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Milk Protein-Based Edible Films: Influence on Mechanical, Hydrodynamic, Optical and Antioxidant Properties

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Abstract: Edible films are thin preformed layers that provide food protection against adverse environmental conditions. Despite milk proteins being functional ingredients that can provide interesting features to films, there is scarce information evaluating their influence on film properties and stability. For this reason, this research work compared the mechanical (thickness, tensile strength, elongation at break), hydrodynamic (moisture content, water solubility, swelling ratio, water vapor transmission rate), color and antioxidant (DPPH) properties of edible films based on casein and whey protein isolate (two types, WPI_1 and WPI_2). Films with casein displayed the highest thickness (0.193 mm), elongation at break (49.67%), moisture content (40.21%) and antioxidant capacity (32.64% of DPPH inhibition), while obtaining the lowest water vapor transmission rate (15.28 $g/m^2 \cdot day$). Significant differences were found in the color properties, mainly between films with casein and those made with WPI. Films containing WPI_1 and WPI_2 were statistically similar in thickness, tensile strength and color properties. The results showed that the properties of the edible films depended on the type of milk protein used. Thus, it is important to evaluate the features provided by different ingredients and formulations for obtaining edible films that properly preserve food.

Keywords: edible films; casein; whey protein isolated; by-product; waste; functional films

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1. Introduction

Proteins are suitable bio-polymeric materials for producing biodegradable films and coatings. Edible films are thin preformed layers of polymers that are applied onto the surface of fresh fruits, vegetables and food. They provide protection against adverse environmental conditions (light, temperature, humidity), acting as physical barriers to mass transfer and preserving food features such as freshness, firmness, color, nutritional and microbiological quality. As a result, they can be used to extend the shelf life of food.

On the other hand, the increasing consumer demand for fresh, high-quality and sustainable food has focused new research on the development of novel technologies for preserving food with renewable and biodegradable materials [1]. Nowadays, edible films and coatings are formulated using natural, biodegradable and non-toxic biopolymers [2]. There are three main biopolymer groups: carbohydrates, lipids and proteins. These represent interesting alternatives to petroleum polymers since they decompose naturally without delivering toxic or harmful substances into the environment [3]. Particularly, edible films based on proteins are of interest since they possess higher nutritional and barrier features, as well as better mechanical properties than those made with either carbohydrates or lipids [4].

In this context, milk is one of the most important sources of proteins. Milk proteins possess high nutritional and functional properties. For instance, they modulate the digestive, immune, cardiovascular, endocrine and central nervous systems and possess activities such as antioxidant, antihypertensive, antimicrobial, antithrombotic, immunomodulatory, mineral binding, and opioid-like activities [5]. Their bioactive peptides regulate functions related to food intake, body weight gain and glucose homeostasis. Several protein types can be obtained from milk such as casein, whey protein concentrate (WPC), whey protein isolate (WPI) and milk protein concentrate (MPC) [6]. Casein is the main protein in milk, representing up to 80% of its composition, while whey proteins make up to 20%.

Overall, whey is a by-product from the cheese industry and is mainly considered to be a waste product. In fact, there is a great concern over its deleterious effects on the environment due to retaining a high content of lactose and its relationship with microbiological fermentation [7]. However, whey proteins have been used as valuable food ingredients with high nutritional content and also as gelling, emulsifier and foam agents [8]. For this, evaluating its suitability in the development of edible films and coatings is compulsory for contributing to the "zero waste" goals from the Food and Agriculture Organization of the United Nations (FAO).

Edible films based on whey proteins show resistance to oxygen permeability and adequate mechanical, sensorial and optical properties, but they are sensitive to moisture [5]. In this sense, the literature has shown some studies evaluating the different features and effects of whey-based edible films. For instance, water vapor diffusion in whey protein films was analyzed by means of macroscopic aspects of moisture transmission [9]. A swelling test was conducted to evaluate the crosslinking effect of whey protein-based films during storage [10]. The antimicrobial efficiency of edible coatings containing WPI, clove and oregano oils on chicken breast fillet shelf life during refrigerated storage has been evaluated [11]. The effects of whey protein active coatings with *Origanum virens* essential oils on the quality and shelf life improvement of processed meat products have been investigated [12]. Additionally, the effect of melanin from watermelon seeds on the physicochemical, mechanical and antioxidant properties of whey protein concentrate/isolate (WPC/WPI) films has been studied [13].

