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REPLACEMENT OF CONVENTIONAL CHEESE COATINGS BY NATURAL WHEY PROTEIN EDIBLE COATINGS WITH ANTIMICROBIAL ACTIVITY

Marta Henriques[1, 2*], Gustavo Santos[1], Ana Rodrigues[1], David Gomes[1], Carlos Pereira[1], Maria Gil[2]

[1]Department of Food Science and Technology, Escola Superior Agrária de Coimbra - Polytechnic Institute of Coimbra, Bencanta, 3040-316 Coimbra, Portugal.
[2]Chemical Engineering Department, CIEPQPF, Faculty of Science and Technology - University of Coimbra, Rua Sílvio Lima – Pólo II, 3030-790 Coimbra, Portugal.

[*]e-mail: mhenriques@esac.pt

Abstract

The present work assesses the efficacy of whey protein edible coatings with antimicrobial properties applied to ripened cheese as alternatives to commercial cheese coatings. Whey protein edible coatings were produced from ovine whey protein concentrate (WPC) with lactic acid and natamycin as antimicrobials. Two methods of coating polymerization were performed separately and in combination; the heat denaturation method (HD) and the innovative UV polymerization method (UV). Their effectiveness was evaluated by measuring the physico-chemical, microbiological and sensorial properties of coated cheeses throughout 45 days.

Coatings produced only by HD did not significantly improve the coating efficiency; however, the UV polymerization in combination with thermal treatment (HD+UV) originated coatings that showed good performance. With regard to physico-chemical evaluation, no significant differences were found between cheeses bearing commercial coatings or edible coatings (UV and HD+UV) in terms of weight loss, fat, protein and salt contents, as well as aw, pH and hardness, revealing that the antimicrobial edible coatings could be used as an alternative to their commercial counterpart(s). Microbiological analysis proved that edible coatings prevented growth of *Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, yeasts and molds what demonstrates their ability to assure the safety of cheese. In fact coatings produced by HD+UV showed better inhibition or reduction in microbial growth as a result of the synergistic effect of the antimicrobials and UV light. The commercial coating had the best performance against yeasts and molds due to its higher amount of natamycin. With regard to sensorial analysis, cheeses with antimicrobial edible coatings did not show significant differences with the commercial coated ones.

***Key words**: Whey protein edible coatings, Antimicrobial coating, UV-polymerization*.

1. Introduction

Food safety depends on hygienic characteristics of foodstuffs during production, storage and commercialization. Several physical and chemical methods to preserve food quality, such as high pressure, sterilization, irradiation, ultrasounds and acidification were developed aiming that goal. However, none of these methods was self sufficient without the use of an appropriate package as the final step of the preservation process. The use of plastic films is appraised due to their efficiency in protecting and reducing the mass transfer between food and its surroundings. Nevertheless, their increasing use and non biodegradability characteristics led to disposal problems. Edible films and coatings are natural and biodegradable products that contribute to the environment protection while reducing residues from agro-industry.

Characteristics facilitating handling and carriage of foods can be improved by the use of edible films and coatings, enhancing their sensorial attributes like color, transparency, roughness or stickiness. Though many functions of edible packaging are identical to the synthetic ones, such as gases, vapor and solute permeability, they appear to be a complementary parameter for the quality of fresh or treated products according to their non toxic character and carrier capacity [1, 2, 3, 4 and 5]. A novel function attributed to edible coatings is their action as carriers of active ingredients and additives (e.g. flavors, pigments, antioxidants and antimicrobial agents) protecting

and improving food quality. Whey protein films and coatings have shown to be poor moisture barriers because of their hydrophilic nature, but present very interesting oxygen barrier properties, comparable to synthetic polymers [6, 7, and 8], and according Miller and Krochta [9] even better mechanical characteristics than polysaccharide-based edible films. The improvement of whey protein edible films and coatings with antioxidants and antimicrobial ingredients enhancing food safety and shelf life is their most promising application [10 and 11].

Antimicrobial performance of whey protein coatings was tested in some applications: fresh or processed meat (Min *et al.* [2], Cagri *et al.* [12], and Zinoviadou *et al.* [4]), fish (Stuchell and Krochta [13], Min *et al.* [14], Neetoo *et al.* [15]) and cheese (Franssen [16] and Ramos [17]). The presence of antimicrobial agents in the coating applied to the surface of these food products may reduce or even prevent growth of pathogenic and spoilage microorganisms allowing the use of antimicrobials at lower initial levels and assuring a constant background of these compounds during storage [18]. Antimicrobial selection is primarily dependent of the food application and their target pathogenic microorganisms that are intended to eliminate or control growth. Hence, wide spectrum antimicrobials or very specific ones can be selected. The addition of lactic acid into edible films and coatings has proven to have high efficiency [17, 19 and 20]. It is frequently added to food for preservation purposes reducing or eliminating pathogenic gram-positive bacteria. However, according Ray [21], it is not considered a wide spectrum antimicrobial due to its inefficiency against yeasts and molds. Natamicyn is a successful antimycotic polyene that prevents yeasts and molds growth at cheese surfaces or slices thereof [22 and 23] and sausages [12]. It is considered as GRAS (Generally Recognized as Safe) by the U.S. Food and Drug Administration and assigned to be the number E-235-natural preservative in European Union.

Formation of protein coatings on food products may involve dipping, spraying, enrobing or panning the food with the coating formulation requiring in all cases drying of the solvent from the protein coating formulation after its application to food [7]. Thermal protein denaturation is the most commonly used polymerization method for whey protein coating production [24, 25, and 26], while protein cross-linking can also be induced chemically [27 and 28], enzymatically [29] or by means of irradiation.

The used of UV/γ-irradiation, presents some advantages: it is a clean and well-known process for the sterilization of goods [30] and less expensive than the use of enzymes. γ-irradiation was used by Mezgheni *et al.* [31], Vachon *et al.* [32] and Lacroix *et al.* [33] to produce edible films from caseinates and whey proteins. The proposed mechanism was radical polymerization through tyrosine with the formation of bityrosine linkages between protein chains. The lack of tyrosine residues in whey proteins can be overtaken by the use of chemicals like photoinitiators that under UV-irradiation create radicals that start the polymerization reaction [33 and 34].

Our assumption for this research was that the combination of both active compounds (lactic acid and natamycin) and UV-irradiation would generate whey protein edible coatings with an improved cohesion and antimicrobial properties, making them suitable for coating traditional cheeses and be an alternative to the existing commercially available cheese coatings (PVA-based). The effect of the protein polymerization method in the production of WPC-based edible coatings and its antimicrobial activity could be assessed via physico-chemical, microbial and sensorial evaluation of coated cheeses, throughout 45 days of ripening, and by comparison with cheeses with a commercial coating or uncoated at all.

