

ORIGINAL ARTICLE

Physical and chemical properties of chitosan salts obtained from common lobster chitin (*Panulirus argus*)

Propiedades físicas y químicas de sales de quitosana obtenidas a partir de quitina de langosta común (*Panulirus argus*)

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Received: 11 February 2024 / Accepted: 13 May 2024 / Published online: 30 July 2024

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Abstract The physical and chemical properties of chitosan salts were evaluated. To produce chitosan acetate and lactate, chitosan solutions at 4% (w/v) were prepared in solutions of acetic and lactic acids at 10% (v/v), respectively. The spray drying was carried out at inlet/outlet temperatures of 160/100 °C. The particle size, shape, surface morphology, and microstructure of the chitosan salts were characterized. Additionally, moisture content and water activity were determined. Analyses of the chemical structure and thermal properties of the compounds were performed. The color of the chitosan and its salts-forming solutions (FPS) was also evaluated. Chitosan lactate exhibited more spherical particles than chitosan acetate, which showed greater particle agglomeration with irregular and sticky shapes, associated with its higher moisture content. Chitosan acetate proved more stable, exhibiting a higher exothermic temperature than chitosan lactate. A partial conversion of the chitosan acetate structure was observed due to the high temperature of the spray drying process. The FPS of chitosan lactate was the least luminous and showed the highest b* value ($p \leq 0.05$), indicating a more intense coloration. No significant differences were found in the values for a* component between the FPS of chitosan and its salts.

Keywords chitosan salts, physical and chemical properties, spray drying, powder properties.

Resumen Se evaluaron las propiedades físicas y químicas de las sales de quitosana. Para la producción de acetato y lactato de quitosana, se prepararon disoluciones de quitosana al 4 % (m/v) en soluciones de ácido acético y láctico al 10 % (v/v), respectivamente. El secado por aspersión se llevó a cabo a temperaturas de entrada/salida de 160/100 °C. Se caracterizaron el tamaño de partícula, la forma, la morfología de la superficie y la microestructura de las sales de quitosana. Además, se determinó el contenido de humedad y la actividad de agua. Se realizaron análisis de la estructura química y propiedades térmicas de los compuestos. También se evaluó el color de las disoluciones formadoras de películas (DFP) de quitosana y de sus sales. El lactato de quitosana presentó partículas más esféricas en comparación con el acetato de quitosana, que mostró una mayor aglutinación de partículas con formas irregulares y pegajosas, lo cual se relaciona con su mayor porcentaje de humedad. El acetato de quitosana resultó más estable, presentando una temperatura exotérmica mayor que el lactato de quitosana. Se observó una conversión parcial en la estructura del acetato de quitosana debido a la alta temperatura del proceso de secado por aspersión. La DFP de lactato de quitosana fue la menos luminosa y mostró el mayor valor de b* ($p \leq 0,05$), lo que indica una coloración más intensa. No se encontraron diferencias significativas en los valores de la componente a* entre las DFP de quitosana y sus sales.

Palabras clave sales de quitosana, propiedades físicas y químicas, secado por aspersión, propiedades de polvos.

How to cite

de la Paz, N.; Fernández, M., Hernández, J.A., & García, M.A. (2024). Physical and chemical properties of chitosan salts obtained from common lobster chitin (*Panulirus argus*). *Journal of Food Science and Gastronomy*, 2(2), 8-16. <https://doi.org/10.5281/zenodo.13996969>

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Introduction

Currently, there is a growing interest in developing materials that enhance the shelf life of food and provide greater microbiological safety. Many natural substances have been studied for this purpose (Teshome et al., 2022). Antioxidants prevent or delay oxidative damage to lipids, proteins, and nucleic acids caused by reactive oxygen species, such as free radicals (Chaudhary et al., 2023). In addition to playing a crucial role in physiological systems, antioxidants are used in the food industry as additives to extend the shelf life of foods, especially those rich in polyunsaturated fats. These components are susceptible to oxidation by reactive oxygen species, contributing to quality degradation, nutritional losses, the development of undesirable flavors, and non-characteristic colorations (Pruteanu et al., 2023). Evidence suggests that using many synthetic antioxidants in food can lead to negative health effects. Due to these concerns, interest in natural antioxidants has increased, among which chitosan is notable (Herdiana et al., 2023).

