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Research Article

Characterization of Colombian Silk Sericin Dehydrated by Spray Drying and Freeze Drying

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Abstract: This study focuses on characterizing the sericin dehydrated by Freeze-drying (-82°C, 0.023 mbar, 72 h) and Spray drying (flow rate: 6.3 mL/min, T_{input}: 160°C, spray flow: 40 m^3/h), in order to evaluate its potential as an additive in developing food. The protein extraction was performed from defective cocoons from Corporación para el Desarrollo de la Sericultura del Cauca-CORSEDA (Popayán, Colombia), using hot water and an autoclave (1:30 p: v, 121°C, 30 min). Characterization was performed by Fourier transform infrared spectroscopy, scanning electron microscopy, thermo-gravimetric analysis, solubility in hot water, color, isoelectric point, protein content, swelling, fat adsorption capacity and water retention capacity. The results showed that the properties and behavior of the protein have a significant dependence on the dehydration method, which not only affects the morphology of the samples, but also their secondary structure. These results suggest that when incorporating sericin in the creation of food, it is necessary to consider not only what properties are intended to be a contribution to this development, but also, what its influence would be in the interrelation with the other components of the food matrix, in order to select the most appropriate dehydration method and application forms.

Keywords: Morphology, protein, silk, secondary structure, sericulture

INTRODUCTION

Silk is a natural filament segregated by the *Bombyx mori L.*, silkworm to make the cocoon during its metamorphosis stage. It consists of two proteins: fibroin, which makes up 70-80% of the cocoon and sericin, which represents approximately 25-30% of the weight. Fibroin is an insoluble, linearly structured protein with high mechanical resistance. On the other hand, sericin is a partially hydrosoluble protein with globular structure which is responsible for holding together the fibroin filaments during cocoon conformation (Campbell and Farrell, 2005; Cenis, 2008; Patel and Modasiya, 2011; Vázquez Martínez *et al*., 2005). Currently, the scientific community has shown great interest in the use of these two proteins, since they come from natural origin, are biodegradable, non-toxic and have great versatility in their uses.

The transformation process of silk, known as sericulture, is still very precarious in Colombia. At present, there are very small communities where sericulture is developed at the artisanal and semi-industrial level, in which the use of the material produced during the transformation of the cocoon to threads (pupae, fibrous material, sericin) is deficient. During this process, the best quality cocoons (Worm cocoons suitable for reeling silk) are used mainly to make silk threads. These cocoons are characterized by an adequate size, for not having stains and perforations and for not having fine tips, thus leaving the cocoons defective (perforated, double, deformed, with fine tips, stained) and other fibrous waste, for the production of coarse yarns or fillings, which are products of lower added value (Pescio *et al*., 2009). In both cases, both sericin and pupae are discarded.

Taking into account the above and that in Colombia knowledge of silk proteins is still very precarious, it is necessary to generate knowledge of their properties and to evaluate their relation with the processes used for their extraction and drying, considering internationally developed studies. In addition, it is important to take into account that the quality and properties of the silk obtained, as well as that of its components, depend on the genetic and modifiable characteristics of the silkworm, which are given by the place of production, (temperature, humidity, air, lighting) and the characteristics of the food available (Pescio *et al*., 2009; Rodríguez *et al*., 2012). In the case of sericin, the relationship between the extraction technique (degumming) and the recovery

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used (Aramwit *et al*., 2012) and the properties and final application of sericin obtained has been extensively studied.

Among the properties attributed to the sericin protein are; oxidation resistance, antibacterial and antimicrobial activity, protection against Ultraviolet (UV) solar radiation, easy absorption and release of moisture, the inhibition of tyrosinase and kinase activity, cellular activity, anticoagulant and anticancer properties. In addition, sericinpromotes cell growth and serves as a cicatrizant (Capar *et al*., 2009; Naskar *et al*., 2014; Ude *et al*., 2014; Vepari and Kaplan, 2007; Zhang, 2002). These properties have led to a growing interest in the use of this protein for developing products of high value; mainly for cosmetic, pharmaceutical and biomedical applications. Despite finding some reports which evaluate sericin as a food additive, there are still few studies (Züge *et al*., 2015)

Taking into account the above, this research was aimed at characterizing sericin extracted from defective cocoons and evaluating their properties in relation to the dehydration method (spray drying and freeze-drying), with the objective of determining if it has characteristics that enable it to be included in food matrices, being able to represent significant economic and social benefit for the Colombian sericulture sector.

