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Electrostatic Interaction of Proteins Improves the Stability of A Food Powder Enriched with β-Carotene

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Abstract. The aim of this work was to determine the effect of hydrolyzed marine collagen and soy protein isolate in the stability of protein food powder enriched with β-carotene. Four powders (SPI-0.99:HMC-0.00, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99) were prepared by freeze-drying with a concentration of 1 % (w/v) of β-carotene, varying the weight fraction of hydrolyzed marine collagen (HMC) and soy protein isolate (SPI). The powders were stored for 3 weeks in a water activity (aw) range from 0.11 to 0.84 at 25 °C and the degradation of β-carotene was determined by UV-vis spectrophotometry. After, it was caried out differential scanning calorimetry (DSC) at aw= 0 and a_w= 0.328. Finally, the powders were analyzed by infrared spectroscopy (FTIR) at a_w= 0.328 and were determined their technological properties. SPI-0.74:HMC-0.25 presented the highest intensity at 3277 cm-1 (N-H stretching), the highest percentage of β-carotene retention (83.84 %) and the highest glass transition temperature value (T_g=147.57 °C) at a_w=0.328. The results showed that there is a greater interaction of marine collagen and soy protein in a specific ratio and water activity, which

J. Mex. Chem. Soc. **2026**, *70(1):e2501*
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allows for the preservation of a greater amount of β-carotene.
Therefore, the development of this study is an advance in identifying new factors that produce better stability of food powders rich in proteins and antioxidants.

Resumen. El objetivo de este trabajo fue determinar el efecto del colágeno marino hidrolizado y del aislado de proteína de soya en la estabilidad de un alimento proteico en polvo enriquecido con β-caroteno. En este trabajo se prepararon cuatro polvos (SPI-0.99:HMC-0, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.5 and SPI-0:HMC-0.99) mediante liofilización con una concentración de 1 % (p/v) de β-caroteno, variando la fracción en peso de colágeno marino hidrolizado (HMC) y aislado de proteína de soya (SPI). Los polvos se almacenaron durante 3 semanas en un rango de actividad de agua (a_w) de 0.11 a 0.84 a 25 °C y la degradación del β-caroteno se determinó por espectrofotometría UV-vis. Después, se realizó calorimetría diferencia de barrido (DSC) a a_w= 0 and a_w= 0.328. Finalmente, los polvos fueron analizados por espectroscopia infrarroja (FTIR) a a_w= 0.328 y se determinaron sus propiedades tecnológicas. SPI-0.74:HMC-0.25 presentó la mayor intensidad a 3277 cm^{-1} (estiramiento N-H), el mayor porcentaje de retención de β-caroteno (83.84 %) y el mayor valor de temperatura de transición vítrea (T_g=147.57 °C) a a_w=0,328. Los resultados mostraron que existe una mayor interacción del colágeno marino y la proteína de soya en una especifica proporción y actividad de agua, lo que permite conservar una mayor cantidad de β-caroteno, por lo tanto, el desarrollo de este estudio es un avance para identificar nuevos factores que producen una mejor estabilidad de los polvos alimenticios ricos en proteínas y antioxidantes.

Introduction

In recent years, society's concern has increased to improve its quality of life and health through the practice of physical activity and the consumption of dietary supplements. This has promoted the development of powdered and liquid food products that offer certain nutritional requirements of consumers, such as proteins, vitamins, minerals and antioxidants. In this regard, a protein powder product enriched with β-carotene can be a new food that offer society a source of amino acids and antioxidants.

The β-carotene is a bioactive compound that the human body cannot synthesize itself, therefore it is important that humans obtain it through diet or taking supplements [1]. Consumption of sufficient amounts of β-carotene has been linked to great health benefits such as reducing the risk of breast cancer, cardiovascular disease, and other chronic disorders [2]. However, despite the multiple benefits that β-carotene brings to human health, there are many challenges that limit its use due to its low chemical stability and degradation when it is exposed to many environmental factors. Therefore, it is necessary to carry out studies of various materials that can protect this compound from degradation. In this regard, the protection of this compound has been carried out with various wall materials such as chitosan, cyclodextrin, whey and soy protein, etc. [3,4].