The seafood processing industry generates a significant amount of solid waste, as 75-85% of the animal mass is considered waste (such as shells, heads, and legs). This waste contaminates the environment and represents an economic burden for the industry, as its disposal can be problematic and costly. Currently, there are technological alternatives to utilize these wastes and convert them into useful products, such as chitin and its derivatives (Santos et al., 2020). Chitin is the most abundant natural polymer after cellulose. It is found in insect wings, fungal cell walls, algae, and the exoskeletons of crustaceans, with the latter being its primary source (Elieh-Ali-Komi & Hamblin, 2016). Chitosan, obtained by deacetylating chitin, is a helical-structured polymer with reactive amino groups, allowing for a wide variety of modifications and ionic interactions (Aranaz et al., 2021).

In recent years, chitosan has attracted researchers' attention due to its extraordinary properties and ease of extraction. As the few cationic polysaccharides, it offers unique properties compared to other polysaccharides, typically neutral or negatively charged (Desai et al., 2023). However, the practical applications of chitosan are limited by its insolubility in water at pH levels above 6. Several chitosan derivatives have been designed to overcome this limitation. Chitosan can form water-soluble salts with organic and inorganic acids, such as hydrochloric, formic, glutamic, lactic, citric, acetic, and ascorbic acids. The reactive amino groups in chitosan can be protonated ($\text{NH}_3^+ \text{OCOR}^-$) by these acids, resulting in a positively charged, water-soluble polysaccharide (Desai et al., 2023). Considering the reasons mentioned earlier, this study evaluated the physical and chemical properties of chitosan salts obtained from the chitin of the common lobster (*Panulirus argus*).

Materials and methods

The materials used to prepare the solutions included chitosan with a molecular weight of 275 kDa and a degree of deacetylation of 75%, obtained from the chitin of the common lobster (*P. argus*) at the Center for Research and Development of Medicines. Additionally, a 90% lactic acid solution (Merck, Germany), glacial acetic acid (Merck, Germany), and distilled water were employed.

Chitosan acetate and lactate were generated with approximately 30% (m/m) chitosan through spray drying at inlet/outlet temperatures of 160/100 °C in a San Young dryer. The analysis of particle size, shape, surface morphology, and microstructure of the salts was conducted using a scanning electron microscope (Zeiss, DSM 962, Germany), capturing micrographs at a magnification of 1000x. The moisture content was determined in triplicate using a Karl Fischer method (Mettler DL35, Switzerland), and water activity was measured with an AquaLab water activity meter (Series 3TE, Sweden), also in triplicate. Fourier-transform infrared (FT-IR) spectra were recorded using an IR spectrometer (FT/IR Jasco 460-Plus, Japan) by processing compressed KBr discs.

The salts thermograms were obtained using a differential scanning calorimeter (DSC 823e, Mettler Toledo, Greifensee, Switzerland), utilizing a nitrogen flow at 50 mL/min. The samples weighed in aluminum pans, were heated from 25 to 220 °C for chitosan lactate and from 25 to 300 °C for chitosan acetate at a heating rate of 10 °C/min.

Thermogravimetric analysis (TGA) was performed using a TGA 850 analyzer (Mettler Toledo, Switzerland), employing nitrogen at 50 mL/min and samples of 5 mg, from 25 to 250 °C at 10 °C/min. The color of the chitosan and its salt film-forming solutions (DFP) at 1% (m/v) was determined spectrophotometrically, following the methodology described by Casariego et al. (2009). A spectrophotometer (Shimadzu UV-2401PC UV-VIS, Japan) was used to measure transmittance between 400 and 700 nm, and the data were transformed into the CIE $L^* a^* b^*$ color space. Luminosity (L^*) and hue components (a^* and b^*) were calculated, using the D65 illuminant and a standard observer viewing angle of 10° (CIE LAB, 1976).

A double ANOVA was conducted using the Statistics software (version 7, 2004, StatSoft, Inc., Tulsa, USA) and Duncan's multiple range test to assess differences between samples, with a significance level of $p \leq 0.05$.