MATERIALS AND METHODS

Materials: Defective cocoons (Dc) were obtained from the Corporación para el Desarrollo de la Sericultura del Cauca-CORSEDA (Popayán, Colombia). The Dc were cut into small segments (approximately 0.5 cm) by removing the dried pupae and other impurities.

Extraction of sericin protein: It was performed in hot water, using a Labtech autoclave equipment at a temperature of 121°C for 30 min, in a 1:30 (w/v) bath ratio. The obtained Sericin Solution (SS) was vacuum filtered to remove impurities and particulate material present in the sample.

Dehydration by freeze-drying: The filtered SS was frozen using liquid nitrogen and was placed into a dehydration procedure for 72 h using Labconco brand equipment, whose condenser temperature and pressure were -82°C and 0.023 mbar respectively.

Dehydration by spray drying: BUCHI brand equipment (B 290) was used. The filtered SS was pumped into the drying chamber at a flow rate of 6.3 mL/min, an inlet temperature of 160°C and a spray flow of 40 m^3/h.

Fourier transform Infrared Spectroscopy (FTIR): The secondary structure of the SS dehydrated by freeze-drying (SS_L) and spray drying (SS_S) was analyzed using a Nicolet 6700 Series spectrophotometer. The equipment has a diamond window mounted on a tungsten carbide support with a sample area of approximately 0.5 mm^2. For each sample, 64 scans were performed at a resolution of 4 cm^{-1} and a wavelength at 4000-400 cm^{-1}. A deconvolution was performed in the region of the amide I (1600-1700) using the software Origin 6.0, effecting a smoothing of Savitzky Golay with 7 points and forms of Gaussian curves.

Scanning Electron Microscopy (SEM): The morphology of both dehydrated samples was observed using a high vacuum scanning electron microscope with a secondary electron detector (JEOL JSM-6490LV brand). Prior to observation, a thin gold coating was performed using a DENTON VACUUM Desk IV brand kit until a thickness of approximately 7 nm was achieved. Subsequently the samples were fixed on graphite tape. The images were captured at 2000x, 5000x and 20000x.

Thermo Gravimetric Analysis (TGA): The thermal behavior of the samples was carried out on a Mettler Toledo TGA Q500 thermo gravimetric scale, where the thermo grams were obtained under an inert atmosphere of nitrogen, with a gas rate of 50 mL/min and a constant heating rate of 10°C/min, with a temperature range of 30 to 800°C.

Solubility index in hot water: It was performed by means of an adaptation of the method given by Anderson (1982) and Phoungchandang and Sertwasana (2010). About 25 mg of the dry sample was weighed into falcon tubes by adding 10 mL of distilled water; these were heated at three different temperatures (30, 60 and 90°C) for 30 min. After this time the samples were centrifuged at 6000 rpm for 15 min. Subsequently, the supernatant was separated from the precipitate, taking the first of them to a forced convection oven (maxthermo brand) at a temperature of 35°C for 24 h until constant weight was reached. This procedure was performed in triplicate. The calculation of the water solubility index of sericin was performed using the following equation:

$$I.S.A = \frac{(W_3 - W_2)}{W_1} * 100$$

where,

W_1 = The weight of the dry powder sample (g)
W_2 = The weight of the capsule (g)
W_3 = The weight of the capsule with the dry supernatant (g)

Color: It was carried out in a portable X-ray spectrophotometer series SP60. The CIEL*a*b Colorimetric system was used. The results were expressed as arithmetic averages, with which the chromatic difference (ΔE), the yellow/blue index (IA)

and the whiteness (IB) were calculated. These indices are calculated using the following equations, reported by Ramírez-Navas(2010), where:

$$\Delta E = \sqrt{(\Delta L^*_{r,s})^2 + (\Delta a^*_{r,s})^2 + (\Delta b^*_{r,s})^2}$$

$$IA = 142.86 \left(\frac{b^*}{L^*}\right)$$

$$IB = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Isoelectric point (pI): An adaptation of the method of Gonçalves *et al*. (1997) was carried out. The solutions obtained were centrifuged at 6000 rpm for 15 min and the supernatants were separated from the sediment. Then 6 aliquots were taken to bring them to different pHs from 6.0 to 3.5 at 0.5 pH unit intervals (Gonçalves *et al*., 1997). After adjusting these pH values, samples were again centrifuged under the same conditions. The supernatants obtained after centrifugation were recovered to determine the protein percentage of each aliquot by the Biuret method.

