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Evaluation of Antioxidant and Physicochemical Properties of Microalgae/Whey Protein-Based Edible Films †

Vasiliki G. Kontogianni [1,*], Alexandra V. Chatzikonstantinou [2], Marios Mataragas [1], Efthymia Kondyli [1], Haralambos Stamatis [2] and Loulouda Bosnea [1]

[1] Dairy Research Department, Hellenic Agricultural Organization-DEMETER, Katsikas, 45221 Ioannina, Greece; mmatster@gmail.com (M.M.); kondyliefi@gmail.com (E.K.); louloudabosnea@gmail.com (L.B.)
[2] Biotechnology Laboratory, Department of Biological Applications and Technologies, University of Ioannina, 45110 Ioannina, Greece; alexandra_xatzi@hotmail.com (A.V.C.); hstamati@uoi.gr (H.S.)
* Correspondence: vgkontog@gmail.com
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Abstract: In the last decades, edible films based on whey proteins have become promising eco-friendly materials that can be used as carriers for some bioactive substances such as nutrients and antioxidant agents. Spirulina, one of the best-known cyanobacteria, is a rich source of nutritional compounds with beneficial health effects. Edible films from whey protein concentrates (WPC) were developed applying different treatments, such as water-bath heating for 30 min at 75 °C and ultrasound treatment in a common ultrasonic bath for 15 min at 70 °C with the addition of a commercial spirulina powder. WPC-based edible films were prepared with the addition of spirulina in different concentrations. Other edible microalgae such as commercial chlorella and ulva have also been examined for the production of edible films. After production, the films were characterized according to their physicochemical properties (thickness, moisture content, solubility in water, degree of swelling), optical parameters (Fourier transform infrared spectrum), tensile properties, and antioxidant activity.

Keywords: whey protein concentrate; edible film; spirulina; microalgae

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1. Introduction

Active edible film made of whey protein enriched with bioactive compounds is one of the latest trends in the area of food technology and innovations. This novel food packaging is intended to extend the shelf-life or to maintain or improve the condition of packaged food by adding an active agent or compounds into the film which can, for example have antioxidant activity, decrease the oxidation of lipids, inhibit the growth of microorganisms, can act as nutraceutical's vehicle and affect the flavor [1]. Bioactive compounds enriched in edible films, are usually extracted from different natural sources and may also improve the functional properties of edible films.

Spirulina and Chlorella are popular blue-green microalgae because of their high protein content (65% and ~60%, respectively) and their great nutritional value. They contain abundant amino acids, vitamins and minerals, unsaturated fatty acids and high-antioxidant components. Especially spirulina, has diverse possible health-promoting beneficial effects [2]. As they are considered as "functional food", much recent interest has been focused on their addition in traditional foods in order to increase functional product characteristics. However, this addition adversely affects the sensory attributes of the final product.

The main objective of the current investigation was to study the effect of incorporating of a commercial spirulina powder (sp.) into edible films prepared with WPC, applying

different treatments. WPC-based edible films were prepared with the addition of spirulina in different concentrations (0.5; 1; 2; 4; 6 and 8% *w*/*w*). Other edible microalgae such as commercial chlorella and ulva have also been examined for the production of edible films. In this study, physicochemical, mechanical, and antioxidant properties along with optical parameters of the films tested were investigated.

2. Materials and Methods

2.1. Chemicals, and Standards

2,2-diphenyl-picrylhydrazyl (DPPH) 95% was purchased from Sigma-Aldrich, (Steinheim, Germany). All solvents were of appropriate purity and were purchased from various suppliers. Whey protein concentrate (WPC) 80% (WheyPro80, Epirus Proteins) and glycerol (Gly) (anhydrous glycerol AG 99.5%, Penta, Prague, Czech Republic) were used to prepare film-forming solutions. Spirulina and Chlorella powder were commercial samples (MegaFoods, Greece). Ulva was also a commercial sample (Hell Seaweed, China) as flakes that was dried with liquid nitrogen and pulverized into a fine powder.

