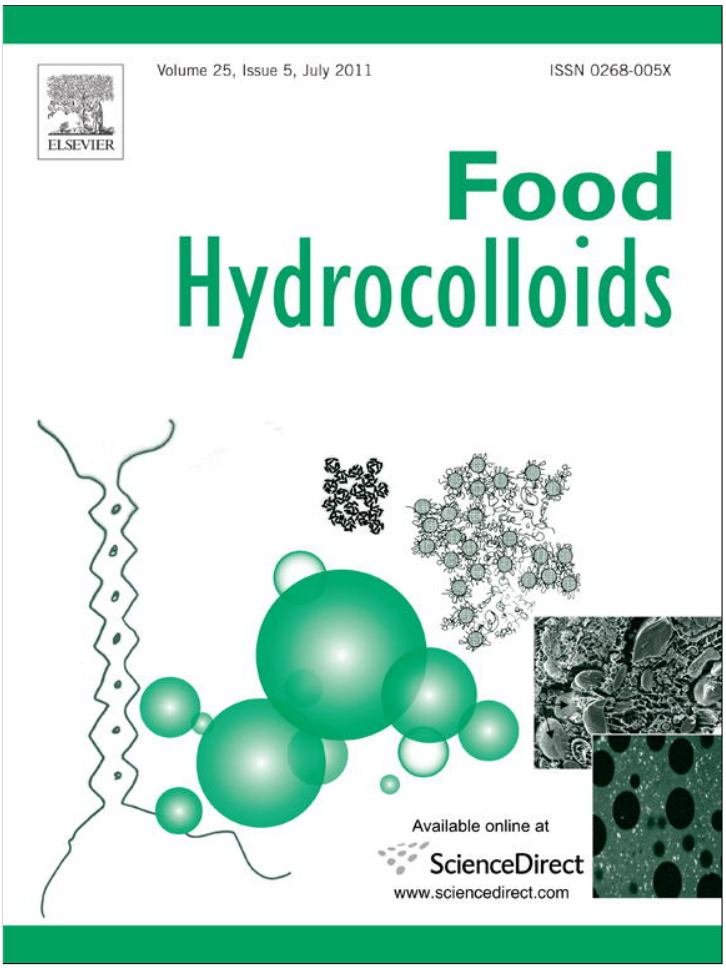

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Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films

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ABSTRACT

The objective of this work was to study the effect of drying temperature and the beeswax (BW) content on the physical properties of whey protein emulsion films. For this purpose, films were obtained by the casting method and dried at two selected temperatures (5 and 25 °C). Film thickness, water vapor permeability (WVP), solubility and mechanical properties were measured. The results showed that the decrease in drying temperature from 25 to 5 °C reduced the WVP and increased the solubility of the films containing BW. The effect of drying temperature on the mechanical properties was significant in the tensile test but not in the puncture test. The addition of BW decreased the WVP and the solubility and also had a significant effect on the evaluated parameters in both mechanical tests. In general, this effect was observed at both drying temperatures studied. Therefore, taking into account the several applications of the coatings the optimization of coating formulations and drying conditions is of vital importance.

1. Introduction

Edible films are defined as a thin layer of material, which can be consumed and contributes to extend the shelf life and to improve the quality of foods by providing a barrier to mass transfer, carrying food ingredients and/or improving the mechanical integrity or handling characteristics of the product (Pérez-Gago & Krochta, 2000; Bourtoom, 2008). The renewed interest in the development of edible films and coatings is the result of the consumers demand for high quality foods and the increasing environmental concern on the disposal of food non-renewable packaging materials.

Whey protein concentrates have excellent nutritional and functional properties in addition to their capacity to form films (Pérez-Gago, Serra, Alonso, Mateos, & del Río, 2003). Therefore they constitute a promising alternative for the manufacture of edible coatings representing an effective means of increasing the utilization of excess whey and improving the whey disposal problem (Banerjee & Chen, 1995). Although whey proteins are commercialized as whey concentrates (WPC) and isolates (WPI), in most of the reviewed literature WPC is scarcely used for the manufacture of edible films.

