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Innovative Intelligent Cheese Packaging with Whey Protein-Based Edible Films Containing Spirulina

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Abstract: The use of edible and biodegradable films and coatings as active packaging for cheese has recently attracted great attention as it meets the concept of sustainability and ensures safety. *Spirulina* is a rich source of high-added-value biocompounds, which could be used as functional ingredients. In the present study, *spirulina* was added in different concentrations (0.5; 1; 2; 4% *w*/*w*) to the edible films produced from whey protein concentrate-based solutions. The films were characterized according to their optical parameters (color); they were studied for their total phenolic content, and the viability of the films in simulated gastric juice was investigated. The possible use of the developed films for intelligent food packaging, as colorimetric pH indicators, was also investigated. Finally, a preliminary evaluation of selected films containing *spirulina* (WPC-based films containing 2% *spirulina*) as packaging for "kefalotyri" cheese was also assessed. The effect of these films, applied as packaging for "kefalotyri" cheese during two months of refrigerated storage, was evaluated. GC-MS analysis was used to evaluate the effect of the *spirulina* odor of the film with *spirulina* incorporated and the cheese products where the film was applied.

Keywords: whey protein concentrate; edible film; *spirulina*; cheese packaging; functional food

Citation: Kontogianni, V.G.; Kosma, I.; Mataragas, M.; Pappa, E.; Badeka, A.V.; Bosnea, L. Innovative Intelligent Cheese Packaging with Whey Protein-Based Edible Films Containing Spirulina. *Sustainability* **2023**, *15*, 13909. https://doi.org/10.3390/su151813909

Academic Editor: Filippo Giarratana

Received: 7 August 2023
Revised: 7 September 2023
Accepted: 14 September 2023
Published: 19 September 2023

1. Introduction

Since cheeses are considered biologically and biochemically to be in the unstable food category, there has been an increase in the demand for innovative proper packaging systems to promote their safety [1]. The need to design bio-based food materials has increased, and the use of biodegradable biopolymers for food packaging has attracted great attention given the lack of sustainability of conventional food packaging [2]. Active packaging such as edible and biodegradable films is suggested in order to avoid undesired microbial growth and lipid oxidation on cheese [3]. Edible films and coatings can enhance the organoleptic and nutritional properties of cheese, either due to their film/coating composition, which may have beneficial properties by itself, or due to their capacity to incorporate active ingredients, which are eaten with the coating [4]. Whey protein (WP), a nutrient-rich byproduct of the cheese industry, has been applied to edible film formulations, which could be consumed with the cheese, generate no waste, lengthen its shelf life, and increase its quality [5]. Active packaging could provide the potential for bioactive compounds to be introduced both into packaged foods [6,7] and into the consumer's gastrointestinal system, at a controlled rate [8]. Another concept, called intelligent or smart packaging materials, is also trending in the same area. Such packaging can detect, monitor, and transmit information about changes occurring inside the food to the outside world [9]. Therefore, consumers can visualize changes in the state of the material and avoid wasting food and resources to a certain extent. This kind of food packaging material is more in line with the requirements of sustainable development.

WP edible films with antimicrobial and antioxidant agents with a natural origin incorporated can have a functional effect on the surface of cheese, as detailed below. Pluta-Kubica et al. [10] developed films produced from furcellaran–whey protein isolate solutions with the addition of yerba mate and white tea extract, which were successfully applied in order to improve the shelf life of soft rennet-curd cheese. Recently, Robalo et al. [11] prepared films produced from WP-based solutions enriched with green tea extracts and implemented them as a packaging material for Latin-style fresh cheeses, which efficiently protected them. In one of our previous works [12], the application of whey protein concentrate (WPC)-based films enriched with rosemary and sage infusions as a packaging material for soft cheese was able to protect the soft cheese from spoilage or pathogenic bacteria.

Spirulina (sp.) is abundant in important human health constituents, such as proteins, vitamins, amino acids, and minerals, and is a natural polyphenol source with many biological functions [13]. The safety and effectiveness of sp. biocompounds in the treatment of many human diseases has led scientists to apply sp. or its bioactive compounds to foods [14,15]. However, its inclusion, owing to the peculiar flavor and aroma associated with sp., has a negative impact on the final product's sensory attributes. A recent review covered studies on the use of sp. biomass and its blue extract for the enrichment of edible films and coatings (gelatin-based, chitosan-based, etc.) and their application to fruits, meat, fish, etc. [16]. Our research group used WPC, a troublesome byproduct of the cheese industry, to develop edible films with the addition of a commercial sp. powder in different concentrations, applying different treatments [17].As far as we know, this research constitutes the first approach toward the production of WPC-based films enriched with sp. powder.

Therefore, the present study expands this research, in which sp. was added in different concentrations (0.5; 1; 2; 4% *w*/*w*) to the edible films produced from WPC-based solutions, and the physicochemical, mechanical, and antioxidant properties, along with the optical parameters of the prepared films were evaluated [17].In the current investigation, the above films were characterized according to their optical parameters (color), and they were studied for their total phenolic content. The possible use of the developed films as an intelligent pH change indicator was also investigated along with the viability of the films in simulated gastric juice. The primary goal of this research was to assess the possible application of the WPC films with the best properties as a packaging material for "kefalotyri" cheese. WPC-based films containing 2% sp. were applied as packaging to "kefalotyri" cheese stored in refrigerated conditions to determine their influence on the cheese's microbiological stability. GC-MS analysis was used to evaluate the effect of sp. odor in the film with sp. incorporated And the cheese products where the film was applied.

2. Materials and Methods

2.1. Chemicals and Standards

All chemical reagents and solvents used in the current study and the ingredients used for the production of the film systems were the same as previously described [17].

Ringer's solution tablets, potato dextrose agar, and plate count agar were purchased from Neogen Culture Media (Heywood, UK).

2.2. Preparation of Film Systems

The films were prepared according to Kontogianni et al. [17]. The film-forming solution was prepared by dissolving 10 g WPC and 5 g glycerol in 100 mL water. The pH of each solution was adjusted to 5.95 using 3% *w*/*v* CH_3COOH. Then, the sp. powder at different concentrations (0.5; 1; 2; 4; 6; 8% *w*/*w*) and 1 drop of Tween 80 were added. The obtained solutions were stirred magnetically and homogenized with an Ultra Turrax homogenizer. The dispersion was placed in a common ultrasonic bath for 15 min at 70 °C, homogenized with the Ultra Turrax, and degassed by sonication for 30 min. After cooling the solutions at room temperature, their pH was adjusted to 5.5, and they were filtrated with a cheese cloth. Finally, the films were dried and stabilized.