There is much research that has demonstrated the effectiveness of soy protein isolate in mixtures with other biopolymers as an encapsulating agent to prevent degradation and improve the stability and bio accessibility of β-carotene [5,6,4,7], but there is no research where the hydrolyzed collagen marine is used with soy protein isolate. Also, soy protein is a source of high-quality vegetable protein because of has similar amino acid composition and digestibility to milk and whey, making it, with high encapsulation performance of bioactive nutrients and ease of obtaining [8,9]. In the case of marine collagen, no studies have been found on its use in the encapsulation of β-carotene, but they have been found in other compounds such as curcumin and polyphenols [10,11]. Collagen is known for its anti-aging effects; it is the main structural protein and represents 30 % of the body's total proteins. Collagen provides resistance and structural stability, and likewise carries out other types of regulatory activities, such as tissue development and repair [12], as well as bioactive peptides from marine collagen have been used as preservatives or antioxidants to prevent food spoilage [13].

Based on what was described above, this work proposes the use of hydrolyzed marine collagen and soy protein isolate as encapsulating agents to prepare a protein food powder rich in amino acids and antioxidants, expecting an improvement of the stability of β carotene during storage due to a synergistic effect between the proteins used. Therefore, the aim of this work was to determine the effect of hydrolyzed marine collagen and soy protein isolate in the stability of protein food powder enriched with β-carotene.

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J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

Experimental

Materials

Hydrolyzed marine collagen (SESEN, Fercel Food Supplements) and soy protein isolate (NATSA, Productos Naturales) with 90 % protein. Synthetic β-carotene > 93 % powder. Lithium chloride (LiCl), potassium acetate (CH3COOK), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), magnesium nitrate (Mg $(NO_3)_2$), sodium nitrite ($NaNO_2$), sodium chloride (NaCl). The reagents and synthetic β-carotene were obtained from Sigma Aldrich, Mexico.

Methods

Obtaining powders

Four different powders were prepared with hydrolyzed marine collagen, soy protein isolate and β-carotene varying the weight fraction of hydrolyzed marine collagen (SPI-0.99:HMC-0, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.5 and SPI-0:HMC-0.99). Suspensions were made with a concentration of solids of 20 % (w/v) [14], and with a 1 % (w/w solids) concentration of β-carotene. The mixtures were homogenized at 1200 rpm for 20 min at 25 °C in a OHAUS GUARDIAN 5000 stirrer. The 0.75 weight fraction was not considered because the collagen precipitated. Once the stirring was completed, the mixtures were poured into plastic containers and covered with aluminum foil to avoid contact with sunlight, then frozen at -70 °C before freeze-drying. The freeze-drying process was carried out at a temperature of -49 °C and a pressure of 0.05 mBar using a benchtop freeze dryer (Labconco Freezone, USA) for 48 h.

Powders appearance

An initial photograph of the powders was taken after freeze-drying with the Poco X3 mobile cellular device (Sony, IMX682, China) with 64 MP camera inside a dark space, and another photograph was also taken after three weeks of storage at 25°C in a laboratory incubator (Fisher Scientific model FFU20F9CW3, USA) in a range of water activities from 0.11 to 0.84.

Determination of β-carotene

β-carotene of SPI-0.99:HMC-0, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.5 and SPI-0:HMC-0.99 were determined after freeze-drying and after storage at 25 °C in a laboratory incubator (Fisher Scientific model FFU20F9CW3, USA) in a range of water activities from 0.1 to 0.9. β-carotene was determined following the approach reported by Li et al. [15] with some modifications. First, β-carotene was weighed and dissolved in a 3:1 binary solution (ethanol and n-hexane, respectively) and then was diluted. The absorbance of β-carotene was measured at 450 nm using a UV-vis spectrophotometer to generate the standard curve. After, the binary solution was added to the powders, the liquid phase was collected, and their absorbance was quantified at 450 nm. The amount of encapsulated β-carotene was determined by the standard curve. The retention percentage (RP) was calculated employing equation 1.

$$\text{RP}\ (\%) = \left(\frac{m_1}{m_2}\right) \times 100 \qquad \text{Eq (1)}$$

where m_1 represents β-carotene concentration after storage, and m_2 represents β-carotene after freeze-drying.

