

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

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Abstract: Functional foods have raised within the industrial production era. 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 platensis* which has recognized as safe (GRAS) for human consumption, provides important properties such as anti-cancer and anti-hypertensive activity, immune system enhancement, high content of antioxidants, vitamins and trace elements 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 platensis* and study its effect on microflora and physicochemical properties of produced cheeses. Feta cheese was prepared with pasteurized sheep milk. The effects of supplemented *S. platensis* (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 was reported higher in cheese samples containing spirulina as a result the starter culture (lactic acid bacterial culture) used in Feta cheese production is not disturbed can be even enhanced by the presence of microalgae. Specifically, *S. platensis* 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 moisture was detected higher as the content of spirulina increased. To conclude, *Spirulina platensis* 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: *Spirulina platensis*; feta cheese; functional foods; sensory properties; nutritional value

1. Introduction

Functional foods have raised within the industrial production era due to the increasing consumers demand 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 platensis* which has recognized as safe (GRAS) for human consumption [1] provides important properties such as anti-cancer and anti-hypertensive activity, immune system enhancement, high content of antioxidants, vitamins and trace elements 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 platensis* 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 platensis* 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 yogurt starter culture (*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) at a rate of 0.02 g kg⁻¹ was added 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 as 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 was added a quantity of powdered spirulina (0.25%) was spread (Figure 1).



Figure 1. Powder *Spirulina platensis* 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 for 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, cut into smaller pieces so that can 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 at the chamber of 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 proved 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) and were analyzed for salt content according to the modified Volhard method (Kosikowski, 1982) and moisture content by drying to constant weight at 102 ± 1 °C (International Dairy Federation, 1982).

Classical microbiological analysis was performed during manufacture and storage of cheese. On each sampling date, ten-gram portions of each cheese sample were added to 90 mL with sterilized Ringer solution $\frac{1}{4}$ 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 $\frac{1}{4}$ strength. Viable counts for staphylococci, lactic acid bacteria, lactic cocci, moulds and yeasts, coliforms and enterobacteria were performed in duplicate. Dilutions' samples from cheese (1-mL or 0.1-mL) were poured or spread on total or selective agar plates for each species and according to instructions given by manufacturer [3]. 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 and according to manufacture guidelines for Feta cheese production (60 days). The evaluation panel parted by 5 experts (25–55 years old) which provided their comments concerning cheese sensory attributes of smoothness, bitterness, dairy-sour taste, microalgae odor and taste, dairy flavour, crumbly texture, colour and overall acceptability. Assessors were non-smokers and they were familiar with the consumption of dairy and fermented milk products.

3. Results & 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 approx. 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 platensis is one of the most nutritious microalgae. The bioactive peptides deriving from Spirulina may posse 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 culture Spirulina nutrient availability and functional value can be promoted [1]. In the present study, the effect of supplemented powder *S. platensis* (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 was

reported higher in cheese samples containing spirulina 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, *S. platensis* showed a positive effect on the growth of lactobacilli while lactococci viability was detected enhanced in cheese samples with incorporated *spirulina*. 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 Lactobacilli	Mesophilic Lactococci	Staphylococci	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 Spirulina 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 [4]. In the present study, odor of incorporated spirulina in feta type cheese samples was characterized as insignificant by evaluators. This results probably came as an outcome of fermentation and Feta cheese volatile by-products 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 graded by evaluators. Finally, all cheese samples received high scores of preferences and were characterized as acceptable for consumption by the expert’s evaluation panel.

4. Conclusions

Spirulina platensis is one of the most nutritious microalgae. The bioactive peptides deriving from Spirulina may posse 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 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 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 products nutritional value in parallel.

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

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