To determinate the isoelectric point by the Biuret method, a calibration curve was performed using a commercial sericin standard solution of the SIGMA brand. The prepared solutions in the color development tube were allowed to stand for 30 min at room temperature. The absorbance of the white was then read at 550 cm^{-1} to adjust for 100% transmittance or 0.0 absorbance.

Protein content: The protein content was performed using the Kjeldahl-Gunning-Arnold method, adapted by Griffin. Based on the data obtained, the percentage of nitrogen in the samples was calculated using the following equation:

$$\text{Nitrogen } (\%) = \frac{V * M * 14 * 100}{P}$$

where, V is the volume in liters of HCl consumed, M is the molarity of the standardized HCl and P the weight of the sample in grams. To determine the protein percentage of the samples the average nitrogen percentage of the samples is taken and multiplied by the factor 6.25 according to the literature (Chemist, 2005).

Determination of amino acid content by mass spectrometry MALDI-TOF: The samples were analyzed on an Ultraflextreme MALDI-TOF-TOF (Bruker Daltonics) mass spectrometer, operating in a positive reflectron mode, in a mass range of 700-3500 m/z.

Swelling (SW): It was determined by measuring the volume of the protein gain after reaching equilibrium with excess water. About 0.5 g of dehydrated sericin (SS_L and SS_S) were weighed in a graduated cylinder, which was hydrated with 10 mL of distilled water. Subsequently, the samples were allowed to stand for 24h at room temperature and thereafter, the final volume of the sample was measured. The results are expressed as mL of water/g of sericin.

Fat Adsorption Capacity (FAC): Approximately 0.5 g of dehydrated sample (SS_L and SS_S) were weighed and poured into a falcon tube, to which 10 mL of oil was added. Samples were allowed to stand for 24 h. After this time it was centrifuged at 4000 rpm for 20 min. The supernatant obtained was decanted and the precipitate was weighed. Lipid adsorption is expressed as g fat/g of sericin.

Water Retention Capacity (WRC): It was determined after centrifuging the water-insoluble residue. Approximately 0.5 g of the dehydrated sample (SS_L and SS_S) were weighed in a falcon tube and 10 mL of distilled water were added, they were allowed to stand for 24 h, after which time it was centrifuged at 4000 rpm for 20 min, the supernatant was decanted and the precipitate was weighed. The water retention is measured as g water/g of sericin.

The results of functional characterization tests (SW, FAC, WRC, protein content and solubility) were performed in triplicate.

Statistical analysis: For the results of the SW, FAC, WRC, protein content and solubility tests, a variance analysis (one-way ANOVA) was performed using Statgraphics, in order to determine the existence of a statistically significant difference between the values obtained for each dehydration method (freeze-drying and spray drying).

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR): Figure 1 shows the spectra of the samples SS_L and SS_S, where both show the presence of the characteristic peaks of the amide groups in the proteins: A, B, I, II and III. The amides A and B (3000, 3500 cm^{-1}) are related to the stretching of N-H links which overlap with the hydroxylated amino acid (OH) residues, such as serine and threonine (Teramoto and Miyazawa, 2005). The amine I (1600-1700 cm^{-1}) represents the stretching of the carbonyl group (C=O), which are involved with the backbone of the polypeptides and are therefore more sensitive to the secondary structure and molecular orientation of the protein. The amide II (1504-1582 cm^{-1}) is associated with the stretching of the C-N links and the deformation of the N-H links. Finally, the amide III (1200-1300 cm^{-1}) is found which relates to the same links as amide II (Aramwit *et al*., 2010; Choudhury and Devi, 2016; Tretinnikov and Tamada, 2001; Wasan and Prasong, 2011).

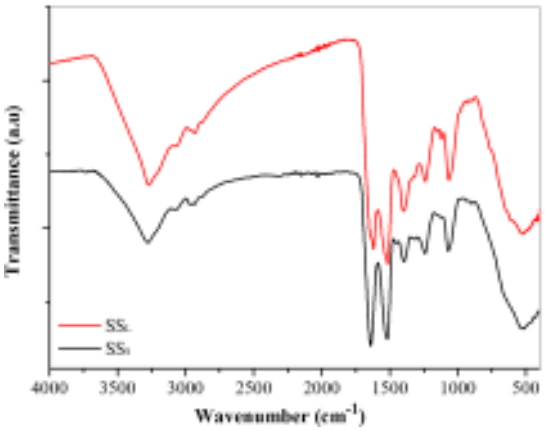


Fig. 1: FTIR of sericin extracted from defective silk cocoons, dehydrated by freeze-drying (SS_L) and spray drying (SS_S)