Determination of T_g of powders

The glass transition temperature (T_g) of the samples were investigated using a differential scanning calorimeter (TA Instruments, Discovery model, USA) following the methodology of Fongin et al. [16] with some modifications. Before carrying out the DSC analysis, the samples were equilibrated inside desiccators with phosphorus pentoxide and saturated solution of magnesium chloride (MgCl) at 25 °C to evaluate the Tg at a_w= 0 and a_w= 0.328, respectively. The measurement was carried out in the temperature range from −20 °C to 200 °C at 3 °C/min. The temperature and heat flux were calibrated with indium and distilled water, while alumina powder was used as a reference. A nitrogen atmosphere was used with a flow rate of 40 mL/min. The samples (5-10 mg) were hermetically sealed in the aluminum tray of the DSC. In order to cancel the enthalpy

J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

relaxation effect of the powder, a second thermal scan of each sample was performed. Tg was determined using TA Instruments Trios 5.1.1 software, which was interfaced with the DSC.

FTIR analysis

The samples equilibrated at a_w=0.328 were characterized by ATR-FTIR spectroscopy according to the methodology described by Van Soest et al. [17]. Spectra were obtained from a FTIR standard spectrometer (Perkin Elmer, Waltham, MA, USA) using an accessory of diamond crystal ATR. FTIR spectra were fitted with the Gaussian and Lorentzian curve fitting algorithm from specific bands by deconvolutions. The average of 32 scans from 400 to 4000 cm^{-1} at 1 cm^{-1} resolution was collected.

Technological properties of powders

Solubility

The study of the solubility of the samples was carried out following the methodology proposed by Alvarez-Ossorio et al. [18] with some modifications. 500 mg of powders were dispersed in 20 mL of water. The protein dispersion was homogenized for 10 minutes at 3000 rpm, and then centrifuged (centrifuge HERMLE Z 206 A, USA) at 1800 rcf for 20 minutes. The supernatant was taken, and the amount of protein was evaluated using the Biuret method.

Emulsification

The emulsifying properties were evaluated following the methodology reported by Alvarez-Ossorio et al. [18] with some modifications. First, the powders were dispersed in water at 2 % (w/v) concentration. After, 15 mL of these suspensions were homogenized with 15 mL of sunflower oil in centrifuge tubes for 10 minutes at 3000 rpm. The tubes were then centrifuged (centrifuge HERMLE Z 206 A, USA) at 1800 rcf for 10 minutes and the emulsifying capacity was measured. The emulsifying capacity (EC) was expressed as the percentage of the emulsified layer of the total volumen remaining after centrifugation, with the following equation:

$$\mathrm{EC}(\%) = \frac{\text{Volume of emulsified layer}}{\text{Total volume}} \times 100 \qquad \text{Eq (2)}$$

Foam capacity

The methodology proposed by Chen et al. [19] was used with some adjustments. First, powders solutions were prepared in water (2 % w/v), after the solutions were vortexed at 3000 rpm for 10 min. The amount of foam was evaluated to calculate the FC (foaming capacity) following equation 3:

$$\mathrm{FC}(\%) = \frac{\text{Foam volume}}{\text{Volume of sample solution}} \times 100 \qquad \text{Eq (3)}$$

Water and oil holding capacity

The water holding capacity (WHC) and oil (OHC) were determined according to the methodology proposed by Alvarez-Ossorio et al. [18] with some modifications. 0.25 g of each sample was left for two nights in tubes previously weighed with 15 mL of distilled water or sunflower oil, centrifuged at 1800 rcf for 20 minutes and the supernatant was discarded. WHC and OHC were determined according to the following equations:

$$\mathrm{WHC} = \frac{(\text{g de protein} + \text{water}) - (\text{g de protein})}{\text{g de protein}} \qquad \text{Eq (4)}$$

$$\mathrm{OHC} = \frac{(\text{g de protein} + \text{oil}) - (\text{g de protein})}{\text{g de protein}} \qquad \text{Eq (5)}$$

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J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

Statical analysis

An one-way ANOVA was performed to determine significant differences between of powders samples with an alpha level of 0.05 ($P \leq 0.05$) by Tukey´s test. The analysis was performed using the statistical analysis package SigmaStat version 3.5.

Results and discussion

Powder appearance

Table 1 shows the images of the four powders after stored at different water activities for 3 weeks at 25 °C and the powders before being stored (initial). Initially, SPI-0.99:HMC-0.00 and SPI-0.74:HMC-0.25 presented a reddish-yellowish tone, while SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 showed gray tones because the concentration of HCM was higher than that of SPI. Visible surface changes with respect to water activity were similar across the four powders after three weeks of storage at 25 °C. At water activities of a_w = 0.113, a_w = 0.328, and a_w = 0.529, the color and texture remained similar before and after storage, while in a_w=0.753 and a_w=0.843 color changes were observed, and a caking process were already evident. This suggests that the physicochemical properties of powders are less affected when stored at water activities lower than 0.529, while have evident plasticization when stored above this water activity. This change was like that reported by Shih et al. [20], who stored soy protein isolate powder in two different a_w (0.25 and 0.75) and temperature (25 and 45 °C) during a period of 224 days to evaluate their effect on the physicochemical characteristics and functional properties of powder. They noted considerable changes in color and texture at water activity of 0.75 after storage, while at a_w = 0.25 the color and texture were very similar at the beginning and end of storage.