2.2. Film Preparation

Films were prepared according to García et al. [3], with some modifications. Briefly, WPC (10 g) was dispersed in water (100 mL) and 5 g of glycerol (as plasticizer) were added to the film-forming solution. After complete dissolution of the film-forming materials, the pH of each solution was adjusted to 5.95 using 3% *w*/*v* CH_3COOH. The film forming solution was incorporated with powder sp. in different concentrations (0.5; 1; 2; 4; 6 and 8% *w*/*w*) and 1 drop of Tween 80 was added. The addition of sp. at concentrations higher than 2% *w*/*w* changed the pH value, so it was adjusted again to 5.95 using 3% *w*/*v* CH_3COOH, after the addition. Chlorella and ulva powder were also tested in concentrations of 0.5; 1 and 2% *w*/*w*. The obtained solutions were stirred magnetically for 10 min and homogenized (10 min; 19,000 rpm) with an IKA Ultra Turrax T25 basic homogenizer (Ika Labortechnik, Germany). Then the solution was heated at 75 °C for 30 min in a water bath or was placed n a common ultrasonic bath for 15 min at 70 °C (Elmasonic S 60 H, Ultrasonic cleaning unit, Elma, Germany), homogenized (3 min; 19,000 rpm) with Ultra Turrax and degassed by sonication for 30 min. After that, the solution was cooled at room temperature, and then the pH of each film-forming solution was adjusted to 5.5 and finally filtrated with a cheese cloth. Control films without microalgae were also prepared for comparison. Petri dishes coated with a layer of rice oil were filled with 14 g of film-forming solution and dried on a leveled surface in a universal oven (Memmert, Germany) for 24 h at 25 °C. The films were stabilized for 48 h at 25 °C and 58% relative humidity.

2.3. Thickness

Film thickness was measured with an electronic digital caliper (Beta 1651 SC, Italy) having a precision of 0.01 mm.

2.4. Water Behavior

Water content was determined at least in three repetitions through the weight loss undergone by the film after 24 h oven drying at 105 ± 1 °C. The water solubility of each film was measured in at least five repetitions according to the method of Garcia et al. [3]. Five randomly selected samples (20 mm × 20 mm) of each type of film were first dried at 105 ± 1 °C for 24 h to determine the initial dry matter. The degree of swelling was determined according to Jamroz et al. [4].

2.5. Determination of Mechanical Properties

Tensile strength (TS), Young's modulus (YM), and elongation at break (εb) of the films 25 mm × 50 mm in size were determined using a Texture Profile Analyser (EZTest, EZ-X Series, Shimadzu Europa Gmbh, Germany) according to the ASTM standard

method D882-02. The initial distance of separation was adjusted to 35 mm. Data were collected using software TRAPEZIUM X (Shimadzu Autograph, Software, Shimadzu Europa Gmbh, Germany).

2.6. Antioxidant Properties

Antioxidant properties of edible films were evaluated according to Pluta-Kubica et al. [5], with slight modifications. The films were cut into small squares with a surface area of about 4–5 mm^2. Then 40 mg of film was dissolved in 10 mL of distilled water, shaken for few minutes using a vortex and placed for 15 min in an ultrasonic bath for homogenization, finally centrifuged at 4500 rpm for 30 min. Afterwards, 2.5 mL of the acquired supernatant was mixed with 1.0 mL of 0.3 mM ethanolic solution of DPPH and allowed to react at room temperature. The blank was prepared by addition of distilled water instead of film extract. Ethanol (1.0 mL) plus extract / pure compound solution (2.5 mL) was used as control. The mixtures were incubated in the dark for 30 min and their absorbance was then measured at 517 nm (UV-1700 Pharmaspec, UV-Visible spectrophotometer, Shimadzu, Japan). The obtained results were expressed as the percentage of radical scavenging effect. The analysis of antioxidant assay was performed in three independent measurements for each sample.