Despite there being some studies evaluating protein-based films, to the best of our knowledge there is no available information analyzing the influence of protein type on the mechanical, physicochemical and antioxidant properties of films. For this reason, the purpose of this research was to compare the mechanical (thickness, tensile strength, elongation at break), hydrodynamic (moisture content, water solubility, swelling ratio, water vapor transmission rate), color and antioxidant (DPPH) properties of edible films based on two of the main milk proteins: casein and whey protein isolate (two types: WPI_1 and WPI_2).

2. Materials and Methods

2.1. Materials and Reagents

Two types of whey protein isolate (WPI), containing 93% of protein, were purchased from Volac International Ltd. (Royston, Hertfordshire, UK): WPI_1 (Volactive Ultrawhey 90 Standard) and WPI_2 (Volactive Ultrawhey 90 Instant with sunflower lecithin). They were carefully selected on the basis of their extraction processing, which according to the manufacturer, was a traditional membrane filtration process within 48 h after cheese manufacture.

Casein from bovine milk and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (St. Louis, MO, USA). Glycerol, hydrochloric acid, sodium hydroxide and calcium chloride, all at analytical grade, were locally acquired.

2.2. Preparation of Edible Films

Edible films were prepared following the methodology of Asdagh et al. [8], slightly modified. Briefly, a solution containing 5% milk protein (casein, WPI_1 or WPI_2) was added to distilled water. Then, the pH was adjusted to 9.0 with 0.1 N NaOH and the mixture was heated at 90 °C for 30 min, until it reached a uniform appearance. The solution was cooled at room temperature and then 5% of glycerol was added. Aliquots of 10 mL of this solution were cast on polystyrene plates (120 mm × 120 mm) and dried at 40 °C for 48 h. Finally, each dry film was carefully removed from the plates, conditioned at room temperature and 50% relative humidity (RH) and evaluated. It is important to state that at least 10 films from each formulation were prepared.

2.3. Thickness

This assay was measured using a Film/Thickness GAUGE (BENETECH, GM210, Shenzhen, China). Prior to the assay, the equipment was calibrated at 49, 102, 255, 491, 992 and 1999 µm ± 1%. Results were averaged from six random points in each sample and reported in millimeters (mm).

2.4. Tensile Test

The tensile properties of the films were evaluated according to the ASTM method D882-10, using a TA.HD Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). Film strips of 7.5 cm × 2.5 cm were conditioned at 23 °C for 48 h and 50% RH. Film strips were placed between grips at an initial grip separation and crosshead speed set at 40 and 50 mm/min, respectively. Tensile strength (MPa) and elongation at break (%) were calculated from the load-deformation curves.

2.5. Hydrodynamic Properties (Moisture Content, Water Solubility, Swelling Ratio and WPTR)

Previous to these assays, edible films were conditioned at 23 °C for 48 h at 50% RH. Afterwards, each assay was carried out following its specific conditions.

2.5.1. Moisture Content

The moisture content was evaluated following the methodology of Łopusiewicz et al. [13]. Samples were weighed before and after drying at 105 °C for 24 h at 1% RH. Results were reported as the change in the weight of the film (%).

2.5.2. Water Solubility

A film disc (1.7 cm diameter) from each sample was carefully placed in an oven at 60 °C for 12 h at around 5% RH to assess the water solubility (WS). Afterwards, it was weighed and dipped in 50 mL of distilled water at 25 °C for 24 h with sporadic shaking. Samples were filtered, dried (105 °C for 24 h and 1% RH) and weighed. Results were calculated as follows (Equation (1)):

$$\mathrm{WS}(\%) = \frac{\mathrm{Wi} - \mathrm{Wf}}{\mathrm{Wi}} \times 100 \quad (1)$$

where Wi is the initial weight of the film and Wf is the weight after 24 h of drying.