Table 1. Appearance of the powders after three weeks of storage at 25 °C.

a_w	SPI-0.99:HMC-0.00	SPI-0.74:HMC-0.25	SPI-0.49:HMC-0.50	SPI-0.00:HMC-0.99
Initial				
0.113				
0.328				
0.529				
0.753				
0.843				

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J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

β-Carotene retention percent

Fig. 1 shows the β-carotene retention percent values of the four powders after being stored for three weeks at 25 °C in a water activity range from 0.11 to 0.84. The highest β-carotene retention values were reflected in the SPI-0.74:HMC-0.25 powder, however, SPI-0.74:HMC-0.25 and SPI-0.49:HMC-0.50 containing soy protein isolate and marine collagen presented retention values higher than 60 % when they were stored at $a_w \leq 0.529$ after three weeks of storage, which is similar to that reported by Geng et al. [7], who encapsulated β-carotene using a nanoemulsion with soy protein isolate-epigallocatechin gallate-maltose conjugate (SPI-EGCG-maltose) by ultrasound. They reported that the retention rate of β-carotene gradually decreased in storage, but the samples maintained a value above 60 % after 30 days of storage. On the other hand, Ma et al. [21] employed the ultrasound-prepared electrostatic complex and covalent conjugate of soy protein isolate (SPI) and citrus pectin (CP) to prepare β-carotene-loaded nanoemulsions. Nanoemulsions stabilized by ultrasound-treated complex/conjugate showed the highest encapsulation efficiency and exhibited the highest stability during 14-day storage at 25 °C. These nanoemulsions also demonstrated the highest absolute values of zeta potential, indicating that both electrostatic complexation/covalent conjugation and ultrasound treatment could significantly improve the stability of the resulting nanoemulsions. Xu et al. [22] demonstrated that modified aggregated insoluble soybean protein hydrolysate-xanthan gum complexes stabilize water-in-oil-in-water emulsions, improving encapsulation efficiency and bioavailability of vitamin C and β-carotene, with optimal stability and carrying capacity achieved at 0.25 % w/v XG concentration. They observed a decrease in the AISPH α-helix and β-sheet content, surface hydrophobicity, and fluorescence intensity all decreased after binding. In this work, the highest β-carotene retention result was given at a_w=0.328 (83.84 %) in SPI-0.74:HMC-0.25 powder after 3 weeks of storage, which may be related to the good interaction between the components providing greater stability in the structure and better protection to the compound, since according to the FTIR results (Fig. 2). Although SPI-0.99:HMC-0.00 and SPI-0.74:HMC-0.25 powders had similar spectrum, a greater intensity is noted at 3277 cm^{-1} corresponding to SPI-0.74:HMC-0.25, which indicates stretching N-H between amides, so the marine collagen interacted with soy protein isolate with greater electrostatic force in SPI-0.74:HMC-0.25 than in the others samples. Furthermore, in Fig. 4, SPI-0.74:HMC-0.25 powder had the highest value of Tg at a_w 0.328 indicating a greater rigidity of the structure than the others samples. Also, in Table 3 it is possible to see an anti-plasticization effect, since the Tg increased as the a_w increases, which can occur in samples with low moisture contents, where the structure becomes more rigid, while the other samples has the opposite effect, i.e., Tg decreased as the a_w increases [23].

SPI-0.00:HMC-0.99 and SPI-0.99:HMC-0.00 presented the lowest percentages of β-carotene retention, suggesting that the combination of collagen with soy protein isolate has a synergistic and protective effect on this compound. Flores-Miranda et al. [24] made oil-in-water emulsions of beta-carotene using stearic acid and Tween 80 as emulsifier, presenting a high degradation of beta-carotene (32-80 %) during 21 days of storage at 25°C. However, Geng et al. [7] and Cui et al. [9] demonstrated that the combination of soy protein isolates with other substances such as salts or disaccharides (maltose or dipotassium glycyrrhizinate) helps to improve the encapsulation of hydrophobic bioactive compounds and their physicochemical properties. In this work, it was demonstrated that the combination of marine collagen (which is basically another protein) and soy protein isolate, also helps to improve the encapsulation of these bioactive compounds, particularly β-carotene.

