



Universitat
de les Illes Balears

DOCTORAL THESIS
2024

GENERATION OF ADDED VALUE FOR NON-
TRADITIONAL CROPS AND AGRI-FOOD WASTE:
USE OF NOVEL RAW MATERIALS TO PRODUCE
BIODEGRADABLE FILMS, 3D FOOD PRINTING,
AND DRY EMULSIONS WITH POTENTIAL
INDUSTRIAL APPLICATIONS

Luis Daniel Daza Ramírez



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Doctoral Programme in Chemical Science and Technology

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Doctor by the Universitat de les Illes Balears

PAPERS LIST

This doctoral thesis titled “**Generation of added value for non-traditional crops and agri-food waste: Use of novel raw materials to produce biodegradable films, 3D food printing, and dry emulsions with potential industrial applications**” whose author is Luis Daniel Daza Ramírez, is presented as papers compendium, which are listed below:

- Daza, L. D., Eim, V. S., & Váquiro, H. A. (2021). Influence of Ulluco Starch Concentration on the Physicochemical Properties of Starch–Chitosan Biocomposite Films. *Polymers*, 13(23), 4232. <https://doi.org/10.3390/polym13234232>. Journal impact factor (2021) 4.967. Q1 (16/90) Polymer Science.
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Based Biodegradable Films. Foods, 12(9), 1824. <https://doi.org/10.3390/foods12091824>. Journal impact factor (2022) 5.2. Q1 (34/142) Food Science & Technology.

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Dr. Valeria Soledad Eim Iznardo, of Universitat de les Illes Balears

I DECLARE:

That the thesis titles *Generation of added value for non-traditional crops and agri-food waste: Use of novel raw materials to produce biodegradable films, 3D food printing, and dry emulsions with potential industrial applications*, presented by Luis Daniel Daza Ramírez to obtain a doctoral degree, has been completed under my supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, I hereby sign this document.

Signature

Palma de Mallorca, June 5th 2024



Dr. Henry Alexander Váquiro Herrera, of Universidad del Tolima

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For all intents and purposes, I hereby sign this document.

Signature

Ibagué, June 5th 2024

*The good thing about Science is that it's true,
whether or not you believe in it.*

Neil deGrasse Tyson

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ABSTRACT

The increase in the world's population, climate change, and the processes associated with food production and distribution have altered consumption trends, increasing both agricultural and industrial production. This situation has generated a greater demand for plant-based products and raw materials, as well as an increase in the generation of agricultural waste. According to FAO data, this imbalance has contributed to food insecurity that affects more than 40% of the world's population. Aware of this situation, various governmental and non-governmental organizations have evaluated and implemented strategies to reduce this problem.

In this context, the valorization of resources through the search for non-commercial crops that can be used as an alternative for obtaining industrially relevant compounds, as well as the transition from a linear to a circular economy that promotes and includes the use of agri-food waste or by-products as raw materials for other processes, has become a priority. Considering the current production needs of biodegradable packaging to replace synthetic ones, as well as the requirements for natural surfactants in the food industry and emerging technologies such as 3D printing that require the incorporation of raw materials to allow their development, this research aimed to valorize non-traditional crops and agri-food waste, implementing them in the production of biodegradable films and evaluating their performance as surfactants and raw materials for 3D food printing with industrial application.

Due to the wide range of crops and agri-food by-products with potential for valorization, this work prioritized those that showed the most potential after a literature review. Therefore, products and agri-food waste from the productive chains of Andean tubers such as ulluco and cubio, oilseeds such as chachafruto, fruits such as Tahiti lime, and aromatic plants such as lemongrass were selected.

The first part of the work focused on the extraction and physicochemical, morphological, and thermal characterization of the starches obtained from chachafruto, ulluco, and cubio, and their comparison with commercial potato starch. The materials showed starch extraction yields of 9.5%, 26.5%, and 43.2% on a dry basis for chachafruto, cubio, and ulluco, respectively. The wet extraction method demonstrated high efficiency in obtaining starches with a high degree of purity, as evidenced by protein, lipid, and ash contents of less than 1% in all starches. The granule integrity was also confirmed using scanning electron microscopy (SEM) and optical microscopy. On the other hand, the apparent amylose content (AM) was 27.9%, 31.2%, 36.6%, and 32.1% for ulluco, cubio, chachafruto, and potato, respectively. These results demonstrated the potential of

these starches for applications such as film production because a high AM promotes retrogradation and leads to the formation of more organized structures. The results associated with the crystallinity of the starches showed that all the materials evaluated presented crystalline polymorphs type B and relative crystallinity values between 16% and 29%, showing an inverse relationship with the AM content. On the other hand, no relationship was observed between particle size and starch pasting properties. The lowest pasting temperature was observed in cubio starch (60.7 °C), followed by potato starches (63.9 °C), ulluco (65.3 °C), and chachafruto (68.2 °C). However, the highest final viscosity was observed in chachafruto starch. Thermal gravimetric analysis (TGA) demonstrated high thermal stability of the starches.

On the other hand, the effect of physical modification by annealing (ANN) on the physicochemical properties of ulluco starch was studied. For this modification, the moisture content (50-70%) and the modification time (8-24 h) were selected as process factors, resulting in 13 treatments. In general, no significant changes were observed in the morphology of the starch granules in any treatment. However, parameters such as solubility, water holding capacity (WHC), oil holding capacity (OHC), and gel hardness increased by up to 400%, 28%, 26%, and 128%, respectively, compared to native starch. Regarding gelatinization properties, no differences were observed in the gelatinization temperatures between the modified starch and the native starch. However, an increase in the final viscosity of all starches modified by ANN was achieved, with values between 125% and 220% of the initial value.

The potential of the extracted materials to prepare biodegradable films was evaluated in three stages. In the first stage, the viability of native starches and modified ulluco starch at different concentrations to prepare the materials was evaluated. The results obtained in this stage suggested that using chachafruto starch for film preparation was only efficient at a 2% concentration of the material; higher or lower concentrations cannot form a film. Regarding cubio starch, films were produced at concentrations of 2%, 2.5%, and 3% starch. Meanwhile, ulluco starch films were prepared at a concentration of 2.6% for both native and modified starches. In general, all native starch treatments showed the formation of morphologically homogeneous structures with some imperfections on their surfaces, which can be associated with good barrier capabilities. In contrast, samples obtained with modified ulluco starch showed cracks on their surfaces. Water vapor permeability (WVP) values ranged from 2.18×10^{-9} to 2.52×10^{-9} g m⁻¹s⁻¹Pa⁻¹, while films prepared with modified starch showed an increase in permeability (2.46×10^{-9} to 3.45×10^{-9} g m⁻¹s⁻¹Pa⁻¹) compared to films obtained with native ulluco starch. High transparency and good thermal resistance were also observed in films with native starches. On the other hand, the modified starch

showed a decrease of up to 48% in swelling power but generated an increase of up to 200% in Young's modulus and 100% in tensile strength compared to films prepared with native ulluco starch.

In the second stage, the viability of using essential oils obtained from Tahiti lime waste (TLEO) and lemongrass (LEO) in combination with chitosan for producing biodegradable films was evaluated. For this purpose, six film formulations were prepared: two controls with chitosan concentrations of 1% and 1.5% w/v, two formulations combining the two chitosan concentrations with 1% LEO v/v, and two formulations combining the two chitosan concentrations with 1% TLEO v/v. The addition of essential oils (EO) to the films promoted a 35-50% decrease in crystallinity, associated with an increase in elasticity (16-35%) and a reduction in tensile strength (9.3-29.2 MPa) and Young's modulus (190-1555 MPa) of the films. Regarding optical properties, the opacity of the films with TLEO increased up to 500% and 439% for chitosan concentrations of 1% and 1.5%, respectively. At the same time, the increase in opacity for films prepared with LEO was 357% and 187%. Thermal degradation of the films using TLEO was higher than those using LEO.

In the last stage associated with the preparation of biodegradable films, the preparation of edible films from ulluco starch in combination with chitosan was evaluated, and the effect of starch concentration on the physicochemical properties of the materials was evaluated. Different concentrations of ulluco starch (2%, 3%, 4%, and 5%) and a fixed chitosan concentration (1.5%) were used to prepare the films. The biodegradable films showed an increase in solubility from 17.5% to 21.7%, swelling power from 38.9% to 267%, tensile strength from 3.69 MPa to 10.7 MPa, Young's modulus from 18.0 Pa to 652 Pa, and thermal stability as the ulluco starch concentration increased. However, samples with low starch concentrations showed higher elongation at break (36.6%) and better barrier properties ($5.61 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$).

Additionally, the viability of unconventional starches as food inks for 3D printing was evaluated. Prints were prepared with different concentrations of ulluco, cubio, and chachafruto starches (8%, 10%, and 12% w/v), and parameters such as microstructure, texture, crystallinity, and rheology of the samples obtained by 3D printing were analyzed. All concentrations used showed printing capability, producing stable structures. For tuber starches, it was observed that concentrations of 8% were the most efficient, which could be due to the high amylose concentration in the samples prepared at 10% and 12%, which increased retrogradation and viscosity of the paste, hindering good printability. On the other hand, prints obtained with chachafruto starch were more efficient at concentrations of 10%. All prints reduced their relative crystallinity but retained the crystalline polymorph type B. Regarding hardness, ulluco prints presented the lowest hardness

values, between 0.41 and 0.85 N, followed by those obtained using cubio, 0.59 to 1.72 N, and chachafruto, 0.93 to 2.3 N.

Finally, the potential of chachafruto flour (CHF) as a stabilizing agent for an oil-in-water (O/W) emulsion and its impact on the emulsion's physicochemical properties after spray drying were evaluated. For this purpose, emulsions were prepared with different CHF concentrations (2%, 3%, and 4%) and compared to a control emulsion. The results from the creaming index and particle size analyses indicated that the highest emulsion stability, even after 168 h, was achieved with 4% CHF, attributed to its high protein content. The resulting encapsulates exhibited spherical and rough surface morphologies but without holes on the surface. Additionally, low moisture (<5%) and water activity ($a_w < 0.2$) contents were associated with excellent powder stability. The encapsulates added with CHF presented good reconstitution properties, mainly characterized by preserving the initial particle size of the emulsions. Fourier-transform infrared spectroscopy (FTIR) confirmed the absence of chemical interactions during encapsulation, contributing to the high stability. Furthermore, the addition of CHF improved the thermal stability of the encapsulates.

In summary, this PhD thesis proposes different alternatives for valorizing crops and agri-food by-products, such as producing biodegradable packaging, surfactants, encapsulants, and raw materials for 3D printing. The findings in this research demonstrate the industrial potential of the products under study and contribute to the value addition to their productive chains.

RESUMEN

El aumento de la población mundial, junto con el cambio climático y los procesos asociados a la producción y distribución de alimentos, ha alterado las tendencias de consumo, resultando en un aumento tanto en la producción agrícola como en la industrial. Esta situación ha generado una mayor demanda de productos y materias primas de origen vegetal, así como un incremento en la generación de residuos agrícolas. Este desequilibrio ha contribuido a una situación de inseguridad alimentaria que, según datos de la FAO, afecta a más del 40% de la población mundial. Conscientes de esta situación, diferentes organizaciones gubernamentales y no gubernamentales han evaluado e implementado estrategias enfocadas a la disminución de esta problemática.

En este contexto, la revalorización de recursos mediante la búsqueda de cultivos no comerciales que puedan emplearse como alternativa para la obtención de compuestos de interés industrial, así como la transición de una economía lineal a una circular que promueva e incluya el uso de residuos o subproductos agroalimentarios como materias primas para otros procesos, se ha convertido en una prioridad. Considerando las necesidades de producción actual de envases biodegradable que sustituyan a los sintéticos, así como los requerimiento de surfactantes naturales en la industria alimentaria y las tecnologías emergentes como la impresión 3D que requiere la incorporación de materias primas que permitan su desarrollo, esta investigación tuvo como objetivo la revalorización de cultivos no tradicionales y residuos agroalimentarios, implementándolos en la producción de películas biodegradables y evaluando su desempeño como surfactantes y materias primas para la impresión 3D de alimentos con aplicación industrial.

Debido a la amplia gama de cultivos y subproductos agroalimentarios con potencial para ser revalorizados, este trabajo priorizó aquellos que mostraron mayor potencial después de una revisión de la literatura. Por lo tanto, se seleccionaron productos y residuos agroalimentarios procedentes de las cadenas productivas de tubérculos andinos como ulluco y cubio, así como de oleaginosas como el chachafruto, frutales como la lima Tahití y plantas aromáticas como el limoncillo.

La primera parte del trabajo se centró en la extracción y caracterización fisicoquímica, morfológica y térmica de los almidones obtenidos a partir del chachafruto, ulluco y cubio y su comparación con almidón comercial de papa (patata). Los materiales mostraron rendimientos de extracción de almidón de 9.5, 26.5 y 43.2% en base seca para chachafruto, cubio y ulluco, respectivamente. El método de extracción húmedo demostró una alta eficiencia para la obtención de almidones con un alto grado de pureza, lo que se comprobó mediante contenidos

de proteínas, lípidos y cenizas menores al 1% en todos los almidones. Además, la integridad del gránulo fue confirmada mediante la utilización de microscopía electrónica de barrido (SEM) y microscopía óptica. Por otro lado, el contenido aparente de amilosa (AM) fue de 27.9, 31.2, 36.6, y 32.1%, para ulluco, cubio, chachafruto y papa, respectivamente. Estos resultados demostraron el potencial de estos almidones para aplicaciones como la elaboración de películas debido a que un alto contenido de amilosa promueve la retrogradación y conduce a la formación de estructuras más organizadas. Los resultados asociados a la cristalinidad de los almidones demostraron que todos los materiales evaluados presentaron alomorfos cristalinos tipo B y valores de cristalinidad relativa entre 16 y 29% guardando una relación inversa con el contenido de AM. Por otro lado, no se observó una relación entre el tamaño de partícula y las propiedades de gelificación de los almidones. La menor temperatura de gelificación fue observada en almidón de cubio (60.7°C), seguido por los almidones de papa (63.9°C), ulluco (65.3°C) y chachafruto (68.2°C). No obstante, la viscosidad final más alta se observó en el almidón de chachafruto. Por su parte, el análisis termogravimétrico (TGA) demostró una alta estabilidad térmica de los almidones.

Por otro lado, se estudió el efecto de la modificación física por recocido o “annealing” (ANN) sobre las propiedades fisicoquímicas del almidón de ulluco. Para esta modificación se seleccionaron como factores de proceso el contenido de humedad (50-70%) y el tiempo de modificación (8-24 h) resultando en un total de 13 tratamientos. En general, no se observaron cambios significativos en la morfología de los gránulos de almidón en ningún tratamiento. No obstante, parámetros como la solubilidad, la capacidad de retención de agua (WHC), la capacidad de retener aceite (OHC) y la dureza del gel aumentaron hasta en un 400, 28, 26 y 128%, respectivamente, en comparación con el almidón nativo. En cuanto a las propiedades de gelatinización, no se observaron diferencias en las temperaturas de gelatinización entre el almidón modificado y el almidón nativo, pero se logró un aumento en la viscosidad final de todos los almidones modificados por ANN, con valores entre 125% y 220% del valor inicial.

La evaluación del potencial de los materiales extraídos para la preparación de películas biodegradables se realizó en tres etapas. En la primera se evaluó la viabilidad de almidones nativos y almidón de ulluco modificado en diferentes concentraciones para la preparación de los materiales. Los resultados obtenidos en esta etapa sugirieron que el uso de almidón de chachafruto para la preparación de películas solo fue eficiente a una concentración del 2% del material, concentraciones superiores o inferiores no son capaces de formar película. Con respecto al almidón de cubio, se lograron producir películas a concentraciones de 2, 2.5 y 3% de almidón. Por su parte, las películas de almidón de ulluco se prepararon a una concentración de 2.6% de los almidones nativo y modificado. En

general, todos los tratamientos de almidones nativos mostraron la formación de estructuras morfológicamente homogéneas con algunas imperfecciones en sus superficies, lo que puede asociarse con buenas capacidades de barrera. Mientras que las muestras obtenidas con almidón de ulluco modificado presentaron fisuras en sus superficies. Los valores de permeabilidad al vapor de agua (PVA) oscilaron entre 2.18×10^{-9} y $2.52 \times 10^{-9} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, mientras que las películas preparadas con almidón modificado mostraron un aumento en la permeabilidad (2.46×10^{-9} a $3.45 \times 10^{-9} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$) respecto a las películas obtenidas con almidón nativo de ulluco. También se observó una alta transparencia y una buena resistencia térmica en las películas preparadas con almidones nativos. Por su parte, el almidón modificado mostró una disminución de hasta el 48% en el poder de hinchamiento, pero generó un aumento de hasta el 200% de la rigidez (YM) y del 100% en la fuerza tensil en comparación con las películas preparadas con almidón de ulluco nativo.

En la segunda etapa se evaluó la viabilidad del empleo de aceites esenciales obtenidos de residuos de lima Tahití (TLEO) y de limoncillo (LEO) en mezcla con quitosano para la obtención de películas biodegradables. Para lo anterior se prepararon seis formulaciones de películas: dos controles con concentraciones de quitosano del 1% y 1.5% v/p, dos formulaciones combinando las dos concentraciones de quitosano con un 1% de LEO v/v, y dos formulaciones combinando las dos concentraciones de quitosano con un 1% de TLEO v/v. La adición de aceites esenciales (EO) a las películas promovió una disminución del 35-50% en la cristalinidad, asociado con un aumento en la elasticidad (16-35%) y una disminución en la resistencia a la tracción (9.3-29.2 MPa) y en el módulo de Young (190-1555 MPa). En cuanto a las propiedades ópticas, la opacidad de las películas con TLEO aumentó hasta un 500% y un 439% para concentraciones de quitosano del 1% y 1.5%, respectivamente. Mientras que el aumento en la opacidad para las películas preparadas con LEO fue del 357% y 187%. La degradación térmica de las películas utilizando TLEO fue mayor que las películas donde se usó LEO.

En la última etapa asociada a la preparación de películas biodegradables, se evaluó la preparación de películas comestibles de almidón de ulluco en mezcla con quitosano y se evaluó el efecto de la concentración del almidón en las propiedades fisicoquímicas de los materiales. Para la preparación de las películas se emplearon diferentes concentraciones de almidón de ulluco (2%, 3%, 4% y 5%) y una concentración fija de quitosano (1.5%). Las películas biodegradables mostraron un aumento en la solubilidad del 17.5% al 21.7%, en el poder de hinchamiento del 38.9% al 267%, en la resistencia a la tracción de 3.69 MPa a 10.7 MPa, en el módulo de Young de 18.0 Pa a 652 Pa, y en la estabilidad térmica, todo esto con un aumento de la concentración de almidón de ulluco. Sin embargo, las muestras con bajas concentraciones de almidón mostraron una mayor elongación a la rotura (36.6%) y mejores propiedades de barrera ($5.61 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$).

Además, se evaluó la viabilidad de almidones no convencionales como tintas alimentarias para la impresión 3D. Se prepararon impresos con diferentes concentraciones de almidón de ulluco, cubio y chachafruto (8%, 10% y 12% p/v), y se analizaron parámetros como la microestructura, textura, cristalinidad y reología de las muestras obtenidas por impresión 3D. Todas las concentraciones empleadas mostraron capacidad de impresión produciendo estructuras estables. Se observó que para almidones obtenidos de tubérculos las concentraciones de 8% fueron las de mejor rendimiento, lo que pudo obedecer a la alta concentración de amilosa en las muestras preparadas a 10 y 12% que aumentaron la retrogradación y la viscosidad de la pasta impidiendo una buena capacidad de impresión. Por su parte, los impresos obtenidos con almidón de chachafruto fueron más eficientes a concentraciones de 10%. Todos los impresos redujeron su cristalinidad relativa, pero conservaron el alomorfo cristalino tipo B. Con relación a la dureza, los impresos de ulluco presentaron los valores de dureza más bajos, entre 0.41 a 0.85 N, seguidos de los obtenidos empleando ulluco, 0.59 a 1.72 N, y chachafruto 0.93 a 2.3 N.

Finalmente, se evaluó el potencial de la harina de chachafruto (CHF) como agente estabilizante para una emulsión aceite-en-agua (O/W) y su impacto en las propiedades fisicoquímicas de la emulsión después del secado por atomización. Para esto, se prepararon emulsiones con diferentes concentraciones de CHF (2%, 3% y 4%) y se compararon con una emulsión control. Los resultados del índice de crema y los análisis de tamaño de partícula indicaron que la mayor estabilidad de la emulsión, incluso después de 168 horas, se logró con un 4% de CHF, atribuido al alto contenido de proteínas. Los encapsulados resultantes mostraron morfologías de superficie esféricas y rugosas, pero sin agujeros en la superficie. Además, bajos contenidos de humedad (<5%) y actividad de agua ($a_w < 0.2$) se asociaron con una excelente estabilidad en polvo. Los encapsulados adicionados con CHF presentaron buenas propiedades de reconstitución, principalmente caracterizadas por preservar el tamaño de partícula inicial de las emulsiones. La espectroscopía infrarroja por transformada de Fourier (FTIR) confirmó la ausencia de interacciones químicas durante el proceso de encapsulación, contribuyendo a la alta estabilidad. Además, la adición de CHF mejoró la estabilidad térmica de los encapsulados.

En síntesis, esta tesis ha propone diferentes alternativas para la valorización de cultivos y subproductos agroalimentarios como la producción de envases biodegradables, como agentes surfactantes y encapsulantes y como materias primas para la impresión 3D. Los hallazgos obtenidos en esta investigación demuestran el potencial industrial de los productos en estudio y sirve como aporte para la agregación de valor a sus cadenas productivas.

RESUM

L'augment de la població mundial, juntament amb el canvi climàtic i els processos associats a la producció i distribució d'aliments, ha alterat les tendències de consum, resultant en un augment tant en la producció agrícola com en la industrial. Aquesta situació ha generat una major demanda de productes i matèries primeres d'origen vegetal, així com un increment en la generació de residus agrícoles. Aquest desequilibri ha contribuït a una situació d'inseguretat alimentària que, segons dades de la FAO, afecta a més del 40% de la població mundial. Conscients d'aquesta situació, diferents organitzacions governamentals i no governamentals han avaluat i implementat estratègies enfocades a la disminució d'aquesta problemàtica.

En aquest context, la revalorització de recursos mitjançant la cerca de cultius no comercials que puguin emprar-se com a alternativa per a l'obtenció de compostos d'interès industrial, així com la transició d'una economia lineal a una circular que promogui i inclogui l'ús de residus o subproductes agroalimentaris com a matèries primeres per a altres processos, s'ha convertit en una prioritat. Considerant les necessitats de producció actual d'envasos biodegradables que substitueixin els sintètics, així com els requeriments de surfactants naturals en la indústria alimentària i les tecnologies emergents com la impressió 3D que requereixen la incorporació de matèries primeres que permetin el seu desenvolupament, aquesta investigació va tenir com a objectiu la revalorització de cultius no tradicionals i residus agroalimentaris, implementant-los en la producció de pel·lícules biodegradables i avaluant el seu rendiment com a surfactants i matèries primeres per a la impressió 3D d'aliments amb aplicació industrial.

Degut a l'àmplia gamma de cultius i subproductes agroalimentaris amb potencial per a ser revaloritzats, aquest treball va prioritzar aquells que van mostrar major potencial després d'una revisió de la literatura. Per tant, es van seleccionar productes i residus agroalimentaris procedents de les cadenes productives de tubercles andins com ullucu i cubiu, així com d'oleaginoses com el chachafruto, fruiters com la llima Tahití i plantes aromàtiques com el llimoncillo.

La primera part del treball es va centrar en l'extracció i caracterització fisicoquímica, morfològica i tèrmica dels midons obtinguts a partir del chachafruto, ullucu i cubiu i la seva comparació amb midó comercial de patata. Els materials van mostrar rendiments d'extracció de midó de 9.5, 26.5 i 43.2% en base seca per a chachafruto, cubiu i ullucu, respectivament. El mètode d'extracció humida va demostrar una alta eficiència per a l'obtenció de midons amb un alt grau de puresa, com es va comprovar mitjançant continguts de proteïnes, lípids i cendres menors del 1% en tots els midons. A més, la integritat del grànul va ser confirmada

Per altre bandes, es va estudiar l'efecte de la modificació física per requisit a

L'avaluació del potencial dels materials extrets per a la preparació de polítics

Mentre que les mostres obtingudes amb midó d'ullucu modificat van presentar fissures en les seves superfícies. Els valors de permeabilitat al vapor d'aigua (PVA) van oscil·lar entre 2.18×10^{-9} i $2.52 \times 10^{-9} \text{ g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$, mentre que les pel·lícules preparades amb midó modificat van mostrar un augment en la permeabilitat (2.46×10^{-9} a $3.45 \times 10^{-9} \text{ g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$) respecte a les pel·lícules obtingudes amb midó nativo d'ullucu. També es va observar una alta transparència i una bona resistència tèrmica en les pel·lícules preparades amb midons nadius. Pel que fa al midó modificat, es va mostrar una disminució de fins al 48% en el poder de inflor, però va generar un augment de fins al 200% de la rigidesa (YM) i del 100% en la resistència a la tracció en comparació amb les pel·lícules preparades amb midó d'ullucu nativo.

En la segona etapa es va avaluar la viabilitat de l'ús d'olis essencials obtinguts de residus de llima Tahití (TLEO) i de llimoncillo (LEO) en barreja amb quitosà per a l'obtenció de pel·lícules biodegradables. Per a això es van preparar sis formulacions de pel·lícules: dos controls amb concentracions de quitosà del 1% i 1.5% v/v, dues formulacions combinant les dues concentracions de quitosà amb un 1% de LEO v/v, i dues formulacions combinant les dues concentracions de quitosà amb un 1% de TLEO v/v. L'addició d'olis essencials (EO) a les pel·lícules va promoure una disminució del 35-50% en la cristal·linositat, associat amb un augment en l'elasticitat (16-35%) i una disminució en la resistència a la tracció (9.3-29.2 MPa) i en el mòdul de Young (190-1555 MPa). Pel que fa a les propietats òptiques, l'opacitat de les pel·lícules amb TLEO va augmentar fins a un 500% i un 439% per a concentracions de quitosà del 1% i 1.5%, respectivament. Mentre que l'augment en l'opacitat per a les pel·lícules preparades amb LEO va ser del 357% i 187%. La degradació tèrmica de les pel·lícules utilitzant TLEO va ser major que les pel·lícules on es va usar LEO. En l'última etapa associada a la preparació de pel·lícules biodegradables, es va avaluar la preparació de pel·lícules comestibles de midó d'ullucu en barreja amb quitosà i es va avaluar l'efecte de la concentració del midó en les propietats fisicoquímiques dels materials. Per a la preparació de les pel·lícules es van emprar diferents concentracions de midó d'ullucu (2%, 3%, 4% i 5%) i una concentració fixa de quitosà (1.5%). Les pel·lícules biodegradables van mostrar un augment en la solubilitat del 17.5% al 21.7%, en el poder d'inflor del 38.9% al 267%, en la resistència a la tracció de 3.69 MPa a 10.7 MPa, en el mòdul de Young de 18.0 Pa a 652 Pa, i en l'estabilitat tèrmica, tot això amb un augment de la concentració de midó d'ullucu. No obstant això, les mostres amb baixes concentracions de midó van mostrar una major elongació a la ruptura (36.6%) i millors propietats de barrera ($5.61 \times 10^{-11} \text{ g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$).

A més, es va avaluar la viabilitat de midons no convencionals com a tintes alimentàries per a la impressió 3D. Es van preparar impresos amb diferents concentracions de midó d'ullucu, cubiu i chachafruto (8%, 10% i 12% p/v), i es van

analitzar paràmetres com la microestructura, textura, cristal·linositat i reologia de les mostres obtingudes per impressió 3D. Totes les concentracions emprades van mostrar capacitat d'impressió produint estructures estables. Es va observar que per a midons obtinguts de tubercles les concentracions del 8% van ser les de millor rendiment, el que va poder obeir a l'alta concentració d'amilopectina en les mostres preparades al 10 i 12% que van augmentar la retrogradació i la viscositat de la pasta impedit una bona imprimibilitat. Per la seva banda, els impresos obtinguts amb midó de chachafruto van ser més eficients a concentracions del 10%. Tots els impresos van reduir la seva cristal·linositat relativa, però van conservar l'alomorf cristal·lí tipus B. Pel que fa a la duresa, els impresos d'ullucu van presentar els valors de duresa més baixos, entre 0.41 a 0.85 N, seguits dels obtinguts emprant ullucu, 0.59 a 1.72 N, i chachafruto 0.93 a 2.3 N.

Finalment, es va avaluar el potencial de la farina de chachafruto (CHF) com a agent estabilitzant per a una emulsió oli-en-aigua (O/W) i el seu impacte en les propietats fisicoquímiques de l'emulsió després de la seqüenciació per atomització. Per això, es van preparar emulsions amb diferents concentracions de CHF (2%, 3% i 4%) i es van comparar amb una emulsió control. Els resultats de l'índex de crema i els anàlisis de mida de partícula van indicar que la major estabilitat de l'emulsió, fins i tot després de 168 hores, es va aconseguir amb un 4% de CHF, atribuït a l'alt contingut de proteïnes. Els encapsulats resultants van mostrar morfologies de superfície esfèriques i rugoses, però sense forats en la superfície. A més, baixos continguts d'humitat (<5%) i activitat d'aigua ($a_w < 0.2$) es van associar amb una excel·lent estabilitat en pols. Els encapsulats afegits amb CHF van presentar bones propietats de reconstitució, principalment caracteritzades per preservar la mida de partícula inicial de les emulsions. L'espectroscòpia infraroja per transformada de Fourier (FTIR) va confirmar l'absència d'interaccions químiques durant el procés d'encapsulació, contribuint a l'alta estabilitat. A més, l'addició de CHF va millorar l'estabilitat tèrmica dels encapsulats.

En síntesi, aquesta tesi ha proposat diferents alternatives per a la valorització de cultius i subproductes agroalimentaris com la producció d'envasos biodegradables, com agents surfactants i encapsulants i com a matèries primeres per a la impressió 3D. Els descobriments obtinguts en aquesta investigació demostren el potencial industrial dels productes en estudi i serveix com a aportació per a l'agregació de valor a les seves cadenes productives

List of Symbols and Abbreviations

Parameters and analysis

Abbreviation	Description
SS	Starch synthase
XRD	X-ray diffraction
1GB	First-generation bioplastics
2GB	Second-generation bioplastics
3D	Three dimensional
3GB	Third-generation bioplastics
A	Mass of the permeability cell (g) at time t (s)
A	Exposed area (m ²)
a*	CIELab coordinate (redness/greenness)
a.u.	Arbitrary Unit
Abs	Absorbance
ADP-Glc	Adenosine-5'-diphosphoglucose
AGPase	ADP-Glc pyrophosphorylase
AM	Amylose
ANN	Annealing
AP	Amylopectin
ATP	Adenosine triphosphate
Aw	Water activity
b*	CIELab coordinate (yellowness/blueness)
BD	Breakdown
BE	Branching enzyme
bioPA	bio-polyamide
bioPE	bio-polyethylene
bioPET	bio-poly(ethylene terephthalate)
bioPP	bio-polypropylene
bioPTT	bio-poly(trimethylene terephthalate)
BU	Brabender units
BVA	Brabender Visco-Amylograph
CI	Color index
C _i	Creaming index (Chapter 8)
D _[3, 2]	Sauter Mean Diameter (μm)
D _[4, 3]	De Brouckere Mean Diameter (μm)
d _{0.1}	Percentile 10 %
d _{0.5}	Percentile 50 %, median droplet diameter
d _{0.9}	Percentile 90 %
Da	Dalton or unified atomic mass unit
DATM	Diacetyl tartaric acid ester of monoglyceride
Db	Dry basis
DBE	Debranching enzyme
DE	Dextrose equivalent
Dm	Dry matter
DMA	Dynamic-mechanical analysis

DP or Xn	Degree of polymerization
DPE	Disproportionating enzyme
DPPH•	(2,2-diphenyl-1-picrylhydrazyl) radical
DSC	Differential scanning calorimetry
DTG	Derivate of thermogravimetric analysis
EB	Elongation at break (%)
EO	Essential oil
FRAP	Ferric reducing antioxidant power
FT	Film thickness (m)
FTIR	Fourier Transform Infrared Spectroscopy
FV	Final viscosity
G'	Storage modulus (Pa)
G''	Loss modulus (Pa)
GBSSI	Granule-bound Starch synthase I
GRAS	Generally Recognized as Safe
HAS	High amylose starch
HLB	Hydrophilic-lipophilic balance
ISA	Isoamylase-type debranching enzyme
L*	CIELab coordinate (Luminosity)
MC	Moisture content
MOS	Malto-oligosaccharides
Mw	Molecular weight
n	Number of samples
n'	Frequency exponent (dimensionless)
O/W	Oil-in-water emulsions
OHC	Oil-holding capacity
OP	Opacity
PBAT	poly(butylene adipate terephthalate)
PBS	poly(butylene succinate)
PCL	Polycaprolactone
PE	Polyethylene
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PLA	Polylactic acid
PP	Polypropylene
PPi	Pyrophosphate
Psat	Saturation vapor pressure at the test temperature (Pa)
PT	Pasting temperature (°C)
PTST1	Protein targeting to starch 1
PV	Peak viscosity (BU)
RH	Relative humidity (%)
RS	Resistant starch
RSD	Rapidly digestible starch
RVA	Rapid Visco-Analyzer
S	Solubility (%)
SD	Standard deviation
SDS	slowly digestible starch

SEM	Scanning electron microscopy
SP	Swelling power (%)
SP	Starch phosphorylase (Introduction)
T _c	Conclusion temperature (°C)
T _g	Glass transition temperature (°C)
TGA	Thermogravimetric analysis
T _m	Melting temperature (°C)
T _o	Onset temperature (°C)
T _p	Peak temperature (°C)
TPC	Total phenolic compounds
TPS	Thermoplastic starch
TS	Tensile strength (Pa)
TV	Trough viscosity
W/O	Water-in-oil emulsions
WHC	water-holding capacity (%)
WI	White Index
WL	Weight loss
WVP	Water vapor permeability (g m ⁻¹ s ⁻¹ Pa ⁻¹)
YI	Yellow index
YM	Young's modulus

Organization and institutions

Symbol	Description
FAO	Food and Agricultural Organization of the United Nations
SDGs	Sustainable Development Goals
GDP	gross domestic product
ONU	United Nations
MADS	Ministry of environment and sustainable development, Colombia
IBRD	International Bank for Reconstruction and Development
ISO	International Organization for Standardization
MITECO	Ministry for Ecological Transition and the Demographic Challenge of Spain
MAPA	Ministry of Agriculture, Fisheries, and Food of Spain
CODEX	Codex Alimentarius

Greek symbols

Symbol	Description
ΔH	Enthalpy of gelatinization (J/g)
2θ	Angle 2Theta
$\tan \delta$	Tangent of the phase angle (δ)
ω	Frequency (Hz)
λ	Wavelength of the incident X-radiation
ρ	Density (kg/m ³ or g/mL)
η	Apparent viscosity (mPa·s)
ΔE	Total color change
Γ	Shear rate (s ⁻¹)

Samples and conditions

Chapter 1

Symbol	Description
CHS	Chachafruto starch
CHS3D1	3D printed with 8% starch
CHS3D2	3D printed with 10% starch
CHS3D3	3D printed with 12% starch
CHSF	Chachafruto starch film

Chapter 2

Symbol	Description
CUS	Cubio starch
FG	Free glucose
G	Glucose
POS	Potato starch
SCF1	Films with 2.0 % w/v of CUS
SCF2	Films with 2.5 % w/v of CUS
SCF3	Films with 3.0 % w/v of CUS
TS	Total starch

Chapter 3

Symbol	Description
PS	Potato starch

Chapter 4

Symbol	Description
Ac	Crystalline peak area
ANNS	Annealing modified starch
At	Total area
FS1	ANNS1 film
FS2	ANNS2 film
FS3	ANNS3 film
FSC	Native ulluco starch film
S1	Annealed starch at 55 °C for 8 h and 50% of water
S2	Annealed starch at 55 °C for 24 h and 60% of water
S3	Annealed starch at 55 °C for 24 h and 70% of water
TD	Degradation temperature

Chapter 5

Symbol	Description
TLEO	Tahiti lime essential oil
LEO	Lemongrass essential oil
Ch1	Control films sample - chitosan 1%
Ch15	Control films sample - chitosan 1.5%
Ch15L	Film with 1.5% of chitosan and lemongrass essential oil
Ch15TL	Film with 1.5% of chitosan and Tahiti lime essential oil
Ch1L	Film with 1% of chitosan and lemongrass essential oil
Ch1TL	Film with 1% of chitosan and Tahiti lime essential oil

Chapter 6

Symbol	Description
US	Ulluco starch
Ch	Chitosan
S2	Film with 0.75 Ch and 2% US
S3	Film with 0.75 Ch and 3% US
S4	Film with 0.75 Ch and 4% US
S5	Film with 0.75 Ch and 5% US

Chapter 7

Symbol	Description
ULS	Ulluco starch
CUS	Cubio starch
CUS10	3D printed with 10% cubio starch
CUS12	3D printed with 12% cubio starch
CUS8	3D printed with 8% cubio starch
ULS10	3D printed with 10% ulluco starch
ULS12	3D printed with 12% ulluco starch
ULS8	3D printed with 8% ulluco starch

Chapter 8

Symbol	Description
CHF	Chachafruto flour
CE	Control emulsion
CE	Control emulsion
CHE1	Emulsion with 2% CHF
CHE2	Emulsion with 3% CHF
CHE3	Emulsion with 4% CHF
E1	Encapsulation with 2% CHF
E2	Encapsulation with 3% CHF
E3	Encapsulation with 4% CHF
EC	Control encapsulation
HS	Height of the serum
HT	Total height
MD	Maltodextrin

INTRODUCTION

1. INTRODUCTION

1.1. Non-traditional crops and agricultural waste

The continuous growth of the world's population, estimated to reach 10 billion inhabitants by 2050, along with increasing urbanization, the implementation of new technologies, climate change, and processes associated with food production and distribution, have altered consumption trends, leading to shortages in the food chain and, consequently, food insecurity (Leisner, 2020; Milião et al., 2022; Molotoks et al., 2021). According to reports from the FAO, in 2022, world hunger, measured by the prevalence of undernourishment, indicator 2.1.1 of the Sustainable Development Goals (SDGs), affected 9.2% of the global population, representing a 1.3% increase compared to 2019 (7.9% of the population affected) (FAO et al., 2023). These figures contradict SDG 2, which aims to eradicate hunger by 2030.

On the other hand, the increase in production in industries that use plant-based raw materials, such as textile, pharmaceutical, paper, adhesive, paint, biodiesel, and bioplastics, among others, has led to a rise in demand for these materials (Zarski et al., 2021). However, this demand cannot be met without compromising food security, as crops intended for human consumption are the primary source used to extract these raw materials. An example of this situation is represented by starch, which is a raw material for various industries and is sourced from crops such as corn, potatoes, wheat, and rice (de Barros Mesquita et al., 2016)

1.1.1 Non-traditional crops as an alternative to industrial processes

It is estimated that there are approximately 30,000 edible plants, yet only 7,000 have been cultivated and utilized as food. Fewer than twenty species supply 90% of the world's food needs, with wheat, corn, and rice being the most representative crops globally (constituting 60% of the human diet) (Chivenge et al., 2015). Non-traditional crops refer to agricultural products such as fruits, vegetables, oilseeds, and aromatic plants, among others, with high nutritional value, which can be native or not. The production of these crops is focused on local distribution and consumption (Carletto et al., 2011; Rosado et al., 2021). The diversification of small producers towards the production of non-traditional crops aims to reduce poverty and food insecurity in the region. However, their contribution to the gross domestic product (GDP) is insignificant. Despite this, these crops can represent a rich source of different compounds with the potential to become a supply for the food chain (Milião et al., 2022). This could result in the promotion of these crops and their industrial exploitation, adding value to the chain and avoiding their extinction.

In an effort to revalue non-traditional crops, several studies have explored the technological properties of molecules derived from unconventional raw materials

(Milião et al., 2022). Among the alternatives of non-traditional crops, compounds such as proteins or protein isolates (Badjona et al., 2023; Burghardt et al., 2024; Chandran et al., 2023; X. Zhang et al., 2024), starches (Maniglia & Tapia-Blácido, 2016; Nogueira et al., 2018; Pascoal et al., 2013; Tessema & Admassu, 2021), pectins (Abe-Inge et al., 2020; Reichembach & Lúcia de Oliveira Petkowicz, 2021; Roman-Benn et al., 2023), among others, have been identified and extracted. All these products have shown high variability in their properties, and prior characterization of their functional and technological potential is necessary before defining their viability for industrial use.

1.1.2 Agricultural waste

On the other hand, the growth of the world's population has also caused an imbalance between production and consumption, leading to increased agricultural production. According to the FAO, this production has increased by almost 300% worldwide in the last 50 years (FAO, 2023). However, this increase has been accompanied by a rise in food losses and waste, indicating deficiencies in food systems. Over 31% of food produced worldwide is estimated to be lost in the food production and distribution chain. These losses are divided into post-harvest losses (14%) and losses at the stages of sale, food services, and consumption (17%) (FAO, 2022; ONU, 2021).

The losses and generation of agri-food waste can affect the sustainability of productive systems by increasing food insecurity due to the reduction in food availability, inequity in access to food, indiscriminate use of resources, and loss of product quality along supply chains (Badran et al., 2017). Additionally, the increase in these wastes can negatively impact the environment through increased greenhouse gas emissions, degradation of fauna through ecosystem degradation, loss of biodiversity, and the use of resources necessary for their production (FAO, 2022).

Previously, losses by product weight in the supply chain have been estimated. It was determined that the highest volumes of loss are concentrated in the fruit and vegetable chain (44% of total mass losses), followed by the chains of roots and tubers (20%), cereals (19%), dairy (8%), meat (4%), oilseeds and legumes (3%), and fish and seafood (2%) (Badran et al., 2017; Blakeney, 2011).

1.1.2.1 Circular economy

To make the food supply chain sustainable, it is crucial to promote waste management by reducing food losses and waste, ensuring the implementation of the three "R's": reuse, recovery, and recycling (Kumar et al., 2023). Adopting the linear economy model has resulted in increased resource consumption, as it needs to consider the reuse and recovery of products (Jamaludin et al., 2022). This situation has led to the need for countries' economies to transition to a circular

economy model in which waste from one process becomes inputs or raw materials for others, reducing consumption and providing comprehensive use of natural resources (Zhang et al., 2022).

Worldwide, different countries have established objectives to achieve the sustainability of the food supply chain and have begun to adopt policies focused on transitioning to a circular economy (Hartley et al., 2020; IBRD, 2022). For example, several countries have established policies aimed at limiting the use of single-use plastics or non-biodegradable plastics (MADS, 2021), have set criteria for the production of products based on eco-design (MITECO, 2018), or have implemented programs focused on reducing losses or waste associated with the agricultural chain, such as the strategy implemented by the MAPA in Spain "More food, less waste" (MAPA, 2015). Alongside implementing these policies, adopting productive strategies that mitigate environmental impacts and reduce food insecurity is necessary. In this context, various research studies have evaluated strategies for valorizing agricultural products and their industrial utilization (Awogbemi & Kallon, 2022; Capanoglu et al., 2022).

1.2. Compounds with industrial potential

1.2.1. Essential oils

Essential oils (EOs) are volatile liquids that some plants synthesize as secondary metabolites. Characterized by their intense odor, they can be found in different parts of the plant, such as the leaves, bark, seeds, flowers, fruits, branches, and rhizomes, depending on the plant species from which they are obtained. These oils can be stored in epidermal cells, cavities, and secretory cells of glandular trichomes. Furthermore, they serve various functions in plants, including protection against environmental stress, pests, viruses, insects, and microorganisms, as well as the attraction of pollinators (Falleh et al., 2020).

According to ISO 9235:2021, essential oils are defined as the "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits or by dry distillation, after separation of the aqueous phase – if any – by physical processes" (ISO, 2021). About 3000 essential oils are known, of which approximately 300 are used commercially (Jugreet et al., 2020). Most of these essential oils have been recognized by the Food and Drug Administration (FDA) as "Generally Recognized as Safe (GRAS)" due to their antibacterial, antifungal, anti-mycotoxin, antioxidant, antiviral, anti-inflammatory, analgesic, and hepatoprotective properties, among others, they have been used in different areas such as food, pharmaceuticals, biomedicine, agriculture, and textiles (Jackson-Davis et al., 2023; Jugreet et al., 2020; Mutlu-Ingok et al., 2020).

1.2.1.1. Chemical composition

Essential oils (EOs) are complex natural mixtures of volatile compounds containing between 20 and 60 compounds of different natures (Baptista-Silva et al., 2020; Singh et al., 2021). Generally, the concentrations of the components are variable, with usually one to three major components present in high concentrations (20-90%) that determine the functional properties of the EO, while the remaining components are present in very low or trace amounts (Haro-González et al., 2021; Paw et al., 2020). For example, one of the parameters used for the classification of EOs extracted from the same species is the chemotype, which is based on identifying the major components to differentiate their bioactive potential (Miladinović et al., 2022; Walia et al., 2020). The components of EOs are divided into two groups, which are differentiated by their biosynthetic origin. The primary group is constituted of terpenes and terpenoids (mevalonate pathway), and the second group of aromatic and aliphatic compounds called phenylpropanoids (shikimate pathway) (Falleh et al., 2020).

The basic unit of terpenes is isoprene, and the condensation of two or more units of this compound results in the formation of hemiterpenes (C₅), monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), triterpenes (C₃₀), and tetraterpenes (C₄₀) (Jugreet et al., 2020; Maurya et al., 2021). On the other hand, phenylpropanoids are less abundant compounds in EOs and correspond to alcohols (cinnamic alcohol), aldehydes (cinnamaldehyde), phenols (chavicol, eugenol), heterocycles, methoxy derivatives (anethole, elemicine, estragole, methyleugenols), and methylenedioxy compounds (apiole, myristicin, safrole) (Figure 1) (Bakkali et al., 2008).

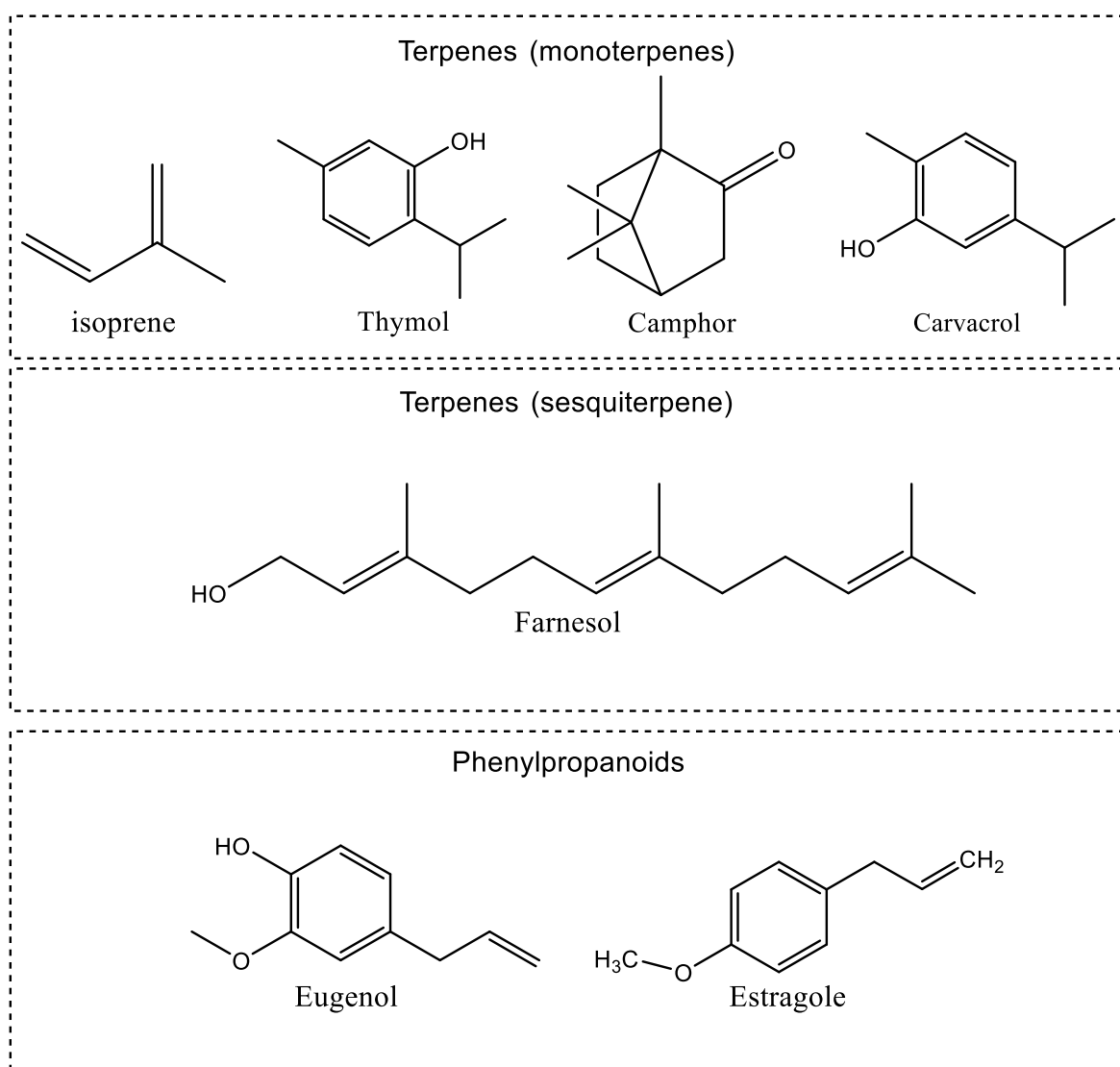


Figure 1. Chemical structures of selected components of essential oils.

1.2.2. Starch

Starch is a semicrystalline, birefringent, and insoluble in cold water homopolymer that is widely distributed in most plants, including cereals, roots, tubers, seeds, and fruits, among others. This macromolecule is fundamental to plants' energy storage, accumulating in cells as granules. Due to its wide distribution, this molecule is the primary source of energy in the human diet and is the second most abundant glucose polymer in nature (Pfister et al., 2020; Zavareze & Dias, 2011). In addition, owing to its renewable, biodegradable, cost-effective, and non-toxic nature, starch finds extensive applications across various industries, including food, textiles, paper, pharmaceuticals, chemicals, and biomedical sectors (Elmi Sharlina et al., 2017). The primary commercial starch sources are corn, wheat, rice, potatoes, and cassava, with an annual production of around 60 million tons worldwide (de Barros Mesquita et al., 2016). Despite the diversity of crops for commercially obtaining

starch, its technological properties vary depending on the composition, the arrangement inside the granule, and the botanical source, among other factors.

In plants, this molecule is primarily synthesized in organs such as leaves, seeds, roots, tubers, and fruits through specialized cellular organelles called plastids. In leaves, synthesis occurs in chloroplasts and is stored temporarily, while in long-term storage organs, it is synthesized in amyloplasts (Pfister & Zeeman, 2016; Wang et al., 2014). Starch biosynthesis involves more than fifteen enzymes regulated mainly by post-translational control mechanisms (Tetlow & Bertoft, 2020). Simplified, three simultaneous and interdependent enzymatic reactions catalyzed by Starch synthase (SS), Branching enzyme (BE), and Debranching enzyme (DBE) are required. Previously, ADP-glucose (ADP-Glc) is formed in a reaction mediated by the enzyme ADP-Glc pyrophosphorylase (AGPase) from glucose-1-phosphate and ATP with the release of pyrophosphate (PPi). Once ADP-Glc is obtained, SS elongates the non-reducing ends of glucose chains using ADP-Glc as the glucosyl donor; then, BE creates branches from existing chains through glucanotransferase reactions; and finally, DBE hydrolyzes some of the branches again (Pfister & Zeeman, 2016) (Figure 2).

Following the described synthesis process, the starch granule comprises two glucose polymers: amylose (AM) and amylopectin (AP). Their contents typically range between 15% and 30% for AM and between 70% and 85% for AP (Tetlow & Bertoft, 2020). Furthermore, there are starches known as waxy, with PA concentrations greater than 99% (Agarwal, 2021; Khlestkin et al., 2018), as well as starches with high amylose content (HAS; >40%) (Obadi et al., 2023; Zhong et al., 2022). The native starch granule morphology and size depend on the extraction source and cultivation conditions, resulting in various shapes such as spherical, oval, polygonal, disc (lenticular), elongated, and kidney geometries with sizes ranging between 1 μm and more than 100 μm (Table 1) (Doblado-Maldonado et al., 2017; Pfister & Zeeman, 2016).

