No Waste from Waste: Membrane-Based Fractionation of Second Cheese Whey for Potential Nutraceutical and Cosmeceutical Applications, and as Renewable Substrate for Fermentation Processes Development

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Abstract: Second cheese whey (SCW) derived from buffalo milk is the main by-product of the mozzarella cheese dairy industry. The objective of this study was to develop a membrane-based purification procedure to obtain specific fractions from SCW and assess their biological and applicative potential. Special interest was paid to the proteins and newly identified health-promoting compounds that could be recovered and used as value-added products in different sectors of food and pharmaceutical industries. SCW has been treated, sequentially, with microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) membranes giving the possibility to obtain three different fractions, namely retentates recovered on 100 and 10 kDa (R100 and R10) and a nanofiltration retentate (RNF). These retentates were compared for their ability to preserve human keratinocytes from dehydration, to form protein-based films by casting, and finally they were used for probiotic cultivations as the main substrate. Results showed that *Lactobacillus rhamnosus* could grow without any further additional nutrient up to $2.2 \pm 0.3 \times 10^9$ CFU/mL in the RNF medium. Dehydration tests on HaCat cells proved R100 as the most efficient fraction in preserving cell viability from this specific stress. R10, after diafiltrations, formed transparent films with improved features when glycerol was added as a plasticizer.

Keywords: buffalo whey; second cheese whey; membrane processes; probiotics; biopolymer film; counteracting skin dehydration

1. Introduction

Whey is the main by-product obtained from cheese production [1]. It derives from all types of milk processing (cow, goat, sheep, and camel), even though bovine milk whey is the most common. It accounts for about 85–90% of the volume of milk and contains about 55% of its nutrients [2]. Whey gives rise to considerable quantities of wastewaters, which cannot be discharged directly and should then be treated, resulting in additional costs for manufacturing companies. In fact, approximately 50% of the total worldwide production of whey is normally disposed of, without being utilized [3,4]. The production of whey worldwide, estimated to be around 180–190 million ton/year in 2010 and 300–320 million ton/year in 2020, is expected to increase by 3.5–4% annually [5]. In Italy, and in particular in the Campania region, cheese production facilities, especially related to mozzarella cheese manufacturing, dispose of large volumes of whey with high seasonal peaks (spring/summer). For example, the total volume of SCW produced in Italy in 2016 was equal to 1 million tons [6], and the amount of ricotta cheese produced in the Campania...
region in 2021 was about 1400 tons (Agri-food Quality Department). Considering a more sustainable approach in the circular bio-economy paradigm, these wastewaters should be considered a resource. Some applications have already been implemented or proposed, but possibly others can be suggested, thus creating added value products/fractions starting from a raw material of high availability, low cost, and especially high environmental impact (COD, salinity, etc.) [7]. Whey consists of lipids, proteins, and minerals, which make it useful for the production of various compounds and highlights the potential for researchers to uncover new applications for whey as an added value product. The chemical, physical, biological, and nutritional characteristics of whey components have been studied, and notably lactose is the main component present. In particular, milk whey proteins represent about 20% of milk proteins, and β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and the so-called macropeptides represent more than 80% of the total [8]. In fact, due to its high biological value and versatile functional properties, whey is widely used in pharmaceutical and food industries [9,10]. The liquid remaining after whey cheese separation represents more than 90% of the original whey and is called second cheese whey (SCW), or Scotta in Italy [11]. Mozzarella soft cheese manufacturing is very often followed by a second curd phase from which ricotta cheese is obtained, recovering most of the proteins and containing less lipids than the first soft cheese produced. During this step of manufacturing, second cheese whey is produced, and as mentioned above, the volumes to be disposed of may increase up to six—sevenfold during late spring and summer. The composition of SCW is very similar to that of whey, namely lactose (4.8–5.0%), salts (1.0–1.13%), and proteins (0.15–0.22%) generally compose SCW resulting from bovine milk [12]. Very often, these larger volumes cause a problem related to biochemical oxygen demand (BOD) and chemical oxygen demand (COD) content when these wastewaters reach small rivers and, following, the sea. For this reason, it may be interesting to assess an easy to scale process to improve the re-use and recycle of the second cheese whey considering the circular economy idea “from waste to value”. Currently, there is growing interest in new applications of second cheese whey and its derivatives for various food products with improved quality that are beneficial to health. Due to its composition, SCW can be used for different purposes including food formulations, nutraceuticals, and biofuels [13,14]. Few papers mention the use of SCW as a substrate for the production of fermented food and drinks, and added value products (e.g., lactic acid, biofuels). For example, Maragkoudakis and collaborators grew three strains of lactobacilli on SCW obtaining $10^9$ cfu/mL, proving that it may have potential as substrate for bacterial growth for the production of a fermented drink [6]. Furthermore, Secchi and collaborators used bovine SCW for the production of lactic acid from mixed cultures of lactic acid bacteria with yields up to 92%, comparable to those obtained on cheese-whey [15]. Many techniques have been developed to separate the sugar components and selectively concentrate whey proteins, for example, in exploiting ultrafiltration and nanofiltration processes. In fact, whey is not a balanced source of nutrients, since compared to that of proteins the concentration of lactose is a lot higher, and thus it does not possess the nutritional benefits of typical protein sources [5]. Concentration by ultrafiltration (UF) is a very attractive technique since it does not require heating, which can change the characteristics of whey’s thermolabile components. Because of this advantage, but also due to the low costs and scale-up ease, membrane processes are the most favoured in whey downstream applications particularly when considering the perspective of reutilizing—at least partial reuse of—water [11,16].