Table 1: Percentages of structures in SS_L and SS_S

Secondary structure	SS_L (%)	SS_S (%)
β-sheet	39.50	22.24
Random coil	20.08	47.90
α- helix	6.57	0.00
Turns	33.85	29.75
Curve adjustment (R^2)	0.99	0.99

For SS_L it was observed that its structure is β-sheet, since the amide I is located at 1623.77 cm^{-1}, the amide II at 1522.52 cm^{-1} and the amide III at 1241.93 cm^{-1} (Aramwit *et al*., 2010; Kim *et al*., 2012; Rajkhowa *et al*., 2011; Tretinnikov and Tamada, 2001). As for the SS_S sample, the amide II was present at 1516.05 cm^{-1} and the amide III at 1236.37 cm^{-1}, where the predominant structure in both is β-sheet. The vibrations of the amide I were observed at 1641.41 cm^{-1}, whose outstanding peak tends to a random coil structure (Tretinnikov and Tamada, 2001; Zhang *et al*., 2011). To confirm the type of secondary structure of both samples (SS_L, SS_S), the amide I was analyzed by a deconvolution of said region (Gupta *et al*., 2013; Tretinnikov and Tamada, 2001). Table 1 shows the percentage of secondary structures found in each of the samples, which were assigned according to other studies (Zhang *et al*., 2011; Hu *et al*., 2006). The results show that SS_L has a predominant β-sheet structure (39.64%), whereas for SS_S it is a random coil (47.90%).

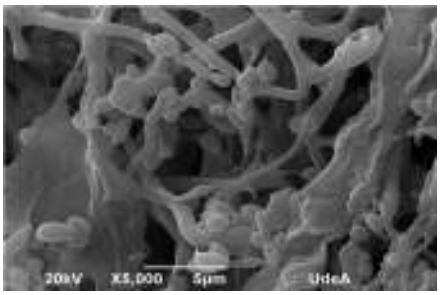
These results show that the dehydration process favors the conformation of some secondary structures in the samples. According to Kim *et al*. (2012), the process of removing water during freeze-drying can cause changes in the secondary and/or tertiary structure of the protein. This is because, during the process, as the water is removed, the chains of the protein enter in proximity, favoring the aggregation of the same and therefore the conformation of structures β-sheet, which show a more stable conformation (Kim *et al*., 2012). In this same research the authors observed that, to obtain a random coil structure, lio-protectors can be added (Aramwit *et al*., 2010; Kim *et al*., 2012; Rajkhowa *et al*., 2011; Tretinnikov and Tamada, 2001), since the hydroxyl groups of these compounds form hydrogen links with the protein, avoiding the aggregation of their chains when the water is sublimated (Kim *et al*., 2012).

The low portion of α-helix structure that is present in the SS_L sample, which is not observed in SS_S, confirms the results found in the literature, where it is reported that sericin extracted from liquid silk and silk cocoons is formed mainly by a random coil structure, followed by β-sheets and no α-helix (Komatsu, 1975; Mondal *et al*., 2007). On the other hand, it has been reported that sericin extracted from liquid silk for 45 min with hot water contains a small fraction (10%) of α-helix (Mondal *et al*., 2007), which shows that some processes favor the appearance of this structure, as observed in the SS_L sample, which, after the freeze-drying process presents 6.57% of these structures, unlike what is observed in SS_S.

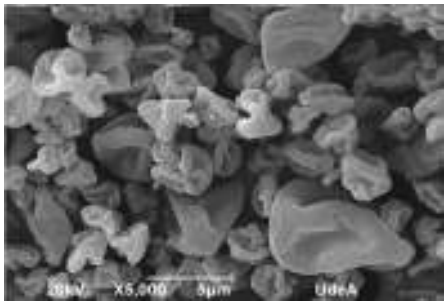
Since the inherent three-dimensional structure of the protein defines the activity of the protein, the changes that it experiences as a consequence of the processes to which it is subjected, can cause the loss of properties or generate different behaviors (Kim *et al*., 2012).