Edible films obtained from heat-denatured whey proteins with the addition of a plasticizer, like glycerol (Gly), become more transparent, bland and flexible. These water-based edible films have excellent oxygen, aroma, and oil barrier properties at low relative humidity (RH) (Pérez-Gago & Krochta, 2002). However, the hydrophilic nature of whey protein films causes them to be less effective moisture barriers. The moisture barrier ability of these films is greatly improved by incorporation of lipids because these materials are good barriers against moisture migration (McHugh & Krochta, 1994; Shellhammer & Krochta, 1997; Pérez-Gago, Nadaud, & Krochta, 1999; Talens & Krochta, 2005). Usually, hydrocolloids are combined with lipids like fatty acids, triglycerides and waxes to form bilayer or emulsion films (Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). As suggested by Talens and Krochta (2005) the addition of a lipid to protein-based films may interfere with polymer chain-to-chain interactions and/or provide flexible domains within the film and because of their lack of cohesive structural integrity they could affect the mechanical properties of the film. In fact, Shellhammer and Krochta (1997) and Pérez-Gago and Krochta (2000) reported that the type and amount of lipid were important in controlling the water vapor permeability of whey protein emulsion films but also had a negative effect on their

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mechanical properties. Consequently, components of edible films should be carefully selected according to the final application of the edible film. An edible film should be resistant in order to withstand manipulation during its application and to maintain its integrity and also its barrier properties (Tanada-Palmu & Grosso, 2003).

One of the most critical steps involved in edible films preparation is drying (Thakhiew, Devahastin, & Soponronnarit, 2010). In the case of protein films, drying conditions may influence the final properties of the material as proteins can change structure as a function of processing parameters (Tapia-Blácido, Sobral, & Menegalli, 2005). Particularly in the case of emulsified films, drying conditions can influence the stability of the film-forming emulsion and thus the final film structure (Morillon et al., 2002). Widespread practice in edible film development involves drying at ambient conditions (Pérez-Gago and Krochta, 2000). However, when the effect of drying temperatures on the properties of edible films was investigated temperatures above room temperature were studied (Alcántara, Rumsey, & Krochta, 1998; Pérez-Gago and Krochta, 2000; Denavi, Tapia-Blácido, Añón, Sobral, Mauri, & Menegalli, 2009; Thakhiew et al., 2010). Taking into account that edible films have shown to be effective to improve the quality of whole and minimally processed fruits (García, Martino, & Zaritzky, 1998; Han, Zhao, Leonard, & Traber, 2004; Pérez-Gago, Serra, & del Río, 2006; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008) and that fruit coating should be performed near storage temperature, the study of the properties of edible films obtained at low temperatures is a challenge.

Accordingly, the objective of this investigation was to study the effect of the decrease in drying temperature from ambient to refrigeration conditions and the beeswax content on water vapor permeability, solubility and mechanical properties of whey proteins emulsion films.

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC) 80% (Arla Food Ingredients S.A., Buenos Aires, Argentina) was used to prepare the film-forming solutions. Beeswax (BW) (yellow, refined, Sigma–Aldrich) was selected as the lipid phase of WPC emulsion films. Gly (Cicarelli, Argentina) was added to all film-forming solutions as a plasticizer, potassium sorbate (Anedra, Argentina) was included in all formulations to avoid the microbial growth and Tween 80 (Anedra, Argentina) was used as emulsifier.

2.2. Preparation of film-forming solutions

Aqueous solutions of 8% (w/w) WPC were prepared according to a modification of the procedure described by Pérez-Gago et al. (2003). Gly (in proportion WPC/Gly 3:1 w/w dry solid basis), potassium sorbate (to obtain a final concentration of 0.12% w/w dry solid basis), and BW (at 0, 20 and 40% w/w dry solid basis in the mixture WPC-Gly) were added. Tween was used as emulsifier in the solutions containing BW. Distilled water was added to obtain a total solid content of 11.5%. Film-forming solutions were heated at 90 °C for 30 min to achieve whey proteins denaturation. Emulsions were obtained by homogenization in a water bath (Dalvo Instruments, Santa Fe, Argentina) at 90 °C using a high-shear probe mixer Ultra-Turrax T25 (IKA Werke, Janke & Kunkel GmbH & Co KG, Staufen, Alemania) for 5 min at 21500 rpm. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of the whey proteins and to crystallize the lipid particles. The emulsions were degassed at room temperature with a vacuum pump and stored at 5 °C until use.