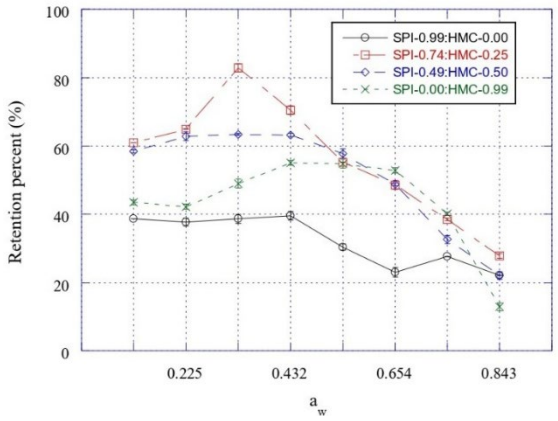

Fig. 1. Retention percentage of powders after 3 weeks of storage.

J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

Functional groups and secondary structure

Fig. 2 shows the results of the FTIR spectroscopy of the samples and Fig. 3 shows the results of FTIR deconvolution in the amide 1 region of the powders. SPI-0.99:HMC-0.00 and SPI-0.74:HMC-0.25 powders have a very similar spectrum, since the SPI-0.74:HMC-0.25 powder has the lowest weight fraction of marine and probably has a greater physicochemical interaction between its components (soy protein and collagen), however, the electrostatic interaction between marine collagen and soy protein isolate is observed at 3277 cm^{-1} in the sample SPI-0.74:HMC-0.25, since is identified a greatest intensity or absorbance in this band, because of there is a greater amount of N-H stretching vibrations between amides [25]. At 3277 cm^{-1} the characteristic band of the proteins is assigned to the Amide type A, this band is attributable to the hydrogen bond between molecules or interactions with water, in this case are indicative of hydrogen bonds between amides [3]. A greater number of hydrogens bonds generate greater rigidity to the structure and provide greater stability to β-carotene at a_w=0.328 (Fig. 1), as well as an increase in Tg (Fig. 3).

On the other hand, at 3101 cm^{-1} the band that represents Amide type B is found. The band located at 2932 cm^{-1} indicates the presence of fatty acids. On the right side of the spectrum, the band corresponding to Amide I can be seen at 1630 cm^{-1}, which is considered very important since from this it is possible to determine the type of secondary structure of a protein since it is associated with the C=O bond of the amide and contributes about 80 % to the configuration of the band, and represents a stretching-type vibration. In the SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 powders, lower intensity was noted in the bands of Amide type A, Amide type B and Amide I compared to the SPI-0.74:HMC-0.25 and SPI-0.99:HMC 0.00 powders, suggesting that the addition of hydrolyzed marine collagen could have altered the secondary structure of the protein. The band found at 1076.5 cm^{-1} is attributed to C-O stretching [26]. On the other hand, at 997.5 cm^{-1} the band is attributed to C-O skeletal stretching [27].

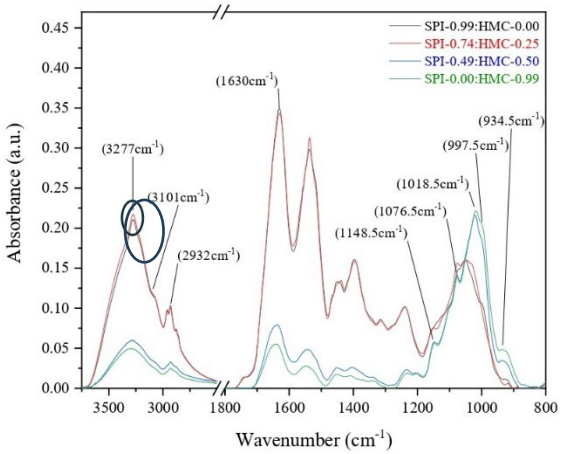

Fig. 2. FTIR spectroscopy of the powders.