Scanning Electron Microscopy (SEM): Figure 2 shows the morphology of dehydrated SS_S and SS_L. The SS_L sample has a structure formed by three-dimensional interconnected fibrils forming a highly porous network (Fig. 2). According to Ribeiro *et al*. (2014), the size of these pores depends on the size of the ice crystals formed during freezing (Ribeiro *et al*., 2014), which depend on the temperature used. For example, frozen samples at very low temperatures have a smaller porosity compared to samples that are frozen at higher temperatures. This is because the freezing process carried out at higher temperatures leads to slow ice nucleation, which produces larger ice crystals, while the freezing process at lower temperatures is particularly faster, leading to obtain smaller pores (Sachlos and Czernuszka, 2003). Another feature observed in the SS_L sample are the globular structures marked in a red circle, which are characteristic of sericin. On the other hand, Mosharraf *et al*. (2007) mentions in his study that the freeze-drying generates an amorphous or partially amorphous structure that increases the stability of the protein, which reduces the mobility and reactivity of the molecule and therefore the protein-protein interactions (Mosharraf *et al*., 2007).

As for the SS_S morphology, the presence of concave spheres of different sizes, partially agglomerated, ranging from 1.75 to 3.98 μm was evidenced (Fig. 2b). According to Genc *et al*. (2009), the particle size depends on the flow rate of solution fed to the equipment and the concentration of the protein in the solution, while the temperature has no effect on this



(a)



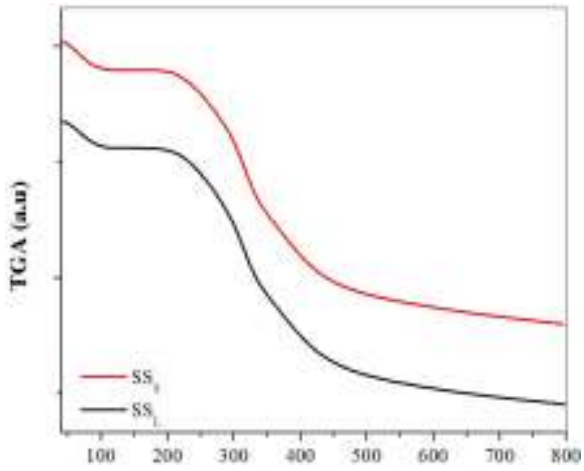
(b)

Fig. 2: SEM of sericin dehydrated by the methods: a) freeze-drying (above) and b) spray drying (down)

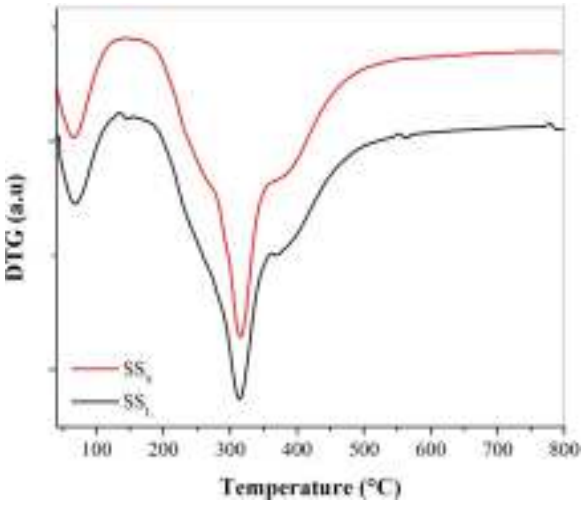
characteristicoron their morphology. The authors also report that particle agglomeration can occur as a consequence of the moisture content of the samples (Genc *et al*., 2009).

Gulrajani *et al*. (2009) show in their study a morphology similar to those obtained in this study in SS_S. They describe that the sample has collapsed spherical shapes, with dents of different depths and sizes, as well as a smooth-looking surface. They further explain that the collapsed structure of the particles is a consequence of the rapid evaporation of the internal water, leaving a concentration of droplets, which tend to form dents (Gulrajani *et al*., 2009). Likewise, Chollakup *et al*. (2015) report that dehydrated sericin particles by spray drying can handle large size distribution and resemble shrunken hollow spheres, which is established as a result of the sample preparation (Chollakup *et al*., 2015).

Thermo Gravimetric Analysis (TGA): Figure 3a shows the thermal behavior of SS_L and SS_S. A first loss of weight is evidenced at a temperature between 50-110°C, related to the evaporation of water remaining in the samples. After this interval no changes are evident until reaching a temperature of approximately 200°C. Subsequently, there is a second thermal event, related to the removal of volatile compounds, followed by the degradation of the side chains of the amino acids and the rupture of the peptide bonds, followed by an almost complete degradation of the sericin at



(a)



(b)

Fig. 3: (a): TGA (above) and (b): DTG (down) of sericin extracted from defective silk cocoons

temperatures of approximately 480°C (Chollakup *et al*., 2015; Ho *et al*., 2012; Khan *et al*., 2013; Zhang *et al*., 2012). The maximum degradation temperature for both samples was approximately 314°C (Fig. 3b), suggesting that the dehydration method and the differences in its secondary structure did not significantly affect the thermal properties of the samples. Both samples present a third thermal event above 360°C, of which no explanation has been found in the literature.

