

Conference Proceedings Paper

Properties of Bio-Materials Obtained from Milk Whey Proteins at Different pH Values and Plasticizer Concentrations

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Received: 15 February 2019; Accepted: 18 February 2019; Published: 28 February 2019

Abstract: Milk whey (MW) represents the major by-product of cheese industry. One possibility to recycle the MW wastes is the use of their globular proteins (MWP) as a polymer source for the production of biodegradable plastic materials. MWP-based films are usually obtained by protein heat treatment in the presence of glycerol (GLY) as plasticizer at pH 7, a method which would require commercially high costing process. In this work it was exploited the possibility to produce manageable MW-derived biomaterials without any heat-treatment but under alkaline conditions. Our results demonstrated that the casting at pH 12 of the unheated MWP film forming solutions (FFSs), containing either 40% or 50% GLY, led to produce more resistant and flexible biomaterials than the ones obtained at pH 7. Also film transparency was observed significantly improved, being lower in the samples obtained at alkaline pH without MWP heating and with higher GLY concentrations. Finally, moisture content decreased with the reduction of GLY content, both in heated and unheated MWP-based films, whereas water uptake of the different films prepared at pH 12 did not significantly change.

Keywords: biodegradable materials; milk whey; protein-based films

1. Introduction

Milk whey (MW) is produced in huge quantities by the dairy industries during the casein coagulation process. MW can be formed from all types (cow, goat, sheep, camel) of milk and, in particular, bovine MW is the most common whey produced in the western countries, sharing about 85%–95% of the generating milk volume and containing about 55% of the whole milk nutrients [1]. The total worldwide MW production is estimated to be more than 180 million tons/year, the major amount (approximately 70%) coming from EU and USA [1]. MW is responsible for relevant environmental problems due to its both large volume and high organic content and, thus, its disposal into municipal sewers is almost everywhere forbidden. On the other hand, land dumping creates severe pollution concerns for the environment by negatively influencing soil physicochemical characteristics. Therefore, since MW components are difficult to degrade, an ecofriendly treatment of MW, when it is not recycled, is required before its disposal, even because the occurrence of numerous nutrients in MW is considered as a potential resource for the production of different value-added products. However, large amounts of MW remain generally unutilized and, thus, MW still deserves attention by researchers to develop further innovative processes able to provide maximal benefits from this by-product and to limit its environmental pollution impact.

Among the various MW treatments (such as heat treatment, chemical modification, pH change, alternating electric field, supercritical carbon dioxide or high hydrostatic pressure treatments, etc.)

heating and pH modification are methods that could have a broader and easier application on industrial scale. Depending on pH, several reactions, such as polymerization due to covalent and/or non-covalent bindings, may occur when a protein solution is heated [2]. As a result of these interactions, MWP are denatured and, then, reactions between the exposed free thiol groups lead to protein aggregation. Since aggregation depends on the repulsive and attractive forces between the particles occurring in solution, the size and the distribution pattern of MWPs depend on both pH and heating temperature, as well as on protein concentration [2,3]. β -Lactoglobulin is the major MW protein of ruminant species and, thus, its molecular characteristics strongly influence MWP aggregation caused by temperature or pH changes. Upon heating, it is capable of self-assembling into a variety of supramolecular structures, existing as an octamer between pH 3.5 and 5.2, as a dimer between pH 5.2 and 7, whereas above pH 8.0 β -lactoglobulin is a monomer with a molecular weight of 18.277 kDa [4]. β -Lactoglobulin is known to resist denaturation at acidic pH while, at alkaline pH, two sequential conformation changes occur in its structure, i.e. the unfolding of α -helix and of exposed β -sheet domains, similarly to its denaturation occurring in the temperature range 50–90 °C, followed by the unfolding of other β -sheets [5]. Therefore, its tunable structuring capacity makes β -lactoglobulin and, consequently MW, a possible interesting source for material science. In fact, one possibility to re-use the MW is to turn its protein content into biodegradable/edible packaging films, that can meet consumer demands for safe, convenient and/or healthy food products with prolonged shelf-life as well as sustainability awareness [6].