2.3. Film formation

Films were prepared by pipetting 8 g of the degassed emulsion on 90 mm diameter disposable polyethylene Petri dishes. Films were dried on a leveled surface in an environmental chamber Tabai Comstar PR 4 GM (Tabai Espec. Corp., Osaka, Japan) at two selected temperatures (5 and 25 °C) and constant RH (58%). The chamber was equipped with a fan that circulated interior air at approximately 60 m/min. After drying, dried films were removed from the plates and were conditioned in the environmental chamber set at 25 °C and 58% RH for 3 days. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles and holes.

2.4. Film thickness

Film thickness was measured with a digital micrometer (Schwyz, China). For each film, nine thickness measurements were taken. Averaged values of thickness measurements were calculated and were used in all calculations. Films were obtained with an average thickness of 0.154 mm.

2.5. Water vapor permeability

A modification of the ASTM E96-95 gravimetric method for measuring water vapor permeability (WVP) known as cup method was employed (McHugh, Avena-Bustillos, & Krochta, 1993). Cups consisted of a cylindrical bottom made of high density polyethylene, a lid of the same material and a rubber O-ring. The bottom had an external diameter of 10 cm. A well of 5 cm in diameter and 3 cm in depth was milled into the bottom. An O-ring was placed into a groove milled around the well. Films were mounted on cups containing 10 mL of distilled water, with the film surface which had been exposed to air during drying facing the low RH environment. A rubber O-ring and six screws located around the cup circumference were used to seal the films in the test cups. The distance between the solution and the film was determined both before and after each experiment using a caliper. Average stagnant air gap heights were calculated. Test cup assemblies were placed in the environmental chamber (25 °C and 58% RH). Weight losses of test cups were recorded until that were made four successive steady-state measurements and were used to calculate the % RH at the film underside and the resulting WVP. Determinations were performed in quintuplicate.

2.6. Solubility in water

A piece of film sized 15 × 7.5 mm was cut, dried in an oven (Dalvo Instruments, Santa Fe, Argentina) at 70 °C for 24 h and then weighed to obtain the initial film dry weight. The piece of film was then placed into a test tube with 10 mL of distilled water and 0.01% potassium sorbate to prevent microbial growth. The test tube was capped and shaken slowly on a shaking platform (Vicking, Argentina) for 24 h at 25 °C. Circular filter papers (qualitative grade, Boeco, Germany) were dried 24 h in an oven at 70 °C. After drying, filters were allowed to reach room temperature in a desiccator to prevent moisture absorption from the air and then quickly weighed to obtain the initial dry filter weight. The solution containing the film was filtered by pouring the contents of test tube onto a filter in a Buchner funnel attached to the neck of a 250 mL Erlenmeyer connected to a vacuum pump. The test tube containing the solution was rinsed at least 3 times with water, and this solution was filtered to ensure that all solids were removed from the tube. The remaining solids on the filter were dried in the oven at 70 °C for 24 h to determine the final filter dry weight. The difference

between the final dry filter weight and initial dry filter weight yielded the final dry film weight. Solubility, expressed as soluble solids (%), was obtained by subtracting the weight of dry matter not solubilized from the weight of initial dry matter and reported on initial dry weight basis (Sothornvit & Krochta, 2000). Determinations were performed in quintuplicate.

2.7. Mechanical properties

Mechanical strengths of the films were evaluated by tensile and puncture tests using an Universal Testing Machine Instron, single column, Series 3340 (Instron, Norwood, MA, United States) with a 10 N load cell. Probes, prepared as explained below for each mechanical test, were conditioned for 1 day at 25 °C and 58% RH and equilibrated to the testing environment for 2 h at 22 °C and 50% RH on average. For each mechanical test ten replications were performed.

In the puncture test films of 9 cm of diameter were fixed to a support with a circular opening and a cylindrical probe of 2 mm diameter was moved perpendicularly to the film surface at a constant speed of 0.8 mm/s until the probe passed through the film. Force-deformation curves were obtained and force (N) and deformation (mm) values at the puncture point were then recorded to represent the puncture strength (N) and deformation (mm) of the films (Chen & Lai, 2008).