2.7. Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-IR) Analysis

ATR-IR measurements of the prepared films were recorded using a Jasco 4700 spectrometer (Jasco, Tokyo, Japan) equipped with a diamond attached to an ATR plate with a horizontal ATR accessory (Jasco ATR PRO ONE). Reflectance was measured as an average of 20 scans over a range of 400–4000 cm^{-1}, with 4 cm^{-1} resolution at room temperature. Background measurements were recorded in the absence of the sample and automatically subtracted from the ATR-IR spectra of the films.

3. Results and Discussion

3.1. Whey Protein Active Packaging Incorporated with Microalgae

First, sp. was added at three different concentrations (0.5; 1; and 2% *w*/*w*) and two different treatments, water-bath heating for 30 min at 75 °C and ultrasound treatment in a common ultrasonic bath for 15 min at 70 °C, were applied in order to develop edible films from whey protein concentrates. All films were flexible, presented a smooth texture without visible pores or cracks and could easily be peeled from the casting plates. Comparing films developed with the two different treatments, the surface of films subjected to heating in waterbath did not display a homogenous texture and uniform appearance (Figure 1). There was not observed a homogenous distribution of sp. in film surface. On the contrary, the distribution was obviously improved in ultrasound-treated films; even in low concentration of 0.5% *w*/*w*. Ultrasound application causes the formation of more compact structure in film. Although the use of ultrasonic bath with a power of ultrasound at 60 Hz, probably does not induce interactions among protein molecules that can influence the physical and mechanical properties of edible protein films increases the solubility of sp. powder in the film forming solution, so it could be better enclosed within the matrix of the edible film.

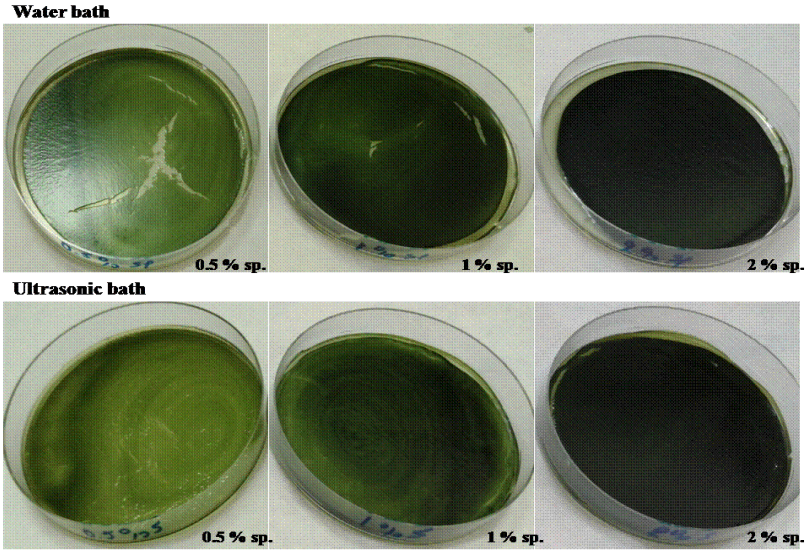

Figure 1. Macroscopic appearance of WPC-based films with spirulina in different concentrations with different treatments.

The same trend was observed with the addition of chlorella powder, too. A drawback with this microalga was its poor solubility in the film forming solution. This phenomenon and even more intense was observed in the case of ulva, too. This is the reason why these microalgae were not further investigated. Control films with both treatments were homogeneous, transparent, and visually presented no differences between them. The treatment with ultrasonic bath was chosen in order to study the addition of higher concentrations of sp. (4, 6 and 8% *w*/*w*). By increasing the concentration of sp., films became stiffer and could easily break into pieces. The WPC films with 6% (*w*/*w*) sp. did not presented a good performance as they were all fragmented. The films with 8% (*w*/*w*) were so stiff once separated from the tested surface were fragmented. The only film that could be considered for further tests was the film with 4% (*w*/*w*) sp. What is interesting is that even at the highest concentration tested films did not retain the characteristic undesirable odor of spirulina. So these WPC films with sp. could be considered as a novel functional product providing all the benefits of a supplement rich in spirulina but also having the consumer's acceptance.