Here, we describe an integrated downstream process to fractionate second cheese whey (SCW), using micro-ultra and nanofiltration. Ultrafiltration membranes with two different cut-offs, namely 10 and 100 kDa, were used, before the nanofiltration process, in order to possibly obtain different fractions to assess the potential use of second cheese whey components in different fields.

In particular, SCW or the derived retentate fractions were evaluated (i) as substrates for growth and biomass production of lactic acid bacteria (LAB), to be used as probiotics or as starters; (ii) as preventive agents for skin dehydration for potential cosmeceutical appli-
cations; and (iii) finally, as protein constituents for biofilm production for food applications. In relation to its protein fraction, whey is reported to be especially used in baby food and as food supplement for athletes; lactose on the other hand can be fermented to produce biomasses and lactic acid. The latter can also be purified to pharma level and may be used as building block for the (bio)synthesis of polylactic acid. In addition, biopolymers derived from whey (mainly proteins) treated under specific conditions can produce films by casting for different applications. The packaging and food industries are joining increasing efforts to use biodegradable materials as environmentally friendly alternatives to non-recyclable traditional plastic materials [17]. One of these is represented by biopolymer films, which are made out of renewable resources containing natural raw materials, such as polysaccharides and proteins. Biodegradable polymers are well indicated for their application as edible films either for individual coating of small food products or placed within a dual texture food to prevent moisture migration and maintain the texture of each of the layers. In this respect, whey has been shown to be a potential suitable starting source for obtaining edible films [18].

2. Materials and Methods

2.1. Materials

Within the research projects PON 03PE00060_2 and BIONUTRA, the dairy factory “La Perla del Mediterraneo” (Battipaglia, Italy) provided the second cheese whey (SCW) sample used in this work. The microfiltration process was performed using a 0.65 µm polyethersulfone hollow fiber membrane with a total filtering area of 0.3 m². Ultrafiltration (UF) and nanofiltration (NF) processes were performed using polyethersulfone spiral membranes of 100 and 10 kDa and 250–300 Da cut-off, respectively, with a total filtering area of 0.3 m² (Fluxa Filtri, Milano, Italy). The system used for the membrane process was a UF-NF system equipped with a 10 L volume steel tank (Idea3 Engineering, Lessona, Italy). Constant and low temperature (8–10 °C) of the sample in the tank and spiral membranes were maintained by using a thermostatic bath.

*L. rhamnous IMC501 and L. brevis SP-48 were provided by “Centro Sperimentale del Latte S.r.L” (Zelo Buon Persico, Italy), while L. fermentum was isolated from buffalo milk previously in our laboratory.

2.2. Downstream Process

For the purification of the SCW, after the microfiltration process, subsequent ultrafiltration and nanofiltration processes were carried out. In particular, the SCW was ultrafiltered on 100 kDa and diafiltered with four volumes of purified water (R100 is 100 kDa retentate; R10 is 10 kDa retentate; and RNF is nanofiltration retentate). The permeate was loaded on 10 kDa membranes and the R10 was diafiltered with eight volumes of purified water. Finally, the recovered permeate was treated on nanofiltration membranes. Analyses of sugars, residual acids, and total proteins were carried out on each retentate or permeate sample obtained. Aliquots (10 mL) of samples of each step of the process were freeze-dried (Beta 2-8 LSC-plus Christ, Germany) for 18 h at −20 °C and at 1.05 mbar and then for 3 h at 20 °C and at 0.040 mbar.