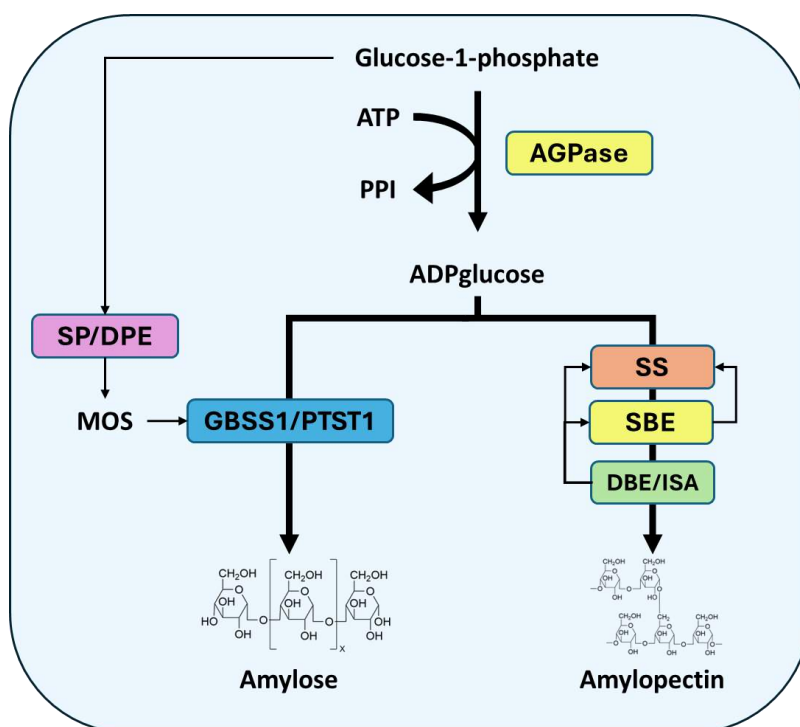


Figure 2. Starch synthesis. Adapted from Pfister & Zeeman (2016)

1.2.2.1. Amylose

AM is an essentially linear polymer composed of chains formed by D-glucopyranose residues linked by α -(1–4) glycosidic bonds and with several α -(1–6) glycosidic bonds less than 1% of the total bonds (branches) (Seung, 2020; Wang et al., 2014) (Figure 3). The α -glucan chains of AM are longer than those of AP, with a degree of polymerization (DP) ranging from 700 to 40,000 units, while the DP of the branched chains can vary from 100 to 700 units (Wang et al., 2014). The average molecular weight of AM can vary widely depending on the source, ranging from 1×10^5 to 1×10^6 Da (Doblado-Maldonado et al., 2017; Seung, 2020). This polymer is synthesized by the action of Granule-bound SS I (GBSSI), which is responsible for elongating AM chains (Han et al., 2019). Likewise, the Protein targeting starch 1 (PTST1) is necessary for the correct localization of the enzyme GBSS. In the presence of ligands like iodine and linear alcohols, AM can undergo conformational changes, forming a left-handed helix that includes 6 to 8 units of D-glucose per turn. In this configuration, the hydroxyl groups point outward while the hydrogen atoms face inward, ensuring stability. However, this structure is unstable in aqueous solutions and tends to form double helices, which precipitate readily (Doblado-Maldonado et al., 2017; Putseys et al., 2010).

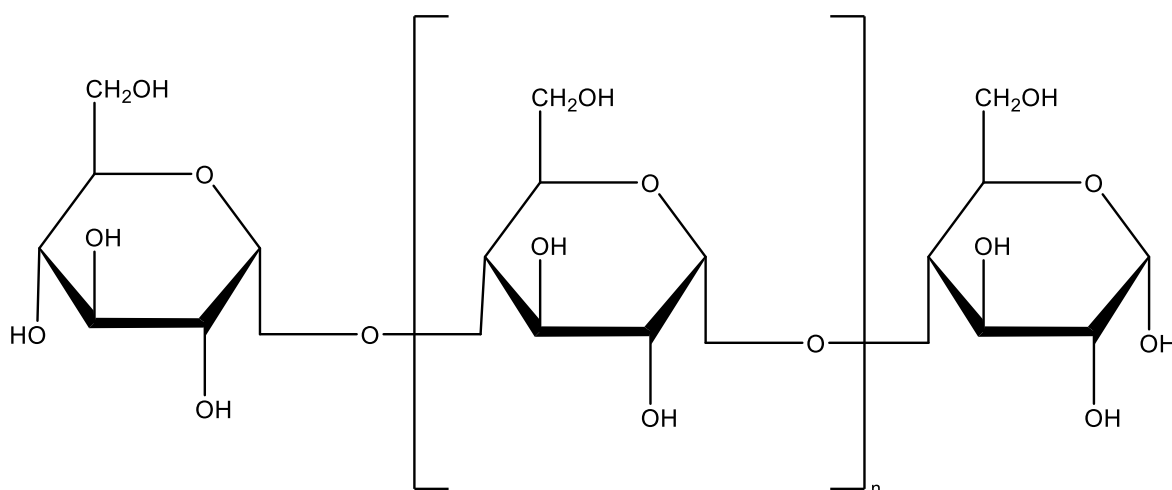


Figure 3. Chemical structure of amylose

It has been shown that the concentration of AM increases with the maturity of the product and is thought to be located in the poorly organized amorphous regions of the AP blocks, with a higher concentration on the granule's surfaces (Chen et al., 2016; Tetlow & Bertoft, 2020). Although AM is part of the amorphous region of the starch granule, it is of great technological importance in the process that occurs after starch gelatinization (retrogradation) due to its ability to align and recrystallize (Galindez et al., 2019). The AM in the granules has been associated with preventing their disintegration and a plasticizing effect on the structure (Vamadevan & Bertoft, 2020). Furthermore, it is postulated that AM fills the spaces within the AP matrix, increasing the density of starch and, thus, the efficiency with which plants can store carbohydrates (Pfister et al., 2020). However, the AM content depends on various factors such as cultivation conditions, botanical source, environmental conditions, and genetics. Thus, for the same product, different concentrations of AM can be found, as well as different average chain sizes. Table 1 shows the reported AM content for various conventional and non-conventional products.

In addition, of the starches with usual AM contents (15-30%), there are starches with AM contents higher than commercial starches (>40%) and which are classified as HAS. These starches have a high nutritional value because they are resistant to digestion and are fermented in the large intestine, which leads to a reduction in blood glucose levels, providing protection against colorectal cancer and diabetes (Li et al., 2023; Zhong et al., 2023). From a technological standpoint, these starches find application in preparing films, coatings, textiles, and paper (Zhong et al., 2023).

Table 1. Literature review of properties of starches obtained from different sources

Sample	Amylose (%)	Crystallinity (%)	Diffraction pattern	Average diameter	Pasting temperature (°C)	Morphology	Reference
Almond	26.25	26	A-type	14.12	52.55	Spherical or hemispherical	(Ren, 2017)
Arrowroot	24.95	NR	A-type	31.4-44.9	75.02	Spherical, oval, and irregular	(Malki et al., 2023)
Avocado seed	39.56	NR	NR	NR	88.5	Oval	(Martins et al., 2022)
Barley	20	28.3	A-type	17.3	NR	Disk	(Martens et al., 2018)
<i>Canna edulis</i> Ker	19.23	26	B-type	31.52	53.45	Spherical and heart	(Ren, 2017)
Cassava	26.2-62	22.5-30	A-type	13.3-14.2	70.9-71.9	Polyhedral, spherical, hemispherical, flat, partially round, semi-ellipsoidal, or irregular	(Huang et al., 2024; Ren, 2017)
Coix seed	25.62	33	A-type	13.31	61.1	Spherical or hemispherical	(Ren, 2017)
Corn	19-24.9	21.5-39	A-type	7.97-17.6	64.45-82.5	Polyhedral, spherical, hemispherical, flat, and squared	(Abelti et al., 2024; Hsieh et al., 2019; Martens et al., 2018; Ren, 2017)
Corn HAS	46-55	28-33.7	A-type	11-13.8	NR	Squared and elongated	(Martens et al., 2018)
Corn waxy	0	28.2	A-type	16	70.2	Squared and elongated	(Hsieh et al., 2019; Martens et al., 2018)
<i>Dioscorea alata</i> L.	36.49-40.24	17.2-18.1	B- and C-type	25.3-32.3	65.02-69.86	Flat oval, triangular, round, and elliptical	(Jiang et al., 2023)
Kiwicha	10.05	NR	NR	1.85	63.56	Polygonal	(Velásquez-Barreto et al., 2021)
Konjac	18.9	31	A-type	11.1	69.45	Polyhedral, spherical, hemispherical, and flat	(Ren, 2017)
Kudzu root	17.37	24	A-type	13.18	67.1	Polyhedral, spherical, hemispherical, and flat	(Ren, 2017)
Lily	28.42	30	A-type	20.05	77.45	Polyhedral, spherical, hemispherical, and flat	(Ren, 2017)
Lotus root	26-27	21	C-type	13.96	53.55	Rod and spherical	(Ren, 2017)
Lotus seed	28.38	20-30	A and B-type	48.99	54.6	Elongated, oval, heart, and triangular	(Ren, 2017; Sun et al., 2023)
Mungbean	23.51-29.1	28	A-type	15.13	72.65	Flat, heart and oval	(Ren, 2017; Roy et al., 2020)
<i>Nymphaea lotus</i>	23.03-24.50	27.03-27.63	A-type	11.19-3.56	71.90-78.27	Polyhedral, angular, and oval	(Abelti et al., 2024)

Table 1 (Continued)

Sample	Amylose (%)	Crystallinity (%)	Diffraction pattern	Average diameter	Pasting temperature (°C)	Morphology	Reference
Papaya	20.41	22	A-type	20.47	68.6	Spherical or hemispherical	(Ren, 2017)
Pea	27	27.3	C-type	24.7	NR	Elongated, round, and bean	(Martens et al., 2018)
<i>Polygonum multiflorum</i> Thunb.	19.23	18	A-type	13.63	56.3	Polyhedral, spherical, hemispherical, and flat	(Ren, 2017)
Potato	18-32	19.7-30	B-type	13.97-45.8	63.8-72.65	Oval, spherical, round, ellipsoidal, cuboidal, elongated	(Abelti et al., 2024; Barandiaran et al., 2024; Martens et al., 2018; S. Ren, 2017)
Potato waxy	0	32.6-33	C-type	45.4	66.4	Elongated and round	(Hsieh et al., 2019; Martens et al., 2018)
Quinoa	13.01-19.05	NR	NR	1.29-1.71	60.37-69.74	Polygonal	(Velásquez-Barreto et al., 2021)
Rice	14-16-21.3	24.2-34.7	A-type	6.2-6.6	93.32	Square, polygonal	(Abelti et al., 2024; Martens et al., 2018)
<i>Semen euryales</i>	18.75	17	C-type	14.34	73.4	Rod and spherical	(Ren, 2017)
Sweet potato	18.7-25.1	30-39.6	A and C-type	13.6	75-82	Spherical or hemispherical	(Ren, 2017; Wang et al., 2020)
Taro	15.93	25.07	A-type	2.55	85.15	Irregular polygonal, cubic	(Huang et al., 2024)
Waxy rice	1	27.1	A-type	8.3	NR	Square	(Martens et al., 2018)
Wheat	9.26-37.09	20.3-38.4	A-type	14.23-20.6	83.1-94.7	Spherical, round, oblate, ellipsoidal	(Huang et al., 2024; X. Liu et al., 2024; Martens et al., 2018; Ren, 2017)
Wheat waxy	NR	33.3	A-type	NR	66.8	Round	(Hsieh et al., 2019)
<i>Wolfiporia extensa</i>	18.51	28	A-type	37.08	69.4	Polyhedral, spherical, hemispherical, and flat	(Ren, 2017)
Yam	22.5-25.8	19-24	B and C-type	14.74	72-84	Spherical or triangular	(Oliveira et al., 2021; Ren, 2017)

NR: not reported

1.2.2.2. Amylopectin

The AP is the main component of starch granules. This molecule comprises short chains linked by α -(1–4) glycosidic bonds connected to side chains through α -(1→6) linkages. The branching level of AP can reach up to 5% at branching points (Bertoft, 2017). This molecule has a DP of $\sim 10^5$, and its molecular weight ranges from 1×10^7 to 5×10^8 Da (approximately 1000 times that of AM) (Figure 4). The main chain of AP can contain 10 to 60 D-glucose units, while the side chains have 15 to 45 D-glucose units (Gamage et al., 2022). The AP has been linked to the starch granule's ability to

swell and form a gel (Doblado-Maldonado et al., 2017; Vamadevan & Bertoft, 2020). Furthermore, the structure of AP and the organization of its double helices can influence other properties of the granule, such as hydrophobicity, viscosity, and crystallinity (Nakamura, 2023).

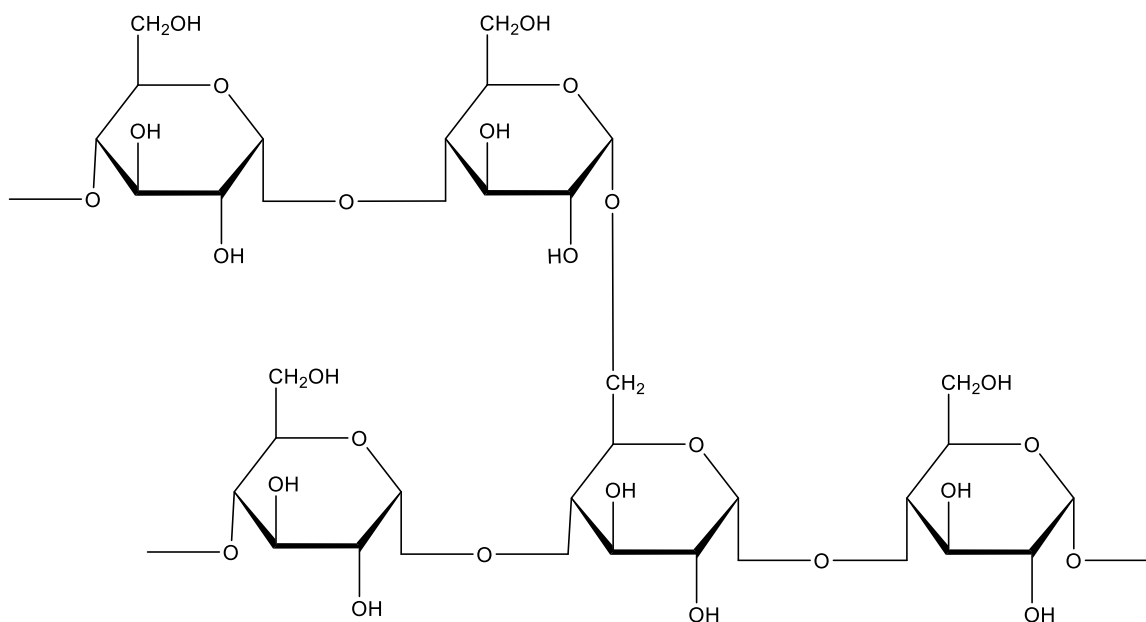


Figure 4. Chemical structure of amylopectin

The structure of AP can be explained by using different models. However, to date, there are two contradictory models, the cluster model and the building block backbone model (Bertoft, 2013, 2017), the first being the most accepted currently (Nakamura & Kainuma, 2022). According to the cluster model, the chains that constitute AP connect and organize themselves into a structure called a cluster (Nakamura, 2023). The A-chains (DP 6-12) are short and unbranched (external chains), while the B-chains (internal chains), which are branched, are subdivided into B1 chains (DP 13-24), short chains present within a single cluster, and B2 (DP 25-36), B3 (≥ 37), and B4 chains, which interconnect 2, 3, and 4 clusters, respectively (Figure 5) (Nakamura, 2023; Nakamura & Kainuma, 2022). The C chain is the only reducing end (Bijttebier et al., 2008).

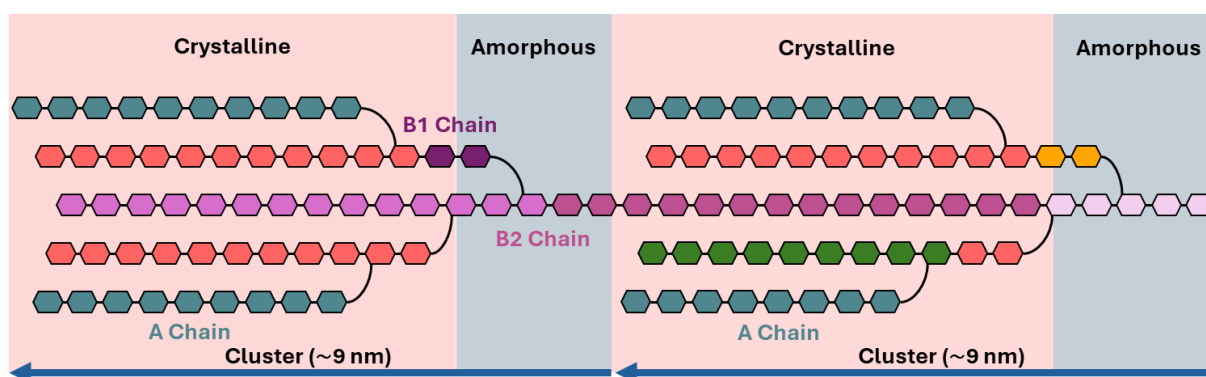


Figure 5. Representation of the structure of amylopectin cluster. Adapted from (Nakamura, 2023).

1.2.2.3. Starch granule architecture

As shown previously, AP exhibits a tree-like architecture, where short chains of AP are oriented within clusters (A- and B1-chains) with lengths of 9 nm (approximately 27-28 glucose units) that are connected by longer chains (B2-, B3-, and B4-chains) (Figure 6). As the number of branch points increases (towards the reducing end), linear segments of sufficient length can form double helices. Furthermore, neighboring double helices can align parallelly to form crystalline lamellae (Pfister et al., 2020). The packing of these double helices can result in two types of allomorphs. On the one hand, the A-type crystalline allomorph has a monoclinic unit cell structure that gives it a higher packing density, limiting the amount of bound water (Doblado-Maldonado et al., 2017). This type of crystalline structure can be observed mainly in starches obtained from cereals. On the other hand, the B-type crystalline allomorph features a hexagonal packing, allowing for greater water retention. This type of structure is generally found in starch obtained from tubers. Two other allomorphs can be found in starches: type C and type V. The first corresponds to a mixture of the first two crystallinity patterns and can be observed in starches from some oilseeds (Bertoft, 2017). The type V crystallinity pattern corresponds to starches that exhibit crystallization or complexes between AM and lipids (Daza et al., 2022).

In addition to the crystalline lamellae (3-6 nm) formed by the alignment of double helices, there are also less dense amorphous lamellae (4-6 nm) that may or may not have branch points and where it has been described that AM is found in the arrangement of simple helices. However, there is evidence that some AM molecules may be present inside the granule and that these can form double helices with the chains of AP or become entangled in the granular structure (Glaring et al., 2006; Roos, 1995; Seung, 2020). Crystall lamellae are more resistant to enzymatic and chemical attacks than amorphous ones. Likewise, the amorphous lamella can absorb more water at lower temperatures than the gelatinization temperature. The alignment of the double helices is associated with the type of crystalline allomorph.

1.2.3.1. Swelling and solubility

Starch granules are insoluble in cold water and even at room temperature. However, when heated with an excess of water, the bonds and binding forces of the granule weaken and break, allowing for new interactions between the hydroxyl groups of the AM and AP chains and water molecules. This process causes swelling and disorganization of the crystalline structures (the birefringence phenomenon disappears) (Cornejo-Ramírez et al., 2018). At the same time, leaching and solubility of AM chains and small AP chains occurs until reaching a temperature at which the granule disintegrates and dissolves in the aqueous medium (Vamadevan & Bertoft, 2020). Solubility, therefore, is mainly represented by the degree of dissolution of AM in water after heating or gelatinization (Zhuang et al., 2024).

Different factors influence the swelling of starch and its solubility, such as the ratio between AM and AP and their molecular structures, the size of the granules, the presence of ligands (such as lipids), and other compounds that may interact with the starch chains (Zhu, 2014). For example, forming lipid-AM complexes can create a water-insoluble layer that prevents the entry or interaction with water molecules, limiting the swelling of the granule (Putseys et al., 2010). Additionally, the effect of adding cations on the swelling of potato starch was evaluated by Zhuang et al. (2024), who observed that the addition of cations (mono-, di-, and trivalent) and the increase in their concentration had an inverse relationship with the swelling of the granule due to the breaking of hydrogen bonds and the cations' ability to compete for or absorb water. However, the opposite effect was observed with starch solubility. The authors noted that adding di- or trivalent cations at a concentration of $5 \mu\text{mol} \cdot \text{g}^{-1}$ increased the solubility of the granule, which was associated with the destruction or reduction of crystalline zones due to the addition of salts. It has been established that the presence of phosphate groups in starch can improve its swelling and solubility by associating with negatively charged phosphate groups that reduce intermolecular binding forces by repelling phosphate groups from adjacent chains, thus favoring water absorption (Bruni et al., 2018; Ding et al., 2024).

Regarding the concentration of AM, it has been established that high concentrations of this molecule decrease the swelling capacity of the granule. Kong et al. (2015) established an inverse correlation between the apparent AM content of 14 rice cultivars and their swelling power. Still, they did not observe a relationship between the AM content and the solubility of the samples. On the other hand, Vamadevan & Bertoft. (2020) evaluated the swelling power of different starches and found that waxy starches were more sensitive to swelling than non-waxy starches from the same botanical source. They also observed that the structure of amylopectin influenced the swelling of the granules; starches with shorter amylopectin chains of types A and B1 showed greater susceptibility to swelling at low temperatures (around 55°C) and loss of integrity above 85°C . In contrast, starches with a higher

number of internal and longer chains of amylopectin were more resistant to swelling and showed greater stability. It was also demonstrated that shorter internal B chains in the amylopectin molecule were inversely related to the leaching of amylose, preventing its solubilization because amylose and internal parts of amylopectin can coexist in the amorphous region and become entangled, avoiding the leaching and swelling process (Li & Zhu, 2017).

There is contradictory evidence regarding the influence of particle size on the swelling capacity of starch granules and their solubility (Jia et al., 2023). Liu et al. (2023) did not find a direct relationship between swelling power and particle size. On the other hand, Hu et al. (2020) found that a smaller particle size of potato starch increased the swelling power, associating it with the expansion rate of potato powder particles. In contrast, increased starch particle size has been observed to increase swelling power (Katyal et al., 2019). Starch granules with type B allomorphs are known to have a low AM content, high crystallinity, and a large surface area per unit weight, making them hydrate and swell more efficiently than granules with type A allomorphs (Cornejo-Ramírez et al., 2018). Particle size alone is not decisive in the swelling capacity of starch granules. It is necessary to consider this parameter along with others, such as the concentration of amylose and its relationship with amylopectin and crystallinity.

1.2.3.2. Pasting properties and retrogradation

As mentioned earlier, subjecting starch to high temperatures will produce a series of structural changes collectively referred to as starch gelatinization, which includes the following changes: swelling of granules due to water absorption, loss of crystallinity due to dissociation of the double helix of amylopectin, solubilization and leaching of amylose, and granule rupture (Obadi et al., 2023). The swelling capacity and solubility of starch granules are linked to the gelatinization and pasting properties of starch. The evaluation of pasting properties is crucial in determining the potential use of the material. The most widely used equipment for assessing pasting properties in laboratory and industrial settings are the Rapid Visco-Analyzer (RVA) and Brabender Visco-Amylograph (BVA) (Zhu, 2014). Figure 7 shows a typical pasting curve of commercial potato starch obtained using a Micro Visco-AmyloGraph Brabender (Daza et al., 2022).

The peak viscosity (PV), also known as the swelling peak, indicates the maximum viscosity starch can reach after gelatinization and before total granule disintegration (Obadi et al., 2023; Ren, 2017). The PV, like granule swelling, is associated with the ratio between AM and AP, their structures, and the presence of ligands. It has been previously reported that waxy starches present a high PV, while HAS present low PV due to the controlled swelling of starch granules (Hsieh et al., 2019; Li, Dhital, et al., 2020; Zhang et al., 2022). The presence of AM and AP structures also influences the

starch granule's thermal stability; a high pasting temperature (PT) is associated with more stability of the starch granules. It has been evidenced that the absence of AM in waxy starch samples can considerably decrease the PT compared to their native counterpart (Table 1) (Hsieh et al., 2019). If the heating process continues, the starch granule disintegrates, generating a viscosity decrease known as breakdown (BD), which measures the paste stability and the granules' tendency to resist shear force during the heating process (Daza et al., 2023). After the viscosity drop, leached AM and linear fractions of AP tend to reorganize or reassociate, a phenomenon known as retrogradation, leading to the formation of starch pastes or gels with a final viscosity (FV) higher than the trough or holding viscosity.

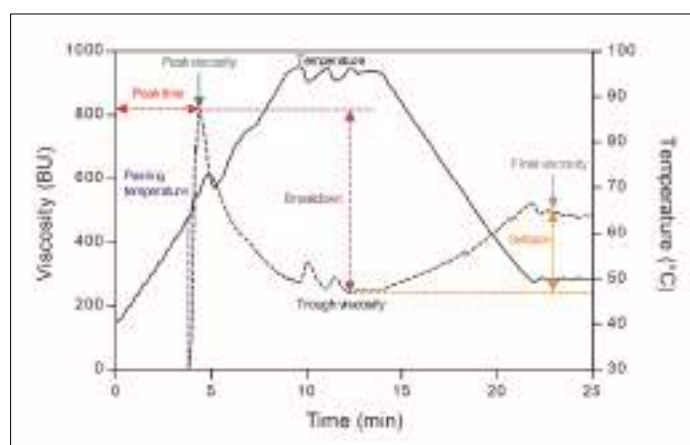


Figure 7. Pasting profile of commercial potato starch

Since starch gels are metastable matrices and non-equilibrium systems, they can undergo changes. Therefore, retrogradation is associated with storing starch-rich products and is influenced by water content and parameters such as storage temperature (Doblado-Maldonado et al., 2017). Retrogradation can lead to water loss, reduced shelf life, and decreased consumer acceptance of the product. Additionally, in this process, amylose tends to form double helix interactions of about 40 to 70 glucose units, which leads to the formation of crystalline AM; thus, the higher the AM concentration, the stronger the tendency to retrograde (Hoover, 2001; Zhao et al., 2021). Starch retrogradation is associated with syneresis, which is defined as a process in which a gel contracts when left at rest and exudes liquid. This phenomenon is related to the stability of the formed gel and can be measured in cycles of freezing and thawing (Mohamed et al., 2022). A low syneresis indicates a low retrogradation associated with the strong interaction between dispersed amylose and amylopectin and water molecules.

1.2.4. Starch modification

Starch, in its native form, does not always exhibit the appropriate physical and chemical properties for use as a raw material in industrial processes. However, modifying this material can significantly improve its technological and functional properties (Suri & Singh, 2023). Starch modifications can be classified as physical (thermal and non-thermal), chemical (oxidation, acetylation, succinylation, acid hydrolysis, and cross-linking), enzymatic, and dual or synergistic (homogeneous and heterogeneous) (Kumari & Sit, 2023).

In general, chemical modifications involve reactions that occur in the hydroxyl groups of the AM and AP chains, leading to cross-linking or substitutions (Zailani et al., 2023). Although chemical modification allows for a broader range of functionalities of the treated starches, the use of reagents such as acids, phosphates, and acetates, among others, can generate materials with trace components, as well as negatively impact the environment (Punia Bangar et al., 2022). On the other hand, enzymatic modifications are mediated by enzymes that can be obtained from different sources, such as humans, animals, bacteria, and fungi (Zailani et al., 2023). Traditionally, enzymatic modification has been divided into four categories based on the type of enzyme used: endoamylases, exoamylases, debranching enzymes, and transferases (Compart et al., 2023). Enzymatic modification aims to change the molecular mass, the distribution of branched-chain length, and the ratio of AM/AP in starch (Park et al., 2018). Despite the increasing use of this methodology in recent years, mainly in the food industry, it presents some limitations, such as processing times for obtaining the modification, structural instability, and low catalytic efficiency of the natural enzymes used (Compart et al., 2023).

Regarding physical methods, it has been established that their environmental impact, compared to chemical methods, is lower. Furthermore, physical modifications of starch are presented as simple, safe, green, or "clean label" and cost-effective methods (Punia, 2020). Physical modifications destroy or change the packing arrangement of the AM and AP chains present in the starch granule. These changes in structure produce altered physical characteristics of the gels and pastes that starches can form after gelatinization (Punia, 2020). The most widely used methods in this category of starch modification are pre-gelatinization, extrusion, thermal hydration, annealing, microwaves (thermal methods), ultra-high pressure, ultrasonication, and pulsed electric field (non-thermal methods) (Amaraweera et al., 2021; Compart et al., 2023; Punia, 2020).

Annealing (ANN) is a physical modification technique that leads to a reorganization of the AM and the double helices of the AP, generating an improvement in the

structural arrangement of the granule. This involves subjecting the sample to an excess of water (>40%) to heating at a temperature above the glass transition temperature (T_g) but below the gelatinization temperature (Parra et al., 2022). The benefits of modifying starches with this method include increased thermal stability, gelatinization temperature, granule size of the starch, texture properties, and molecular mobility while decreasing structural relaxation (Fonseca et al., 2021; Punia, 2020). ANN modification of sweet potato starch for 1, 3, and 5 days showed an increase in the gelatinization temperature and paste viscosity of the starch, as well as the particle size of the granule (Hu et al., 2020). Similar observations were made for potato and amaranth starches (Siwatch et al., 2022). However, in both cases, a decrease in crystallinity was observed, which can be explained by a reduction in inter-crystalline space due to the increased interaction of the AM and AP chains (Siwatch et al., 2022). However, Zheng et al. (2023) observed that the increase or decrease in the crystallinity of starches modified by ANN depends on the botanical source, the type of allomorph, and the incubation time to generate the modification. For example, they observed that at 24 and 48 h, the starch crystallinity increased for corn starch, but treatments equal to or longer than 48 h decreased crystallinity. Meanwhile, they observed an increase at all treatment times for potato starch and a decrease for pea starch. The authors attribute the increase in crystallinity in annealed potato starch to a rise in the mobility of amorphous regions to a crystalline state. On the other hand, they suggest that the decrease in corn and pea starches after 48 h of annealing occurs because the alteration of the crystalline structure and reorganization of the double helices of AP and the alteration of the amorphous zones coincide.

1.2.5. Starch applications

Starch is a raw material with great potential for use in different industries due to its low cost, renewability, and abundant availability (Agarwal et al., 2023). Because of its characteristics and versatility, starch is widely used as a raw material in food, adhesives, binders, paper production, the textile industry, chemical production, and fermentation processes (Ogunsona et al., 2018).

In the food industry, starch has been widely used in battered and breaded foods (adhesion), formed meat, snacks seasonings (binding agent), beverages (stabilizer and clouding agent), fried foods (crispness), ice creams, salad dressings (fat replacer), and mainly in baked foods (Imbachí-Narváez et al., 2019; Le-Bail et al., 2017; Liu et al., 2022; van Rooyen et al., 2023). However, in recent years, new alternatives to using starches have been sought in response to market demands, food safety, and sustainable development goals. Among these new alternatives are processes associated with nanotechnology, where the production of nanoparticles from native starches from different sources has been sought (Gujral et al., 2021;

Gutiérrez et al., 2020; Jiang et al., 2022). These nanoparticles have been mainly used as carriers of bioactive compounds (Jiang et al., 2022), as stabilizing agents in emulsions (Ko & Kim, 2021; Yao et al., 2023), and as fillers in biodegradable films (Campelo et al., 2020). Furthermore, using starches for 3D food printing has been gaining importance due to the possibility of customizing diets. Although this process is in its early stages in the food industry, the properties of 3D prints obtained from potato, rice, corn, cassava, wheat, sweet potato, and buckwheat starches have been evaluated (Bugarin-Castillo et al., 2023; Chen et al., 2019; Cui et al., 2022; Ji et al., 2022; Maniglia et al., 2020; Martínez-Monzó et al., 2019; Theagarajan et al., 2020; Xu et al., 2020)

One of the most interesting applications of starch that have garnered significant interest is the preparation of biodegradable packaging or coatings that can replace commercially available ones, which are generally made from petroleum-derived materials, making them materials with low degradability rates (Agarwal et al., 2023). Starch has a remarkable ability to form films mainly due to its concentration of AM. Because the characteristics of starch depend primarily on its botanical source, different authors have evaluated the physicochemical properties of films obtained using conventional and unconventional starches such as arracacha (Sánchez Riaño et al., 2022), sweet potato (Song et al., 2021), cassava (Lim et al., 2020; W. Liu et al., 2020), mung bean, water chestnut (Suh et al., 2020), anchote (Abera et al., 2020), corn (Dai et al., 2015), potato (Zavareze et al., 2012) among others. Films prepared from starch exhibit high transparency, low oxygen permeability, odorless and colorless nature, high degradability, and good thermal properties. However, they have some limitations, such as their high affinity for water, which increases their solubility and water vapor permeability (WVP), and deficient mechanical properties compared to conventional plastics (Gonçalves et al., 2024; Jiménez et al., 2012).

As mentioned, the heterogeneity of starch characteristics, even in starches of the same species, creates the need to evaluate and characterize the potential of each starch as a film former, as well as the best process alternative for packaging production. Dai et al. (2019) evaluated starches from different botanical sources (waxy maize, cassava, sweet potato, potato, wheat, maize, and three modified cassava starches) with varying AM concentrations. The authors did not observe a correlation between AM content and transparency. Regarding the surface morphology of the films, it was observed that lower AM content produced more uniform and dense films, which was inversely associated with WVP values. In addition, elongation values ranged from 13 to 181%, with modified starches showing the highest elongation compared to native starches. On the other hand, Luchese et al. (2018) prepared films from potato, wheat, maize, and cassava starches and demonstrated that different starch concentrations affected the

properties of the films. They also associated the AM content with the ability to form films but could not relate it to the general properties of the prepared films.

Unlike previous studies, Suh et al. (2020) observed a relationship between AM content and the mechanical properties, crystallinity, and solubility of films prepared with mung bean, water chestnut, sweet potato, and cassava starches. Additionally, differences have been reported between materials prepared with starches of the same variety and similar AM contents. Song et al. (2021) prepared films from starch obtained from five sweet potato cultivars and found differences in morphology, thickness, mechanical properties, water, and oxygen permeability among the films.

To improve the characteristics of films prepared from native starch, the inclusion of different materials that can interact with starch has been employed, among which the use of plasticizers, nanomaterials, crosslinking agents, other biopolymers such as gums, proteins, chitosan, gelatin, waxes, oils, essential oils, and even modified starches or starch nanoparticles stand out (Thakur et al., 2019). For example, Dutta & Sit (2022) prepared films from native potato starch and starch modified by annealing and heat-moisture treatment and observed an improvement in the mechanical properties, solubility, transparency, and WVP of the materials prepared.

1.3. Bioplastics

Bioplastics are materials of biological origin, biodegradable, or that can exhibit both properties (Fredi & Dorigato, 2021). The term "bioplastic" applies to a varied range of materials that can be produced from renewable sources of plant origin and refined fossil fuels to be converted into biodegradable materials such as polycaprolactone (PCL) (Boisseaux et al., 2023). The classification of these materials suggests three main groups. The first corresponds to "biologically based" materials produced from biological resources, such as polylactic acid (PLA), polyhydroxyalkanoates (PHA), thermoplastic starch (TPS), and poly(butylene succinate) (PBS). The second corresponds to biodegradable plastics from petrochemical resources, such as poly(butylene adipate terephthalate) (PBAT) and PCL. The third group corresponds to non-biodegradable or partially biodegradable plastics produced from monomers of biological origin, such as bio-polyethylene (bioPE), bio-poly(ethylene terephthalate) (bioPET), bio-polypropylene (bioPP), and also bioderived technical polymers, such as bio-poly(trimethylene terephthalate) (bioPTT) or some bio-polyamides (bioPA) (Coppola et al., 2021; Fredi & Dorigato, 2021; Jayakumar et al., 2023) (Figure 8).

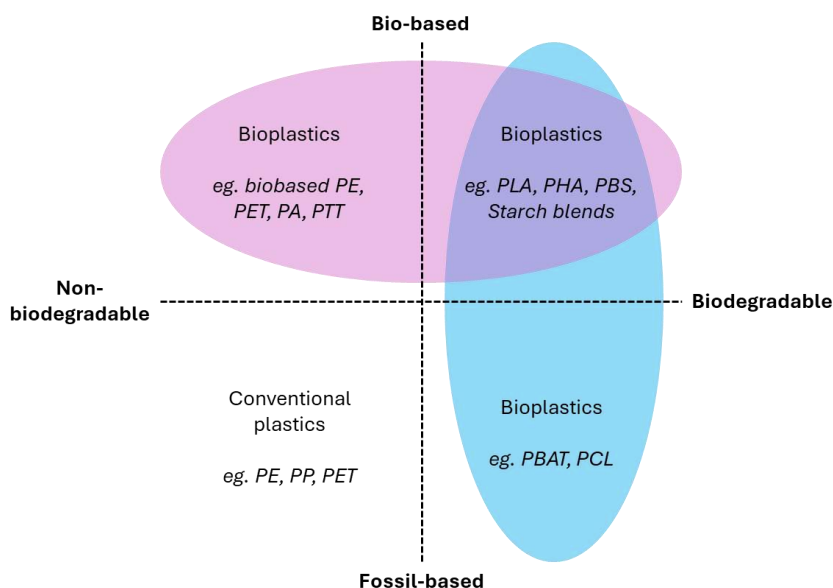


Figure 8. Classification of bioplastics. (Philp et al., 2013).

In 2021, global plastics production reached 390.7 million tons, primarily destined for the packaging and construction sectors (Figure 9). Despite the increasing production of bioplastics in recent years, it is estimated that the share of these materials in global production was only 1.5% (Plastics Europe, 2022), of which approximately 60% is used in the packaging industry and the rest in other sectors such as agriculture and textiles (European Commission, 2019). From a commercial point of view, the market has favored the production of bioplastics from renewable plant sources, with PLA obtained from corn and BioPE based on sugarcane being examples of commercial bioplastics (Boisseaux et al., 2023). However, these materials are classified as first-generation bioplastics (1GB), produced from plant sources primarily used for human consumption, affecting food security (Jayakumar et al., 2023). The current trend is towards second-generation (2GB) and third-generation (3GB) bioplastics, which are obtained from non-commercial crops, agricultural, agro-industrial, or food waste (2GB), and non-food resources such as algae, bacteria, and fungi (3GB) (Boisseaux et al., 2023).

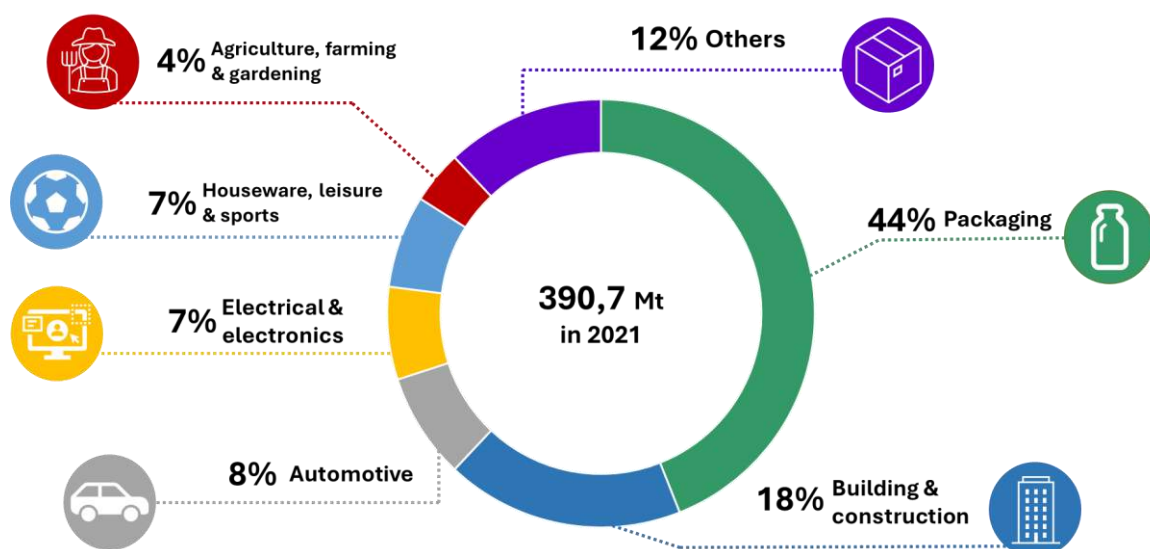


Figure 9. Main plastic use sectors

Different authors have obtained and evaluated the properties of 2GB from various raw materials. Among the characteristics of these bioplastics, those most relevant are the micro and macrostructural, optical, mechanical, thermal, barrier, bioactive, and functional properties (Cheng et al., 2021). Zhang et al. (2023) extracted and mixed pectin and cellulose from different citrus peels to form a film-forming suspension and thus produce biodegradable films. Once prepared, they were immersed in a CaCl_2 solution to cross-link the pectin chains. The bioplastic films exhibited a structure of dense layers, which was related to the inter- and intramolecular "entanglement" between the cross-linked pectin and cellulose fibers. Additionally, excellent mechanical properties such as elongation ($> 25\%$) and tensile strength ($> 40 \text{ MPa}$) were observed. Likewise, the evaluated films showed a high degradation rate and excellent ability to act as a barrier against water. The authors concluded that using these residues in the preparation of bioplastics is an environmentally friendly alternative to the use of plastics derived from the petrochemical industry.

On the other hand, Kharb & Saharan (2022) evaluated the potential of cucumber residues mixed with corn starch, acetic acid, and glycerol or sorbitol to obtain bioplastic. The samples showed good thermal stability and physical interaction between the components determined by FTIR. However, the samples exhibited heterogeneous microstructures. This demonstrates that 2GB has excellent potential to be used in different industries, including the food packaging industry, due to their barrier, mechanical, and thermal properties.

1.3.1. Methods for the preparation of bioplastics

The design and production of bioplastics depend on different parameters, such as the material's final use, the technology employed, the raw materials, and the process conditions. Currently, the preparation of bioplastics, specifically

biodegradable films, can be carried out using two different methods: the wet method or solvent casting processes and the dry method or extrusion method (Suhag et al., 2020).

1.3.1.1 Casting method

The solvent casting method is the most widely used technique in the production of biodegradable and edible films because it is easy to operate, cost-effective, and environmentally friendly (Table 2) (Wang et al., 2021). According to various authors, this technique can be divided into three stages: i) solubilization or dispersion of the biopolymer in a suitable solvent, ii) heating, melting, and casting of the solution, and iii) drying of the molten solution (Prakoso et al., 2023; Suhag et al., 2020). Once the drying stage is completed, the material is removed or delaminated from its mold and stored for later use (Figure 10).

Table 2. Main advantages and disadvantages of the casting method

Advantages	Disadvantages
- Easy preparation. - Does not require the use of specialized equipment. - Low processing cost - More effective interaction between particles results in a more homogeneous packing, reducing the incidence of defects, especially smaller ones. - Optical purity of materials	- The shapes of the materials are limited - Denaturation of some molecules - Low production volumes - Product heterogeneity

In the solubilization stage, the preferred solvent in the food industry is water. However, different solvents or solvent mixtures can be used depending on the components employed and their solubility. This selection will impact the physicochemical properties and performance of the resulting film. A study conducted by Cetindag et al. (2020) evaluated the use of different solvents in the preparing films based on hydroxypropyl methylcellulose or hydroxypropyl cellulose and with the addition of fenofibrate as an active ingredient. The study found that the selection of solvent and polymer significantly affected the tensile strength (TS), Young's modulus (YM), elongation at break (EB), and crystallization of the films due to the solubility of the materials in the solvent system used.

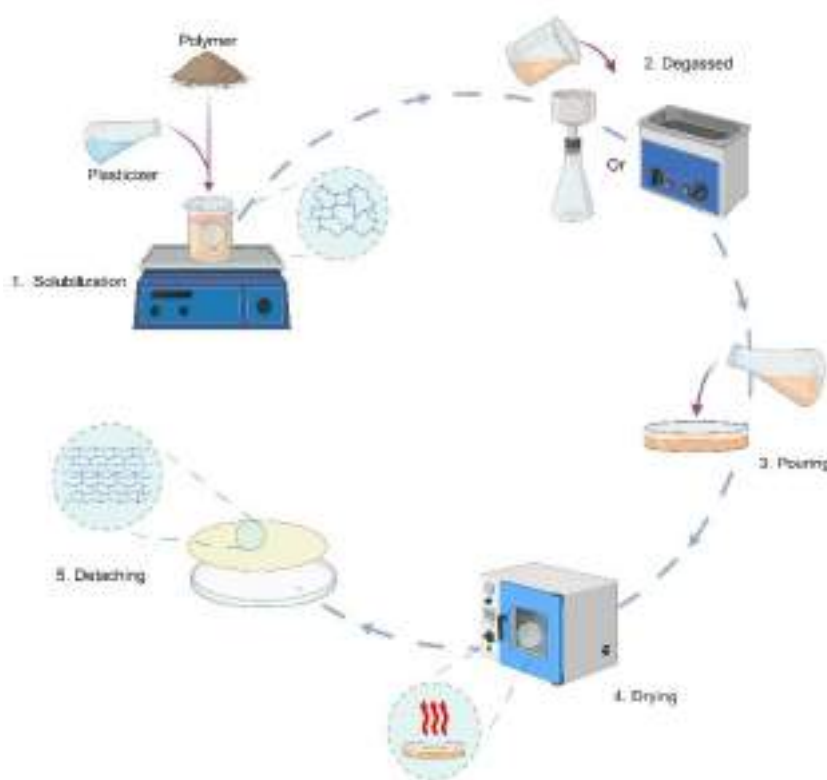


Figure 10. Process for preparing films by the casting method

The solubilization process of the materials is accompanied by thermal and agitation processes that seek to reduce the intramolecular interactions or bonds of the polymers to increase their solubility. Likewise, the thermal process can lead to the gelatinization or gelation of the components. The temperature of this stage varies according to the polymers used in the preparation, but always avoiding subjecting the thermolabile compounds to high temperatures (Kumari et al., 2021; Lin et al., 2022).

Once the incorporation and homogenization of all components have been achieved, it is necessary to degas the solution to remove excess bubbles and achieve a homogeneous film. This degassing stage has been done through filtration, sonicators (Yuan et al., 2021), or vacuum (Li, Pei, et al., 2020). After this, the mixture is poured onto a support or mold that can be made of different materials, such as plastic, glass, and silicone resin, among others, which have predefined dimensional characteristics (Wang et al., 2021).

Finally, in the drying stage, the solvent evaporates, and the film forms on the mold. In film production, this stage is one of the most complex. Inadequate conditions in the application of the thermal process can result in morphological defects and poor performance of the films obtained. The relationship between drying temperature and time provides the conditions for polymer chains to interact and achieve the highest organization configuration. Thus, it has been observed that high drying

temperatures and short processing times can lead to deficiencies in the prepared materials. On the other hand, long drying periods can generate defects such as blisters, deformations, and cracks (Li et al., 2019; Suhag et al., 2020).

Since each polymer or combination of polymers has different properties, it is essential to determine the drying conditions that provide the best material yields. (Li et al., 2019) evaluated different drying temperatures (40, 50, 60, 70, and 80 °C) in the preparation of konjac glucomannan-zein blend films and observed that films obtained at a temperature of 60 °C improved the interaction of the components, resulting in better properties of hydrophobicity, TS, EB, and water vapor permeability (WVP). On the other hand, Homez-Jara et al. (2018) prepared chitosan films using different drying temperatures (2, 25, and 40 °C) and found that films obtained at a drying temperature of 40 °C showed the best swelling power values (SP), while films prepared at 25 °C showed the lowest values in this parameter. Regarding the mechanical properties, the authors observed that an increase in temperature led to an increase in TS, which intensified with increased chitosan concentration. However, the EB of the films showed an inverse behavior, which was attributed to the fact that a lower drying temperature allowed for greater water incorporation between the polymer chains and, thus, greater plasticity of the material. Finally, the authors observed an inverse relationship between drying temperature and WVP, explaining that the higher moisture contents reached in films prepared at 2 °C allowed for greater plasticization and formation of amorphous zones that allowed the passage of water vapor.

Similarly, Daza et al. (2018) evaluated the effect of the drying temperature of starch-based films on water affinity, barrier, optical, and mechanical properties. Their findings demonstrated a high dependence of EB, moisture content (MC), and opacity of the films on the drying temperature. By optimizing the process, they determined an optimal temperature of 60 °C and an optimal concentration of starch and plasticizer of 2.6% and 1%, respectively.

Once the stages described above have been completed, the samples must be stored under controlled relative humidity and temperature (usually room temperature). Different salts have been used to adjust the relative humidity to values between 30 and 58%. During this storage period, the polymer chains finish their reorganization to impart the final properties to the materials.

1.3.1.2. Use of plasticizers in the preparation of films by the casting method

A relevant aspect in the formulation of films is the addition, concentration, and selection of the plasticizer. Plasticizers are low molecular weight and low volatility compounds (Eslami et al., 2023). It has been observed that in the absence or at low concentrations of plasticizer, the resulting films are brittle, while an excess of plasticizer can lead to the obtainment of soft and weak films, which is explained by

the antiplasticization effect (Janjarasskul et al., 2020; Mascia et al., 2020). There are two types of plasticizers: hydrophilic ones, such as polyols, monosaccharides, disaccharides, and oligosaccharides, and hydrophobic plasticizers, such as citrate esters, the latter mainly used in the pharmaceutical industry (Suderman et al., 2018).

Different theories have attempted to explain the lubrication or plasticization mechanism, among which the "lubricity theory," "gel theory," and "free volume theory" can be mentioned, with the latter being the most accepted today. The free volume theory postulates that there is unoccupied space between polymer chains that can vary in size depending on the type of polymer; for example, a very rigid polymer will have less free volume than a less rigid one. According to this theory, plasticizers function by increasing this free volume, which reduces the interaction between the chains (intermolecular forces), thus increasing their mobility (Eslami et al., 2023). The interaction between glycerol (plasticizer) and starch is shown in Figure 11.

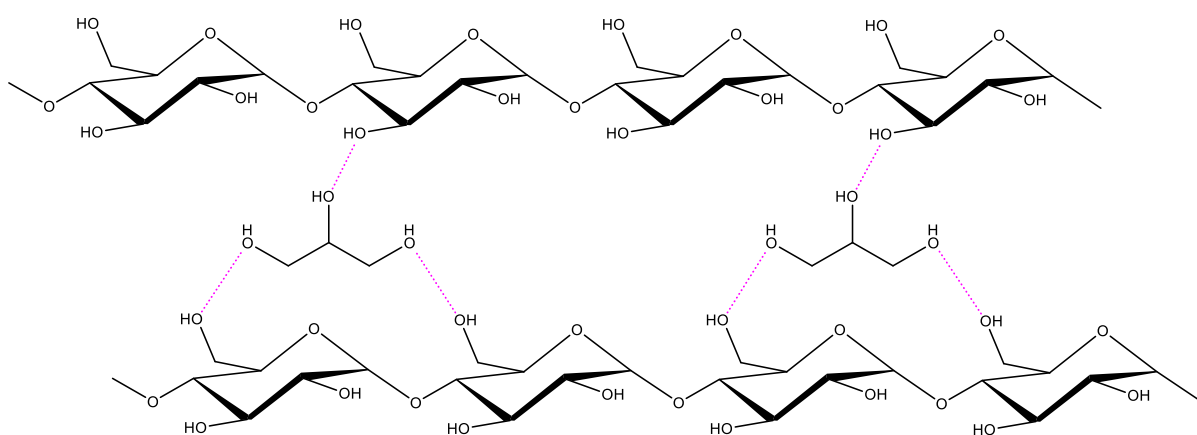


Figure 11. Hydrogen bonds in glycerol plasticized starch

It has been demonstrated that the mechanical, thermal, and water affinity properties are associated with the type and concentration of plasticizer added to the formulation. Ibrahim et al. (2019) evaluated the effect of adding three plasticizers (fructose, sorbitol, and urea) at different concentrations (0%, 25%, 40%, and 55%) on the physicochemical properties of corn starch-based films. In general, a rise in the plasticizer concentration led to an increase in water solubility. This effect was more noticeable in films prepared with the inclusion of urea, followed by sorbitol and fructose. Moreover, an increase in the plasticizer concentration increased the thickness of the films, which was attributed to the greater spacing between polymer chains. Regarding thermal stability, the authors observed that the addition of plasticizers at different concentrations did not significantly affect the stability of the films. However, the concentration of plasticizer affected the residue after decomposition. In terms of morphology, the authors observed that an increase in the concentration of any plasticizer resulted in films with more irregular surfaces.

They also established an anti-plasticizing effect at plasticizer concentrations lower than 15% of the starch weight and segregation at concentrations higher than 60%. Finally, the authors observed that the addition of plasticizer at high concentrations reduced the TS of the films, attributing this decrease to the fact that at higher concentrations of plasticizers, the hydrogen bonds between the plasticizer and polymer chains decrease.

In addition to the properties mentioned above, the concentration and type of plasticizer also affect the barrier properties. It has been observed that an increase in plasticizer up to critical concentrations can increase WVP and oxygen permeability associated with an increase in free volume between chains that allows and facilitates the passage of water and oxygen (Sanyang et al., 2015; Wang et al., 2022). Furthermore, the increase in plasticizers has also been associated with decreased T_g of starch-based films (Sanyang et al., 2015).

1.3.2. Properties of biodegradable films

The production of biodegradable films aims to replace some petroleum-derived products for use in the food industry. Consequently, these films should facilitate the handling and transportation of products. Additionally, they must serve as a barrier and protect the matrix against environmental conditions such as temperature, humidity, light, and oxygen, thus extending their shelf life (Sánchez Riaño et al., 2022). To meet these requirements, the materials must have properties that allow them to perform in adverse environments. These properties mainly include mechanical, optical, barrier, and thermal properties.