Results and discussion

The scanning electron micrographs of chitosan are shown in Figure 1. The chitosan originally derived from lobster chitin consisted of scales with irregular size and shape. In contrast, the chitosan salts exhibited more spherical particles,

with small particles observed around larger ones, which may be related to the drying process temperatures. Similar results have been reported in previous studies on chitosan salts prepared by spray drying (Nunthanid et al., 2008).

It was observed that chitosan lactate was composed of more spherical particles than chitosan acetate, which exhib-

ited a greater agglomeration of particles that were irregular and sticky. This phenomenon may be attributed to an increase in the humidification of the sample, leading to particle clustering. No significant differences were found in these characteristics between the salts obtained at the laboratory scale and those produced at the industrial scale.

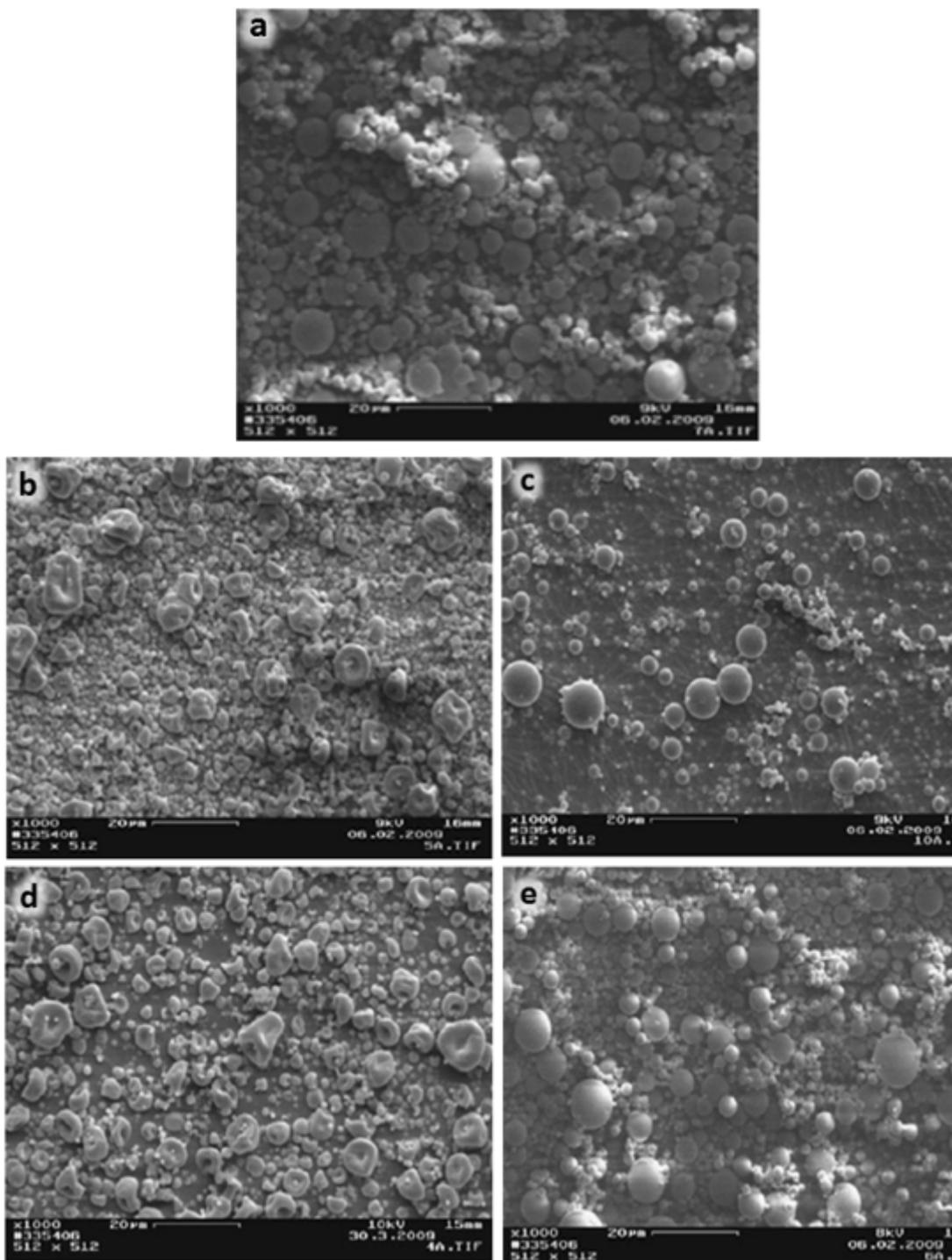


Figure 1. Scanning electron microscopy of (a) chitosan monomer; (b) chitosan acetate; and (c) chitosan lactate at the laboratory scale; (d) chitosan acetate and (e) chitosan lactate at the industrial scale.