2.3. Analytical Methods

The analyses of lactose, glucose, galactose, and organic acids were carried out using a UHPLC Dionex Ultimate 3000+ chromatograph (Thermofisher, Milano, Italy) equipped with a UV/Vis and RI detector. The standards and the samples, previously ultrafiltered on centricron systems with a 3 kDa cut off, were analyzed according to the following operating conditions: an LC Column Phenomenex RezexTM ROA-organic Acid H+ (8%) 300 × 7.8 mm, 6µ; isocratic elution with a buffer 0.1% sulphuric acid in water and a flow of 0.8 mL/min; temperature: 40 °C; concentration range: 30–0.01 mg/mL; and acquisition time: 25 min.
2.4. Protein Assay

The total protein content was obtained by using the Kit Protein assay Biorad (Bio-Rad Laboratories Inc., Hercules, CA, USA), which allowed the researchers to analyze the samples by UV/Vis spectrophotometry at 595 nm using a Beckmann DU-730 spectrophotometer. Standard calibration curve was obtained by Bovine serum albumin (Sigma Aldrich, Milano, Italy) ranging from 10 to 0.01 mg/mL.

2.5. Protein Molecular Weight Analyses

The analyses of protein molecular weight were performed using the UHPLC Dionex previously described. The standards and the samples, previously centrifuged and filtered on a 0.22 µm filter, were analysed according to the following operating conditions: a PolySep-GFC-P 2000 column 300 × 7.8 mm, 5 µm (Phenomenex, Milano, Italy); buffer A: water 0.1% trifluoracetic acid; buffer NaCl 0.05 M; flow of 0.6 mL/min; temperature: 35 °C; protein MW range: 66 kDa (Bovine serum albumin), 25 kDa (alpha-Casein), 6 kDa (Insulin); wave lengths: 214 and 280 nm; and acquisition time: 60 min.

2.6. Growth of Lactic Acid Bacteria in Small-Scale Bottle Experiments

One hundred mL screw-cap bottles with 90 mL of working volume, incubated at 37 °C and 150 rpm in a rotary shaker incubator (model Minintron, Infors, Bottmingen, Switzerland), were used for all small-scale experiments of growth of L. rhamnosus IMC501, L. fermentum, and L. brevis SP-48 that lasted 41 h. A first set of experiments was performed on sterilized (121 ºC, 1 bar of pressure for 20 min) whey proteins, on R10 and RNF. The pH of these growth tests was not controlled and started from 4.05 ± 0.06. Samples were withdrawn at times 0, 17, and 41 h to analyze optical density (600 nm) carbon sources consumption and acid and ethanol production. All bottle experiments were performed at least in triplicate. Viability was evaluated by serial dilution of the samples, which were plated on an MRS-agar medium. Plates were incubated at 37 °C for 36 h before counting viable cells. Each sample was analyzed in triplicate.

A second set of small-scale bottle experiments was performed on the R100, R10, and RNF using pasteurization instead of sterilization at 121 ºC. Pasteurization was conducted at 80 ºC for 2–3 min with a temperature ramp of 18 min. For all growth experiments (sterilized or pasteurized media), a single exponential phase stock solution of L. rhamnosus IMC501 was added to each bottle, in order to start from 0.5 OD600.

2.7. Whey Protein Films Preparation

Films were prepared by dissolving the fractions derived from the R100 and R10 cut off after further extensive diafiltration and in ultrapure water at 4.5% w/v protein in 10 mL [19]. Solutions were stirred for 90 min at room temperature and incubated at 80 °C in a vortemp for 30 min to denature proteins. The solutions were cooled to room temperature. To a set of solutions, glycerol was added as plasticizer to obtain a final concentration of 4.5% w/v, and then the solutions were stirred for 30 min to be homogenized. In order to eliminate the air bubbles, the solutions were centrifuged at 2300 rpm for 10 min. The casting solutions were put in 140 mm-diameter Petri dishes and dried at 37 °C for 24 h.