- **Mechanical properties:** The mechanical properties of films are decisive in their use in the industry, as they determine the material's resistance to external factors and, therefore, its suitability as packaging material. Among the most important parameters, we can mention TS, EB, and the modulus of elasticity or YM. The YM is associated with the stiffness of the samples; a high value of this modulus is characteristic of a harder material. On the other hand, EB is a measure of elasticity and is associated with materials that can stretch. TS refers to the maximum force a material can withstand before breaking (Daza et al., 2018).
- **Barrier properties:** Films are expected to have good barrier properties to prevent the interaction of the matrix with the environment. Various parameters affect this property, such as the addition of plasticizer, drying temperature, or polymer concentration. Among the analyses conducted to evaluate the barrier capacity of the films, the WVP method, oxygen permeability, and carbon dioxide permeability can be mentioned (Cazón et al., 2022). WVP is one of the most commonly used tests for evaluating barrier properties, given the significant role water plays in microbial growth and the physicochemical properties of food

(Bangar et al., 2021). The method employed by ASTM is a gravimetric method where the amount of water passing through the material per unit area, time, and pressure difference is measured ($\text{g s}^{-1} \cdot \text{m}^{-2} \cdot \text{Pa}^{-1}$) (ASTM, 2016).

- **Light barrier property:** According to ASTM, transparency is the ability of a material to transmit light that forms images (ASTM, 2023). The measurement of this parameter can be associated with the degree of opacity of the films and largely determines their use in packaging. If the material has high opacity, it can be used, for example, to protect food matrices susceptible to lipid oxidation. On the other hand, low opacity or high transparency will allow the material to be used in products that do not require protection against light (Guzman-Puyol et al., 2022).
- **Thermal properties:** Thermal properties are associated with the resistance of films to temperature. These properties are measured using thermogravimetric analyzers and differential scanning calorimetry. The first technique determines the degradation of the material associated with an increase in temperature and is calculated from the loss of mass of the material (Wu et al., 2024). The methodology of differential scanning calorimetry allows for determining the T_g and melting temperatures (T_m) (Wang et al., 2021).

1.3.3. Biodegradable bioplastics as food packaging

Based on the recognition of the properties of 2GB and their potential use in the preparation of biodegradable packaging, different authors have explored and evaluated alternative sources for obtaining compounds that form these materials (films or coatings), which also have improved properties and performance. Khalil et al. (2022) extracted pectin from grapefruit and combined it with a lemon peel extract, encapsulated by atomization, to produce biodegradable films. The characterization results demonstrated that the prepared films exhibited good dispersion and compatibility of the ingredients. Likewise, the evaluated materials showed good mechanical and barrier properties, enhanced by incorporating the encapsulated extract. Additionally, a 90% degradation of the total weight of the material was observed. Other non-conventional sources have been evaluated for obtaining film-forming materials, including plum residues (Sheikh et al., 2023), tamarind seeds (Chowdhury et al., 2023), Andean tubers (Daza et al., 2018; Galindez et al., 2019), among others.

The application of these bioplastic food packaging materials to extend the shelf life of food can be achieved through two strategies: a) as edible coatings to form a film on the surface of the matrix, or b) as bioplastic film in the form of a container or protective film. Coatings made from ulluco starch were used as a covering material on goldenberries, and the coating successfully reduced the fruit respiration rate, which translated into an extension of their shelf life. Additionally, the authors observed a decrease in microorganism growth in the goldenberries. The authors

concluded that coatings made from ulluco starch could extend the shelf life of perishable products (goldenberries) (Galindez et al., 2021).

On the other hand, a previous study used biodegradable films with antioxidant capacity obtained from the orange peel as a bag-type container, with heat-sealable properties for protecting against the browning of apple slices. Browning evaluation showed that the samples coated with the bag-type container delayed this process, increasing the shelf life of the apples, which was associated with the UV light-blocking properties and the barrier properties against oxygen (Quilez-Molina et al., 2022).

1.3.4. Active and reinforcing components as additives in biodegradable packaging

Among the strategies employed to improve the physicochemical and mechanical properties of biodegradable packaging, fillers or reinforcement agents and compounds with bioactive potential can be mentioned. Regarding reinforcement agents, the current trend has focused on working with nanocomposites obtained from cellulose or even starch, which can act as fillers and improve the properties of the packaging. Wang et al. (2019) used nanocrystals obtained from cotton waste in the manufacture of PLA films. The addition of these compounds improved the film properties, such as relative crystallinity, TS, YM, and TS. Additionally, the use of bioactive compounds such as essential oils or extracts rich in phenolic compounds has been evaluated to improve mechanical, barrier, and antimicrobial properties, as well as the protection of food matrices (Hernández et al., 2023; Kaur et al., 2023). Liu et al. (2023) increased the shelf life of beef sauce packaged in a bag prepared using cassava starch and the essential oil of *Zanthoxylum armatum*. The increase in shelf life observed with the use of the bioactive and biodegradable packaging was six days, which was associated with the delay of lipid oxidation and microbial growth in the food matrix.

1.4 Microencapsulation

Microencapsulation is a process in which a material in a solid, liquid, or gaseous state, called the core (active ingredient, internal phase, or fill), is coated or trapped in a continuous matrix of encapsulating polymeric materials (wall, layer, shell, coating, wall material, or membrane), resulting in the formation of microcapsules with sizes that, depending on the technique used, range between 1 to 1000 μm (Arenas-Jal et al., 2020; Huang et al., 2023; Yadav et al., 2020). The implementation of this technology aims to protect the active ingredient from exposure to factors such as humidity, temperature, pH, oxygen, and other harmful substances. Additionally, it can be used to mask some organoleptic properties of substances, achieve controlled release, and even safely handle toxic materials (Jyothi et al., 2010; Vivek et al., 2023).

Encapsulation techniques can be classified into three groups. The first corresponds to physical methods such as spray drying, freeze drying, supercritical fluid precipitation, and solvent evaporation. The second group corresponds to physicochemical methods such as coacervation, liposomes, and ionotropic gelation. The third group corresponds to chemical methods and includes interfacial polymerization and molecular inclusion complexation (Ozkan et al., 2019).

1.4.1. Spray drying

Spray drying is among the most widely used techniques at an industrial level for microencapsulating many ingredients (Piñón-Balderrama et al., 2020). According to various authors, spray drying offers several significant advantages, such as low process costs, short drying times (5-100 s), the ability to obtain samples with constant quality throughout the process, suitability for thermosensitive and thermoresistant materials, applicability to a wide range of materials such as suspensions, emulsions, pastes, and molten masses, availability of equipment, and feasibility of scaling up the process (Ziaee et al., 2019). It has been demonstrated that the spray-drying process is 4 to 8 times more economical than the freeze-drying method due to its short drying period and lower electricity consumption (Shishir & Chen, 2017).

Spray drying is a unit operation divided into three stages: atomization of the feed sample, drying, and recovery of the sample, as shown in the schematic diagram in Figure 12 (Baumann et al., 2021; Shishir & Chen, 2017). The first stage aims to generate small particles to increase the surface area and thus increase the efficiency of mass transfer and energy transfer between air and liquid (Gharsallaoui et al., 2007). This is possible thanks to an atomizer providing the pressure or centrifugal energy necessary for particle generation. Different atomizers have been tested at industrial and laboratory levels, including pneumatic atomizers, pressure nozzles, spinning disk configurations, sonic nozzles, two-fluid nozzles, and pressure-swirl nozzles. However, the choice of atomizer depends on the properties of the feed liquid (Baumann et al., 2021). In the food industry, the typical atomizers are the pneumatic atomizer and the pressure nozzle (Samborska et al., 2022).

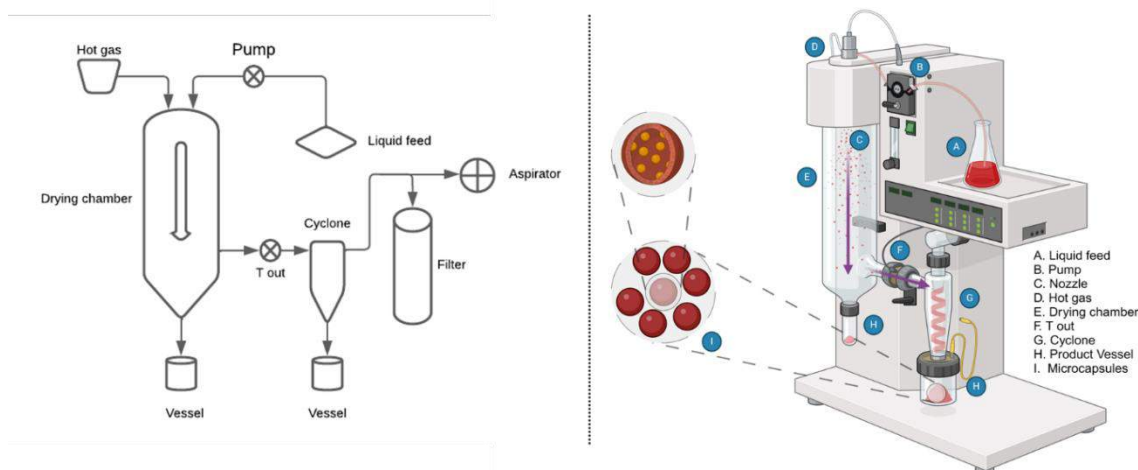


Figure 12. Spray drying process

During the second stage of the spray drying process, the drying chamber is where the interaction between the atomized liquid and the hot gas stream takes place, resulting in the drying of the particles (Baumann et al., 2021; Gharsallaoui et al., 2007). The interaction between particles and drying gas is highly influenced by the equipment configuration. There are three possible configurations in spray dryers: co-current, where the atomizer is at the top of the equipment, and the liquid is atomized in the same direction as the drying gas; counter-current, where the atomizer is located at the top of the equipment, while the drying gas inlet is at the bottom, allowing the liquid to be atomized in the opposite direction to the drying gas; and mixed flow, where the atomizer is located in the central part of the drying chamber, and the solution is fed upwards or downwards, while the airflow enters from the top of the equipment (Mohammed et al., 2020; Ziaee et al., 2019). Once atomization and interaction between the fluids are achieved, the drying process occurs in two phases. First, free and easy evaporation of the particle surface is achieved, known as the constant rate period. Then comes a delay phase, called the falling rate period, where the evaporation of the liquid is hindered by the formation of a solid phase or membrane on the surface (Piñón-Balderrama et al., 2020). The process ends theoretically once the temperature of the particles equals that of the drying gas (Gharsallaoui et al., 2007).

In the final stage of the process, the separation of the humid air and the sample is carried out, as well as the collection of the material. This is possible in most cases by using a cyclone outside the dryer. The larger and denser particles are collected at the base of the drying chamber, while the finer ones pass through the cyclone and are separated from the air moisture. Generally, the particles obtained in this process have a spherical morphology with surfaces and sizes that vary according to process parameters such as drying temperatures, type of atomizer, and equipment configuration (Ziaee et al., 2019).

1.4.2. Spray drying of hydrophobic compounds

Different lipophilic compounds, such as vegetable oils, essential oils, fatty acids, vitamins, phytosterols, and dyes, are relevant to the food and pharmaceutical industries. Most of these compounds are highly susceptible to factors such as pH variation, exposure to light, changes in ionic strength, and oxidative conditions that are common in industrial processing, as well as in storage processes (Lee et al., 2024; Turek & Stintzing, 2012, 2013). Spray drying has been a successful method of preserving encapsulated sour cherry oil using a mixture of Arabic gum and maltodextrin (Başyigit et al., 2020). The authors observed that the peroxide value did not increase significantly after 30 days of storage of the encapsulated product. Similarly, Wangkulangkool et al. (2023) evaluated the storage stability of encapsulated chia oil in whey protein and Arabic gum in different proportions. They did not observe a significant change in the main fatty acids after 60 days of storage. The authors observed an increase in acidity and peroxide value of the encapsulated chia oil without exceeding the limits established in the CODEX, proving the effectiveness of encapsulation. Other lipophilic compounds, such as essential oils and beta-carotene, have also been successfully encapsulated and stabilized by the spray drying method (Correia-Filho et al., 2019; Rahim et al., 2024)

In addition to the stages that comprise the spray drying process mentioned above, there is a fourth stage corresponding to the preparation of the feed solution, which must be considered in any spray drying process (Samborska et al., 2022). Generally, the samples processed by this method are dissolved in aqueous solutions, limiting the selection of the encapsulating agent and the compound that needs to be encapsulated. In most cases, hydrophilic active ingredients require only one stage of homogenization or mixing with the encapsulating material before atomization and do not require the inclusion of additional materials (Acosta-Vega et al., 2023; Banožić et al., 2023; Feihrmann et al., 2024). However, the encapsulation of lipophilic molecules involves the preparation of oil-in-water emulsions(O/W) with the inclusion of emulsifying agents or surfactants that provide stability to the mixture during the process (Encina et al., 2016; Rahim et al., 2024; Umaña et al., 2021).

1.4.3. Emulsion preparation and stability

An emulsion is a colloidal mixture of two or more immiscible liquid phases. One of these components, called discontinuous or internal phase, is dispersed in tiny droplets throughout the other liquid phase, which is continuous and surrounds the droplets (Øye et al., 2023). Simple emulsions can be prepared using high-energy methods that require equipment such as pressure homogenizers, sonicator ultrasonic homogenizers, high-speed mixers, colloid mills, and membrane homogenizers, which provide intense mechanical forces. Additionally, low-energy

methods can be employed, such as phase inversion emulsification, which alters the physicochemical properties of the system to generate the emulsion (Safaya & Rotliwala, 2020). In the preparation of emulsions used for spray drying, the most employed methods are those of high energy due to their ease of operation (Teixé-Roig et al., 2023).

One of the limitations of emulsions is that they are thermodynamically unstable systems due to the incompatibility of the continuous and dispersed phases present at the interface (Akbari & Nour, 2018). This incompatibility generates interactions between the droplets, which can lead to different types of emulsion destabilization. Several destabilization mechanisms include coalescence, Ostwald ripening, flocculation, phase inversion, sedimentation, and creaming (Kupikowska-Stobba et al., 2024). Coalescence is the mechanism in which two or more droplets of the dispersed phase merge to form larger droplets, leading to the final separation of the emulsion. This mechanism occurs when the thickness of the liquid film between the droplets decreases to a critical thickness (Dinh et al., 2020). Ostwald ripening is the process in which larger droplets grow at the expense of smaller ones, thanks to the molecular diffusion of oil molecules through the continuous phase (McClements et al., 2012). In flocculation, the droplets cluster together to form larger groups or flocs because the repulsive forces are weaker than the attractive forces between them, including van der Waals forces. Creaming and sedimentation involve the movement of the dispersed phase to the upper or lower layer of the emulsion, respectively. This results from the difference in density between the dispersed and continuous phases (Kupikowska-Stobba et al., 2024). In the phase inversion mechanism, there is interconversion between water-in-oil (W/O) and O/W emulsions (Chen et al., 2020). All these mechanisms lead to a decrease in the product's shelf life and a loss of its physicochemical properties.

1.4.3.1. Role of emulsifiers in the emulsion preparation

Amphiphilic compounds called emulsifiers or surfactants are usually employed to prevent instability in emulsions. These compounds can adsorb onto the surface of the oil droplets, reducing their resistance to deformation and the interfacial tension between phases, facilitating droplet rupture (Bai et al., 2017). Laplace pressure, which corresponds to the pressure difference inside and outside the droplet, is inversely associated with the energy required to break it. Therefore, for droplet formation to occur, the pressure or shear must exceed at least the critical pressure, and there must also be interaction with a surfactant that decreases the interfacial tension (Van Der Graaf et al., 2004). Simultaneously with droplet formation, the surfactant is deposited or adsorbed on its surface, creating a membrane stabilizing the system (Kupikowska-Stobba et al., 2024).

There is a wide variety of synthetic emulsifiers available commercially, such as glycerol fatty acid esters, polysorbates, sorbitan anhydride fatty acid esters, and

sucrose fatty acid esters, among others (Nagtode et al., 2023; Soni et al., 2023). Different classifications of surfactants have been established. According to Norn (2014), food emulsifiers can be classified according to their nature as non-ionic, cationic, anionic, and zwitterionic. On the other hand, Kupikowska-Stobba et al. (2024) describe a classification into three groups: i) low molecular weight surfactants such as Tween® 20 and 80, Span 20, 40, 60, and 80, and diacetyl tartaric acid ester of monoglyceride (DATEM) among others; ii) macromolecular surfactants, such as polysaccharides and proteins; and iii) solid particles or Pickering particles such as hydroxyapatite, starch, and chitosan particles. An alternative for the classification or selection of the emulsifying agent is the use of the hydrophilic-lipophilic balance (HLB), which is a numerical value indicating the ratio between the weight percentage of hydrophilic groups to the weight percentage of lipophilic groups in the molecule. The HLB value varies between 0 and 20, and its choice depends on the continuous phase. A low HLB value corresponds to a surfactant with a strong affinity for oil (W/O emulsions), whereas a high value corresponds to a surfactant that exhibits a high affinity for water (O/W emulsions) (Rahaman et al., 2023). Additionally, the HLB value of the surfactant directly influences the surfactant aggregation behavior on the surface of the dispersed phase particles. Thus, high HLB values result in micelle-type aggregations, while at low HLB values (W/O emulsions), reverse micelle-type aggregates are formed. For intermediate HLB values (7-10), the formation of a lamellar phase can be observed (Figure 13) (Rahaman et al., 2023).

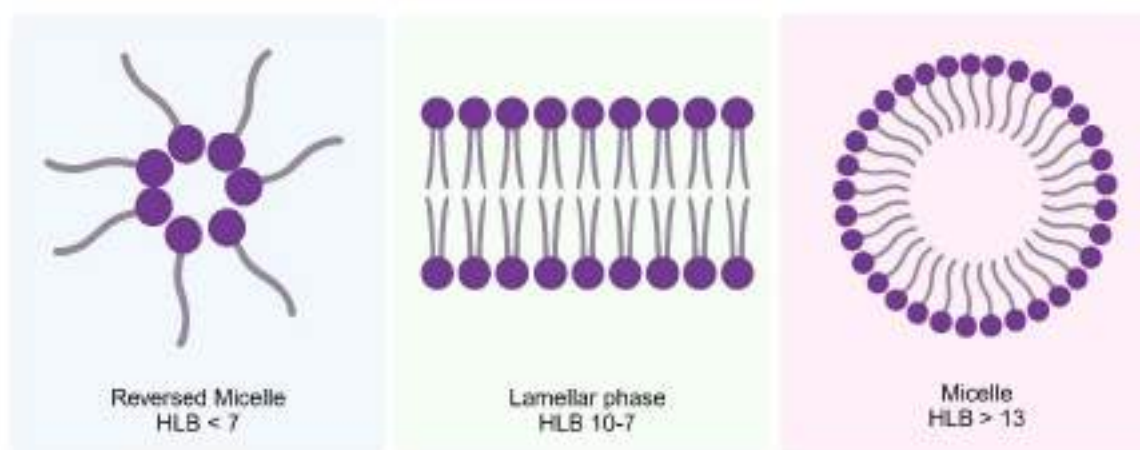


Figure 13. Models of self-aggregation of biosurfactant molecules. Adapted from Rahaman et al. (2023).

The formation of aggregates on the surfaces of oil droplets leads to the overall stability of the emulsion. Furthermore, stability is affected by the surfactant adsorption mechanism. It has been observed that high molecular weight (M_w) polymeric emulsifiers adsorb slowly, allowing for greater droplet growth, but upon adsorption, they result in highly stable droplets. In contrast, low molecular weight

polymers adsorb more quickly, forming smaller but unstable droplets that rapidly coalesce into larger droplets (Manga et al., 2023). Four mechanisms have been established by which an emulsion can be stabilized: i) electrostatic repulsion, ii) steric repulsion, iii) Marangoni-Gibbs effect, and iv) thin film stabilization (Sullivan & Kilpatrick, 2002).

1.4.3.2. Natural surfactants

Despite their high emulsifying capacity, the addition of synthetic surfactants for the preparation of emulsions included in food products can lead to consumer rejection, either due to changes in the sensory properties of the food or due to potential adverse effects on consumer health (Gao et al., 2016; Partridge et al., 2019). Consequently, the interest in the search for new sources of natural, renewable, clean-label, and environmentally friendly emulsifiers has become a challenge for researchers (Burgos-Díaz et al., 2018; Francisco et al., 2020; Umaña et al., 2021). Table 3 shows a review of molecules obtained from plant materials that have been tested for emulsion stabilization. Among the most studied and most relevant materials are proteins of plant origin. The emulsifying properties of proteins obtained from different botanical sources such as soybeans, peas, and cereals have been widely studied, showing great emulsion stabilizing capacity mainly due to their surface activity and rapid interfacial adsorption kinetics (Francisco et al., 2020; Ribeiro et al., 2021; Rosenberg et al., 2018).

Materials containing natural emulsifiers and other molecules and macromolecules such as fibers and starches have been evaluated to reduce processing time and the extraction of compounds that can increase energy consumption and mass loss. Sridharan et al. (2020) evaluated pea flour (50% starch and 20% crude protein) as an emulsifying agent and compared it with a protein concentrate obtained from the same material. The study results showed that the protein was the principal stabilizing agent. Additionally, a monomodal droplet distribution and similar rheological properties were observed regardless of the material used for emulsification. The authors concluded that a protein purification stage is unnecessary for obtaining materials with good emulsifying properties, thus reducing production costs and decreasing the environmental impact of water use in intermediate stages.

Table 3. Natural emulsifiers obtained from plant sources

Natural emulsifier	Source	Oil	Method of emulsion formation	Reference
Arabinoxylans	Corn and wheat brans	Rapeseed oil	High-speed homogenizer	(Wang et al., 2023)
Catechin-dextran-protein	Protein from egg white	Sunflower oil	High-speed homogenizer	(Gu et al., 2023)
Cellulose nanocrystals	Litchi peels	Grape seed oil	Sonicator	(Ma et al., 2023)
Dietary fiber	Pomelo peel	Olive oil	Ultrasonic generator	(Gao et al., 2022)
Flour and protein concentrate	Pea	Rapeseed oil	High-speed rotor-stator homogenizer and high-pressure homogenizer	(Sridharan et al., 2020)
Gum Arabic and Pectin	Pectin from sugar beet	Medium-chain fatty acid triglyceride	High-speed homogenizer	(Niu et al., 2022)
Mucilage	Okra (<i>Abelmoschus esculentus</i> L.)	Corn oil	Homogenizer	(Noorlaila et al., 2015)
Mucilage	<i>Dioscorea opposita</i>	Medium-chain triglycerides	Homogenizer	(Z. Ren et al., 2022)
Pectin	<i>Premna puberula</i> Pamp.	Corn oil	High-speed homogenizer	(N. Yang et al., 2022)
Pectin	<i>Nicandra physaloides</i> (Linn.) Gaertn seeds	Soybean oil	High-speed homogenizer	(Liu et al., 2022)
Pectin	Apple	Soybean oil	High-speed homogenizer	(Liu et al., 2022)
Pectin	Citrus	Soybean oil	High-speed homogenizer	(Liu et al., 2022)
Pectin	Cherry pomaces (<i>Prunus cerasus</i> and <i>Prunus avium</i>)	Corn sprout oil	High-speed homogenizer	(Zhang, Waterhouse, et al., 2023)
Pectin	Dragon fruit peel	Corn germ oil	High-speed homogenizer	(Liu et al., 2023)
Pectin	Citrus	Sunflower oil	High-pressure homogenization	(Humerez-Flores et al., 2022)
Protein and mucilage	Flaxseed	Tricaprylin oil	High shear blender	(Nikbakht Nasrabadi et al., 2019)
Protein concentrate	Spirulina (<i>Arthrospira platensis</i>)	Olive oil	High-speed homogenizer	(Silva et al., 2022)
Protein concentrate	whey, soy, peanut, and zein	Rapeseed oil	High-pressure homogenization and ultrasound	(Taha et al., 2019)

Table 3 (Continued)

Natural emulsifier	Source	Oil	Method of emulsion formation	Reference
Protein isolate	Lupine	Sunflower oil	Homogenizer	(Burgos-Díaz et al., 2019)
Protein isolate	Pea, soy, lentil, and canola	Canola oil	Homogenizer	(Chang et al., 2015)
Protein isolate	Soy and Pea	Soy oil, vitamin A	Two-stage homogenizer	(Li et al., 2019a)
Protein isolate	Pea	Soybean oil	Two-stage homogenizer	(Li et al., 2019b)
Saponins	<i>Quillaja saponaria</i> Molina	Avocado oil	High-speed homogenizer	(Riquelme et al., 2019)

1.4.3.3. Spray drying of emulsions stabilized by natural emulsifiers

In addition to acting as emulsifiers, plant-based materials can also act as encapsulating agents (Table 4). Several authors have evaluated the influence of adding components or macromolecules obtained from different sources on the stability of the emulsion and the properties of encapsulated materials obtained by the spray drying method. Generally, their use is accompanied by commercially recognized encapsulating agents such as maltodextrin, Arabic gum, inulin, and plant-based proteins, among others.

Proteins, mainly obtained from soybeans and peas, appear to be the most relevant raw material for fulfilling the dual function of stabilizing and encapsulating lipophilic compounds (Can Karaca, 2020; Francisco et al., 2020; Gomes & Kurozawa, 2020). In addition to their great emulsifying potential, it has been observed that they can encapsulate and mask odors that are not attractive to consumers. Di Giorgio et al. (2019) evaluated the addition of soybean protein to stabilize and encapsulate fish oil. They found that the particles obtained could mask unpleasant odors and textures. Additionally, they obtained particles with spherical geometries and without holes on their surface, which can help maintain the integrity of the capsule and, therefore, its shelf life. On the other hand, high encapsulation efficiencies have been observed with the inclusion of proteins. In a study on fish oil encapsulated by spray drying using soy protein isolate in a mixture with maltodextrin, high encapsulation efficiencies were observed, exceeding 90% in some treatments (Linke et al., 2020). The authors evaluated the oxidation rate of encapsulated and non-encapsulated oil and determined that the latter degraded, on average, seven times faster than the encapsulated oil. The high capacity of proteins to produce high encapsulation yields and retention of the core component has been confirmed in other studies where black pepper seed oil (Karaca, 2020), orange essential oil (Francisco et al., 2020), and soybean oil (Li et al., 2019b) were encapsulated.

Table 4. Plant materials used in spray drying

Natural emulsifier/wall material	Encapsulated compound	Conditions	References
Brea gum	Corn oil	Emulsion: combination with or without gum Arabic and inulin. Temperature: 150°C. Atomization pressure: 0.1 Mpa	(Castel et al., 2018)
Cellulose nanocrystals from hardwood	Hempseed oil	Temperature: 220°C Nozzle: two-fluid nozzle Feed flow: 2.8 mL min ⁻¹	(Esparza et al., 2020)
Green tea (matcha) powder	Tuna oil	Emulsion: combined with maltodextrin and pectin Temperature: 185°C Nozzle: twin fluid nozzle, 1.5 bar Feed flow: 12 g min ⁻¹	(Shi et al., 2020)
Jackfruit Seed Starch	Rice Bran Oil	Emulsion: combined with whey protein isolate Temperature: 140-160°C Atomizer pressure: 2.5 kg/cm ² Feed flow: 2.5 mL min ⁻¹ Pressure: 115 psi	(Murali et al., 2017)
Modified rice starch	Orange essential oil	Emulsion: combination with maltodextrin and hydrolyzed protein syrup solid solution Temperature: 127-187°C Nozzle: two-fluid nozzle, 0.45 mm Feed flow: 20 kg h ⁻¹	(Márquez-Gómez et al., 2018)
Mushroom (<i>Agaricus bisporus</i>) by-product	Sunflower oil	Emulsion: combination with maltodextrin Temperature: 170 Feed flow: 30 g min ⁻¹ Pressure: 5 bar	(Umaña et al., 2020)
Pea and soy proteins	Orange essential oil	Temperature: 150 Nozzle: double fluid nozzle atomizer of 0.7 mm Feed flow: 10-13 g min ⁻¹	(Francisco et al., 2020)
Pea protein	Black pepper seed oil	Emulsion: combination with maltodextrin and gum Arabic Temperature: 140°C Nozzle: NR Feed flow: 12 g min ⁻¹ Pressure: 6 bar	(Can Karaca, 2020)
Pea protein isolate	Soy oil, vitamin A palmitate, and β -carotene	Emulsion: combination with sodium caseinate and corn syrup solid solution Temperature: 127-187°C Nozzle: two-fluid nozzle, 0.45 mm Feed flow: 20 kg h ⁻¹	(X. Li et al., 2019b)

Table 4 (Continued)

Natural emulsifier/wall material	Encapsulated compound	Conditions	References
Rice protein isolate and rice protein hydrolyzed	Linseed oil	Emulsion: combined with maltodextrin Temperature: 180°C Nozzle: two-fluid nozzle 1 mm, 28 bar Feed flow: 485 mL h ⁻¹	(Gomes & Kurozawa, 2020)
Soy protein isolate	Rapeseed oil	Emulsion: combined with maltodextrin Temperature: 140-220°C Nozzle: two-fluid nozzle Drying air flow: 200 kg h ⁻¹	(Linke et al., 2021)
Soy protein isolate	pink pepper essential oil	Emulsion: combined with maltodextrin and pectin Temperature: 140°C Nozzle: two-fluid nozzle, 0.7 mm diameter Feed flow: 2 mL min ⁻¹ Drying air flow: 819 L h ⁻¹	(Locali Pereira et al., 2019)
Soy protein isolate	Fish Oil	Emulsion: combined with maltodextrin Temperature: 160°C Nozzle: two-fluid nozzle 2 mm Feed flow: 11.42 kg h ⁻¹ Drying air flow: 200 kg h ⁻¹	(Linke et al., 2020)
Soybean protein	Fish oil	Temperature: 180°C Nozzle: 0.7 mm Feed flow: 3.1 mL min ⁻¹ Pressure: 6-8 bar Drying air flow: 357 L h ⁻¹	(Di Giorgio et al., 2019)
Taro starch	Almond oil	Temperature: 145°C Nozzle: two-fluid nozzle Feed flow: 10 g min ⁻¹	(Hoyos-Leyva et al., 2019)
Wheat Protein isolate	Soy oil	Emulsion: high-pressure homogenizer Temperature: 160 Nozzle: centrifugal atomizer	(Rosenberg et al., 2018)

1.5. 3D printing

The 3D printing, also known as additive manufacturing, solid freeform fabrication, or rapid prototyping, is an emerging technology that allows the production of physical structures from computer-aided design printing routes and the layer-by-layer aggregation of a material or a mix of materials, resulting in a product with predetermined size and shape characteristics (Ji et al., 2022). This technology has been implemented in different areas such as biomedicine (Mamo et al., 2023), aerospace industry (Karkun & Dharmalingam, 2022), construction (Chabaud et al.,

2019), water treatment (Ghosal et al., 2022) among others. The advantages that make this technology attractive include product customization in shape, size, mechanical properties, process optimization and automation, waste reduction, and improved profitability (Dankar et al., 2018; J. Zhang et al., 2022).

1.5.1. 3D printing in the food industry

The 3D printing has been implemented for over a decade in the food industry and has become a widely used tool in restaurants and other food applications (Dankar et al., 2018; Lee, 2021). There are four main types of technologies for 3D food printing:

- **Extrusion-based printing:** This technique is considered one of the simplest and can be carried out by hot melt or at room temperature. The method generally involves a robotic arm attached to a syringe with movement in all directions, capable of extruding material through the syringe nozzle. The path followed by the robotic arm is determined by a previously established 3D model (Le-Bail et al., 2020; Zhang et al., 2022). Extrusion 3D printers may or may not have temperature control on the manufacturing platform, syringe container, or cartridge.
- **Selective sintering (laser sintering/hot air sintering):** This technique uses heat sources to sinter powder materials. Initially, a layer of material or material mixture is deposited and leveled. The implementation of the laser fuses the particles until reaching a certain thickness. After this, a new material layer is deposited, and the process is repeated until the desired design is achieved (Jonkers et al., 2022).
- **Binder jetting:** In this method, 3D structures are formed by binding powders such as starch. The powder material is distributed on the production platform and then sprayed with liquid binders injected into a powder bed following a predetermined pattern (Le-Bail et al., 2020; Pulatsu & Lin, 2021).
- **Inkjet printing:** This technique is primarily used for printing digital images in graphic decoration format, surface filling, or cavity deposition. It is characterized by low height due to the use of low-viscosity materials. In this technique, tiny drops of ink are sprayed using a series of pneumatic membrane jets onto the medium along a specific path (Le-Bail et al., 2020; Zhang et al., 2022).

Among the mentioned technologies, extrusion printing is the most widely applied and implemented in the food industry (Carvajal-Mena et al., 2024; Huang et al., 2024; Qiu et al., 2024). Figure 14 presents a general scheme of the 3D food printing process using extrusion technology and the characterization of the obtained product. The use of this technique has allowed the development of personalized foods that can meet specific needs, such as feeding the elderly or patients with

dysphagia (Pant et al., 2021), diabetes (Huang et al., 2024), among other diseases (Liu et al., 2023). Kim et al. (2022) designed a low-calorie formulation using a protein analog for 3D printing of surimi. The authors observed that the printed products showed texture and salinity similar to commercial surimi. Meanwhile, Huang et al. (2024) produced 3D prints using white quinoa and brown rice. The authors carried out a modification process (dry heating) of the flours and their constituents. They obtained products with good mechanical characteristics and increased the materials' resistance to enzymatic degradation. This was associated with the possibility that these products do not increase consumers' blood glucose concentration.

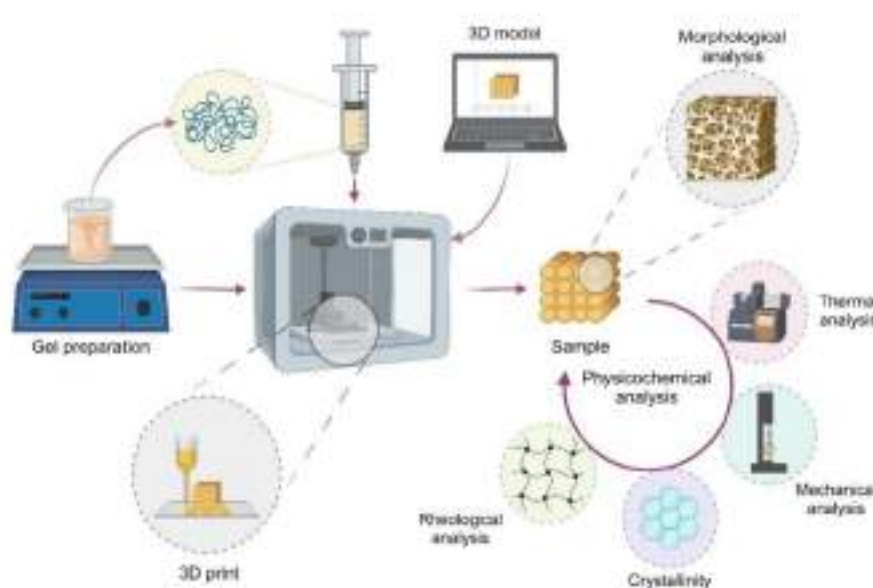


Figure 14. 3D print process

Although 3D food printing technology offers various advantages to the food industry, it has limitations. These limitations include low production volume, lack of software that considers food properties, copyright issues, food safety, printing time and speed, and printing accuracy (Dankar et al., 2018). As a result, there has been a search for solutions to improve the 3D food printing method by extrusion.

One of the aspects that has received the most attention is the design of "food inks" that meet the nutritional requirements and necessary characteristics to guarantee physical and structural integrity, avoiding food collapse during production, transportation, and storage. Therefore, the materials used for this purpose must have malleability, self-support, rapid solidification, and a specific level of flowability that can generate smooth extrusion from the printer's extrusion head. Additionally, the materials must form a stable and resistant structure on the printing platform (Ji et al., 2022). Different materials have been studied to develop and optimize food

inks (Bulut & Candoğan, 2022; Liu et al., 2019; Yang et al., 2018). Zhang et al. (2022) optimized the preparation of inks based on carrot flour and xanthan gum by adding different proteins (proteins obtained from Black soldier fly larva, cricket, soybean, sericin, whey, and spirulina). The results obtained in the factorial design allowed for the optimization and validation of the printability and syneresis of all evaluated material systems. Additionally, the optimized samples that used soy protein showed the highest values of adhesiveness and gumminess without affecting their flowability. On the other hand, the optimized spirulina formulation showed the lowest printability, which was associated with its poor texture characteristics.

The availability of suitable base materials for 3D food printing by extrusion is limited, generating the need for alternatives that meet the requirements of food design and allow for the complete industrial utilization of this technology (Zheng et al., 2023). Several compounds can be used as material for 3D food printing, including proteins, fibers, starches, and pectin (Zhang et al., 2022). However, many of these products are obtained from sources primarily intended for human consumption, affecting food safety. It is necessary to search for alternatives to these raw materials. Considering that the new materials may present different characteristics according to their chemical composition, it is necessary to evaluate the rheological, physical, chemical, structural, and thermal properties that the material may present individually and in a mixture with other components. Unconventional sources have been evaluated for obtaining materials such as protein (Lee & Hashimoto, 2024; Zhang et al., 2022; Zhang et al., 2022), starches (Liu et al., 2023), flours (Pant et al., 2021), and agrifood residues (Chen et al., 2024; Nida et al., 2023) that can be used in 3D food printing.

1.6. Research hypotheses

After reviewing the literature and considering the issues previously outlined, the following research hypotheses were proposed as the starting point for the experimental work:

- Some unconventional crops and agri-food byproducts are potential sources of industrially relevant compounds such as starch, essential oils, and proteins.
- Starches obtained from unconventional crops have suitable technological characteristics to replace commercial starches and contribute to reducing food insecurity.
- The physical modification of starches obtained from unconventional crops can improve the characteristics of native starch and increase its industrial potential.
- The compounds obtained from non-conventional crops and agri-food byproducts can be used as raw material in the preparation of biodegradable films, emulsions, 3D food prints, and as encapsulating agents.

1.7. References

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RESEARCH OBJECTIVES

2. RESEARCH OBJECTIVES

The research presented in this thesis was conducted as part of four distinct projects undertaken by the Agri-Food Engineering Group at the University of the Balearic Islands. These projects received financial support from various organizations, including the l'Oficina de Cooperació al Desenvolupament i Solidaritat (OCDS) of Universitat de les Illes Balears (UIB), Direcció General de Coperació del Govern de les Illes Balears, the National Institute of Agri-Food Research and Technology (INIA), ERDF, the Spanish Research Agency (AEI), and the Ministry of Science and Innovation (MCI). The projects are detailed below:

- *Revalorización de subproductos agroalimentarios. Uso potencial de biocompuestos como material de recubrimiento* (OCDS-CUD2018/05).
- *Implementación de estrategias para la valorización de cultivos no tradicionales y residuos agroindustriales con potencial uso industrial* (OCDS-CUD2022/02).
- Integration of revalued agrifood by-products in a circular economy model. Vegan food with added value for the agroindustrial and HORECA sectors (PID2019-106148RR-C43).
- Digital twins to optimize lignocellulosic and protein waste upcycling. Process modeling (PID2022-136889OBC21).

These projects focused on the transition of agri-food by-products, from a linear to a circular economy, by using them as renewable raw matters in the food industry; and the implementation of strategies for the valorization of non-traditional crops and agro-industrial waste and agro-industrial waste with potential industrial use.

Within this context, this research aimed to add value to non-traditional crops and revalorize agri-food waste by using them to produce materials to develop biodegradable packaging, encapsulates, and 3D food prints for industrial applications.

To achieve this objective, the following specific objectives were proposed:

- Extract and characterize compounds obtained from different unconventional products (ulluco, cubio, chachafruto) and agri-food residues (*Cymbopogon citratus*, Tahitian lime, chachafruto).
- Evaluate the impact of incorporating unconventional starches and essential oils on the physicochemical and technological properties of biodegradable films.
- Assess the impact of thermal modification (ANN) of ulluco starch on the preparation of biodegradable films and its influence on their properties.

- Evaluate the feasibility of using alternative starch sources as raw materials for preparing edible inks for food production by 3D printing.
- Evaluate the utility of flour obtained from chachafruto byproducts in the microencapsulation of lipids and essential oils, determining its effect on the stability of oil-in-water emulsions and the encapsulated compounds obtained in the drying process.

3. RESULTS AND DISCUSSION



CHAPTER 1

Non-conventional starch from cubio tuber (*Tropaeolum tuberosum*): Physicochemical, structural, morphological, thermal characterization and the evaluation of its potential as a packaging material

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Non-conventional starch from cubio tuber (*Tropaeolum tuberosum*): Physicochemical, structural, morphological, thermal characterization and the evaluation of its potential as a packaging material

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ABSTRACT

This work aimed to characterize the physicochemical, structural, morphological, and thermal properties of a non-conventional starch obtained from cubio (*Tropaeolum tuberosum*), as well as to evaluate the potential use of this native Andean tuber in the preparation of biodegradable packaging. The cubio starch (CUS) showed an intermediate apparent amylose content (31.2 %) accompanied by a high CEI whiteness index (90.8). About the morphology and particle size, the CUS exhibited irregular oval and round shapes and a smooth surface with a mean particle diameter of $14.64 \pm 0.1 \mu\text{m}$. Although it showed good stability regarding pasting properties, the final viscosity was low. Native CUS exhibits a typical B-type diffraction structure, with a relative crystallinity of 16 %. The resistant starch (RS) fraction of the CUS was 94 %, indicating a low susceptibility to enzymatic hydrolysis. The thermal analysis demonstrated that the CUS showed good thermal stability. Additionally, the films prepared using CUS as raw material showed continuous surfaces without porosities, good thermal stability, and high transparency. The results of this work demonstrate the industrial potential of the CUS as it presents characteristics comparable to commercial potato starch.

1. Introduction

Starch is the main carbohydrate reserve in plants. It consists of two glucose polymers, namely amylose and amylopectin, which form a semi-crystalline granule. Amylopectin consists of α -(1,4)-D-glucan short chains linked through α -1,6-linkages, and amylose, which essentially is a linear chain of α -(1,4)-D-glucopyranose subunits with a small number of branches [1]. Starch is an inexpensive, non-toxic, biodegradable, and renewable material used in the food, pharmaceutical, biomedical and plastic industries for different purposes [2]. Currently, starch for industrial use is primarily obtained from corn, potato, wheat, and rice; around sixty million tons of starch are obtained annually worldwide from these crops [3]. Despite its importance for both the food and non-food industries, and considering the growth in world population, industrial demand for starch cannot be met without compromising food security. In addition, the use of starches varies according to their physicochemical properties, which mainly depend on their botanical

source [4]. Consequently, researchers are constantly looking for new sources of starch to evaluate its physicochemical properties and determine its industrial potential, adding value to non-conventional crops and promoting socio-economic development [5].

The use of plastics derived from the petrochemical industry has generated worldwide concern due to its negative impact on the environment. Only in 2019, the highest demand for plastic was from the packaging industry, comprising 39 % of European production (50.7 Mt) [6]. In this regard, different policies have been implemented to reduce the impact of non-biodegradable packaging [7]. However, more strategies are necessary to mitigate the problem. Biodegradable packaging appears as an environmentally friendly alternative to replace petroleum-based packaging. This type of material is prepared from polymers obtained from nature that present high biodegradability, which makes it necessary to search for new raw materials to obtain them [8,9].

Although tubers are used primarily in human nutrition, either as raw or processed products, mainly for their carbohydrate content, potatoes

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and sweet potatoes are also commonly used for starch extraction for industrial purposes. However, many tuber crops have not yet been studied because these materials are not well known worldwide. Besides, there is no information about the functional and technological characteristics of the starches obtained from them, limiting their use to in natura consumption. Hence, the analysis of these starches could further increase the value of these raw materials.

Cubio (*Tropaeolum tuberosum*) also known as Mashua, is a native Andean tuber with a high starch content (41.3 % d.b.) [10,11], the species being native to Peru and Bolivia. Its cultivation has spread from Venezuela to northern Argentina, mainly at altitudes between 1500 and 4300 m above sea level (m.a.s.l.) [12]. This crop can grow in poor soils without adding fertilizers or pesticides, which reduces its production costs [12], hence its potential as a source of starch for industrial purposes. However, one factor limiting its use is the lack of knowledge of its physicochemical and technological characteristics.

Different non-conventional starches have been used alone or in combination to obtain biodegradable films, including ulluco, chachafuto, chayote/telte [13], mung bean [14], banana [15] and arracacho [16] starches. The main characteristics of these films have been low oxygen permeability, high transparency, good thermal stability and flexibility, which demonstrates the great potential of these materials to replace commercial sources of starch in the preparation of biodegradable films. Additionally, it has been shown that the application of edible

coatings obtained with these starches has increased the shelf life of different food matrices [17].

Considering the unexplored industrial potential of cubio starch, and the demand for new natural materials for the manufacture of biodegradable films. This study aimed to characterize the physicochemical, functional, structural, morphological, thermal properties, and technological characteristics of the starch obtained from cubio tubers as novel material for edible film production.

2. Materials and methods

2.1. Raw materials and starch isolation

Cubio tubers were acquired at a local market in the city of Ibagué (Colombia). Samples were selected and washed, and those exhibiting any mechanical damage were discarded. For the extraction process, samples were cut into cubes (around 5 mm). Then, the cubes were milled using a food blender (BRLV07-Zoo, Oster, Colombia) for 1 min with distilled water in a ratio of 1:2. The resulting sample was filtered using a cloth sieve; the filtrate was collected, and the solid was re-extracted and filtered again. The collected filtrate containing the starch was left to stand at 4 °C for 24 h until complete starch separation occurred. Then, the starch was separated from the supernatant and re-suspended in water. This washing procedure was repeated six times. Finally, samples

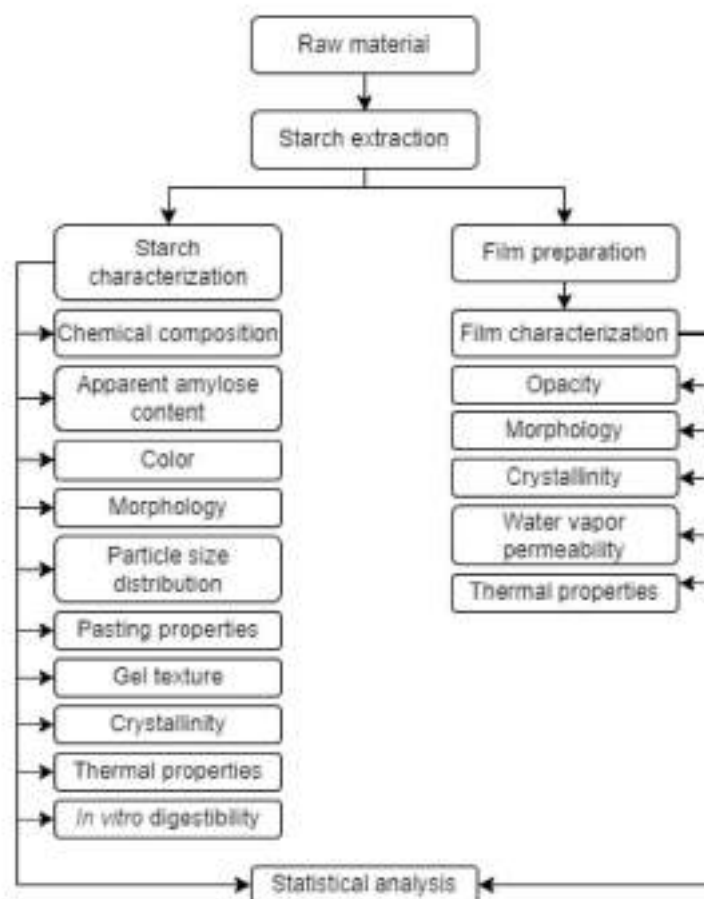


Fig. 1. Methodology flowchart.

were dried at 40 °C in an oven (Thermolab, DiEs, Antioquia, Colombia) with forced air circulation and stored in a desiccator at room temperature (–25 °C). Commercial potato starch (POS) (Tecnas S.A, Colombia) was used as a control in the physicochemical characterization.

2.2. Chemical composition, apparent amylose content, and color

The methodology of this work is summarized in Fig. 1. Samples were analyzed to determine its protein (A.O.A.C Method 920.87), lipid (A.O. A.C Method 920.85), moisture (A.O.A.C Method 925.10), and ash (A.O. A.C Method 923.03) contents, according to the methods of the AOAC [18]. The total carbohydrate content was obtained by subtracting the sum of the protein, lipid, moisture, and ash content from 100. The apparent amylose content was determined using the method reported by Hoover & Ratnayake [19]. The color was evaluated using a Konica Minolta colorimeter (CR 410, Minolta Co., Japan). Results were obtained according to the CIELAB color system (L*: darkness to lightness; a*: greenness to redness; and b*: yellowness to blueness). The Whiteness Index (WI) was estimated according to Daza et al. [9].

2.3. Morphological characteristics

2.3.1. Scanning electron microscopy (SEM)

The morphological characteristics of the starches were examined by scanning electron microscopy (S-3400N, Hitachi, Germany). The samples were placed on stubs using double-sided carbon tape and covered with a gold layer using a sputtering system (E5400, Polaron, UK). Images were obtained at an accelerating voltage of 15 kV and under a vacuum pressure of 40 Pa at varying degrees of magnification.

2.3.2. Optical microscopy

The starch was placed on a glass slide, mixed with 1–2 drops of distilled water, and fixed to the slide with a coverslip. Samples were observed at 40× magnifications, with and without polarized light in an optical microscopy (BX60, Olympus, Japan) coupled with a camera Moticam 3.0 MP Color Digital Camera (3.0MP, Motic, Hong Kong).

2.4. Particle size distribution

The size distribution of the starch granules was determined by laser diffraction analysis using a Laser Particle Analyzer (Sync Microtrac MIRA, wet operation FlowSonic, Montgomeryville, PA, USA) according to Lin et al. [20]. The starches were suspended in distilled water and stirred at 2000 rpm. The refractive indexes of water and starch were adjusted to 1.33 and 1.53, respectively. The samples were sonicated in the equipment at 25 Hz for 15 s before analysis, to avoid aggregation of the starch granules. Diameters at the 10th percentile [$d_{(0.1)}$], median [$d_{(0.5)}$], and 90th percentile [$d_{(0.9)}$] were reported.

2.5. Paste clarity and pasting properties

The paste clarity of the samples was analyzed at 640 nm using an ultraviolet-visible spectrophotometer (Genesys 10S UV-vis, Thermo Fisher Scientific, USA) at intervals of 24 h for 5 days, according to Paramakrishnan et al. [21]. Pasting properties of starches were determined in a Visco-Amylo-Graph (Mileo Visco-Amylo-Graph®, Brabender Instrument Inc., Germany) according to the methodology described by Li et al. [22]. Pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown (BD), setback (SB), and final viscosity (FV) were recorded. Viscosity was expressed in Brabender Units (BU).

2.6. Gel texture analysis

The texture of the gels was measured using a texture analyzer equipped with a load cell of 20 N (LS1, Lloyd Ltd., USA) according to the method described by Li et al. [22] with some modifications. Starch gels

were transferred to plastic containers (25 mm of diameter; 50 mm of height). Samples were kept at 4 °C for 24 h before analysis. The gels were analyzed by being compressed twice to a distance of 10 mm (trigger force = 0.05 N) at a speed of 0.5 mm/s by a flat-ended cylindrical probe (6 mm of diameter). Hardness, adhesiveness, and cohesiveness were determined using Nexygen Plus software (Lloyd Ltd., Version 3.0).

2.7. X-ray diffraction (XRD)

The XRD analysis of the samples was carried out using a Bruker D8 ADVANCE diffractometer (Bruker, Billerica, USA) operating with Ni-filtered Cu-K α radiation at 40 kV and 40 mA and a specific wavelength of 1.5406 Å. Data collection was carried out in the 2 θ range of 3.5–70°, with a step size of 0.02035° (2 θ) and a counting time of 0.8 s/step. Relative crystallinity was calculated using the DIFFRAC.EVA software (Bruker, Billerica, USA).

2.8. Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of the samples were obtained using a Fourier – transform infrared spectroscopy (Nicolet 380, Thermo Scientific Inc., USA). The measurements were recorded over the range of 4000 to 500 cm^{–1}, with a resolution of 4 cm^{–1} and 40 scans at room temperature (–20.4 °C). For the analysis, pellets were prepared by mixture and homogenization of the sample and potassium bromide (KBr).

2.9. Thermal properties

2.9.1. Differential scanning calorimetry (DSC)

Thermal properties were performed using a differential scanning calorimeter (2920, TA Instruments, Japan) according to the methodology described by Li et al. [22]. The obtained thermograms were examined using Universal Analysis 2000 V3.9A software (TA Instruments, Japan). Onset (T_0), peak (T_p) and conclusion (T_c) temperatures, and enthalpy of gelatinization (ΔH) were reported as the mean of three replicates.

2.9.2. Thermogravimetric analysis (TGA)

TGA was performed using a thermogravimetric analyzer (SDT 2960, TA Instruments, Japan) following the methodology described by Galindez et al. [8]. The weight loss (WL) and thermal degradation temperature of the samples were determined over the temperature range of 20–600 °C at a heating rate of 20 °C min^{–1} and under a stream of nitrogen.

