrospira* (Spirulina) *platensis* on the Growth of Microflora and Physicochemical Properties of Feta-Type Cheese as Functional Food. *Proceedings* **2021**, *70*, 97. https://doi.org/10.3390/foods_2020-07659

Published: 9 November 2020

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The prevalence of functional foods has increased within the industrial production era due to the increasing demand of consumers for nutritious and safe foods with health

benefits, particularly of natural origin. This field is growing a lot lately since bioactive natural compounds, which are parts of several natural foods, exert pharmacological effects, and therefore practically add their “functionality” to food products. In this context, microalgae have become an innovative and promising resource of nutritional supplements as they are commercially cultivated to produce valuable compounds, including protein, pigments, lipids, essential amino acids, monounsaturated and polyunsaturated fatty acids, carotenoids, steroids, and vitamins, among others. Specifically, *Spirulina*, which has been recognized as safe (GRAS) for human consumption [1], provides important properties such as anticancer and antihypertensive activity; immune system enhancement; high content of antioxidants, vitamins, and trace element; and belongs to the category of superfoods. Concerning the above, the main target of the current study was to produce a novel functional feta-type cheese with incorporated *Spirulina* and study its effect on microflora and physicochemical properties of the produced cheeses. Fermentation is known to break down the polymers and release micromolecules, which can be absorbed more easily by consumers [1]. This is achieved via microbial metabolism and enzyme production. Likewise, the applied starter culture of cheese is expected to promote nutrient availability and functional value of added *Spirulina*. In addition, *Spirulina* has been reported to enhance growth and viability of lactic acid bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*) [2], therefore it is also expected to enforce the starter culture during feta-type cheese production.

2. Materials and Methods

2.1. Production of Feta-Type Cheese Enriched with *Spirulina* Microalgae

Fresh raw ewe milk standardized to 6% fat content was filtered and pasteurized at 63 °C for 30 min. After cooling (37 °C), a direct vat of yogurt starter culture (*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) at a rate of 0.02 g kg⁻¹ was added at a rate of 0.02 g kg⁻¹, as per producer’s instructions, and left undisturbed for 30 min. Then, 0.02 mL kg⁻¹ of liquid rennet and 0.02 mL kg⁻¹ of CaCl₂ (40% w/v) was added so that the coagulation could be achieved in about 30 min. The cheese curd was cut into small pieces (cubes of 2 cm side) and transferred into 2 rectangular molds. In one of the molds, between the layers of the curd that were added, a quantity of powdered *Spirulina* (0.25%) was spread (Figure 1).



Figure 1. Powder *Spirulina* added during feta-type cheese production.

After draining the curd for 30 min, the molds were turned upside down and the same procedure was repeated 3 times. Then, the molds were placed in a chamber of 16 °C for 24 h. The next day, the curd was removed from the molds and cut into smaller pieces so that it could be placed in tin vessels. Granular recrystallized NaCl (type 3), equivalent to 25.0 g kg⁻¹ of cheese, was added and the curd was placed in the tin vessel and placed in a chamber at 16 °C for another 24 h. Then, the aqueous phase (released whey from the curd)

was removed and replaced by 70.0 g kg⁻¹ NaCl solution in a ratio of brine volume to cheese weight of 1:4. The tin vessels were sealed and left for ripening (16–18 °C) for about 15 days. Subsequently, the cheeses were transferred into the storage rooms (3–4 °C) and remained there until proven microbiologically as ready for consumption.

2.2. Physicochemical, Microbiological, and Sensory Analysis of Feta-Type Cheese

Cheese samples were examined for pH electrometrically (Micro pH 2002; Crison, Barcelona, Spain), analyzed for salt content according to the modified Volhard method [3] and analyzed for moisture content by drying to constant weight at 102 ± 1 °C [4].