To evaluate tensile properties films were cut into strips, 7 mm wide and 60 mm length, using a scalpel. Strip ends were mounted with double sided tape and squares of 30 mm of cardstock. The exposed film strip length, between cardstock ends, was 30 mm. The cardstock pads were placed on the ends of film strips to prevent tearing and slippage in the testing device (Shellhammer & Krochta, 1997). The initial grip distance and crosshead speed were 3 cm and 0.05 mm/s, respectively. The parameters obtained from stress–strain curves were: tensile strength (TS) calculated by dividing the peak load by the cross sectional area (thickness of film × 0.7 cm) of the initial film; elongation (E) calculated as the percentile of the change in the length of specimen respect to the original distance between the grips (3 cm), and elastic modulus (EM) calculated from the initial slope of the stress–strain curve (Han, Seo, Park, Kim, & Lee, 2006).

2.8. Statistical analysis

A full factorial design was performed in this investigation. Two factors were chosen as variables (drying temperature and BW content) and were tested at two (5 and 25 °C) and three levels (0, 20 and 40%), respectively. Analysis of variance was used and when the effect of the factors was significant ($p < 0.05$), the test of multiple ranks honestly significant difference (HSD) of Tukey was applied (95% of confidence level). The statistical analysis was performed using Minitab 13.20 (Minitab Inc., State College, PA).

3. Results and discussion

3.1. Characteristics of WPC emulsion films

In agreement with Pérez-Gago and Krochta (2000) scarcely emulsion separation was observed during drying. Pérez-Gago and Krochta (2000) observed this emulsion separation in almost all the studied WPI-lipid emulsion film compositions when they measured WVP placing the film surface which had been exposed to air during drying facing either “up” or “down” in the test cup. Therefore the film side exposed to air during drying was marked and was considered in both WVP tests and mechanical properties. Another phenomena observed during the drying process, consistent with Pérez-Gago and Krochta (2000), was a slight lipid migration towards the edges of the casting plate. The extent of this migration could affect the final appearance and the properties of the films. Pérez-Gago and Krochta (2000) attributed this migration to the fact that as the film started drying from the edges of the casting plates to the center, the lipid phase tends to migrate towards the more hydrophobic (lower moisture content) area. Our results showed that the decrease in drying temperature slowed the lipid migration phenomena and thus films had more homogeneous lipid distribution when dried at 5 °C.

3.2. Water vapor permeability

The WVP of edible WPC films with different formulations dried at 5 and 25 °C are shown in Fig. 1. The change of the drying temperature from 25 to 5 °C significantly decreased the WVP of the films containing BW in their formulations. This advantage can be attributed to lesser extension of lipid migration and thus a more homogeneous distribution of BW at low temperature. Possibly, this fact determined a higher effective concentration of lipid in the films dried at 5 °C compared with those obtained at 25 °C. Fig. 1 also shows that the addition of BW to the film significantly lowered film WVP because of an increase in hydrophobicity imparted by the wax. These results are comparable to those obtained by Talens and Krochta (2005) and Shellhammer and Krochta (1997). In films dried at 5 °C the decrease in WVP was dependent of the BW content in the film, but when the drying temperature was 25 °C only a level of 40% of BW had a significant effect on the WVP. In general, both drying at 5 °C and the addition of BW significantly improved the barrier properties of WPC emulsion films.

3.3. Solubility in water

Fig. 2 shows the water solubility of WPC emulsion films. All films were partially soluble (solubilities ranging 13.6–34.4%) maintaining their integrity during immersion. This was an indicative of highly stable protein-polymer network. Solubility is an important protein functional property and in the case of edible films solubility in water affects the usage of the film (Sothornvit & Krochta, 2000). For example, films on high-moisture foods must be insoluble, while films for water soluble pouches must be readily soluble.