2.10. In vitro digestibility

The in vitro digestibility expressed as rapidly digestible starch (RSD) and slowly digestible starch (SDS) was determined by using the method described by Dong et al. [23]. Starch samples (200 mg, d.b.) were mixed in sodium acetate buffer (7.5 mL; 0.1 M; pH 5.2). Then, 2 mL of enzyme solution [1.2 g of porcine pancreatin (8 USP), and 0.385 mL of amyloglucosidase (260 U/mL) in sodium acetate buffer] were added to the mixture and incubated in shaking water bath at 37 °C during 120 min. Hydrolyzed samples were collected at 20 and 120 min and then mixed with 10 mL of absolute ethanol to denature the enzymes. The hydrolyzed samples were centrifuged at 4000 rpm for 5 min, and glucose content was measured using the glucose oxidase/peroxidase (GOPOD) assay kit. The RDS, SDS and resistant starch (RS) were calculated as follows:

$$\%RDS = [(G_{30} - FG) \times 0.9] / TS \times 100 \quad (1)$$

$$\%SDS = [(G_{120} - G_{30}) \times 0.9] / TS \times 100 \quad (2)$$

$$\%RS = (TS - RDS - SDS) / TS \times 100 \quad (3)$$

where, G_{20} and G_{120} are glucose content at 20 and 120 min, respectively; FG is the free glucose in the native starch; TS is the weight of the total starch sample.

2.11. Evaluation of the technological feasibility of cubio starch

2.11.1. Cubio starch-based films preparation

The films were prepared via casting, following the method described by Daza et al. [9], with some modifications. Cubio starch was dissolved in distilled water, at different concentrations (films with 2.0 % w/v of cubio starch (CUS): SCF1; films with 2.5 % w/v of CUS: SCF2; and films with 3.0 % w/v of CUS: SCF3 w/v) and agitated by magnetic stirring for 10 min. The mixture was heated to 95 °C and maintained at this temperature until a viscous gel was obtained. The mixture was then cooled to 50 °C, and glycerol was added at a fixed concentration of 1.0 % (w/v). The prepared solution was transferred to a Petri dish with a diameter of 8.5 cm (~0.53 g of the film solution per cm²). The films were dried at 40 °C for 48 h in an oven under forced air. Finally, the samples were stabilized in a desiccator at a relative humidity (RH) of 54 % (using a saturated solution of magnesium nitrate hexahydrate) and room temperature (~23 °C) for 24 h, before the analyses.

2.11.2. Edible films characterization

The morphology, crystallinity and temperature stability of biodegradable films were analyzed using the methodologies described in previous sections (2.3.1, 2.6, and 2.9.2) [8].

For opacity, the absorbance of the films was measured at 600 nm using an ultraviolet–visible spectrophotometer (Genesys 10S UV–vis, Thermo Scientific, Thermo Fisher Scientific, USA). The opacity (OP) was calculated using Eq. (4):

$$OP = Abs_{600}/FT \quad (4)$$

where, Abs_{600} is the absorbance at 600 nm, and FT is the film thickness (mm).

The water vapor permeability (WVP) was evaluated according to the method described by Galindez et al. [8]. The film samples were loaded into stainless steel permeability cells, in which 6 mL of distilled water was deposited with an exposed area of 9.6 cm². It was assumed that RH was maintained at 100 % within the cell. The cells were placed in a controlled chamber at 25 ± 2 °C and 75 ± 2 % RH. Every hour, the mass of the cells was recorded using an analytical balance (±0.0001 g) for 8 h. A fan was placed inside the chamber to ensure uniform conditions within the chamber. The WVP (g m⁻¹ s⁻¹ Pa⁻¹) was calculated using Eq. (5):

$$WVP = aFT/(\Delta P_{sat}(RH_1 - RH_2)) \quad (5)$$

where, a is the mass of the permeability cell (g) at time t (s); FT is the film thickness (m); A is the exposed area (m²); P_{sat} is the saturation vapor pressure at the test temperature (Pa); RH_1 is the RH of the water contained in the cells, expressed as a fraction; and RH_2 is the RH of the chamber, expressed as a fraction.

The dynamic-mechanical thermal behavior of film samples was evaluated using a dynamic mechanical analyzer (DMA, Q800, T.A. Instruments, USA) following the method described by Tynnes et al. [24] with some modifications. Film specimens (30 mm × 9 mm) were loaded and submitted to a range of temperatures between -80 °C and 150 °C with a heating rate of 10 °C, at a constant frequency of 1 Hz and strain amplitude of 10 μm. For thermal analysis, the result were reported as the mean of three repetitions.

2.12. Statistical analysis

The obtained results were reported as the mean and standard deviation of at least three replicates. Analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) between the samples for

each property of the biodegradable films. Multiple range tests (MRTs), with the Tukey–Kramer range test, were used to determine which means were significantly different ($p < 0.05$).

3. Results and discussion

3.1. Chemical composition, apparent amylose content, and color

The chemical composition of the extracted CUS in comparison to commercial POS is shown in Table 1. Low contents of protein, lipids, and ashes were observed in both samples, indicating high purity related to the efficiency of the extraction process. The samples showed values lower than 20 % in the moisture content, which is considered a limit value in starch samples [25]. The apparent amylose content of CUS and POS was in agreement with the reports for different tuber starches [26,27] and they can be classified as normal starches according to the classification reported by Felisberto et al. [28]. However, the amylose content reported for CUS in this work was higher than previously reported for the same species [10]. The amylose concentration is related to the functional properties of the starch; thus, high amylose content tends to promote retrogradation leads to the formation of a more organized structure, which is a desirable characteristic to the production of packaging materials or gels [8,29].

Color is an essential feature of starch since it is directly related to its purity and, therefore, its acceptance by the consumer. Boudries et al. [30] reported that luminosity values (L^*) equal to or >90 were associated with high extraction purity, which is in agreement with the results obtained for the analyzed starches, as can be seen in Table 1 and Fig. 2A and B. Also, the low values of a^* and b^* are associated with low

Table 1
Chemical composition, color characteristics, particle size, pasting properties, gel texture, and thermal properties of cubio starch and commercial potato starch.

Parameter	CUS	POS
Chemical composition		
Moisture (%)	8.20 ± 0.33 ^a	15.91 ± 0.23 ^b
Protein (% dm)	0.16 ± 0.01 ^a	0.17 ± 0.02 ^a
Lipids (% dm)	0.25 ± 0.01 ^b	0.06 ± 0.01 ^b
Ash (% dm)	0.21 ± 0.02 ^a	0.23 ± 0.02 ^a
Carbohydrates (% dm)	99.4 ± 0.2 ^a	99.6 ± 0.2 ^a
Amylose (% dm)	31.2 ± 0.9 ^a	32.1 ± 0.6 ^a
Color characteristics		
L^*	93.5 ± 0.0 ^a	95.3 ± 0.0 ^a
a^*	1.54 ± 0.02 ^b	1.32 ± 0.06 ^b
b^*	-3.19 ± 0.04 ^a	-3.06 ± 0.09 ^a
WI	40.8 ± 0.5 ^a	92.2 ± 0.4 ^a
Particle size distribution		
$d_{(0.1)}$ (μm)	6.8 ± 0.2 ^a	20.7 ± 1.0 ^b
$d_{(0.5)}$ (μm)	14.0 ± 0.1 ^a	40.1 ± 0.4 ^b
$d_{(0.9)}$ (μm)	21.9 ± 0.1 ^a	65.7 ± 1.1 ^b
Pasting properties		
Pasting temperature (PT, °C)	66.7 ± 0.20 ^a	63.9 ± 0.10 ^b
Peak viscosity (PV, BU)	635 ± 12 ^a	812 ± 16 ^b
Trough viscosity (TV, BU)	239 ± 4 ^a	267 ± 10 ^a
Breakdown (BU, BU)	396 ± 15 ^a	545 ± 6 ^b
Setback (SB, BU)	143 ± 4 ^a	207 ± 4 ^b
Final viscosity (FV, BU)	372 ± 4 ^a	483 ± 5 ^b
Gel texture		
Hardness (gf)	34.7 ± 8.2 ^a	54.5 ± 17.2 ^b
Adhesiveness (g s)	50.0 ± 1.5 ^a	30.5 ± 4.6 ^a
Cohesiveness	0.07 ± 0.01 ^a	0.07 ± 0.02 ^a
Thermal properties		
T_g (°C)	60.9 ± 0.3 ^a	63.9 ± 0.3 ^b
T_p (°C)	63.8 ± 0.2 ^a	67.0 ± 0.3 ^b
T_c (°C)	67.0 ± 0.2 ^a	71.2 ± 0.4 ^b
ΔH (J/g)	4.26 ± 0.78 ^a	3.35 ± 0.08 ^a

CUS: cubio starch; POS: potato starch; dm: dry matter; a^* : CIELab coordinate (redness/greenness); b^* : CIELab coordinate (yellowness/blueness); L^* : CIELab coordinate (luminosity); WI: white index; d : diameter at different percentiles; BU: Brabender Units; T_g : onset temperature; T_p : peak temperature; T_c : conclusion temperature; ΔH : enthalpy of gelatinization.

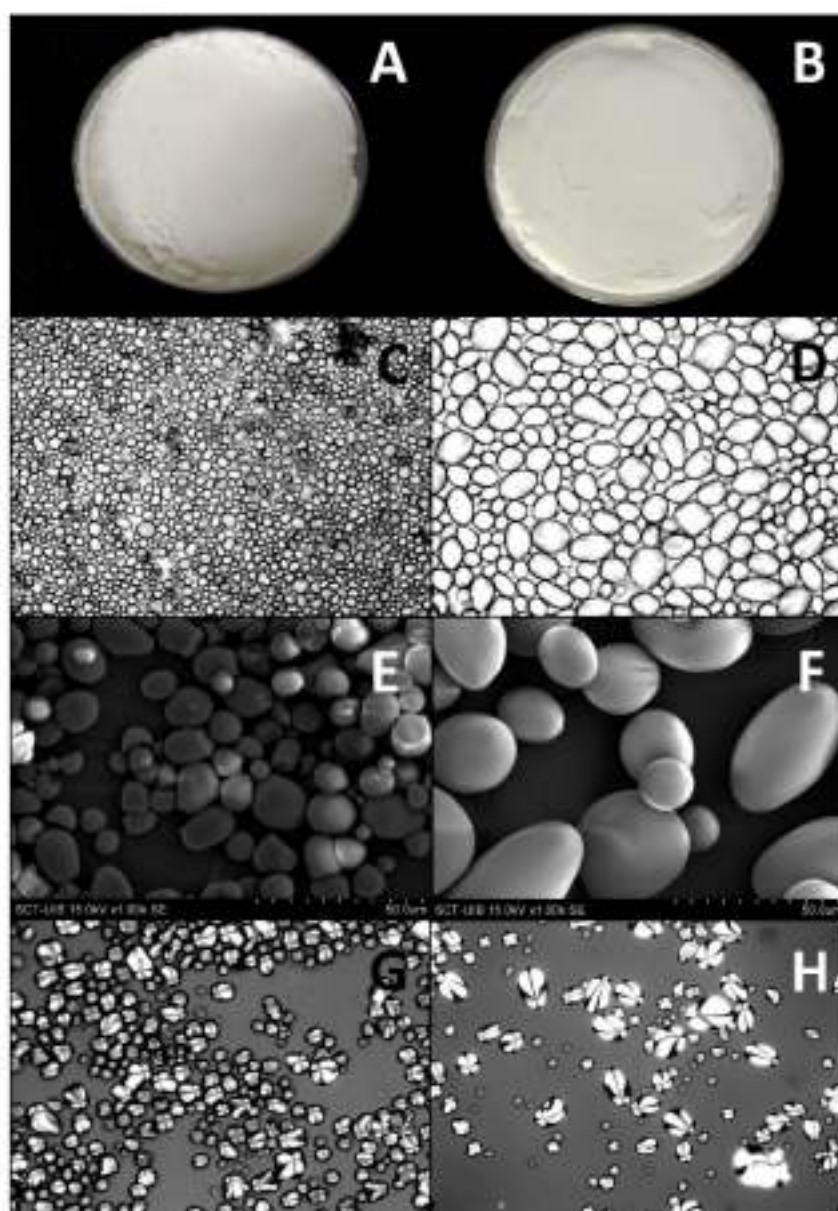


Fig. 2. Native starch, light microscope, scanning electronic microscope, and polarized light microscope images of cubio starch (CUS) (A, C, E, G) and commercial posito starch (POS) (B, D, F, H).

concentrations of pigments or contaminating material that can affect the color of the material. The WI is a parameter related to consumer acceptance, and the values obtained for CUS and POS are similar, indicating that the extraction process did not affect the visual characteristics of the CUS. In addition, the high WI can be related to the high purity of the starches analyzed.

3.2. Morphological characteristics

The morphologies of CUS and POS as evaluated by light microscopy, SEM, and polarized light are shown in Fig. 2. In general, CUS and POS granules exhibited irregular oval and round shapes, with a smooth

surface and without fissures (Fig. 2C–F). When the starches were observed with the polarized light microscope, the Maltese cross was observed, which is related to the birefringence of starches generated by the anisotropy properties of the semi-crystalline structure of the granules (Fig. 2G, and H) [31]. The presence of the Maltese cross and the lack of fissures on the surface of the starches granules is evidence that the extraction method did not affect the integrity of the samples [28]. Furthermore, a higher intensity in the birefringence signal can be observed in POS granules than CUS granules, which may be due to the differences in granule size or crystallinity index [32].

3.3. Particle size distribution

As can be seen in Fig. 3, the particle size distribution of CUS and POS showed a bimodal distribution. The first granule size population, corresponding to a small population, was in the ranges of 1.37–3.89 μm for CUS and 1.37–6.54 μm for POS. The second and the main size population was in the ranges of 4.62–53.3 μm for CUS and 11–148 μm for POS (Fig. 3A). Furthermore, as shown in Table 1, the values for $d_{(0.1)}$, $d_{(0.5)}$, and $d_{(0.9)}$ were about three times higher for POS than for CUS ($p < 0.05$). The bimodal distribution has also been observed in starch from *Cucurbita argyrea* [4] and *Boehmeria nivea* [33]. Differences in the size of the granules can influence the technological properties of starches, such as hydration and pasting properties, since a larger granule size leads to greater hydration [34]. The mean diameters of CUS and POS are in agreement with the reports for native potatoes of the Andean region [35]. However, to date, there are no reports of the particle size distribution of the Cubio starch.

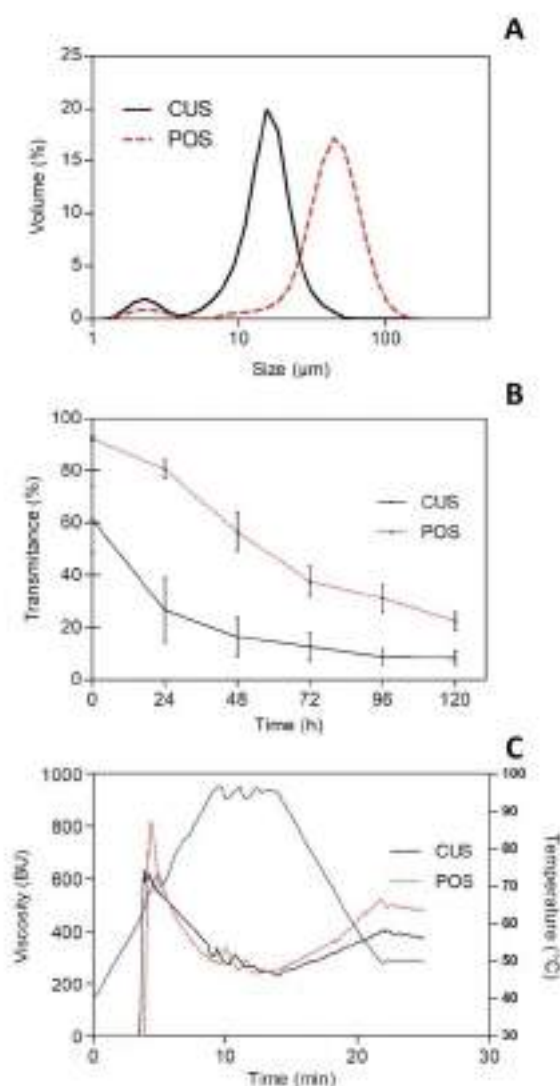


Fig. 3. (A) Particle size distribution, (B) paste clarity and (C) pasting properties of cubio starch (CUS) and potato starch (POS).

3.4. Paste clarity

Results of paste clarity are shown in Fig. 3B. Initial transmittance (0 h) was significantly ($p < 0.05$) higher in POS (92.2 %) than in CUS (61.3 %). A decrease in transmittance was observed as storage time increased (Fig. 3B). After 120 h of storage, transmittance decreased to 22.4 % in the POS sample and to 8.3 % in the CUS sample. After gelatinization, low storage temperature induces retrogradation of the amylose molecules and debranched amylopectin in the starch gels leading to the formation of crystals, which can reduce the clarity of the gels [35]. As Kaur et al. [36] described, the differences in the paste clarity observed between CUS and POS may be mainly due to the size of the granules.

3.5. Pasting properties

Pasting properties are shown in Table 1. The analyzed samples showed typical pasting profiles (Fig. 3C). The CUS showed lower peak viscosity (PV) than the POS; this parameter measures the capacity of the starch granules to hold water regarding the resistance of swollen granules under normal or heated conditions [4]. The differences in PV can be related to the particle size of the granules, which confers a higher hydration capacity to the POS than to the CUS, since larger size distribution leads to the granule having a greater filling space and, therefore greater water retention [34]. The resistance of the granules to swelling can be confirmed with the values of pasting temperature (PT), which was $\sim 4^\circ\text{C}$ lower for CUS ($p < 0.05$). After reaching the PV, the samples showed a marked decrease in viscosity associated with the granule disruption, followed by the leaching of amylose and amylopectin. The degree of disruption and the decrease in viscosity is related to the stability of the granules. The CUS showed the lowest breakdown (BD) value in relation to the decrease in viscosity, which indicates more granule stability than in the POS. As Li et al. [37] mentioned, the ability of starch to resist heating and shear stress is an aspect of interest to determine its technological potential. In this study, the through viscosity (TV) of CUS and POS was not significantly different ($p > 0.05$). Finally, the retrogradation process produced by a reassociation of the leaching amylose and amylopectin molecules leads to an increment in the viscosity of the samples. Although the TV was similar for both samples, the setback (SB) and the final viscosity (FV) were significantly ($p < 0.05$) lower for CUS. Differences in SB and FV may be due to the amount of amylose released or the amylose chains' molecular weights. The smaller the size of the amylose chains, the greater the reassociation [8]. Although the FV of the CUS was lower than that of the commercial POS analyzed in this study, the FV shown by the CUS was higher than that of the ulluco starch (117 BU) [38].

3.6. Gel texture

Textural properties of the CUS and POS gels are summarized in Table 1. The hardness of the CUS gel was $\sim 36\%$ lower than that of the POS gel, which also showed the highest adhesiveness ($\sim 64\%$ higher). With regard to cohesiveness, no significant differences were observed between both starches ($p > 0.05$). The hardness of the gels can be related to the FV obtained in the pasting properties evaluation. Additionally, this property can be related to the size of the granule, since a large granule size leads to an excess of water absorbed by the starch, forming a hard, and dense gel network. Li et al. [37] evaluated the hardness of starch gels isolated from twelve *Lycopers* species and observed that, in general, a larger granule size was associated with a higher gel hardness. The hardness of the CUS gel showed higher values than those reported for starches isolated from yellow yuca, purple yuca, cassava, and potato starches with hardness values of 21.1 g, 25.0 g, 16.5 g, 27.5 g, respectively [39]. As well as some varieties of rice [40], and twelve *Lycopers* species [37].

3.7. X-ray diffraction (XRD) of starch and edible films

The X-ray diffraction patterns of CUS and POS are shown in Fig. 4A. Starches can exhibit four significant diffraction patterns of crystallinity called A-, B-, C-, and V-types. According to Jiang et al. [1] and Miranda et al. [41], the A-type starches show strong diffraction peaks at around 15° and 23° 2θ , and a dual peak at around 17° and 18° 2θ . The B-type starches exhibit a characteristic peak at around 5.6° 2θ and a strong diffraction peak at about 17° 2θ , with small peaks at around 15° , 20° , 22° , and 24° 2θ . The C-type crystal starch is a mixture of both A- and B-types; while type-V starch shows intensity peaks at diffraction angles of 2θ at approximately 12.6° , 13.2° , 19.4° and 20.6° , which is characteristic of the crystallization of amylose with lipids. Native CUS and POS exhibited a typical B-type diffraction structure, with the characteristic peaks. The B-type structure is found mainly in materials cultivated under cool and wet environmental conditions, as in roots and tubers [42], and is more resistant to enzymatic hydrolysis [35]. These findings

agree with the results reported for starches isolated from cubio and Andean potatoes [6,35]. In addition, the relative crystallinity of the CUS and POS was $16.0 \pm 0.13\%$ and $18.6 \pm 0.4\%$, respectively. A low crystallinity may be associated with the high apparent amylose content, since the crystallinity is related to the amylopectin content [43]. However, crystallinity values were within the range reported for starches obtained from different *Dioscorea* plants [1].

3.8. Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of starches are presented in Fig. 4B. Broadband was observed between 3000 cm^{-1} and 3600 cm^{-1} , corresponding to vibrational stretches of the free and inter- and intramolecular bound hydroxyl groups [4]. The peaks observed at 2940 cm^{-1} and 1650 cm^{-1} were associated with $-\text{CH}_2$ stretching and absorption by water in the amorphous region of the starch [8]. In addition, bands corresponding to C–C, C–OH, C–H stretching vibrations were observed between 800 cm^{-1} and 1400 cm^{-1} [44]. Bands at 1022 cm^{-1} and 1047 cm^{-1} are related to the amorphous and crystalline regions of the starch, respectively [43]. The ratio ($R_{1047/1022}$) of the deconvoluted spectra obtained for CUS and POS was 0.74 ± 0.02 and 0.82 ± 0.01 , respectively. These values are associated with the degree of short-range molecular orders, which is in agreement with the X-ray results, and indicate the higher degree of crystallinity shown by POS in comparison with CUS.

3.9. Thermal analysis

Starch gelatinization is a process in which there is a breakdown of the organized structure of starch granules leading to a greater interaction with water molecules in the presence of heat. As a result, double helix de-coiling, melting of crystalline areas, loss of birefringence, viscosity development and starch granule swelling were observed [45]. Table 1 shows the gelatinization temperatures (T_0 , T_p , and T_c) and the gelatinization enthalpy of CUS and POS. The gelatinization temperatures corroborate the results obtained for PT in the pasting properties analysis, indicating a lower resistance to swelling of the CUS granules ($p < 0.05$). The enthalpy of gelatinization was low for both samples, which is related to their crystallinity values (see Section 3.7). According to Valcárcel-Yamani et al. [10], a low enthalpy value is associated with a lower degree of organization or lower stability of the crystals. Results of gelatinization enthalpy reported in this study were in agreement with those reported for some *Dioscorea* pyriformis tubers [2].

Thermograms of CUS and POS are shown in Fig. 4C. Three stages of degradation were observed for the analyzed starches with a total weight loss of $82.53 \pm 0.52\%$ for CUS and $84.21 \pm 0.33\%$ for POS. The first stage occurs between 40°C and 150°C and corresponds to the evaporation of the bound water of the samples. The loss of mass of CUS (8.87%) and POS (17.7%) in this first stage agrees with the values obtained for the sample's moisture. The second stage occurs in a temperature range of 250°C – 350°C and involves the depolymerization of the constituents of the starch granule [8]. A small transition was observed in the temperature range of 261°C to 274°C for CUS, which could be related to the decomposition of proteinaceous material. This behavior was also observed for *C. angustifolia* starch [4]. Finally, the formation of inert carbonaceous residues occurs in the third stage (above 350°C). Elmi et al. [2] established that if the stability of the starch granule (peak of maximum degradability of the second decomposition stage) exceeds 190°C , it can be considered that the starch shows good thermal stability. Considering that the peak of maximum degradation of the analyzed samples was higher than 190°C in both cases (298.5°C for CUS and 298.8°C for POS), we can conclude that the CUS and POS showed good thermal stability.

3.10. In vitro digestibility

Rapidly digestible starch (RSD) and slowly digestible starch (SDS)

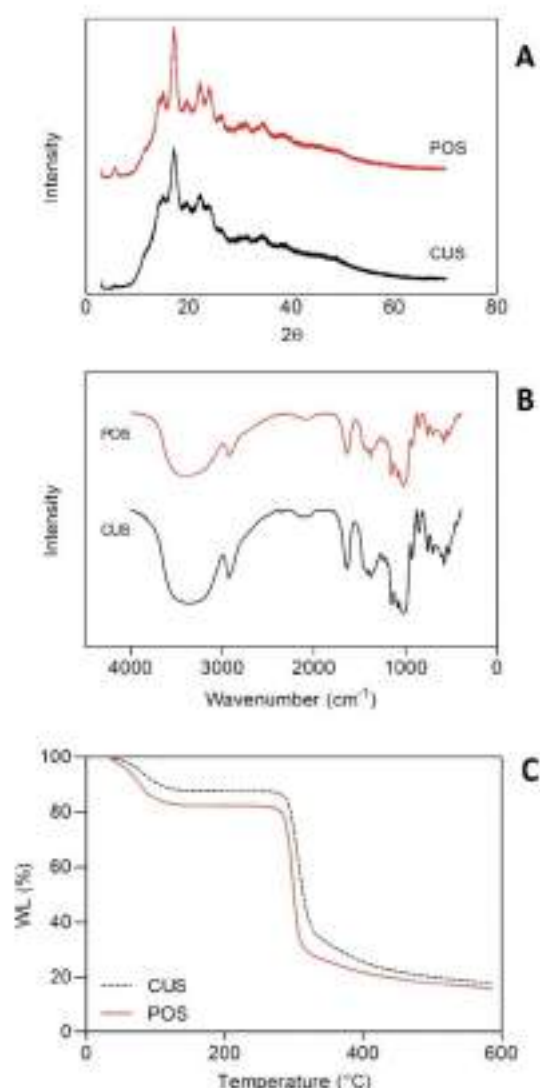


Fig. 4. (A) XRD diffractograms, (B) FTIR spectra and (C) weight loss curves of cubio starch (CUS) and potato starch (POS).

fractions of CUS were of $0.18 \pm 0.13\%$ and $5.42 \pm 1.10\%$, respectively, and for POS were 0 and $0.16 \pm 0.04\%$, respectively. Regarding resistant starch (RS), CUS and POS showed high RS fractions, $94.4 \pm 1.06\%$ and $99.8 \pm 0.04\%$, respectively. This behavior is typical of starches isolated from tubers, which present a characteristic B-type X-ray diffraction pattern [35]. Polymorphs of B-type starches show a high carbohydrate density in the amorphous region, which makes digestibility difficult [1]. The high RS fraction in the analyzed samples can also be related to the high apparent amylose content. Also, it has been observed that samples with lower amylose content were more susceptible to enzymatic degradation [46]. High RS fractions have been observed in other tubers such as *Amilla Blanca*, and *Amilla Negra*, two varieties of potato cultivated in the Andean region [36]. The difference in enzymatic susceptibility of the starches analyzed can be related to the botanical source and the size of particles, since smaller granules have a greater effective surface area for the action of enzymes, similar to observations previously made by Dong et al. [33]. The low susceptibility to enzymatic degradation shown by CUS demonstrates its potential as a food ingredient with possible prebiotic effects.

3.11. Evaluation of the technological feasibility of cubio starch as food packaging material

The suitability of CUS for use as a raw material in the production of biodegradable films was evaluated. About the morphology, all the films presented a continuous surface without porosities, which can be related to an improvement in the barrier properties of the films helping to reduce the passage of gases (Fig. 5). The presence of cracks on the surface of materials can increase the permeability of water vapor and other gases, as was observed in hydroxypropyl methylcellulose/hydroxypropyl starch films [47]. The morphology of the samples analyzed may be due to the concentration of amylose, which leads to a higher degree of reordering in the retrogradation process and prevents the fracture of the film surface.

Cubio-starch-based films (SCF) showed crystallinity patterns with signals at 5.6° , 17° and 20° 2θ (Fig. 6A), which means a partial loss of the native crystallinity structure of CUS after gelatinization of the materials to obtain the films. Crystallinity of samples was of $5.26 \pm 0.45\%$, $5.16 \pm 0.15\%$, and $5.43 \pm 0.35\%$ for SCF1, SCF2 and SCF3, respectively ($p > 0.05$). These values of crystallinity can be related to the retrogradation of the starch granules and associated to the resistance of the films against the passage of water, due to the organization of the molecular chains. Similar behavior was reported for films obtained with alluco starch [8], and potato starch films containing mesoporous nano-silica [48]. The opacity of films were 1.23, 0.98 and 0.72 for SCF1, SCF2 and SCF3, respectively, which indicates the high transparency of the samples (Fig. 5D). The low opacity of films may be due to the low crystallinity values observed, since materials with high crystallinity have a high refractive index and therefore a high dispersion of light [49]. Since there are no significant differences with the crystallinity values, the differences in opacity between the samples evaluated may be related to the content of starch and therefore amylopectin. A higher concentration of amylopectin makes a film more transparent as its branched structure makes a film matrix less reordered and compact [49]. Similar results for opacity and transparency were reported for films prepared using mung bean starch [14]. However, opacity values obtained for the samples analyzed were lower than those reported for films from banana starch and curcumin-loaded nanoemulsions [15]. These differences can be related to the botanical source of the starch and its particular physico-chemical characteristics which affect the light dispersion of the material.

The WVP of films were $2.29 \times 10^{-9} \pm 8.92 \times 10^{-11}$, $2.52 \times 10^{-9} \pm 3.19 \times 10^{-11}$, and $2.51 \times 10^{-9} \pm 1.97 \times 10^{-11}$ $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$ for SCF1, SCF2, and SCF3, respectively. No statistically significant differences were observed between the samples. The films evaluated in this work showed WVP similar to those reported for chachafruto starch films [50]. However, the samples displayed high WVP compared to films obtained

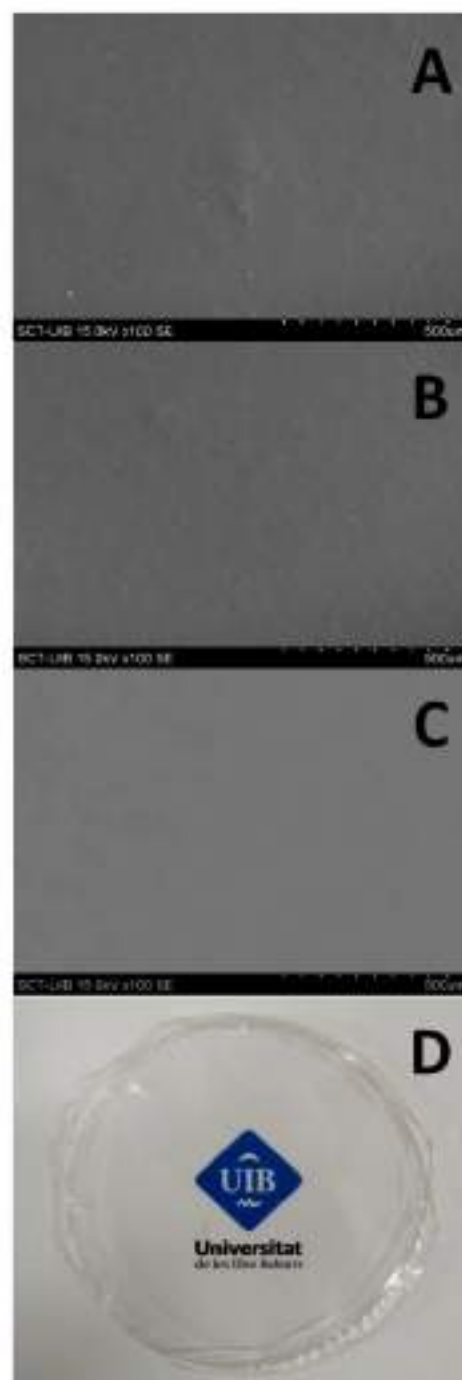


Fig. 5. Surface micrographs of cubio starch-based film samples: (A) SCF1 (films with 2.0 % w/v of cubio starch (CUS)), (B) SCF2 (films with 2.5 % w/v of CUS), (C) SCF3 (films with 3.0 % w/v of CUS), and (D) photograph of SCF3.

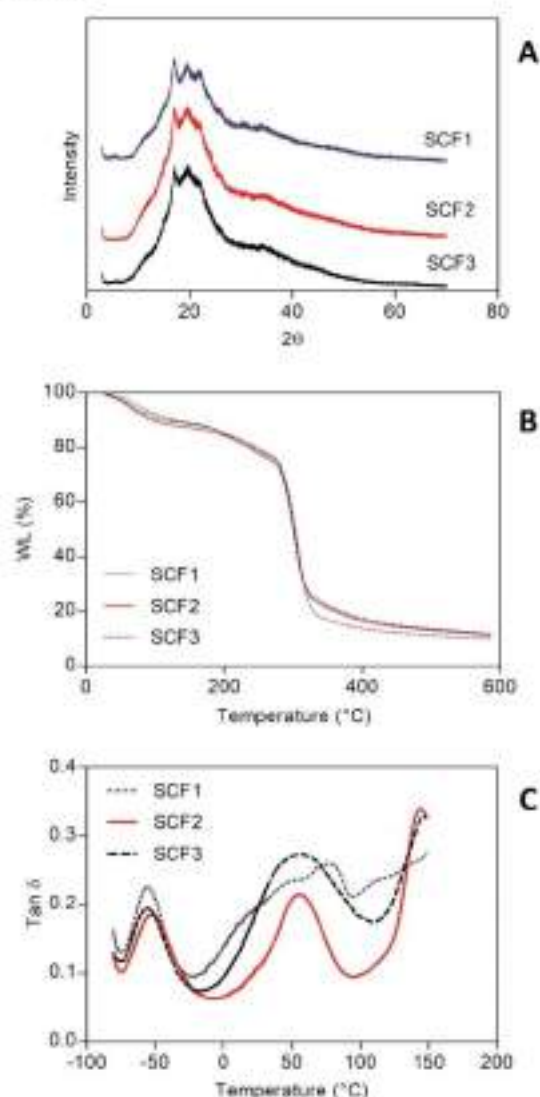


Fig. 6. (A) XRD diffractograms, (B) weight loss curves, and (C) $\tan \delta$ curves of cubão starch-based films samples. SCF1 (films with 2.0 % w/v of cubão starch (CUS)), (B) SCF2 (films with 2.5 % w/v of CUS) and (C) SCF3 (films with 3.0 % w/v of CUS).

with starch from other Andean tubers [8]. The high permeability of the films may be related to the low crystallinity that allows the passage of water molecules through the network formed by the components in the films.

As can be seen in Fig. 6B, the total weight loss of films was 89.5 ± 1.5 % for SCF1, 89.5 ± 0.9 % for SCF2, and 87.9 ± 0.7 % for SCF3 ($p > 0.05$). Three main stages of decomposition were observed for the analyzed samples, which is in agreement with previous reports for starch-based films (Fig. 6B) [51,52]. The first (between 50 °C and 180 °C) and second (between 180 °C and 270 °C) stages of degradation correspond to the loss of water and the degradation of glycerol. Meanwhile, the third stage (between 270 °C and 360 °C) corresponds to the decomposition of the starch. A displacement of the peak of the maximum degradation temperature of the films can be observed in comparison with the CUS (Fig. 4C), which implies an increase in the stability of the

analyzed samples due to the interaction of the structural components.

Fig. 6C shows the results obtained from the DMA analysis. All the samples showed a dual structure that could be explained due to the partial miscibility of glycerol and starch. The first peak of starch-based films appears at temperatures between -54.6 °C and -53.11 °C and is related to the β -relaxation of the films, which corresponds to the relaxation of the glycerol-rich phase. The second transition was in the range of 55.3 °C and 75.4 °C, which is associated with the α -relaxation of the starch-rich phase [53,54]. These results are in agreement with those reported for corn starch films [53]. Additionally, a marked shift in the second peak was observed for SCF1. This shift may be explained because of the higher amount of starch in SCF2 and SCF3 that can interact with the plasticizer, generating a decrease in the intermolecular forces due to an increase in molecular mobility. On the contrary, in SCF1, the low amount of starch is quickly saturated by the presence of the same concentration of the plasticizer.

4. Conclusions

The extraction of the CUS was successful considering the low concentration of lipids, proteins, and ashes, as well as the integrity of the granules observed under the microscope. The CUS showed an intermediate apparent amylose content and good color characteristics, positive for consumer acceptance. Additionally, low particle size was observed, influencing the pasting properties and gel texture of the starch. Native CUS exhibited a typical B-type diffraction structure with a relative crystallinity of 16 %, which is associated with low susceptibility to enzymatic degradation, indicating its potential as a food ingredient with possible prebiotic effects. The thermal analysis demonstrated the good thermal stability of the starch, a desirable characteristic for industrial purposes. Concerning the potential as packaging material, the films based on CUS showed satisfactory characteristics such as good morphology related to the barrier properties, high transparency, useful for products that require visualization, and good thermal stability related to its potential as the packaging of products that may be subjected to different temperature ranges. However, the starch concentrations studied did not markedly affect the properties of the materials. Therefore, it could be concluded that the CUS showed industrial potential, mainly as a novel, biodegradable, and cheap material for the preparation of biodegradable packaging that can have wide use in the food industry, which implies a novel alternative to the use and exploitation of this crop. However, more studies on the modification of the CUS should be developed, to improve some of its physicochemical characteristics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 2

Chachafruto starch: Physicochemical characterization, film forming properties, and 3D printability

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Chachafruto starch: Physicochemical characterization, film-forming properties, and 3D printability

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ABSTRACT

This work aimed to characterize the physicochemical, film-forming properties, and 3D printability of a nonconventional starch from chachafruto. The chachafruto native starch (CHS) presented an excellent extraction yield (10 % db) and purity (99 % db), along with an oval and round morphology, a smooth surface with few defects, and a mean diameter of 15.4 µm. The typical X-ray diffraction pattern was observed in the CHS with a crystallinity of 17.4 %. The starch presented a paste temperature of 66.1 °C, an enthalpy of 11.5 J g⁻¹, and a final viscosity of 596 Brabender Units. The thermal analysis demonstrated good thermal stability. The evaluated film presented a reduction in crystallinity (8.18 %) to the CHS, which generated a good elasticity in the material. Likewise, it presented a continuous structure without cracks, providing good barrier properties (2.5 × 10⁻⁴ g cm⁻¹ s⁻¹ atm⁻¹) and high transparency. Meanwhile, 3D prints prepared with CHS showed good tensile properties and high consistency. The morphological analysis showed that the prints generated organized cell structures. However, high concentrations of CHS were not efficient in obtaining 3D prints. The results of this work demonstrate the tremendous industrial potential of chachafruto as an unconventional source of starch and were alternative uses for adding value to the crop.

1. Introduction

Starch is a natural polysaccharide in cereal grains, legumes, tuber, and root crops. This macromolecule is consumed worldwide and is used as a raw material in different industries such as pharmaceuticals, food, biomedical, and plastics [1]. By 2026, the production of this material is expected to reach 120 million tons, with corn, wheat, cassava, and potatoes being its primary sources [2]. This situation has generated concern worldwide about the possible impact on food security, which has led to the search for alternative sources of starch in its native form [3–5]. However, the chemical composition of the starches obtained from these alternative sources, as well as their industrial potential, may vary according to the crop's botanical source and agro-ecological conditions. Consequently, it is necessary to characterize the physicochemical properties and evaluate their industrial potential.

The chachafruto (*Drychrisa edulis* Triana) is a legume plant native to South America, cultivated mainly at altitudes between 1600 and 2000 m above sea level. The plant has a broad spectrum of uses ranging from

animal feed (as fodder) to soil recovery due to its nitrogen fixation capacity in soils [6]. The seeds have a high protein content with values ranging from 18 % to 24 %, as well as a high content of carbohydrates (70 %), mainly starch (28.6 % db) [7–9]. Despite its nutritional potential, its use in the human diet is limited and is restricted to consumption by local populations. The lack of a chachafruto industry represents an opportunity for exploitation and adding value to this crop. Recent studies have focused on obtaining chachafruto protein fractions with different biological potentials [7,10]. However, the studies focused on evaluating the starch obtained from its seeds, and its use is limited. In order to promote the integral use and exploitation of the chachafruto, as well as generating a circular economy around this crop, it is necessary to carry out studies focused on the complete characterization of the macromolecule obtained from the starch and its potential use at industrial level.

Among the alternative uses of starch in the food industry, the production of biodegradable films that can partially replace petroleum-derived plastics, has been a topic of interest and development in

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recent years largely due to its environmental impact. Different starch sources have been evaluated for preparing these biomaterials, such as sweet potato, rice, pumpkin, lentil, and quinoa starches, showing great potential for the packaging industry mainly due to their low oxygen permeability, flexibility, and optical properties [11–13]. However, the material's performance depends on the processing conditions and the starch's physicochemical properties. The characteristics of starch-based films rely on the ratio of amylose and amylopectin in the starch. Boslák et al. [14] compared biodegradable films made from wheat (25 % amylose), corn (27 % amylose), and potato (20 % amylose) starches and found differences in their barrier, mechanical and hydration properties. They reported that films with lower amylose content had lower water permeability, solubility, and better mechanical properties.

3D printing is an innovative technology that can produce food products with defined shapes by depositing one or more materials layer by layer [15,16]. This technology offers several benefits for food production, such as diet customization, process automation, time and waste reduction, as well as enhanced sensory attributes [17]. The materials used for printing must be malleable, self-supporting, quickly solidifying, and have a certain flowability that allows them to pass through the nozzle, which means that only some food materials can be used for this purpose [16]. Starch is a material that meets all these characteristics due to its ability to gelatinize and form viscoelastic gels. However, the printability properties depend on the type and properties of the starch used [17]. Zheng et al. [18] compared the effects of amylose content on 3D printing using potato, wheat, and corn starches. They found that the microstructure and texture of the 3D prints varied significantly, even with the same starch concentration (25 % w/v). Furthermore, printability was associated with the lowest final viscosity observed in wheat starch preparations. Starch concentration in 3D printing preparation influenced the technological properties of the materials. Qiu et al. [20] reported that higher starch concentration in gels increased the gel viscosity and the interaction of the amylose and amylopectin chains, resulting in more defective and less printable materials. Similar observations were reported for 3D prints prepared with potato starch [21]. The authors found that intermediate starch concentrations enhanced printability and the resistance of the monolayer of the 3D prints.

Considering the growing demand for new starch sources and the unexplored industrial potential of chachafruto starch, this study aimed as well, to evaluate the physicochemical characteristics and the technological potential of starch obtained from chachafruto as a novel material for edible films production and food 3D printing. The study hopes to contribute to the generation of a circular economy for the cultivation of chachafruto, as well as being the basis for added value on an industrial scale, of the starch obtained from this material.

2. Materials and methods

2.1. Materials

Chachafruto (*Erythrina edulis* Triana) was harvested on the farm "El Mirador" located in the village "La India", Coello Cocora, Ibagué city (4°22'14.2"N 75°19'16.4"W). Samples were selected and washed, and those exhibiting mechanical damage were discarded.

2.2. Starch isolation

The chachafruto samples were selected, discarding any that showed mechanical damage, washed with tap water and peeled manually. They were then cut into thin slices (approx. 2 mm) and milled using a food blender (BRLY07 - Zon, Oster, Colombia) for 1 min with distilled water at a ratio of 1:2. The resulting mixture was filtered using a cloth sieve. Then, the solid was re-extracted, and the filtrates pooled and stored at 4 °C and left to stand for 18 h until phase separation. After this, the supernatant was discarded, and water was added to re-suspend the sediment. The mixture was filtered again using a cloth filter. This

procedure was repeated four more times until a translucent supernatant was obtained. The last two washes were performed using distilled water. The chachafruto starch (CHS) obtained was dried in a forced convection oven (Thermolab, DfA, Colombia) at 40 °C and stored at room temperature (–25 °C) in a vertical desiccator cabinet.

2.3. Chemical composition, apparent amylose content, color, and morphological characterization of chachafruto starch

The moisture (Method 925.10), protein (Method 920.07), lipids (Method 920.05), and ash (Method 923.03) contents were determined according to the methods of the A.O.A.C. [22]. The total carbohydrate content was obtained by subtracting the sum of the protein, lipid, moisture, and ash content from 100. Apparent amylose content analysis was carried out according to the method reported by Galindez et al. [23]. The color parameters were obtained according to the CIELAB color system using a Konica Minolta colorimeter (G-410, Minolta Co., Japan). Morphological characterizations were examined with and without polarized light in optical microscopy (BX60, Olympus, Japan) coupled with a camera Motikam 3.0 MP Color Digital Camera (3.0MP, Moric, Hong Kong), as well as with scanning electron microscopy (SEM/S-3400 N, Hitachi, Germany) as reported previously by Daza et al. [13].

2.4. Particle size distribution

The particle size distribution of the CHS was evaluated using a Laser Particle Analyser (Sync Microtec MRB, wet operation Flowtec, Montgomeryville, PA, USA) according to Lin et al. [24]. For this purpose, the CHS was suspended in distilled water and stirred at 2000 rpm. For the analysis, water and starch refractive indexes were adjusted to 1.33 and 1.53, respectively. The samples were sonicated in the equipment at 25 Hz for 15 s before analysis, to avoid aggregation of the granules. Results were reported as diameters at 10th percentile ($d_{0.1}$), median ($d_{0.5}$), and 90th percentile ($d_{0.9}$).

2.5. Pasting properties, gel texture, and paste clarity

The pasting properties of CHS were evaluated using a Visco-Amylograph (Micro Visco-Amylograph®, Brabender Instrument Inc., Germany) according to [3]. Pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown (BD), setback (SB), and final viscosity (FV) were calculated. Viscosity was expressed in Brabender Units (BU). The gel's texture was measured according to the method described by [3] using a texture analyzer with a load cell of 20 N (LS1, Lloyd Ltd., USA). The CHS gels obtained in pasting properties analysis were transferred to plastic containers (25 mm diameter, 50 mm height) and stored at 4 °C for 24 h before analysis. For the analysis, the sample gels were subjected to two compression cycles at a distance of 10 mm (0.05 N trigger force) at a speed of 0.5 mm s⁻¹ by a flat-ended using a cylindrical probe (6 mm diameter). Hardness, adhesiveness, and cohesiveness were determined using Nexygen Plus software (Lloyd Ltd., Version 3.0) [25].

2.6. X-ray diffraction (XRD)

The X-ray diffraction patterns of the samples were obtained using a Bruker D8 ADVANCE diffractometer (Bruker, Billerica, USA) with Ni-filtered Cu-K α radiation (40 kV and 40 mA) and a specific wavelength of 1.5406 Å. Data was collected in the 2 θ range of 3.5–70° (0.02035° of step size) and a counting time of 0.8 s/step. Relative crystallinity was calculated using the DIFFRAC.EVA software (Bruker, Billerica, USA).

2.7. Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of the CHS were obtained using a Fourier – transform Infrared spectroscopy (Nicolet 300, Thermo Scientific Inc., USA).

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The measurements were recorded over the 4000 to 500 cm^{-1} , with a resolution of 4 cm^{-1} and 40 scans at room temperature ($\sim 20.4^\circ\text{C}$).

2.8. Thermal properties

The Onset (T_0), peak (T_p), and conclusion (T_c) temperatures and enthalpy of gelatinization (ΔH) of the CHS were measured using a differential scanning calorimeter (2920, TA Instruments, Japan) according to the methodology described by Lin et al. [3]. The obtained thermograms were examined using Universal Analysis 2000 V3.9A software (TA Instruments, Japan).

CHS's thermal degradation temperature and weight loss (WL) were measured using a thermogravimetric analyzer (SDT 2960, TA Instruments, Japan) over the temperature range of 20–600 $^\circ\text{C}$ at a heating rate of 20 $^\circ\text{C min}^{-1}$ and under a stream of nitrogen.

2.9. Potential as food packaging of CHS

2.9.1. The CHS - based films preparation

The CHS was dissolved in distilled water at a concentration of 2.0 % (w/v, dry basis d.b.) (CHSF) (this concentration was selected after preliminary analysis, data not shown) and agitated for 10 min using a magnetic agitator. The mixture was heated to 95 $^\circ\text{C}$ and maintained at this temperature for 5 min. Then, glycerol (1.0 % w/v) was added to the mixture when it reached 50 $^\circ\text{C}$. The prepared solutions were cast in a Petri dish ($\sim 0.53 \text{ g}$ of the film solution per cm^2). The films were dried at 40 $^\circ\text{C}$ for 24 h in a forced convection oven. Before the analyses, the samples were stabilized in a vertical desiccator cabinet at room temperature ($\sim 23^\circ\text{C}$) and relative humidity (RH) of 54 % for 24 h.

2.9.2. CHS - based films characterization

The CHS - based films were characterized with regard to swelling power (SP; %), solubility (S; %), opacity (OP), mechanical properties [Young Modulus (YM; MPa), elongation at break (EB; %), tensile strength (TS; MPa)], morphology and water vapor permeability (WVP) according to the methodologies described previously by Gomez-Jara et al. [26]. The XRD, FTIR, and TGA were carried out according to the methods described in previous sections.

2.10. CHS potential for 3D printing

2.10.1. Sample preparation and 3D printing

For the preparation of the starch gels, the CHS was mixed with distilled water at three concentrations [8 % (CHS3D1), 10 % (CHS3D2), and 12 % (CHS3D3) w/v d.b.] (selected from preliminary analysis). Then, the mixture was heated to $90 \pm 2^\circ\text{C}$ with constant stirring using a mechanical stirrer (RZR 2021, Heidolph, Germany) at 180 rpm for 10 min. Then, the samples were left to stand until they reached a temperature of 60 $^\circ\text{C}$ and then transferred to a print cartridge (nozzle of 1.5 mm). The 3D printing was carried out in an extrusion-based 3D printer (FoodiniTM, Natural Machines, Spain) based on preliminary analysis and the manufacturer's specifications [27]. A rhoid model (geometry given by the machine by default) with dimensions of 20 mm $\times$ 20 mm $\times$ 10 mm was printed with a monolayer height and nozzle movement speed of 1.4 mm and 500 mm/min. Printed specimens with dimensions of 10 mm $\times$ 10 mm $\times$ 5 mm were used for the dynamic-mechanical analysis (DMA).

2.10.2. Characterization of 3D prints

The microstructural characteristics of the samples were analyzed using scanning electron microscopy (SEM/S-3400 N, Hitachi, Germany) in freeze-dried and cold-broken samples using liquid nitrogen [1,28]. Textural analysis was performed using a universal test machine (Zwick/2100, Zwick Roell, Germany). For this purpose, each printed sample was placed in the center of a steel plate and submitted to compression (40 % of the total sample height) using a cylindrical probe at a 5 mm/min

compression rate with an initial gap separation of 13 mm. The XRD parameters were adjusted according to the methodology described in the previous section (Section 2.6). Before the analysis, the samples were dried by lyophilization and ground using an analytical grinder universal mill (Yellowline A10, IKA, Germany), according to Liu et al. [29]. The DMA was evaluated according to Yang et al. [30] using a dynamic mechanical analyzer (DMA, Q000, TA Instruments, USA) with a parallel-plate geometry (diameter of 20 mm and a gap of 10 mm). The specimens (10 mm $\times$ 10 mm $\times$ 5 mm) were loaded and submitted to a compression test in a "frequency sweep" mode ranging from 0.1 to 30 Hz at a fixed strain amplitude of 1 ± 0.004 % of the total sample height and at room temperature ($24 \pm 2^\circ\text{C}$). The storage modulus (G') and loss modulus (G'') of the materials were plotted and analyzed using Universal Analysis 2000 V3.9A software (TA Instruments, Japan).

2.11. Statistical analysis

The results were reported as the mean and standard deviation of each property based on at least three replicates for each sample. Analysis of variance (ANOVA) and Tukey–Kramer range tests at a significance level of 0.05 were performed to compare the properties of the biodegradable films.

3. Results and discussion

3.1. Chemical composition, apparent amylose content, color, and morphological characterization

The yield and chemical composition of the chachafruto starch (CHS) is shown in Table 1. The yield of starch obtained from chachafruto was slightly lower than the 13 % reported for the same species [31], which could be related to differences in the extraction method and agro-ecological conditions. The CHS showed acceptable moisture content considering that the maximum value for industrial use is 20 % [32]. The low content of protein, fat, lipids, and ash in the material analyzed (< 1.0 % of the total composition) is related to the efficiency of the extraction process and the purity of the material extracted [33]. Similar

Table 1
Chemical composition, color characteristics, and particle size of chachafruto starch.