The results regarding the moisture content and water activity of the spray-dried chitosan salts are presented in Table 1. All chitosan salt samples contained water, as the acid solvent used in their preparation did not completely evaporate during the drying process. Chitosan acetate showed the highest moisture percentages, which may be related to the fact that acetic acid has a lower molecular weight ($M(\text{acetic acid}) = 60.06 \text{ g/mol}$ and $M(\text{lactic acid}) = 90.08 \text{ g/mol}$) and a lower boiling point (acetic acid: $118.2 \text{ }^\circ\text{C}$ and lactic acid: $122 \text{ }^\circ\text{C}$) (Demarger-Andre et al., 1994), making it the most volatile acid among those studied in this work.

The moisture content of the salts is linked to the inlet temperature of the spray-drying process, during which there is

an increase in the energy of the adsorbed water molecules. This phenomenon facilitates the release of these molecules from the active sites of the chitosan salts, resulting in a reduction in water absorption. Water activity reflects the active part of the moisture, meaning the fraction that, under normal conditions, can be exchanged between the product and its environment (Desai et al., 2023). Similar to moisture content, the water activity of chitosan salts also depends on the temperatures used in the drying process. It was observed that the water activity values were higher for chitosan lactate than those of chitosan acetate, which could be attributed to the ability of lactate ions to compete with water molecules, thereby affecting their dehydration potential.

Table 1. Moisture content, water activity (a_w), and mass loss by TGA of chitosan salts ($n = 3$)

Chitosan salts	Scale	Moisture content (%)	a_w	Mass loss by TGA (%)
Chitosan acetate	Laboratory	8.09 (0.2)	0.267 (0.002)	7.08
	Industrial	7.99 (0.2)	0.273 (0.0)	7.13
Chitosan lactate	Laboratory	3.14 (0.03)	0.352 (0.007)	3.06
	Industrial	3.8 (0.2)	0.364 (0.01)	2.23

The FT-IR spectra of the chitosan salts are presented in Figure 2. In the FT-IR spectrum, broad bands are identified in the range of $3450\text{-}3400 \text{ cm}^{-1}$, indicating the presence of intermolecular hydrogen bonds. Additionally, the stretching of the bands corresponding to the NH groups may overlap in this same spectrum region. Amino groups exhibit characteristic absorption bands at 1597 and 1615 cm^{-1} in the FT-IR spectrum of chitosan. With both chitosan salts, these bands are reduced, suggesting that the NH groups are protonated. The carboxylate -COO- band at 1556 cm^{-1} was detected in all salts, allowing us to infer an ionic interaction between chitosan and the acids.

The FT-IR spectra of chitosan acetate exhibit intense peaks in the range of $1550\text{-}1600 \text{ cm}^{-1}$ and weak peaks near 1400 cm^{-1} , which are attributed to the carboxylate anion, suggesting that this salt is adequately formed as a result of spray drying. In contrast, the FT-IR spectra of chitosan lactate reveal certain spectral changes. As shown in the figure, the spectrum of chitosan lactate displays a prominent NH_2 band at 1630 cm^{-1} . This shift of the vibration to higher wavenumbers, compared to typical amino group values, indicates the formation of a carboxylate between the -COO- groups of the acid and the -NH_3^+ groups of chitosan (Di Foggia et al., 2023).

Additionally, absorption bands for carbonyl groups are observed at 1700 cm^{-1} or higher, indicating the presence of carboxylic acids. The band around 1730 cm^{-1} corresponds to the ester of the carboxylic group of the oligo (lactic acid) either in a chain or free form (Cervera et al., 2011), suggesting the presence of free lactic acid in the chitosan lactate salt. This finding also aligns with previous studies (Yao et al., 2003).

These results suggest that organic acids may interact with chitosan at the positions of the amino groups during the spray-drying process, thus forming chitosan salts. The FT-IR spectra of all chitosan salts showed ammonium and carboxylate bands, although free carboxyl groups in chitosan lactate are evident at wavelengths above 1700 cm^{-1} .