Classical microbiological analysis was performed during the manufacture and storage of cheese. On each sampling date, ten-gram portions of each cheese sample were added to 90 mL sterilized Ringer solution ¼ strength and mixed with a stomacher (Bag-mixer 400, Model VW, Interscience) for 120 s at room temperature. Subsequent dilutions were made in sterilized Ringer's solution ¼ strength. Viable counts for *Staphylococci*, lactic acid bacteria, lactic cocci, molds and yeasts, coliforms, and enterobacteria were performed in duplicate. Dilution samples from cheese (1 mL or 0.1 mL) were poured or spread on total or selective agar plates for each species according to instructions given by manufacturer [5]. Unless otherwise stated, all media and supplements were purchased from Neogen Culture Media (Heywood, UK).

A consumer acceptance test was conducted after the cheese was microbiologically verified as ready for consumption according to manufacturer guidelines for feta cheese production (60 days). The evaluation panel consisted of 5 experts (25–55 years old), who provided their comments concerning cheese sensory attributes of smoothness, bitterness, dairy-sour taste, microalgae odor and taste, dairy flavor, crumbly texture, color, and overall acceptability. Assessors were non-smokers and they were familiar with the consumption of dairy and fermented milk products.

3. Results and Discussion

3.1. Cheese Production and Physicochemical Analysis

The moisture and salt composition were determined during storage of produced cheese (Table 1). Moisture content was determined to be approximately 2% lower compared to commercial feta cheese. This result can be explained as power Spirulina is added in dry form and can absorb a significant content of water during initial incorporation with cheese curd. As a result, the final moisture content of Spirulina-fortified cheese is lower compared to commercial feta cheese.

Table 1. Chemical composition of cheese during maturation for 60 days.

Cheese Type	Days of Storage	Moisture (%)	Salt
Control (Feta)	1	64.45	3.69
	15	63.29	2.8
	60	62.27	2.46
Spirulina 0.25%	1	62.71	3.25
	15	62.47	2.56
	60	59.33	2.22

3.2. Cheese Microbiological Profile

Spirulina is one of the most nutritious microalgae. The bioactive peptides deriving from Spirulina may possess antibacterial, antihypertensive, antitumor, antiallergic, and immune modulation properties, while its protein content can reach up to 70%. Fermentation is a procedure known to break down polymers into micromolecules through microbial metabolism and enzyme production. Through fermentation of feta cheese starter cul-

ture, *Spirulina* nutrient availability and functional value can be promoted [1]. In the present study, the effect of supplemented powder *Spirulina* (0.25%) was studied during manufacture and storage (4 °C) of feta-type cheese for 60 days and compared with commercial feta cheese. Growth and viability of *Lactobacilli* and *Lactococci* were more highly reported in cheese samples containing *Spirulina* and, as a result, the starter culture (lactic acid bacterial culture) used in feta cheese production is not disturbed and can even be enhanced by the presence of microalgae. Specifically, *Spirulina* showed a positive effect on the growth of *Lactobacilli*, while *Lactococci* viability was enhanced in cheese samples with incorporated *Spirulina* (Table 2). Finally, no pathogenic microorganisms were detected after the 30th day of production in all cheese samples and, as a result, cheeses were characterized as safe-for-consumption.

Table 2. Microbiological profile of feta-type cheese during maturation and storage at 4 °C.

Cheese	Days	Mesophilic <i>Lactobacilli</i>	Mesophilic <i>Lactococci</i>	<i>Staphylococci</i>	Total Coliforms	Total Enterobacteria	Molds and Yeasts
Feta	1	9.00	9.98	0.00	3.42	3.45	0.00
	15	6.70	8.08	0.00	2.66	2.71	1.70
	30	4.60	7.40	1.00	1.30	1.60	3.34
	60	4.48	7.81	0.00	0.00	0.00	2.34
Feta with <i>Spirulina</i> 0.25%	1	9.65	9.86	1.30	1.78	2.36	0.00
	15	6.28	8.85	1.30	1.48	1.60	2.81
	30	5.11	8.69	0.00	0.00	0.00	4.18
	60	4.85	8.61	0.00	0.00	0.00	2.08