Parameter	Value $\pm$ SD
Chemical composition	
Moisture (%)	8.70 $\pm$ 0.08
Yield (%) d.b.	9.34 $\pm$ 0.71
Protein (%) d.b.	0.88 $\pm$ 0.07
Lipid (%) d.b.	0.01 $\pm$ 0.00
Carbohydrate (%) d.b.	99.2 $\pm$ 0.1
Ash (%) d.b.	0.03 $\pm$ 0.00
Amylose (%) d.b.	36.5 $\pm$ 1.2
Color characteristics	
L*	98.0 $\pm$ 0.6
a*	5.91 $\pm$ 0.04
b*	-1.27 $\pm$ 0.08
W	93.8 $\pm$ 0.5
Particle size distribution	
$d_{w,10}$ (μm)	9.0 $\pm$ 0.0
$d_{w,50}$ (μm)	13.4 $\pm$ 0.5
$d_{w,90}$ (μm)	25.8 $\pm$ 0.7
D [3, 4] (μm)	15.3 $\pm$ 0.5
D [3, 3] (μm)	18.8 $\pm$ 0.2

d.b.: dry basis; L*: CIE Lab coordinates (lightness/greenness); a*: CIE Lab coordinates (yellowness/blueness); b*: CIE Lab coordinates (blueness); W: white index; μ : diameter at different percentiles. Values are expressed as mean $\pm$ standard deviation of at least three repetitions.

purity results were observed in the extraction of other starches [3]. The apparent amylose content of CHS was in agreement with that of the mung bean [5] and the common bean [34]; however, it was greater than tamarind and moth bean starches [4,35]. Amylose content can influence the film-forming properties and printability of starches. Thus, starches with a high content of this molecule can produce films with more homogeneous morphologies and greater tensile strength, this being related to the organization of the network due to the retrogradation of amylose [36]. Similar to the chemical composition, the color parameters can also be associated with the purity of the extracted starch. According to Beaudies et al. [20], high luminosity (L^* > 90) and high whiteness index (WI) values (WI > 90) correspond to starches with high purity. In addition, the low values in parameters a^* and b^* are associated with the absence of pigments (Table 1), which can interfere with the CHS's coloration and the consumer's final perception.

The CHS granules exhibited mainly elongated and spherical shapes with smooth surfaces (Fig. 1 A-C). However, some irregular granules were observed, which may be due to a physical influence of the extraction method on some of the analyzed granules (red arrows in Fig. 1B). The morphology of the CHS is in agreement with the morphology of the bean [34], tamarind kernel [4], moth bean [35], and kidney bean [37] starches. In addition, the birefringence of the granules was confirmed with the Maltese cross observation using polarized light microscopy (Fig. 1C). The presence of the Maltese cross can be related to the integrity of the granules [38].

3.2. Particle size distribution

The starch granules obtained from the charachafuto showed a bimodal distribution (Fig. 2A). The first granule size population, corresponding to 6.70 % of the granule population and ranged between 1 and 4 μm with a mean diameter of $2.33 \pm 0.31 \mu\text{m}$ (Fig. 2A). Meanwhile, the second and the main population corresponded to 93.2 % of the granule population and ranged between 6 and 44 μm with a mean diameter of $15.8 \pm 0.5 \mu\text{m}$. The bimodal distribution has also been observed in starches obtained from legumes such as mung bean [39] and kidney bean [40]. In addition, the mean granule diameter obtained for the analyzed starch (Table 1) is in accordance with the results reported for Yard-long bean seed starch [41]. However, the values obtained for CHS were lower than those observed in starches from different bean starches [42]. The differences in size particle distribution are related to the botanical sources and associated with the techno-functional properties of starches [39].

3.3. Pasting properties and gel texture

Fig. 2B shows a low pasting temperature (PT) ($58.2 \pm 0.2^\circ\text{C}$), with a maximum increase in the CHS viscosity at peak viscosity (PV) of $666 \pm 13 \text{ BU}$ (Brabender units) (Table 2). The PV shows the capacity of CHS to hold water and the resistance of the swollen granules to rupture. The values of PV obtained in this work could be related to the particle size distribution, since low particle size distribution leads to a lower hydration capacity due to a limited filling space [43]. With regard to PV, CHS showed lower values than that reported for potatoes (1421 BU) but higher than wheat (333 BU) and corn starches (476 BU) [44]. As a consequence of the swelling, a rupture of the granule occurs, which generates a decrease in the viscosity of the paste; this phenomenon is known as breakdown (BD) and measures the stability of the pastes and the tendency of the granules to resist the shear force during the heating process [34]. The BD obtained for CHS was $290 \pm 14 \text{ BU}$, reducing about 44 % of the maximum observed viscosity (PV). This decay value was similar to those observed in mung bean starch, for which a viscosity reduction of approximately 43 % was reported in the BD stage [39]. The setback (SB) and final viscosity (FV) of CHS were $220 \pm 6 \text{ BU}$, and $596 \pm 6 \text{ BU}$, respectively. The SB and FV are related to the retrogradation tendency to amylose release from the BD stage starch granule, which has

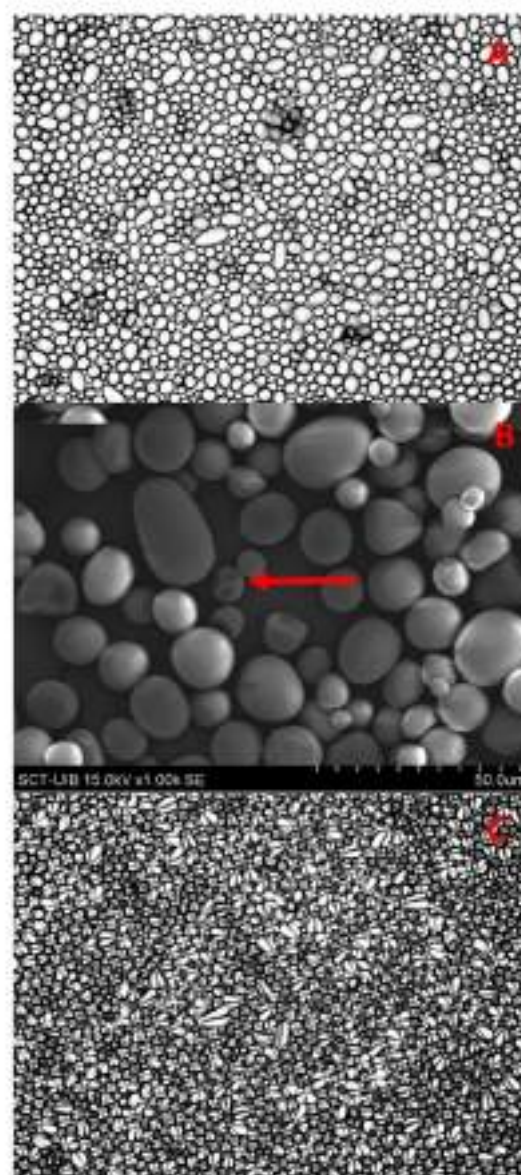


Fig. 1. Light microscope (A), scanning electronic microscope (B), and polarized light microscope (C) images of charachafuto starch.

strong reassociation capabilities [45]. The FV obtained for the analyzed starch was higher than for wheat starch [46].

The hardness, cohesiveness, and adhesiveness of the charachafuto starch gels were $49 \pm 7 \text{ N}$, $0.07 \pm 0.01 \text{ N}$, and $0.59 \pm 0.05 \text{ N s}^{-1}$, respectively. The hardness of the charachafuto starch gel can be related to the starch's FV and apparent amylose content since high viscosity is partially associated with a molecular re-association in the BD process. In addition, amylopectin chain length can influence the hardness of the paste [33]. The hardness of the starch gel analyzed was higher than

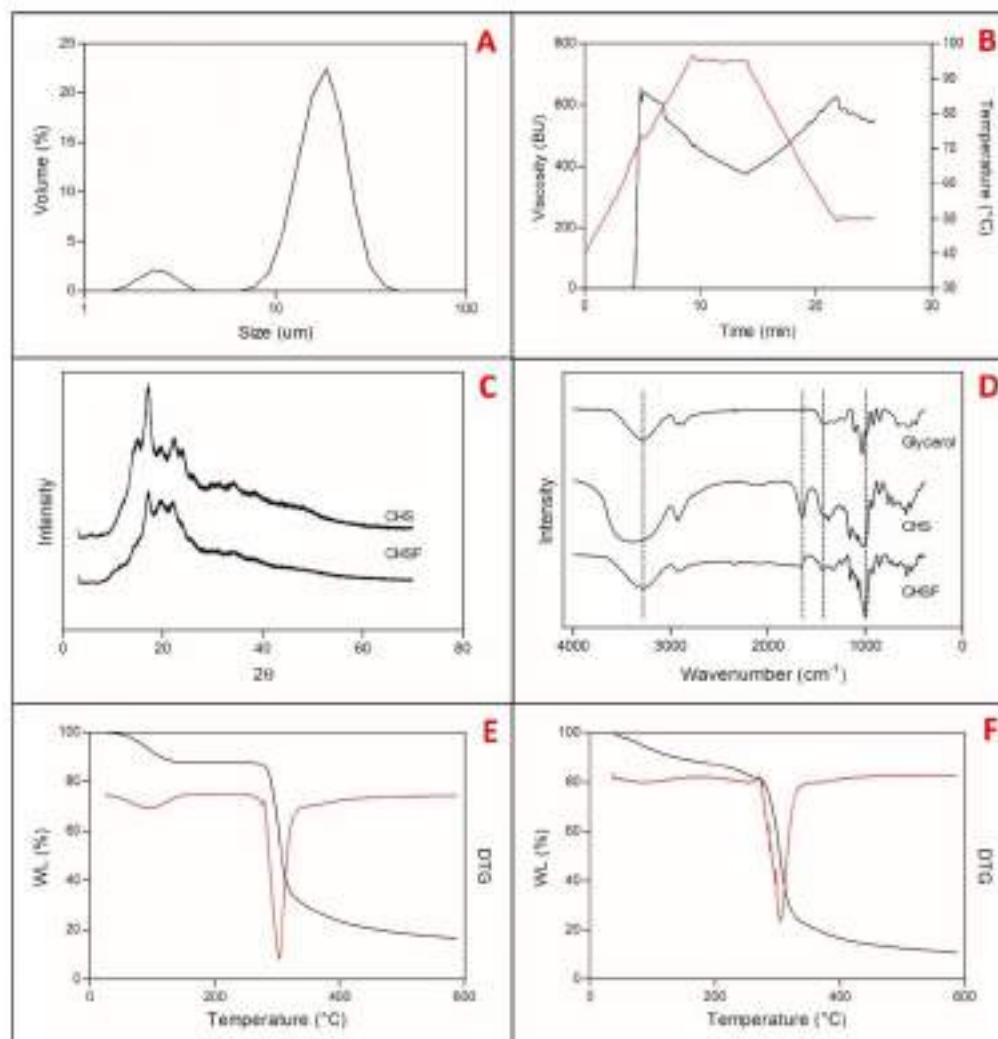


Fig. 2. (A) Particle size distribution, (B) pasting properties of chachafruto starch (CHS), (C) XRD diffractograms of CHS and film (with 2 % of CHS w/v), (D) FTIR spectra of glycerol, CHS and film, (E) weight loss curve of CHS, (F) weight loss curve of film.

Table 2
Pasting properties of chachafruto starch.

Pasting properties	Value $\pm$ SD
Pasting temperature (°C)	68.2 $\pm$ 0.2
Peak viscosity (PV, BU)	666 $\pm$ 13
Breakdown (BV, BU)	38 $\pm$ 2
Final viscosity (FV, BU)	598 $\pm$ 14
Setback (SB, BU)	528 $\pm$ 4
Road viscosity (RV, BU)	596 $\pm$ 6

BU, Brabender Units. Values are expressed as means $\pm$ standard deviation of at least three repetitions.

values obtained for wheat starches [47]. Meanwhile, adhesiveness was higher than those reported for kidney bean starch, but the cohesiveness showed lower values than for the same species [48]. The results

obtained in this work show a high capacity to form elastic pastes with the ability to retain their initial structure under mechanical stress.

3.4. X-ray diffraction (XRD) of starch

The X-ray diffraction of the starch is shown in Fig. 2C. The CHS exhibited a typical B-type pattern with peaks at 5.6, 15, 17, 20, 22, and 24° at diffraction angles of 2 θ [23]. Although the C-type pattern is expected in starches extracted from legumes, different authors have reported the presence of a B-type pattern for starches obtained from various legumes such as wrinkled peas [45] and faba beans [49]. The X-ray diffraction pattern depends on the botanical origin and the organization of crystalline lamellae of amylopectin [41]. In addition, the starch showed relatively low crystallinity of 17.4 $\pm$ 1.1 %, which is in accordance with the crystallinity values reported for wrinkled peas (17.7 %)

[50], black bean (17.0 %), lentil (18.7 %), and chickpea (17.6 %) starches [48]. The relative crystallinity of the starch was low, which could be partly related to the intermediate apparent amylose content since it's amylopectin that mainly contributes to the crystalline structure in starch [41]. The crystallinity of starches also can be influenced by the length of the amylopectin chain, the orientation of the double helices, and the interaction between them [41].

3.5. Fourier-transform infrared (FTIR) spectroscopy

The Fourier-transform infrared (FTIR) spectrum of the native starch is shown in Fig. 2D. Native starch showed a broad band at 3400 cm^{-1} associated with the stretching of free hydroxyl groups [41]. The absorption peak exhibited at about 2900 cm^{-1} is related to the C–H bond stretching [35]. At the same time, the peak at 1650 cm^{-1} corresponds to the absorption of water molecules in the amorphous region of the starch [51]. Bands between 900 and 1100 cm^{-1} are sensitive to starch structure changes [32]. Thus, the bands observed at 970 cm^{-1} are associated with the bending vibration of C–O–H groups (water–starch interactions). Meanwhile, signals at 1025 cm^{-1} and 1090 cm^{-1} are related to the amorphous and crystalline structure of the starch [32]. Similar spectra have been reported for other starches obtained from legumes [30,41].

3.6. Thermal properties

The gelatinization temperatures [Onset (T_o), peak (T_p), and conclusion (T_c) temperatures] and the gelatinization enthalpy (ΔH) of the CHS are shown in Table 3. The T_p of CHS was similar to those reported for the same species and kidney bean [40], sorghum [36], and common bean (73–76 °C) starches [34]. However, this value was lower than those reported for starches obtained from young bamboo culm (75–80 °C) [20] and Talipot palm (82 °C) [52]. Compared with the same starches, the CHS showed an intermediate ΔH value. As Boudries [36] mentioned, low gelatinization temperature can indicate the low stability of starch crystallites in the granule and lower resistance to swelling. In addition, the ΔH is related to the energy required for the gelatinization process and the loss of molecular order for the dissociation of double helices [40]. These results are in accordance with the low degree of crystallinity of the CHS analyzed.

The thermogram of CHS is shown in Fig. 2E. For the analyzed starch, the degradation took place in three stages with a total weight loss of $83.7 \pm 0.4\%$. The first stage of degradation is related to water loss, which occurs in a temperature range between 55 °C to 134 °C with a maximum degradation peak at 93 °C and a weight loss of $12.1 \pm 0.0\%$, which is consistent with the moisture content of the sample. The second stage occurs in a temperature range of 278 °C and 321 °C (maximum degradation peak at 301 °C) with a loss of mass of $66.3 \pm 0.2\%$. This degradation is associated with the depolymerization of the carbonic chain [41]. The final stage occurs in temperatures above 330 °C and is associated with the formation of inert carbonaceous residues. The degradation of the analyzed starch agrees with the behavior reported for other starches [46,53].

Table 3
Thermal properties of chachafruto starch.

Fasting properties	Value $\pm$ SD
T_o (°C)	60.1 ± 0.6
T_p (°C)	71.3 ± 0.5
T_c (°C)	76.4 ± 0.9
ΔH (J g ⁻¹)	11.5 ± 1.8

T_o : onset temperature; T_p : peak temperature; T_c : conclusion temperature; ΔH : enthalpy of gelatinization. Values are expressed as means $\pm$ standard deviation of at least three repetitions.

3.7. Potential as food packaging of CHS

The chachafruto starch film (CHSF) exhibited peaks at 17, 20, and 22° at diffraction angles of 2θ , which indicates a partial loss of crystallinity due to the heating of the starch that generates the disruption of the granules (Fig. 3C). However, the reorganization of amylose and amylopectin chains in retrogradation provided a relative crystallinity value of $8.2 \pm 0.4\%$. The presence of an organized structure can positively impact the increasing barrier properties of the films, due to the increase in the organization of the structure, which will make the passage of water molecules more tortuous [23]. The crystallinity value observed for the film analyzed in this work is similar to that obtained for sweet potato starch films [11].

The CHSF and glycerol FTIR spectra are shown in Fig. 2D. Similar to native starch, CHSF presented bands at 3400 cm^{-1} , 2900 cm^{-1} , 1650 cm^{-1} , and 970 cm^{-1} . However, the signal at 1625 cm^{-1} in CHS had a slight shift towards 1618 cm^{-1} with an increase in peak intensity, which is related to an increase in the amorphous character of the film. In addition, a shift of the band corresponding to the stretching of the free hydroxyl groups towards a lower wavelength was observed, which can be associated with hydrogen interactions, as reported by Mantovan et al. [34]. The band at 3400 cm^{-1} also presented an intermediate intensity between that observed for glycerol and CHS, associated with the decrease in free hydroxyl groups by the interaction between glycerol and native starch and the reduction of the moisture content in the film analyzed. Similar observations have been made for films prepared with arrowroot starch, glycerol, and PLA [55]. The CHSF results agree with those obtained in films prepared using non-conventional starches from different sources [23,56].

Regarding the thermal stability of the CHSF, the decomposition occurred in four stages with a total loss of mass of $59.1 \pm 0.3\%$. The first stage ranged from 55 °C to 128 °C related to the loss of water with a loss of mass of $10.3 \pm 0.5\%$. The second, ranging from 203 °C to 262 °C , is associated with the decomposition of glycerol used as a plasticizer (Fig. 2F) and exhibited a mass loss of $9.34 \pm 1.01\%$. The third stage occurred between 275 °C and 326 °C and showed the highest weight loss of the analyzed material (64.1%), which is related to the depolymerization of carbonic chains. As in the degradation of pure starch, inert carbonaceous residue formation occurs at temperatures above 330 °C . Considering that the maximum degradation peak of the starch and the biodegradable film occurs at temperatures above 200 °C , it can be established that both materials exhibit high thermal stability [57].

The film obtained using CHS exhibited a continuous surface and cross-sectional without porosities, which could be related to the homogeneity of the material's interaction (Fig. 3). However, some roughness can be observed on the film surface, which can be attributed to the production method (casting method). The presence of continuous surfaces in the films has been reported as a desirable feature in the production of packaging materials as it helps to improve the barrier properties of the materials [58]. With regard to the properties of affinity for water, the CHSF showed values of swelling power (SP) and solubility (S) of $101 \pm 1\%$ and $18.1 \pm 0.9\%$, respectively. The S is associated with the ability of films to resist degradation and loss of structure in an aqueous environment. CHSF S were lower than those observed in films prepared using chitosan mixed with cassava and potato starch [59] and similar to the values for ulluco starch [23]. The SP is the ability of a film to retain water in its structure, which can be improved by the presence of hydrophilic groups [20]. The SP was higher than that observed for films obtained from arrowroot starch and Polylactic acid (PLA) [55] and similar to those reported for ulluco-based films [23].

The opacity of packaging materials is important in relation to their acceptability and the perception of the consumer. Thus, this parameter can define the final use of a material [56]. The CHSF showed an opacity value of 0.729 ± 0.05 , demonstrating the high transparency of the material. The results obtained for the analyzed samples confirm that the films prepared with starch are highly transparent [12]; however, the

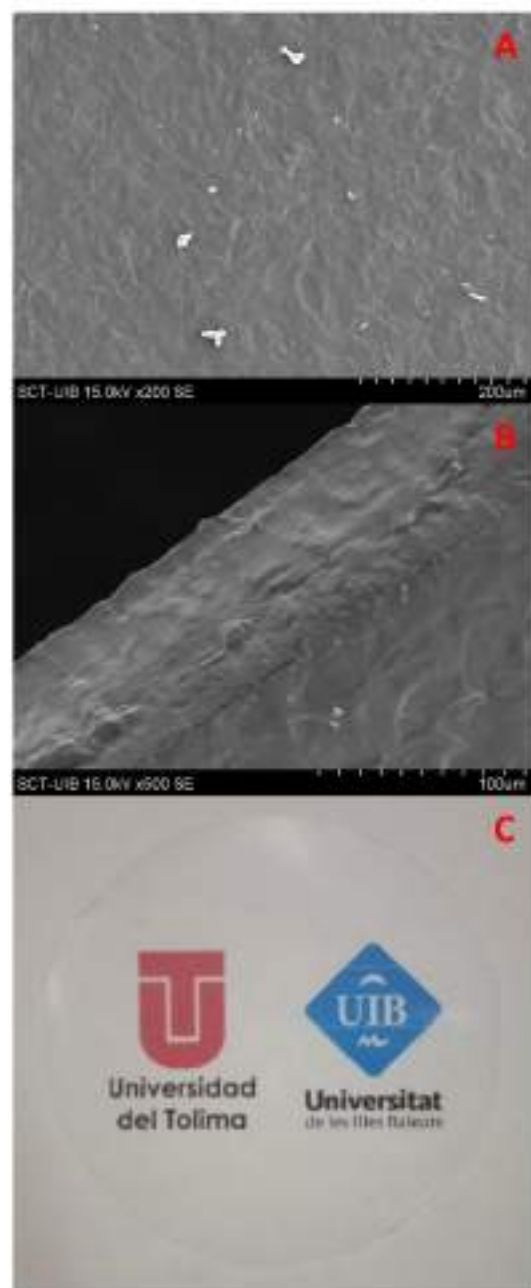


Fig. 3. SEM micrograph of surface (x 200) (A), cross-section (x 500) (B), and photograph (C) of chachaluto starch (CHS)-based film (prepared with 2 % of CHS w/v).

mixture of starch with another type of material, such as gums, proteins, and other polysaccharides, can reduce transparency [30].

The water vapor permeability (WVP) value of the CHSF was $2.3 \times 10^{-9} \pm 0.8 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$. This result is in accordance with those obtained for films prepared using cabio, pumpkin, lentil, and quinoa starches [1,13]. However, the WVP obtained was lower than those obtained using the same starch mixed with different concentrations of commercial oil [9]. The difference between the permeability of films obtained with starch extracted from the same species may be due to the amylose content, since a greater amount of amylose can help rearrange the chains after retrogradation and cause a consequent increase in the final crystallinity of the film [13]. The relationship between film crystallinity and permeability has been demonstrated in previous studies [1].

The CHSF showed elongation at break (EB), tensile strength (TS), and Young Modulus (YM) values of $40.1 \pm 2.65 \%$, $3.68 \pm 0.57 \text{ MPa}$, and $21.9 \pm 3.4 \text{ MPa}$, respectively. The EB and TS results were higher than those reported for films prepared with starch of the same species and different commercial oil concentrations [9]. The differences observed may be due to the different concentrations of starch and plasticizer in the formulations, but mainly to the presence of higher amylose content in the starch analyzed in this work, which, as mentioned above, produces a greater ordering of the amylose/amylopectin chains after retrogradation. Additionally, the analyzed sample presented a higher EB than the potato, pumpkin, lentil, and quinoa starch films but a lower tensile strength [13].

3.B. Potential as food ink for 3D printing of CHS

3.B.1. Printability and morphology of 3D printing of CHS

The appearance and the microstructure of the 3D-printed pastes based on CHS are shown in Fig. 4. All concentrations of starch allowed good printability and produced stable structures. Despite this, it was observed that the lowest concentration of starch (3D printed at a CHS concentration of 8 %; CHS3D1) produced a structure with rounded corners but with well-defined and smooth lines. Meanwhile, increased starch concentration in the gels (3D printed at a CHS concentration of 10 %; CHS3D2, and 3D printed at a CHS concentration of 12 %; CHS3D3) produced a structure with regular corners but with irregularities on the four sides of the geometrical form (lines with some indentations). The ease of printability with the lower concentration gel paste can be associated with its lower viscosity, due to the lower concentration and, therefore, less interaction between amylose chains, amylopectin, and water molecules. Similar behavior was observed in 3D-printed oxidized maize starch at different concentrations (11 % to 19 % of starch) [30]. The authors reported that the best 3D prints were achieved at 11 % and 13 % starch concentrations; however, higher concentrations generated print defects possibly related to greater interaction between oxidized starch and water molecules. In addition, Liu et al. [21] showed that a low viscosity in potato starch gels improved printability but negatively affected the stability of 3D prints because the structure of the first layer was not strong enough to support subsequent layers and could collapse. The printability and degree of printing accuracy of the tested materials can affect consumer perception. In the food production process, low printing precision and sample collapse due to the low stability of the product can generate rejection by the consumer. Because of the low degree of gelatinization of CHS3D3, defects such as the structure collapse of the printed food could be caused by itself or in combination with other ingredients. On the other hand, CHS3D1 and CHS3D2 would present a greater resistance and printability.

With regard to the microstructure, it was observed that an increase in the starch concentration produced structures with a smaller pore size (higher cell density). In comparison, the lower concentration of CHS produced a larger pore size (lower cell density). Cells in the structure suggest that the gels and the 3D-printed geometries were formed under

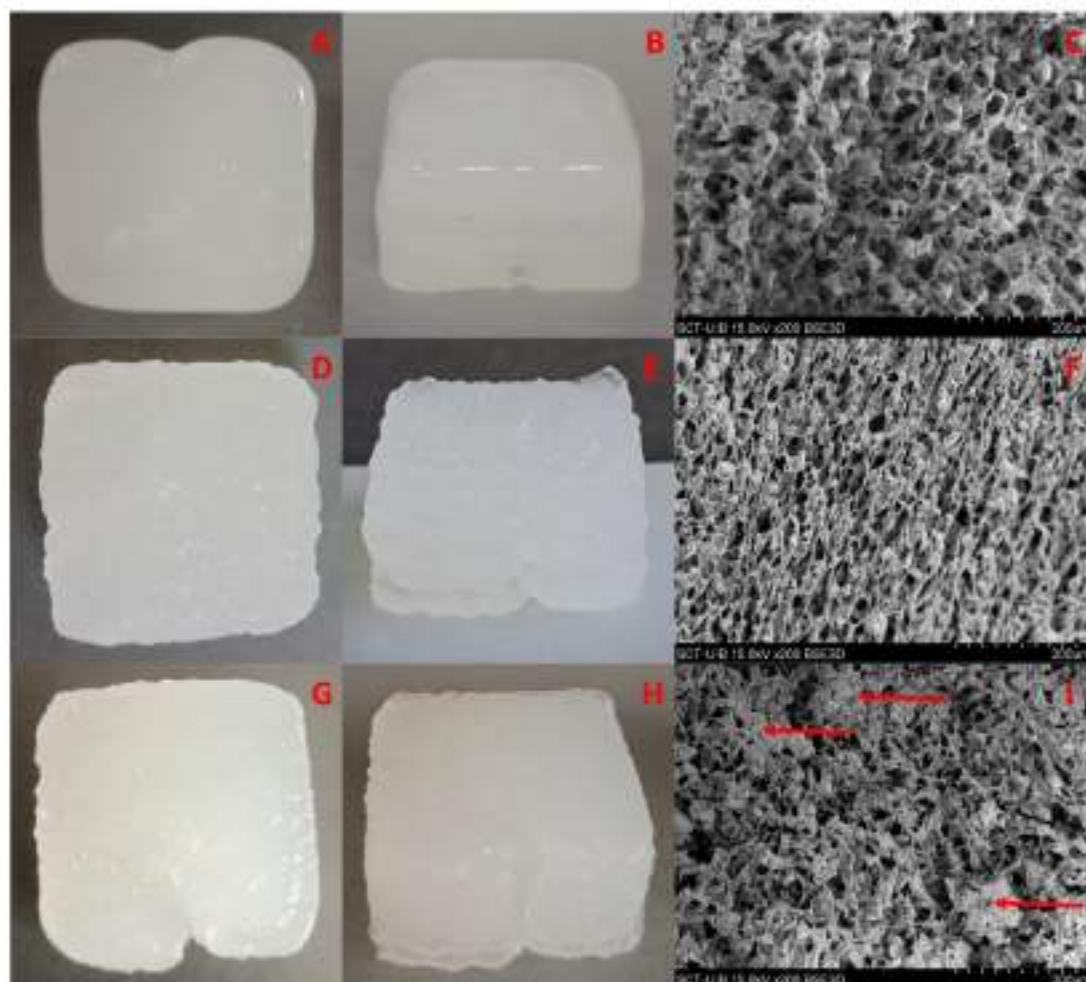


Fig. 4. Top, front view (photographs) and SEM micrographs of the structure of 3D prints based on chachafruto starch (CHS) prepared at different starch concentrations: 8 % (A–C), 10 % (D–F), and 12 % (G–I).

conditions that enabled CHS to gelatinize fully or partially and form a cross-linked network structure [29]. Despite the observation of network formation in sample CHS3D3, agglomeration of material could be observed due to the low degree of gelatinization, which may be because of the high concentration of starch and the short processing time (red arrows, Fig. 4(F)). The starch granules used for 3D printing undergo different degrees of gelatinization depending on their properties and the process parameters. These parameters include gelatinization time, gelatinization enthalpy, amylose content, and process temperature. Previous studies have demonstrated that the process temperature used for the gelatinization of corn starch affected the structure of the 3D prints [61]. At low temperatures ($<70^{\circ}\text{C}$ and a gelatinization time of 15 min), the starch granules do not gelatinize sufficiently to form a cross-linked network structure. Meanwhile, high temperatures ($>80^{\circ}\text{C}$ and a gelatinization time <15 min) generated greater leaching of short chains in the retrogradation process, which have a lower capacity for interaction, leading to the formation of structures with microfractures. The formation of cross-linked network structures has also been

described in 3D prints from potato and corn starches [30,29]. These structures were associated with 3D print properties such as water retention capacity and porosity. The authors found that lower starch concentrations produced more uniform network structures, enhancing water absorption. A higher starch concentration leads to denser networks related to the aggregation and entanglement of molecular chains. Although higher starch concentrations can form a more robust network, this also increases the fluid viscosity, hindering the sample flow through the nozzle and affecting printability. High starch concentrations, as in the case of CHS3D3, can generate this type of defect in the printed products.

3.8.2. Crystallinity of 3D printing of CHS

The 3D prints showed a crystallinity pattern similar to the CHS with signals at 5.6° , 15° , 17° , 20° , 22° , and 24° at diffraction angles of 2θ (Fig. 5). The crystallinity values of the samples were 3.4 %, 3.5 % and 5.6 % for CHS3D1, CHS3D2, and CHS3D3, respectively. The reduction of crystallinity compared with the native starch is due to the partial loss of the

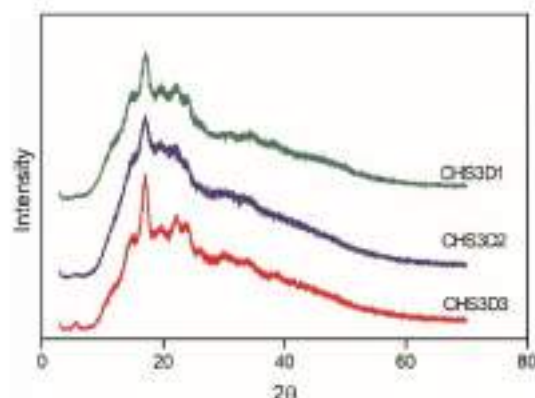


Fig. 5. XRD diffractograms of 3D prints based on chachafruto starch (CHS) prepared at different starch concentrations: 8 % (CHS3D1), 10 % (CHS3D2), and 12 % (CHS3D3).

organized structure because of a partial gelatinization of the starch. The differences between the crystallinity values of the prints may be because the temperature and time used in the preparation of the gels were insufficient for a total gelatinization of the starch. The decrease in the intensity of the peaks could be evidence of this. Similar observations were made for the crystallinity of 3D prints prepared with corn and potato starch [39,62]. In both cases, a decrease in crystallinity was observed after gelatinization and the printing of the gels. In the case of 3D prints prepared with corn starch, a gelatinization temperature of 90 °C was used for 25 min, achieving a partial decrease in crystallinity. However, the remaining crystallinity of the material prepared with the highest starch concentration, was associated with the re-crystallization of the starch chains during retrogradation. On the other hand, in the preparation of 3D prints obtained from potato starch, it was observed that both the starch concentration and the temperature affected the final value of crystallinity. Low starch concentrations (10 %) at an intermediate temperature (70 °C) led to the loss of the original crystalline structure. However, low treatment temperatures (<70 °C) with intermediate concentrations of starch (15 %) partially retained the original crystalline structure.

3.3.3. Textural properties of the 3D printing of CHS

Texture analysis can be related to sensory acceptability, as well as to the printability and overall stability of the product. Thus, a product that is poorly printed will exhibit poor textural properties. In addition, these textural properties are an essential sensory attribute of food acceptability [63]. The hardness of the samples analyzed was 2.1 ± 0.2 N (21.3 $\pm$ 2.3 g), 2.3 ± 0.4 N (23.1 $\pm$ 4.1 g), and 0.93 ± 0.02 N (9.6 $\pm$ 3 g) for CHS3D1, CHS3D2, and CHS3D3, respectively. Statistical differences were observed between CHS3D3 and samples CHS3D1 and CHS3D2 ($p < 0.05$). The low degree of hardness shown by the sample prepared with the highest starch content may be associated with the low degree of starch gelatinization, which limited the formation of the crosslinked network in the gel and subsequently in the 3D print. These results can be associated to the microstructure results where the deficient formation of the cells in the material is observed. The hardness values presented by samples CHS3D1 and CHS3D2 may be related to a greater starch gelatinization, leading to its retrogradation, and forming a more organized cross-linked network. Since the hardness of the samples is associated with the force necessary to deform them, it was observed that samples CHS3D1 and CHS3D2 showed an excellent ability to retain their initial shape after being subjected to a compressive force, in comparison with samples prepared from potato (105 g), wheat (367 g) and corn starches

(105 g) [18,39,64]. According to Zheng et al. [26], starch synthesis and subsequent recrystallization of amylose and amylopectin were the main factors affecting the firmness of 3D prints from wheat starch.

Springiness is related to the elasticity of the samples and their ability to return to their original shape after being subjected to initial compression [18]. The values obtained for the samples analyzed were 0.92 ± 0.01 , 0.91 ± 0.01 , and 0.75 ± 0.01 for CHS3D1, CHS3D2, and CHS3D3, respectively ($p < 0.05$). Similar to the hardness values, the sample with the highest starch content (CHS3D3) presented the lowest values of springiness, which can be associated with the degree of gelatinization, leading in turn to low water retention. The samples obtained with CHS showed higher springiness values than those reported for samples printed from cassava, wheat, corn, sweet potato, potato, and buckwheat starches [17] and similar to those reported for native and annealed wheat starch [18].

Cohesiveness is a parameter related to the strength of the internal bonds of the sample and the degree to which they can be deformed before the break. Cohesiveness showed a similar trend to springiness and hardness, with values of 0.88 ± 0.10 , 0.59 ± 0.07 , 0.59 ± 0.03 for CHS3D1, CHS3D2, and CHS3D3, respectively ($p < 0.05$). As explained above, the partial gelatinization of starch in sample CHS3D3 generated a low release of amylose and amylopectin, affecting the network structure's generation and reducing the sample's cohesiveness. Samples CHS3D1 and CHS3D2 showed cohesiveness values comparable to those reported for 3D printing from potato, corn, and wheat starch [19] and annealed wheat starch [18]. This indicates a high consistency of the samples obtained with low and medium CHS concentrations.

3.3.4. Dynamic mechanical analysis of the 3D printing of CHS

The behavior of the storage modulus (G') and the loss modulus (G'') of the 3D samples is presented in Fig. 6. In all the samples, the value of G' increases as the frequency value increases. In contrast, the value of G'' tends to increase but then decreases. This behavior agrees with that reported for 3D gels printed using potato starch [15]. Additionally, it was observed that the value of G' was greater than G'' throughout the analysis, indicating that the samples presented a predominantly elastic character. This can be related to the ability of the material to maintain its shape [16]. Similar observations were made for gels used for 3D printing prepared from cassava, corn, wheat, potato, and buckwheat starches [17]. Among the samples evaluated, sample CHS3D1 presents the highest value of G' , followed by sample CHS3D2 and sample CHS3D3. These results corroborate the observations made in the texture analysis for hardness. As previously mentioned, the sample with the highest starch content presented low gelatinization, negatively impacting the material's microstructure and, therefore, the dynamic-mechanical properties. On the other hand, the values of G' in the samples CHS3D1 and CHS3D2 can be related to a greater gelatinization and, therefore, a greater release of amylose [17]. However, the difference between these two samples may be due to cell structure and packing.

4. Conclusions

The native starch from chachafruto has a high extraction yield and low lipids, proteins, and ashes content. These characteristics indicate a high purity level and potential for consumer acceptance. The wet extraction method preserves the morphology of the starch granules without affecting their integrity. The technological properties of the material, such as texture and printing properties, are influenced by its small particle size. The apparent amylose content of this starch was another factor that determined its physicochemical properties and industrial potential. The X-ray diffraction analysis revealed that the starch presents the typical characteristics obtained from legumes. Moreover, the thermal analysis indicated that the starch has excellent thermal stability, making it desirable for industrial use. Regarding the feasibility of producing food packaging, the chachafruto starch exhibited good mechanical, thermal, and barrier characteristics. These properties and

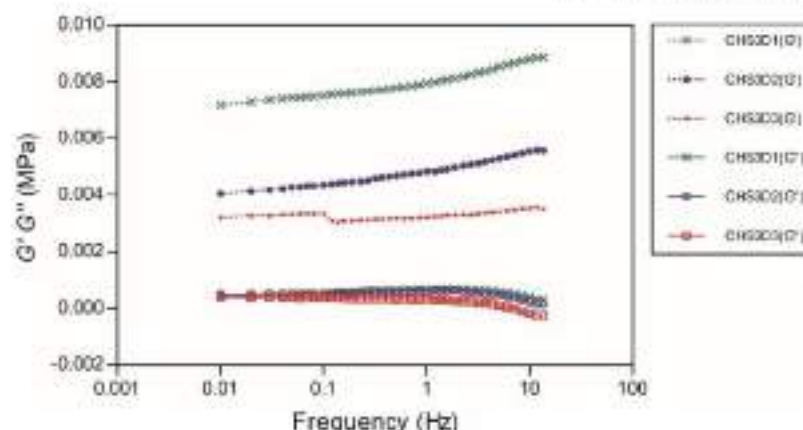


Fig. 6. Storage Modulus (G') and Loss Modulus (G'') curves of 3D prints based on chachafruto starch (CHS) prepared at different starch concentrations: 8 % (CHS08), 10 % (CHS10), and 12 % (CHS12).

their affinity for water suggested that chachafruto starch-based films could suit food matrices with intermediate moisture contents. In addition, the high transparency of the starch-based films made them a potential material for products that are not very susceptible to oxidation and for products that require visualization by the consumer. Starch concentration affected the physical properties of the 3D prints under the process parameters used in this study. Although low-quality 3D prints were produced at the highest starch concentration, at the other concentrations chachafruto starch was a suitable ingredient for 3D printing food, as it showed good printability, integrity, texture, and microstructure. Further research should focus on optimizing the printing process; exploring the interaction of chachafruto starch with other ingredients and its impact on film preparation and 3D printing; as well as a complementary characterization that could determine the molecular distribution and lamellar ordering of the amylose and amylopectin chains within the granule. Considering these findings, chachafruto emerged as an unconventional source of starch with tremendous industrial potential; this study demonstrated viable strategies for adding value to chachafruto crops.

CRediT authorship contribution statement

Luis Daniel Daza: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Visualization. **Miguel Ángel Montalegre:** Formal analysis, Writing – original draft. **Cristina Roche:** Formal analysis, Writing – original draft. **Angélica Sandoval Aldana:** Conceptualization, Funding acquisition, Supervision, Writing – original draft. **Valeria Soledad Elmi:** Conceptualization, Funding acquisition, Supervision, Writing – original draft. **Project administration.** **Henry Alexander Viqueiro:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 3

Annealing treatment of ulluco starch: Effect of moisture content and time on the physicochemical properties

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Annealing treatment of ulluco starch: Effect of moisture content and time on the physicochemical properties

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Abstract

Annealing is a modification method that can improve the physicochemical properties of starch. The effect of moisture content (MC) and time (t) on the physicochemical properties of ulluco starch obtained by annealing (ANN) modification was studied. The results showed the ulluco-modified starches improved physicochemical properties such as solubility, water-holding capacity (WHC), oil-holding capacity (OHC), pasting properties, and the texture of the gel formed. Furthermore, the ordered structure and granules shape of starches were not significantly affected by the ANN modification. Regarding the influence of the process parameters on the properties of the modified starches, MC was the one that influenced the most, followed by t. The ANN treatment applied to ulluco starch proved to be an efficient, low cost, and green method for improving its physicochemical properties. This study showed that the native and physically modified ulluco starch is an industrial alternative to starches obtained from traditional crops, contributing to food security.

Novelty impact statement: Annealing is a physical method used to modify starch that can improve the physicochemical characteristics of the material. It is also a low-cost and environmentally friendly method. The modification of starches is an alternative to add value to different crops.

1 | INTRODUCTION

Ulluco (*Ullucus tuberosus* Caldas), the only species of the monotypic genus *Ullucus* from the Basellaceae family, is an Andean tuber usually cultivated around 2,800 m.a.s.l. (Cejudo-Bastante et al., 2014). The tubers showed smooth and waxy skins with spherical (between 2 and 10 cm diameter) or curved and elongated shapes (between 2 and 15 cm in length). It has a wide range of bright colors on the skin, including white, yellow, orange, red, magenta, and green. In South America, its sowing and harvesting occur throughout the year, mainly in rainy seasons (Parra-Quijano et al., 2012). Its use is currently limited to consumption in nature, as an ingredient in preparing typical dishes, by the local population due to their nutritional composition, mainly its high starch content (~65% dry basis). However, its limited use and industrial exploitation result from the lack of information about its technological potential. As a result of the above, in recent

years, the interest in this crop has increased. Various studies have demonstrated their biological potential due to secondary metabolites, mainly flavonoids and betalains (Cejudo-Bastante et al., 2014; Pacheco et al., 2019). Nevertheless, the most interesting approach is obtaining native starch, which has demonstrated technological potential for its use in the food industry as raw material for preparing edible films or coatings. Galindez et al. (2019) demonstrated that edible films prepared with ulluco starch showed good mechanical properties, low water vapor permeability, and good stability against thermal degradation, which could be promising for their potential use in the transportation of food in various environmental conditions, these characteristics were associated with the amylose content of the starch (35.3%) (Pérez & Bertoft, 2010; Wang et al., 2014).

Although the starch obtained from ulluco has a high industrial potential for the food or packaging industry, it may show limited functional properties in its native form. Low thermal, shear, and acid stability, and

high retrogradation must be improved for industrial purposes (Wang & Copeland, 2015). In this sense, physical, chemical, and enzymatic modifications appear as an alternative to improving or promoting specific functional properties to the native ulluco starch (Jayakody & Hoover, 2008). Among these methods, the physical modifications have generated interest both in the industry for its low cost, as in the consumer because it is more natural complying with the "clean label" trend that seeks chemical-free ingredients, safe for consumption, and obtained by environmentally friendly methods (Samarasekera et al., 2020). Modifications can also increase starch utilization in nonfood industries, such as textiles, paper, and pharmaceuticals (Zavareze & Dias, 2011).

Annealing (ANN) is one of the most widely used physical modification technique since it changes starch physicochemical properties without destroying the granular structure (Sudheesh et al., 2019). ANN process leads to a reorganization of starch molecules and amylopectin double helices such that the structure acquires a more organized configuration (Zavareze & Dias, 2011). ANN takes place with excess (> 60%) or intermediate (> 40%) moisture contents at a temperature above the glass transition but below the gelatinization temperature of starch (Fonseca et al., 2021; Wang et al., 2018). Various studies have been focused on the effect of these treatments on the physicochemical properties of starches from diverse botanical sources (Ji et al., 2019; Sudheesh et al., 2019; Wang et al., 2018). The findings of these investigations demonstrated the importance of selecting temperature and moisture content in the annealing modification process since the optimal conditions differ according to the starch source species. In addition, annealing as a method of modifying starch has improved pasting properties mainly by reducing the breakdown of the modified Kithul palm starch, which is a sign of an increase in the stability of the starch showing better performance than acetylation and oxidation modification (Sudheesh et al., 2019). Besides, Wang et al. (2018) demonstrated that the annealing modification significantly improved the swelling power, solubility, and gel texture of rice starch. In this sense, the annealing modification is emerging as an alternative to improve the physicochemical properties of native ulluco starch and to add value to this crop by using its modified starch for industrial applications. In this way, it expands botanical sources and contributes to food security due to the overuse of traditional corn, potato, and rice starches. However, there is no literature related to the modification of this material by the annealing method. Therefore, there is a lack of knowledge of the appropriate conditions for this modification in ulluco starch. Based on the above considerations, this work aims to evaluate the effects of the moisture content (MC) and time (t) on the physical properties of ulluco starch modified by the annealing method.

2 | MATERIALS AND METHODS

2.1 | Ulluco starch extraction

Ulluco was acquired at a local market in the city of Ibagué (Colombia). The native ulluco starch was extracted according to the methodology described by Valcárcel-Yamani et al. (2013).

2.2 | Composition of the native ulluco starch

The native ulluco starch was analyzed to determine its lipid (Method 920.85), moisture (Method 925.101, and ash (Method 923.03) contents according to the Association of Official Analytical Chemists methods (AOAC, 1995). The amylose content was determined using the method reported by Hoover and Ratnayake (2001).

2.3 | Physical modification by annealing (ANN)

The annealing (ANN) modification of the ulluco starch was carried out following the methodology described by Wang et al. (2018) with some modifications. In brief, the native ulluco starch moisture content was adjusted to values between 50% and 70% (Table 1) according to preliminary analysis (data not shown) and stored at 4°C for 12 hr to ensure uniformity. According to preliminary analysis, the samples were heated at 55°C (this temperature was chosen according to the T_g and the gelatinization temperature of the native ulluco starch, data not shown) for times ranging between 8 hr and 24 hr. The starches obtained from these treatments were dried in an oven with forced air convection (Thermolab, DfEs, Antioquia, Colombia) at 35°C until reaching a moisture content less than or equal 30%. Starches were milled and stored in a desiccator cabinet (DH-1002, Aquealab, Colombia) at a relative humidity of 57% and a room temperature (<25°C) until the analyses. Each treatment was prepared in triplicate.

2.4 | Morphology of starch

The morphological characteristics of the native ulluco starch and annealed starch were examined by scanning electron microscopy (SEM; Quanta 650 FEG, FEI Company, Hillsboro, OR, USA). The shape of starch granules was analyzed. The samples were placed on stubs using double-sided carbon tape and covered with a gold layer using a rotary-pump coating system (Q150R, Quorum, Lewes, UK). Images were obtained at an accelerating voltage of 15 kV.

2.5 | Solubility (S)

One gram (w_0 , d.b.) of the samples was mixed with 25 ml of distilled water in previously preweighed centrifuge tubes. The mixture was heated at 95°C for 30 min in a water bath. Then, the suspension was cooled at room temperature and centrifuged at 4,000g for 20 min. The supernatant was transferred and dried in capsules at 105°C until constant weight (w_1). The solubility (S, %) was calculated using Equation (1) (Li & Yeh, 2001).

$$S = (w_1/w_0) \times 100 \quad (1)$$

TABLE 1 Experimental design and physicochemical properties of native uluco and annealed uluco starches

Sample	t (h)	MC (%)	S (%)	WHC (%)	OHC (%)	WI	Gel hardness (N)	Ratio 1.047/1.022 cm ⁻¹
T1	8	50	2.77 ^{a,de} ± 0.20	56.3 ^{de} ± 1.2	47.7 ^{bc} ± 2.2	90.9 ^d ± 0.2	1.67 ^{de} ± 0.17	0.96 ^d ± 0.00
T2	16	50	2.53 ^{a,de} ± 0.45	57.0 ^{de} ± 0.0	50.2 ^{abc} ± 0.9	91.0 ^d ± 0.1	2.03 ^{de} ± 0.53	0.97 ^d ± 0.00
T3	24	50	1.98 ^{a,de} ± 0.11	57.5 ^{de} ± 0.3	51.6 ^a ± 1.2	91.3 ^{de} ± 0.1	2.21 ^{bc,de} ± 0.23	0.98 ^d ± 0.01
T4	10	53	2.92 ^{a,d} ± 0.47	62.1 ^{ab} ± 0.6	49.6 ^{abc} ± 1.4	91.1 ^{de} ± 0.0	2.01 ^{de} ± 0.28	0.97 ^d ± 0.00
T5	22	53	5.49 ^{b,b} ± 0.75	61.2 ^{ab} ± 0.2	49.6 ^{abc} ± 0.6	91.3 ^{de} ± 0.1	2.35 ^{bc,d} ± 0.12	0.97 ^d ± 0.01
T6	8	60	2.15 ^{a,de} ± 0.14	62.7 ^a ± 0.2	47.1 ^a ± 0.9	91.2 ^{de} ± 0.0	1.69 ^{de} ± 0.37	0.97 ^d ± 0.00
T7	16	60	3.25 ^c ± 0.49	58.9 ^{cd} ± 0.9	51.4 ^{ab} ± 1.4	91.5 ^d ± 0.1	2.09 ^{cd,de} ± 0.28	0.99 ^d ± 0.02
T8	24	60	2.26 ^{a,d} ± 0.23	57.6 ^{de} ± 1.2	49.5 ^{abc} ± 1.3	91.3 ^{de} ± 0.1	2.97 ^a ± 0.30	0.96 ^d ± 0.01
T9	10	67	1.83 ^{a,de} ± 0.20	59.3 ^{cd} ± 2.4	48.9 ^{bc} ± 1.3	91.3 ^{de} ± 0.0	2.59 ^{bc} ± 0.36	0.97 ^d ± 0.02
T10	22	67	2.80 ^{a,de} ± 0.28	56.9 ^{de} ± 0.4	48.8 ^{abc} ± 1.5	91.5 ^{d,de} ± 0.0	2.09 ^{cd,de} ± 0.51	0.96 ^d ± 0.01
T11	8	70	6.11 ^b ± 0.58	54.9 ^d ± 0.6	49.3 ^{bc} ± 0.7	91.6 ^c ± 0.0	2.61 ^{ab} ± 0.37	0.95 ^d ± 0.00
T12	16	70	1.80 ^{a,de} ± 0.22	58.5 ^d ± 1.1	51.0 ^{bc} ± 1.8	91.4 ^{de} ± 0.2	2.72 ^{ab} ± 0.24	0.98 ^d ± 0.02
T13	24	70	5.37 ^b ± 0.41	59.0 ^{cd} ± 0.8	51.6 ^{ab} ± 1.4	91.5 ^{d,de} ± 0.1	2.67 ^{ab} ± 0.25	0.97 ^d ± 0.01
Native uluco starch	NA	11	1.23 ^d ± 0.14	48.8 ^d ± 0.6	40.8 ^d ± 0.4	91.7 ^d ± 0.1	1.30 ^d ± 0.23	0.96 ^d ± 0.00
P5	NA	13	4.54 ^b ± 0.45	56.5 ^{de} ± 1.9	35.3 ^d ± 0.9	92.6 ^c ± 0.0	2.39 ^{bc,d} ± 0.42	0.97 ^d ± 0.00

Note: Values are expressed as means ± standard deviation (n = 6). Different letters in the same column indicate significant differences between samples (p < .05).

Abbreviations: MC, moisture content; OHC, oil-holding capacity; S, solubility; SP, swelling power; t, time; WHC, water-holding capacity; WI, white index.

2.6 | Water-holding capacity (WHC) and oil-holding capacity (OHC)

The water-holding capacity (WHC) and oil-holding capacity (OHC) of the samples were analyzed according to the procedure described by Chau et al. (1997) with some modifications. The samples were weighed (W_0) and then mixed in a vortex with distilled water or edible oil in a ratio of 1:10 (w/w) for 4 min. After that, the samples were centrifuged at 3,000g for 25 min, and the wet precipitate was weighed (W_1). The WHC (%) and OHC (%) were calculated using Equations (2) and (3):

$$\text{WHC} = (W_1/W_0) \times 100 \quad (2)$$

$$\text{OHC} = (W_1/W_0) \times 100 \quad (3)$$

2.7 | Gel texture and colorimetric properties

The hardness of the gel formed by the native and modified starches was evaluated according to the methodology described by Han et al. (2011) using a texture analyzer (0.51, Lloyd Ltd., Largo, FL, USA). Results were calculated using the Nexygen Plus Software (Lloyd, Version 3.0) and expressed in Newtons. The colorimetric properties of the powdered starches and their gels were analyzed with a Minolta colorimeter (Cr 410, Konica Minolta, Japan) on the CieLab scale. The results were expressed according to the CieLab color system (L^* : lightness, a^* : redness, and b^* : greenness). The whiteness of the samples was calculated using Equation (4):

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

2.8 | Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy spectra of the samples were obtained using a Fourier-transform infrared spectroscopy (Nicolet 380, Thermo Scientific Inc., Waltham, MA, USA). The measurements were recorded over the range of 4,000 to 500 cm⁻¹, with a resolution of 4 cm⁻¹, and 40 scans at room temperature (20.4). For the analysis, pellets were prepared by mixture and homogenization of the sample and potassium bromide (KBr).