Edible films, endowed with a low environmental impact, are been progressively improved to effectively protect various food products through tailored mechanical and/or barrier properties, as well as a controlled release of active ingredients [7]. In addition, these biomaterials might replace the petroleum-based plastics, considered as a major threat of pollution of the environment because they are not easily degradable. More than 35 million of wastes deriving from different plastic items are produced each year in the world and only 7% of them are recycled, the remaining waste being deposited in the landfills or dispersed in the oceans [8]. Therefore, manufacturers are trying to reduce the application of plastic materials for food packaging and to develop innovative biodegradable films and edible coatings [9]. Hence, in this research the film forming capacity of MWPs denatured both at high temperatures and alkaline pH were specifically investigated and several properties of the derived biomaterials obtained at different concentrations of glycerol (GLY), used as plasticizer, were analyzed.

2. Materials and Methods

2.1. Materials

Commercial MWP isolate (~90% dry basis protein) was obtained from BioLine (Auckland, New Zealand), whereas GLY and all other reagents were purchased from Sigma (Steinheim, Germany).

2.2. Film Preparation

Film forming solutions (FFSs) of MWP isolate in distilled water (1% MWPs, *w/v*) were prepared adjusting the pH at the different values by using 0.1 N NaOH. FFSs were heated or not at 80°C for 25 min under continuous stirring. After cooling down at room temperature, GLY (30%, 40%, 50% *w/w* of MWPs) was added just before casting 50 mL FFSs into 8 cm diameter polyester Petri dishes. The derived films were then obtained by drying at 25 °C and 45% RH for 48 h.

2.3. Zeta-Potential and Particle Size Measurements

1.0 mL of each MWP containing FFS was analyzed for zeta-potential and the particle size by using a Zetasizer Nano-ZSP (Malvern®, Worcestershire, UK). The device was equipped with a helium-neon laser of 4 mW output power operating at the fixed wavelength of 633 nm (wavelength of laser red emission). The instrument software programmer calculated the zeta-potential through the electrophoretic mobility by applying a voltage of 200 mV and using the Henry equation [10].

2.4. Film Mechanical Properties

All dried films were cut into 1 cm × 8 cm strips by using a sharp scissor, conditioned at 25°C and 50% RH for 2 h by placing them into a glass chamber over a saturated solution of Mg(NO₃)₂ before being tested. Film thickness was measured in six different points with a micrometer (Electronic digital micrometer, DC-516, sensitivity 0.001 mm) and film tensile strength (TS), elongation at break (EB) and Young's modulus (YM) were determined on five specimens of each sample (5 cm gage length, 1 kN load and 1 mm/5 min speed) by using an Instron universal testing instrument model No. 5543A (Instron Engineering Corp., Norwood, MA, USA).

2.5. Film Transparency

The opacity analysis was performed as described by Galus and Kadzinska [11].

Opacity was calculated as follows:

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

where $A_{600\text{nm}}$ was the absorbance at 600 nm and X was the film thickness (mm).

2.6. Film Moisture Content

Moisture content test was performed by evaluating the mass loss of the film sample after 24 h at 105 °C as previously described [11]. Analyses in triplicate of each film were made and the results were expressed as mean value ± standard deviation.

Film moisture content was calculated as:

$$\text{Film moisture content (\%)} = (W_1 - W_2)/W_1 \times 100 \quad (2)$$

where W_1 is the initial weight of the film and W_2 is the film weight after drying.

2.7. Film Moisture Uptake

Film moisture uptake tests were carried out by a gravimetric method described by Manrich et al. [12]. The analysis was performed by determining the mass of film samples after drying at 105°C for 24 h and the mass after the samples were put in a conditioning environment at RH 50% (saturated solution of Mg(NO₃)₂) for other 24 h. The moisture uptake was finally calculated as:

$$\text{Film moisture uptake (\%)} = (W_s - W_d)/W_s \times 100 \quad (3)$$

where W_s and W_d are the weight of swollen and dried films, respectively.