2.3. Color Measurements

The Hunter color (L*, lightness; a*, redness; b*, yellowness) values were measured using a HunterLab, Model D25 L optical sensor (Hunter Associates, Reston, VA, USA). Each sample was analyzed using three independent repetitions with four to six measurements taken on each repetition (n = 3 × 4). The results reported are the mean of the above determinations.

2.4. Total Phenolic Content

For the preparation of the film solutions, we used the procedure followed for the antioxidant properties' evaluation as previously described [17]. The total phenolic content (TPC) was determined according to the method described by Jamróz et al. [18], expressed as mg of gallic acid/g of film. The analysis of each sample consisted of two independent measurements.

2.5. Viability of Films in Simulated Gastric Juice and Intelligent Material Analysis

For simulated gastric juice (SGJ), we dissolved pepsin in a 0.5% NaCl (*w*/*v*) solution to a concentration of 3 g/L. The pH was adjusted to 2.0 with a 1.0 M HCl solution. The gastric juice was freshly made for use on the same day. Next, 1 g of Control films and the WPC-based edible films incorporating six different concentrations of sp. powder were suspended in 9.0 mL of SGJ solution, placed in glass Petri dishes, and incubated at 37 °C for 3 h under orbital shaking in a formal orbital shaker (Thermo Electron Corporation, Waltham, MA, USA). Optical observation was made at regular time intervals, every 5 min for the first hour of observation and every 30 min for the 2 h left until the films were fully disintegrated.

The trial of the intelligent material assay was according to Jamróz et al. [18].

2.6. Application as Packaging for "kefalotyri" Cheese Pieces

The WPC-based films containing 2% sp. powder were selected as the best concentration for the evaluation of their potential as a packaging material for "kefalotyri" cheese portions. "Kefalotyri" is a traditional hard Greek cheese, manufactured from a mixture of sheep and goat milk. It has a flat cylindrical shape, firm texture, salty taste, and strong flavor. The cheese composition according to the label was: maximum moisture 38.0%; minimum dry fat 40%.; protein 26%; and salt 2.7% (the "kefalotyri" cheese was a commercial product purchased from local supermarket).Two cheese pieces in a trapezium shape (~5 cm long, 2.5 and 3.5 cm wide, 0.5 cm thickness) were layered with one film (Ø 90 mm), placed in a glass Petri dish, and vacuum packaged and sealed in sterile LDPE granule extruded bags (18 × 30 cm size). Furthermore, Control films were used to cover the cheese pieces in the same way for comparison purposes. The packages were stored at 4 °C for 7 days. Then, they were left at room temperature (23 °C) for 2 h before opening and evaluation. The sensory evaluation was performed by three analysts individually. The experiments were repeated three times. Each judge evaluated three packages with edible films as packaging for "kefalotyri" cheese pieces. The observations about the wholeness of the films after the storage and their removal from the cheese, about changes of color or odor in the cheese pieces after storage, and the smell and appearance of the film were recorded by questionnaires based on the respective analysis performed by [19]. Actually, they recorded the film wholeness after opening the package of the LDPE bag, expressed as the percentage of broken films to the total number of films, and easiness of the separation of the film from the cheese, expressed as the percentage of each evaluation related to the total evaluated surfaces. The samples used for microbiological analyses were prepared as follows. Pieces of cheese,12–14 g, in a trapezium shape were placed in sterile Petri dishes and distributed into three groups. The first group was kept without a surface film (Control, containing only "kefalotyri" cheese); the second group was layered with the edible film without sp., while the third group was layered with edible films containing 2% (*w*/*w*) sp., respectively. Films before use were sterilized as previously described [12]. The above samples were kept

for two months of refrigerated storage (stored at 4 °C), and proper cheese samples were collected for analysis at 0, 30, and 60 d of storage.

2.7. Microbiological Analyses

For the microbiological analyses of the cheese samples, we used the procedure as previously described [12].Viable counts for total mesophilic counts (TVCs), molds, and yeasts were performed in duplicate. More specifically, total mesophilic counts were enumerated on plate count agar (PCA) (30 °C for 72 h), and yeasts and molds were enumerated on potato dextrose agar (PDA) (30 °C for 72 h). All counts were recorded as logcfu/g.

2.8. Analysis of Volatile Compounds: Identification and Percentage Ratio of Volatile Compounds Using SPME–GC/MS

For the volatile analysis, the method described by Vatavali et al. [20] was used with minor modifications. Briefly, three grams of sample ("kefalotyri", film, or sp. powder) were used for analysis. SPME was performed with the fiber as previously described [20]. The samples were placed in an 80 °C water bath and stirred at 800 rpm. There were 30 min used for the sample to equilibrate and 15 min for the exposure of the fiber. The column was initially maintained at 40 °C for 2 min, heated to 170 °C at a rate of 5 °Cmin^{-1}, heated to 260 °C at a rate of 10 °Cmin^{-1}, and held for 2 min. For the volatile compounds of sp., the method according to Ozogul et al. [21] was used. Peak identification was performed by the comparison of the mass spectra of the eluting compounds to those of the Wiley library [22]. The retention indices (RIs) of the volatile compounds were calculated using-n-alkane (C8–C20) standard solution (Fluka, Buchs, Switzerland).

All determinations were carried out in triplicate.

2.9. NIR Spectroscopy: Spectra Acquisition

The homogenized "kefalotyri" sample and the "kefalotyri" cheese sample layered with film containing 2% sp. after storage at 4 °C for 7 days were subjected to NIR spectroscopy using a FoodScan 2 spectrophotometer (FOSS Analytical, Hillerod, Denmark).

2.10. Statistical Analysis

ANOVA was applied to the results using SPSS software [23]. Tukey's test was used to assess differences between means, and differences were considered significant at the level of $p < 0.05$.