3.2. Moisture Content, Solubility in Water, Swelling Index and Film Thickness

Thickness, moisture content, solubility in the water and swelling index of films are shown in Table 1. The thickness of films incorporated with sp. varied between the range of 0.181 and 0.250 mm. The control film presented a thickness of 0.188 mm (±0.038). The slight increase in thickness when sp. was added up to 2% suggesting that sp. could be distributed in the film matrix without affecting the film thickness. Moisture content value (MC) of WPC films with sp. reduced by increasing the level of addition of sp. with respect to control, as expected, due to the addition of hydrophobic compounds in the polymeric matrix. A phenomenon that could be ascribed to the cleavage of film matrix, which increases the amount of water molecules found between polymer chains by hydrogen bonding. Solubility in water (S) is an indicator of the resistance of the film to water. The S of films incorporated with sp. varied between the range of 18.91 and 21.60%. Control film presented a S value of 18.82% (±0.33). The small increase in S values observed when adding sp. r in the matrix of the WPC film may be attributed to the establishment of protein-protein interactions between WPC proteins and constituents of sp. (proteins, lipids, etc.) which weaken the interactions of the polymeric network and limit the interaction of glycerol with the protein matrix. Finally, an increase on the degree of swelling was observed as a trend with the increase incorporation of sp.

Table 1. The influence of addition of sp. on the water properties and mechanical properties of WPC films. Values (average ± standard deviation) of moisture content (MC), solubility (S), swelling degree (SW), tensile strength (TS), Young's modulus (YM) and elongation at break (E).

Film	Thickness (mm)	MC (%)	S (%)	SW (%)	TS (MPa)	YM (MPa)	E (%)
Control	0.188 ± 0.038	26.48 ±1.71	18.82 ± 0.33	8.78 ± 0.90	0.69 ± 0.07	10.16 ± 1.79	51.09 ± 5.69
0.5% sp.	0.181 ± 0.015	27.71 ± 0.47	18.91 ± 0.17	8.48 ± 1.02	0.89 ± 0.04	11.9 ± 1.73	22.76 ± 2.78
1% sp.	0.207 ± 0.044	22.74 ± 1.40	20.68 ± 1.48	10.90 ± 0.92	1.35 ± 0.09	19.00 ± 1.75	17.88 ± 0.91
2% sp.	0.193 ± 0.005	21.88 ± 0.46	21.06 ± 0.47	14.60 ± 1.04	2.55 ± 0.36	23.15 ± 2.82	12.10 ± 1.40
4% sp.	0.250 ± 0.018	15.70 ± 0.39	21.60 ± 0.08	18.75 ± 1.45	1.43 ± 0.27	28.31 ± 0.35	9.44 ± 1.38

3.3. Mechanical Properties of the Edible Films

Table 1 shows the mechanical properties of films. By increasing the sp. content in WPC-films, tensile strength and modulus elasticity tends to increase and elongation during the break tends to decrease compared to the control film. These results indicate that the addition of sp. had a positive effect on the mechanical properties of WPC-films. A phenomenon ascribed to strong interaction between constituents of sp. (proteins, lipids, etc.) with the functional group of WPC, which resulted in the rigid structure of the films. Control film presented the lowest TS values (0.69 MPa) and the highest E values (51.09%), exhibiting weaker but flexible structure, than films with sp.