2. Materials and Methods

2.1 Materials

Ovine freeze dried WPC obtained by ultrafiltration as described by Henriques *et al.* [35] was used as protein source for whey protein based coating formulations (61.53 g protein 100 g^{-1} and 28.28 g lactose 100 g^{-1}). Glycerol (99% purity) supplied by JM Vaz Pereira Lda. (Portugal) was the plasticizer used, guar gum was added as natural thickener and emulsifier (Formulab Aditivos Alimentares, LDA, Portugal), sunflower oil (Olimambo) was provided by Cidacel S.A. (Portugal) and the surfactant tween 20 was supplied by Fluka Chemika (Spain). The photoinitiator 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) was used for coatings produced by UV polymerization and was supplied by Ciba Specialty Chemicals. The active antimicrobial compounds were: lactic acid (90% purity, JM Vaz Pereira Lda. Portugal) and natamycin (50% purity, Enzilab Lda., Portugal).

The commercial coating (Readom D, Enzilab Lda., Portugal) was composed of polyvinyl acetate (as base material) and ca. 0.25 g 100 mL^{-1} natamycin as active component (indicated by the supplier).

Microbial analysis of *Staphylococcus* spp. was performed in Baird-Parker Agar Base, BPA (HIMEDIA) supplemented with egg yolk and telurite emulsion (Fluka Chemika); *Pseudomonas* spp. in Pseudomonas Agar F, PAF (DIFCO Laboratories); *Enterobacteriaceae* in Violet Red Bile Glucose Agar, VRBGA (HIMEDIA) with Triptona Soya Agar, TSA (HIMEDIA) and yeast and molds in Rose-Bengal Chloramphenicol Agar, RBC

(Merck). Peptone water (Merck, Germany) was used for the sample decimal dilutions. All other chemicals were reagent-grade or better, and were used without further purification.

2.2 Cheese

Cylindrical and semi-hard bovine cheeses of approximately 120 g were produced in Escola Superior Agrária (ESAC) facilities (Coimbra, Portugal) and used as traditional Portuguese raw milk cheese food model material for whey protein-based coating application. Standardized (3.5 ± 0.1 g fat 100 g^{-1}) bovine milk was heated and maintained at (30 ± 0.5 °C) during coagulation (45 min) in the presence of 0.04 g 100 mL^{-1} $CaCl_2$ solution (36 g 100 g^{-1}, Betegeux), 10 mg L^{-1} of Mesófilo Plus Starter (Abiasa, Spain), 25 mg L^{-1} of Lysozyme and 20 mg L^{-1} of rennet (> 92 g 100 g^{-1} chimosin, Tecnilac-Portugal) previously diluted in tap water. The curd was molded in plastic molds and stored at 5 ± 1°C and 80 ± 3% relative humidity during one day, before coating application.

2.3 Coating production and application

The coating formulation developed by Ramos [17] was the basis for the coating formulation used in this research with some specific adaptations. The protein base material, WPI in the former, was replaced by ovine WPC at the same protein content (10 g 100 g^{-1}) and the amount of natamycin as antimicrobial was reduced from 0.025 g 100 mL^{-1} to 0.0125 g 100 mL^{-1}, in order to better evaluate the antimicrobial effect of UV-irradiation. All the remaining coating ingredients were maintained at the optimized concentration achieved by Ramos [17]: glycerol was added at 50 g 100 g^{-1} (protein basis), guar gum (0.7 g 100 g^{-1}), sunflower oil and tween 20 (10 g 100 g^{-1} and 0.2 g 100 g^{-1}, respectively), and the antimicrobial agent lactic acid at 0.6 g 100 mL^{-1}.

Three different types of whey protein-based antimicrobial coatings were made for cheese application according to each polymerization method: heat denaturation (HD), UV polymerization (UV) and both methods combined (HD+UV). Cheeses coated with the whey protein coatings were compared to the negative control (uncoated cheeses) and positive control (cheeses with commercial coating).

The production of WPC coatings by the HD technique implies that the thermal treatment and consequently protein denaturation occurs in the coating solution before the coating application to the cheese surface. Glycerol and WPC were homogenized in deionized water until complete dissolution. Subsequently the solution was heated in a water bath at 80 ± 2 ºC for 20 min., under continuous agitation, and cooled down to approximately 30 ºC. Guar gum, sunflower oil and tween 20 were added under stirring for ca. 20 min. at room temperature. Afterwards the antimicrobial agents; lactic acid and natamycin were added and pH was adjusted to 7.0 using NaOH (40 g L^{-1}). The coating solution was homogenized using a T25 Ultra-Turrax® (IKA from Staufen, Germany) at 10000 - 13000 rpm for ca. 2 min before application into cheeses.

In the UV polymerization method, protein polymerization by UV irradiation only occurs after the coating solution application to the cheese surface. The coating solution was prepared as described in the aforementioned method, without any heat treatment. During coating solution preparation the photoinitiator at 3.5 g 100 g^{-1} (protein basis) was also added under continuous stirring. Antimicrobial compounds were incorporated into the edible coating matrix followed by pH adjustment. The coating solution homogenization was performed in the same conditions mentioned previously and applied to the cheese surface that was exposed for 10 min. on each side at direct UV-irradiation using UV lamps (G8 T5 - 8W, PHILIPS®) at ca. 23 cm from the cheeses.

The combination of both polymerization techniques implies the protein denaturation in the solution by heat treatment, before coating application, and the UV protein polymerization, after the coating application to the cheese surface. The coating composition and preparation steps were similar to the aforementioned methods.

The latter and the commercial coating (Readom D) were directly applied on the cheese surface one day after cheese manufacture. Coatings were manually applied by brushing until all cheese surfaces were covered – with the residual coating being allowed to drip off. Cheeses were then stored in an appropriate chamber for 45 days, at 11 ºC and 85% RH by turning them from time to time. The coated cheeses were compared with their uncoated counterparts.

2.4 Coating solutions rheological analysis

Before application to cheese, the rheological behavior of the coating solutions was evaluated based on their apparent viscosity determined on a controlled stress rheometer (Rheostress 1 - RS1, Haake, Thermo Fisher Scientific, Germany) using a parallel plate sensor (TP20 Ti, 20 mm diameter, 115 μm gap). Flow tests were carried out at 20 ± 0.1 ºC with upward and downward linear shear rate ramps between 0.1 and 10 s^{-1}. For each thixotropic cycle, the shear rate was increased over a period of 100 s, held at the upper limit for 20 s and then decreased again over a period of 100 s. Thixotropy was recorded as the resultant area between the upward and

downward shear stress (σ) curves as function of shear rate (γ). For each coating solution three measurements were performed. The upward shear rate ramp was used to determine the consistency index (*k*) and the power law factor (*n*) according to the Power Law model equation:

$$\eta = k(\gamma)^{n-1} \quad (1)$$

where η is the apparent viscosity (Pa.s) and γ is the shear rate (1/s).