2.8. Dehydration Test

The dehydration test was accomplished using HaCat cells provided by the Zooprophilatic Institute (Brescia, Italy), cultured in Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 10% (v/v) heat inactivated Fetal Bovine Serum (FBS), penicillin 100 U/mL, and streptomycin 100 µg/mL. Dulbecco’s Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin-streptomycin, PBS, and Trypsin were provided by Gibco Invitrogen (Milan, Italy). The cells were grown on tissue culture plates (BD Bioscience-Falcon, San Jose, CA, USA) in a humidified atmosphere (95% air and 5% CO2, v/v) at 37 °C. HaCat cells were cultured in a standard 24-well culture plate, until a confluence of about 50% was reached and then treated for 2 h with different samples: SCW, R100, and R10 at two
concentrations, 4 mg/mL and 2 mg/mL. After 2 h, the treatments were removed (except for the positive control, CTR+, where DMEM 10% FBS was present), and the plates were then left open in the incubator (at 37 °C and 5% humidified air) for 20 min to mimic a stress condition [20]. Negative control (CTR-) was denoted by the cells that were subjected to dehydration without adding any treatment (only medium). Cell vitality was evaluated after dehydration using the MTT test. The MTT assay is a colorimetric assay based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to measure cellular metabolic activity as parameter of cell viability. Viable cells contain NAD(P)H-dependent oxidoreductase enzymes that reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple color. Then, MTT substrate was prepared in a physiologically balanced solution at a concentration of 0.5 mg/mL in white DMEM without FBS, added to cells in culture, and incubated for 3 h. The formazan crystals were solubilized in HCl 0.1 M in isopropanol. The amount of formazan (presumably directly proportional to the number of viable cells) was measured by recording changes in absorbance at 570 nm using a spectrophotometer. An optical microscope was used to observe morphological changes of the cells before the dehydration test and after the suffering status.

3. Results

3.1. Downstream Process

The downstream process reported by Alfano and collaborators was slightly modified [21]. After the microfiltration process, SCW was first concentrated on 100 kDa membranes, and the permeate obtained was treated on 10 kDa membranes. The 10 kDa permeate was successively concentrated on nanofiltration membranes. The results of membrane processes are reported in Table 1. With this approach, different retentate fractions were obtained and used for this application, such as to make biofilms (R100 and R10) or to serve as a main substrate for probiotic cultures (R100, R10, and RNF) and finally for dehydration tests on HaCat cells (R100 and R10). Table 2 reports the concentration of the main carbon sources, of lactic acid, and of proteins present in the SCW sample and in the different fractions obtained during the course of the purification process (retentates). Figure 1 shows the molecular weight distribution of proteins found in the fractions obtained from the downstream process.

Table 1. Parameters used during filtration processes.

<table>
<thead>
<tr>
<th>Process</th>
<th>Concentration Factor</th>
<th>Diafiltration Factor</th>
<th>Initial Transmembrane Pressure (Bar)</th>
<th>Final Transmembrane Pressure (Bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF 100</td>
<td>9.4</td>
<td>4</td>
<td>3.1</td>
<td>3.3</td>
</tr>
<tr>
<td>UF 10</td>
<td>5.5</td>
<td>8</td>
<td>4.0</td>
<td>5.2</td>
</tr>
<tr>
<td>NF</td>
<td>4.8</td>
<td>/</td>
<td>11.0</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 2. Concentration of carbon sources (lactose, glucose, and galactose), lactic acid, and proteins found in the initial whey sample, in SCW, and in ultrafiltration and nanofiltration retentates. n.a. not analyzed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lactose (g/L)</th>
<th>Glucose (g/L)</th>
<th>Galactose (g/L)</th>
<th>Lactic Acid (g/L)</th>
<th>Proteins (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey</td>
<td>34.0</td>
<td>n.a.</td>
<td>n.a.</td>
<td>4.6</td>
<td>8.0</td>
</tr>
<tr>
<td>SCW</td>
<td>33.1</td>
<td>5.3</td>
<td>6.7</td>
<td>4.5</td>
<td>0.84</td>
</tr>
<tr>
<td>R100</td>
<td>7.9</td>
<td>1.2</td>
<td>1.6</td>
<td>1.1</td>
<td>0.22</td>
</tr>
<tr>
<td>R10</td>
<td>28.2</td>
<td>3.5</td>
<td>4.4</td>
<td>3.2</td>
<td>1.53</td>
</tr>
<tr>
<td>RNF</td>
<td>41.9</td>
<td>3.8</td>
<td>5.1</td>
<td>5.1</td>
<td>0.67</td>
</tr>
</tbody>
</table>
3.2. Small-Scale Bottle Experiments

