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The Development of Novel Edible Films from Single-Cell Protein Produced by the Biotechnological Valorization of Cheese Whey

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Abstract: The production of value-added products from microorganisms, such as single-cell protein (SCP), through the valorization of agricultural byproducts enhances circular economy while offering alternative solutions for waste treatment. In this study, SCP was obtained through the biotechnological treatment and valorization of cheese whey, the main byproduct of the dairy industry, for the development of novel edible films. To the best of the authors' knowledge, this is the first report examining SCP as a biopolymer for edible film production. Specifically, *Kluyveromyces marxianus*, which has gained QPS and GRAS status, strain EXF-5288 cultivated in deproteinized cheese whey (DCW) lactose (10.0 g/L) in a 3 L fed-batch bioreactor, resulting in a SCP_{max} of 2.63 g/L with a protein content of up to 49.1% *w*/*w*. The addition of increased glycerol concentrations (30, 40, and 50% *w*/*w* of dry cells) as plasticizers was examined to develop SCP-based edible films. Regarding physicochemical characterization, increased glycerol concentration significantly increased moisture content (MC%) and solubility (S%), but there was not a significant difference in other parameters. Regarding wettability, SCP-based films could be described as oleophilic surfaces since the degree of oil contact angle (OCA) ranged between 46.7° ± 1.3 and 54.0° ± 0.5. The proposed holistic approach could contribute to the development of sustainable packaging materials through waste treatment.

Keywords: fed-batch; bioprocess; cheese whey valorization; edible films; sustainable packaging; single-cell protein

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1. Introduction

Annual plastic production volumes are expected to continue rising in the following decades, rising to approximately 590 million metric tons by 2050, which would be an increase of more than 30% compared to 2025 [1]. The European Commission [COM(2018) 340:1–2] [2], in its proposal to limit the impact of certain plastic products on the environment, defined plastic as a material with toxic and other hazardous effects (plastic residues are found in marine life and consequently end up in the food chain, affecting the biosphere in general) [3]. Therefore, the development of biobased materials (biodegradable and/or edible) has been gaining importance to replace synthetic packaging materials. Edible films present great potential in packaging materials due to their non-toxicity, environmental friendliness, and biodegradability [4], as well as the fact that no waste remains after their use, which outweighs them over other packaging materials (degradable or non-degradable). Furthermore, they can be designed to improve the quality and extend the shelf life of packaged foods by controlling water transfer, gas exchange, presenting mechanical

and rheological properties, and inhibiting oxidation [4,5]. Edible films are usually produced from biopolymers such as proteins (i.e., whey protein and zein) [6,7] or polysaccharides (i.e., chitosan) [8] and are often reinforced with bioactive ingredients including polyphenols [9], essential oils [10], natural pigments [7], bioactive compounds derived from renewable resources [11], or even with microorganisms fortifying the antifungal activity of films [12] and probiotics [13]. Recently, the use of single-cell protein (SCP) as a biopolymer was studied for the development of biodegradable films [14]. SCP refers to dry cells of microorganisms representing an alternative protein source for multiple applications, including human food [15,16] and animal feed [17]. Koukoumaki et al. [18] reported a plethora of studies indicating that yeasts are ideal candidates for SCP production. Specifically, the nonconventional yeast species *Kluyveromyces marxianus*, which is classified as a "Qualified Presumption of Safely" (QPS) and "Generally Recognized as Safe" (GRAS) microorganism and thus is considered suitable to produce food-grade enzymes and proteins [19] arises interest due to its ability to produce high yields of SCP through the valorization of low-cost substrates, such as cheese whey [19,20]. Cheese whey is the main by-product of cheese production and is considered the most important pollutant of the dairy industry due to its high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) [21]. Major volumes of cheese whey are produced annually, considering that 1 kg of cheese production leads to 9 L of whey, thus demanding treatment.

In the framework of sustainability and eco-friendliness, this study presents a holistic approach regarding the development of an alternative packaging that is potentially edible due to the use of a GRAS yeast strain and subsequently does not constitute a pollutant after use. Moreover, the utilization of an agricultural by-product as a substrate for the growth of a yeast strain in order to develop SCP-based edible films contributes to this aspect. To the best of the authors' knowledge, this is the first time that SCP derived from bioprocess is used as biopolymer for edible film production.