2.5 Physico-chemical analyses

Physico-chemical analyses of cheeses were assessed in triplicate, by 1, 15, 30 and 45 days after coating application.

Weight loss was determined by individually cheese weighing with a Mettler Toledo PB102 (Switzerland) analytical balance at the beginning and during the storage period. The percentage of the relative weight loss (*ΔW*) was calculated based on equation 2.

$$\%\,\Delta W = \frac{I_{wo} - F_{wi}}{I_{wo}} \times 100 \quad (2)$$

where I_{wo} is the initial cheese weight and F_{wi} is the final cheese weight at time *i*.

Cheese moisture content was gravimetrically determined according to the Portuguese standard method (NP 3544 [36]). Protein evaluation was performed by the Kjeldahl method (ISO 8968-1 [37]) at the first day of storage and after 45 days. The fat content was determined using the Van Gulik method (NP 2105 [38]). Total chloride in cheese was determined using the official AOAC method 935.43 [39]. The pH of cheeses was measured directly using a pH meter (PHM61 Laboratory pH Meter, Denmark) equipped with a probe for reading solids and the titratable acidity expressed as g lactic acid 100 g^{-1} according to the method AOAC [40].

Water activity (a_w) of cheese samples (representative from the bulk and cheese surface) was measured after temperature stabilization (20ºC) using an hygrometer (Rotronic Hygroskop BT, Zurich, Switzerland) coupled with a DMS 100H device and equipped with a WA-14TH probe connected to a thermostatic bath.

2.6 Texture

Cheese hardness was determined in a Stable Micro Systems Texture Analyzer, model TA.XT Express Enhanced used to perform textural analysis, after data treatment by the Specific Expression PC Software. Cheese texture profile analysis (TPA) was performed with a penetration distance of 15 mm at 2 mm/s test speed, using an acrylic cylindrical probe with a diameter of 5 mm and 38.1 mm of height. Three penetrations were performed per cheese at distinct locations.

2.7 Color

Cheese color was determined by a portable colorimeter HP-2132, Zheijang Top Instruments Co, Ltd., previously calibrated with a standard white plate of known parameters ($L^*_{standard}$ = 97.03; $a^*_{standard}$ = -0.67; $b^*_{standard}$ = 5.57), using C illuminant in the color space CIEL*a*b*. The color of cheeses was expressed by the individual three coordinates of CIEL*a*b* and by the total color difference (*ΔE*). For each type of coating and ripening time, three cheese samples were measured and three readings per cheese were made.

2.8 Microbiological analysis

Microbiological development on the cheese surface was evaluated via enumeration of viable cells, by 1, 15, 30 and 45 days after application of said coatings. 10 g of cheese were aseptically removed from the upper surface area of each cheese into a stomacher bag, and accordingly diluted to 1:10 (w/v) in sterile 1 g 100 mL^{-1} sodium citrate (Merck) and blended in a stomacher (Masticator IUL Instruments) for 1.5 min at 260 rpm. Subsequently, decimal dilutions were prepared with 0.1 g 100 mL^{-1} peptone water and plated, in triplicate, on the corresponding media.

Staphylococcus spp. were enumerated on Baird-Parker Agar Base, BPA supplemented with egg yolk and telurite emulsion, as originally proposed by Baird-Parker [41]. *Pseudomonas* spp. were counted on Pseudomonas Agar F, PAF. Both media were incubated aerobically at 37 ºC for 48 h. *Enterobacteriaceae* were counted on Violet Red Bile Glucose Agar, VRBGA with Tripton Soya Agar, TSA after incubation at 30 ºC for 48 h. Yeasts and molds were determined after 5 days of incubation at 25 ºC on Rose-Bengal Chloramphenicol Agar, RBC. Except for the enumeration of *Enterobacteriaceae* on VRBGA (for which the pour plate technique was used) the surface plating technique described by Miles *et al.* [42] was followed for all other samples and growth media.

2.9 Sensorial analysis

Sensorial tests were carried out at the end of the ripening period (45 days), in the sensory room of ESAC by a trained panel of 12 members, from both genders - and familiar with traditional Portuguese cheeses. Two tests were performed by each panelist; the first for the evaluation of the global cheese appearance, where

the whole cheese was first analyzed; and the second for the evaluation of cheese characteristics, using cheese slices of ca. 1 cm thickness that were placed on individual plastic Petri dishes coded using a random tree-digit-code.

A 5-point scale was used by panelists to evaluate all the attributes used to classify whole cheeses samples and sliced cheese samples. For whole cheese evaluation, the sensorial attributes assessed were: shape (1 = not characteristic and 5 = ideal); rind color (1 = white and 5 = dark yellow); color homogeneity (1 = heterogenic and 5 = homogeneous); hardness (1 = very soft and 5 = very hard). For cheese slices evaluation the sensorial attributes were: differences between paste and ring color (1= imperceptible and 5= intense); odor (1 = imperceptible and 5 = intense); consistency (1 = very soft and 5 = very hard); flavor (1 = imperceptible and 5 = intense) and overall acceptability (1 = less accepted and 5 = most accepted). For better definition of coating attributes, panelists were asked to include useful information in the "observations" section included on each evaluation card.

2.10 Statistical analysis

Statistical analysis of the data was carried out employing analysis of variance (ANOVA) package included in StatSoft Statistica 8.0 (Hill and Lewicki [43]). Tukey-HSD post-hoc test, with a 95% confidence level, was applied to assessed differences between physico-chemical, microbiological and sensory properties of cheeses coated with WPC-based coatings, commercial coating and uncoated cheeses.

One-Way ANOVA tests were performed to compare the means of the coating solutions rheological properties (thixotropy and apparent viscosity) and the cheese samples attributes used for the sensorial evaluation of the whole cheese and sliced cheese.

Two-way ANOVA with interaction was employed to determine the effects of both storage time and coating type on physico-chemical properties (moisture, fat and salt contents; weight loss, water activity, titratable acidity, pH; hardness and color) and microbiological properties (*Staphylococcus* spp.; *Pseudomonas* spp.; *Enterobacteriaceae* and yeast and molds) of ripened cheeses.