2.9 | Pasting properties

Pasting properties were determined in a Brabender Micro Visco-Amylograph (C.W. Brabender Instrument Inc., South Hackensack, NJ). An aqueous suspension of the starch (10% w/v) was prepared and analyzed with a defined temperature profile starting at 40°C, then increased to 95°C at 1.5°C/min and held for 20 min. Finally, the sample was cooled to 50°C at 1.5°C/min and held for 10 min. Pasting temperature (PT), peak viscosity (PV), breakdown (BD), setback (SB), and final viscosity (FV) were obtained. The viscosity of the starch suspension was reported in Brabender Units (BU) according to the method ISI 19-6e (2000).

2.10 | Experimental design and statistical analysis

Moisture content (MC) and time (*t*) were combined using a central composite design to evaluate the interaction between these factors with a reduced number of treatments, compared to a full factorial design. A design matrix with 13 treatments was obtained, combining MC and *t* levels between 50% and 70%, and 8 hr and 24 hr, respectively. Also, control treatments of native ulluco starch without physical modification and potato starch (PS) were included. PS was used as a control because it is one of the most industrially used starches due to its technological characteristics. One-way analysis of variance (ANOVA) at the 95% confidence level was used to identify statistically significant differences between the mean results associated with each property and treatment. Also, only the data that include main effects and interactions at all levels (nine treatments) were subjected to a two-way ANOVA to test the effect of the factors on the mean of the physicochemical properties of modified ulluco starch. Multiple range tests (MRTs), using the Tukey–Kramer range test, were used to determine which mean results were significantly different.

3 | RESULTS AND DISCUSSION

3.1 | Extraction and composition of native ulluco starch

The native ulluco starch extraction yield was $7.33 \pm 0.98\%$ (wet basis; or 43.2% d.b.). The native ulluco starch showed low lipid content $0.11 \pm 0.01\%$, ash content $0.12 \pm 0.02\%$, and MC of $11.0 \pm 1.0\%$, which can be associated with a high purity extraction product. These components are considered impurities due to the limited ability to bind covalently to the polysaccharides making up the starch granules, affecting the starch's technological performance (de Almeida et al., 2020). These results agree with the values reported by various Andean tubers (Cruz et al., 2016). The native ulluco starch showed an amylose content of $27.9 \pm 2.0\%$, similar to the value obtained for commercial PS ($27.3 \pm 0.3\%$). The amylose content from native ulluco starch obtained in this work was similar to the results for ulluco starch reported by Valcárcel-Yamani et al. (2013). However, it differed from the results reported by Cruz et al. (2016) and Galíndez et al. (2019). The agroecological crop conditions can explain the variation in amylose content in the same botanical source. Besides, native ulluco starch showed higher amylose content than commercial maize starch (Wang et al., 2014) and rice starch (Yang et al., 2021).

High amylose content in starch is known to form solid and flexible films widely used in food and pharmaceutical industries (Tejavathi et al., 2020). It has been reported that amylose content provides heterogeneity and increases the gelatinization temperature and starch retrogradation which may negatively affect the starch quality. Higher starch amylose content is correlated with structural and physical properties of gels, pastes, and stronger resistance to enzymes (Yang et al., 2021). Thus, the composition of starch in terms

of amylose and amylopectin ratio is essential in determining its functional properties.

3.2 | Morphology of starch

The SEM micrographs of native ulluco starch and modified starches are shown in Figure 1. The native ulluco starch granules showed mainly ellipsoid, oval smooth, and irregular morphologies similar to previous reports for the same tuber species (Galíndez et al., 2019; Valcárcel-Yamani et al., 2013). In general, the ANN starches appear to have retained the same shape as native ulluco starch. However, in the modified starches, the surfaces showed noticeable dents (Figure 1b–f, red arrows). This phenomenon can be explained since during the annealing process, the starch granules adhered to each other, thus generating dents on the surfaces of the adjacent granules. A similar alteration was observed for ANN pea starch (Wang et al., 2013) and ANN potato starch (Xu et al., 2018). There is no effect of MC or *t* on the morphology of the modified starches.

3.3 | Solubility (S)

Solubility is an important functional property of starch in determining its use in food processing and other industrial applications. An increase in native ulluco starch solubility means that it can be used in various food and nonfood matrices since its native form is insoluble at low temperatures, limiting its use. Modification of ulluco starch numerically improved the solubility of all treatments compared to native ulluco starch (Table 1). Furthermore, a significant increase regarding native ulluco starch in solubility values was observed in T5, T7, T11, and T13, with values of 5.49%, 3.25%, 6.11, and 5.37%, respectively. The samples showed the highest values of *S* were mainly influenced by the combination of the MC and *t* ($p < .05$) (Table 3). Compared to commercial PS, a significant increase in solubility value was observed only in T11 ($p < .05$). The increase in solubility agreed with the results obtained for starches modified by annealing for acorn (Molavi & Razavi, 2018), potato (Xu et al., 2018) and sweet potato (Trung et al., 2017). The authors attribute this behavior to weak interactions formed by the starch's functional groups during heat treatment. However, the results contradict other authors, who reported that solubility is reduced in the starch after annealing due to the strengthening of the bonds between amylose and amylopectin or between amylopectin molecules, avoiding leaching out of granular components (Devil, 2019).

3.4 | WHC and OHC

The WHC and OHC of native and modified starches are presented in Table 1. In all cases, the annealed process improved significantly the WHC of the native ulluco starch ($p < .05$), ranging between 54.9% and 62.7% for T11 and T6, respectively. However, no differences

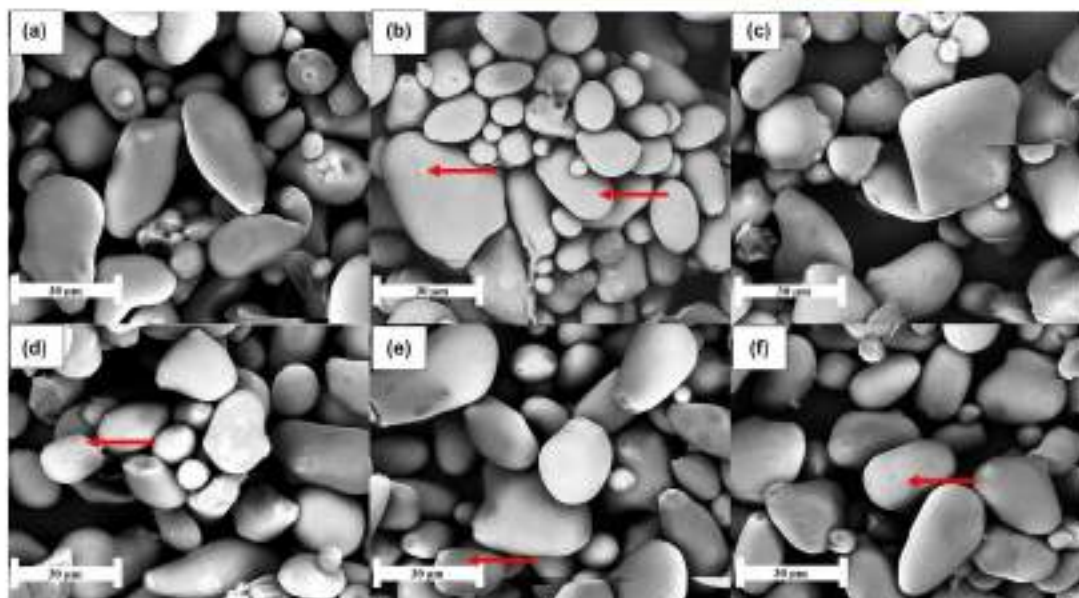


FIGURE 1 Micrographs of native ulluco starch (a) and annealing starch obtaining at different parameter conditions: T1 (8 hr and 50% MC) (b), T5 (22 hr and 53% MC) (c), T6 (24 hr and 60% MC) (d), T11 (8 hr and 70% MC) (e) and T13 (24 hr and 70% MC) (f) at magnification of 3,000x

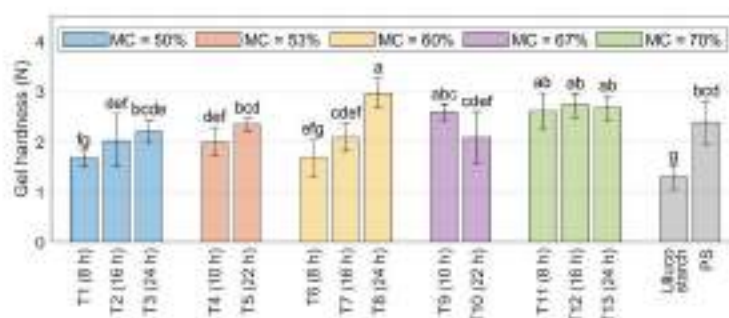


FIGURE 2 The gel texture of native ulluco starch, annealing ulluco starch, and potato starch (PS)

were observed between the treatments. In comparison to PS, except for T1 and T11, all treatments showed higher WHC values. About the influence of the factors evaluated, the combination of MC and *t* showed the more significant influence in the WHC parameter (Table 3), which means treatments with higher MC and shorter *t* or lower MC and long *t* process presented the highest values of WHC. The high WHC values shown by the treatments T6 and T4 can be associated with short annealing times which prevent an efficient reorganization of the starch molecules, allowing the presence of a greater number of amorphous regions, which are related to the swollen of granules at lower temperatures (Xu et al., 2018). The ability to absorb water or oil is an important property concerning beverage applications, bakery, and other products since it indicates

the amount of water available in the gelatinization processes (Ignat-Gutiérrez et al., 2020).

The OHC of modified starches ranged between 47.1% and 51.6% (Table 1). All samples showed an improvement in OHC compared with native ulluco starch (40.8%) and PS (35.3%) ($p < .05$). This functional property is important when the starch will be incorporated in a food formulation that includes oil or fat since it can indicate the emulsifying potentials of the starch (Astuti et al., 2018). Concerning the factors evaluated, the *t* showed a greater influence on the OHC of modified starches (Table 3), while MC and the combination of MC and *t* did not show a strong influence on the OHC property. In general, it was observed that treatments T4, T11, T9, T1, and T6 with short annealing times (8 and

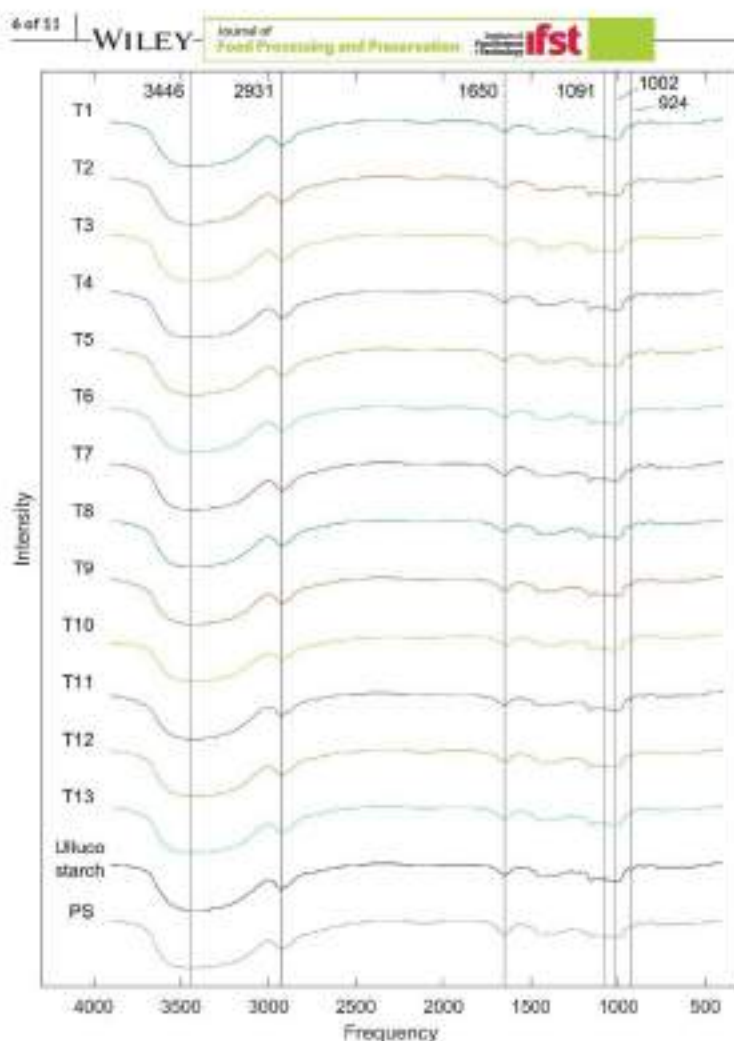


FIGURE 3 FTIR spectra of native uluco starch, annealing uluco starch, and potato starch (PS)

10 hr) presented the lowest values of OHC, while treatments T3 and T13 with the longest process times showed the highest values of OHC.

3.5 | Gel texture and colorimetric properties

A valuable quality in food applications is the starch ability to easily form a gel when heated, especially in food products like jams and ice cream (Wang et al., 2020). The gel texture and colorimetric properties of all the samples are summarized in Table 1. In all cases, the gel hardness was higher than the values obtained for native uluco starch (Figure 2). Also, T8, T12, T13, T11, and T9 showed higher gel hardness than commercial PS. Improvement of the native uluco starch gel hardness after modification by ANN is caused by the rearrangement of starch molecules (Chung et al., 2000) which can be associated with an increase in mobility of the amorphous part facilitates the ordering

of double helices and probably greater ordering in that region, resulting in more interaction and, therefore, gel hardness (Zavareze & Dias, 2011). In addition, the increase in gel hardness can be associated with the FV, since the amylose molecules leaching in the gelatinization have strong reassociation capabilities.

Regarding process parameters, gel hardness was mainly influenced by the MC (Table 3). In general, the higher the moisture content, the harder the gel, which means that a greater amount of water will facilitate the molecules' mobility and, therefore, their reorganization. The obtained results agree with those reported for rice (Homdok & Nookhorn, 2007) and water chestnut (Yadav et al., 2013) starches modified by annealing treatment.

White index results showed that modified starch presented lower values than native uluco starch and PS (Table 1). However, there were no statistically significant differences, indicating that the conditions of the annealing process used in this work did not strongly affect the starch color. The prediction of the influence of

the evaluated factors on WI indicated that this parameter was mainly influenced by MC, which means that treatments with low MC values tend to decrease WI. Results obtained in this work showed similar behavior to that of rice modified by annealing (Wang et al., 2018). The authors attribute the decrease in WI to the processing temperatures, which can increase the Maillard reaction rate, leading to a reduction in this property.

3.6 | Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy was used to observe if the physical modification of the starch induced changes at the level of functional groups. The Fourier-transform infrared spectral data on ANN starches, native uluco starch, and PS are presented in Figure 3. The spectra of native uluco starch, PS, and all the treatments exhibited a broad band at $3,446\text{ cm}^{-1}$, associated with energy absorption by the free hydroxyl groups (Figure 3). The spectrum also showed a peak at $2,931\text{ cm}^{-1}$ related to the $-\text{CH}_2$ stretching (Waman et al., 2016). A single peak was observed at about $1,650\text{ cm}^{-1}$ corresponding to absorption by water molecules in the amorphous region due to the starch hygroscopic nature (Pozo et al., 2018). The bands at about 924 cm^{-1} and $1,091\text{ cm}^{-1}$ are characteristic of the crystalline starch structure, while the band at about $1,002\text{ cm}^{-1}$ is associated with the amorphous region (He et al., 2020). Also, the IR bands around $1,047$ and $1,022\text{ cm}^{-1}$ are sensitive to changes in the crystalline and amorphous starch regions (Liu et al., 2020).

In this study, ANN did not result in significant changes in the short-range molecular order of native uluco starch. Results showed that FTIR ratios of $1,047/1,022\text{ cm}^{-1}$ were not statistically significant between native uluco starch and annealed starches ($p > .05$) (Table 1). In this sense, we coincide with Wang and Ren (2020) who reported that annealing treatment did not alter the ordered structure of potato and yam starches. Also, Samarakoon et al. (2020) reported slightly increased $1,047/1,022\text{ cm}^{-1}$ ratio in waxy starches after annealing. However, the increase was not significant ($p > .05$), showing that the double-helical arrangement at the granule surface was not affected by the hydothermal treatment. Thus, physical modification as ANN does not alter starch functional groups (Tester & Debon, 2000).

3.7 | Pasting properties

The pasting properties of uluco and potato native starches and modified uluco starches are presented in Table 2 and Figure 4. In comparison to the native uluco starch, all treatments (annealed starches) showed an increase in the PV, SB, and FV properties ($p < .05$). The highest pasting temperature was observed in T8 and T13; this increase in PT is related to an increased temperature necessary for the starch gelatinization and increased resistance to swelling. Xu et al. (2018) reported a similar effect for annealing potato starch and attributed this behavior to a strengthening of the starch granules bonds during the annealing process. Regarding annealed starches, PV ranged between 191 and 343 BU (Table 2). A marked

TABLE 2 Pasting properties of native uluco and annealed uluco starches

Sample	t (h)	MC (%)	PT (°C)	PV (BU)	BD (BU)	SB (BU)	FV (BU)	Th (°C)
T1	8	50	$88.5^a \pm 2.5$	$343^b \pm 24$	$97.0^b \pm 16.9$	$144^{ab} \pm 7$	$357^{ab} \pm 28$	$70.2^b \pm 1.3$
T2	16	50	$92.9^a \pm 2.8$	$290^{ab} \pm 19$	$58.5^{bc} \pm 13.4$	$144^{ab} \pm 2$	$340^{ab} \pm 0$	$72.4^{ab} \pm 0.2$
T3	24	50	$94.6^a \pm 0.7$	$275^{cd} \pm 0$	$43.0^c \pm 1.4$	$143^{ab} \pm 14$	$347^{ab} \pm 5$	$72.4^{ab} \pm 1.6$
T4	10	53	$88.4^a \pm 1.8$	$320^{ab} \pm 3$	$71.0^{bc} \pm 1.4$	$145^{ab} \pm 12$	$375^a \pm 8$	$70.3^b \pm 0.8$
T5	22	53	$90.9^a \pm 2.3$	$261^{cd} \pm 5$	$49.0^{bc} \pm 4.2$	$132^{ab} \pm 2$	$337^{ab} \pm 3$	$71.2^{ab} \pm 0.1$
T6	8	60	$94.8^a \pm 0.3$	$291^{ab} \pm 15$	$62.0^{bc} \pm 12.7$	$137^{ab} \pm 6$	$340^{ab} \pm 4$	$71.6^{ab} \pm 0.5$
T7	16	60	$95.1^a \pm 0.2$	$192^d \pm 24$	$38.5^c \pm 17.7$	$116^{bc} \pm 6$	$264^c \pm 4$	$72.5^{ab} \pm 0.4$
T8	24	60	$94.8^a \pm 0.2$	$288^{ab} \pm 14$	$38.5^c \pm 6.4$	$158^a \pm 16$	$372^{ab} \pm 6$	$74.6^{ab} \pm 0.2$
T9	10	67	$94.9^a \pm 0.3$	$282^{cd} \pm 5$	$44.5^c \pm 0.7$	$147^{ab} \pm 12$	$359^{ab} \pm 2$	$72.6^b \pm 0.2$
T10	22	67	$94.7^a \pm 0.2$	$294^{ab} \pm 7$	$46.0^c \pm 4.2$	$144^{ab} \pm 7$	$375^a \pm 1$	$73.1^{ab} \pm 0.5$
T11	8	70	$92.9^a \pm 3.3$	$191^d \pm 10$	$28.0^c \pm 2.8$	$109^c \pm 5$	$267^c \pm 3$	$73.0^{ab} \pm 0.9$
T12	16	70	$94.9^a \pm 0.1$	$274^{cd} \pm 10$	$45.0^c \pm 9.9$	$135^{ab} \pm 0$	$344^{ab} \pm 2$	$73.7^{ab} \pm 0.8$
T13	24	70	$94.7^a \pm 0.8$	$234^{cd} \pm 1$	$35.7^c \pm 2.9$	$132^{ab} \pm 0$	$324^b \pm 9$	$74.0^a \pm 0.4$
Native uluco starch	NA	11	$92.8^a \pm 2.9$	$108^d \pm 4$	$42.5^c \pm 26.2$	$99.5^c \pm 9.2$	$117^d \pm 43$	$66.0^c \pm 2.1$
PS	NA	13	$70.1^b \pm 0.1$	$619^a \pm 28$	$475.3^a \pm 25.4$	$129^{ab} \pm 7$	$265^a \pm 4$	$65.3^a \pm 0.3$

Note: Values are expressed as means $\pm$ standard deviation ($n = 6$). Different letters in the same column indicate significant differences between samples ($p < .05$).

Abbreviations: BD, breakdown; FV, final viscosity; MC, moisture content; PT, pasting temperature; PV, peak viscosity; SB, setback; t, time; Th, gelatinization temperature.

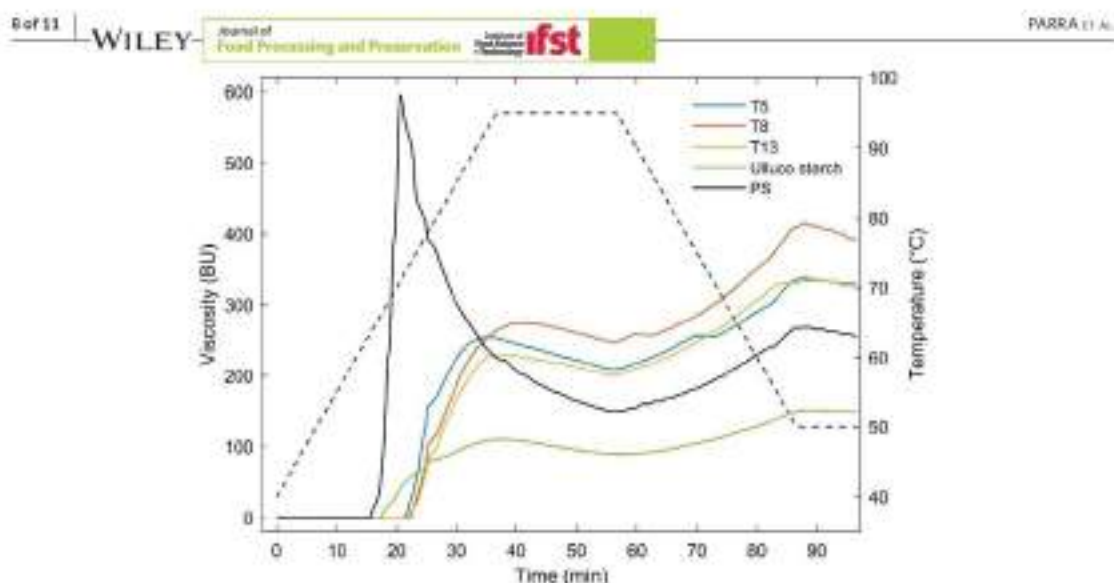


FIGURE 4 Pasting curve of native ulluco starch, annealing ulluco starch, and potato starch (PS)

Parameter	Factor	p-value	Parameter	Factor	p-value
S (%)	MC	3.55×10^{-34}	BD	MC	1.59×10^{-01}
	Time	7.25×10^{-08}		Time	8.76×10^{-03}
	MC × time	2.14×10^{-14}		MC × time	1.18×10^{-01}
WHC (%)	MC	3.09×10^{-06}	SB	MC	6.33×10^{-08}
	Time	8.55×10^{-01}		Time	1.61×10^{-06}
	MC × time	1.35×10^{-02}		MC × time	4.86×10^{-02}
OHC (%)	MC	2.64×10^{-31}	FV	MC	4.07×10^{-34}
	Time	3.11×10^{-04}		Time	5.99×10^{-34}
	MC × time	3.77×10^{-01}		MC × time	7.36×10^{-06}
WI	MC	8.20×10^{-24}	Th	MC	6.73×10^{-02}
	Time	1.03×10^{-01}		Time	4.00×10^{-08}
	MC × time	6.83×10^{-02}		MC × time	2.48×10^{-01}
Hardness (N)	MC	1.17×10^{-10}	Ratio	MC	6.36×10^{-1}
	Time	4.20×10^{-09}		Time	3.31×10^{-2}
	MC × time	3.77×10^{-06}		MC × time	1.76×10^{-1}
PV	MC	2.67×10^{-09}			
	Time	6.81×10^{-02}			
	MC × time	2.70×10^{-09}			

TABLE 3 Multiway ANOVA to determine the influence of factors on physicochemical parameters of the annealed ulluco starch

effect of the MC and t on the PV results of the treatments ($p < .05$) (Table 3) was observed. In general, treatments with the lowest MC associated with short times in the ANN process showed the highest pasting viscosity. The highest PV for treatments obtained using short ANN times can be explained due to the limited reorientation of the starch granules. In contrast to the starches subjected to long ANN times, it will increase the crystallinity region due to the granules reorientation (Zavareze & Dias, 2011). Despite the increase in

viscosity of modified starches, the highest viscosity was presented by commercial potato starch.

The breakdown is related to the starch granules' stability during continuous stirring and heating by measuring swollen granules' disruption, leading to amylose leaching (Wang et al., 2013). This can be associated with the fact that starches with high PV will be more susceptible to disruption of their granules. As we expected, the starches with the highest PV presented the highest BD values

as follows $P5 > T1 > T4 > T6 > T2$ (Table 2). Meanwhile, the modified starches with the lowest PV, $T11 < T7 < T13$, showed the highest stability to stirring and heating with BD values of 28.0, 38.5, and 35.7 BU, respectively. Compared to the native uluco starch, some treatments showed an improvement in the stability associated with the better reorganization within the granule, which is mainly influenced by the MC of the treatment ($p < .05$) (Table 3). The increased stability of uluco starch by hydrothermal modification agrees with that reported for rice, African yam bean, and sweet potato modified starches (Adebawale et al., 2009; Trung et al., 2017; Wang et al., 2018). The final and total setback viscosities of the modified starches were in the range of 109 and 158 BU; and from 264 to 375 BU, respectively (Table 2). In both cases, the values of modified samples were higher than the values of native uluco starch and, in most cases, higher than the values of the native potato starch. An increase in final viscosity represented the stability of the cooling paste associated with the aggregation of the amylose molecules during cooling (Xu et al., 2018) and was mainly influenced by the combination of the MC and time factors ($p < .05$). The SB is indicative of the retrogradation tendency of amylose release from the starch granule in the BD stage, which undergoes a rearrangement (Karim et al., 2000). In general, the SB was influenced mainly by the MC, the lowest the MC, the highest the SB ($p < .05$) (Table 3). SB and FV results agree with those reported for yam and potato starches modified using annealing methodology (Wang et al., 2017).

4 | CONCLUSIONS

The ANN treatment applied to uluco starch in this study proved to be an efficient method for improving physicochemical parameters such as solubility, water retention capacity, and texture of the gel formed, which are valuable properties for potential applications. Also, the ANN treatment improved the pasting properties of the native uluco starch, which are related to the starch's stability in thermal processes. In general, MC was the most influenced parameter in the annealing process. Considering that the differences observed between the treatments were low, it is necessary to carry out studies that broaden the range of conditions to be evaluated. Besides, annealed modified native uluco starch was shown to have good properties compared to commercial potato starch, highlighting its potential for use in the agro-alimentary and nonalimentary industries. This study showed that the native and physically modified uluco starch is an alternative to starches obtained from traditional crops, contributing to food security and generating added value to the uluco crop.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Daniela Oliveras-Parrá: Conceptualization; Formal analysis; Investigation; Methodology; Writing—original draft. **Luis Daniel Daza Ramírez:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Writing—original draft; Writing—review & editing. **Angélica Piedad Sandoval:** Methodology; Resources; Writing—review & editing. **Valeria Elm:** Conceptualization; Methodology; Writing—review & editing. **Henry Alexander Vázquez:** Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing—review & editing.

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CHAPTER 4

Influence of Ulluco Starch Modified by Annealing on the Physicochemical Properties of Biodegradable Films

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Article

Influence of Ulluco Starch Modified by Annealing on the Physicochemical Properties of Biodegradable Films

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Abstract: This work aimed to evaluate the use of annealing (ANN) ulluco starch in the preparation of biodegradable films and its impact on the physicochemical properties of the materials. Three film samples (PS1, PS2, and PS3) were prepared at a fixed starch concentration (2.6% w/v) using glycerol as a plasticizer and then compared to a control sample (F9C) prepared with native ulluco starch. The physical, mechanical, and thermal properties of the films were evaluated. The use of ANN starch decreased the solubility (from 21.8% to 19.5%) and the swelling power (from 299% to 153%) of the film samples. In addition, an increase in opacity and relative crystallinity (from 7.54% to 10.5%) were observed. Regarding the thermal properties, all the samples presented high stability to degradation, with degradation temperatures above 200 °C. However, the samples showed deficiencies in their morphology, which affected the barrier properties. The use of ANN starch has some advantages over native starch in preparing films. However, more analysis is needed to improve the barrier properties of the materials. This work reveals the potential of the ANN ulluco starch for biodegradable film preparation. In addition, the use of modified ulluco starch is an alternative to add value to the crop, as well as to replace non-biodegradable materials used in the preparation of packaging.

Keywords: *Ullucus tuberosus* Cakile; biopolymer; biodegradable films; packaging; starch modification



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1. Introduction

Plastics are inexpensive materials with exceptional characteristics, such as great strength, versatility, water impermeability, low conductivity, corrosion resistance, and durability, making them an essential raw material for different industries. As a consequence of its industrial importance, in 2020, the worldwide production of plastic reached 367 million metric tons [1]. Production is expected to increase twofold by 2040 [2]. However, plastics are non-biodegradable materials synthesized from non-renewable resources. Their post-consumer disposal, mainly of single-use plastics, can cause air, water, and soil pollution, severely impacting the environment and human health [3]. Consequently, different policies and strategies have been implemented to reduce their environmental impact [4]. Among these strategies, the search for biodegradable and renewable materials that can be used to prepare alternative substitutes for plastic has become highly relevant [5].

Biodegradable packaging materials are sustainable and eco-friendly materials based on synthetic (polylactic acid (PLA), polyvinyl alcohol (PVA), and polycaprolactone (PCL)) or natural (proteins, polysaccharides, and lipids) polymers, which can serve as substitutes for non-biodegradable plastics [3]. Among the natural polymers, starch is one of the most widely used in preparing biodegradable packaging due to its abundance, biodegradability, low cost, and renewability. Different studies have evaluated the potential of starches obtained from non-conventional sources to produce biodegradable packaging [6] to ensure

food security and generate added value to underexploited crops. Although starch-based films are characterized by low oxygen permeability, they have disadvantages such as poor mechanical properties and higher solubility. Starch can be enzymatically, chemically, or physically modified to overcome these drawbacks. Annealing (ANN) is a physical modification in which a reorganization of starch granules occurs at temperatures between the glass transition (T_g) and the onset of gelatinization (T_0) and with an excess of moisture [7]. The main effects of annealing on the material are increased crystallinity, thermal stability, granule size and molecular mobility, and reduced water absorption capacity [8].

In recent years, starch obtained from ulluco (*Ullucus tuberosus*), an Andean tuber, has gained attention due to its high extraction yield [43–65% dry basis (d.b.)] along with its amylose content (between 28% and 36% d.b.), which makes it suitable for use in the production of biodegradable packaging due to the high transparency, excellent stability against temperature, and good texture and water vapor permeability characteristics [9,10]. Additionally, ulluco starch has shown excellent compatibility with chitosan to produce biodegradable packaging [11].

Ulluco starch was even successfully tested as a coating for goldenberries, increasing the shelf life of the fruits [9]. More recently, ANN modification of ulluco starch has been shown to improve the physicochemical characteristics of native starch. However, there is no information related to the use of modified ulluco starch in the preparation of biodegradable packaging. Based on the above considerations, this work compares the physicochemical properties of biodegradable films prepared with native ulluco starch and ulluco starch modified by ANN.

2. Materials and Methods

The methodology of this work is summarized in Figure 1.

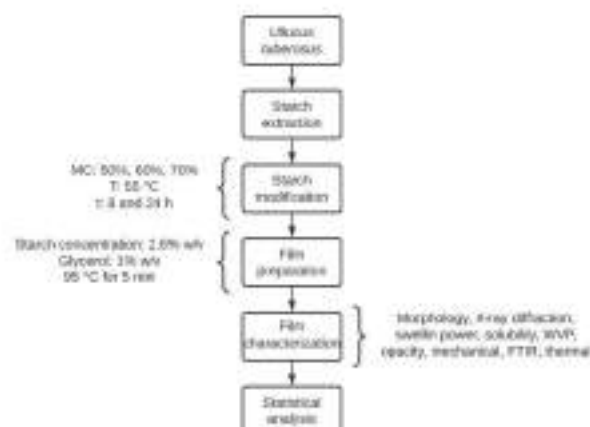


Figure 1. Methodology flowchart.

2.1. Raw Material and Starch Extraction

Ulluco tubers (*Ullucus tuberosus* Caldas) were acquired at a local market in Itagué (Colombia). The native starch (amylose content 27.9%) and the annealing modified starch (ANNS) were obtained according to the methodology described by Valcárcel-Yamani [12] and modified by Parra et al. [7]. In brief, after adjusting the moisture content (MC) at 50% (S1), 60% (S2), and 70% (S3), the starch was heated at 55 °C for 8 h (S1) or 24 h (S2 and S3). Then, the samples were dried at 35 °C until reaching a moisture content less than or equal to 10%. Starches were ground with a mortar and stored at room temperature (~25 °C). Three annealed starches were obtained ANNS1 (50% MC and 8 h at 55 °C), ANNS2 (60% MC

and 24 h at 55 °C), and ANNS3 (70% MC and 24 h at 55 °C). The treatments were selected considering the best physicochemical properties of modified starch to obtain biodegradable films. These properties were evaluated and discussed in a previous study [7].

2.2. Film Preparation

The films were prepared using the casting method following the methodology described by [10]. Native ulluco starch, ANNS1, ANNS2, and ANNS3 were dissolved in distilled water, at a concentration of 2.6% (w/v), with magnetic stirring for 5 min. The mixture was heated to 95 °C and maintained at this temperature for 5 min. After that, the mixture was cooled to 50 °C, and glycerol was added at a concentration of 1.0% (w/v). The solutions were stirred for 10 min and then transferred to a Petri dish with a diameter of 8.5 cm (~0.53 g of the film solution per cm²). The films were dried at 40 °C for 24 h. After the drying process, native ulluco starch film (FSC), ANNS1 film (FS1), ANNS2 film (FS2), and ANNS3 film (FS3) were obtained and stabilized at a relative humidity (RH) of 57 ± 3% and a room temperature (−25 ± 2 °C) for at least 24 h, before the analyses.

2.3. Morphology of Edible Films

Micrographs of the surface of the samples were acquired using scanning electron microscopy (S-3400N, Hitachi, Düsseldorf, Germany) at an accelerating voltage of 15 kV under vacuum pressure of 40 Pa and different magnifications. For the analysis, samples were placed on stubs using double-sided carbon tape and covered with a gold layer.

2.4. X-ray Diffraction (XRD) of Edible Films

X-ray diffraction patterns were analyzed using a Bruker D8 Advance diffractometer (D8 advance, Bruker, San Jose, CA, USA) operating with Nickel filtered and Cu-Kα1 radiation, 40 kV and 40 mA. The wavelength used was 1.5406 Å. Data collection was carried out in the 2θ range of 3.5–70°, with a step size of 0.02035° (2θ) and a counting time of 0.8 s/step. Relative crystallinity was determined after deconvolution of peaks from diffractograms by the relationship between the crystalline peak area (*A_c*) and the total area (*A_t*) using Equation (1) [13]. Results were reported as the average of three measurements.

$$\text{Relative crystallinity (\%)} = A_c / A_t \times 100 \quad (1)$$

2.5. Swelling Power and Solubility

Swelling power (SP) and solubility (S) were calculated using the methodology reported by Baron et al. [14] with some modifications. Films specimens (2 cm × 2 cm) were weighed within pre-weighed aluminum capsules. Subsequently, the pieces were dried at 110 °C for 24 h and weighed (w1). The dried films were placed in glass bottles containing 50 mL of distilled water and stored for 24 h at room temperature (−25 ± 2 °C). Then, excess water was gently removed from the surface of the samples, and the mass gain of the swollen films was measured by weighing (w2). Finally, the pieces were dried at 110 °C for 24 h, and the final weight was recorded (w3). The moisture content (MC, %), swelling power (SP, %), and solubility in water (S, %) of the edible films were calculated using Equations (2) and (3), respectively.

$$SP = [(w_2 - w_1) / w_1] \times 100 \quad (2)$$

$$S = [(w_1 - w_3) / w_1] \times 100 \quad (3)$$

2.6. Water Vapor Permeability

The water vapor permeability (WVP) was determined according to [15]. Results were reported as the mean of four repetitions.

2.7. Opacity

The absorbance of US edible films was measured at 600 nm using an ultraviolet–visible spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific, Thermo Fisher Scientific, Waltham, MA, USA). The OP was calculated using Equation (4).

$$OP = Abs_{600} / FT \quad (4)$$

where Abs_{600} is the absorbance at 600 nm and FT is the film thickness (mm). Results were reported as the average of twelve repetitions.

2.8. Mechanical Properties

Samples with dimensions of 5.0×1.0 cm were loaded in a texturometer (LS1, Lloyd Ltd., Largo, FL, USA) and their mechanical properties were evaluated according to [16]. The crosshead speed and initial distance were 5 mm/min and 4 cm, respectively. The tensile strength (TS, MPa), Young modulus (YM, MPa), and elongation at break (EB, %) were calculated using the Nexygen Plus software (Lloyd Ltd., Version 3.0). Results were reported as the average of eight measurements.

2.9. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The Fourier-transform infrared spectroscopy (ATR-FTIR) spectra of samples were determined using a Fourier transform infrared spectroscopy (Tensor 27, Bruker Optics, Ettlingen, Germany) equipped with the Platinum ATR accessory (Bruker). The spectra were obtained with a resolution of 4 cm^{-1} and 40 scans over the range of $4000\text{--}700 \text{ cm}^{-1}$.

2.10. Thermal Analysis of Starch and Edible Films

The weight loss (WL) and thermal degradation temperature (TD) of film samples were assessed using a thermogravimetric analyzer (SDT 2960, TA Instruments, Tokyo, Japan) according to the method described by [11]. A temperature range between 30°C and 600°C was used, with a heating rate of 10°C , under a nitrogen stream.

The dynamic-mechanical thermal behavior of film samples was evaluated using a dynamic mechanical analyzer (DMA, Q800, TA Instruments, New Castle, DE, USA) following the method described by [17] with some modifications. Films specimens ($30 \text{ mm} \times 9 \text{ mm}$) were loaded and submitted to a range of temperatures between -80°C and 150°C with a heating rate of 10°C , at a constant frequency of 1 Hz and strain amplitude of $10 \mu\text{m}$. For thermal analysis, the result was reported as the mean of three repetitions.

2.11. Statistical Analysis

The mean and standard deviation of the results for at least three replicates under each treatment were reported. Analysis of variance (ANOVA) at the 95% confidence level was used to determine any statistically significant differences. Multiple range tests (MRT), using the Tukey–Kramer range test, were used to determine which mean results differed significantly from others. ANOVA and MRT were performed using MATLAB® software (Version R2019b, The MathWorks Inc., Natick, MA, USA).

3. Results

3.1. Morphology of Biodegradable Films

Micrographs of the films evaluated are shown in Figure 2. In general, all the samples presented roughness on the surface. Only FSC showed a continuous morphology without cracks or porosities. In contrast, the FS1 and FS3 samples showed macrocracks on their surface. However, FS2 shows only some minor cracks (microcracks). These defects may be due to shrinkage of the starch phase after drying and may negatively affect material properties such as water vapor permeability and mechanical properties. A similar phenomenon was observed in hydroxypropyl methylcellulose/hydroxypropyl starch films [18]; the authors related the presence of the micro and macrocracks on the films' surface with the decrease in

elongation and increase of gas permeability. These micro and macro fractures may also be associated with the high degree of ordering of the modified starch compared to the native starch. This organization can reduce the interaction between the polymer chains and the plasticizer (glycerol), which would generate more fragile and brittle films, which agrees with previous reports [19]. A possible strategy to mitigate the appearance of these fractures in films prepared with modified starch may involve using higher plasticizer concentrations or a longer heating time at 95 °C.

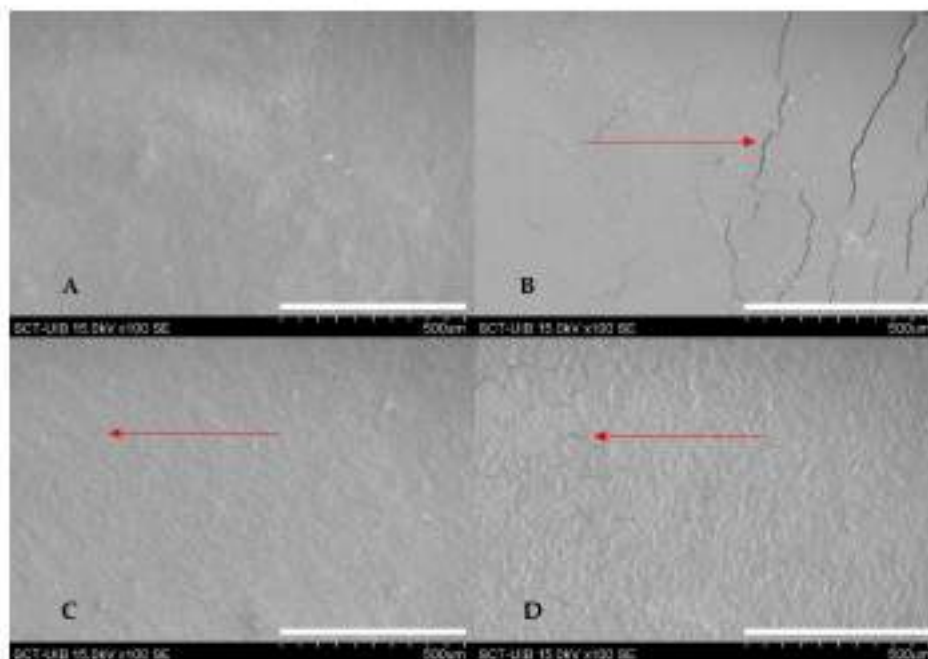


Figure 2. SEM micrograph of PSC (A), FS1 (B), FS2 (C), and FS3 (D) at magnification of 100 $\times$.

3.2. X-ray Diffraction (XRD) of Edible Films

The XRD patterns of biodegradable films are presented in Figure 3. All samples showed a typical B-type crystal structure similar to the native ulluco starch [7]. This structure showed characteristic peaks at around 5.6° 2 θ and 17° 2 θ (peak with strong diffraction intensity) and with small peaks at around 15°, 20°, 22°, and 24° 2 θ . The crystalline regions in the evaluated films are characteristic of semicrystalline materials and agree with the results obtained for guinea arrowroot starch-based films [20]. In addition, relative crystallinity of the biodegradable films was $7.5 \pm 0.8\%$, $10.5 \pm 1.2\%$, $8.03 \pm 0.19\%$ and $8.5 \pm 0.8\%$ for PSC, FS1, FS2, and FS3, respectively, with statistical differences between FS1 and the other samples ($p < 0.05$). The difference between the relative crystallinity values of the films can be associated with the reorganization of the starch granule structure in the modification process [8]. Other researchers have also observed the recrystallization process in starch films stored under different temperature and relative humidity conditions. They have associated this behavior with the process of retrogradation and realignment of the polymer chains, which depends on factors such as the amylose/amylopectin ratio, the presence and concentration of plasticizers, and the glass transition temperature [19]. High relative crystallinity of the materials is associated with a low permeability to gases, which is due to a greater organization of the polymer chains that will prevent the passage of water

molecules. However, as mentioned above, the presence of micro and macrocracks on the surface of the films increases their passage.

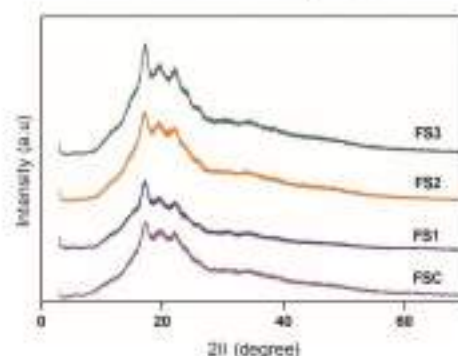


Figure 3. XRD diffractograms of native and modified starch-based films samples.

3.3. Solubility and Swelling Power of Biodegradable Films

The solubility and swelling ability of the films obtained with annealed starch were lower than the FSC (Table 1). However, only samples FS2 and FS3 presented statistical differences for solubility compared to FSC ($p < 0.05$). The decrease in these parameters may be because the ANN process produces a reorganization of the molecular structures in the starch granule, strengthening the interactions between amylose and amylopectin, thus generating a more stable structure, or increasing the crystallinity of the starch [8]. Since solubility is related to the affinity of the materials to water, a low value of this parameter could be related to a better resistance or structural integrity of the films. The solubility values obtained in this work were similar to those reported for films obtained using heat-moisture treatment (HMT) on modified rice starch [21] and potato starch [22] but lower than those reported for films based on modified pearl millet starch [23]. Despite having been subjected to thermal modification, starches may present differences in their physicochemical properties due to differences in the botanical source of the material, the amylose content, and the parameters of the modification process.

Table 1. Physicochemical properties of biodegradable films.

Sample	S (%)	SP (%)	WVP $\times 10^{-9}$ (g m ⁻¹ s ⁻¹ Pa ⁻¹)	Thickness	Opacity	EB (%)	YM (MPa)	TS (%)
FSC	21.8 $\pm$ 0.8 ^a	299 $\pm$ 39 ^a	2.38 $\pm$ 0.10 ^c	0.13 $\pm$ 0.01	0.96 $\pm$ 0.07 ^b	25 $\pm$ 2 ^b	288 $\pm$ 42 ^c	5.1 $\pm$ 0.3 ^c
FS1	20.7 $\pm$ 0.9 ^{ab}	191 $\pm$ 16 ^b	3.45 $\pm$ 0.21 ^a	0.11 $\pm$ 0.01	0.95 $\pm$ 0.09 ^b	22 $\pm$ 4 ^b	892 $\pm$ 89 ^a	10.5 $\pm$ 0.8 ^a
FS2	20.6 $\pm$ 0.8 ^b	207 $\pm$ 22 ^b	2.46 $\pm$ 0.18 ^b	0.13 $\pm$ 0.01	1.35 $\pm$ 0.12 ^a	50 $\pm$ 3 ^a	237 $\pm$ 61 ^c	5.5 $\pm$ 0.7 ^c
FS3	19.6 $\pm$ 0.8 ^b	155 $\pm$ 11 ^c	2.88 $\pm$ 0.10 ^b	0.11 $\pm$ 0.01	0.83 $\pm$ 0.04 ^c	22 $\pm$ 3 ^b	567 $\pm$ 77 ^b	9.4 $\pm$ 0.5 ^b

S: solubility; SP: swelling power; WVP: water vapor permeability; EB: elongation at break; YM: Young modulus; TS: tensile strength. Values are expressed as means $\pm$ standard deviation. Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

The FSC showed the highest swelling power ($p < 0.05$), followed by FS2, FS1, and FS3. A decrease in the swelling power was observed in the films prepared with ANN starch compared to FSC. Such decreases in swelling power in films prepared with modified starch are related to the reduction of free volume in the film structure, which is a consequence of the crystallinity increase [24]. Although a reduction in the swelling power of the films obtained with modified starch was observed, the values obtained in this work were higher than those reported for films based on acha (93.1%) and iburu (99.5%) starch [25]. As mentioned above, the differences in the physicochemical properties between films obtained

with different starches may be due to the type of starch used (native or modified), botanical source, amylose content, and crystallinity.

3.4. Water Vapor Permeability of the Films

The purpose of food packaging materials is to isolate the food matrix from environmental factors such as light, oxygen exposure, and water vapor, thus increasing its shelf life. The permeability of a film is defined as the amount of water vapor that passes through a unit area of the material per unit of time [9]. The WVP values of biodegradable films are shown in Table 1. The highest permeability was observed in films FS1 and FS3, while FS2 and FSC presented the lowest values ($p < 0.05$). The differences in permeability in the analyzed samples can be related to the defects (macrocracks) in the morphologies of the films obtained with ANN starch. An increase in permeability related to defects in morphology was also observed in films prepared using a mixture of hydroxypropyl methylcellulose/hydroxypropyl starch [18]. In addition to the presence of macrocracks, the microcracks produced by the recrystallization of the modified starch could also influence the increase in permeability [21].

3.5. Opacity

Transparency is a parameter that can help define the final use of food packaging since it is related to sensory perception by the customer as well as product protection. The opacity of biodegradable films is shown in Table 1. The highest opacity was observed in FS3, followed by FS1 ($p < 0.05$), while FS2 and FSC presented low opacity. A similar increase in the opacity of films obtained with HMT-modified rice starch was observed compared to those obtained with native starch [21]. The authors attributed the lower transparency of the films obtained with modified starch to conformational changes in the starch granule structure due to the reorganization by the modification process.

3.6. Mechanical Properties

The mechanical properties of edible films are shown in Table 1. FS2 showed the highest EB ($p < 0.05$). The higher value of EB can be related to the low relative crystallinity of the sample (Section 3.6), which indicates a lower reorganization, compared to samples FS1 and FS3, of the internal structure and, therefore, a greater elasticity. In addition, the high crystallinity decreases the interaction between the plasticizer (glycerol) and polymer chains, limiting the mobility of these chains in the amorphous region of the films due to the low intercalation of glycerol between the polymeric structure [26]. In contrast to the EB, the FSC and FS2 samples showed the lowest values of TS and YM, which is expected because the tensile strength and the modulus increase with an increase in the organization of the crystalline structure of the materials, providing greater rigidity to the films [27]. Similar behavior was observed in corn starch-based films. Generally, films with high crystallinity degrees presented high TS and YM values [28–30]. Another determining factor is the presence or absence of macro and microcracks in the morphology of starch annealed films which can influence the mechanical properties reducing its elasticity and others, such as barrier properties [18].

3.7. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The ATR-FTIR spectra of samples analyzed are shown in Figure 4. All samples exhibited a band at 3300 cm^{-1} corresponding to the stretching of hydroxyl groups (O-H) [11]. A previous report observed that the native ulluco starch and the modified starches presented the same band at 3446 cm^{-1} [7]. However, there was a slight shift in the signal in the films analyzed. This behavior was previously reported in films based on cassava starch [31]. It may be associated with the interaction between the hydroxyl groups of glycerol and native or modified starch [9]. The asymmetrical and symmetrical stretching of bonds C-H is associated with bands between 2900 cm^{-1} and 2950 cm^{-1} [32].

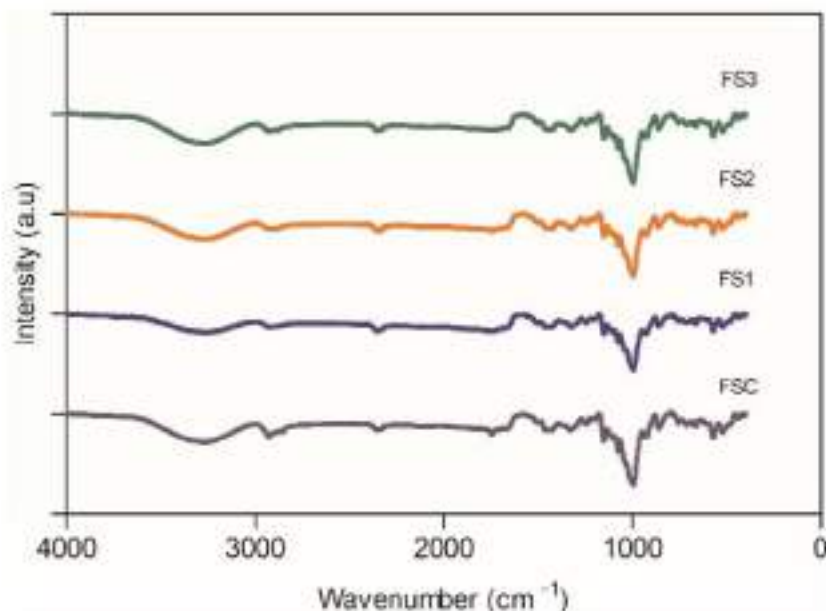


Figure 4. FTIR spectra of native and modified starch-based films samples.

Additionally, bands at 1320 cm^{-1} and 1430 cm^{-1} were observed, corresponding to the bending vibration of $-\text{CH}$ groups [31]. The bands observed at 958 cm^{-1} and 1078 cm^{-1} are related to the crystalline structure, whereas the band observed at 997 cm^{-1} is associated with the amorphous region correlated to the presence of starch in the film composition [9]. The high intensity of the signal corresponding to the amorphous region corresponds to the low relative crystallinity of the films (see Section 3.6).