The change of the drying temperature from 25 to 5 °C significantly increased the solubility of the films with BW but had no

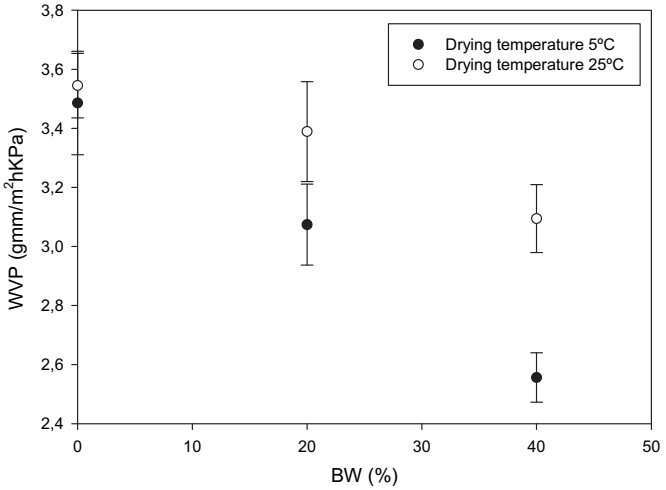

Fig. 1. Effect of drying temperature and BW content of WPC emulsion films on WVP. Bars are based on standard deviations.

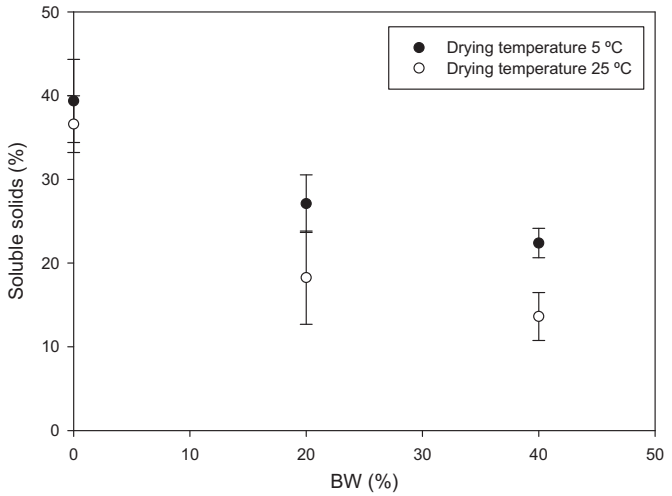

Fig. 2. Effect of drying temperature and BW content of WPC emulsion films on water solubility. Bars are based on standard deviations.

significant effect on the solubility of films without BW. Incorporation of BW reduced the solubility of the WPC emulsion films. Gennadios, Cezeirat, Weller, and Hanna (1998) and Kim and Ustunol (2001) reported that the adding of a lipid compound to the formulation determined a reduction of the solubility of soy protein films and whey protein films, respectively. In the formulation of films total solids level remained constant therefore increasing the amount of BW decreases total soluble matter, which translates into a decrease in the solubility of the film.

3.4. Mechanical properties

Tables 1 and 2 show the parameters calculated from force-deformation and stress–strain curves, respectively. The measurement of the mechanical properties of edible films is important because they relate their durability as well the ability to enhance the mechanical integrity of foods.

The decrease on drying temperature from 25 to 5 °C had no significant effect on any parameter evaluated with the puncture test. On the other hand, the decrease on drying temperature showed a significant effect in all the parameters evaluated by the tensile test in formulations without BW, but when BW was included in the formulations, drying temperature had no significant effect in most the evaluated parameters.

Although the drying behavior of thin films has been extensively studied for synthetic polymers, drying behavior of edible films have yet to be explored (Alcántara et al., 1998). The mechanical properties of methycellulose films and peanut protein concentrate films have been shown to improve when drying temperatures were increased (Donhowe & Fennema, 1993; Jangchud & Chinnan, 1999). Results were attributed to changes in film morphology and to the fact that higher drying rates induced crystallinity of methylcellulose and cross-linking of peanut protein concentrate. In contrast, Debeaufort and Voilley (1995) that studied emulsified methylcellulose-lipid films at several drying conditions observed that longer drying times of film-forming emulsion, produced by lower temperatures and higher relative humidities, provided better barrier and mechanical properties. Concerning whey protein films, Alcántara et al. (1998) observed that more rapid drying of WPI (95 °C and 30% RH compared to 21 °C and 50% RH) resulted in stiffer, less flexible films (higher Young's Modulus), but had smaller apparent effect on film tensile strength and elongation. In this case, higher drying rates formed denser WPI films. On the other hand, Pérez-Gago and Krochta (2000) drying WPI and WPI emulsion films at 25, 40 and 80 °C found that the drying temperature had no significant effect on tensile strength, elongation and elastic moduli of the films.