SCW, as well as the ultrafiltration and nanofiltration fractions obtained, were tested for their ability to prompt probiotic cell growth. A preliminary screening in bottle experiments was performed to evaluate growth of three different probiotic strains, namely *L. rhamnosus* IMC501, *L. fermentum*, and *L. brevis* SP-48, on SCW. Cell viability was estimated at different time points. As shown in Figure 2, after 17 h of growth, *L. rhamnosus* and *L. brevis* reached a concentration of about $1.5 \times 10^9$ CFU/mL of viable cells in the broth, whereas no increase was found for *L. fermentum* cell titer. However, after 41 h of growth, an increase of viable cells was observed for *L. fermentum* ($1.1 \pm 0.5 \times 10^9$ CFU/mL) and *L. rhamnosus* ($1.9 \pm 0.3 \times 10^9$ CFU/mL), whereas *L. brevis* viability decreased to $2.3 \pm 0.7 \times 10^8$ CFU/mL.

Tests on SCW fractions were performed in two series of experiments in order to identify the possible use of retentates obtained from membrane processes. The first experiments were carried out with standard sterilization of the medium at 121 °C for 20 min at 1 bar of pressure. Growth of *L. rhamnosus* on RNF, R10, and R100 produced $2.2 \pm 0.3 \times 10^9$, $9.5 \pm 3 \times 10^8$, and $4.9 \pm 2 \times 10^8$ CFU/mL, respectively (Figure 3). Biomass and lactic acid production was also evaluated on pasteurized fractions, as shown in Figure 4. Experiments lasted 16 h to avoid contamination issues.
Figure 2. Growth comparison on SCW of *L. rhamnosus* IMC501, *L. brevis* SP-48 and *L. fermentum*.

Figure 3. Growth of *L. rhamnosus* IMC501 on sterilized retentate fractions. Data were analyzed by two-tailed non homoscedastic Student’s *t*-test. * indicates *p* < 0.05 (Viability results); ** indicates *p* < 0.005 (Viability results).
process. Only R10 DF + glycerol led to the formation of a removable and reusable film with a thickness of 100 µm (Figure 6). The surface of the casted films resulted quite smooth, and the section is presenting a quite compact material that seems to be amorphous, as expected due to the presence of glycerol in the matrix together with the purified/dialysed proteins.

3.3. Evaluation of Casting Conditions of Whey Proteins

Whey protein films were initially prepared using 450 mg of proteins recovered from ultrafiltered samples. All films were casted in petri dishes and incubated for 24 h at 37 °C. Figure 5 shows films obtained in the presence or absence of glycerol after the diafiltration process. Only R10 DF + glycerol led to the formation of a removable and reusable film with a thickness of 100 µm (Figure 6). The surface of the casted films resulted quite smooth, and the section is presenting a quite compact material that seems to be amorphous, as expected due to the presence of glycerol in the matrix together with the purified/dialysed proteins.
3.4. Dehydration Test

The potential protective activity of SCW and of its derived fractions was evaluated in a particular type of tissue damage provoked from dehydration, as previously described in the model [19]. Cell viability (Figure 7) was reported as absorbance at 570 nm normalized over unstressed cell monolayers as a positive control. Two concentrations, namely 4 and 2 g/L, for each fraction were tested.

![Figure 6. SEM image of the R10 fraction.](image)

![Figure 7. Cell viability after dehydration. * p < 0.05 vs. CTR, ** p < 0.01 vs R10 2 g/L, § p < 0.01 vs. R10 2 g/L, § § p < 0.005 vs. R100 2 g/L, + p < 0.05 vs. whey R10 4 g/L.](image)
4. Discussion

As consumer demand for milk-derived products increases, so will the production of whey, which poses a serious wastewater management problem. To overcome this issue, various technological approaches have been employed to transform and exploit whey components [3]. In fact, by implementing innovative downstream and biotechnological processes, using membranes, rather than being viewed as a waste product whey may be considered a resource to obtain high added-value products [4–22]. Several methods, such as UF, NF, and ion exchange chromatography, have been used to concentrate and separate proteins and lactose from other components present in second cheese whey. MF, UF, NF, and RO have been reported by different authors [23–25] for valorisation of SCW, recovery of lactose, and of protein-rich fractions.

This project involved the design of a downstream process, using membranes, to obtain powders containing bioactive molecules from second cheese buffalo whey to be applied in the cosmeceutical, food, and pharmaceutical industries. Currently, only applications of whey from cow’s or goat’s milk are numerous and well-known. Whey from buffalo milk is typical in the south of Italy; buffalo milk is characterized by a protein and lipid content higher than 4.2% and 7.5%, respectively (specification of the Agri-food Quality Department), which is roughly double compared to the other milk sources. The greater amount of proteins allowed us to obtain reusable fractions with high added value. In particular, three fractions, R100, R10, and RNF, were obtained and used for dehydration tests, probiotic cultivations, and production of whey protein films.