2.5.3. Swelling Ratio

Firstly, the films were weighed and introduced into 50 mL of distilled water for 1 h at 23 °C and 50% RH. Afterwards, the supernatant was carefully removed using a Pasteur pipette and filter paper was used to remove the final drops. Equation (2) was used for calculating the swelling ratio (SR) results:

$$\mathrm{SR}\ (\%) = \frac{\mathrm{Wf} - \mathrm{Wi}}{\mathrm{Wi}} \times 100 \quad (2)$$

where Wi is the initial weight of the film and Wf is the weight after 1 h of submersion.

2.5.4. Water Vapor Transmission Rate

The water vapor transmission rate (WVTR) was performed based on the gravimetric method described by Łopusiewicz et al. [14], slightly modified. Briefly, dry $CaCl_2$ (9 g) was placed inside a container (0% RH) and sealed with a film (8.86 cm^2). Containers were placed in a desiccator containing saturated NaCl at 25°C and 33% RH. The weight of the containers was registered daily for four days to measure the absorption of water vapor through the films. Results were calculated from averaged values from each day and expressed as g/(m^2·day).

2.6. Color and Optical Properties

The color of the films was measured with a colorimeter (Minolta CR-300, Osaka, Japan). Results from parameters L*, a* and b* were reported as the mean ± standard deviation. Additionally, color difference (ΔE), whiteness index (WI), opacity and transparency were also calculated (Equations (3)–(6), respectively).

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (4)$$

where $\Delta L^* = L^*_{control} - L^*_{sample}$; $\Delta a^* = a^*_{control} - a^*_{sample}$; $\Delta b^* = b^*_{control} - b^*_{sample}$. The control consisted of a white plaque.

$$\mathrm{Opacity} = (A_{500})(t) \quad (5)$$

$$\mathrm{Transparency} = A_{600}/t \quad (6)$$

where A is the absorbance at 500 or 600 nm for opacity and transparency, respectively; t is the thickness of the film.

2.7. Antioxidant Capacity

This assay was performed by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity as follows: 40 mg of film was dissolved with 10 mL of methanolic-DPPH (0.025 g/L). Afterwards, the films were placed in a dark room for 30 min, filtered and the absorbance was measured (515 nm) [15]. Additionally, the absorbance of the solution (methanolic-DPPH) was measured as a control. Results were reported as % of DPPH inhibition.

2.8. Statistical Analysis

Analysis of variance (ANOVA) of the results followed by the least significant difference test (LSD) were carried out to determine significant differences ($p < 0.05$) in the milk protein-based edible films. The statistical analyses were performed by the Statgraphics Plus 5.1 program (Statistical Graphics Corporation, Inc., Rockville, MD, USA). The results were reported as mean ± standard deviation from at least five repetitions.

3. Results and Discussion

3.1. Thickness

The thickness is a property of edible films related to its tensile, mechanical and light barrier properties—thus influencing the shelf life of food products [16]. In this research, the edible films showed a thickness between 0.17 and 0.19 mm, as shown in Table 1. Significant statistical differences ($p < 0.05$) were observed between the thicknesses of the films with casein versus those of WPI. In this sense, the greatest thickness value was observed in films with casein, while films with WPI_1 and WPI_2 were statistically similar ($p > 0.05$). Differences between the thicknesses of these films could be explained by the composition of their ingredients. In this case, casein possessed a higher solid content in the film solution than the whey protein isolate [17]. Casein is the main protein in milk, making up 80% of cow's milk. Casein is mainly extracted by ultrafiltration, increasing the concentration of bioactive peptides [6]. Thus, the placement of large molecules in the surrounding film matrix leads to an increase in the thickness [8].

Table 1. Thickness and mechanical properties of milk protein-based edible films. [1]

Films	Thickness (mm)	Tensile Strength (MPa)	Elongation at Break (%)
Casein	0.19 ± 0.01a	0.70 ± 0.06b	49.67 ± 5.51a
WPI_1	0.18 ± 0.01b	2.32 ± 0.23a	11.57 ± 0.67c
WPI_2	0.17 ± 0.01b	2.25 ± 0.13a	28.17 ± 2.39b

[1] Results are reported as the mean ± standard deviation. Different lowercase letter within each column indicates significant statistical differences ($p < 0.05$) between films.