Solubility index in hot water: The highest solubility of the SS_L and SS_S samples was observed at a temperature of 90°C (91%) and 60°C (86%) respectively (Fig. 4). The variance analysis showed that there is no uniform trend and that the results are dependent on both the dehydration method and the solubilization temperature. The behavior of the samples is due to the fact that the β-sheet structure, present

Table 2: CIEL*a*b parameters and indexes of yellow / blue and whiteness

	Parameters CIEL*a*b			Indexes	
Sample	L*	a*	b*	IA	IB
SS_S	90.63±0.09	-1.00±0.02	3.90±0.01	6.14	89.80
SS_L	89.19±3.52	-0.37±0.22	4.35±0.57	6.97	88.34

L*: brightness and takes values from 0 (black) to 100 (white); a*: green chromaticity (-60) to red (+60); b*: chromaticity of blue (-60) to yellow (+60). The yellow/blue index (IA) and the whiteness (IB)

Table 3: CIEL*a*b parameters of the CMC

CEIL*a*b Parameters	L*	a*	b*
CMC	86.77	-0.26	8.16

L*: brightness and takes values from 0 (black) to 100 (white); a*: green chromaticity (-60) to red (+60); b*: chromaticity of blue (-60) to yellow (+60)

Table 4: Chromatic difference of SS_S and SS_L with respect to CMC

	Deltas from the CIEL*a*b parameters of the SS_L, SS_S and CMC sample			
Sample				ΔE*
SS_S	3.86	-0.73	-4.26	5.80
SS_L	2.42	-0.11	-3.81	4.51

ΔE*: chromatic difference

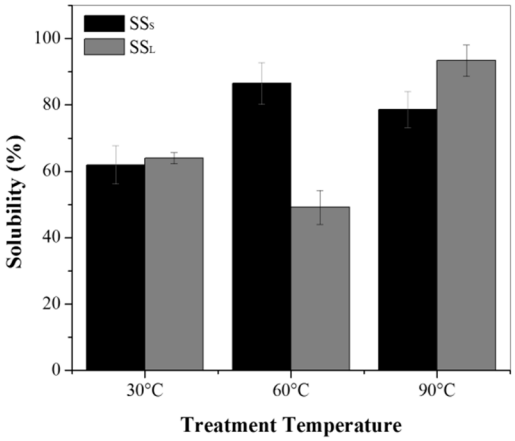


Fig. 4: Solubility of sericin extracted from defective silk cocoons

mainly in the SS_L sample, has a lower solubility compared to the random coil structure, predominant in SS_S (Kim *et al*., 2012). Solubility is closely related to the molecular conformation of the protein, where the β-sheet structure provides little solubility at low temperatures, unlike the random coil, which is readily soluble in water at room temperature (Padamwar and Pawar, 2004; Tsukada, 1980).

Similar results were observed by Kim *et al*. (2012), who obtained sericin with a β-sheet structure after freeze-drying without using lio-protector and they observed that the sample had a solubility of 60% at room temperature, while a dehydrated sample with the addition of lio-protectors, by having a higher content of random coil structures, has a higher solubility (Kim *et al*., 2012).

Color: In Table 2 the CIEL*a*b parameters of the dehydrated sericin samples can be seen, with the values of yellow/blue (IA) and whiteness (IB) being calculated. The results show that the parameter L* does not show a dependence of the dehydration method and takes values close to 100, which indicates a white luminosity. In contrast to L*, the parameters a* and b* are affected by the dehydration method, where both b* values are positive (yellow chromaticity) and both a* values are negative (tendency to green).

Table 5: Relationship between the observer's judgment and the color difference ΔE* (Ramírez-Navas, 2010)

Sensory perceived difference	Value ΔE* (instrumentally)
None	0-0.7
Light	0.7-2.5
Remarkable	2.5-3.0
Appreciable	3.0-6.0
Considerable	6.0-12.0
Extraordinary	12.0

Based on the report by Ramírez-Navas (2010), an IB value of 100 shows an ideal white and any difference with this value suggests an approximation to the yellow/blue index (IA). When the IA value grows on the positive scale (+) indicates an approximation towards yellow and if it goes towards the negative scale (-) indicates a tendency towards the blue (Ramírez-Navas, 2010). According to the obtained indexes for IB and IA, both samples are slightly distant from the ideal white and have a tendency towards yellow.