3. Results and Discussion

3.1. Zeta-Potential and Particle Size

Zeta-potential measurements indicated that both heated and unheated MWP FFSs prepared at pH 7 and 12 were quite stable. In particular, as reported in Table 1, the value of zeta-potential detected for the FFSs prepared at pH 12 was around -35 mV with an average particle size between 400 and 600 nm regardless GLY concentration and heat-treatment. Conversely, all the zeta-potential values measured for FFSs prepared at pH 7 resulted significantly lower than those observed for the FFSs prepared at pH 12. Moreover, a higher Z-average value was detected, with a concomitant significant decrease in zeta-potential, when FFSs were not heated and added with lower amounts of GLY (30%).

Table 1. Zeta-potential and Z-average measurements of MWP FFSs prepared at pH 7 or 12 and subjected or not to heat treatment.

MWP FFSs	Z-Average (d. nm)		Zeta-Potential (mV)	
	pH 7	pH 12	pH 7	pH 12
+30% GLY, heated	147.1 ± 18.4	418.9 ± 31.9	-27.0 ± 1.0	-35.3 ± 2.1
+30% GLY	1127.0 ± 167.4	610.2 ± 56.5	-21.6 ± 0.4	-38.8 ± 2.6
+40% GLY, heated	110.5 ± 21.1	415.4 ± 6.7	-29.1 ± 0.6	-35.4 ± 2.8
+40% GLY	522.6 ± 102.5	519.2 ± 30.8	-22.9 ± 0.3	-35.9 ± 2.4
+50% GLY, heated	350.1 ± 13.8	403.9 ± 19.7	-27.0 ± 0.1	-36.2 ± 3.3
+50% GLY	516.3 ± 23.1	526.1 ± 23.1	-24.0 ± 0.4	-35.6 ± 2.9

3.2. Film Mechanical Properties

After the analyses of the stability of all the MWP FFSs prepared under different experimental conditions, the mechanical properties only of the handleable films obtained were investigated. More in detail, TS, EB and YM of the films derived from the FFSs, heated and unheated, prepared at pH 12 in the presence of different amounts of GLY were determined. Conversely, it was possible to evaluate the mechanical properties only of the films derived from the FFSs heated and prepared at pH 7 in the presence of 40% or 50% GLY. In fact, brittle films were obtained under the same experimental conditions in the presence of 30% GLY, whereas unmanageable sticky materials were obtained with unheated FFS at the same pH and with all GLY concentrations. The results reported in Table 2 indicate that the unheated FFSs, prepared at pH 12 and containing 50% GLY, led to obtain more flexible biomaterials, as demonstrated by the highest elongation at break and the lowest Young's module detected. In addition, these values were also much higher and lower, respectively, than those observed with counterpart films obtained at pH 7 from heated FFSs in the presence of 50% GLY (Table 3).

Table 2. Mechanical properties of MWP films obtained at pH 12.

MWP films	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)	Thickness (µm)
+30% GLY, heated	3.40 ± 0.90	8.3 ± 4.8	71.6 ± 14.2	48 ± 7
+30% GLY	3.72 ± 0.61	4.6 ± 0.8	185.1 ± 16.2	38 ± 4
+40% GLY, heated	1.41 ± 0.09	21.3 ± 5.5	34.0 ± 7.4	44 ± 4
+40% GLY	2.72 ± 0.12	33.9 ± 8.5	86.2 ± 3.2	66 ± 2
+50% GLY, heated	0.60 ± 0.11	36.9 ± 10.8	22.7 ± 5.1	85 ± 5
+50% GLY	1.11 ± 0.12	61.6 ± 8.6	24.1 ± 4.7	83 ± 2

Table 3. Mechanical properties of MWP films obtained at pH 7.