The addition of BW significantly decreased all the parameters evaluated in tensile and puncture tests at both studied temperatures. Shellhammer and Krochta (1997) and Talens and Krochta. (2005) found similar results for tensile test of whey protein emulsion films prepared with different lipids. Navarro-Tarazaga, Sothornvit and Pérez-Gago (2008) explained the negative effect of the addition of BW in hydroxypropyl methylcellulose (HPMC) films considering the BW caused the disruption of the HPMC continuous matrix inducing the development of a heterogeneous film structure; decreased the water affinity, which reduced the water plasticizer effect on film mechanical properties; and as the total solids content remained constant, reduced the HPMC content, which acts as film structural matrix. All this factors resulted in enhanced film brittleness and decreased tensile strength and elongation.

Table 1
Effect of drying temperature and BW content of WPC emulsion films on parameters derived from the puncture test.

Film sample			
Drying temperature (°C)	BW (%)	Puncture strength (N)	Deformation (mm)
5	0	1.863 ± 0.341^{a}	1.752 ± 0.140^{a}
5	20	0.207 ± 0.030^{b}	0.500 ± 0.091^{b}
5	40	0.188 ± 0.045^{b}	0.436 ± 0.074^{b}
25	0	2.067 ± 0.418^{a}	1.871 ± 0.204^{a}
25	20	0.243 ± 0.054^{b}	0.552 ± 0.103^{b}
25	40	0.178 ± 0.026^{b}	0.568 ± 0.098^{b}

Data corresponds to mean values and standard deviations of ten samples.
Values with different letters in each column are significantly different ($p < 0.05$).

Table 2
Effect of drying temperature and BW content of WPC emulsion films on parameters derived from the tensile test.

Film sample				
Drying temperature (°C)	BW (%)	TS (MPa)	E (%)	EM (MPa)
5	0	4.918 ± 0.778^{a}	3.499 ± 0.981^{b}	229.213 ± 21.618^{a}
5	20	2.629 ± 0.359^{c}	2.378 ± 0.356^{c}	184.670 ± 11.939^{b}
5	40	1.645 ± 0.329^{d}	1.797 ± 0.422^{c}	149.403 ± 11.939^{cd}
25	0	3.315 ± 0.433^{b}	4.360 ± 0.833^{a}	155.747 ± 20.630^{c}
25	20	2.133 ± 0.228^{cd}	2.390 ± 0.466^{c}	155.568 ± 9.113^{c}
25	40	1.646 ± 0.074^{d}	2.413 ± 0.529^{c}	132.891 ± 16.842^{d}

Data corresponds to mean values and standard deviations of ten samples.
Values with different letters in each column are significantly different ($p < 0.05$).

4. Conclusions

Our results showed that combination of two factors such as drying under refrigeration temperature and the addition of a lipid like BW to the WPC-Gly matrix could be appropriate for the coating of fruits in order to preserve their quality and prolong their shelf life. Particularly, a key parameter like WVP, which is involved in moisture retention, was improved by the decrease in drying temperature and by the addition of BW and is fundamentally related to fruit quality preservation. With reference to solubility the addition of BW was beneficial to the maintenance of film integrity while the decrease on drying temperature was detrimental but only at high BW contents. Finally, taking account the effect of the studied factors on mechanical properties the decrease in drying temperature was beneficial for maintaining both puncture strength and

deformation of the films in the puncture test and both tensile strength and elongation of the films in the tensile test. However, the addition of BW was not beneficial for the preservation of the mechanical properties.

People involved with food research and industry have to keep in mind that every change in the composition and in the production process results in the improvement of some physical property but can be detrimental to another's. Therefore, the search for improved compositions and process conditions is a matter of permanent concern and is related to the desired application of the edible film.

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