3. Results and Discussion

3.1 Coatings rheological properties

The apparent viscosity of the WPC-based coatings produced by the different polymerization methods (HD, UV and HD+UV) was analyzed and compared with the commercial coating matrix viscosity, before its application into the cheese surfaces in order to better understand in what extent this property would influenced the coating phenomenon. These results are shown in Figure 1. For the entire range of shear rates it was observed similar flow behavior for all the produced coating solutions; being however clearly visible the distinction between individual batch viscosities over the tested shear rate range. The shape of coating solutions flow curves exhibited shear-thinning behavior, characteristic of pseudoplastic fluids, and showed decreasing apparent viscosity with increasing shear rate which is typical of weakly aggregating dispersion systems [46]. Coating matrixes subjected to heat denaturation (HD+UV and HD) had the highest apparent viscosity values, while the commercial and UV coating solutions displayed the lower viscosity values. These differences can be related to the amount and nature of polymeric base material (e.g. whey proteins or PVA), coating additives and the method used to perform coating polymerization. It is important to notice that in UV coating solution, the polymerization process is induced only after its application to the cheese surface and UV irradiation exposure. The smaller viscosity obtained for this case corroborates that, until this stage only colloidal particles are present in the coating solution, without any indication that polymerization has started. On the other hand, the application of a thermal process to the whey protein coating solutions promotes protein denaturation and the beginning of polymerization before coating application leading to higher viscosity values.

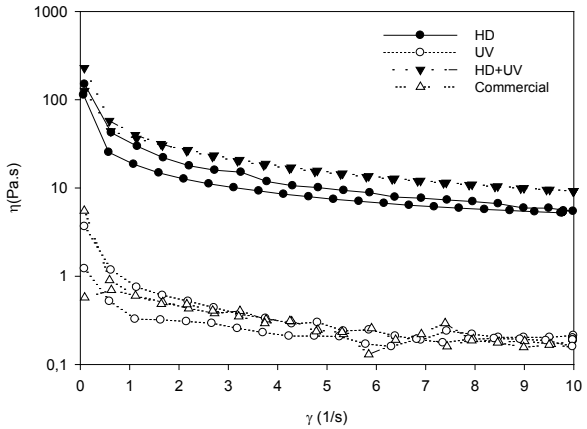


Figure 1. Apparent viscosity (η) as a function of shear rate of the antimicrobial WPC coating solutions produced by (●) HD; (○) UV and (▼) HD+UV polymerization compared with (△) commercial coating solution, before its application to the cheese surface

Thixotropic or "hysteresis" loops were generated for the tested coating samples as shown in Figure 2 and the thixotropy (Pa/s) plotted in Table 1. It was observed that HD coating solution showed the higher thixotropic

behavior. The presence of the photoinitiator in HD+UV formulation may probably contribute to a more effective and accurate molecular rearrangement of denatured proteins after heat treatment, explaining the distinct rheological behavior between both samples.

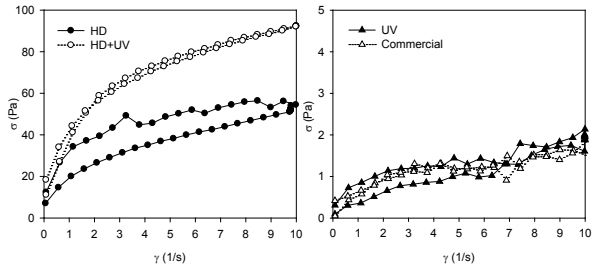


Figure 2. Thixotropic loops of the antimicrobial WPC coating solutions produced by (●) HD, (▲) UV and (○) HD+UV polymerization compared with the (Δ) commercial coating solution, before its application to the cheese surface

The power law model (equation 1) was applied in order to describe the flow behavior of coating solutions at the cheese surfaces by determining the consistency index (*k*) and the power law factor (*n*). The consistency index (Table 1) was in agreement with the aforementioned observations. Significant higher values ($P < 0.05$) were obtained for HD+UV and HD coating solutions (36.68 and 29.30 $Pa.s^n$, respectively) against 0.81 $Pa.s^n$ for the UV and 0.65 $Pa.s^n$ for commercial coating solution. According to the power law factor no significant differences were observed between samples and the pseudoplastic flow behavior was confirmed ($n < 1$) for all the coating solutions.

According to these findings it was possible to predict that during coating application more viscous solutions would exhibit higher adherence to cheese surfaces than the less viscous ones, which could result in very distinct coating thickness among cheeses if a dipping process is used. In order to produce coatings with similar thickness it was decided to apply the coating solutions manually with a brush until all the cheese surfaces were covered.

3.2 Physico-chemical profile

Physico-chemical properties of cheese were assayed by 1, 15, 30 and 45 days of storage and are displayed in Figure 3 (weight loss, water activity (a_w), moisture, fat, protein and salt (NaCl) contents), Table 2 (titratable acidity and pH) and Figure 4 (hardness).

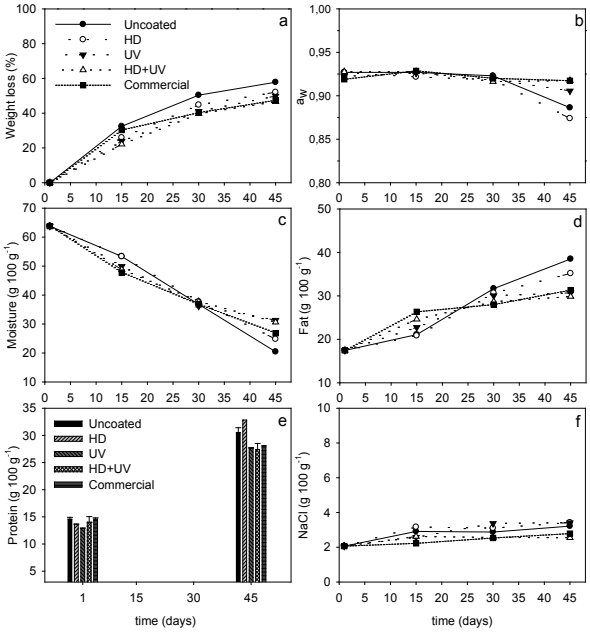


Figure 3. Weight loss (n = 3), water activity (a_w) (n = 3), moisture (n = 9), fat (n = 3), protein (n = 9) and salt (NaCl) (n = 6) contents of cheese samples coated with antimicrobial whey protein edible coatings produced by (○) HD, (▼) UV and (Δ) HD+UV polymerization compared with (●) uncoated cheese and (■) cheese with commercial coating, during 45 days of ripening at 11 °C and 85% RH

Table 1. Thixotropy, consistency index (k) and power law factor (n) of the power law model of the antimicrobial WPC coating solutions produced by HD, UV and HD+UV polymerization compared with the commercial coating solution