Table 2 presents the coformations of the secondary structure of the protein, which were studied in the powders, where it is observed that both β-Sheet and β-Turn predominate in the SPI-0.99:HMC-0.00 and SPI-0.74:HMC-0.25 , while Random Coil and α-Helix have a minor contribution compared to the SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 samples, indicating that the composition of the powders directly impacts the secondary structure of the proteins. The β-carotene retention percents in the powders show that the adequate mass fraction of collagen to be mixed with soy protein corresponds to 0.25 that corresponding to the SPI-0.74:HMC-0.25 powder, because in this mixture the highest retention percent was presented, which may be due to the fact that the collagen was well integrated into the suspension with the soy protein and β-carotene, since its secondary structure did not change drastically compared to SB. According to other studies [28,29] it is explained that the increase in Random Coil and α-Helix indicates protein aggregation and denaturation, while the decrease in β-Sheet and β-Turn indicate an increase in hydrophobicity, this behavior occurred in samples

J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 , which indicates that in sample SPI-0.49:HMC-0.50 the collagen did not integrate well into the suspension, so its structure is more similar to sample SPI-0.00:HMC-0.99 where the collagen weight fraction was 0.99.

Table 2. FTIR results for the secondary structure contents of the powders.

Powder	B-Sheet (%)	Random Coil (%)	A-Helix (%)	B-Turn (%)
SPI-0.99:HMC-0.00	33.90 ± 0.95^{a}	29.91 ± 0.17^{a}	23.65 ± 1.15^{a}	12.55 ± 0.79^{a}
SPI-0.74:HMC-0.25	35.16 ± 0.26^{a}	29.12 ± 1.52^{a}	22.75 ± 1.28^{a}	12.97 ± 0.39^{a}
SPI-0.49:HMC-0.50	17.15 ± 1.02^{b}	33.20 ± 1.41^{b}	44.68 ± 0.72^{b}	4.97 ± 0.94^{b}
SPI-0.00:HMC-0.99	11.59 ± 0.72^{c}	35.71 ± 0.84^{c}	47.35 ± 1.20^{c}	5.36 ± 0.42^{b}

Values are expressed as mean ± standard deviation for three determinations. Values with different superscript in the same column are significantly different ($p < 0.05$).

Glass transition temperature

Fig. 3 and Fig. 4 represent the DSC thermograms of powders equilibrated at a_w=0.328 and a_w=0, respectively. At a_w=0, Tg for SPI-0.99:HMC-0.00, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 powders were 149.7 °C, 146.7 °C, 146.8°C and 77.9 °C, respectively. Tg value of soy protein isolate powder was close to the normal anhydrous Tg values in soybeans, which commonly range between 150 and 199 °C [30], the differences are due to fact that the Tg value depend on the varies factors such the composition of the soy protein isolate, the temperature at which they were conditioned, the a_w and the moisture content present in the powders. In Fig. 3, at a_w=0.328, SPI-0.99:HMC-0.00, SPI-0.74:HMC-0.25, SPI-0.49:HMC-0.50 and SPI-0:HMC-0.99 had the following values of Tg: 145.6°C, 147.5 °C,142.9 and 61.9°C, respectively, where SPI-0.74:HMC-0.25 had the highest Tg. Tg is an important parameter in the processing, storage stability and texture control of dehydrated foods, and the water content and water activity have a great influence on it, therefore the results suggest that SPI-0.74:HMC-0.25 has better stability than the other powders at a_w=0.328 because in this water activity had the highest value of Tg and the highest value of β- carotene retention. This result suggests that the increase in the glass transition temperature of sample SPI-0.74:HMC-0.25 was due to the addition of HCM to the powder in a proportion of 0.25, providing it with greater stability and thus better protection of the β-carotene. The SPI-0.99:HMC-0.00, SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 powders presented Tg values lower at a_w=0.328 than at a_w=0, which was expected because Tg decrease when water activity increases, but in the case of SPI-0.74:HCM-0.25 the Tg increased slightly. The transition from a glass to a rubbery state can occur because of an increase in water content or water activity, even at a constant temperature, since the Tg of solids decreases with an increase in water content [16]. If the solute molecules can move easily then a smaller amount of heat will be required for their chains to begin to vibrate and thus be able to change from a glassy to a rubbery state, an increase in water content increases the mobility of the molecules and causes a decrease in Tg, and in addition, the loss of nutrients is facilitated by the increase in the rate of deterioration reactions. However, SPI-0.74:HMC-0.25 presented an opposite effect, since Tg and the percent of β-carotene retention values were higher at a_w=0.328, which coincides with an antiplasticizing effect, as explained by Seow et al. [23], who argue that in some polymers at water activities higher than zero this type of phenomena can occur because the structure becomes more rigid, so in this case this effect could be related to the stretching N-H vibrations at 3277 cm^{-1}(Fig. 2).