In line with the results obtained in this research, a similar thickness of 0.14 mm from a film made with WPI was obtained by Łopusiewicz et al. [13]. The previous author also reported that the addition of melanin did not significantly change the thickness of WPI films when applied at a low concentration (0.1% to 0.5%). On the contrary, the addition of coconut essential oil (0%, 0.4%, 0.8% *w*/*v*) and paprika extract (0%, 0.03%, 0.06% *w*/*v*) to WPI films significantly affected the thickness [8].

3.2. Tensile Test

The tensile test consisted of evaluating the mechanical properties of films: tensile strength and elongation at break, as reported in Table 1. The analysis of these features allows edible films with appropriate mechanical properties for protecting fruits and vegetables to be obtained.

Overall, tensile strength is the maximum stress that a film can withstand while being stretched before breaking. This property depends on the interactions between film constituents [18]. It has been reported that as the protein concentration increases, the films become stronger, with high tensile strength [19]. In this research, all films contained 5% milk protein. However, when they were compared, films containing casein had the lowest tensile strength (0.70 MPa), being up to 3.3 times lower than those made with WPI. When the tensile strength of films with WPI_1 and WPI_2 were compared, they did not show significant differences ($p > 0.05$).

On the other hand, all analyzed films displayed different elongation at break, where films with WPI_1 showed the lowest % of elongation, while those with casein had the highest. Similar to tensile strength, the elongation at break depends on the interactions between film constituents. In this case, the % of elongation decreased as the protein concentration increased [19].

Additional chemical treatments with acid, alkali or crosslinking components can be added to edible films for improving the permeability and tensile strength of films as the chain structure is modified [20]. However, these treatments can also modify the color, flavor and acceptance of fresh coated fruit and vegetables. For this, it is necessary to evaluate the formulation of edible films in order to achieve desirable features. As an example, films composed of red pomelo peel pectin, casein and egg albumin at ratios of 50:50:0 and 50:25:25 showed higher tensile strength in comparison to other formulations [21].

3.3. Hydrodynamic Properties (Moisture Content, Water Solubility, Swelling Ratio and WPTR)

3.3.1. Moisture Content

The moisture content was in the range from 21.85% to 40.21% in the films analyzed in this research (Table 2). The three formulations showed significant differences, the film with casein being the most wet, followed by WPI_2 and WPI_1. In this case, films with casein showed 1.84-times higher moisture content than those containing WPI_1. When comparing films with WPI_1 and WPI_2, the latest was 1.13-times more wet than WPI_1.

Table 2. Hydrodynamic properties of milk protein based edible films. [1]

Films	Moisture Content (%)	Water Solubility (%)	Swelling Ratio (%)	WVTR g/(m²·day)
Casein	40.21 ± 1.91a	34.71 ± 2.01b	39.79 ± 3.08c	15.28 ± 0.35c
WPI_1	21.85 ± 1.63b	36.46 ± 4.02ab	109.08 ± 8.41a	23.32 ± 1.80a
WPI_2	24.75 ± 0.80c	41.54 ± 1.23a	82.71 ± 8.36b	19.94 ± 0.50b

[1] Results are reported as the mean ± standard deviation. Different lowercase letter within each column indicates significant statistical differences ($p < 0.05$) between films. WVTR = Water Vapor Transmission Rate.

Evaluation of moisture content is important, since this parameter is related with changes in the stability and quality of products [12]. This feature also limits the long-term stability of films, with a greater sensitivity to moisture content and a greater weakness to mechanical properties [22]. In this sense, the lowest moisture content was obtained in films containing WPI_1, which could be also related to their lower water activity, and thus were the most stable films with the lowest deterioration [23]. Depending on the film structure and composition, the moisture content can be modified; films with closed structures possess strong interactions between polymeric molecules, thus decreasing the moisture content [21].

3.3.2. Water Solubility

The results showed that the percentage of water solubility (WS) was 34.71, 36.46 and 41.54 for films with casein, WPI_1 and WPI_2, respectively (Table 2). According to the statistical analysis, similar water solubility was observed between films containing casein and WPI_1. In the same line, no statistical differences were obtained between the WS from films with WPI_1 and WPI_2.