To calculate the chromatic difference (ΔE*) of the sericin samples, the CIEL*a*b parameters of the CMC were used as white (Table 3), considering that this is one of the most used additives in the food industry. The results of this test are shown in Table 4. According to the National Institute of Agricultural Technology (INTA), values of ΔE* equal to, or greater than 2.70 will be appreciable to the human eye (Table 5) (Casassa and Sari, 2006), therefore and based on Table 4 and 5, it can be concluded that the color differences between SS_L and SS_S, with regard to the CMC, are appreciable to the human eye; being ΔE* the highest in the SS_S sample.

Isoelectric point (pI) and protein content: The results presented in Table 6 show that the pI of the dehydrated

Table 6: Protein content and isoelectric point of extracted sericin

Sample	Protein content	Isoelectric point
SS_S	94.22 ± 1.09	4.54
SS_L	97.32 ± 0.33	5.5

Table 7: Amino acid content in the sericin of defective cocoons

AA	Percentage	HFo*	HFi**
Alanine	7.91	X	
Arginine	4.28		X
Asparagine	2.31		X
Aspartic acid	6.59		X
Cysteine	5.77		X
Glutamine	3.29		X
Glutamic acid	9.72		X
Glycine	2.80		X
Histidine	2.80		X
Isoleucine	2.47	X	
Leucine	10.71	X	
Lysine	9.88		X
Methionine	0.82	X	
Phenylalanine	4.94	X	
Proline	4.61	X	
Serine	5.27		X
Threonine	5.60		X

sericin samples have dependence with the dehydration method, where the highest pI is present in the sample dehydrated by freeze-drying. The higher isoelectric point of SS_L may be related to the predominant structure of this sample (β-sheet), which affects properties such as solubility. Similar isoelectric points are reported for proteins with this type of structure; For example, fibroin has a pI of 5 (Patil *et al*., 2015; Tang *et al*., 2015), as well as proteins such as β-lactoglobulin (pI 5,2) and bovine serum albumin (pI 5,3) (Badui Dergal, 2006). As for SS_S, it presented a behavior similar to that reported by authors such as Jo and Um (2015), which indicate apI for sericin extracted in hot water of 4.3 (a fact which is consistent with that published by other authors (Sookne and Harris, 1939).

The results of the protein content show that this characteristic does not depend on the dehydration method (Table 6). However, Gulrajani *et al*. (2009) have reported that the extration method can affect the different properties of the sericin obtained, as well as the protein content. These authors found that sericin extracted with hot water (HTHP autoclave) has a highest content (98.7%), compared to sericin powder obtained from alkaline liqueurs (92%) and degummed with alkaline soap (58%), it is due to the presence of residues of the chemical agents used. In a study of sericin extraction using hot water (Gulrajani *et al*., 2009; Wu *et al*., 2007), cited by Gulrajani *et al*. (2009), found a 90% of protein content.

Determination of amino acid content by mass spectrometry MALDI TOF: According to the results, the dehydration method does not affect the chemical composition of the samples and therefore their amino acid content is the same (Table 7). Taking into account the characteristics of each amino acid, it can be said that sericin obtained from defective cocoons has a 32.22% content of hydrophobic amino acids and 61.78% hydrophilic. On the other hand, 51.88% of them are considered essential amino acids.

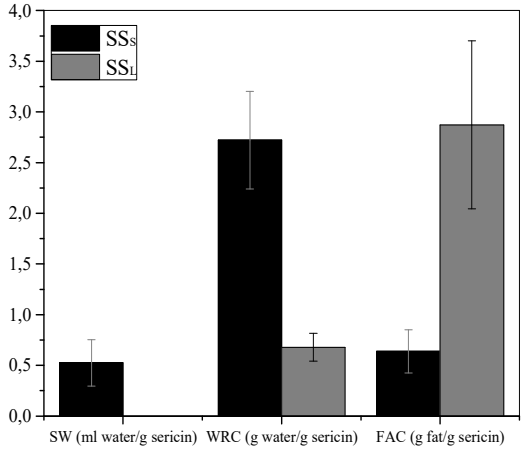


Fig. 5: Behavior of SW, FAC, WRC for the extracted sericin sample

Swelling (SW), Fat Adsorption Capacity (FAC) and Water Retention Capacity (WRC): As shown in Fig. 5, the dehydration method also has a significant influence on the capacities of the samples. The results indicate that the FAC of SS_L is higher than SS_S. This suggests that both the type of structure (β-sheet and random coil) and the samples morphology could be affecting this property.