2. Materials and Methods

2.1. Raw Materials, Microorganisms, and Growth Media

Cheese whey was kindly provided by local cheese manufacturing (Hrysafis, Lemnos, Greece) and was frozen at -20 ± 2 °C until further use. Deproteinized cheese whey (DCW) was obtained by sterilization (121 °C, 20 min), followed by centrifugation (9000 rpm, 15 min, and 4 °C) (Universal 320R Hettich, Tuttlingen, Germany) and filtration. The DCW contained approximately ~50.0 g/L lactose and a pH value of ~6.7. The *K. marxianus* strain EXF-5288 was kindly provided by the Infrastructural Centre Mycosmo, MRIC UL, University of Ljubljana, Slovenia. The yeast strain was conserved in YPDA medium (20 g/L glucose, 10 g/L yeast extract, 20 g/L peptone, and 20 g/L agar) at 4 ± 2 °C. Mineral salts were added into the media (concentration in g/L): KH_2PO_4 7.00; Na_2HPO_4 2.50; $MgSO_4 \cdot 7H_2O$ 1.50; $FeCl_3 \cdot 6H_2O$ 0.15; $CaCl_2 \cdot 2H_2O$ 0.15; $ZnSO_4 \cdot 7H_2O$ 0.02; and $MnSO_4 \cdot H_2O$ 0.06. As a nitrogen source, urea was used at a concentration of 0.22% (*w*/*w*).

2.2. Fed-Batch Experiment

The fed-batch bioreactor experiment was conducted in a 4.6 L total volume bioreactor (Minifors 2, INFORS HT, Surrey, UK) with a working volume of 3.0 L using sterilized DCW as the sole carbon source (initial DCW lactose concentration of 10.0 g/L). An inoculum of 2% (*v*/*v*) of a 24 h preculture (YPD) of the strain was used. The fermentation temperature was set at 20.0 °C, the agitation rate at 350 rpm, and the aeration rate at 2 VVM, and the pH value was maintained at 3.5. Concentrated DCW (~270 g/L) was used as the sole feeding solution during the fed-batch experiment.

2.3. Biomass, SCP, Ethanol Determination, and Substrate Consumption

Yeast cells were harvested by centrifugation (9000 rpm, 10 min, and 4 °C) using a Universal 320R-Hettich centrifuge (Tuttlingen, Germany), and the supernatant was collected at −20 °C for substrate consumption and ethanol production analysis. Centrifugation

was applied two more times under the same conditions. The biomass concentration was determined from the dry weight (~85 °C until constant weight). The protein content of dry cells was determined by the Biuret method, as described in Dourou et al.'s study [22], where the concentration of protein content was determined with the assay of $CuSO_4.5H_2O$ and SCP was expressed as albumin equivalents at 540 nm (Shimadzu, UV-1900 i, Kyoto, Japan). The lipid content of yeast cells was determined using Folch's extraction method [23]. Substrate consumption and ethanol production were analyzed via high-performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan) equipped with a refractive index (RI) detector (RID-10A; Shimadzu Corp., Kyoto, Japan) and a ReproGel H column (250 × 8 mm, 9 μm; Dr. Maisch, Ammerbuch, Germany). The column flow rate was 0.6 mL/min at T = 40 °C, while the mobile phase consisted of 5 mM H_2SO_4. The sample volume was 20 μL. Lactose and ethanol were quantified using the standard calibration curves.

2.4. Preparation of Edible Films

Edible films were prepared according to Papadaki et al. [11], with some modifications. Briefly, 7% (*w*/*w*) of dry biomass was dispersed in deionized water, and the pH was adjusted to 8.0 using 0.5 M NaOH. The solution was denatured under 80 °C for 30 min and then rapidly cooled to prevent further denaturation. Different glycerol concentrations (30, 40, and 50% based on dry cells, 30GLY, 40GLY and 50GLY, respectively) were added into the solution following homogenization (4000 rpm, 15 min) (HG-15D, Witeg, Germany). Then, the solution was degassed using an ultrasonic bath (P70H, Elma Ultrasonic, Weinfelden, Switzerland) for at least 20 min. Film casting was performed on Petri dishes (9 cm), which were left to dry in an environmental-controlled chamber at 25 ± 1 °C with a relative humidity (RH) 55 ± 2% for 24 to 48 h. Then, the films were peeled off and conditioned in a test chamber at 25 ± 1 °C.

2.5. Characterization of Edible Films

2.5.1. Film Thickness

Film thickness (mm) was measured at five different points, and the average film thickness resulted from the measurement of three independent replicates using a digital micrometer (F.F. GROUP TOOLS, Frankfurt, Germany) with an accuracy of 0.002 mm.