Similar to moisture content, the water solubility is influenced by the film structure and composition, and stronger polymeric interactions within the matrix lead to lower solubility. Depending on the application, films with a high water solubility could be desirable for the following situations: (a) when films are created to avoid changes in quality, nutritional and sensorial features of food; (b) when films are consumed together with food; (c) when films are dissolved after food cooking or processing [24]. On the other hand, films with low water solubility are necessary when they must act as a barrier to gas permeability, when film must protect foods, and, finally, when coating fatty foods [25].

3.3.3. Swelling Ratio

Among the three types of films analyzed in this research, the swelling ratio showed values from 39.79% to 109.08%. Films with WPI_1 had an around 2.74-times higher swelling ratio than films with casein, and 1.32-times higher than films with WPI_2.

Overall, the swelling ratio is considered the fraction of gain weight in the film due to water adsorption. In this sense, swelling is related to the multimolecular adsorption of water, inducing changes in the spatial structure of macromolecules due to high relative humidity [6]. Other factors, such as the material features, porosity and cross-linking, also affect this parameter [2].

Thus, it could be stated that the addition of WPI to films increases porosity and cross linking, which is reflected in a high swelling ratio in tested films. Similar to water solubility, a high or low swelling ration could be desirable, depending on the film application. For instance, films containing whey protein concentrate (WPC) show up to a 4.27-times higher percentage of swelling ratio than those with whey protein isolate (values from 324.30% and 75.85% for WPC and WPI, respectively) [13].

3.3.4. Water Vapor Transmission Rate (WPTR)

In the edible films analyzed in this research, the water vapor transmission rate (WVTR) was between 15.28 and 23.32 g/(m^2·day). This property depends on both temperature and relative humidity. Generally, the higher the temperature, the higher the water vapor diffusion through edible films. There is an enhanced motion of polymer segments and an increase in the energy levels of permeating molecules due to increased temperatures, showing that permeability increases as the temperature increases [26].

The relative humidity of the environment also plays an important role in the WVTR. The higher the relative humidity, the higher the effect on WVTR [27]. As indicated in the material and methods section, the desiccator was saturated with $CaCl_2$ at 25 °C and 33% RH. The sorption of humidity by $CaCl_2$ and the increase of weight of the film showed that water vapor passed through the films [8]. It is also important to state that both effects (temperature and relative humidity) on water vapor solubility in the polymer can be deduced from water sorption isotherms. It has been reported that sorption curves are typical of water vapor-sensitive polymers, including those that came from protein-based films, amylose corn starch and cellulose [26,28].

Thus, the WPTR assesses the quantity of water vapor mass that can penetrate an area of a material in a specific period of time, providing an overview of the film performance as a barrier to permeants. For this, WVTR is given in units of mass per area per unit of time. A high WVTR is not desirable, as it indicates a high moisture loss of food during storage [29], with the subsequent consequences of weight, firmness and quality losses [30]. In the case of this research, the film containing casein showed the lowest WVTR, which may lead to higher protection and better storage of foodstuffs than those containing WPI.

On the other hand, films containing WPI_2 had 15% lower WVTR than those with WPI_1. This could be related with the fact that WPI_2 was formulated with the addition of sunflower lecithin. It is probable that lecithin may avoid the water vapor transmission through the film due to its lipophilic nature, explaining why WPI_2 showed lower WVTR as compared with WPI_1.

A comparison of the results obtained in the present research work with those reported in the literature are difficult due to the differences in film formulation and thickness, as well as in temperature and RH. For instance, it has been reported that the WVTR values are in the range of 130–160 g/(m^2·day), but the film thicknesses must also be considered and must not be normalized to 100 μm [31]. The WVTR was in the range of around 125 to 160 g/(m^2·day) in WPI-based films, with different degrees of protein denaturalization at 23 °C and 50% to 0% of RH [28]. On the other hand, a WVTR of 1618.57 g/(m^2·day) was found in edible films containing WPI, but it significantly decreased by 3.2% and 7.9% when melanin isolated from watermelon (*Citrullus lanatus*) seeds was added at concentrations of 0.1% and 0.5%, respectively [13]. However, the previous authors did not provide information regarding temperature and RH.

Therefore, it is important to find edible films that are able to control the WPTR in order to avoid moisture loss or condensation, as their composition greatly influences the physicochemical and mechanical properties of films.