3. Results

3.1. Color Measurements

One of the most-important factors of edible films developed for food products is color. It can influence both the product appearance and consumer acceptance. The color properties of WPC-based films with sp. incorporated are shown in Table 1. As was expected, the L* value, which gives the lightness, was higher in Control films, and its value decreased as the sp. powder concentrations increased from 0.5 to 4% ($p < 0.05$). The reduced L* value with increased sp. concentrations indicated that the films with sp. became darker. The color value between red and green is expressed by the a* value; the highest was found in the film with 1% sp. (-21.57 ± 1.04); the lowest was found in film with 4% sp. (0.90 ± 0.03), recording statistically significant differences. The color value between yellow and blue, which is expressed by the b* value, was found to be the highest in the film with 0.5% sp. (24.86 ± 0.45) and the lowest in the film with 4% sp. (-0.27 ± 0.05), also statistically significant ($p < 0.05$). Yellowness, which is expressed by the b* value, increased with the addition of 0.5% sp. and, then, with increasing sp. concentrations, decreased. In accordance with the findings of Balti et al. [24], the addition of sp. had a remarkable impact on the color of the resulting WPC films and could be attributed to the presence of phenolic compounds and colored substances.

Table 1. Color of WPC films with the addition of sp. and total phenolic content of the films.

Film	L *	A *	B *	TPC (mg Gallic/g Film)
Control	75.17 ± 0.44 ^{e,*}	−3.36 ± 0.39 ^{d}	15.91 ± 0.43 ^{c}	62.00 ± 4.35 ^{b}
0.5% sp.	51.88 ± 0.29 ^{d}	−18.80 ± 1.44 ^{b}	24.86 ± 0.45 ^{e}	41.00 ± 2.22 ^{a}
1% sp.	29.99 ± 0.23 ^{c}	−21.57 ± 1.04 ^{a}	21.73 ± 0.47 ^{d}	86.75 ± 5.11 ^{d}
2% sp.	14.75 ± 0.29 ^{b}	−8.95 ± 0.66 ^{c}	6.22 ± 0.44 ^{b}	100.75 ± 6.21 ^{e}
4% sp.	7.08 ± 0.13 ^{a}	−0.90 ± 0.03 ^{e}	−0.27 ± 0.05 ^{a}	74.75 ± 3.52 ^{c}

* Values with different letters in the same column indicate statistically significant differences; $p < 0.05$.

3.2. Total Phenolic Content

Film containing 0.5% sp. showed the lowest total phenolic content (TPC) (41.00 ± 2.22 mg gallic acid/g), even lower than the Control film (62.00 ± 4.35 mg gallic acid/g). In general, the TPC of films with sp. incorporated increased with an increasing amount of sp. powder until the concentration of 2% sp.($p < 0.05$).The DPPH assay results were in accordance with these results [17], where the edible film containing 2% sp. powder showed the highest percentage of radical scavenging activity(higher than the films containing 4, 6, and 8% sp.).

Miranda et al. [25] reported that the major phenolic compounds identified in sp. were salicylic, trans-cinnamic, synaptic, chlorogenic, quinic, and caffeic acids. Our results are in agreement with those of Balti et al. [24], who found that the TPC in crab chitosan films made with sp. extract increased with increasing sp. extract concentration. However, they reported that the TPC in crab chitosan films made with sp. ranged from 5.62 to 21.85 mg GAE/g film, quite lower values compared to our films.

3.3. Simulated Gastric Juice Test and Intelligent Material Analysis

The incubation of WPC-based films containing 0.5; 1; 2; and 4% sp. powder and the incubation of the Control films in SGJ imitating the low-pH and highly digestive conditions in the stomach resulted in the digestion of the films (Figures 1 and 2). The above films can be hydrolyzed by pepsin in the simulated gastric juice. According to optical observations, the WPC-based films containing 0.5; 1; 2; and 4% sp. powder and the Control films disintegrated within 1 h (60 min), and only small fragments of the films were still detectable. Especially, the WPC-based films containing 4% sp. disintegrated faster than the others and almost disappeared entirely within 60 min. On the contrary, the WPC-based films containing 6 and 8% sp. remained intact throughout the treatment with simulated gastric juice, even after 2 h (Figure 2).

The food transit time in the stomach has been found to be between 2 and 4 h, while the emptying time of liquid foods from the stomach is about 20 min. Therefore, WPC-based films containing 0.5; 1; 2; and 4% sp. and the Control films could be used as a primary packaging (the packaging that is in direct contact with the food), which is contained in a secondary or tertiary packaging and can probably be consumed safely with the food as a food additive and work as a functional food. According to Nourmohammadi et al. [26], who encapsulated sp. using alginate and whey protein concentrate (WPC) by the emulsification method and investigated its addition to non-fat stirred yogurt during storage, the total release of the sp. from the microcapsules was observed in simulated intestinal fluid. Sp. as a prebiotic source enhances the population of *Lactobacillus* in the human intestine by producing growth factors such as extracellular carbohydrates. The *Lactobacillus* population in the human intestinal tract is enhanced using sp. as a source of prebiotic substances by liberating growth substances such as extracellular carbohydrates [27].

Intelligent packaging is a crucial aspect of food technology that can help to improve food quality control. Considering that the change in the acidity of foods is common when they begin to deteriorate, this simplistic, optical measurement can be utilized to detect pH changes in food products. The color response of the pH indicator of the WPC films with sp. and the Control films was tested by immersing them in different pH solutions (pH 3.0 and

pH 12.0). The color of the WPC films with the addition of sp. in all different concentrations did not change after being placed either solution.

Recently, a research group developed films with C-phycocyanin as a pH indicator for food quality control and confirmed that, at an alkaline pH above 8, the films lost their blue and green tones, giving a color inclining toward white [28]. Such a phenomenon was attributed to some natural pigments present in sp. that are susceptible to pH changes, such as β-carotene, tocopherols, phycoerythrin, and chlorophylls. Furthermore, according to Kuntzler et al. [29], who incorporated sp. biomass into nanofibers, color changes occurred between pH 5 and 7, a pH value range that includes an extensive diversity of fresh foods.