2.5.2. Color Analysis

The color of the films was analyzed using a Lovibond LC100 Spectrocolorimeter. L* (0 = black; 100 = white), a* ([+] value = red; [−] value = green), b* ([+] value = yellow; [−] value = blue), h* (hue angle), and C* (chroma) values were recorded.

2.5.3. Film Opacity

Film strips of 1 × 4 cm were attached to the internal side of a cell, and opacity was measured in a UV–VIS spectrometer (Shimadzu, UV-1900 i) and calculated by the following equation [24]:

$$\text{Opacity} = A_{600}/x \quad (1)$$

where A_{600} is the absorbance at 600 nm and x is the film thickness (mm). Three triplicates were performed.

2.5.4. Moisture Content, Solubility, and Swelling Index

The moisture content of film strips with dimensions of 2 cm × 2 cm was determined in an oven at 105 °C (LDO-060S, LabTech, Namyangju, South Korea) for 24 h [25]. Dried films were cooled in desiccators and weighed until a constant weight was obtained. The MC (%) was calculated as the percentage difference between wet and dry weight.

Solubility (S%) and swelling index (S.I%) were determined according to Papadaki et al. [11]. Briefly, the solubility of the film strips (2 cm × 2 cm) was measured by immersing them in 30 mL of deionized water for 24 h at room temperature, and the insoluble solids

of the films were collected and dried (60 °C) until constant weight. Then, solubility was calculated using the following equation:

$$S\ (\%) = [(S_0 - S_1)/S_0] * 100 \quad (2)$$

where S_0 = the initial film weight (g) and S_1 = weight of dried film (g).

The S.I was determined using film strips (2 cm × 2 cm) that were immersed in deionized water for 2 min. The excess water was removed, and the sample was weighed. Then, the S.I was calculated as follows:

$$S.I\ (\%) = [(S.I_1 - S.I_0)/S.I_0] * 100 \quad (3)$$

where $S.I_0$ = the initial film weight (g) and $S.I_1$ = weight of dried film (g).

2.5.5. Water Vapor Permeability

Water vapor permeability (WVP) of films was gravimetrically assessed, according to Protocol B of the American Society for Testing and Materials (ASTM) (1995) [26] and Ramos et al. [5], with some modifications. Briefly, test cups were filled with deionized water, and film was used to seal the cups. Then, the cups were stored in an environmental chamber at 25 °C with RH = 50 ± 2% for 144 h. The water vapor transmission rate (WVTR) was calculated from the slope of the linear regression of the weight gained over time in days (d) ($R^2 \geq 0.99$). The WVP (g·mm/m^2·d·kPa) was then calculated using the following equation:

$$WVP = WVTR * x/\Delta p \quad (4)$$

where WVTR (g/m^2 d) is the slope (g/d) divided by the transfer area of the film (m^2); x (mm) is the film thickness; and Δp (kPa) is the partial water vapor pressure across the film.

2.5.6. Mechanical Properties

Mechanical properties such as tensile strength (TS) and elongation at break (E) were measured according to protocols of the ASTM D882 [27] and Piccirilli et al. [28], using the Texture Analyzer (TA.XT. plus C, Stable Micro Systems, Surrey, UK) equipped with A/MTG Mini Tensile grips. Data were evaluated using Texture Exponent Software (Version 6.1.18.0, Stable Micro Systems, Godalming, UK). Briefly, film strips were cut into 10 × 60 mm, with an initial distance between the grips of 30 mm. The crosshead speed was 0.05 mm/s, and stress–strain curves were obtained. The values of TS (MPa) and E (%) were calculated as follows:

$$TS\ (MPa) = F/a * x * 10^6 \quad (5)$$

$$E\ (\%) = (d/l) * 100 \quad (6)$$

where F refers to the maximum force (N); a = film thickness (m); x = width of film (m); d = elongation at the moment of rupture (mm); and l = the initial exposure length of the test film (mm).

2.5.7. Wettability

Film surface wettability was assessed by measuring the contact angle (θ) using a goniometer (L2004A1, Ossila, UK). The films were cut into 1 cm × 1 cm and attached to the equipment, and a 2 µL drop of olive oil or deionized water was released on the surface of the films using a 25 µL precision syringe (Hamilton, Bonaduz, Switzerland).

2.6. Statistical Analysis

All measurements in films were conducted in three independent replicates (N = 3), and in each independent replicate, the average value was calculated in triplicate. Data were collected in Microsoft Excel and analyzed using XLSTAT software (Version, 2018.1., Addinsoft, Lumivero, Denver, CO, USA) via one-way analysis of variance (ANOVA). Mean

comparisons were performed using Tukey's HSD test adjustment at significance level $\alpha = 0.05$ ($p \leq 0.05$). In the fed-batch experiment, the analysis of each point was conducted in duplicate.