MWP Films	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)	Thickness (µm)
+ 40% GLY, heated	2.81 ± 0.72	3.6 ± 0.7	350.5 ± 63.4	96 ± 6
+ 50% GLY, heated	3.20 ± 0.21	20.8 ± 4.4	164.7 ± 77.5	129 ± 35

3.3. Film Transparency

Since the appearance of the coated products plays an important role in consumer acceptability, the opacity of the obtained MWP films was evaluated by measuring light transmission through the films at a wavelength of 600 nm [13]. Table 4 clearly indicates that only slight differences were detected between the films prepared at pH 12 and pH 7. Nevertheless, opacity was observed to significantly change, being higher in the films obtained at pH 12 without FFS heating and, when FFS was heated, at higher GLY concentrations. The opacity values were also compared with ones exhibited by traditional commercial plastics such as cellulose triacetate and polypropylene, resulting more and much less transparent, respectively, than the MWP films.

Table 4. Opacity of MWP-based biomaterials obtained under different experimental conditions.

MWP Films	Opacity (A_{600}/mm)
+30% GLY, heated, pH 12	18 ± 0.64
+ 30% GLY, pH 12	2.65 ± 0.11
+40% GLY, heated, pH 12	1.23 ± 0.05
+40% GLY, pH 12	2.07 ± 0.25
+50% GLY, heated, pH 12	1.57 ± 0.13
+50% GLY, pH 12	2.20 ± 0.81
+40% GLY, heated, pH 7	1.66 ± 0.01
+50% GLY, heated, pH 7	1.27 ± 0.01
Polypropylene *	32.02 ± 3.35
Cellulose triacetate *	0.54 ± 0.09

*: Values from Giosafatto et al. [13].

3.4. Film Moisture Content and Moisture Uptake

The prepared MWP films were also analyzed for moisture content and moisture uptake, as these features are important for food packaging applications, particularly when the water activity is high or when the film should act as a food protective barrier [14]. In fact, a high moisture content of the coating material considerably limits its use for packaging foods. The results reported in Table 5 showed that the moisture content of the films prepared at pH 12 decreased when the FFS was heated, as well as when the amount of GLY was lower in both heated and unheated samples. Conversely, film water uptake did not seem to significantly change in all films cast at pH 12. Finally, the moisture uptake values were found significantly lower when the films were prepared at pH 7.

Table 5. Film moisture content and uptake of MWP-based biomaterials obtained under different experimental conditions.

MWP film	Moisture Content (%)	Moisture Uptake (%)
+30% GLY, heated, pH 12	15.24 ± 1.32	13.31 ± 1.00
+30% GLY, pH 12	20.35 ± 1.20	15.05 ± 0.73
+40% GLY, heated, pH 12	18.39 ± 1.94	15.70 ± 0.04
+40% GLY, pH 12	25.65 ± 0.69	15.98 ± 2.10
+50% GLY, heated, pH 12	18.93 ± 3.30	14.90 ± 0.66
+50% GLY, pH 12	29.50 ± 2.30	16.56 ± 0.77
+40% GLY, heated, pH 7	21.43 ± 0.32	9.12 ± 0.85
+50% GLY, heated, pH 7	33.27 ± 0.50	9.01 ± 0.72

4. Conclusions

Since it is known that denaturation and aggregation of MWPs are pH dependent, with strong alkalis producing rod-like microstructures able to form fine-stranded fiber-like matrices, it was investigated and demonstrated the possibility to obtain GLY-plasticized biomaterials by using MWP isolate treated at pH 12 without heating. Conversely, at pH 7 it was necessary not only to previously heat at 80 °C for 25 min the MWP-containing FFS, but also to increase to at least 40% the GLY concentration to obtain handleable biomaterials. The developed experimental conditions allowed to produce hydrocolloid films with higher flexibility and transparency with respect to the MWP-based films so far obtained at pH 7 and following FFS heat treatment, probably because MWPs denatured under alkaline conditions form small primary aggregates able to combine into large clusters [15].

Author Contributions: Conceptualization, R.P. and C.V.L.G.; Methodology, M.A.; Writing–Original Draft Preparation, M.A and C.V.L.G.; Writing–Review & Editing, R.P.; Supervision, C.V.L.G. and R.P.

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

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