J. Mex. Chem. Soc. **2026**, *70(1):e2501*
Regular Issue

ISSN-e 2594-0317

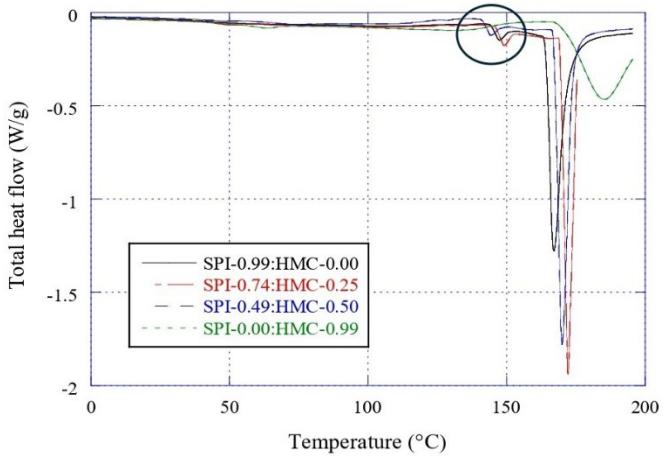

Fig. 3. DSC thermograms of the powders at aw= 0.328.

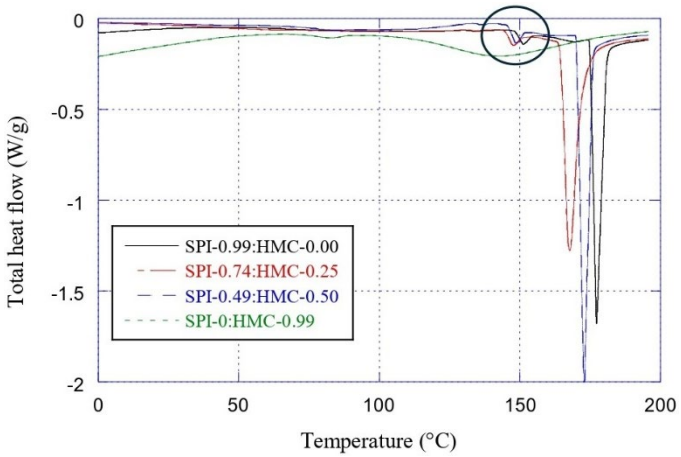

Fig. 4. DSC thermograms of the powders at aw= 0.

Technological properties of food powders

The results of the functional properties of the powders are shown in Table 3. Solubility stands out among the functional properties of protein hydrolysates, since other functional properties such as emulsification, foam formation and water absorption are affected by this [13]. The SPI-0.99:HMC-0.00 powder recorded the highest solubility value with 52.67 %, then the SPI-0.74:HMC-0.25 powder with 41.71 %, followed by the SPI-0.49:HMC-0.50 powder with 9.71 % and the lowest value was for SPI-0:HMC-0.99 powder with 7.75 %. The SPI-0.99:HMC-0.00 powder showed the lowest water absorption with 0.78 g water/g protein while the other powders showed a notable increase and similarity in their values since the SPI-0.74:HMC-0.25 powder registered 1.03 g water/g protein, the SPI-0.49:HMC-0.5 and SPI-0:HMC-0.99 powders absorbed 1.07 g water/g protein, which is related to the low solubility presented by collagen. In addition, the water retention capacity also depends on multiple factors, such as the hydrophilic and hydrophobic regions of the protein, which dictate its water absorption and retention capacity [31]. Regarding the oil retention capacity, the SPI-0.74:HMC-0.25 and SPI-0.99:HMC-0.00 powders registered a value of 0.11 g oil/g protein, while the SPI-0.49:HMC-0.50 and SPI-0.00:HMC-0.99 powders were the ones that showed the highest oil retention with 0.85 g oil/g protein, which may be related to the exposure of more hydrophobic sites in the