3. Results and Discussion

3.1. K. marxianus Strain EXF-5288 Growth in Fed-Batch Condition

In general, *K. marxianus* strains are characterized as Crab-tree negative, not having the ability to produce ethanol using glycose as substrate [29]; however, *K. marxianus* overall metabolic pathway in glycolysis and the tricarboxylic acid (TCA) cycle remain unclear [30]. Regarding this, previous studies have reported ethanol production by *K. marxianus* strains [19,31,32]. In the present study, the selection of the initial DCW lactose concentration at 10.0 g/L was based on preliminary experiments [33] to enhance respiration over alcoholic fermentation of *K. marxianus* strain EXF-5288. As shown in Figure 1, the exponential phase of the strain started at 8 h, while by 13 h, it was able to assimilate total substrate, reaching a biomass value of 2.58 ± 0.04 g/L ($Y_{X/Laccons}$ = 0.32 ± 0.00 g/g). The first feed (at 14 h) fortified ethanol production from 1.9 ± 0.00 to 5.5 ± 0.3 g/L (Figure 1), reaching a yield of ethanol $Y_{Eth/Laccons}$ = 0.36 ± 0.02 g/g. Further addition of DCW lactose in the medium (second and third feeds) favored the production of ethanol, with Eth_{max} = 9.8 ± 0.2 g/L and a respective yield of lactose consumed of 0.42 ± 0.01 g/g (Table 1), indicating that strain *K. marxianus* EXF- 5288 could be described as Crab-tree positive. Similar results regarding high ethanol yield were observed by Ozmihci and Kargi [34], where ethanol production by *K. marxianus* strain DSMZ 7239 valorizing cheese whey powder in a fed-batch bioreactor (1 L) was studied. The ethanol yield of the consumed substrate reached constantly 0.52 ± 0.02 g/g between initial sugar concentrations of 25 and 150 g/L. In the present study, fed-batch cycles increased biomass and SCP production (Figure 1). Specifically, maximum values of biomass and SCP were observed at 28 h (after 3rd feed), reaching 5.36 ± 0.02 and 2.63 ± 0.04 g/L, respectively. In addition, the protein content of dry cells reached high yields (42.0–50.0% *w*/*w*), indicating that *K. marxianus* strain EXF-5288 is an ideal candidate for SCP production. Yadav et al. [20] assessed SCP production by *K. marxianus* through batch and continuous mode trials using cheese whey (T = 40.0 °C; pH = 3.5; ~50.0 g/L lactose) in a 10L bioreactor. The results showed that protein content reached 42% *w*/*w*, which is in agreement with the findings of the present study regarding protein content (% *w*/*w*).

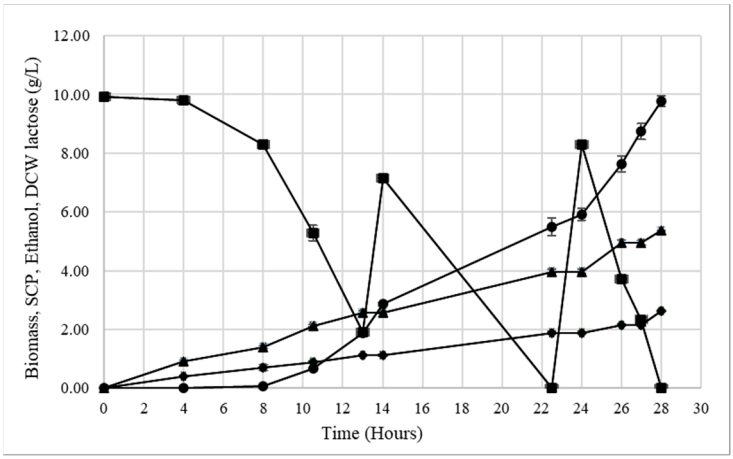


Figure 1. Biomass, SCP, and ethanol production and lactose consumption of *Kluyveromyces marxianus* strain EXF-5288 cultivated in an initial deproteinized cheese whey (DCW) lactose concentration of 10.0 g/L in a 3 L fed-batch bioreactor. Culture conditions: pH = 3.5; incubation temperature T = 20.0 °C; and 2 VVM. The analysis for each point was conducted in duplicate. Biomass (g/L) ( ), SCP (g/L) ( ), ethanol (g/L) ( ), and DCW lactose (g/L) ( ).