3.4. Evaluation of Films as Packaging Material of "kefalotyri" Cheese Pieces

In order to select a film containing sp. in a concentration that showed the best characteristics as a packaging material for "kefalotyri", we compared all the parameters evaluated in our previous work [17] and in the present study. The ultrasound-treated WPC films with 2% sp. had the best uniform appearance and homogeneous texture. The incorporation of sp. had a beneficial impact on the mechanical properties of the edible films; the film with 2% sp. presented the highest TS value (TS for Control: 0.69 ± 0.07; for 2% sp.: 2.55 ± 0.36; for 4% sp.: 1.43 ± 0.27), exhibiting a strong structure and being more flexible than the film with 4% (presenting a higher E% value, E% for Control: 51.09 ± 5.69; for 2% sp.: 12.10 ± 1.40; for 4% sp.: 9.44 ± 1.38). It also had the highest percentage of radical scavenging activity. Based on the results of the current study, the film with 2% sp. showed good color properties and the highest total phenolic content and could be hydrolyzed by pepsin in the simulated gastric juice, which disintegrated within 1 h. Therefore, films with 2% sp. were selected to be applied as packaging for "kefalotyri" cheese surfaces.

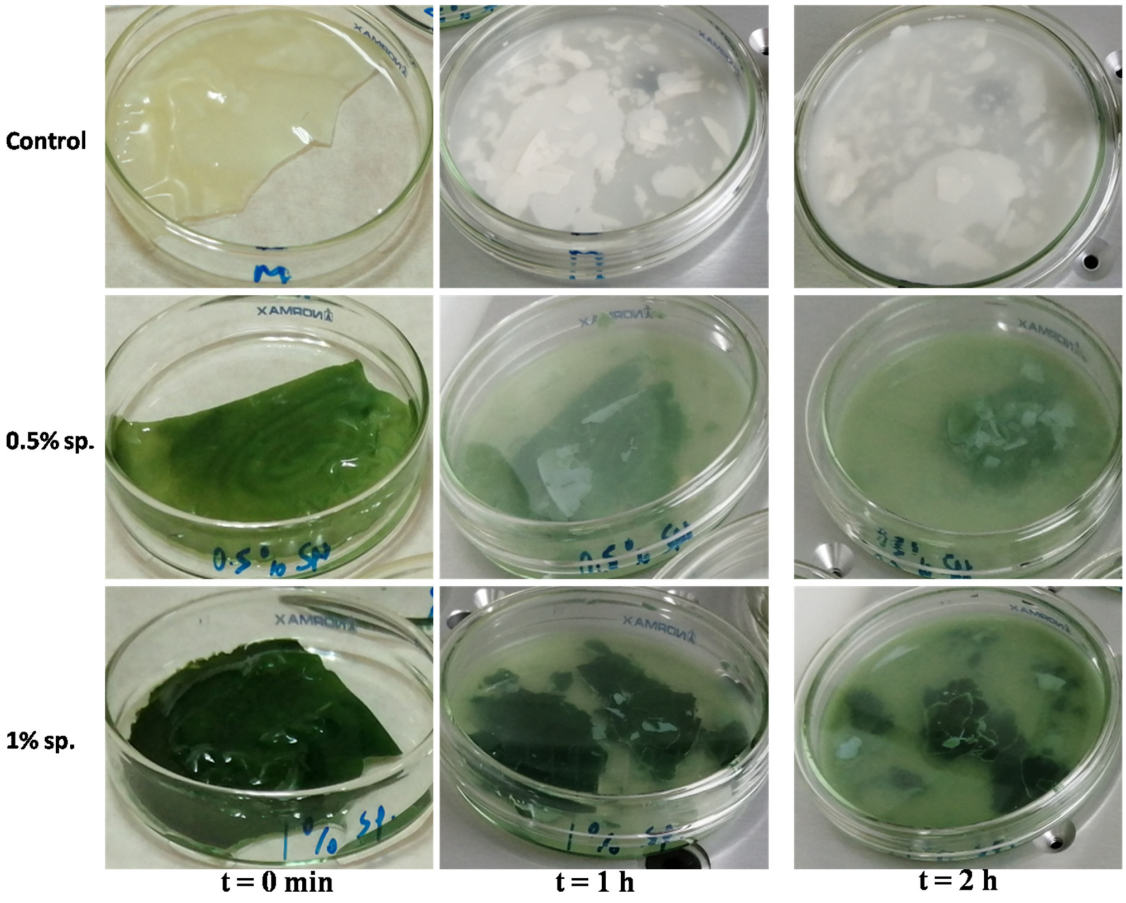

Figure 1. Photographs of WPC-based films (control, 0.5% sp., and 1% sp.) containing different concentrations of sp. immersed in SGJ for different times.