For dehydration tests, SCW, R100, and R10 were employed to investigate—using an in vitro model—the potential activity of whey fractions to counteract skin damage or preserve skin integrity and to reduce drawbacks relative to dermal dryness. Results showed that the treatments protected the cells; however, better protection was guaranteed by R100 at both concentrations. In fact, the percentage of cell viability after dehydration in the presence of R100 4 g/L and R100 2 g/L fractions increased compared to the negative control (de-hydrated cells). Tong and collaborators indicated that high molecular weight whey fractions inhibit TBARS and lipid peroxide formation [26]. In the present study, the molecular weight distribution and amount of proteins and lactose in SCW and R100 samples are quite similar yielding, however, different results. The difference might be attributed to the type of proteins retained on the 100 kDa or to sample conductivity (SCW conductivity: 14 mS/cm, R100 conductivity: 4.5 mS/cm).

SCW, R100, R10, and RNF were also used as a main substrate for probiotic cultures. In fact, thanks to the carbon sources content (lactose, glucose, galactose) in addition to proteins (as reported in Table 1), all are potentially complete substrates for the cultivation of different microorganisms [7–21] and for the production of value-added chemicals (e.g., succinic acid, lactic acid), antimicrobial peptides, besides the biomass itself [27–29]. In the first trial reported in Figure 2, three different Lactobacillus strains were grown on an SCW-based medium, showing that after 17 h, a comparable viability for L. rhamnosus IMC501and L. brevis SP-48 was obtained whereas a far lower concentration of viable cells was found for L. fermentum. After 41 h of growth, L. rhamnosus showed the highest titers of viable cells and was therefore selected for subsequent experiments. In the next set of experiments, L. rhamnosus was grown on all fractions collected during the downstream process. Results shown in Figure 3 demonstrate the ability of this strain to grow on all tested media (up to 41 h); the highest viability of about 2.2 ± 0.3 × 10⁹ CFU/mL was obtained on the RNF fraction, in fact titres obtained on R10 and R100 where 2.3 and 4.5-fold lower, respectively. The increase in viability is probably due to the higher concentration of carbon sources present in the RNF medium. Furthermore, by comparing growth of L. rhamnosus IMC501 on SCW and RNF data are very similar, proving that it is possible to divide the sample into different fractions without losing the potential of the initial sample to sustain LAB growth. Many studies have confirmed the ability of L. rhamnosus to grow on dairy industry waste, producing for example lactic acid, which can be used in the food and pharmaceutical fields and within chemical industries as a bio-based molecule for bio-
In order to better simulate the dairy industrial process, the fractions were pasteurized, instead of being autoclaved, and the probiotic strain showed the capability to grow on all UF-NF retentates. Here, significant differences were observed among media; higher lactate production (5.4 ± 0.14 g/L) was observed on RNF with an increase of about 20% and 35%, in respect to results obtained on R10 and R100 fractions, respectively. Finally, only extensively diafiltered fractions containing proteins at 50% of dry weight proved to form films in the presence of glycerol.

5. Conclusions

In this research work, an integrated process was assessed to convert second cheese whey from buffalo mozzarella cheese manufacturing into selected purified fractions, which showed different potentialities. The developed downstream process for these specific types of wastewaters may reduce its environmental impact, especially considering the peculiar seasonal peaks of one of the best-selling products in the Campania region—buffalo mozzarella cheese. The typical and more widespread cheese manufacturing facility gives the possibility to apply similar strategies to other similar milk processing plants. From a second cheese whey, it was possible to recover three fractions that showed valuable characteristics for specific potential applications. In fact, R100 can be a valuable active principle in cosmeceutical preparations counteracting skin dehydration; R10 showed to be suitable for producing by casting protein based films, which may have diverse uses such as packaging for food industries, coatings for foods such as cheese, and even production of cosmetic masks; and RNF is a renewable substrate to obtain lactic acid bacterial biomass, either as probiotics or as starters, metabolic primary products such as lactic acid, an example of building block for bio-plastics production.

Author Contributions: C.S. conceived the study; A.A. drafted the manuscript; A.A. conducted downstream processes; S.D., D.C. and L.F. conducted bottle experiments; M.D. conducted dehydration test; R.F. conducted HPLC analyses and whey protein films preparation. All authors have read and agree to the published version of the manuscript.

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