Figure 3 presents the DSC curves of the chitosan salts dried by spray drying. All samples exhibit significant endothermic peaks in the temperature range of $70\text{-}120 \text{ }^\circ\text{C}$. In particular, chitosan lactate shows a broad second endothermic peak between $150\text{-}200 \text{ }^\circ\text{C}$, which can be attributed to the loss of crystallization water and the melting point of the samples. Similar behavior has been reported in previous studies on chitosan lactate microcapsules and films.

Chitosan acetate exhibited an additional endothermic peak in the temperature range of $150\text{-}200 \text{ }^\circ\text{C}$. According to the literature, these endothermic peaks of chitosan acetate are associated with mass loss of the salt (de la Paz N et al., 2021). The correlation between the higher ΔH values and the moisture contents of the chitosan salt samples suggests differences in their polymer-water interaction and water retention capacity. The ΔH values for chitosan acetate and lactate dried at $160/100 \text{ }^\circ\text{C}$ were 105.2 and 45.8 J g^{-1} , respectively.

The thermal decomposition of chitosan is an exothermic process that involves the contraction of the crystalline structure of the material, starting after dehydration (Muralidharan et al., 2015). In this study, exothermic peaks of the chitosan salts were observed around $290 \text{ }^\circ\text{C}$ (Figure 3). Chitosan lactate demonstrated lower stability than chitosan