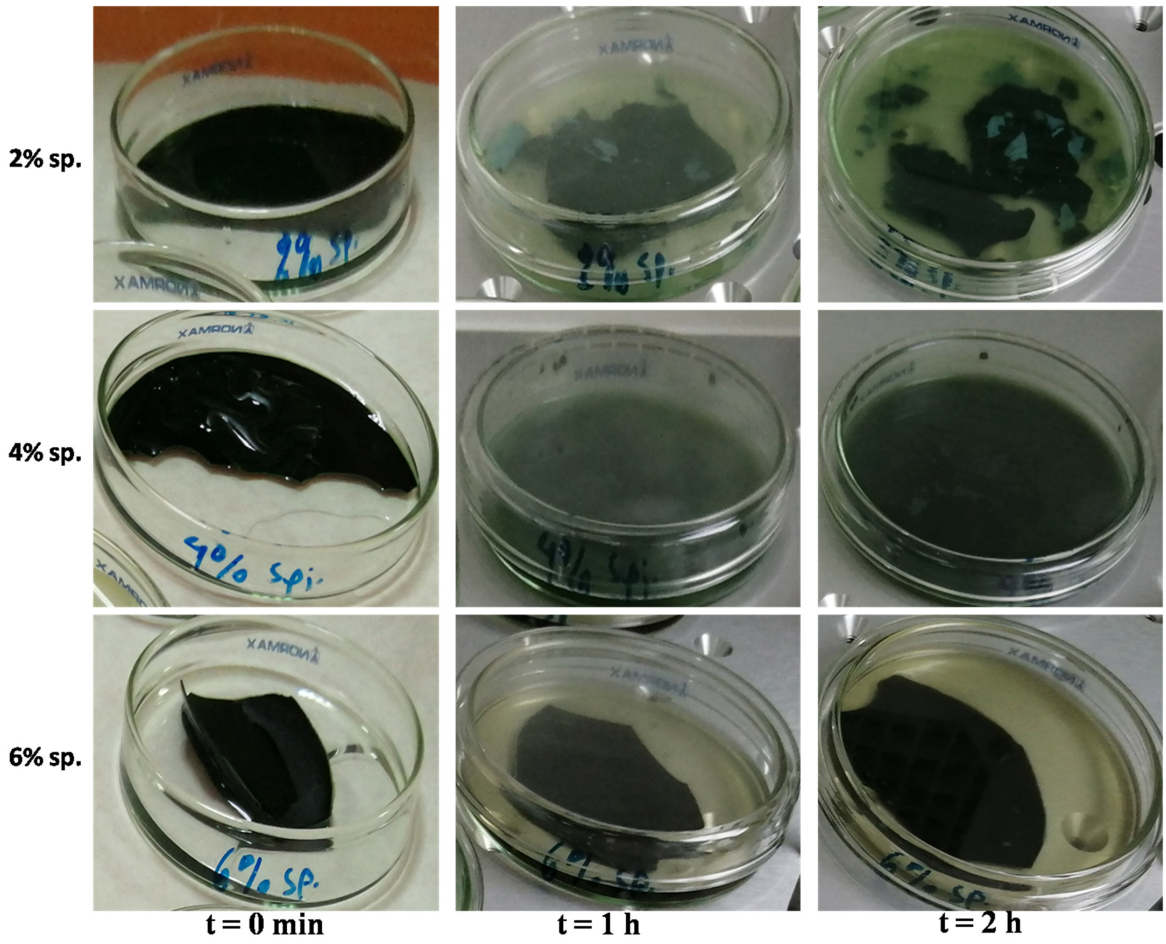

Figure 2. PPhotographs of WPC-based films (2% sp., 4% sp, and 6% sp.) containing different concentrations of sp. immersed in SGJ for different times.

In the evaluation of the films' wholeness after storage with cheese pieces at 4 °C for 7 days in LDPE bags (after opening the bag), the Control films were stuck to the bag when the package was opened, and small pieces of the film stayed on the surface of the cheese (Figures 3a and S1). The color of the Control film was almost the same as that of the cheese, so it is necessary to focus on Figure S1a in order to detect the broken parts of the Control film material that stayed stuck to the bag. In the evaluation of the film wholeness after opening the bag, 98.71% of the Control films broke during opening the package and stayed stuck to the bag. On the contrary, all the WPC-based films containing 2% sp. stayed intact on the surface of the cheese after 7 days of storage at 4 °C (Figure 3b). Regarding the ease of the film separation from the cheese both for the Control films and the films with sp. added, it was not possible to separate them from the cheese surface. The packaging material did not affect the smell of the cheese, neither for the Control film nor the film with sp. The evaluators did not find any changes to the odor of the cheese caused by the packaging material laid on the surface of the cheese. As they indicated, the odor of the Control film and the film with sp. added was the same and did not resemble the unwanted aroma of sp. The evaluators reported a slight change in the tonality on the surface of the cheese after the removal of the WPC-based films containing 2% sp. It turned darker, but it did not stain the cheese surface green (Figure 3c; the same piece after removing the film containing 2% sp. (on the right side) with a freshly cut piece of cheese (on the left side) for comparison).

Figure 3. (**a**) "Kefalotyri" cheese layered with Control film after storage at 4 °C for 7 days; (**b**) cheese layered with WPC-based films containing 2% sp. after storage at 4 °C for 7 days; (**c**) the same piece after removing the film containing 2% sp. (on the right side) with a freshly cut piece of cheese (on the left side) for comparison.

The above packaging material (film with sp.) was unable to be separated from the cheese surface after a storage time of 7 days at 4 °C intact, so probably, we should consider and evaluate the possibility of the film being consumed with the cheese and constituting a functional food consumed together with the cheese. WP is an animal protein with great nutritional value that closely matches the human body's requirements for all eight essential amino acids; thus, WP-based films have nutritional properties themselves [4]. WP- based films are non-toxic, biodegradable, safe to use, tasteless, odorless, and biocompatible with cheese products [30]. Since all the film ingredients are safe for consumption, this does not threaten the health of the final consumer, but the consumer could benefit from the health-promoting effects of sp. and its great nutritional value. *Spirulina* has received Generally Recognized As Safe(GRAS) certification from the Food and Drug Administration (FDA), allowing it to be used as a food or food supplement [31]. Both the WPC and sp. powder used in this study were commercial products sold as dietary supplements, accompanied by toxicological tests and all the necessary tests by national organizations. This packaging material possesses trustworthy characteristics. It is non-toxic, has minimal manufacturing expenses, and is completely compatible with the packaged product. Utilizing a film with sp. incorporated as a packaging material for a food that could be consumed with it could prove an effective method to suppress the unwanted aroma and even taste of the core material. It could be used probably to cover the undesirable taste of sp. and enhance the customer's satisfaction. Further analysis of the odor of the cheese after the removal of the film containing 2% sp. and the odor of the film on its own using instrumental analysis would prove this speculation. Furthermore, the organoleptic evaluation of the cheese after the removal of the film with sp. and the combination of the films and the cheese is recommended.

Cruz-Diaz et al. [19] evaluated films from whey proteins as a separation material for cheese slices; the films exhibited similar results regarding slice separation ease and slice wholeness after separation with those of the commercial material, without modifying the cheese color and odor. Pluta-Kubica et al. [10] assessed the organoleptic quality of soft rennet-curd cheese wrapped with films produced from furcellaran–whey protein isolate solutions with the addition of yerba mate and white tea extract. They concluded that the application of the films as packaging positively influenced the consistency and the overall quality of the cheese samples, although it had a negative impact on the appearance of the film, the appearance after removing the film, and the smell of the cheese samples.

3.5. Microbiological Changes of "kefalotyri" Cheese with Edible Films Applied

The results of the total mesophilic counts, molds, and yeasts are shown in Table 2. Until the 30th day of storage, no spoilage bacteria were found in any of our cheese samples. In the Control samples (cheeses without film), 5.3 logcfu/g of yeasts were counted on the 30th day of storage and persisted to rise until they amount to 7.6 logcfu/g on Day 60. Yeast spoilage was also obvious from Day 30 on those samples as colorful stains were observed on the surface of the cheeses without films. No spoilage bacteria were evident until the end of the storage days in cheese samples layered with films containing 2% sp. (Table 2).

Table 2. Total TVC and yeast/mold levels of "kefalotyri" cheese during two months of refrigerated storage.