3.8. Thermal Analysis of Starch and Edible Films

The weight loss of films was $86.9 \pm 1.9\%$, $88.8 \pm 0.7\%$, $86.6 \pm 1.1\%$, and $86.3 \pm 0.5\%$ for FSC, FS1, FS2, and FS3, respectively ($p > 0.05$). The samples showed four stages of decomposition (Figure 5): The first stage ranged between $40\text{ }^{\circ}\text{C}$ and $150\text{ }^{\circ}\text{C}$, the second stage ranged between $214\text{ }^{\circ}\text{C}$ to $269\text{ }^{\circ}\text{C}$, the third stage ranged between $264\text{ }^{\circ}\text{C}$ to $324\text{ }^{\circ}\text{C}$, and the fourth stage occurred above $324\text{ }^{\circ}\text{C}$. The first stage corresponds to the water evaporation from the films, which is related to the moisture content of samples. The second stage corresponds to the degradation of the glycerol-rich phase, which is characteristic of starch films plasticized with glycerol [33]. The depolymerization of the constituents of the starch takes place in stage three, which is associated with the greatest decomposition of the analyzed samples, with weight losses between 59% and 63% at maximum decomposition temperatures between $301\text{ }^{\circ}\text{C}$ and $304\text{ }^{\circ}\text{C}$. Finally, in stage four, the formation of inert carbonaceous residues occurs. No statistical differences were observed between the samples. These results agree with those reported for biodegradable films obtained using wheat, corn, and potato starch [34].

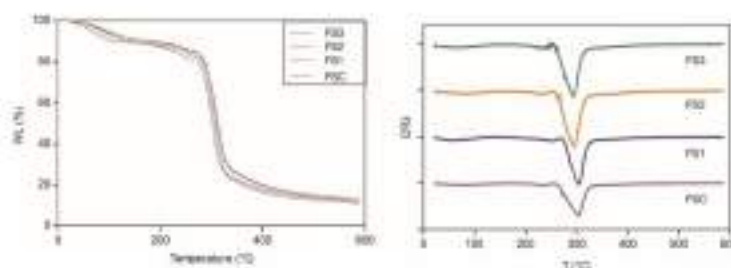


Figure 5. Weight loss curves of native and modified starch-based films sample.

4. Conclusions

Ulluco starch modified by ANN showed potential as a material to produce biodegradable films. However, a significant influence of the modification process on the physicochemical characteristics of the materials evaluated, mainly in the morphology, was evidenced. In general, the modified ulluco starch improved film characteristics such as solubility and swelling power, providing greater resistance to moisture. In addition, an increase in relative crystallinity was observed, associated with a decrease in the transparency of the films. This fact may contribute to the consideration that a reduction of the transparency of the films could be related to a lower passage of light and greater protection of the food matrix. Although an increase in the crystallinity of the samples is observed, there is no evidence of a relationship between this characteristic and the barrier properties of the materials, which could be influenced by the micro and macrocracks observed on the surface of the samples. This observation suggests that more tests should be carried out to determine the optimal concentration of modified starch and plasticizer in the preparation of biodegradable films as well as the starch gelatinization time and thus obtain continuous and more consistent morphologies.

On the other hand, all the samples presented high thermal stability. However, no impact on this property was observed using modified starch. In general, ulluco starch modified by ANN offers as a viable alternative for producing biodegradable films with improved physicochemical properties. However, additional testing is required to optimize its performance.

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CHAPTER 5

Influence of Ulluco Starch Concentration on the Physicochemical Properties of Starch–Chitosan Biocomposite Films

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Article

Influence of Ulluco Starch Concentration on the Physicochemical Properties of Starch–Chitosan Biocomposite Films

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Abstract: This work aimed to prepare ulluco starch (US)/chitosan (Ch) edible films and evaluate the effect of the concentration of US on their physicochemical properties. The use of edible films is a means of adding value to the ulluco crop and evaluating the viability of using new sources to produce packaging materials. Different samples were prepared at different US concentrations (2%, 3%, 4%, and 5% w/v) and a fixed chitosan concentration (1.5% w/v); then, samples were analyzed, considering their physical, mechanical, and thermal properties. The US/Ch edible films showed an increase in solubility from 17.5% to 21.7%, swelling power (SP) from 38.9% to 267%, tensile strength (TS) from 3.69 MPa to 10.7 MPa, Young modulus (Ym) from 18.0 Pa to 632 Pa, and thermal stability as the US concentration increased. However, samples with low US concentrations showed higher elongation at break (EB) (36.6%) and better barrier properties (WVP) (3.61×10^{-11} g/(m × Pa)). The films evaluated in this work presented good physical, mechanical, and barrier properties, revealing their potential as packaging material ensuring food security, and demonstrating the technological potential of US.

Keywords: packaging; chitosan; biopolymer; films; *Ullucus tuberosus* Cárdenas



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1. Introduction

One of the main strategies of the food industry for product preservation, conservation, storage, and transportation is the use of packaging. Over the last 50 years, conventional synthetic or petroleum-based plastic packaging such as polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) has adequately accomplished this task [1,2]. However, there are now new concerns regarding the use of these types of plastic, such as the fact that they are single-use, which, added to their low biodegradability, can generate pollution [3]. Their impact on the environment has been such that different policies have been implemented worldwide to reduce the use of single-use plastics [4]. These policies create significant challenges for the industry, and the development and design of eco-friendly packaging is one of the alternatives most explored by researchers [5]. Different investigations have been conducted to evaluate film-forming materials and to study the effect on the physicochemical properties of these films, in order to obtain materials that have similar characteristics to the existing petroleum-based plastics and also provide additional benefits such as antimicrobial and antioxidant properties [6]. However, the huge demand for packaging for the industry requires the search for new sources of materials for the development of this biodegradable packaging; sources which must have certain characteristics such as biodegradability, non-toxicity, biocompatibility, film forming properties, and low cost. Starch is the main carbohydrate reserve in plants and consists of amylose composed of α-(1,4)-D-glucopyranose subunits and amylopectin composed

of α -(1,4)-D-glucan short chains linked through α -1,6 linkages. In addition, starch is a renewable, low-cost, and biodegradable material, which makes it a raw material with high potential in the packaging industry.

Ullucus tuberosus Caldas, commonly known as ulluco, olluco, chugua, or papalisa, is an Andean tuber belonging to the Basellaceae family cultivated at high altitudes from Bolivia to Ecuador and Colombia [7]. This crop presents a high starch content, which displays good film-forming properties due to the high amylose content [8,9]. Previous studies have reported the physicochemical properties of ulluco starch (US) and the obtaining of edible films based on US, which presented good mechanical properties, low opacity, and intermediate solubility and swelling properties, indicating its potential as a packaging material [10,11]. On the other hand, these edible films showed high water vapor permeability (WVP) and low antimicrobial capacity, highlighting the need to look for alternatives to improve the barrier and active properties of US-based films. The addition of different active ingredients such as essential oils [12], natural extracts [5], or the mixture with other polymers such as gelatin [13], gums [14] or chitosan [15] are strategies to overcome these problems and have been successfully tested.

Chitosan (Ch) is a linear polycationic amino-polysaccharide extracted from crustaceans or mushrooms, consisting of (1,4)-linked-2-amino-deoxy- β -D-glucan obtained by the partial deacetylation of chitin [16,17]. It is considered a biodegradable, non-toxic, renewable, and biocompatible material, which shows antimicrobial capacity [18]. Some studies have demonstrated the compatibility of starch and Ch for the preparation of film, and the improvement in their mechanical and active properties [15,19]. Currently, there is no evidence of the use of US blended with Ch for the preparation of edible films, which is due to the low exploitation of ulluco as a raw material for industrial processes. Although the use of other starches with chitosan for the preparation of biodegradable films has been evaluated, the interaction of each of these materials is different, which is due to factors such as amylose content, starch purity, and structural configuration of the granule, among others. It is therefore necessary to evaluate the behavior of new starches with chitosan and thus be able to establish the potential of the mixture of these materials for use as packaging.

In this sense, as an alternative to non-biodegradable packaging, this work aims to prepare edible films based on US mixed with Ch and evaluate the effect of starch concentration on their physicochemical and thermal properties. This work will contribute to understanding the properties and US potential as a raw material for the industry.

2. Materials and Methods

2.1. Materials

Ch (Low molecular weight 50–190 kDa, Deacetylated 75–85%) was purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). US (amylose content: 35.3%) was previously obtained as reported by Galindez et al. [10]. Food grade acetic acid was purchased from Cimpa S.A.S (Cimpa S.A.S, Bogotá, Colombia), glycerol, and Tween® 80 (Density at 20 °C, 1060 kg m⁻³) were purchased from Merck (Merck, Darmstadt, Germany).

2.2. Edible Films Preparation

Ch solution: Ch was dissolved in 1% v/v acetic acid under agitation for 1 h at 25 °C to obtain a final solution of 1.5%. The chitosonium acetate solution was allowed to stand overnight and stored at 4 °C [20].

US solution: US was dissolved in distilled water at different concentrations 2%, 3%, 4% and 5% (w/v). US mixtures were heated to 95 °C and maintained at this temperature for 5 min. Then, the mixtures were cooled to 60 °C.

For edible film preparation, the Ch solution was heated to 60 °C and mixed with each US solution in a 1:1 (v/v) ratio. The mixtures were stirred, and then glycerol (1% v/v) and Tween® 80 (0.1% v/v) were added (Table 1). After 10 min of agitation, the solution was passed through a peristaltic pump (to eliminate the bubbles). The prepared solutions (30 g) were poured into Petri dishes (Diameter: 8.5 cm) and dried at 40 °C for 24 h using

a forced air-flow oven. Before the analysis, the samples were stabilized in a desiccator cabinet (DH-1002, Acoquillab, Bogotá, Colombia) at a relative humidity (RH) of 54% and a room temperature ($\sim 20^{\circ}\text{C}$) for at least 24 h.

Table 1. Ulluco starch /Chitosan edible film formulations.

Sample	Initial Concentration of Solutions		Final Concentration of Films			
	US (%)	Ch (%)	US (%)	Ch (%)	Glycerol (%)	Tween 80® (%)
S2	2.0	1.5	1.0	0.75	1	0.1
S3	3.0	1.5	1.5	0.75	1	0.1
S4	4.0	1.5	2.0	0.75	1	0.1
S5	5.0	1.5	2.5	0.75	1	0.1

US: ulluco starch; Ch: chitosan.

2.3. Moisture Content, Swelling Power, and Solubility

Moisture content (MC) g of water/100 g of sample (% w.b.), swelling power (SP, %), and solubility (%) of edible films were determined by following the gravimetric method described by Hornez-Jara et al. [20]. Results were reported as the average of five repetitions.

2.4. Color and Transparency

The color parameters were assessed using a Minolta colorimeter (Cr 410, Konica Minolta, Tokyo, Japan) according to [11]. Results were expressed as yellow index (YI) and color index (CI). The opacity of the films was assessed following the methodology described by [11]. Measurements were performed on the film samples at least eight times, and the average results were reported.

2.5. Water Vapor Permeability

The water vapor permeability (WVP) was evaluated according to the ASTM method [21]. The film samples were loaded into stainless steel permeability cells, in which 6 mL of distilled water was deposited with an exposed area of 9.6 cm^2 . It was assumed that the RH was maintained at 100% within the cell. The cells were placed in a controlled chamber at $25 \pm 2^{\circ}\text{C}$ and $75 \pm 2\%$ RH. Every hour, the mass of the cells was recorded using an analytical balance ($\pm 0.0001\text{ g}$) for 8 h. A fan was placed inside the chamber to ensure uniform conditions within the chamber. The WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated using Equation (1):

$$\text{WVP} = \frac{aFT}{tAP_{\text{sat}}(RH_1 - RH_2)} \quad (1)$$

where a is the mass of the permeability cell (g) at time t (s); FT is the film thickness (m); A is the exposed area (m^2); P_{sat} is the saturation vapor pressure at the test temperature (Pa); RH_1 is the RH of the water contained in the cells, expressed as a fraction; and RH_2 is the RH of the chamber, expressed as a fraction. Measurements were performed on the film samples at least four times.

2.6. Mechanical Properties

The tensile properties of the films were evaluated following the ASTM methodology [22] using a texturometer (LS1, Lloyd Ltd., Largo, FL, USA). The films were cut into $5\text{ cm} \times 1.0\text{ cm}$ strips, and the crosshead speed and initial grip separation were set to 5 mm/min and 4 cm , respectively. The tensile strength (TS, MPa), Young modulus (YM, MPa), and elongation at break (EB, %) were determined using Nexygen Plus software (Lloyd Ltd., Largo, FL, USA, Version 3.0). Results were reported as the average of six measurements.

2.7. Morphology of Edible Films

The surface morphology of US and edible films was examined by scanning electron microscopy (SEM; Quanta 650 FEG, FEI Company, Hillsboro, OR, USA). The samples were placed on stubs using double-sided carbon tape and covered with a gold layer using a rotary-pump coating system (Q150R, Quorum, Lewes, UK). Images were obtained at an accelerating voltage of 10 kV and varying degrees of magnification.

2.8. X-ray Diffraction of Edible Films

X-ray diffraction (XRD) analysis of the US, Ch, and films was carried out using a Bruker D8 ADVANCE diffractometer (Bruker, Billerica, MA, USA) operating with Ni-filtered Cu-K α 1 radiation at 40 kV and 40 mA and a specific wavelength of 1.5406 Å. Data collection was carried out in the 2 θ range of 3.5–70°, with a step size of 0.02035° (2 θ) and a counting time of 0.8 s/step. Relative crystallinity was determined using the relationship between the peak area and total area.

2.9. Fourier-Transform Infrared (ATR-FTIR) Spectroscopy of Starch and Edible Films

The Fourier-transform infrared spectroscopy (ATR-FTIR) spectra of pure components and edible films were recorded in Fourier transform infrared spectroscopy (FTIR, IRAffinity-1S, Shimadzu, Japan). For the analysis of solid samples, pellets were prepared by a mixture and homogenization of the sample with potassium bromide (KBr), and for liquid samples, a liquid cell was used. The measurements were taken at room temperature, in triplicate, and recorded over the range of 4000–700 cm^{−1}, with a resolution of 4 cm^{−1} and 20 scans.

2.10. Contact Angle Measurement

The contact angle (θ) of samples was determined by the sessile drop method using an automated drop tensiometer (Tracker S, TRACKER™, Teclis Scientific, France). A droplet of 5 μ L of distilled water was deposited on the surface of the films. The contact angle was measured after 5 s and expressed in degrees [23]. The results were expressed as the mean of at least five measurements taken at random positions on the samples.

2.11. Thermal Analysis

Differential scanning calorimetry (DSC) analysis was performed using a differential scanning calorimeter (DSC 3+, star system, Mettler Toledo, Columbus, OH, USA). Approximately 3 to 6 mg of the samples were placed into aluminum pans and hermetically sealed. The pans containing the samples were cooled to −60 °C at 20 °C/min and then heated from −60 °C to 250 °C at a constant rate of 20 °C/min. The whole process was carried out in a nitrogen atmosphere. An empty pan was used as a reference. The obtained thermograms were examined using Universal Analysis 2000 V3.9A software (TA Instruments, Tokyo, Japan). The melting temperature (T_m) of each sample was determined. Thermal analyses were carried out in triplicate.

Thermogravimetric analysis (TGA) was performed according to Galindez et al., [10] using a thermogravimetric analyzer (SDT 2960, TA instruments, Tokyo, Japan). The weight loss, thermal degradation temperature (T_{deg}), onset temperature (T_{onset}), and total loss of pure components and edible films were determined over the temperature range of 20–600 °C at a heating rate of 20 °C/min and under a stream of nitrogen. Thermal analyses were carried out in duplicate.

2.12. Statistical Analysis

Analysis of variance (ANOVA) at a 95% confidence level was performed to determine significant differences in the properties between samples and storage time. Multiple range tests (MRTs), using the Tukey–Kramer test, were used to determine significant differences between groups. Statistical analysis was performed using the MATLAB® R2017b software (The MathWorks Inc., Natick, MA, USA).

3. Results

3.1. Moisture Content, Swelling Power, and Solubility

The MC of US/Ch biodegradable films ranged from 15.2% to 33.4% (Table 2). Results showed an inverse correlation between the starch concentration and the MC. Samples S2 and S5 showed the highest and the lowest MC ($p < 0.05$), respectively. This behavior can be explained since a higher concentration of starch is related to a greater amount of hydrophilic groups, which can bind more strongly the water molecules and thus prevent their evaporation. In contrast, its hydrophilic groups interact rapidly with the hydrophilic groups of chitosan and glycerol at low starch concentrations. These will reduce the hydrophilic groups available to interact with the water molecules through hydrogen bonds, leading to a greater amount of free water molecules and consequently greater evaporation and mass loss [20].

Table 2. Physicochemical properties of alluco-starch-chitosan edible films.

Sample	MC (%)	Solubility (%)	SP (%)	WVP $\times 10^{-11}$ (g/m s Pa)	Opacity	YI
S2	33.4 $\pm$ 1.2 ^a	17.5 $\pm$ 1.0 ^c	39 $\pm$ 5 ^d	5.6 $\pm$ 0.3 ^c	0.40 $\pm$ 0.06 ^a	5.7 $\pm$ 1.7 ^a
S3	21.1 $\pm$ 1.6 ^b	19.6 $\pm$ 0.7 ^b	116 $\pm$ 20 ^e	6.9 $\pm$ 0.5 ^b	0.32 $\pm$ 0.02 ^b	5.3 $\pm$ 0.9 ^a
S4	17.9 $\pm$ 1.2 ^c	19.7 $\pm$ 0.8 ^b	177 $\pm$ 22 ^b	6.4 $\pm$ 0.7 ^{bc}	0.32 $\pm$ 0.03 ^b	4.3 $\pm$ 0.4 ^b
S5	15.2 $\pm$ 0.8 ^d	21.7 $\pm$ 1.1 ^a	267 $\pm$ 18 ^a	8.5 $\pm$ 0.9 ^a	0.31 $\pm$ 0.01 ^b	4.3 $\pm$ 0.4 ^b

MC: moisture content; S: solubility; SP: swelling power; WVP: water vapor permeability; WI: white index; YI: yellow index; Values are expressed as means $\pm$ standard deviation. Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

The solubility of the samples analyzed increased with a rise in the concentration of US (Table 2), indicating that films with a higher content of US showed greater affinity for water molecules or low water resistance. The solubility values ranged between 17.5% and 21.7%, the highest solubility being shown by S5, followed by S4, S3, and S2 ($p < 0.05$). A possible explanation for this behavior could be that the interactions between the components of the films do not generate steric hindrance due to the formation of a disorganized network gel structure, allowing the entry of water. In addition, the low moisture content of the films with a higher concentration of US increased the susceptibility to interact with molecules of water through hydrogen bonds. The increase in solubility showed similar behavior to the films prepared from gelatin and casein phosphopeptides [24]. In addition, our results are similar to those reported for solubility of films obtained using corn starch and Ch [25] but differ from the high solubility reported for pea starch and Ch [26]. These differences can be attributed to the botanical source of starch, amylose content, the final concentration of the polymers in the film-forming solution, and the molecular weight of the Ch. In our case, the use of Ch with a low molecular weight can contribute to a more significant interaction between polymers.

Moreover, results obtained in this work agree with those reported previously for alluco edible films [11], indicating that the chitosan did not affect the films' affinity towards water. The solubility of materials can help determine their applicability to foods. In some applications, such as the coating of products with high moisture content, it is necessary to use materials with low solubility to guarantee their integrity. On the other hand, the coating of products with low moisture content, such as candies, requires materials with high solubility [27].

The SP showed a similar behavior to solubility; as the concentration of starch increased, the ability of the biodegradable films to swell significantly increased ($p < 0.05$) (Table 2). This behavior is characterized by more functional groups of a hydrophilic nature that can interact with water molecules; thus, the higher the US concentration, the greater the SP. Furthermore, as explained above, the degree of disorder of the gel network structure and the lack of steric hindrance increases the interaction capacity between the constituents (US, Ch, and glycerol) of the biodegradable films and the water. In addition, glycerol and

chitosan can interact with starch molecules, enhancing free volume in the film network [28]. Similar behavior was reported for films produced using potato starch and Ch [29]. The SP of edible films evaluated in this study was higher than that reported for edible films obtained with starch from the same species and dried at different temperatures [10,11]. The differences observed between films obtained with the same type of polymer are mainly due to the concentration of starch. US edible films showed similar swelling capacities to other films obtained with lentil (126 to 133%) and quinoa starch (255 to 297%), but lower than those obtained using pumpkin starch (387 to 500%) [26].

3.2. Water Vapor Permeability

A key characteristic of food packaging is to act as a barrier that prevents the exchange of water and gases between the food matrix and the surroundings, thus delaying the deterioration of the product. The WVP values of US/Ch biodegradable films are shown in Table 2. In general, an increase in the WVP of the samples was observed with an increase of US concentration, which is related to the higher solubility and SP of films with higher US content. However, samples S3 and S4 did not differ in their WVP values ($p > 0.05$). The results obtained in this work were better than those obtained for tapioca starch [30] and the US films [11]. In addition, the results obtained in this work were better than those reported for corn starch and Ch with low or medium molecular weight, but similar to the films prepared using Ch with high molecular weight [31]. Considering that Ch with low molecular weight was used in this work, the observed differences may be due to the amylose content of starches, which is higher in the US (35.3%) than in corn starch (25%). The low WVP of US/Ch biodegradable films indicates its great potential for extending the shelf life of foods. The differences in WVP between pure starch edible films and UC/Ch films may be due to the addition of Ch to the blend, which displayed hydrophobic characteristics.

According to Minh and Yoksan [32], the addition of chitosan to the mixture with starch can decrease the WVP values due to two factors: a) the presence of acetyl groups at the C2 position of the chitosan pyranose ring provides less hydrophilicity to chitosan as compared with the existence of OH— groups of starch; and b) β -D-(1-4) glucopyranosyl linkages of chitosan give it better packing structure or higher crystallinity than α -D-(1-4) glucopyranosyl linkages of starch, due to the stronger hydrogen bond formation, which leads to less moisture/water absorption and more difficulty in hydrolysis. Since the US concentration in film S5 was higher than in the others, the amount of available hydrophilic groups was greater, which increased the possibility of interacting with water molecules, allowing the migration of water vapor through the films. Different works have shown that the decrease in the starch / Ch ratio has reduced the WVP of the films [27].

3.3. Contact Angle

The θ is related to the hydrophilicity or hydrophobicity degree of the edible film surface [33]. The θ of the samples were $51.39^\circ \pm 2.17$, $62.54^\circ \pm 5.58$, $56.03^\circ \pm 4.98$, and $63.22^\circ \pm 2.11$ for S2, S3, S4, and S5, respectively. According to Vogler [34], US/Ch edible films can be classified as hydrophilic surfaces ($\theta < 65^\circ$). The lowest θ was shown by S2, indicating the highest hydrophilicity ($p < 0.05$). An increase in US concentration greater than 3% in the edible film preparation leads to an increase in the θ , which means a decrease in the hydrophilicity of the samples. The increase in the θ in the S3, S4, and S5 may be related to the fact that an increase in the concentration of US generated an increase in intermolecular interactions, reducing the availability of hydroxyl groups and therefore the interaction of polymers with water molecules [35]. A similar explanation was pointed out for the θ shown by films prepared using native cassava starch and Ch with high molecular weight [32]. In addition, the US retrogradation after the heat treatment to generate the films can help to reduce the hydrophilicity of the samples [36]. It is important to note that the film with the highest θ shows the highest WVP value. Although sample S5 presents lower hydrophilicity than S2, the number of hydroxyl groups available in S2 is greater.

Therefore, these can bind water molecules and entrap them in the structure, preventing its passage (lower WVP value). The opposite occurs with S5.

3.4. Optical Properties

Color and transparency are important factors when evaluating packaging, as they will influence consumer perception and acceptability of the product. In addition, the optical properties will serve as selection criteria in defining the end use of the food packaging evaluated [37]. The opacity of the US/Ch edible films ranged between 0.31 and 0.40 (Table 2). It was observed that a decrease in the starch concentration or the starch/chitosan ratio yielded films with higher opacity values, which means that S5 showed the highest transparency among the evaluated samples. The results obtained in this work showed that the opacity of the films is related to the MC, which was similar to the behavior reported for edible films obtained from yuba protein [38]. This phenomenon can be related to the intermolecular agglomeration produced by the MC increase, rising the turbidity, and, therefore, the light scattering of films [38]. The behavior of the opacity from US/Ch was contradictory to the results reported for corn starch and Ch films [27]. The differences observed can be attributed to the deacetylation degree of the Ch used in the film's preparation, the amylose content of the starch or the thickness of films obtained.

YI values are presented in Table 2. S2 showed the highest YI, followed by S3, S4, and S5 ($p < 0.05$). An increase in the US/Ch ratio was characterized by a decrease in the YI of the films, which is associated with the addition of the Ch, and the development of a yellowish coloration due to the presence of β -1-4 linked 2-amino-2-deoxy-D-glucopyranose repeat units [39]; thus, higher US concentration limited the development of this coloration. Similar behavior was reported for the YI of edible films of Ch and tapioca starch [40]. The values of WI ranged between 87.3 and 87.8. As expected, low US/Ch ratios generated films with low WI, resulting from the development of the yellow color mentioned above. However, no statistical differences were observed between the WI of the treatments with an increase in the concentration of starch.

3.5. Mechanical Properties

Figure 1 shows the EB, TS, and YM values obtained for US/Ch edible films. It was observed that the EB of the evaluated samples decreased as the US concentration increased; however, no differences were observed between samples S3, S4, and S5. The decrease of the EB with an increase in US can be related to the MC since water molecules can act as a plasticizer and thus improve the flexibility of the edible films. Therefore, adding greater amounts of starch will reduce the EB due to the interactions between starch and water molecules [41,42]. In contrast, the samples with the highest values of MC show the lowest values of TS, indicating that the higher the US concentration, the higher the TS of the films. The above is associated with an increase in the number of hydrogen bond interactions between the NH_3^+ groups, formed from protonation in an acid medium (acetic acid solution) of the amino groups of chitosan backbone, and the OH^- of the US exposed by the destruction of the ordered crystalline structure in the gelatinization process [27]. YM is related to the stiffness of a material, which means that materials with high values of this parameter will be rigid. In contrast, materials with low YM values will present more flexible characteristics. As expected, the YM of the US/Ch edible films increased at higher US concentration, which can be explained due to the interaction between the NH_3^+ and the OH^- groups mentioned above. US/Ch films showed higher EB, TS, and YM than those films obtained using starch from brown rice and Ch [15]. In addition, US/Ch films were more elastic than the films obtained using pea starch and Ch, but in contrast, it presented lower values of YM and TS [26].

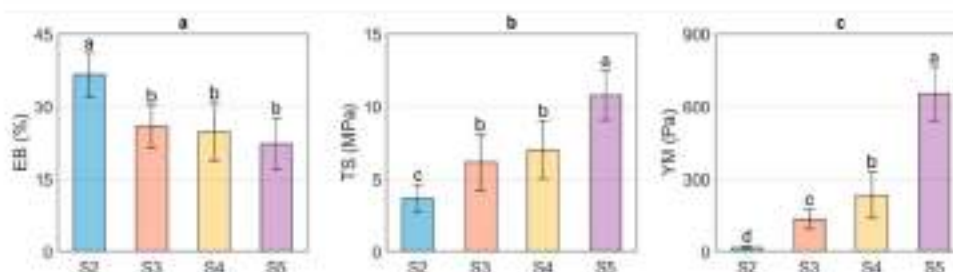


Figure 1. Mechanical properties of the ulluco starch-chitosan edible films. Elongation at break (EB, %) (a), tensile strength (TS, MPa) (b), and Young Modulus (YM, Pa) (c). Different letters indicate significant differences between samples ($p \leq 0.05$).

3.6. Morphology of Edible Films

SEM micrographs of US granules and edible films are shown in Figure 2, where the US granules showed irregular shapes and sizes. However, most granules are characterized by an elongated and mounded shape, with a smooth surface and without evidence of pores or fissures (Figure 2a). The morphology of the US analyzed in this work was similar to that reported for the same species harvested in Argentina [8]. Edible films presented continuous surfaces with no fractures or porosities, implying that the materials were homogeneously mixed. The crowds observed in the figures may be related to problems in the serving process in the Petri dishes. The homogeneity of the mixture of starch and Ch and its effect on the morphology of edible films have been previously reported for pea [41] and corn starches [27].

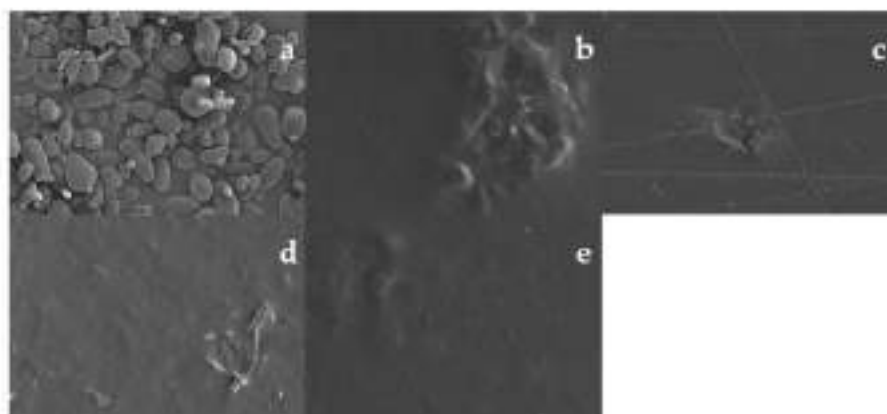


Figure 2. Micrographs of ulluco starch at magnification of 500 $\times$ (a), and micrographs of surface from ulluco starch-chitosan edible films samples: S2 (b), S3 (c), S4 (d) and S5 (e) at magnification of 200 $\times$.

3.7. X-ray Diffraction

Figure 3 presented the XRD patterns of US/Ch edible films, Ch and US. A typical B-type starch diffraction pattern is shown by the native US used in the preparation of the films, which presented peaks at 5.6 $^{\circ}$, 15 $^{\circ}$, 17 $^{\circ}$, 20 $^{\circ}$, 22 $^{\circ}$ and 24 $^{\circ}$ 2 θ . This type of starch is obtained commonly by extraction from tubers [27]. Ch presented a characteristic peak at about 21 $^{\circ}$ 2 θ , which agrees with the literature [43]. Relative crystallinity of the samples analyzed was 16.0%, 13.9%, 15.7%, 16.0%, 36.9 and 32.7% for S2, S3, S4, S5, US and Ch,

respectively. The mixture of US and Ch to form a film-forming solution decreased the crystallinity of the films, which indicates that the blend was miscible [44]. This behavior agreed with Liu et al. [45] for films prepared using high amylose cornstarch and Ch. The authors attribute the reduction in the film crystallinity to the fact that the hydrogen bonds formed by the interaction between the polymers (US and Ch) can reduce the mobility of the molecules and, therefore, reduce the ability of the components to crystallize.

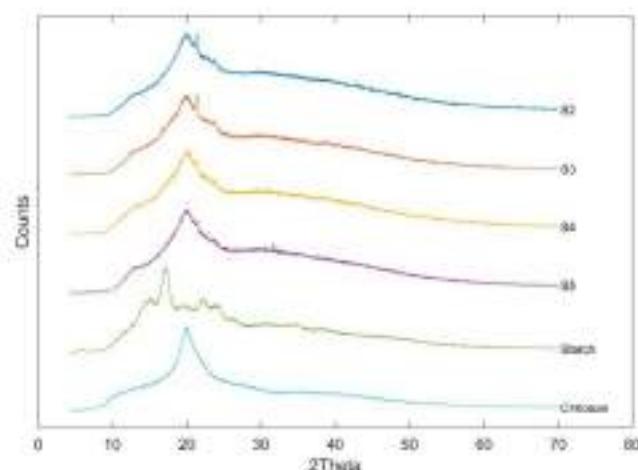


Figure 3. XRD diffractograms of ulluco starch, chitosan, and ulluco starch–chitosan edible films.

3.8. Fourier-Transform Infrared (ATR-FTIR) Spectroscopy of Starch and Edible Films

Interaction of the components of edible films was studied using the ATR-FTIR technique. Figure 4 shows the ATR-FTIR spectra of the US, Ch, glycerol, Tween[®] 80, and edible films. The absorption bands between 3600 and 3000 cm^{-1} observed in pure samples and edible films could be associated with the stretching of free hydroxyl groups [10]. However, this absorption region can also be related to the stretching of amide (N-H) present in the Ch [20]. Spectra also showed peaks at 2900 cm^{-1} associated with stretching-CH and -CH₂ and bending vibrations [46]. The interactions between components of a system are related to changes in the wavenumbers of the spectral peaks [45]. Compared to the ATR-FTIR spectra, a shift of the peaks of hydroxyl groups from US 3386 cm^{-1} and Ch 3427 cm^{-1} to 3288 cm^{-1} in the edible films was observed, which can be related to the formation of inter and intra-molecular hydrogen bonding between the components [27]. Peaks at 1645 and 1562 cm^{-1} in the edible films indicate hydroxyl-amino-group interaction (starch–Ch interaction) and Ch primary amine ionization, respectively [47]. Interaction of the components can be confirmed due to the shift of the peaks at 1656 cm^{-1} of Ch and 1650 cm^{-1} of the starch to 1645 cm^{-1} in the edible films [45]. In addition, it was observed that an increase in starch concentration reduces the intensity of the peaks at 1562 and 1645 cm^{-1} . Furthermore, edible films showed peaks at 1089 and 901 cm^{-1} characteristics of the crystalline starch structure and at 1043 cm^{-1} associated with the amorphous region [10].

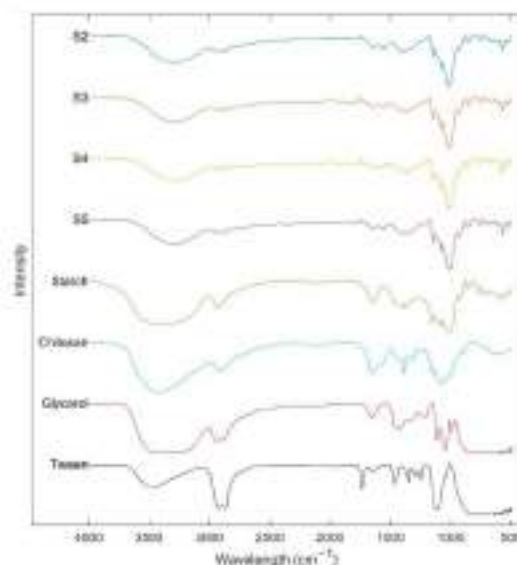


Figure 4. FTIR spectrums of uliaco-starch, chitosan, glycerol, tween 80, and uliaco starch-chitosan edible films.

3.9. Thermal Analysis

The DSC thermograms of US-Ch edible films and pure components are shown in Figure 5. Melting points (T_m) of US, Ch, glycerol, and Tween were 128.2, 113.4, 145.2, and 115.4 °C, respectively. As shown in Figure 5a, the endothermic peaks corresponding to T_m were 119.9, 121.1, 114.5, and 126.3 °C for S2, S3, S4, and S5, respectively (Table 3). Except for S4, the T_m of the samples showed an increase with the US concentration, which can be associated with the higher concentration of starch, resulting in greater interactions between the hydroxyl and the $\sim\text{NH}_3$ groups of the Ch [45]. This phenomenon can also be explained by MC, since water molecules can act as a plasticizer and weaken the intermolecular forces by hindering the alignment of the polymer chains [48]. Results obtained in this work were similar to those reported for edible films obtained from potato starch and Ch with peanut shell and skin extracts [49].

The thermal decomposition of the films and pure components was evaluated using a TG analysis. Figure 5b,c shows the weight loss and the first derivative of weight loss curves of samples and individual components. Thermal decomposition of film samples took place in three stages. The first stage occurred between 47.7 and 178.9 °C with a weight loss between 8.42% and 10.59%, and the maximum decomposition rate between 87.3 and 99.2 °C (Table 3). This loss of mass is associated with water evaporation inside the film [6]. The second stage of weight loss took place at a temperature between 259.9 and 389.3 °C with a weight loss between 60.74% and 62.57% at the maximum decomposition rate at temperatures between 313.8 and 319.8 °C, which is associated with the depolymerization of starch and Ch molecules and decomposition of functional groups of the Ch, glycerol, and starch, such as hydroxyl and amine groups [10,15]. The third stage occurred between 391.1 and 463.9 °C, with a weight loss between 4.80% and 5.20% and a maximum degradation rate between 418.5 and 419.2 °C; this stage can be associated with the decomposition of the Tween 80, which has a maximum decomposition temperature of 419.5 °C (pure component) (Figure 5c). Decomposition stages one and two showed a shift in the onset and maximum temperatures compared to the pure compounds, probably related to the interaction between Ch/US/water (stage 1) and interactions between Ch/US/Glycerol

(stage 2). In addition, an increase in the maximum decomposition temperature of the films was observed, related to an increase in the starch composition, which is due to the increased number of hydroxyl groups. The above improves the interaction between starch functional groups, water molecules, and functional groups of Ch, reinforcing the film network formed. This rise in the onset temperature of film degradation due to the interactions of its components indicates an increase in the stability of the edible films [6,15].

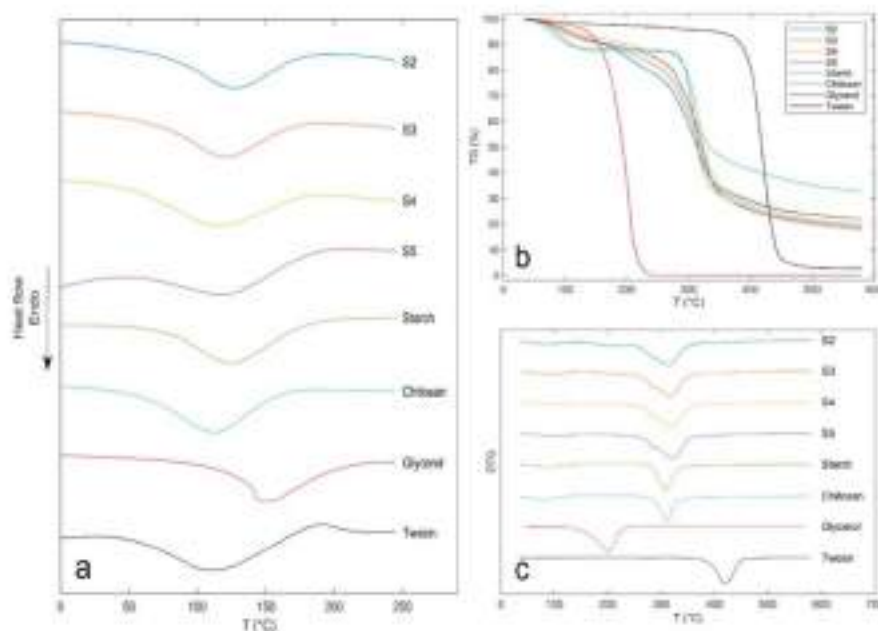


Figure 5. DSC thermogram (a), TG (b) and DTG (c) curves of ullico starch, chitosan, glycerol, tween 80 and ullico starch-chitosan edible films.

Table 3. Thermal degradation temperature of ullico starch-chitosan edible films and pure components.

Sample	T_{D} (°C)	Thermal Degradation Temperature (°C)						Total Loss (%)
		Peak 1		Peak 2		Peak 3		
		T_{onset} (°C)	T_{max} (°C)	T_{onset} (°C)	T_{max} (°C)	T_{onset} (°C)	T_{max} (°C)	
S2	119 ± 9.52	47.7	87.3	259.9	313.8	398.6	418.5	79.7
S3	121 ± 0.98	47.7	91.9	263.9	316.5	391.1	419.2	80.3
S4	114 ± 4.16	52.1	99.2	261.9	318.6	396.5	418.5	78.1
S5	126 ± 5.60	52.1	98.4	263.9	319.8	393.5	418.4	81.5
Glycerol	145 ± 1.99	60.2	100.9	230.7	273.4	-	-	97.5
Tween 80®	115 ± 9.34	392.4	419.5	-	-	-	-	110
Starch	128 ± 5.26	50.0	89.3	281.9	308.5	-	-	78.3
Chitosan	113 ± 0.66	45.7	82.6	282.4	312.2	-	-	67.7

4. Conclusions

Edible films based on US and Ch were successfully prepared, indicating a good interaction between the two polymers used. In general, an improvement in mechanical and thermal properties was observed with an increase in US concentration. In contrast, the

increase in starch led to a decrease in barrier properties, limiting the number of applications of US-rich packaging. On the other hand, low and medium concentrations of US together with Ch generated films with high barrier capacity. Concerning the visual attributes, all the films evaluated presented low opacity and yellow index, which facilitates their use in products that do not require high protection from light exposure and require the product to be viewed by the consumer. The good thermal properties of the US/Ch films are associated with their potential for use in a wide range of temperatures. The US presents great technological potential as a material for preparing environmentally friendly packaging focused on the food industry. The above can be affirmed due to its high extraction performance, low production costs, high amylose content, and ease of interaction with other polymers such as Ch. However, more studies are needed to better understand the US interaction with other polymers and bioactive compounds.

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CHAPTER 6

Effect of Essential Oils from Lemongrass and Tahiti Lime Residues on the Physicochemical Properties of Chitosan-Based Biodegradable Films

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Article

Effect of Essential Oils from Lemongrass and Tahiti Lime Residues on the Physicochemical Properties of Chitosan-Based Biodegradable Films

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Abstract: This work aimed to evaluate the impact of adding two essential oils (EO) from lemongrass (LEO) and Tahiti lime (TLEO) on the physical, mechanical, and thermal properties of chitosan-based biodegradable films. Six film formulations were prepared: two controls with chitosan concentrations of 1% and 1.5% w/v; two formulations combining the two chitosan concentrations with 1% LEO w/v; and two formulations combining the two chitosan concentrations with 1% TLEO w/v. The films' morphological, water affinity, barrier, mechanical, and thermal properties were evaluated. The films' surface showed a heterogeneous morphology without cracks, whereas the cross-section showed a porous-like structure. Adding EO to the films promoted a 35–50% decrease in crystallinity, which was associated with an increase in the elasticity (16–35%) and a decrease in the tensile strength (9.5–28.2 MPa) and Young's modulus (190–355 MPa) on the films. Regarding the optical properties, the opacity of the films with TLEO increased up to 500% and 430% for chitosan concentrations of 1% and 1.5%, respectively. While the increase in opacity for the films prepared with LEO was 307% and 187%, the reduction in crystallinity also reduced the resistance of the films to thermal processes, which could be explained by the reduction in the enthalpy of fusion. The thermal degradation of the films using TLEO was higher than those where LEO was used. These results were indicative of the great potential of using TLEO and LEO in biodegradable films. Likewise, this work showed an alternative for adding value to the cultivation of Tahiti lime due to the use of its residues, which is in accordance with the circular economy model. However, it was necessary to deepen the study and the use of these essential oils in the preparation of biodegradable films.

Keywords: biopolymers; food packaging; biodegradable films; bio-based material

1. Introduction

The high demand for plastic packaging derived from petroleum has generated worldwide concern due to the environmental impact that its use can produce. In this sense, the production of biodegradable and ecological packaging becomes an alternative to reduce the impact generated by non-biodegradable packaging since they offer characteristics such as a low cost of production, bioavailability, sustainability, and biodegradability [1]. In the food industry, different materials, such as starch, gelatin, proteins, and chitosan, have been evaluated as raw materials to produce edible films and coatings [2]. Among the materials used for this purpose, chitosan has been one of the most studied due to its different properties, such as its ability to form films, antimicrobial activity, odorless quality, tastelessness, and biodegradability [3]. Other authors have reported successfully preparing biodegradable films and edible coatings from chitosan and its application as packaging in different food

matrices [4–6]. However, chitosan-based coatings and films have some limitations that are mainly related to their mechanical properties due to high brittleness [7]. This has led researchers to seek alternatives to enhance the properties of chitosan films and coatings [8].

In addition, new consumer trends toward clean-label products and natural additives to the detriment of synthetic additives have awakened interest in active packaging with functional characteristics such as antioxidants, antibacterial, and antifungal [2]. Essential oils are secondary metabolites of plants and are primarily aromatic volatile organic compounds with biological activity [9]. These materials are highly recognized for their use in the cosmetic and medical industry due to their antiradical and antimicrobial potential. The above characteristics make essential oils (EO) an alternative to the synthetic additives used in the packaging industry [9]. Recently, different authors have evaluated the potential of chitosan films by including the EO of ginger [10], cinnamon, clove, and mustard [11], thus demonstrating the compatibility of materials and the improvement in some of their physicochemical and functional properties. However, the results observed in the literature are not conclusive, and each material and its properties depend to a large extent on the concentration and type, or source of the EO used. On the one hand, it was reported that the inclusion of orange EO, in different concentrations, to chitosan and gelatin films decreased tensile strength while increasing elongation at the break of the evaluated materials [3]. On the other hand, an increase in tensile strength was reported with a decrease in the elongation of chitosan films with the inclusion of different concentrations of cinnamon EO [12]. Therefore, there is a need to characterize and evaluate the effect of adding EO on the physicochemical properties of chitosan-based films.

The cultivation of Tahiti lime represents a productive sector of great importance worldwide, with an approximate production of 15 million tons per year [13]. However, its use and industrial exploitation generate a large volume of waste (approximately 50% of the total weight). Among the possible uses of the agro-industrial residues of Tahiti lime, the extraction of EO using different methodologies has been studied successfully [13,14]. Regarding the use of this EO as an active ingredient in the formulation of edible coatings, evaluations have been made due to its incorporation into chitosan-gelatin systems for the preservation of rainbow trout fillets [15] and in the preparation of gelatin-based coatings for shrimp life extension [16]. In both cases, an increase in the shelf life of the food matrices was observed. Despite the above, there are very few reports on the evaluation of edible films based on chitosan and Tahiti lime EO, which makes the evaluation of this type of formulation necessary.

On the other hand, the lemongrass (*Cymbopogon citratus*) EO (LEO), due to its high extraction yield and bioactive properties, has emerged as a profitable option for its use as an active principle in the preparation of biodegradable packaging with active potential [17]. Lemongrass essential oil has been successfully used as an active ingredient in the preparation of cassava starch films. Lemongrass essential oil has been successfully used as an active principle in the preparation of films. The addition of essential oil to a cassava starch film-forming solution improved the mechanical properties of the obtained films [18]. Perdana et al. [19] prepared biodegradable films based on starch (from cassava or sago) and chitosan (at different concentrations) with the inclusion of LEO and glycerol. The authors reported a reduction in the mechanical properties of the films with LEO inclusion. Additionally, the use of this coating on chilies increased the shelf life of the product by reducing weight loss and delaying ripening during storage.

Based on the availability of the Tahiti lime residues and lemongrass, as well as the high extraction yield and potential of LEO and TLEO as ingredients for the preparation of biodegradable films, this work aimed to evaluate the impact of the addition of these two EOs on the physical, mechanical, and thermal properties of chitosan-based films and compared the two ingredient systems.

2. Materials and Methods

2.1. Materials

The chitosan (Low molecular weight 50–190 kDa, Deacetylated 75–85%) was purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). The acetic acid (food grade) was purchased from Cimpa S.A.S. (Cimpa S.A.S., Bogotá, Colombia), and glycerol was purchased from Merck (Merck, Darmstadt, Germany). Lemongrass and Tahiti lime were purchased at a local market in the city of Ibagué (Colombia). The EO (LEO and TLEO) were obtained from the hydrodistillation method using a Clevenger apparatus.

2.2. Films Preparation

Chitosan at two concentrations (1.0% and 1.5% w/v) was dissolved in 1% v/v acetic acid under agitation for 1 h at 25 °C. Then, the chitosonium acetate solution was stored at 4 °C and allowed to stand overnight.

The films were prepared using the casting method described previously by Homez-Jara et al. [5], with some modifications. Six film formulations were prepared: the chitosonium solutions at 60 °C were mixed only with glycerol (1% w/v) to prepare the control samples (Ch1 and Ch15 films); they were also mixed with the EO of lemongrass (1% v/v) (Ch1L and Ch15L films) or Tahiti lime (1% v/v) (Ch1TL and Ch15TL films). Mixing was carried out for 2 min at 10,000 rpm with a homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany). Then, the solutions were vacuum filtered to remove excess bubbles. The solution (30 g) was poured into Petri dishes (diameter 8.5 cm) and dried at 40 °C for 24 h. The obtained films were stabilized for at least 24 h within a desiccator cabinet (DH-1002, Acequilab, Bogotá, Colombia) under a relative humidity (RH) of 54% (using magnesium nitrate salt) before analysis.

2.3. Morphology of Films

The surface and cross-sectional morphology of the films was examined using a microscope scanning electron microscopy (S-3400N, Hitachi, Düsseldorf, Germany), which operated at an accelerating voltage of 15 kV under a vacuum pressure of 40 Pa. The samples were placed on stubs over double-sided carbon tape. Then, the samples were covered with a gold layer using a rotary-pump coating system (Q150R, Quorum, Laughton, UK). The micrographs were obtained at different magnifications. For cross-sectional images, the samples were cryo-fractured under liquid nitrogen.

2.4. X-ray Diffraction (XRD) of Films

The X-ray diffraction patterns of the samples were obtained using a Bruker D8 ADVANCE diffractometer (Bruker, Billerica, MA, USA) with Ni-filtered Cu-K α 1 radiation (40 kV and 40 mA) and a specific wavelength of 1.5406 Å. Data collection was carried out in the 2 θ range of 3.5–70° (0.02035° of step size) and at a counting time of 0.8 s/step. Relative crystallinity was calculated using the DIFFRAC-EVA software (Bruker, Billerica, MA, USA).

2.5. Moisture, Swelling Power, and Solubility

The determination of the moisture content (MC), swelling power (SP), and solubility (S) were carried out by the gravimetric method reported by Homez-Jara et al. [5].

2.6. Water Vapor Permeability

Water vapor permeability (WVP) was determined using the ASTM method [20]. The samples were loaded into stainless steel permeability cells, where 6 mL of distilled water was deposited with an exposed area of 9.6 cm². The cells were placed in a controlled chamber (25 $\pm$ 2 °C and 75 $\pm$ 2% RH), and the weight was recorded every hour for 8 h. To ensure a uniform condition, a fan was placed inside the chamber. The RH was assumed to be maintained at 100% within the cells. The WVP (g m⁻¹ s⁻¹ Pa⁻¹) was calculated using Equation (1)

$$WVP = \pi FT / (AP_{sat}(RH_1 - RH_2)) \quad (1)$$

where m is the mass of the permeability cell (g) at time t (s); FT is the film thickness (m); A is the exposed area (m^2); P_{sat} is the saturation vapor pressure at the test temperature (Pa); RH_1 is the RH of the water contained in the cells, expressed as a fraction; and RH_2 is the RH of the chamber, which can be expressed as a fraction.

2.7. Optical Properties

The color parameters were assessed using a Minolta colorimeter (Cr 410, Konica Minolta, Tokyo, Japan). The results were expressed as a yellow index (YI) (CIELAB color space). Opacity (OP) was calculated using the absorbance of the material measured at 600 nm using a spectrophotometer (Genesys 10S UV-VIS, Thermo Fisher Scientific, Waltham, MA, USA) and Equation (2):

$$OP = Abs_{600} / FT \quad (2)$$

where Abs_{600} is the absorbance at 600 nm, and FT is the film thickness (mm).

2.8. Mechanical Properties

The mechanical properties of the films were evaluated using the ASTM D882 methodology [21]. Specimens with dimensions of 5.0 cm $\times$ 1.0 cm were loaded in a texturometer (LS1, Lloyd Ltd., Largo, FL, USA) equipped with a 1 kN load cell. The initial distance between the grips was 4 cm, and the speed of the analysis was 5 mm/min. The analysis was carried out at room temperature (-22 ± 2 °C). The tensile strength (TS, MPa), Young's modulus (YM, MPa), and elongation at break (EB, %) were calculated using Nexygen Plus software (Lloyd Ltd., Largo, FL, USA, Version 3.0).

2.9. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Fourier-transform infrared spectroscopy (ATR-FTIR) analysis was carried out using a Fourier-transform infrared spectroscopy (Tensor 27, Bruker Optics, Ettlingen, Germany) equipped with the Platinum ATR accessory (Bruker). The measurements were recorded over a range of 4000 to 500 cm^{-1} , with a resolution of 4 cm^{-1} and 40 scans at room temperature (-22 ± 2 °C).

2.10. Thermal Analysis

Thermogravimetric analysis (TGA) was performed using a thermogravimetric analyzer (SDT 2960, TA Instruments, Tokyo, Japan). Samples (5–10 mg) were loaded into porcelain microcapsules. The samples were heated at a temperature range between 30 °C and 600 °C, with a heating rate of 20 °C, under a nitrogen stream. The samples' weight loss (WL) and thermal degradation temperature (TD) were calculated using Universal Analysis 2000 V 4.5 A software (TA Instruments, Japan).

The differential scanning calorimetry (DSC) analysis was performed using a differential scanning calorimeter (DSC 3+, star system, Mettler Toledo, Columbus, OH, USA). The sample (approximately 5 mg) was placed into aluminum pans, which were hermetically sealed. Then, the samples were subjected to the following temperature program: cooling from room temperature to -60 °C at a rate of 20 °C min^{-1} , followed by heating up to 250 °C at a rate of 20 °C min^{-1} . The process was carried out in a nitrogen atmosphere using an empty pan as a reference. The melting temperature (T_m) and melting enthalpy (ΔH) of each sample were determined using Mettler-Toledo STAR[®] software, version 14.00 (Mettler Toledo, Columbus, OH, USA).

2.11. Statistical Analysis

The results were reported as the mean and standard deviation of at least three replicates. An analysis of variance was used to determine significant differences ($p < 0.05$) between the samples. Multiple range tests, with the Tukey–Kramer range test, were used to determine which means were significantly different ($p < 0.05$).