Coating type	Thixotropy (Pa/s)		Power law model			
			k ($Pa.s^n$)		n	
HD	43.30 ± 0.35	c	29.30 ± 13.39	b	0.35 ± 0.13	a
UV	3.35 ± 0.70	a	0.81 ± 0.08	a	0.37 ± 0.02	a
HD+UV	24.08 ± 12.7	b	36.68 ± 0.32	b	0.44 ± 0.01	a
Commercial	4.40 ± 0.84	a	0.65 ± 0.13	a	0.41 ± 0.08	a

[a,b,c] means (n = 3) ± standard deviation with different letters within a column are significantly different ($P < 0.05$)

Table 2. Titrable acidity (g lactic acid) and pH of cheese samples coated with antimicrobial whey protein edible coatings produced by HD, UV and HD+UV polymerization compared with uncoated cheese and cheese with commercial coating, during 45 days of ripening at 11 °C and 85% RH

	Coating type	Ripening time (days)			
		1	15	30	45
Titratable acidity $^{\alpha}$ (g lactic acid)	Uncoated	0.42 ± 0.09 ^{aA}	0.97 ± 0.03 ^{aB}	0.95 ± 0.02 ^{aB}	1.08 ± 0.15 ^{aB}
	HD	0.42 ± 0.09 ^{aA}	0.96 ± 0.07 ^{aB}	0.98 ± 0.16 ^{aB}	1.13 ± 0.40 ^{aB}
	UV	0.42 ± 0.09 ^{aA}	1.04 ± 0.05 ^{aB}	1.05 ± 0.02 ^{abB}	1.16 ± 0.16 ^{aB}
	HD+UV	0.42 ± 0.09 ^{aA}	1.29 ± 0.06 ^{bB}	1.54 ± 0.05 ^{cC}	1.40 ± 0.09 ^{bBC}
	Commercial	0.42 ± 0.09 ^{aA}	1.06 ± 0.07 ^{aB}	1.17 ± 0.03 ^{bB}	1.10 ± 0.14 ^{aB}
pH $^{\beta}$	Uncoated	5.19 ± 0.23 ^{aB}	4.62 ± 0.04 ^{aA}	4.69 ± 0.10 ^{abA}	4.76 ± 0.19 ^{aA}
	HD	5.19 ± 0.23 ^{aB}	4.56 ± 0.04 ^{aA}	4.37 ± 0.06 ^{aA}	4.69 ± 0.07 ^{aA}
	UV	5.19 ± 0.23 ^{aB}	4.49 ± 0.02 ^{aA}	5.03 ± 0.05 ^{cB}	4.81 ± 0.06 ^{aAB}
	HD+UV	5.19 ± 0.23 ^{aC}	4.49 ± 0.01 ^{aA}	4.93 ± 0.03 ^{bcB}	4.70 ± 0.05 ^{aAB}
	Commercial	5.19 ± 0.23 ^{aB}	4.73 ± 0.03 ^{aA}	4.62 ± 0.03 ^{abA}	4.57 ± 0.02 ^{aA}

$^{\alpha}$ means (n = 6) ± standard deviation. $^{\beta}$means (n = 9) ± standard deviation. $^{A,B,\cdots}$means ± standard deviation with different capital letters are significantly different (P < 0.05) during ripening time for the same coating type (line). $^{a,b,\cdots}$means ± standard deviation with different small letters are significantly different (P < 0.05) between coating types at the same ripening day (column).

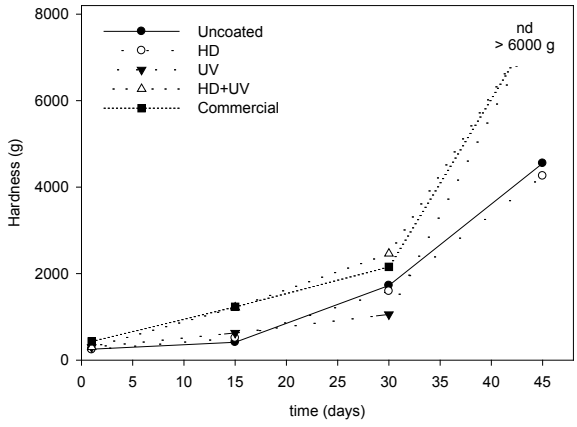


Figure 4. Hardness of cheese samples coated with antimicrobial whey protein edible coatings produced by (○) HD, (▼) UV and (△) HD+UV polymerization compared with (●) uncoated cheese and (■) cheese with commercial coating, during 45 days of ripening at 11 °C and 85% RH

Our analyses provided information on how cheese weight loss throughout 45 days was affected by the presence of a coating, its type and the influence of the polymerization method used. In Figure 3a, one finds an increase in weight loss for all cases throughout storage; such increase is statistically higher (P < 0.05) during the first 30 days, exception made for the commercial coating whose weigh loss is less pronounced after 15 days. No differences in weight loss (P > 0.05) were observed among coated cheeses. However, WPC coating produced by HD+UV method and the commercial coating had the best performance. Cheeses covered with WPC edible coatings produced by HD+UV polymerization showed statistically (P < 0.05) lower values than uncoated cheeses, which presented the higher weight loss.

Water activity (a_w) is the main factor affecting cheese stability during ripening displaying relatively high values (0.87 - 0.92) in some way expected since this type of cheese generally has a_w close to unity [45]. This property remains practically constant during the entire storage period for all tested cheeses, with no significant differences (P > 0.05) during the first 30 days. Only for uncoated cheeses and cheeses coated with WPC produced by HD and UV polymerization (Figure 3b) a significantly decrease (P < 0.05) between the 30th and 45th day of study was observed. The water loss (Figure 3c) is the main factor pointed as responsible for that decrease though protein degradation by release of carboxyl and amino groups may also contribute to water activity decrease [46]. Cheese moisture significantly decreases (P < 0.05) during storage, displaying moisture losses from ca. 33.4 to 40.0% for coated cheeses. The moisture loss profile (Figure 3c) was distinct between cheeses during the ripening period, with significant differences (P < 0.05) between them at the 15th and 45th day. For uncoated cheese

and cheese with HD coating, the moisture loss was less pronounced during the first 15 days, while the remaining samples showed the opposite trend. At the end of the ripening period cheeses coated with WPC coatings produced by the UV and HD+UV method presented the highest levels of humidity, followed by the commercial coated cheese and finally by HD coated and uncoated cheese. The faster decrease in moisture observed at the beginning of the storage for cheeses coated with WPC when the UV polymerization was used, and its similarity to the moisture profile of commercial coated cheese may suggest analogous water availability conditions to microbial proliferation which is crucial at the first days of ripening. These results prove that the presence of a coating, its nature and polymerization method influence the water transfer phenomenon between cheeses and their surroundings during ripening. Moreover, after 45 days, the presence of whey protein antimicrobial edible coatings decreased the weight loss and moisture loss of cheese in 5.8% and 4.4% respectively, if the coating was prepared by HD; 8.0% and 10.9% when only UV polymerization was used; 11.2% and 10.2% using coatings prepared by HD + UV; while in the case of commercial coating those values decreased by 10.4% and 6.4% respectively. Thus, whey protein edible coatings had better or similar performances compared to the commercial one, exception made to the HD coating.