	Kefalotyri log cfu/g		**Kefalotyri + Film Control log cfu/g**		**Kefalotyri + Film *spirulina* log cfu/g**	
Time (days) of Storage	**TVC**	**Yeasts/Molds**	**TVC**	**Yeasts/Molds**	**TCV**	**Yeasts/Molds**
0	$7.2 \pm 0.0^{a,*}$	$3.2 \pm 1.1^{A,**}$	7.2 ± 0.1^{b}	3.1 ± 0.0^{A}	7.4 ± 0.1^{b}	2.6 ± 0.5^{A}
30	8.0 ± 0.1^{b}	5.3 ± 1.6^{A}	7.2 ± 0.1^{a}	3.6 ± 1.9^{A}	7.8 ± 0.3^{b}	3.2 ± 1.4^{A}
60	9.2 ± 1.0^{b}	7.6 ± 1.3^{B}	8.2 ± 0.4^{b}	5.6 ± 1.0^{B}	7.7 ± 0.1^{a}	3.6 ± 0.2^{A}

* Values with different lowercase letters in the same row indicate statistically significant differences, $p < 0.05$; ** values with different capital letters in the same row indicate statistically significant differences, $p < 0.05$.

Balti et al. [24] showed that chitosan edible films with sp. extract incorporated exerted good antibacterial activity, exhibiting better results against Gram-positive bacteria (*L. monocytogenes*) than Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. typhimurium*). The chlorogenic acid contained in sp. extract was the bioactive substance to which the antimicrobial properties of the above films could be attributed. Closely related to this study, hake fillets packaged in gelatin films containing *Spirulina platensis* protein concentrate [32] showed enhanced storage stability. According to the results, the aerobe mesophiles, psychrotrophs, proteolytics, lipolytics, and *Enterobacteriaceae* counts were significantly decreased. Phycocyanin exhibited a significant in vitro antibacterial activity against food-borne pathogens such as *S. aureus*, *M. luteus*, *E. coli*, and *Pseudomonas* spp. [33].

Recently, Stejskal et al. [34] observed a remarkable antimicrobial effect of a gelatine-based packaging film with a protein concentrate from sp. applied on the quality of refrigerated Atlantic mackerel. According to Martelli et al. [35], the antimicrobial activity of the crude sp. extract was attributed to the synergistic effect between the wide diversity of bioactive phenolic compounds and other compounds found in sp., such as carotenoids, C-phycocyanin, and chlorophyll (a and b).

3.6. Analysis of Volatile Compounds of Films, sp. Powde r, etc.

The volatile compounds of the films are shown in Table 3. A total of 43 compounds were identified in the WPC-based film containing 2% sp. and 30 in the Control film. There were 28 common compounds among these films. The same major compounds were identified in the films, namely glycerin (44.21% for Control film and 33.25% for 2% sp.); acetic acid (18.10% for Control film and 11.55% for 2% sp.); nonanoic acid (8.92% for Control film and 11.51% for 2% sp.); octanoic acid (5.83% for Control film and 6.35% for 2% sp.); nonanal (3.84% for Control film and 2.96% for 2% sp.); hexanal (3.29% for Control film and 3.31% for 2% sp.); decanoic acid (3.01% for Control film and 2.12% for 2% sp.); heptadecane (2.51% for Control film and 11.15% for 2% sp.); and hexanoic acid (2.04% for Control film and 2.84% for 2% sp.). The 2% sp.film had 15 compounds more than the Control film (pentanal; disulfide dimethyl;(E)-2-hexenal;(E)-2-heptenal; heptanoic acid; 1-octanol; cyclohexanol, 2,6-dimethyl-;9H-pyrrolo[3',4':3,4]pyrrolo[2,1-a]phthalazine-9,11(10H)-dione,10-ethyl-8-phenyl; 1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-; 1-cyclohexene-1-carboxaldehyde; 2,6,6-trimethyl-;alpha-ionone; 4,5,6,7-tetrahydro-7,7-dimethyl-1(3H)-isobenzofuranone; beta-ionone epoxide; 6(Z),9(E)-heptadecadiene; 4-oxo-

beta-ionone), and the Control film had 2 more compounds than the 2% sp. film (butanoic acid and 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one).

Table 3. Percentage ratio of volatile compounds of the films.

				Control (%)	2% sp. (%)
A/A	**RI Exp ***	**RI Lit ****	**Compound**		
1	571	606	Acetic acid	18.1	11.55
2	690	695	Pentanal	-	0.16
3	780	785	Disulfide, dimethyl	-	0.03
4	790	784	Butanoic acid	0.43	-
5	793	810	Hexanal	3.29	3.31
6	848	854	(E)- 2-hexenal,	-	0.07
7	890		Oxime-, methoxy-phenyl-	0.33	0.27
8	895	893	Styrene;	0.3	0.23
9	895	899	n- heptanal	0.39	0.34
10	925	915	Pyrazine, 2,5-dimethyl-	0.27	0.43
11	933	963	2(E)-heptenal	-	0.88
12	955	970	Hexanoic acid	2.04	2.84
13	989		Glycerin	44.21	33.25
14	1067	1083	Heptanoic acid	-	0.74
15	1067	1062	2 octenal	0.41	1
16	1070	1070	1-octanol	-	0.36
17	1100	1099	Nonanal	3.84	2.96
18	1110	1088	Maltol	0.62	0.4
19	1110	1110	Cyclohexanol, 2,6-dimethyl-	-	0.75
20	1184	1193	Octanoic acid	5.83	6.35
21	1200		9H-pyrrolo[3′,4′:3,4]pyrrolo[2,1-a]phthalazine-9,11(10H)-dione,10-ethyl-8-phenyl	-	0.1
22	1210	1203	Decanal	0.57	0.37
23	1218	1198	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	-	0.13
24	1230	1280	1H-pyrrole-2,5-dione, 3-ethyl-4-methyl-	0.18	0.78
25	1233	1208	1-cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	-	0.13
26	1238	1237	Nonanoic acid	8.92	11.51
27	1289	1300	Tridecane	0.22	0.14
28	1356	1371	Decanoic acid	3.01	2.12
29	1405	1400	Tetradecane	0.15	0.13
30	1425	1617	Tetradecanal	1.35	0.94
31	1475	1437	alpha-ionone	-	0.08
32	1485	1454	(E)-geranylacetone	0.32	0.24
33	1493	1478	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	0.06	-
34	1498		Cyclododecane	0.38	0.18
35	1501		4,5,6,7-tetrahydro-7,7-dimethyl-1(3H)-isobenzofuranone	-	0.08
36	1510	1493	.beta.-Ionone	0.85	2.23
37	1515		beta.-Ionone epoxide	-	0.73
38	1585	1538	(2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone	0.43	1.54
39	1595	1600	Hexadecane	0.48	0.95
40	1620	1811	Hexadecanal	0.26	0.15
41	1685	1648	Methyl dihydrojasmonate	0.16	0.11
42	1691	1668	6(Z),9(E)-heptadecadiene	-	0.04
43	1694	1694	1-heptadecene	0.1	0.19
44	1695	1659	4-oxo-beta-ionone	-	0.05
45	1699	1700	Heptadecane	2.51	11.15