3.4. Color and Optical Properties

The color parameters (L*, a*, b*), color difference (ΔE), whiteness index (WI), opacity and transparency are presented in Table 3.

Table 3. Color and optical parameters of milk protein-based edible films. [1]

Film	L*	a*	b*	ΔE	WI	Opacity	Transparency
Casein	95.76 ± 0.78a	-0.94 ± 0.06b	2.36 ± 0.13a	2.65 ± 0.27c	95.04 ± 0.65a	0.32 ± 0.03a	3.35 ± 0.40c
WPI1	92.67 ± 0.80b	0.36 ± 0.04a	1.14 ± 0.07b	7.36 ± 0.80b	92.57 ± 0.79b	0.16 ± 0.01b	6.23 ± 0.51b
WPI2	89.34 ± 1.15c	0.35 ± 0.04a	0.92 ± 0.08c	10.67 ± 1.15a	89.29 ± 1.14c	0.16 ± 0.02b	4.90 ± 0.54a

[1] Results are reported as the mean ± standard deviation. Different lowercase letter within each column indicates significant statistical differences ($p < 0.05$) between films.

Overall, films with casein showed significant differences in all these parameters with respect to films with WPI$_1$ or WPI$_2$ (Figure 1). Both WPI$_1$ and WPI$_2$ were colorless and bright films, while those with casein were whitish and opaque. For this, the value of the L* (lightness) parameter was around 3%–7% higher in films with casein, regarding that observed in films with WPI. The low value of the a* (red to green) and b* (yellow to blue) parameters in the any of the analyzed samples could be explained by the fact that, overall, the evaluated films were white (films with casein) or colorless (films with WPI$_1$ or WPI$_2$).

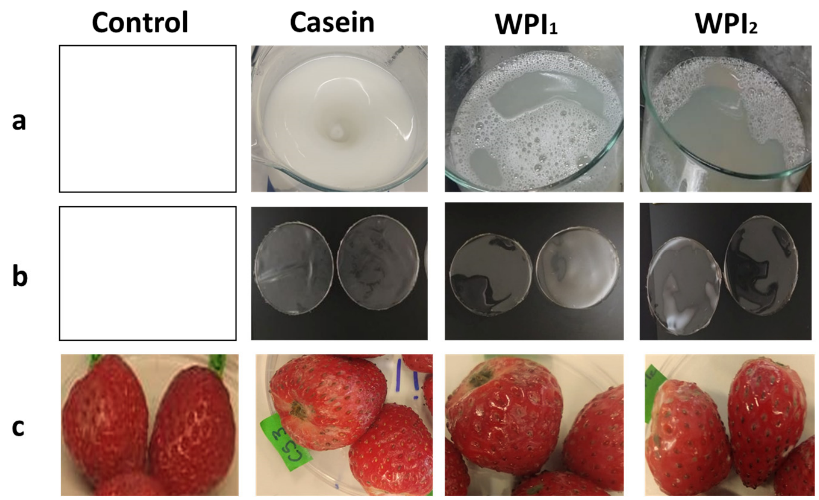

Figure 1. (**a**) Solutions used for preparing milk protein-based films. (**b**) Films based on milk proteins (casein, WPI$_1$ and WPI$_2$). (**c**) Example of film appearance on a fruit (strawberry).

When the color difference (ΔE) was calculated, films containing casein showed the lowest value, followed by films with WPI$_1$ and finally those containing WPI$_2$. This means that films with casein showed less differences with the color of the standard (a white plaque), as compared to samples containing WPI. In line with the ΔE results, the whiteness index (WI) was the highest in films with casein, indicating that that these films were whitish. Regarding the opacity and transparency, it was observed that films with casein were between 1.98 and 2.03-times more opaque than films with WPI, and between 32% and 46% less transparent than WPI-based films. No significant differences were found in the opacity of WP$_1$ and WPI$_2$ ($p > 0.05$), while WPI$_1$ obtained the highest transparency as compared to films with casein or WPI$_2$. Opacity and transparency are inversely related parameters—higher opacity indicates lower transparency—and this trend was also observed in the results obtained in this research. However, it is important to take into consideration that opaque edible films are desirable for preserving light-sensitive constituents of food. Similar results were found in red pomelo peel-based edible films, where the transparency was in the range of 0.89 to 6.97 depending on the ratio of pectin, casein or egg albumin [21]. A transparency of 2.05 in edible films with a ratio of 100% casein has also been reported, which is similar to that obtained in the present research (transparency of 3.35) [21].