3.3. Sensory Evaluation: Consumers Acceptance Test

Spirulina is a blue-green filamentous prokaryotic cyanobacterium known for its algae-like odor [6]. In the present study, odor of incorporated *Spirulina* in feta-type cheese samples was characterized as insignificant by evaluators. These results probably came as an outcome of fermentation and the volatile by-products of feta cheese, which eliminated the algae odor of *Spirulina*. Incorporation of *Spirulina* affected the color and mouthfeel of produced cheeses. Moreover, the green color of the novel *Spirulina*-enriched feta-type cheese was positively rated by evaluators. Finally, all cheese samples received high scores of preferences and were characterized as acceptable for consumption by the expert evaluation panel.

4. Conclusions

Spirulina is one of the most nutritious microalgae. The bioactive peptides deriving from *Spirulina* may possess antibacterial, antihypertensive, antitumor, antiallergic, and immune modulation properties, while its protein content can reach up to 70%. Fermentation generally breaks down the polymers into micromolecules through microbial metabolism and enzyme production, promoting nutrient availability and the functional value of *Spirulina*. As a result, *Spirulina* can be successfully applied as functional fortification supplement in dairy products. In the present study, the moisture, salt, microbiological stability, and consumers' acceptance were tested after the production of cheese containing powder *spirulina* (0.25%) and compared with traditional feta cheese, which was used as control. The novel cheese achieved exceptional organoleptic, physicochemical, and microbiological characteristics. As a result, *Spirulina* has great industrialization potential as an additive in feta-type cheese, while enhancing the product's nutritional value in parallel.

Author Contributions: Conceptualization, L.B. and A.T.; methodology, L.B.; software, M.M.; validation, G.M., A.T. and L.B.; formal analysis, A.T.; investigation, L.B. and A.T.; resources, L.B.; data curation, M.M. and A.T.; writing—original draft preparation, A.T.; writing—review and editing, A.T.; visualization, L.B. and A.T.; supervision, L.B.; project administration, L.B.; funding acquisition, L.B., G.M. and M.M. 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 micro-algae) 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.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Ovando, C.A.; Carvalho, J.C.D.; Vinícius de Melo Pereira, G.; Jacques, P.; Soccol, V.T.; Soccol, C.R. Functional properties and health benefits of bioactive peptides derived from *Spirulina*: A review. *Food Rev. Int.* **2018**, *34*, 34–51, doi:10.1080/87559129.2016.1210632.
2. Çelekli, A.; Alslibi, Z.A.; Bozkurt, H.ü. Influence of incorporated *Spirulina platensis* on the growth of microflora and physicochemical properties of ayran as a functional food. *Algal Res.* **2019**, *44*, 101710, doi:10.1016/j.algal.2019.101710.
3. Fernandez, A.; Kosikowski, F.V. Low Moisture Mozzarella Cheese from Whole Milk Retentates of Ultrafiltration. *J. Dairy Sci.* **1986**, *69*, 2011–2017, doi:10.3168/jds.S0022-0302(86)80630-0.
4. Terpou, A.; Bosnea, L.; Kanellaki, M.; Plessas, S.; Bekatorou, A.; Bezirtzoglou, E.; Koutinas, A.A. Growth Capacity of a Novel Potential Probiotic *Lactobacillus paracasei* K5 Strain Incorporated in Industrial White Brined Cheese as an Adjunct Culture. *J. Food Sci.* **2018**, *83*, 723–731, doi:10.1111/1750-3841.14079.
5. Terpou, A.; Gialleli, A.I.; Bosnea, L.; Kanellaki, M.; Koutinas, A.A.; Castro, G.R. Novel cheese production by incorporation of sea buckthorn berries (*Hippophae rhamnoides* L.) supported probiotic cells. *LWT Food Sci. Technol.* **2017**, *79*, 616–624, doi:10.1016/j.lwt.2016.11.021.
6. 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.