* Experimental retention indices' values based on the calculations using the standard mixture of alkanes; ** retention indices of the identified compounds according to the literature data cited in the NIST MS library.

The volatile compounds for the sp. powder are shown in Table 4. A total of 83 compounds were identified. The major compounds identified were heptadecane (39.32%), pen-

tadecane (14.27%), hexadecane (9.04%), beta-ionone epoxide (5.10%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (3.46%), 4(5)-acetyl-2-(2-propyl)-1H-imidazole (2.96%), and 8-heptadecane (2.88%).

Table 4. Percentage ratio of volatile compounds of the sp. powder.

				sp. Powder (%)
A/A	**RI Exp ***	**RI Lit ****	**Compound**	
1	<500	<500	Acetaldehyde	0.30
2	<500	<500	2-propanone	0.26
3	569	606	Acetic acid	0.02
4	610	605	Furan, 2-methyl-	0.02
5	656	650	Butanal, 3-methyl-	0.02
6	659	661	1-butanol	0.02
7	690	695	Pentanal	0.07
8	735	736	1-butanol, 3-methyl-	0.04
9	738	740	1-butanol, 2-methyl-	0.05
10	750	766	1-pentanol	0.40
11	785	798	Hexanal	0.87
12	809	827	Pyrazine, methyl-	0.21
13	825	920	Formic acid, hexylester	1.68
14	901	890	Pyridine, 2,6-dimethyl-	0.09
15	903	899	2-heptanone	0.17
16	910	896	2-heptanol	0.09
17	911	899	n- heptanal	0.16
18	928	915	Pyrazine, 2,5-dimethyl-	1.29
19	959	954	2-heptanone, 6-methyl-	0.10
20	965	963	(E)-2-heptenal,	0.03
21	980	963	Benzaldehyde	0.33
22	981	983	1-octen-3-ol	1.12
23	984	985	6-methyl-5-hepten-2-one	0.40
24	987	992	2-Octanone	0.12
25	987	998	Furan, 2-pentyl-	0.54
26	989		2-heptanol, 6-methyl-	0.06
27	990	1006	Pyrazine, 2-ethyl-6-methyl-	0.04
28	991	976	Benzene, 1,3,5-trimethyl-	0.03
29	1000	1192	Phenol, 2-methoxy-4-methyl-	0.03
30	1025	1035	Benzenemethanol	0.15
31	1030	1047	Cyclohexanone, 2,2,6-trimethyl-	0.59
32	1039		2,3,4,5-tetramethyl-2-cyclopenten-1-one B	0.43
33	1045	1054	(Z)-2-octen-1-ol	0.73
34	1047	1120	2-cyclohexen-1-one, 3,5,5-trimethyl-	0.48
35	1075	1091	Pyrazine, 3-ethyl-2,5-dimethyl-	0.22
36	1079	1198	2-cyclohexen-1-one, 3,4,4-trimethyl-	0.18
37	1085	1110	6-methyl-3,5-heptadien-2-one	0.86
38	1091	1282	Phenol, 5-methyl-2-(1-methylethyl)-	0.08
39	1094		2-(t-butyl)-3-methylthiophene	0.39
40	1100	1110	Cyclohexanol, 2,6-dimethyl-	0.06
41	1115	1123	2-cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	0.18
42	1130		4(5)-acetyl-2-(2-propyl)-1H-imidazole	2.96
43	1201	1208	Benzaldehyde, 2,5-dimethyl-	0.34
44	12010	1200	Dodecane	0.39
45	1210	1139	Ethanone, 1-(2-methylphenyl)-	0.25
46	1215	1202	Pyridine, 2-pentyl-	0.49
47	1220	1213	Undecane, 2,6-dimethyl-	0.09
48	1225	1206	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	0.77
49	1240	1280	1H-pyrrole-2,5-dione, 3-ethyl-4-methyl-	0.53

Table 4. *Cont.*

				sp. Powder (%)
A/A	**RI Exp ***	**RI Lit ****	**Compound**	
50	1249	1226	beta-cyclocitral	0.89
51	1259		Cyclododecane	0.03
52	1289	1275	4,8-dimethyl-nona-3,8-dien-2-one	0.08
53	1290	1251	1-cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-	0.08
54	1299		3-cyano-2,4,4-trimethyl-2-cyclohexenone	0.16
55	1301	1300	Tridecane	0.17
56	1303		2-cyclopenten-1-one, 2-pentyl-	0.30
57	1310		2-butyl-3-methylpyrazine	0.14
58	1325		m-cresol, 6-tert-butyl-	0.07
59	1390	1355	2-octenal, 2-butyl-	0.17
60	1394		alpha-ionene	0.27
61	1401		Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester	0.08
62	1405	1400	Tetradecane	0.64
63	1440	1408	2-undecanone, 6,10-dimethyl-	0.21
64	1452	1437	alpha-ionone	0.54
65	1465		5,9-undecadien-2-one, (E)-6,10-dimethyl-,	0.6
66	1476	1800	Octadecane	0.12
67	1481		4,5,6,7-tetrahydro-7,7-dimethyl-1(3H)-isobenzofuranone	0.39
68	1486	1587	1-hexadecene	0.22
69	1492	1485	4-(2,6,6-trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.47
70	1495	1500	Pentadecane	14.27
71	1499		beta-ionone epoxide	5.10
72	1569	1539	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	3.46
73	1575		(Z)-7-hexadecene	0.28
74	1581		(E)-2-nonadecene	0.05
75	1592	1600	Hexadecane	9.04
76	1599	2000	Eicosane	0.02
77	1671	1668	6(Z),9(E)-heptadecadiene	0.62
78	1679	1677	8-heptadecene	2.88
79	1681		(Z)-3-heptadecene	0.2
80	1692	1700	Heptadecane	39.32
81	1798	1800	Octadecane	0.15
82	1813	1846	2-pentadecanone, 6,10,14-trimethyl-	0.17
83	1890	1900	Nonadecane	0.04

* Experimental retention indices' values based on the calculations using the standard mixture of alkanes; ** retention indices of the identified compounds according to the literature data cited in the NIST MS library.