Table 1. The production of added value metabolic compounds of *K. marxianus* strain EXF-5288 cultivated under an initial DCW lactose concentration of 10.0 g/L in a 3 L fed-batch bioreactor.

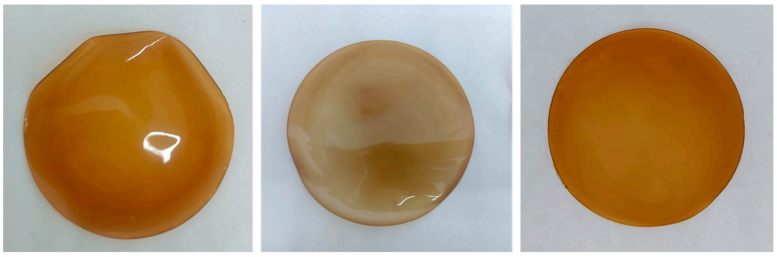
Feed	Hours	Biomass (g/L)	SCP (g/L)	$Lactose_{cons}$ (g/L)	Ethanol (g/L)	$Y_{SCP/X}$ (g/g)	$Y_{X/Laccons}$ (g/g)	$Y_{Eth/Laccons}$ (g/g)
Lac = 9.9 ± 0.2 (g/L)	0.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00
	4.0	0.90 ± 0.05	0.41 ± 0.02	0.1 ± 0.0	0.0	0.46 ± 0.00	8.18 ± 1.00	0.00
	8.0	1.41 ± 0.05	0.70 ± 0.10	1.6 ± 0.1	0.1 ± 0.0	0.50 ± 0.05	0.87 ± 0.01	0.05 ± 0.00
	10.5	2.13 ± 0.04	0.89 ± 0.04	4.6 ± 0.2	0.6 ± 0.0	0.42 ± 0.01	0.46 ± 0.01	0.14 ± 0.00
	13.0	2.58 ± 0.04	1.12 ± 0.04	8.0 ± 0.2	1.9 ± 0.0	0.44 ± 0.00	0.32 ± 0.00	0.23 ± 0.00
1st Lac = 0.0 ± 0.0 (g/L)	14.0	2.58 ± 0.04	1.12 ± 0.04	8.0 ± 0.2	2.9 ± 0.1	0.44 ± 0.00	0.32 ± 0.00	0.36 ± 0.01
	22.5	3.97 ± 0.06	1.89 ± 0.04	15.2 ± 0.3	5.5 ± 0.3	0.48 ± 0.00	0.26 ± 0.00	0.36 ± 0.02
	24.0	3.97 ± 0.06	1.89 ± 0.04	15.2 ± 0.3	5.9 ± 0.2	0.48 ± 0.00	0.26 ± 0.00	0.39 ± 0.02
2nd Lac = 3.7 ± 0.3 (g/L)	26.0	4.95 ± 0.09	2.15 ± 0.05	19.7 ± 0.1	7.6 ± 0.3	0.43 ± 0.00	0.25 ± 0.00	0.39 ± 0.02
	27.0	4.95 ± 0.09	2.15 ± 0.05	21.1 ± 0.5	8.7 ± 0.3	0.43 ± 0.00	0.23 ± 0.00	0.41 ± 0.02
3rd Lac = 0.0 ± 0.0 (g/L)	28.0	5.36 ± 0.02	2.63 ± 0.04	23.5 ± 0.1	9.8 ± 0.2	0.50 ± 0.01	0.23 ± 0.00	0.42 ± 0.01

Lac = concentration of lactose (g/L); $Lactose_{cons}$ = consumed lactose (g/L); $Y_{SCP/X}$ = total SCP on a dry basis (g/g); $Y_{X/Laccons}$ = biomass yield on lactose consumed (g/g); and $Y_{Eth/Laccons}$ = ethanol yield on lactose consumed (g/g). Culture conditions: pH = 3.5; incubation temperature T = 20.0 °C; and 2 VVM. The analysis for each point was conducted in duplicate.

3.2. The Development and Characterization of SCP-Based Edible Films

3.2.1. Color Film Opacity

The presence of dry yeast cells led to a brownish film color (Figure 2), while regarding color parameters, no significant differences were observed (Table 2). On the contrary, another study showed that an increase in glycerol from 40 to 60% (*w*/*w*) led to a decrease in ΔE values of whey protein isolate and whey protein concentrate films [5]. Regarding film opacity, no statistical differences were observed. This is in agreement with Ramos et al. [5], since an increase in glycerol concentration in edible films did not lead to significant differences in opacity at 600 nm. In this study, film opacity values ranged from 3.5 ± 0.7 to 6.0 ± 2.2, which is similar to another study, where the presence of *Debaryomyces hansenii* in chitosan-based edible films led to opacity values from 3.35 to 7.45 [12].