Fat (Figure 3d) and protein content (Figure 3e) increased ($P < 0.05$) during the ripening period for all cheese samples independently of the presence of coating. However, this evolution was coating type dependent and varies in an opposite way to the cheese moisture content. Dried cheeses have higher percentage of fat. It was also observed that the protein nature of the WPC edible coatings did not affect the protein composition of cheese, since no differences ($P > 0.05$) were observed between cheeses after coating. The salt content (Figure 3f) was not affected by the presence of a coating ($P > 0.05$); however during ripening it was observed a significantly ($P < 0.05$) increase. Cheese with WPC coating produced by HD+UV had the lower increase (0.43%) and very similar values to commercial coated cheese. WPC coated cheese produced by HD showed the more pronounced variation (1.36%).

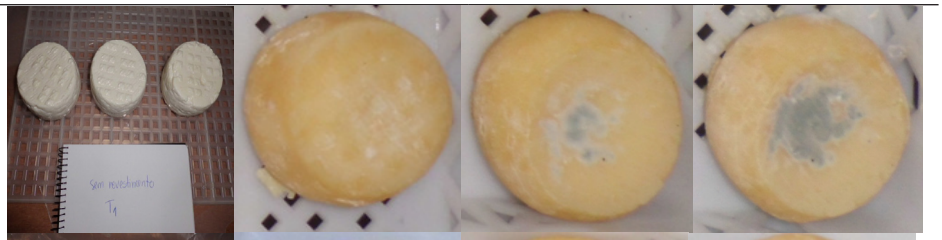
Table 2 shows results for cheese titratable acidity and pH variation throughout ripening. During the first 15 days, titratable acidity significantly increased ($P < 0.05$) both for coated and uncoated cheese samples. Cheese owing WPC coating produced by HD+UV presented significantly higher values (1.40 g lactic acid) than its counterparts. The activity of indigenous cultures of lactic acid bacteria that metabolize lactose to lactate is responsible for the production of acids which results in acidity increase and consequent pH reduction. The observed pH decrease between the 1st and the 45th day of storage (Table 2) showed some variations and did not follow the opposite behavior of titratable acidity. However, at the 30th day of storage significant differences were obtained ($P < 0.05$) between pH among samples.

Figure 4 presents the hardness values for cheese samples throughout storage showing a significant increase ($P < 0.05$) in all cases. It was observed that the presence of coating, its nature and the method of polymerization considerably influenced this parameter. Some authors (Cerqueira et al. [47], Ramos [17]) pointed that cheeses with lower moisture content are harder. This reason could justify our results during the first 15 days of ripening when cheeses with higher moisture content (uncoated, HD and UV) (Figure 3c) had lower hardness values (Figure 4). The moisture content for the different cheese samples become similar at the 30th day of storage but hardness values were significantly different at this point and at the end of the ripening period (45th day). The most dehydrated cheeses (uncoated and HD) had the lower hardness values, while cheeses that had significantly higher humidity (UV, HD+UV and commercial) showed higher hardness values. It is important to notice that cheese hardness determination does not depend exclusively of the cheese bulk consistency but it is also influenced by the rind consistency; where the polymeric material used in coating formulation and the type of chemical interactions that occurred during the coating formation play an important role. The similarity in hardness profiles obtained for cheeses coated with commercial coating and coated by WPC coating produced HD+UV method (Figure 4) may indicate that the molecular and chemical interactions have the same nature, despite the distinct polymeric base material (PVA for the commercial coating). The faster drying and rind formation during the first days of ripening that occurred in the aforementioned cheeses, led to a harder crust which prevented further dehydration from the cheese bulk.

3.3 Cheese appearance

The appearance of cheeses coated with antimicrobial WPC-based coatings produced by the three different polymerization methods (HD, UV and HD+UV) was compared with uncoated cheese and commercial coated cheese during the 45 days of ripening (Table 3).

Table 3. Appearance of cheeses of *ca.* 120 g, coated with antimicrobial whey protein edible coatings produced by HD, UV and HD+UV polymerization compared with uncoated cheese and cheese with commercial coating, during 45 days of storage at 11 ºC and 85% RH. Arrows – presence of molds

Coating	Ripenning time (days)			
	1	15	30	45
Uncoated				
HD				
UV				
HD+UV				
Commercial				

During coating application it was observed that coating solutions with lower viscosity, especially the UV coating solution, resulted in lower adherence to the cheese surface originating a significant drainage from it. Heat denaturation (HD and HD+UV) was responsible for increasing coating solutions viscosity contributing to good adherence. The adopted coating procedure (spreading the coating solution with a brush) is reasonably efficient for the coatings with higher viscosities, since it allowed for the production of thinner coatings, less sticky and with higher drying rates during storage. However, the use of this method in low viscosity coatings may probably compromise the coating efficiency as a result of the extremely thinner coatings produced. For this reason the use of an alternative dipping procedure could be a better alternative to improve coating thickness in these cases.

Identical and homogeneous appearance of the various cheese surfaces (top, bottom and lateral surfaces) was observed one day after coating application with no visual differences between coated and uncoated cheese, neither among the different types of coated cheeses (commercial and WPC-based coatings). The most relevant visual changes in cheese throughout ripening occurred during the first 15 days (Table 3).

The initial white color of cheese and wet appearance gave rise to a dry appearance and a light yellow color. By visual inspection it was not easy to detected color differences between uncoated and coated cheeses, but with respect to microbial growth, uncoated cheese presented a significantly amount of molds at its surface after 30 days of storage, which increased until the 45th day. The presence of some molds on WPC coatings produced by HD or UV polymerization was also observed but in a much smaller extension (Table 3). Possible reasons include the lesser antimicrobial efficiency of these coatings or probably the insufficient amount of coating deposited on the cheese surface. It was not possible to visually detect the presence of molds in commercial coated cheese and in cheese with antimicrobial WPC coating produced by HD+UV polymerization during the 45 days. In both cases a similar good appearance was achieved.