3.4. Antioxidant Properties

The antioxidant activity of WPC-based films with sp. was monitored using DPPH assay. Control film exhibited a percentage of radical scavenging effect of 26.41 ± 3.45. The percentage of radical scavenging effect of films containing sp. were equaled 35.33 ± 2.76, 28.74 ± 2.66, 29.00 ± 5.65 and 29.95 ± 3.88, for 2%, 4%, 6% and 8% of sp. containing films, respectively. The WPC- based film containing 2% sp. powder had the highest percentage of radical scavenging effect. Films with lower than 2% concentrations of sp. were not tested because of their small sp. content. The antioxidant activity of microalgae, like spirulina, derives from their wide diversity in free radical scavengers. According to Barkallah et al. [6], the incorporation of spirulina into yogurt considerably improved the antioxidant activity of the new formulated yogurt, thanks to its high content in pigments, specifically the content in chlorophylls, carotenoids and phycocyanin.

3.5. Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-IR) Analysis

ATR-IR spectra of WPC-based films formulated with the addition of sp. are shown in Figure 2. Similar peaks can be observed for control film and films formulated with sp., thus indicating that the presence of the sp. powder did not alter the chemical structure of the films. There can be observed the absorption peaks located in the spectral range 800–1150 cm^{-1}, attributed to absorption bands of glycerol. Also, the strong absorption bands located at 3000–3600 cm^{-1}, which correspond to the stretching vibration of free and bound –OH and –NH groups, can be intensified by the addition of glycerol, too. The band centered at 1626 cm^{-1} is associated to amide I group of proteins, while, the band at 1540 cm^{-1} is associated to amide II group of proteins and both are characteristic of whey proteins. The band at 1453 cm^{-1} corresponds to stretching of –C-H in the CH_2 groups. Finally, the signal at 1743 cm^{-1} was associated with carbonyl stretching vibration [3].

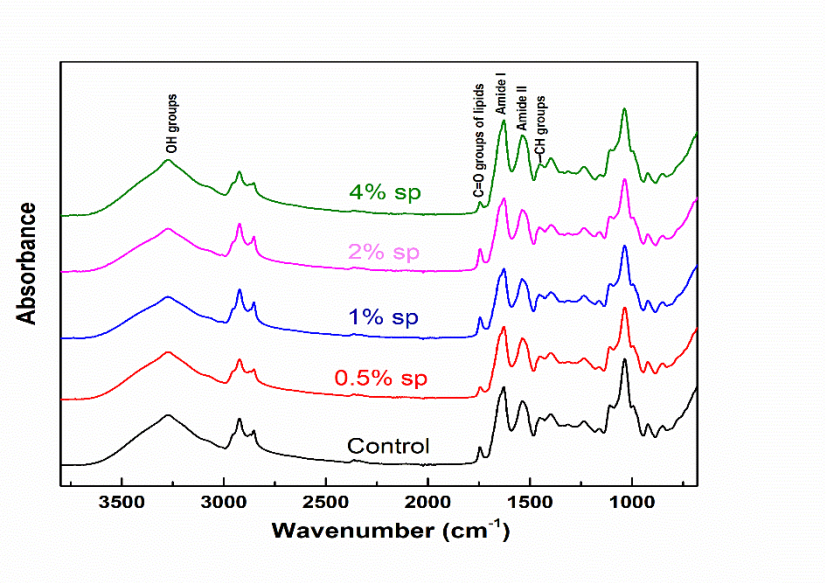

Figure 2. ATR-IR absorbance spectra of WPC-based films (control: corresponds to films without sp., 0.5; 1; 2 & 4% sp: correspond to films incorporated with different concentratios of sp. powder.

4. Conclusions

Results showed that the ultrasound treatment increased the homogenous distribution of sp. during the denaturation process and enabled its entrapment within the film matrix. Furthermore, the incorporation of other microalgae in WPC films needs further research as it was difficult to homogenously distribute them in the film matrix because of their poor solubility, and they were not further investigated.

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