The sp. powder and WPC-based film containing 2% sp. had seven common compounds not found in the Control film. Six of them were minor compounds of sp. powder, namely pentanal;(E)-2-heptanal; cyclohexanol,2,6-dimethyl-; 1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-; alpha-ionone; 4,5,6,7-tetrahydro-7,7-dimethyl-1(3H)-isobenzofuranone; and 6(Z),9(E)-heptadecadiene (0.03–0.77%). Only beta-ionone epoxide was a major compound of sp. powder (5.10%).

The main components of the *S. platensis* extract that Ozogul et al. [21] determined were dioctylamine (67.64%), monoterpene (7.32%, pentadecane), terpene (6.33%, hexadecane), dodecane (2.34%), jasmololone (2.05%), isopulegone (1.63%), and cymarin (0.79%). In another research, the main volatile constituents of *S. platensis* were heptadecane (39.70%) and tetradecane (34.61%) based on the findings of Ozdemir et al. [36]. Finally, the volatile compounds of a "kefalotyri" cheese sample and of a "kefalotyri" cheese sample layered with film containing 2% sp. after storage at 4 °C for 7 days (after the removal of the film) are shown in Table S1. A total of 12 compounds were identified in the "kefalotyri" cheese sample, which were also identified in the cheese sample layered with the film. Two more compounds were found in this sample, namely 2,3-butanedione and 2-octene, not being compounds of the sp. powder. The above findings support that the WPC-based film containing 2% sp. does not sustain the characteristic odor of sp.

3.7. NIR Spectroscopy: Chemical Composition of "kefalotyri" Cheese Sample with Film

The chemical composition of the "kefalotyri" cheese sample layered with film containing 2% sp. after storage at 4 °C for 7 days after the removal of the film and the cheese with the film homogenized together, as determined by the application of NIR spectroscopy, was fat % 27.73 ± 0.08, moisture % 36.22 ± 0.06, protein % 27.79 ± 0.13, salt % 3.00 ± 0.01, SFA % 18.46 ± 0.92, fat in dry matter % 43.5 and total solids % 63.78 ± 0.06 and fat % 27.83 ± 0.09, moisture % 36.07 ± 0.19, protein % 29.66 ± 0.07, salt % 2.08 ± 0.12, SFA % 17.92 ± 0.90, and fat in dry matter % 43.5 and total solids % 63.93 ± 0.19, respectively. Therefore, consuming cheese together with the WPC-based film containing 2% sp. can increase the protein value and intake of the product, and in general, such packaging can maintain the cheese's nutritional value.

4. Conclusions

The film with 2% sp. showed good color properties and the highest total phenolic content and could be hydrolyzed by pepsin in the simulated gastric juice, disintegrating within 1 h. In conclusion, films with 2% sp. were selected to be applied as packaging of "kefalotyri" cheese surfaces. The spoilage from yeasts was optically evident from Day 30 on the surface of the cheeses without films. Until the end of the storage days, no spoilage bacteria were found in cheese samples layered with films containing 2% sp. The antimicrobial activity of sp. is potentially attributed to the synergistic effect between the wide diversity of bioactive phenolic compounds, such as chlorogenic acid, and other compounds found insp., such as carotenoids, C-phycocyanin, and chlorophyll (a and b). Such a cheese packaging material can be an important and sustainable alternative in the preservation of cheese quality and safety. GC-MS analysis of WPC-based film containing 2% sp. showed that the film does not sustain the characteristic odor of sp. The same major compounds were identified in the Control films and films containing 2% sp. The sp. powder and WPC-based film containing 2% sp. had seven common compounds, not found in the Control film; six of them were minor compounds of sp. powder, and only one was among its major compounds. The WPC-based films containing 2% sp. could be consumed with "kefalotyri" cheese and constitute a functional food. Consuming a film containing sp. can improve consumer acceptance due to the flavor- and color-masking effect of films and could probably prove a good alternative for the wider acceptance of functional products enriched with edible microalgae. Finally, it can increase the protein value and intake of the product, and in general, such packaging can maintain the cheese's nutritional value. However, further research needs to be conducted, and the organoleptic evaluation of the cheese after the removal of the film with sp. and the combination of the films and the cheese is recommended to obtain consumers' acceptance.

Supplementary Materials: The following Supporting Information can be downloaded at: https://www.mdpi.com/article/10.3390/su151813909/s1, Table S1: Volatile compounds of "kefalotyri" cheese and "kefalotyri" cheese covered with 2% sp. film after storage at 4 °C for 7 days after the removal of the film, Figure S1: (**a**) "Kefalotyri" cheese layered with Control film after storage at 4 °C for 7 days; (**b**) cheese layered with WPC-based films containing 2% sp. after storage at 4 °C for 7 days; (**c**) the same piece after removing the film containing 2% sp. (on the right side) with a freshly cut piece of cheese (on the left side) for comparison.

Author Contributions: Conceptualization, V.G.K., I.K., and L.B.; methodology, V.G.K., I.K., and L.B.; formal analysis, V.G.K. and L.B; investigation, V.G.K. and L.B; data curation, V.G.K. and L.B; writing—original draft preparation, V.G.K. and L.B; writing—review and editing, M.M., E.P., and A.V.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 was funded by the research program entitled "Innovative utilization approaches and comparative advantages of cheese whey of ovine/caprine origin from the region of Epirus" (MIS number: 5033108), supported by the action "Strengthening of small and medium-sized enterprises for research programs in the fields of agro-nutrition, health and biotechnology",

co-financed by the European Union (European Regional Development Fund) and Greece, under the "Operational Program Epirus 2014–2020" of the National Strategic Reference Framework.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are contained within the article.

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

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