3.5. Antioxidant Capacity

The % of DPPH inhibition is presented in Figure 2. According to the obtained results, films containing caseins showed the highest antioxidant capacity for scavenging DPPH radicals, with 32.64% of DPPH inhibition. Films with WPI_1 displayed 1.22-times higher antioxidant capacity than WPI_2 but, at the same time, 26% lower antioxidant potential as compared to casein.

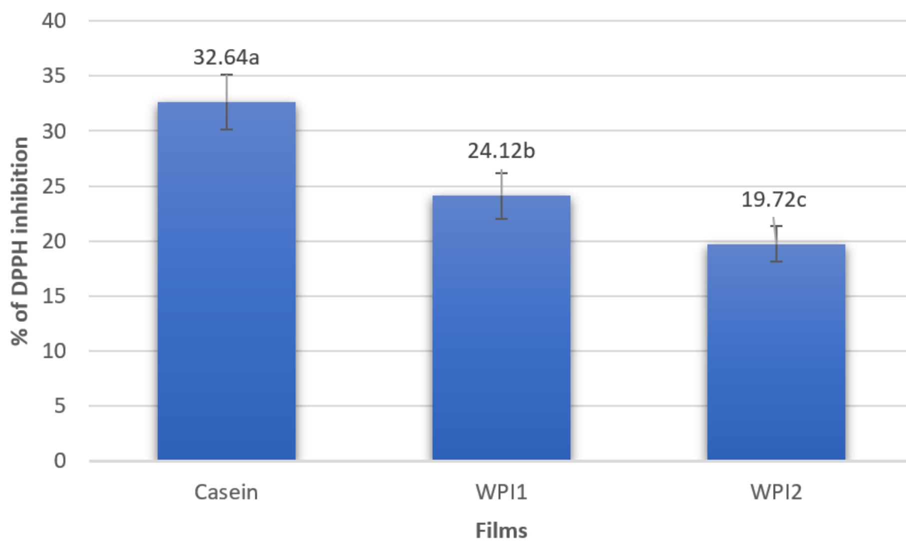

Figure 2. Antioxidant capacity of milk protein-based edible films reported as % of 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition. Different letter indicates significant differences ($p < 0.05$) between films.

Despite the significant differences in the scavenging activity of the films, they all presented antioxidant capacities. Both caseins and WPI contain compounds such as proteins, peptides and amino acids that can act as antioxidants. In particular, casein phosphopeptides have transition metal ion sequestering activity and free radical quenching activity; thus, they are considered primary and secondary antioxidants [32]. Whey proteins have bioactive peptides with biological activities such as immunomodulation, anticancer, hypocholesterolemic, antioxidant, antihypertensive and antimicrobial activity [33]. Additionally, whey proteins may protect against hydrogen peroxide-induced cytotoxicity on C_2C_{12} cells [34].

Considering that films were made with 5% of proteins, they possess an important scavenging activity and thus, they could contribute in the antioxidant capacity of food that will be covered with these films.

4. Conclusions

Milk proteins are suitable ingredients for obtaining functional edible films. The results obtained in this research showed that the mechanical, hydrodynamic, optical and antioxidant properties of the edible films were influenced by the type of milk protein. Among the evaluated samples, those containing casein displayed important film properties such as the highest thickness (0.19 mm), elongation at break (49.67%) and antioxidant capacity (32.64% of DPPH inhibition), while obtaining the lowest water vapor transmission rate (15.28 g/m^2·day) and transparency (3.35). Films with WPI_1 and WPI_2 were similar in thickness, tensile strength and color properties. However, significant differences were found in the color between films with casein and those made with any type of WPI.

Thus, the proper selection of ingredients and formulation has a large impact on the film matrix, on the film's features and also on the preservation of food. Future studies are necessary for evaluating the degree to which these milk protein-based edible films can preserve the quality of fresh covered food, as well as their impact on its shelf life.

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