30GLY 40GLY 50GLY

Figure 2. SCP-based films with increased glycerol concentrations. 30GLY (30% glycerol (*w*/*w* of dry cells)), 40GLY (40% glycerol (*w*/*w* of dry cells)), and 50GLY (50% glycerol (*w*/*w* of dry cells)).

Table 2. The effects on color parameters and film opacity of SCP-based edible films with increased glycerol concentrations.

SCP-Based Edible Films	L*	a*	b*	C*	h*	Film Opacity
30GLY	57.8 ± 1.7 [a]	17.8 ± 2.6 [a]	49.6 ± 4.7 [a]	52.8 ± 5.3 [a]	70.5 ± 1.3 [a]	3.5 ± 0.7 [a]
40GLY	58.3 ± 2.8 [a]	17.0 ± 0.3 [a]	52.3 ± 3.7 [a]	55.2 ± 3.4 [a]	72.0 ± 1.5 [a]	6.0 ± 2.2 [a]
50GLY	58.0 ± 0.7 [a]	17.2 ± 1.4 [a]	48.6 ± 2.4 [a]	51.6 ± 2.4 [a]	70.5 ± 1.6 [a]	5.1 ± 0.6 [a]

Means in a column followed by the same letter ([a]) are not significantly different ($p > 0.05$). 30GLY (30% glycerol (*w*/*w* of dry cells)), 40GLY (40% glycerol (*w*/*w* of dry cells)), and 50GLY (50% glycerol (*w*/*w* of dry cells)). All measurements were conducted in three independent replicates (N = 3).

3.2.2. Moisture Content, Solubility, and Swelling Index

An increase in glycerol concentration increased the MC in edible films from 11.2 ± 0.7% (30GLY) up to 25.9 ± 0.3% (50GLY), showing a typical behavior for hydrophilic materials (Table 3). Glycerol is a hydrophilic plasticizer that loses the structure of films by exposing their hydroxyl groups and provides more active sites where moisture molecules could be adsorbed [35]. Previous studies have shown that an increase in the amount of plasticizer led to an increase in the moisture content of protein-based edible films [5,36]. The results of this study are in line with previous studies, since the MC of 40GLY SCP-based edible films was 15.0 ± 2.7%, while in protein-based films fortified with *Trametes versicolor* biomass reached 10.3 ± 1.6% [37].

Table 3. The effect of glycerol concentration on the moisture content, solubility, swelling index (S.I.), and water vapor permeability (WVP) of SCP-based edible films.

SCP-Based Edible Films	Moisture Content (%)	Solubility (%)	S.I (%)	WVP ($g \cdot mm/m^2 \cdot d \cdot kPa$)
30GLY	11.2 ± 0.7 [a]	48.5 ± 2.5 [a]	33.2 ± 2.5 [a]	20.5 ± 4.1 [a]
40GLY	15.0 ± 2.7 [a]	75.0 ± 1.3 [b]	34.4 ± 1.8 [a]	19.2 ± 3.8 [a]
50GLY	25.9 ± 0.3 [b]	58.2 ± 2.8 [c]	38.8 ± 1.1 [a]	21.4 ± 1.2 [a]

Means in a column followed by the same letters ([a], [b], and [c]) are not significantly different ($p > 0.05$). 30GLY (30% glycerol (w/w of dry cells)), 40GLY (40% glycerol (w/w of dry cells)), and 50GLY (50% glycerol (w/w of dry cells)). All measurements were conducted in three independent replicates (N = 3).

Solubility is an important factor for the application of edible films. To improve product integrity and water resistance, potential applications could require water insolubility. On the other hand, film's solubility in water prior to use may be advantageous in certain cases [38]. Regarding the results, it seems that SCP-based edible films' integrity in water is affected by glycerol concentration. Specifically, the increase of glycerol from 30 to 40% w/w increased S up to 75.0 ± 1.3% (Table 3); however, further increases in plasticizer concentration decreased S to 58.2 ± 2.8%. Ramos et al. [5] proposed that the partial insolubility of protein-based films may be attributed to their highly stable proteinaceous polymeric network, since only small molecules (i.e., monomers, nonprotein materials, and small peptides) are totally soluble. In the present study, the solubility rate of SCP-based edible films indicates that their use as packaging materials in high-moisture foods may not be favorable; however, their application in foods characterized by lower moisture and low water activity (i.e., flour, spices, nuts, and herbs) [39] may be feasible, providing light protection since SCP-based films were characterized by high values of film opacity (transparent food packaging is those with an opacity < 5 [40]).