According to the findings of Mirhosseini *et al*. (2013), the FAC depends on the content of the hydrophobic fraction and on the non-polar side chains present in the samples, thus, when there is a greater presence of amino acids with these characteristics, the sample tends to interact and absorb more greasy material as it can join to the oil's hydrocarbon chains, resulting in a greater FAC (Mirhosseini *et al*., 2013). In spite of this, the results of this investigation show that there is no relationship with the content of hydrophobic amino acids, since as mentioned above; both samples have the same composition. This suggests that β-sheet structures and/or the freeze-drying technique are favoring the exposure of these hydrophobic groups and therefore the higher FAC in the SS_L samples.

As for WRC, it was observed that it is higher in the sample dehydrated by spray drying, which could be due, as mentioned above, to the greater content of random coil structures, or that this method and the morphology obtained from the samples favors the exposure of hydrophilic groups. Like the behavior observed in the FAC, there is no evidence of an effect of the relation of hydrophilic/hydrophobic amino acids on this capacity, as well as to the partial dissociation and unfolding that occurred in the protein, which led to the exposure of hydrophilic/hydrophobic amino acid residues, thereby increasing surface activity and water/oil adsorption (Ghribi *et al*., 2015).

According to Chavan *et al*. (2001), WRC depends on the conformational characteristics, the hydrophilic/hydrophobic balance of amino acids and the thermodynamic conditions of the process and the environment, as well as the solubility of protein molecules (Chavan *et al*., 2001). This latter characteristic confirms the results found in the solubility tests of SS_S and SS_L, which shows that sericin dehydrated by spray drying when having a higher content of random coils, has a higher solubility, which favors WRC.

Regarding SW, a dependence of the dehydration method and therefore, a relation with the secondary structure of the samples is evidenced, as analyzed previously. According to the results of the FTIR, freeze dried sericin has a β-sheet structure, while the sample dried by spray drying is randomly coil, so that the latter have a greater capacity to capture water and therefore to increase its volume in its presence.

In the specific case of sericin, no previous studies on these properties are available to analyze the behavior obtained. However, studies reported by Modercay and Silva Bermudez (1994) show that the properties of bean protein concentrates dehydrated by different techniques depend on the methods used for dehydration (hot air, freeze-drying, parallel rolls). The results showed that WRC and SW of the dehydrated product by freeze-drying and in rolls; are similar and can eventually be used as ingredients in baked products (Modercay and Silva Bermudez, 1994).

The variance analysis for SW, WRC and FAC confirms that there is a statistically significant difference between the dehydration methods with 95% accuracy.

CONCLUSION

Proteins are some of the components responsible for the properties of foodstuffs. Studies have shown the influence of drying on the properties of these molecules, where it is shown that variables such as time, temperature and humidity play a fundamental role (Dehnad *et al*., 2016). As noted in this study, the dehydration method can modify properties such as solubility index, the isoelectric point, WRC, FAC and SW, whereas characteristics such as color, thermal stability, protein content and amino acid composition are not affected. According to the results, one of the main causes for these changes is the secondary structure adopted by the dehydrated samples by the methods used, mainly random coils (SS_S) and β-sheet (SS_L). The characteristics of these structures determine the stability of the protein, understood as its mobility and reactivity and therefore affect its activity and properties and therefore its final application.

Another factor that affects the dehydration method is the morphology of the samples. From the remarks made it is suggested that this may favor the exposure of hydrophobic and/or hydrophilic groups, which play an important role in solubility and the isoelectric point and may be determinant in properties such as WRC, FAC and SW.

Although no influence of the dehydration method on color, thermal stability, protein content and amino acid composition was seen, it is important to emphasize the importance of knowing these characteristics for the formulation of food products, since they determine working conditions, as in the case of thermal stability and organoleptic and nutritional quality factors in the case of the other three mentioned characteristics.

As a final conclusion, it was observed that the dehydration method determines the properties of the dehydrated sample and to use sericin as an ingredient in a food formulation, it is necessary to know the properties that it brings to each method, or the properties that are required in said formulation, in order to make a selection of the most indicated method.

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