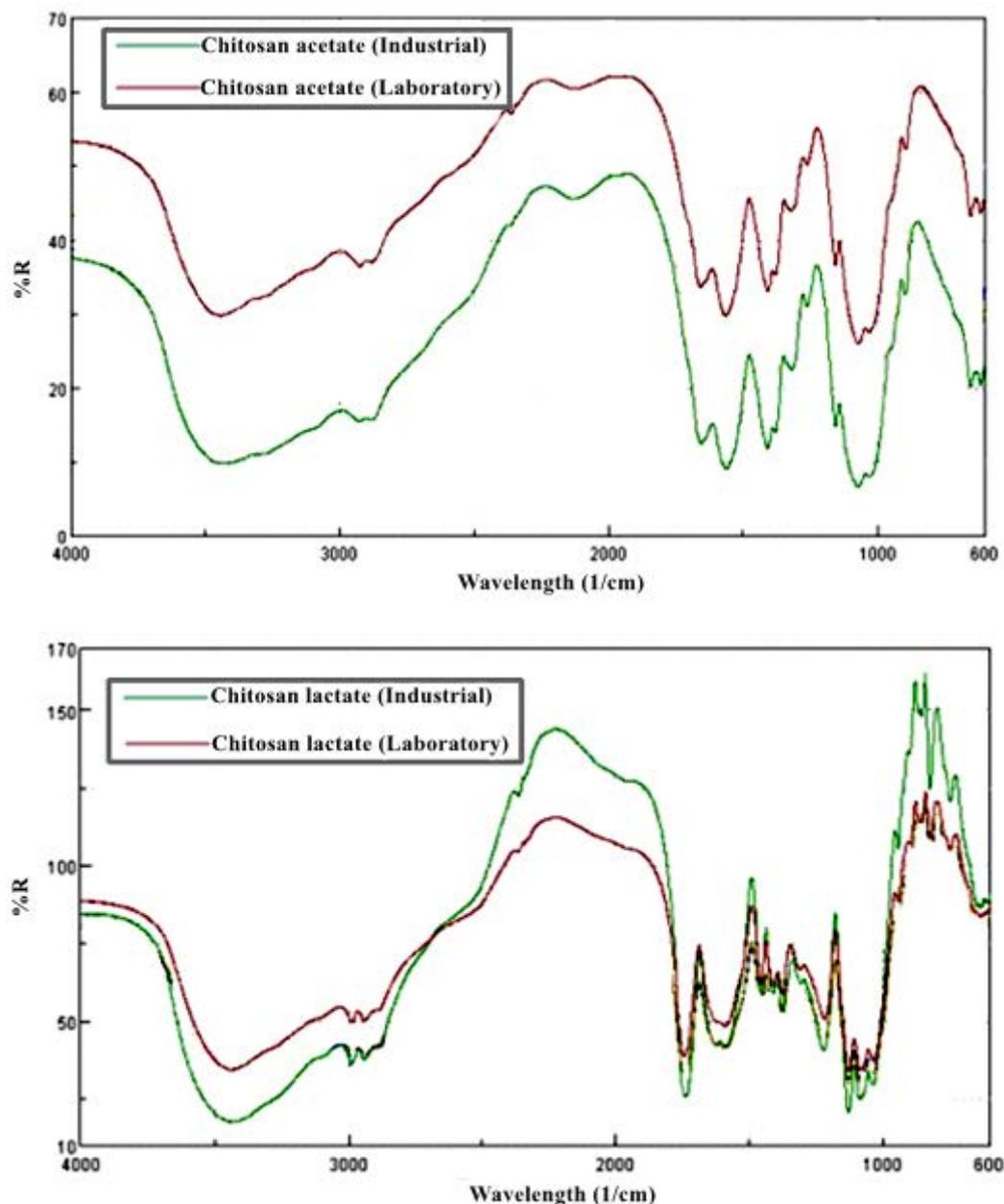


Figure 2. FT-IR spectra of chitosan acetate and chitosan lactate.

and chitosan acetate, as evidenced by its lower degradation temperature. An irreversible change in the structure of chitosan lactate was detected above 220 °C.

These results indicate a higher thermal stability of chitosan acetate compared to chitosan lactate. At elevated temperatures, carboxylic acids can be protonated and react slowly with the amine to form an amide (Lu et al., 2022). This amidation process reduces the amount of hydrophilic groups, which in turn increases water absorption (Lu et al., 2022). In this study, the chitosan lactate samples showed lower moisture content compared to chitosan acetate, possibly due to intramolecular and intermolecular condensations between the carboxylic acid and chitosan.

Figure 4 shows the values for the mass loss of chitosan salts during heating. According to TGA analyses, the mass loss is associated with the endothermic changes observed in the differential scanning calorimetry analysis.

Chitosan acetate exhibited a higher moisture content compared to chitosan lactate. The results of mass loss at elevated temperatures, around 150-200 °C, are consistent with previous findings on chitosan salts (de la Paz N et al., 2021). The mass loss was more pronounced in chitosan lactate than in chitosan acetate, while this change in mass loss was not recorded for pure chitosan.

The values of L^* , a^* , and b^* for the film-forming solutions (FFS) of chitosan are presented in Table 2. It is noteworthy