Color analysis based on L*, a*, b* coordinates and color difference (ΔE^*) (Figure 5) confirmed that all cheese samples changed their color throughout storage; with statistically significant differences ($P < 0.05$) recorded between them. The most pronounced color change occurs during the first 15 days of storage. Lightness (L*) decreased significantly from near 95 (white samples) to approximately 70; a* values change from negative (green) to positive values (red) and b* increased in the positive axis direction from ca. 10 to 20 (yellow direction) (data not shown). After that period and during the following 30 days, color changes were not so pronounced; however the presence of coating and the coating polymerization method plays an important influence on L* and ΔE^* values (Figure 5). It was observed that the use of heat denaturation to produce coatings (HD and HD+UV) leads to darker cheeses than uncoated ones. A possible reason is that the high lactose amounts (28.28 g 100 g^{-1}) present in WPC, when exposed to thermal treatments induce chemical Maillard browning reactions. On the other hand, cheeses coated with commercial coating and WPC coatings produced only by UV polymerization exhibited lower color differences than uncoated cheeses. Said color change for uncoated cheese can be attributed in part, to oxygen and light oxidation, which is lower in coated cheeses due to the reduction in oxygen permeability and higher opacity [47]. Cheese dehydration rate during ripening, that was lower for cheeses with commercial and UV coating, may also be associated to a less dry and therefore less dark cheese rind at the end of the study. It was also mentioned by Cagri et al. [48]) that coatings with lactic acid incorporation and its acidulant feature have the capacity to reduce color change. However, our results did not show that behavior by comparing WPC based coatings (which contained lactic acid as antimicrobial agent) with the commercial counterparts, probably due to the use of WPC instead WPI. The presence of higher lactose concentrations in WPC and its tendency to become darker during time probably masked the lactic acid effect [49].

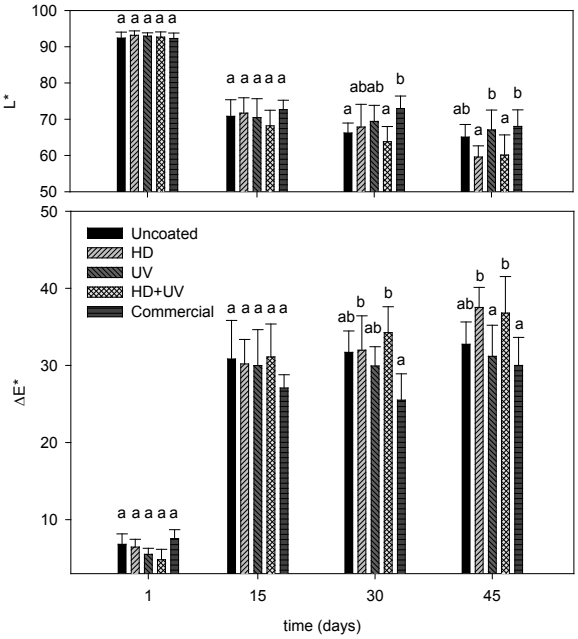


Figure 5. Color coordinate L* and color difference ΔE* of cheese coated with antimicrobial whey protein edible coatings produced by HD, UV and HD+UV polymerization compared with uncoated cheese and cheese with commercial coating, during 45 days of storage at 11 ºC and 85% RH. ^{a,b} means with different letters differ significantly (P < 0.05) between coating types at the same ripening day

3.4 Microbiological profile

The antimicrobial performance of the various WPC-based coatings, with lactic acid and natamycin as bioactive agents, was ascertained using a set of spoilage/pathogenic microflora frequently found on the cheese surface, especially in raw milk cheeses. *Staphylococcus* spp. working as a gram-positive bacterium model; *Pseudomonas* spp. and *Enterobacteriaceae* as gram-negative bacterium model and finally yeasts and molds. The microbiological results of the cheese samples during ripening are presented in Figure 6. These data shows that all the evaluated types of pathogenic or contaminant microorganisms were detected at the cheese surface. Gram-positive bacteria (*Staphylococcus* spp.) were found in lower levels (< 6.5 log (cfu g^{-1})) than gram-negative bacteria (*Pseudomonas* spp. and *Enterobacteriaceae*) or even yeasts and molds. Microbiological analysis also indicated that by 45 days of storage, there are statistical differences ($P < 0.05$) between uncoated cheese and cheeses with the tested antimicrobial coatings.

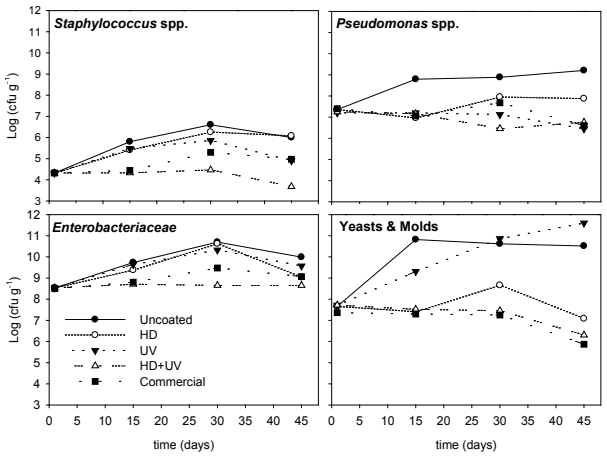


Figure 6. Viable cell counts (log(cfu g^{-1})) (n =3) of *Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae* and yeasts and molds in cheese samples coated with antimicrobial whey protein edible coatings produced by (○) HD, (▼) UV and (△) HD+UV polymerization compared with (●) uncoated cheese and (■) cheese with commercial coating, during 45 days of storage at 11 ºC and 85% RH

The best results for microbial growth control or inhibition were found for cheese coated with antimicrobial WPC-based edible coating produced by the HD+UV polymerization method, independently of the evaluated microorganism. It was very clear the microbial inhibition of *Staphylococcus* spp. and the growth control of *Enterobacteriaceae* (Figure 6) in this coating type in contrast to the performance of the remaining coatings against gram-positive and gram-negative bacteria. The results obtained for yeasts and molds showed similar performance ($P > 0.05$) for HD+UV and commercial coating. In both cases it was not displayed growth of yeasts and molds by comparison with uncoated cheeses or cheeses with UV and HD coatings. Furthermore, a significant reduction ($P < 0.05$) of these types of microorganisms was recorded after 30 days of storage. This outcome was somehow expected for the commercial coating, since it includes natamycin as active compound that has a well-established success in preventing growth of yeasts and molds on cheese surfaces [23]. However the effectiveness against this type of microorganisms by the application of the HD+UV coating which had a relative lower amount of natamycin (0.0125 g 100 mL^{-1} against 0.25 g 100 mL^{-1} in the case of the commercial coating) turns it extremely attractive as an efficient substitute. According to these results the first postulated hypothesis for that behavior was the possible germicidal effect of UV-irradiation promoted during the UV polymerization process. The UV irradiation has been studied as an efficient method of bacterial growth inactivation. The germicidal effects are mainly due to DNA mutations induced through absorption of UV light by DNA molecules [50 and 51]. It was also mentioned that gram-positive bacteria shows higher resistance than gram-negative bacteria to UV-irradiation exposure [52 and 53]. However it was not clear if this difference in susceptibility to UV-radiation is caused by the difference in the cell-wall structure between the two types of bacteria, because gram-positive bacteria have many layers of peptidoglycan, forming thick and rigid cell walls, while gram-negative bacteria have only a single layer or a few layers of peptidoglycan [54].