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Regular Issue

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collagen due to its extraction process. The results were lower than those reported by Acosta-Domínguez et. al [32] who, in their study on the effect of cryogenic treatment on the microstructure and functional properties of soy protein isolate, reported an oil retention capacity of 3.2 ± 0.01 g oil/g protein. The results were also lower than those obtained by Foh et al. [33] who investigated the physical-functional and chemical properties of acidified soy protein isolate (AcPi), alkalized soy protein isolate (AlPi) and soy protein isolate (SPI) made from tilapia muscle and defatted soybean meal; they reported 2.81 ± 0.50 mL oil/g in SPI. Regarding the foaming capacity, the protein plays a major role, since the foaming capacity and stability are usually dependent on the interfacial film formed by proteins, which maintains the suspension of air bubbles and slows down the coalescence rate [34]. The SPI-0.99:HMC-0.00 powder had the highest foaming capacity with 34.78 %, followed by the SPI-0.74:HMC-0.25 powder with 33.50 %, then SPI-0.49:HMC-0.50 with 31.41 % and the SPI-0.00:HMC-0.99 showed the lowest foaming capacity with 20.94 %. The reported data were higher than those reported by Acosta- Domínguez et al. [32], who report a foaming capacity of 3.3± 0.1 % in a SPI sample. On the other hand, Zamorano-Apodaca et al. [35] hydrolyzed collagen from a by-product of different fish species to obtain peptide fractions and calculate their biological and functional activities and reported a higher foaming capacity (78 %). Emulsions are part of a general class of two-phase food systems of matter called colloids [36], where the proteins can function as emulsifiers, however, its emulsifying capacity depend on the extraction process and the processing conditions which are subjected. Emulsification capacity was determined in the food powders, i.e., after freezer drier. Where SB had the highest emulsification with 46.01 %, followed by CSB-I with 37.60 %, CSB-II presented 27.82 % and CB showed the lowest value with 17.06 %.

Table 4. Technological properties of the powders.

Property	**SPI-0.99:HMC-0.00**	**SPI-0.74:HMC-0.25**	**SPI-0.49:HMC-0.50**	**SPI-0.00:HMC-0.99**
Solubility (%)	52.67 ±0.00a	41.71 ±0.09b	9.71 ±0.02c	7.75 ±0.00d
WHC (g water/g protein)	0.78 ±0.00a	1.03 ±0.00b	1.07 ±0.04c	1.070±0.00c
OHC (g oil/ g protein)	0.11 ±0.02a	0.11 ±0.00a	0.85 ±0.00b	0.85 ±0.00b
FC (%)	34.78 ±0.00a	33.50 ±3.49b	31.41 ±4.72c	20.94 ±1.04d
EC (%)	46.01 ±0.00a	37.60 ±0.07b	27.82 ±0.31c	17.06 ±0.07d

Values are expressed as mean ± standard deviation for three determinations. Values with different superscripts in the same row are significantly different ($p < 0.05$). WHC: water holding capacity, OHC. Oil holding capacity, FC: foaming capacity, EC: emulsifying capacity.

These results suggest that emulsifying capacity decreased as a higher concentration of collagen was added. This effect can be attributed to the formation of new hydrogens bond in the sample SPI-0.74:HMC-0.25 because they are observed at 3277 cm^{-1}, where the greatest intensity or absorbance in this band is identified, since there is a greater amount of N-H stretching vibrations between amides [25], which caused a decrease in its emulsifying capacity. On the other hand, in the case of the other powders, it can be attributed to excessive exposure of hydrophobic sites in the marine collagen that reduces its emulsifying capacity. When comparing these results with those reported by Wang et al. [36] in their research on the emulsifying and physicochemical properties of soybean husk hemicellulose conjugates and soy protein isolate, a similarity with this work is found since the higher SPI fraction in the samples corresponded to the better emulsification levels.

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Regular Issue

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Conclusions

The analysis of β-carotene degradation in the powders stored at different a_w at 25 °C revealed that SPI-0.74:HMC-0.25 powder had the best retention percentage of β-carotene than the other three powders, giving the highest retention value at a_w=0.328 with 83.84 % retention, which suggests that the powder with a rate of 0.74-0.25 weight fraction (SPI:HMC) is recommended to preserve the highest amount of β carotene in a_w=0.328 at 25 °C and this is according to UV-vis spectrophotometry, FTIR and DSC analysis, since this powder showed the highest value of Tg and a better electrostatic interaction. The results showed that the interaction of marine collagen and soy protein increased the stability of β-carotene due to a synergistic effect between these proteins, so the development of this study is an advance to identify new factors that produce a better stability of the food powders rich in proteins and antioxidants.

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