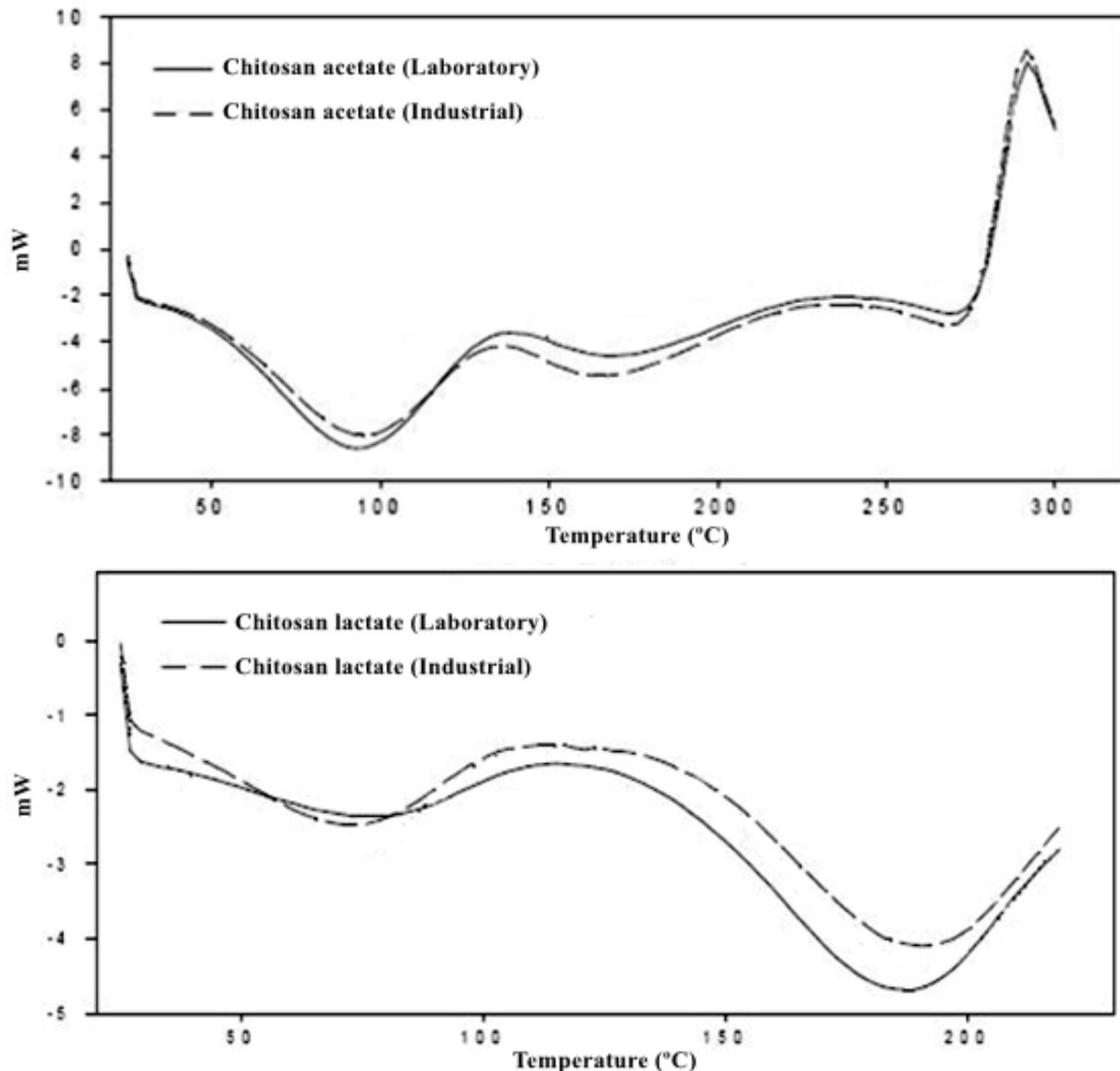


Figure 3. Differential scanning calorimetry of chitosan acetate and chitosan lactate.

that the chitosan solution was the most luminous ($p \leq 0.05$), while the FFS obtained from chitosan lactate was the least luminous.

The b^* value is the parameter that describes the color of the chitosan film-forming solutions (FFS), given that chitosan has a yellow color, and this chromatic component has the most significant influence on the total color difference observed in the FFS. The FFS made with chitosan lactate showed the highest b^* value, which, combined with its lower luminosity, resulted in the solution with the most intense coloration. It is important to note that the development of color in the solutions is linked to the amount of chitosan used in their preparation, with the salts consisting of 30% (m/m) chitosan.

It is widely recognized that color changes may be related to chemical and biological alterations in a substance; the yellow tones in chitosan solutions could be attributed to the carbonyl groups (C=O) present in its structure (Mohan et al., 2019). Additionally, both the origin of the chitosan and the extraction process influence its color. Regarding the values of the component a^* , no significant differences were observed between chitosan and its salts, with values ranging between -2 and 2, indicating a negligible contribution of this component to the color development of the FFS. The chromaticity values (C^*) showed a behavior similar to that observed for the b^* component.