The swelling index refers to structural alterations that impact the internal structure of a biopolymer caused by water absorption. The S.I values did not seem to be affected significantly by the increase in plasticizer concentration and ranged from 33.2 ± 2.5 to 38.8 ± 1.1%. Papadaki et al. [11] developed whey protein edible films under different pH values, resulting in a swelling index value of 39.5 ± 2.1% at pH 7 with 40% glycerol as the plasticizer, which is in line with the S.I values of the present study.

3.2.3. Water Vapor Permeability

Water vapor permeability refers to a property of food packaging materials that determines food quality during preservation and storage through moisture loss or gain control. Several factors affect the WVP of protein-based films, including the presence of polar and non-polar amino acids, pH value, surface charge, and non-homogeneity of the film [14]. In this study, WVP values ranged from 19.2 ± 3.8 to 21.4 ± 1.2 ($g \cdot mm/m^2 \cdot d \cdot kPa$). These findings indicate higher WVP values compared to studies examining protein-based edible films, including whey protein films [41] and gelatin–casein phosphopeptides [42]. These results indicate that the durability of SCP-based edible films regarding water barriers requires

further investigation. Specifically, the factors that need to be considered are the storage time (to investigate the effect of ambient humidity in edible films) and the application of enclosed products with high or low water activity to investigate if microbial spoilage and undesirable organoleptic characteristics will occur [43]. Furthermore, previous studies have shown that an increase in plasticizer concentration (such as glycerol) normally leads to an increase in WVP [38,44], since the hydrophilic nature and amount reduces internal hydrogen bonding and increases intermolecular spacing [45]. However, in this study, increased glycerol concentrations did not increase the WVP of the SCP-based films (Table 3). Those findings could be explained due to the presence of lipids in SCPs (yeast cells are not defatted, containing approximately 15% *w*/*w* of lipids in the present study), which reinforce hydrophobicity in the film [14]. Moreover, the SCP is characterized by a high content of non-polar amino acids [14,46], which contribute to hydrophobicity.

3.2.4. Mechanical Properties

Mechanical properties are important factors of films because they indicate the resistance of the films during their shelf life. Tensile strength refers to film resistance due to the cohesion between the chains, while the elongation at break refers to its plasticity, which is the capacity of the film to extend before fracturing [36]. As shown in Table 4, SCP-based edible films did not result in high mechanical resistance since the TS value did not exceed 1.3 ± 0.6 Mpa, while their E (%) ranged from 4.7 ± 0.5 to 6.9 ± 2.0%. Regarding TS values, similar results were observed by Papadaki et al. [11] for protein-based films reinforced with 40% *w*/*w* glycerol. Specifically, TS values ranged from 0.7 to 1.8 Mpa under different pH treatments. Glycerol increases did not significantly affect mechanical parameters. This is not in line with previous studies, since it is expected that an increase in the water content of films decreases the protein–protein interactions, resulting in higher flexibility and less resistance [47]. In this study, SCP-based edible films presented poor mechanical properties compared to plant-based (i.e., soy protein, gliadin, and zein) and animal-based edible films (i.e., gelatin and casein) [48], and in order to improve the mechanical properties of the examined edible films, reinforcement with active compounds could be applied, as has been carried out in other protein-based films [25,49]. Moreover, it has to be mentioned that the degree of protein denaturation could possibly alter the physicochemical characteristics of edible films; therefore, the study of different denaturation times requires further investigation.

Table 4. The effect of glycerol concentration on the mechanical properties (tensile strength (TS) and elongation at break (E)) and wettability of SCP-based edible films.

SCP-Based Edible Films	TS (Mpa)	E (%)	OCA°
30GLY	1.3 ± 0.6 [a]	6.9 ± 2.0 [a]	47.1°±0.5 [a]
40GLY	0.3 ± 0.1 [a]	5.6 ± 0.8 [a]	46.7° ± 1.3 [a]
50GLY	0.4 ± 0.1 [a]	4.7 ± 0.5 [a]	54.0° ± 0.5 [a]

Means in a column followed by the same letter ([a]) are not significantly different ($p > 0.05$). 30GLY (30% glycerol (*w*/*w* of dry cells)), 40GLY (40% glycerol (*w*/*w* of dry cells)), and 50GLY (50% glycerol (*w*/*w* of dry cells)). OCA—Oil contact angle. All measurements were conducted in three independent replicates (N = 3).