From the observation of the microbiological results (Figure 6) the germicidal effect of UV irradiation is not clear since the antimicrobial efficiency in UV coating, in which only UV polymerization was applied, was extremely poor, concerning to the growth prevention of *Staphylococcus* spp., *Enterobacteriaceae* and especially for yeasts and molds. A possible explanation for that can be the very short time of exposure to UV-irradiation (only 10 min at the beginning of the ripening period) that may be not sufficient for the germicidal effect, but essential for the UV polymerization process starts to occur. Another possibility that can be pointed was the extremely low thickness of this coating type as a result of the reduced adhesion to the cheese surface during coating, which limited the coating effectiveness.

3.5 Sensorial profile

The results observed in sensory analysis of cheese samples coated with antimicrobial whey protein edible coatings and with commercial coating (or none) are presented in Table 4. Sensory assessment was performed for the external attributes (whole cheese evaluation) and also for the internal cheese attributes (sliced cheese). After the external evaluation, all the cheese samples were manually washed and dried at ambient temperature in order to eliminate any contaminant from the cheese surface to use them for internal sensory evaluation.

Table 4. Whole cheese and sliced cheese sensorial evaluation in a 5 point scale (means ± standard deviation) between cheese coated with antimicrobial whey protein edible coatings produced by HD, UV and HD+UV polymerization compared with uncoated cheese and cheese with commercial coating, after 45 days of storage

Sensorial test Attributes	Coating type				
	Uncoated	HD	UV	HD+UV	Commercial
Whole cheese					
shape	3.21±0.78 ^{a}	3.58±0.72 ^{a}	3.46±0.96 ^{a}	2.90±0.87 ^{a}	2.77±0.89 ^{a}
rind color	2.94±0.59 ^{a}	3.43±0.81 ^{a}	3.46±0.68 ^{a}	2.85±0.40 ^{a}	3.82±0.69 ^{a}
color homogeneity	3.30±0.85 ^{ab}	4.02±0.64 ^{b}	3.07±0.92 ^{a}	2.91±0.75 ^{a}	2.56±0.91 ^{a}
hardness	4.33±0.34 ^{ab}	4.13±0.43 ^{ab}	4.59±0.27 ^{b}	4.03±0.59 ^{a}	4.43±0.52 ^{a}b
Sliced cheese					
differences between paste and ring color	3.75±0.83 ^{a}	3.44±0.70 ^{a}	3.47±0.66 ^{a}	3.30±0.80 ^{a}	2.96±0.82 ^{a}
odor	3.06±0.83 ^{a}	3.19±0.82 ^{a}	3.19±1.13 ^{a}	3.23±1.11 ^{a}	2.69±0.78 ^{a}
consistency	3.90±0.67 ^{a}	3.93±0.47 ^{a}	3.49±0.56 ^{a}	3.65±0.64 ^{a}	3.43±0.66 ^{a}
flavor	3.17±0.83 ^{a}	3.23±0.90 ^{a}	3.19±0.63 ^{a}	3.43±0.87 ^{a}	3.22±0.39 ^{a}
overall acceptability	2.60±0.88 ^{a}	2.88±0.93 ^{ab}	2.72±1.02 ^{ab}	3.30±0.89 ^{ab}	3.73±0.72 ^{b}

$^{a,b,\cdots}$means (n = 12) ± standard deviation with different small letters in the same line are significantly different ($P < 0.05$) between coating types.

With respect to external cheese evaluation (Table 4) no differences were observed ($P > 0.05$) between cheeses concerning shape and rind color by visual inspection. Only, for color homogeneity and hardness, sensorial differences were found ($P < 0.05$). It was observed that commercial coated cheese had the lowest score in color homogeneity whereas the cheese with the coating produced by HD polymerization was classified as the most uniform. These results are consistent with the ΔE^* values obtained above (Figure 5) regarding color measurement indicating that commercial coating was the lighter and HD coating was the darker at the end of the ripening period. In some applications, cheeses bearing darker coatings could benefit in terms of homogeneity because smaller defects at the cheese surface may be masked, making them more attractive to consumers. Tested cheeses were classified as hard by panelists scoring this attribute with values higher then 4 in a 5-point scale (very hard). These results corroborate the behavior of those cheeses during hardness measurements with values higher than 3000 g (Figure 4). 45 days of ripening, induced higher levels of dehydration on cheeses (about 40%), indicating that the ripening period was too long for the small cheeses (120 g) used in this study. During ripening it was observed that after 30 days cheeses displayed the ideal texture.

Concerning the internal cheese evaluation, no statistically significant differences ($P > 0.05$) were obtained among cheeses according to the color difference between paste and rind, odor, consistency and flavor. Nevertheless, it was observed that for the first three attributes the cheese bearing the commercial coating had the lower classification. In fact, the lighter rind color obtained for the commercial cheese (Figure 5) lead to a lower color difference between the rind and paste. Cheese coated with WPC edible coatings had more intense odor, probably due to the presence of lactose in the coating. However, this difference does not influence significantly the cheese flavor ($P > 0.05$). Ramos [17] reported that cheeses coated with whey protein edible coating solutions exhibited a bitter flavor and high astringency, but this was not observed in our study.

Finally, in terms of overall acceptability, the uncoated cheese was the less accepted by the panelists and cheeses coated with edible coatings showed a statistically similar ($P > 0.05$) acceptability to the commercial coated cheese.

4. Conclusions

- The distinct rheological behavior of the whey protein coating solutions, as a result of the applied protein polymerization method is determinant for the coating adhesion to the cheese surface. More viscous coating solutions were produced when heat denaturation was applied leading to better adhesion.
- The polymerization method significantly influences the performance of WPC-edible coatings in the physico-chemical, microbiological and sensorial characteristics of cheeses. The application of UV-irradiation enhanced whey protein coating properties, especially in terms of protein cross-linking during the coating formation.
- Antimicrobial edible coatings based on WPC showed that can be used as a less expensive and

suitable alternative to edible coatings based on WPI or commercial coatings since cheese samples coated with either coatings displayed similar ($P > 0.05$) values in terms of physico-chemical, microbiological and sensorial properties, particularly in the case of the whey protein edible coating produced by heat denaturation and UV polymerization (HD+UV). UV-treatment of WPC-based coatings may display improved functionality and provide opportunities for increased utilization of this technology in the food industry, deserving therefore research attention.

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