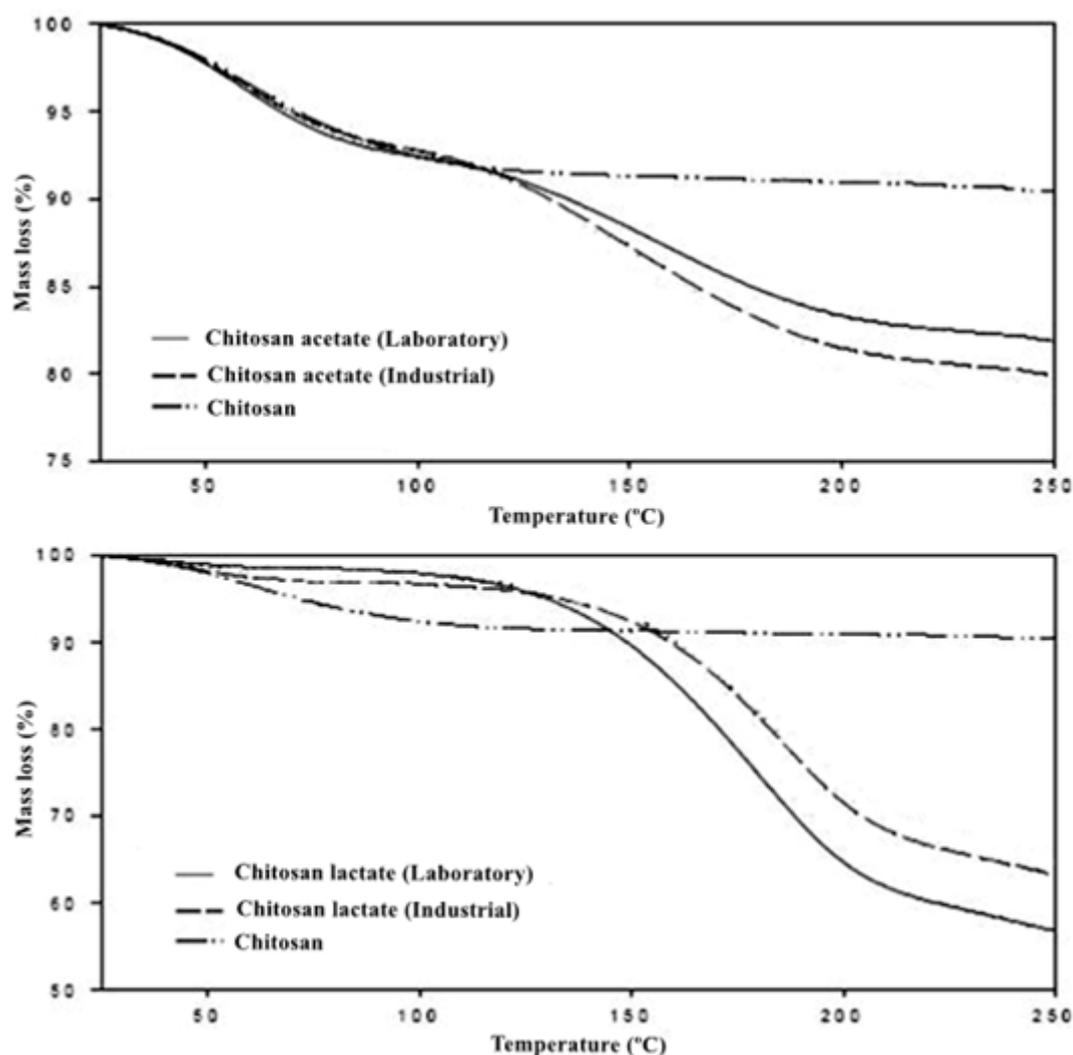


Figure 4. Thermogravimetric analysis of chitosan acetate and chitosan lactate.

Table 2. Parameters related to the color of the FFS.

Compound	L*	a*	b*	C*
Chitosan	91.9 (0.1) a	-1.80 (0.11) c	5.41 (0.26) c	5.71(0.27) c
Chitosan acetate	89.6 (0.1) a	-1.87 (0.02) bc	11.28 (0.04) b	11.43(0.03) b
Chitosan lactate	79.7 (2.2) b	-0.09 (1.47) ac	24.02 (1.15) a	24.06(1.14) a

Mean (Standard deviation); n = 3.

Different letters indicate a significant difference ($p \leq 0.05$) by Duncan's multiple range test.

Conclusions

Chitosan lactate was characterized by more spherical particles than chitosan acetate, which showed greater particle agglomeration with irregular and sticky shapes, correlating with its higher moisture content. Furthermore, chitosan acetate proved more stable, exhibiting a higher exothermic temperature than lactate. A partial conversion in the structure of chitosan acetate was identified, attributed to the high temperatures used during spray drying. Regarding the film-forming solutions (FFS), chitosan lactate was the least

luminous, showing the highest value in component b^* , indicating a more intense coloration. However, no significant differences were found in the values of the component a^* between the chitosan FFS and its salts.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Nilia de la Paz, Mirna Fernández, Jarol A. Hernández and Mario A. García: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, drafting the original manuscript and writing, review, and editing.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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