3.2.5. Wettability

Wettability refers to a general term used to define the spreading behavior of any liquid over a surface. In this study, the wettability of SCP-based edible films was tested with different liquids (water and olive oil). Since water droplets were rapidly absorbed by the surface of SCP-based edible films and penetrated them, as shown in Figure 3, analysis was performed only with olive oil. The Wenzel model predicts that the hydrophilicity of a roughened surface, indicated by a reduced apparent contact angle, will increase when its smooth surface's initial contact angle (θ_0) is less than 90°. Conversely, if $\theta_0 > 90°$, the reverse effect is expected. Since oils typically have lower surface tension and smaller

contact angles than water, Wenzel's model suggests that, in general, most surfaces would be oleophilic [50]. An oil droplet that wets a textured surface is in the "Wenzel state" and tends to leave an oily trail or stain as it slides and spreads [51]. Regarding this, SCP-based edible films could be described as having oleophilic surfaces since the degree of OCA ranged from 46.7° ± 1.3 to 54.0° ± 0.5 (Table 4), as well as the fact that the oil droplets were spread across the films (Figure 3). The surface behavior of protein-based edible films could be associated with the orientation of functional groups of hydrophilic and hydrophobic amino acids [52], as well as the kind of protein. For example, in a study examining gelatin-based edible films, the contact angle was 112.15° ± 2.98, indicating a hydrophobic surface [53]. On the contrary, when soy protein isolate edible films were studied, the degree of water contact angle was 60.9° ± 1.8, indicating a hydrophilic surface [54]. In the present study, an increase in glycerol concentration did not significantly affect the degree of the OCA. Likewise, when Kokozka et al. [45] increased their glycerol concentration from 30 to 50–60% in WPI-edible films, there was no significant difference in contact angle degrees (ranging from 37.8° ± 2.7 to 42.5° ± 9.8).

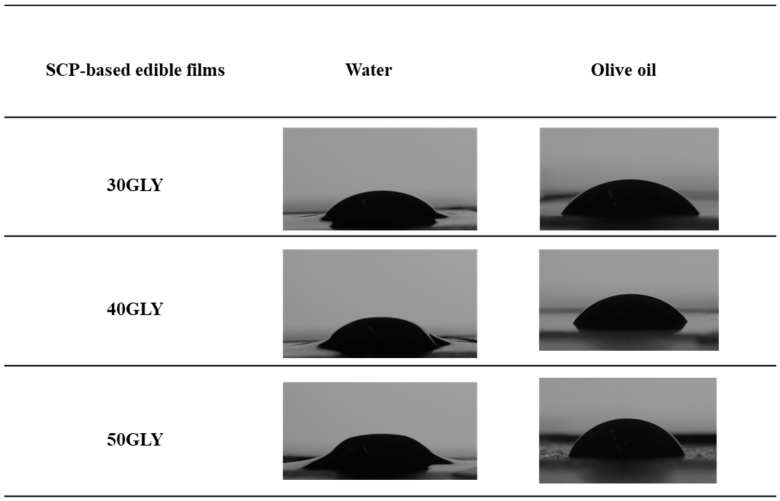


Figure 3. Wettability on SCP-based edible films with different liquids (water and olive oil). 30GLY (30% glycerol (*w*/*w* of dry cells)), 40GLY (40% glycerol (*w*/*w* of dry cells)), and 50GLY (50% glycerol (*w*/*w* of dry cells)). All measurements were conducted in three independent replicates (N = 3).

4. Conclusions

In the present study, novel edible films derived from SCP through the biotechnological treatment and valorization of cheese whey were developed, proposing an eco-friendly packaging material. The yeast strain *K. marxianus* EXF-5288 was cultivated in fed-batch bioreactor mode, valorizing DCW lactose, and totally consumed the substrate with parallel production of added-value compounds including SCP and ethanol. The combined physicochemical results and surface properties lead to the conclusion that SCP-based edible films derived from bioprocesses could be applied as packaging materials, depending on the product enclosed and mainly in low-moisture foods. Focusing on the zero-food waste strategy, the present study demonstrated the development of SCP-based edible films. It could be proposed that the next steps include optimization and property enhancement of SCP-based edible films (i.e., improved quality and shelf life of packaged foods through the optimization of water transfer, gas exchange, mechanical and rheological properties, and inhibition of oxidation) in order to be used as a sustainable approach to substitute or totally replace non-biodegradable materials.

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administration, D.S; funding acquisition, D.S. All authors have read and agreed to the published version of the manuscript.

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