3. Results and Discussion

3.1. Morphology of Films

SEM micrographs of the film are shown in Figure 1. The control films obtained from chitosan and glycerol mixtures (Ch1 and Ch15) showed continuous, smooth surfaces with some irregularities and impurities. Likewise, the cross-section of these samples was homogeneous and without cracks. This morphology has been reported previously for films prepared with these ingredients, demonstrating high compatibility between these two materials [5]. Adding EO to the chitosan and glycerol mixture produced films with a surface without cracks but with heterogeneous morphology (bubble-like surface), indicating the partial separation of hydrophobic EO from the aqueous phase during drying. Additionally, the cross-section showed a porous-like structure, which was caused by the oil-in-water (o/w) emulsion. This phenomenon was more evident in films prepared with TLEO, which may be influenced by the chemotype of the EO or by the more significant interaction of the components of the films prepared with LEO. Similar observations on the morphology of the films prepared with chitosan, nisin, and *Perilla frutescens* EO (at 0.5%, 1.0% and 1.5% v/v) using an ultrasonic frequency and power of 40 kHz and 50 W have been previously reported [22]. The authors observed that with an increase in the EO concentration, the surface heterogeneity and porosity observed in the cross-section of the films increased. Additionally, a similar behavior was reported in chitosan films that had added lemon, cinnamon, and thyme essential oils. Although a homogeneous distribution of the EO droplets was observed in the materials, high concentrations of EO generated defects such as flocculation and coalescence, which led to an increase in pore size [23].

3.2. X-ray Diffraction (XRD) of Edible Films

The X-ray diffraction patterns and relative crystallinity values of the films are shown in Figure 2. All samples showed peaks at around 9.8° and 20° 2θ (Figure 2), which were associated with the anhydrous crystalline (crystals forms I) and aqueous crystal character (crystals forms II), respectively [24]. It was observed that the intensity of the peak corresponding to crystal form I increased in the samples prepared with both EOs compared to the control samples (Ch1 and Ch15), which presented a higher intensity in the peak corresponding to crystal form II. The increase in the intensity of the type I peak in the samples prepared with EOs. This may be due to the separation and partial disorder of the chitosan chains due to the addition of some chemical groups that were present in the EOs. These results confirm those obtained for films prepared by chitosan and *Perilla frutescens* (L.) Britt. EO, in which an increase in the separation of chitosan chains could be observed with an increase in the concentration of EO added [25]. As a result of the loss of order in the chains and internal structures, a decrease in relative crystallinity could be observed. In this work, the addition of TLEO and LEO in chitosan films caused a decrease in crystallinity (Figure 2). This effect was more evident in the films prepared with the lowest chitosan concentration (1%), which could be associated with the lower ratio of chitosan:EO. The reduction in the relative crystallinity of the films can have an effect on the physical properties of the material, such as mechanical and barrier properties [19], due to the lower reorganization of the polymer chains [26].

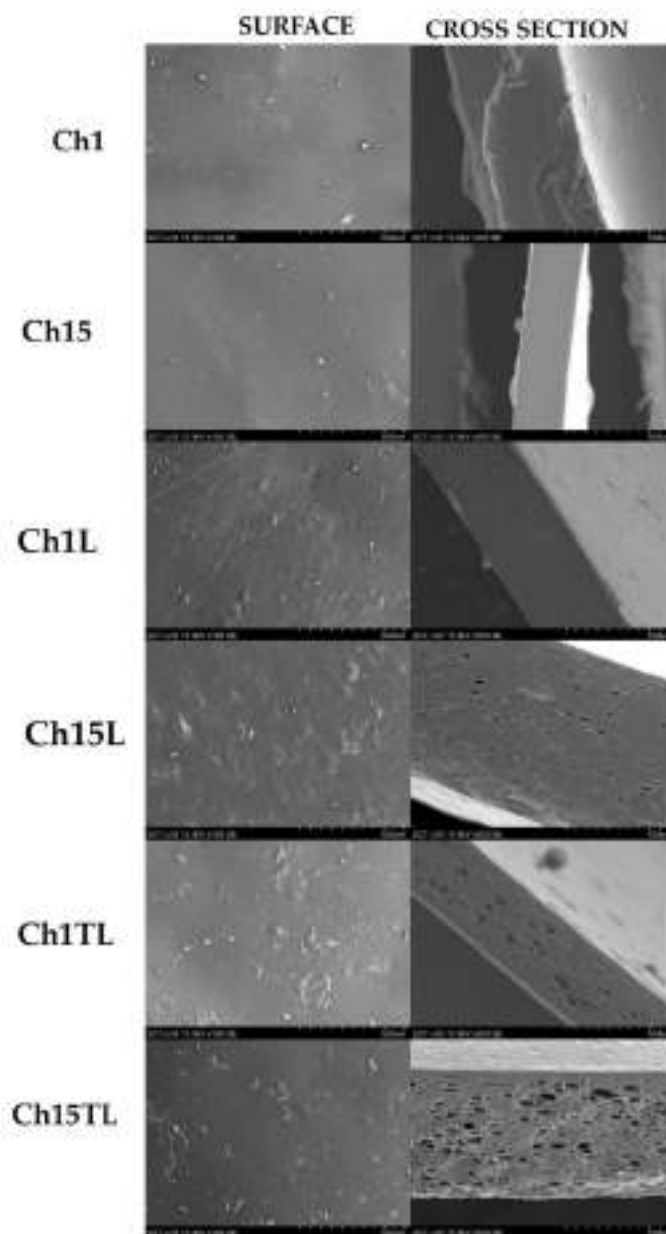


Figure 1. SEM micrograph of surface ($\times 100$) and cross-section ($\times 500$) of control films (Ch1 and Ch15); films with LEO (Ch1L and Ch15L); films with TLEO (Ch1TL and Ch15TL).

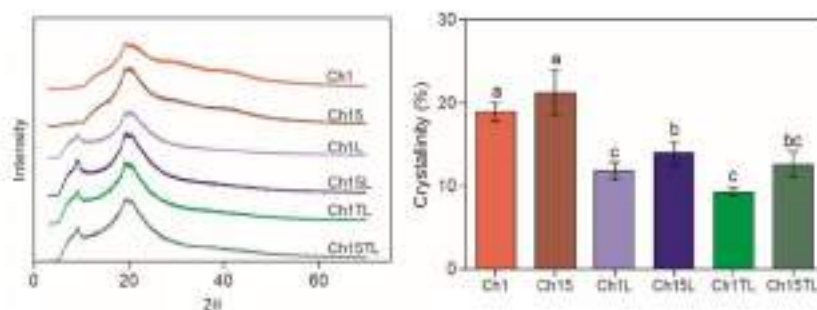


Figure 2. XRD diffractograms and crystallinity (%) of chitosan-essential oils films. Different letters for the same characteristic indicate significant differences among the samples ($p < 0.05$).

3.3. Moisture Content, Swelling Power, and Solubility

MC, SP, and S are properties related to the potential use of films as they can affect barrier and degradation properties [1]. The MC of the samples ranged between 23.9% to 33.1% (Figure 3). In all the films with a chitosan concentration of 1% (Ch1, Ch1L and Ch1TL), the MC was higher than the MC observed for films prepared with the highest chitosan concentration (Ch15, Ch15L and Ch15TL). A higher concentration of chitosan produced a more significant interaction between the components and the rearrangement of the structures due to hydrogen bonding and Van der Waals forces, which produced a more significant entrapment of the water molecules and decreased free water in the films. Similar improvements in water-holding capacity observations were made for films based on chitosan, sodium caseinate, and rosemary EO [27]. The authors attributed an increase in the film's moisture due to the existence of free hydrophilic groups from chitosan since the main interaction of EO was with caseinate.

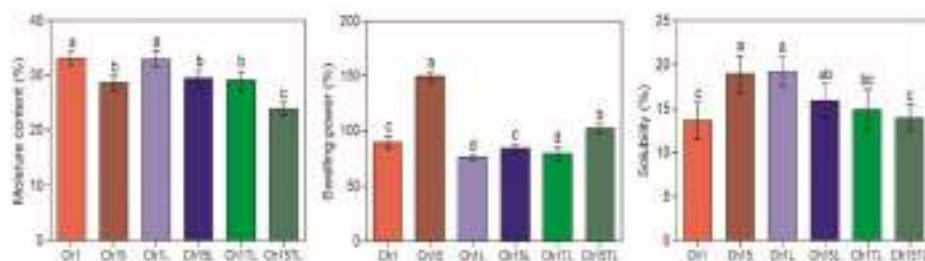


Figure 3. Moisture content (% wet basis), swelling power (%), and solubility (%) of chitosan-essential oil films. Means and standard deviations. Different letters for the same characteristic indicate significant differences among the samples ($p < 0.05$).

The SP of the films showed a decrease between 12% and 15% in the films prepared with 1% chitosan and LEO or TLEO, respectively, and between 33% and 44% in films prepared with 1.5% chitosan and LEO or TLEO, respectively ($p < 0.05$). SP has been related to the ionization of amino or carboxyl groups, the dissociation of hydrogen and ionic bonds, polymer relaxation, and crosslinking interactions between chitosan and EOs [23]. In addition, a greater SP was observed in samples prepared with a higher concentration of chitosan (1.5%) in both systems (LEO and TLEO). A reduction in SP was also observed for the inclusion of different concentrations of EO from lemon, thyme, and cinnamon in chitosan biodegradable films, which could be associated with the increase in cross-linked interactions between chitosan and the oil phase [23,28].

Regarding S , this parameter decreased in films prepared with the highest concentration of chitosan and EOs compared to the control. In contrast, the opposite happened for the films prepared with the lowest chitosan concentration (Figure 3). However, no statistical differences were observed between the same systems (LEO or TLEO) at any chitosan concentration. The non-specific trend of S may be due to heterogeneity due to the creaming or coalescence that EOs may undergo during the drying process of the films. Additionally, it was observed that the addition of TLEO generated a reduction in S compared to the materials, including LEO or the controls, which could be associated with greater resistance to dissolution in aqueous systems or in the presence of matrices with high moisture content. This behavior was also observed in films prepared with chitosan and with the addition of *Plectranthus amboinicus* EO. The authors observed that an increase in the concentration of the EO in films showed a tendency to decrease S up to a critical concentration where this parameter increased again [29].

3.4. Water Vapor Permeability

The WVP values of control films were $2.5 \pm 0.3 \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ (Ch1 film) and $2.41 \pm 0.19 \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ (Ch15 film), without observing significant differences ($p > 0.05$) between both samples. Similarly, adding EO to the formulation did not significantly affect the WVP ($p > 0.05$) of films. Despite adding a lipophilic component to the matrix that could decrease the affinity for water in the material (increase the hydrophobic nature), the barrier properties of the films depended on the sum of different factors such as crystallinity, oil distribution in the matrix, oil concentration, film homogeneity, and microstructure [27]. As observed in previous sections, the addition of LEO and TLEO increased the hydrophobic nature of the films, which led to an increase in the free space between the chitosan chains (more open structure) and a decrease in the crystallinity of the material. Thus, the net effect of adding lipophilic components to the films could be neutralized. Similar results were reported by Di Giuseppe et al. [27] for films based on chitosan, sodium caseinate, and EO from rosemary. The authors concluded that the permeability of the materials evaluated did not depend solely on the addition of lipophilic components but also on their microstructure, which could be affected by the physical state of the EO and its distribution in the polymer matrix.

3.5. Optical Properties

Optical properties such as OP and YI are helpful when evaluating packaging materials as they determine their potential use and consumer acceptability. It was observed that the addition of either of the two EOs significantly affected the optical properties of the films (Figure 4) ($p < 0.05$). Regarding opacity, the greatest increase in this parameter was observed in the Ch1TL sample, with an increase of more than 500% for the value observed by the control, followed by Ch15TL, Ch1L, and Ch15L, with gains of 439%, 357%, and 187%, respectively. The increase in opacity of these materials could be associated with a decrease in transparency due to the emulsion formation process causing a high turbidity blocking effect in the casting solution and, therefore, in the film [30]. This reduction in transparency with the addition of EOs has been previously reported for films prepared with chitosan and EOs of *Curat copticum* (340% opacity increase) [30], clove (119% opacity increase) [31], and rosemary (112% opacity increase) [27]. Although food packaging with low transparency can be associated with the presence of defects or with consumer rejection due to a negative evaluation of products, this type of packaging presents benefits such as barriers against UV-Vis light [31]. The packaging that offers this type of barrier represents an alternative that can be used in products susceptible to lipids and protein oxidation [32].

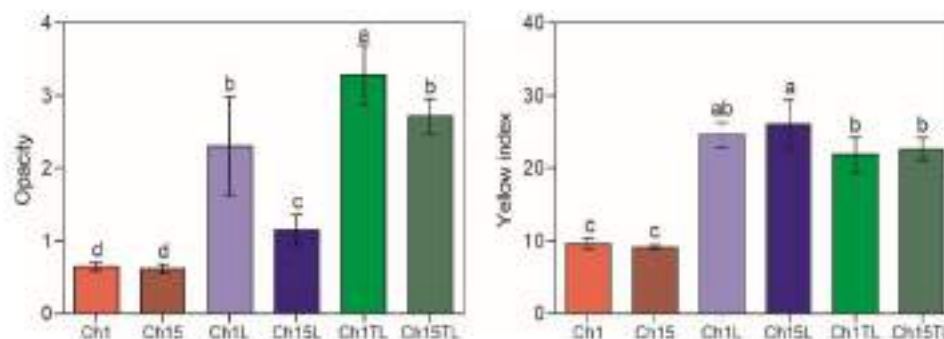


Figure 4. Opacity and yellow index of chitosan-essential oil films. Means and standard deviations. Different letters for the same characteristic indicate significant differences among the samples ($p < 0.05$).

The tendency to form films with a yellowish coloration is characteristic of samples prepared with chitosan and can be associated with β -1-4 linked 2-amino-2-deoxy-D-glucopyranose repeated units in its structure [33,34]. Similar to opacity behavior, the YI values of the analyzed samples significantly increased with the addition of EO (Figure 4). As expected, the greatest increase in YI was observed in samples prepared with EO and the largest chitosan concentration. The rise in YI could be associated with the color of EOs, the interactions of these with chitosan, and the higher opacity of the films. The values obtained for the control samples agreed with those reported for films prepared with pure chitosan [35]. However, they presented lower values than those reported by Jaleel et al. [30] for chitosan films, including 5% *Corum corymbosum* EO, but similar to those observed for films with the inclusion of nail EO at concentrations below 1% [31].

3.6. Mechanical Properties

The results obtained in the determination of EB, TS and YM are shown in Figure 5. The addition of EOs (LEO and TLEO) in the formulation improved the EB of tested samples compared to the control films by up to 200% ($p < 0.05$). Films prepared with LEO and TLEO showed better EB results than the control samples (Figure 5). The increase in EB with the inclusion of EOs was also observed in chitosan films prepared with the inclusion of D-limonene and orange EOs, with increases of up to 230% and 167% for films with the highest EO content (1% w/v in the case of D-limonene and v/v in the case of orange oil), respectively [3,36]. This suggests that adding EOs and forming an emulsion produces deformable droplets that can penetrate the film's matrix and increase the free space between the chitosan chains, thus increasing flexibility. Likewise, variable changes have been reported in the mechanical properties of films prepared with chitosan and the different EOs associated with these materials' chemical composition and source [23]. Peng and Li, [16] prepared chitosan films, which were added with lemon, thyme and cinnamon EOs, and observed an increase in TS in the films with lemon oil, while they observed a reduction in films with the other two types of EOs.

In contrast to EB, the addition of EOs to the formulation generated a decrease in the values for TS and YM. In the case of TS, the greatest reduction was observed in the samples obtained with LEO, with a decrease of 57% (1% chitosan samples) and 45% (1.5% chitosan samples) for the value of the control samples ($p < 0.05$). At the same time, the samples with TLEO showed a reduction in TS by 40% (1% chitosan samples) and 30% (1.5% chitosan samples) ($p < 0.05$). Regarding the YM, a significant decrease between 46% and 83% for the value of the control samples was observed ($p < 0.05$) (Figure 5). A reduction in these parameters was expected because they were associated with greater rigidity in the films. Therefore, a material with a higher elasticity could generally display a lower YM value. This

inverse relationship between elasticity with TS and YM has been previously reported for chitosan films enriched with the EO of lemon, thyme, cinnamon, and orange [3,12,23]. Thus, adding EO could reduce the interaction force between the polymer chains and, therefore, reduce their rigidity, which leads to a decrease in YM.

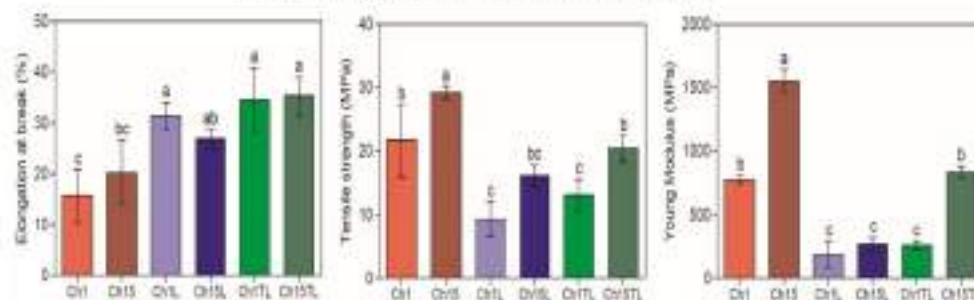


Figure 5. Elongation at break (%), tensile strength (MPa) and Young's modulus (MPa) of chitosan-essential oil films. Means and standard deviations. Different letters for the same characteristic indicate significant differences among the samples ($p < 0.05$).

3.7. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR was used to evaluate the possible interaction between materials in the formulation of chitosan-based biodegradable films. Figure 6 shows the spectra obtained for the samples analyzed. All samples presented a broad band between 3000 and 3700 cm^{-1} (for films, the band is between 3000 cm^{-1} and 3600 cm^{-1}), which is related to the stretching of intra and intermolecular hydroxyl groups ($-\text{O}-\text{H}$) and $-\text{CH}_2-\text{OH}$ stretching vibrations [30]. This band also can be related to the $-\text{N}-\text{H}$ stretching mode of chitosan, indicating an overlapping of these two vibration modes [5]. The bands observed at 2930 and 2870 cm^{-1} could be assigned to C-H bonds' asymmetric and symmetric stretching modes [29,35]. While the bands observed at 1640 cm^{-1} and 1585 cm^{-1} could be related to C=O stretching vibrations (amide I) and the N-H bending vibration mode (amide II), respectively. Absorption bands at 1405 cm^{-1} and 1374 cm^{-1} are related to C-N, N-H stretching modes, whereas bands at 1153 cm^{-1} and 1023 cm^{-1} correspond to the absorption of C-O-C groups [5,30]. The intermolecular interaction between chitosan and glycerol in the films can be evidenced because the inclusion of the latter generated the intensification of absorption bands in the range of 600–750 cm^{-1} corresponding to the bending vibration of hydroxyl groups. In addition, it produces a sharpening of the band, which can be related to the stretching vibration of the OH group (3000 cm^{-1} to 3700 cm^{-1}) and a shift in the band corresponding to the C-O stretching vibration from 1083 cm^{-1} in pure chitosan to 1023 cm^{-1} in the samples evaluated. These observations agree with the report for chitosan films and the inclusion of *Coriari copticum* EO [30]. The same authors also reported that the shift in the NH bending absorption peak of pure chitosan (1670 cm^{-1}) toward a shorter wavelength in the films might be due to the interaction between chitosan and EO, which could be observed in the films, including LEO and TLEO. The latter also confirmed what was observed in chitosan films, including *Ruta graveolens* EO [37].

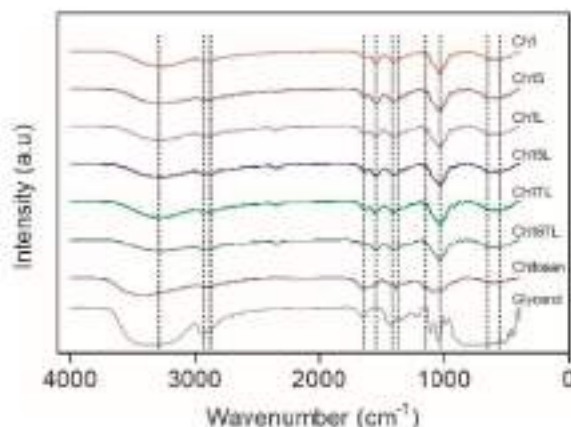


Figure 6. FTIR spectra of chitosan-essential oil films.

3.8. Thermal Analysis

The weight loss curves of chitosan-EO films are shown in Figure 7, and the total weight loss calculated from these curves is represented in Figure 8. The total weight loss due to thermal decomposition for the analyzed samples was from 78.1% to 86.3% (Figure 8). The decomposition took place in three stages for the control films (Ch1 and Ch15), which could be associated with the evaporation of absorbed water and residual acetic acid (49–116 °C) [30], the decomposition of glycerol (166–246 °C) [5], and the depolymerization of chitosan structures with the decomposition of functional groups such as hydroxyl and amine groups [22,33]. A significant increase in the total degradation was observed only in samples of TLEO inclusion (Ch1TL and Ch15TL) ($p < 0.05$). However, the inclusion of LEO did not significantly affect the total decomposition of films Ch1L and Ch15L.

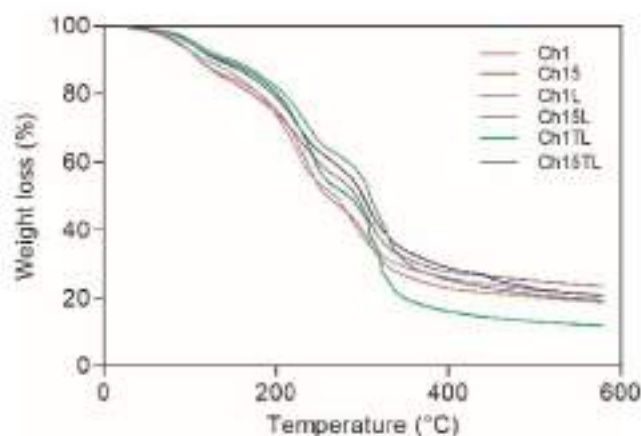


Figure 7. Weight loss curves of chitosan-essential oil films.

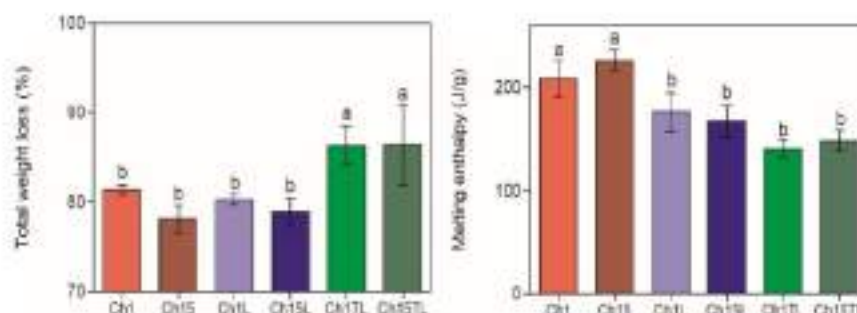


Figure 8. Total weight loss (%) due to thermal decomposition and melting enthalpy (J/g) of chitosan-essential oil films. Means and standard deviations. Different letters for the same characteristic indicate significant differences among the samples ($p < 0.05$).

The samples with EO added did not present significant changes in degradation stages I and II. However, the addition of TLEO in films Ch1TL and Ch15TL generated a degradation in two stages of decomposition stage III; this may be due to the interaction between EO and the chitosan chains since the latter could act as an encapsulating agent for the oil droplets once they had degraded, been released and allowed for the degradation of EO. While in the case of the samples added with LEO, a weak peak appeared above 422 °C (more evident in Ch15L than in Ch1L), which could have been related to the presence of aromatic residues from the EO. This same behavior was observed in chitosan films added with *Perilla frutescens* EO, and the intensity of the degradation peak could be associated with the EO concentration [22,25]. The results obtained in this work are consistent with those reported for chitosan films and *Curat copiticum* EO [30].

An endothermic peak corresponding to the melting of the samples was observed in all the samples. No significant differences were observed among samples regarding the melting temperature, and the average figure considering the samples was 124.0 ± 6.3 °C. Thus, including EOs in the preparation of films did not generate a significant shift in T_m . The estimated melting enthalpy (ΔH) of the films is also shown in Figure 8. Despite the above, it can be observed that the films with added EO showed a decrease in the ΔH value, which can be associated with the fact that the inclusion of EO in the samples generated a reduction in the degree of order, and therefore, in the degree of crystallinity (see previous section). Thus, the higher the crystallinity value, the greater the resistance to temperature the samples have [38]. These results are in agreement with the results obtained for chitosan films dried at different temperatures [5]. However, there are differences with those obtained for chitosan films by including ginger EO at concentrations of 0.1, 0.2, and 0.3% (v/v) [38]. Those differences could be due to the concentrations used to prepare the materials.

4. Conclusions

The physicochemical properties of biodegradable films based on a low molecular weight chitosan were affected by including EOs from lemongrass (LEO) and Tahiti lime (TLEO) in the formulation. However, these EO's had a neutral effect on the solubility and barrier properties. On the other hand, a reduction in the swelling power and crystallinity was observed, the latter associated with the loss of ordering of the polymer chains, which directly impacted the mechanical properties reducing the tensile strength and Young's modulus but also increasing the elasticity of the material. However, a marked effect was not observed in relation to the concentration of chitosan in the evaluated system. Considering the high brittleness of the films prepared only with chitosan, the increase in their elasticity is a desired characteristic for their use in the food industry. Additionally, the inclusion of LEO and TLEO demonstrated an increase in both the opacity and the yellow index. Regarding

opacity, the inclusion of TLEO presented a greater increase in this parameter compared to films prepared with LEO. Although these characteristics can be associated with negative consumer perception, they could be beneficial when preventing the damage or alterations of foods that are sensitive to photooxidation. Regarding the thermal properties, it was observed that the energy necessary for the casting of the films decreased with the inclusion of essential oils, which could be related to the loss of an organized structure or crystallinity. On the other hand, the addition of TLEO increased the total weight loss of the films, which may be associated with higher degradability. The use of TLEO as an active component in chitosan films demonstrated the potential of this EO and provided an alternative to the use of waste obtained from this material, which is in line with the circular economy trend. In general, the use of LEO and TLEO in the preparation of biodegradable films based on chitosan showed great potential. However, an optimization study is necessary to determine the best concentrations of both EOs, as well as the best concentration of chitosan for the preparation of biodegradable films.

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microstructural, textural, and rheological properties

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CHAPTER 7

3D printing of unconventional starches from Andean tubers: microstructural, textural, and rheological properties

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3D printing of unconventional starches from Andean tubers: microstructural, textural, and rheological properties

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Abstract

This work evaluated the potential of two unconventional starches in obtaining 3D prints. Different starch concentrations (8%, 10%, and 12% w/v) were assessed, and the microstructure, texture, crystallinity, and rheology were analyzed in the printed samples. The starches demonstrated good printability due to their moderate viscosities. The printed biomaterials at low and medium concentrations had good structural characteristics and formed cross-linked networks, which enhanced their water retention and textural properties, such as hardness. The printed samples also exhibited good resilience and low crystallinity, indicating a loss of the native starch diffraction pattern. The rheological properties of the printed samples presented an elastic behavior and a good fit to the power law model. The cubio and ulluco starches used in this study demonstrated great feasibility as an ingredient for 3D printing applications for the food industry.

Keywords: printability, gelatinization, non-conventional starches

1. Introduction

Three-dimensional (3D) printing is an emerging technology that produces physical structures by depositing a material or a material blend layer by layer, resulting in a product with predetermined size and shape characteristics (Carvajal-Mena et al., 2023; Ji et al., 2022). This technology offers various production advantages for the food industry, including product customization in shape, size, sensory and nutritional properties; optimization and automation; waste reduction; and cost-effectiveness improvement (Daza et al., 2023; Zheng et al., 2023). For instance, Kim et al. (2022) designed a low-calorie formulation to produce surimi by 3D printing with a protein analog, obtaining texture and salinity characteristics similar to those of commercial surimi. Similarly, Bebek et al. (2023) produced 3D printed snacks from strawberry products with antioxidant and antimicrobial potential, meeting nutritional needs and ensuring stability of the printed food during storage.

However, 3D food printing requires the design of “food inks” that meet nutritional requirements and the necessary characteristics to ensure physical and structural integrity by avoiding food collapse during production, transport, and storage. Therefore, the materials used for this purpose must have malleability, self-support, rapid solidification, and a specific level of fluidity that can generate a smooth extrusion from the printer’s extrusion head. Additionally, the materials must form a stable and resistant structure on the printing platform (Ji et al., 2022). Due to the limited availability of materials suitable for 3D printing of food, there is a need for alternative options that can meet the requirements of food design and enable the total industrial leverage of this technology (Zheng et al., 2023).

Starch is a primary carbohydrate in the human diet, as it is widely present in various plant-based foods and often accounts for 50-70% of dietary energy intake (Punia et al., 2020). Several authors have reported that starches from different botanical sources exhibit the typical shear-thinning behavior of a pseudoplastic fluid. This attribute is closely related to the ease of extrusion, which makes starch an attractive raw material for 3D printing (Rong et al., 2023; Singh & Kaur, 2017). Moreover, the retrogradation process that occurs after heating and gelatinizing the starch leads to

the leaching of amylose and amylopectin and their subsequent reorganization, resulting in improved hardness and shape retention of the 3D print (Galindez et al., 2019; Rong et al., 2023). The printability of starch is influenced by different factors such as amylose content, granule size, amylose: amylopectin ratio, pasting properties, and starch concentration, among others, which vary depending on the botanical source.

Although different types of starch have been tested for 3D printing of food products and their effects on rheological (Chen et al., 2019; Theagarajan et al., 2020), textural and printability properties (Bugarin-Castillo et al., 2023; Zheng et al., 2019) have been assessed, most studies have focused on commercial starches and there is still limited information about the potential of new or unconventional starchy materials that could offer advantages regarding food safety and added value.

Considering the need for new materials for the growing 3D food printing industry and the lack of information on the impact of these materials on the final product, this study aimed to prepare 3D prints at different concentrations of two unconventional starches obtained from ulluco (*Ullucus tuberosus*) and cubio (*Tropaeolum tuberosum*) and evaluate their physical and rheological properties.

2. Materials and methods

2.1 Raw material

Ulluco starch (ULS; apparent amylose content of 27%; relative crystallinity: 17.1%) and Cubio starch (CUS; apparent amylose content of 31%; relative crystallinity: 16%) were obtained and characterized as previously reported (Daza et al., 2022; Parra et al., 2022).

2.2 Sample preparation and 3D printing

The starch gels pastes were prepared by mixing the material at different concentrations (8%, 10%, and 12% w/v) with distilled water. The mixture was heated to 90 ± 2 °C with constant stirring using a mechanical stirrer (RZR 2021, Heidolph,

Germany) at 180 rpm for 10 min. After the sample reached a temperature of 60 °C, it was transferred to a print cartridge (nozzle of 1.5 mm). The 3D printing was carried out in an extrusion-based 3D printer (Foodini™, Natural Machines, Spain). A cuboid model with dimensions of 20 mm × 20 mm × 10 mm was printed with a monolayer height and nozzle movement speed of 1.4 mm and 500 mm × min⁻¹. For the dynamic-mechanical analysis (DMA), specimens with dimensions of 10 mm × 10 mm × 5 mm were printed. The samples were coded according to the starch source; ulluco starch was identified as ULS and cubio starch as CUS, followed by the corresponding concentration (Thus, the 3D printing of ULS at 8% will be called ULS8).

2.3 Microstructure of 3D prints

The microstructural characteristics of the 3D print samples were visualized using scanning electron microscopy (SEM/S-3400N, Hitachi, Germany). Prior to analysis, samples were lyophilized for 72 h and freeze-fractured using liquid nitrogen. Then, the samples were placed on stubs using double-sided carbon tape. Images were obtained at an accelerating voltage of 15 kV and under a vacuum pressure of 40 Pa at varying degrees of magnification.

2.4 X-ray diffraction

The X-ray diffraction XRD was carried out using a Bruker D8 ADVANCE diffractometer (Bruker, USA) operating with Ni-filtered under the condition of Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$ and at 40 kV and 40 mA). The 3D-printed samples were lyophilized and grounded using an analytical grinder universal mill (Yellowline A10, IKA, Germany). Data collection was carried out in the 2θ range of 3.5 – 70°, with a step size of 0.02035° (2θ) and a counting time of 0.8 s step⁻¹. Relative crystallinity was calculated using the DIFFRAC.EVA software (Bruker, USA).

2.5 Texture analysis

Textural analysis was performed using a universal test machine (Zwick/Z100, Zwick Roell, Germany). The gels were analyzed by being compressed twice to 40% of the

total sample height at a speed of $6 \text{ mm} \times \text{min}^{-1}$ using a cylindrical probe with an initial gap of 13 mm. The hardness, springiness, and cohesiveness were reported as the mean of at least five repetitions.

2.6 Dynamic-mechanical analysis

The dynamic mechanical analysis (DMA) was conducted using a dynamic mechanical analyzer (DMA, Q800, TA Instruments, USA) equipped with a parallel plate geometry (20 mm diameter and 10 mm gap). The samples with dimensions of $10 \text{ mm} \times 10 \text{ mm} \times 5 \text{ mm}$ were subjected to compression testing in a “frequency sweep” mode, ranging from 0.1 to 30 Hz at a fixed strain amplitude of $1 \pm 0.004 \%$ of the total sample height. The test was performed at room temperature ($24 \pm 2 \text{ }^{\circ}\text{C}$) (Yang et al., 2021). The resulting storage modulus (G') and loss modulus (G'') were obtained and analyzed using Universal Analysis 2000 V3.9A software (TA Instruments, Japan). The power law equation (Eq. 1) (Dong et al., 2019) was used to model the dependence of G' on frequency.

$$G' = K \cdot \omega^{n'} \quad (1)$$

where K is related to the viscoelastic properties of the material (power law constant) (MPa Hz^{-1}); ω is the frequency (Hz), and n' is the frequency exponent (dimensionless).

2.7 Statistical analysis

The results were reported as each property's mean and standard deviation based on at least three replicates for each sample. Analysis of variance (ANOVA) and Tukey–Kramer range tests at a significance level of 0.05 were performed to compare the properties of the 3D prints.

3. Results and discussion

3.1 Printability and morphology of 3D printed starch

The microstructure and visual aspect of the 3D prints extruded gel pastes are shown in Fig. 1. Samples prepared using ULS and CUS presented good stability and printability. However, all ULS samples showed poorly defined geometries with rounded shapes (low precision with the cubic model used). In contrast, the CUS samples had better-defined geometries, as evidenced by the presence of straight lines. However, rounded corners were also observed. Using either starch, the best geometry was obtained with the lowest material concentrations (ULS8 and CUS8). This behavior could be related to the higher viscosity of pastes with higher starch concentration, which could limit the ease of extrusion and formation of the printed figure. Similar observations were made for 3D printing of potato starch at different concentrations (0%, 1%, 2%, and 4%) combined with mashed potatoes, where it was evidenced that the sample without starch was easy to print but quickly lost structure. Moreover, the authors observed that low starch concentrations (1% and 2%) improved the printability of the gel pastes, which was related to the values of shear stress and the storage modulus of the gel paste (Liu et al., 2018).

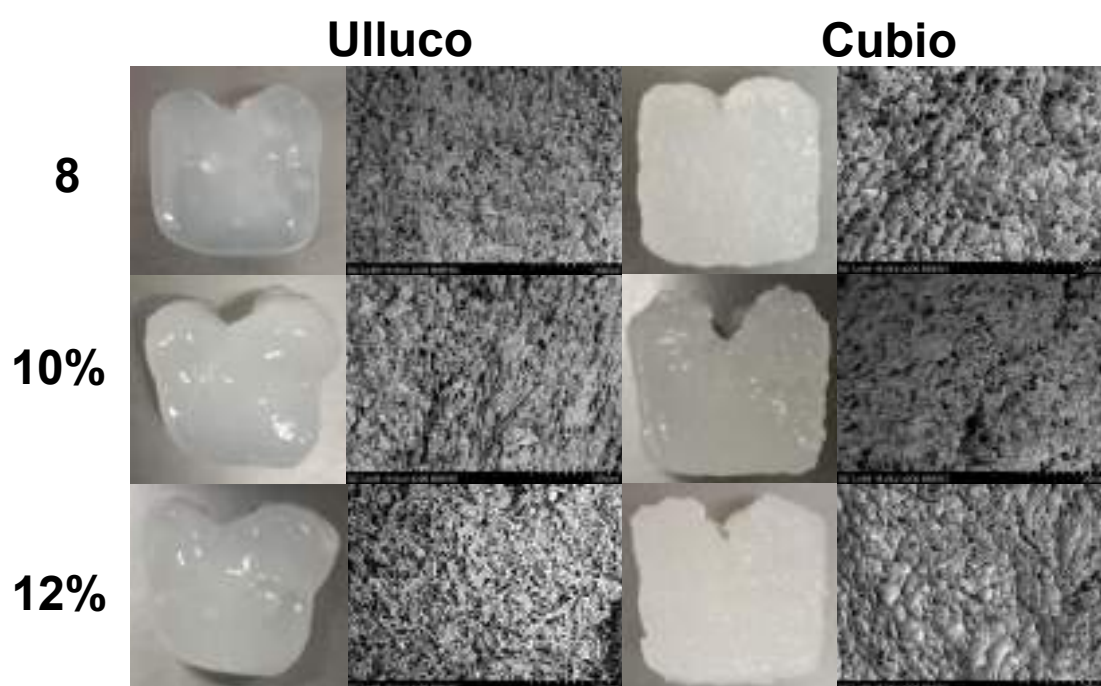


Fig. 1. 3D prints and SEM micrographs of 3D prints based on ulluco and cubio starches prepared at different starch concentrations: 8%, 10%, and 12%.

Micrographs of the samples showed the formation of reticulated, lamellar, or modular cell-like structures. Increasing the starch concentration in the samples prepared with ULS resulted in higher cell density in the structure. These structures can be associated with complete or partial starch gelatinization and cross-linked interactions between polymer chains (Liu et al., 2020). However, higher starch concentration also hindered gelatinization, and structures with incomplete gelatinization were observed, leading to material agglomeration. The degree of gelatinization depends on gelatinization time and process temperature and can affect the microstructure of the print. Cheng et al. (2023) demonstrated that low or high processing temperatures could affect the microstructural integrity of 3D-printed corn starch (15 min of gelatinization). The authors found that a temperature of 65 °C generated a partial gelatinization of the starch, and thus, the structure could not achieve adequate crosslinking; on the contrary, temperatures above 80 °C caused microfractures in the structure, which was associated with the presence of short chains that have less interaction capacity. Samples prepared with CUS had a similar cell structure to samples prepared with ULS but with a larger size. Except for the CUS10 sample, which presented a high cell density and porosity, the other samples prepared with CUS had a highly compact microstructure with decreased observed pores. Similar structures were observed in 3D printing using corn, wheat, and potato starches at a concentration of 25%. According to Zheng et al. (Zheng et al., 2019), more compact structures result in firmer prints with more resistance to loss of integrity. The microstructure of 3D prints can also affect other properties, such as the mechanical properties and overall integrity of the structure. However, the microstructure of 3D prints prepared with starches depends on different factors such as the processing conditions (temperature, equipment, printing cartridge) and the physicochemical properties of the starch (amylose and amylopectin content, pasting properties, size, or degree of polymerization (DP) of the chains, gelatinization temperature among others).

3.2 Crystallinity of 3D printed starch

The crystallinity patterns of the samples are shown in Fig. 2. The relative crystallinity values of samples prepared using ULS were 1.8%, 1.9%, and 2.0% for ULS8, ULS10, and ULS12, respectively. The relative crystallinity for samples obtained with CUS were 1.3%, 1.7%, and 1.9% for CUS8, CUS10, and CUS12, respectively. All samples displayed signals at 17° at a diffraction angle of 2θ . The presence of a single low-intensity signal associated with crystallinity patterns indicates the loss of the ordered structure of the native starch granule, which can be attributed to the gelatinization of starch granules during the thermal process, as well as the retrogradation of the amylose and amylopectin chains. Previous studies reported that the diffraction pattern of ULS and CUS corresponds to type-B with diffraction peaks at 5.6° , 15° , 17° , 20° , 22° and 24° 2θ (Daza et al., 2021; Galindez et al., 2019). Liu et al. (2020) evaluated the impact of printing temperature on the crystallinity of potato starch-based 3D prints. They observed that low printing temperatures (60°C) partially preserved the type-B diffraction pattern in the samples regardless of the starch concentration. However, at 70°C , the signals corresponding to the crystalline regions of the samples vanished completely. Ahmadzadeh & Ubeyitogullari (2022) also reported relative crystallinity (ranging from 5.64% to 8.78%) in 3D prints based on corn starch at different concentrations. This relative crystallinity is associated with the recrystallization of the amylose and amylopectin chains, and it can be related to the textural properties since a higher crystallinity implies a higher ordering of the polymer chains. However, other factors also affect the properties of the 3D printed materials, such as the size or degree of polymerization of amylopectin and gelatinization properties. In this work, no relationship was established between the relative crystallinity value of the samples obtained with ULS and the microstructure since a higher ordering was expected in the samples with higher starch concentration. However, the ULS10 and ULS12 samples showed partial gelatinization, so their relative crystallinity value was similar to that of ULS8, which may affect the textural properties (as observed in section 3.3). Additionally, the samples prepared with CUS showed a direct relationship between the relative crystallinity and the starch concentration.

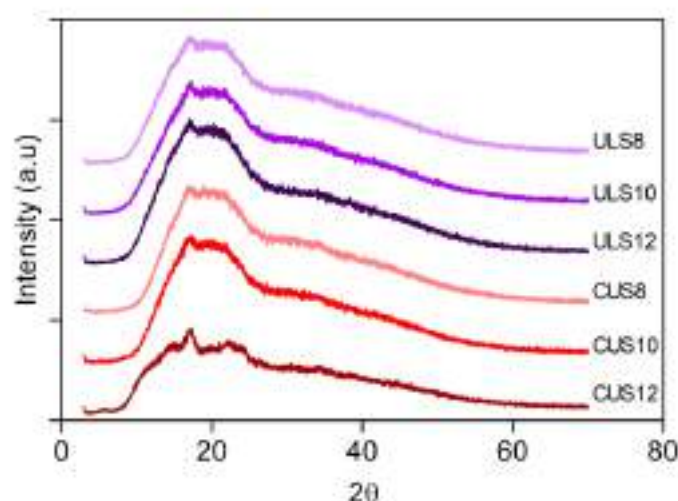


Fig. 2. XRD diffractogram of 3D prints based on ulluco and cubio starches prepared at different starch concentrations: 8%, 10%, and 12%.

3.3 Textural properties of 3D printed starch

The hardness, springiness, and cohesiveness of 3D samples based on ULS and CUS are shown in Fig. 3. Hardness is a parameter associated with shape retention and softness of the 3D material, which is important for sensory and technological aspects (Maniglia et al., 2020). The samples prepared with ULS had hardness values between 0.41 ± 0.02 N and 0.85 ± 0.10 N. In comparison, the samples prepared with CUS presented hardness values between 0.59 ± 0.03 N and 1.72 ± 0.24 N, with statistical differences observed between samples ($p < 0.05$). In both cases, intermediate starch concentrations (samples ULS10 and CUS10) resulted in the lowest hardness values, which could be related to a partial or insufficient gelatinization of the material, deficient formation of reticulated, lamellar, or modular microstructure, or a low interaction of the material chains. In addition, the samples based on ULS showed the highest hardness in ULS8 (the lowest concentration of starch), while the samples prepared with CUS showed the highest hardness in CUS12 (the highest starch concentration). The hardness results for the ULS samples can be explained by considering that the microstructural analysis revealed segments associated with the partial gelatinization of the ULS10 and ULS12 samples, which hindered the reorganization of the structures and led to low

compaction of the 3D printed. On the other hand, ULS8 had a more organized microstructure, indicating a higher degree of gelatinization of the material. For the samples prepared with CUS, the higher hardness of CUS12 could be attributed to the high degree of gelatinization achieved by the process conditions, which resulted in more amylose and amylopectin leaching and facilitated the chains' reorganization. As observed in the morphological analysis, this sample showed high compaction in the microstructure, leading to its greater hardness. Previous studies have associated the hardness of starch-based 3D printed materials with the retrogradation and subsequent recrystallization of amylose and amylopectin chains (Ji et al., 2022; Zheng et al., 2021). In addition, comparable hardness values were reported for 3D prints obtained with cassava (1.10 N), wheat (0.58 N), corn (1.21 N), sweet potato (0.64 N), potato (1.88 N), and buckwheat (1.56 N) starches (Ji et al., 2022). The authors associated the hardness of the materials with the presence of

amylopectin chains with low and high DP, finding a direct relationship between the DP of amylopectin and the hardness of the material.

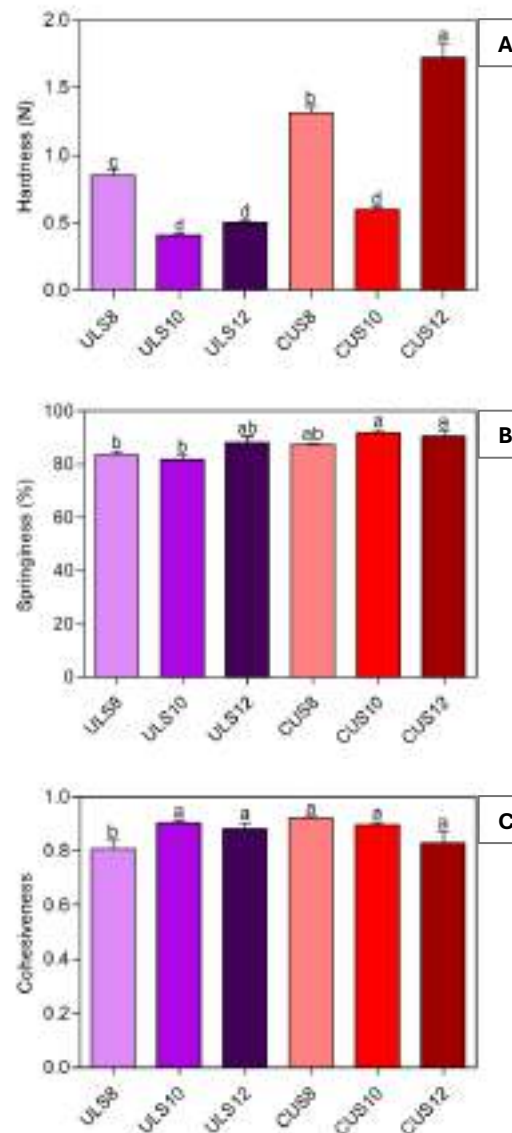


Fig. 3. Textural properties of 3D prints based on ulluco and cubio starches prepared at different starch concentrations: 8%, 10%, and 12%. Hardness (A), Springiness (B) and Cohesiveness (C)

The springiness attribute is related to the capacity of the 3D-printed material to recover its original shape after being subjected to a deforming force (Ji et al., 2022). No significant differences were found between the springiness values of 3D printed samples obtained using ULS or CUS ($p > 0.05$). However, differences between ULS8 and ULS10 were observed regarding the CUS10 and CUS12 samples ($p < 0.05$). All

samples generally had values above 0.8, indicating good material resilience, which could benefit the final product (Xu et al., 2020). The results obtained for the analyzed samples were in agreement with values reported for 3D printing (gelatinized starch at 85 °C for 20 min) obtained from native wheat starch (0.41) and modified using a heat treatment method for 2 h (0.41) and 4 h (0.41) (Maniglia et al., 2020). However, our results were higher than those obtained for 3D samples prepared with 6% potato starch, different alginate, and xanthan gum concentrations, with springiness values ranging between 0.29 and 0.65 (Cui et al., 2022).

Cohesiveness is associated with the force that maintains the structure of the 3D printed material through interactions or internal bonds between the amylose and amylopectin chains (Zheng et al., 2023). No statistical differences were found between the cohesiveness values of the evaluated samples ($p > 0.05$), demonstrating that using both starches in the established concentrations formed structures with resistance to fragmentation. The values reported in this work were similar to those obtained for 3D printing obtained from native wheat starch (0.7) and annealing wheat starch (0.8) but higher than the cohesion value of hydrothermally modified wheat starch (0.55), which the authors associated with the decreased in cohesiveness of hydrothermally modified starch due to a degradation of amylopectin chains (Zheng et al., 2023).

3.4 Dynamic mechanical analysis of the 3D prints

The storage (G') and loss modulus (G'') of the analyzed samples can be observed in Figure 4. Overall, all the samples exhibited a viscoelastic behavior, with a predominant elastic character, as indicated by the higher values of G' compared to G'' throughout the analysis. Previous studies have reported similar observations regarding 3D gels produced from commercial potato puree and whole milk (Martínez-Monzó et al., 2019). In this study, the authors evaluated three different formulations prepared to varying ratios of the components. They observed that the G' and G'' of these materials depended on the frequency. The G' observed for the analyzed samples presented a direct relationship with the hardness values

observed in the texture analysis, except for CU12, which showed a low G' value, indicating a possible syneresis process in this sample due to its high starch content. Previously, a direct relationship was reported between the values of G' and the hardness of 3D gels prepared with chachafruto starch and potato puree (Daza et al., 2023; Martínez-Monzó et al., 2019). This relationship was attributed to an increase in the strength of the internal structures of the 3D prints, as well as to the degree of gelatinization of the starch granules. The parameters K and n' were obtained by fitting G' to the frequency sweep (Table 1). The results showed that increasing the starch concentration in both starch systems leads to a lower value of K , which reflects the behavior of G' and the strengthening of the interactions among starch chains attributed to a higher degree of gelatinization. Regarding the parameter n' , similar values were observed for all the samples except for CU8. This can be attributed to the fact that the frequency dependence of the materials was mainly determined by the matrix or material (starch) rather than its concentration. This finding is consistent with previous observations made for 3D printed PLA materials with cellulose nanofibers, with or without annealing treatment (Dong et al., 2019). The authors reported that adding nanocellulose improved the elastic and viscous properties proportionally to its concentration. At the same time, the values of n' did not differ among the treatments, indicating that frequency dependence was not affected by the addition of nanocellulose.

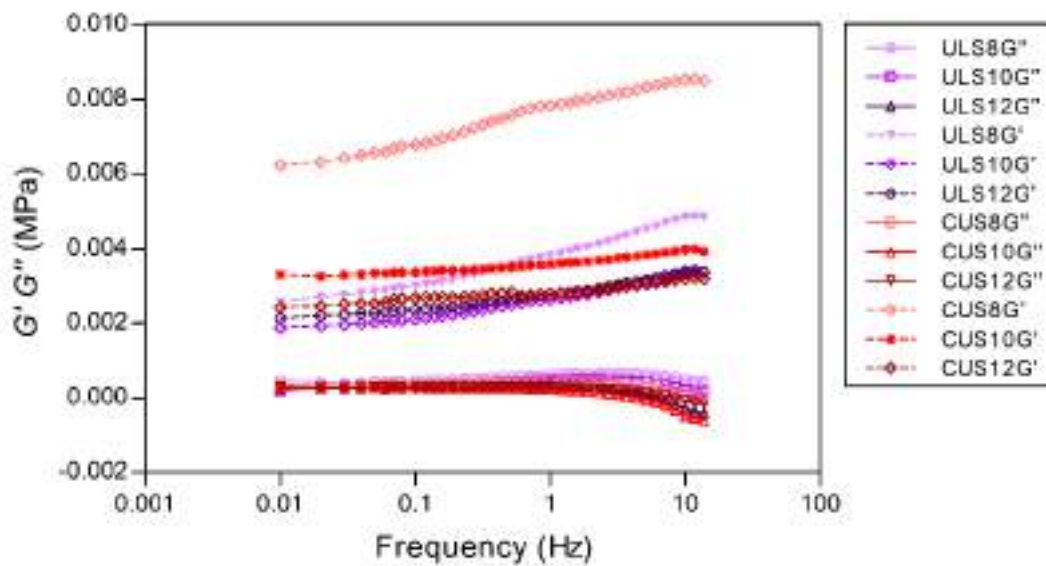


Fig. 4. Storage Modulus curves, and Loss Modulus curves of 3D prints based on ulluco and cubio starches prepared at different starch concentrations: 8%, 10%, and 12%.

Table 1. Power-law parameters for frequency sweep results of 3D prints based on ulluco and cubio starch.

Sample	K (MPa Hz ⁻¹)	n'	R^2
ULS8	3713 ± 15^b	0.151 ± 0.001^a	0.996 ± 0.00
ULS10	2512 ± 153^c	0.152 ± 0.001^a	0.988 ± 0.01
ULS12	2260 ± 393^c	0.152 ± 0.001^a	0.993 ± 0.00
CUS8	7895 ± 247^a	0.046 ± 0.003^b	0.985 ± 0.00
CUS10	4159 ± 583^b	0.151 ± 0.001^a	0.984 ± 0.00
CUS12	2497 ± 481^c	0.152 ± 0.001^a	0.975 ± 0.01

4. Conclusions

The unconventional starches of ulluco and cubio present great 3D printability associated with the pseudoplastic fluid characteristics of the formed gels. The concentration of starch within the formulations significantly influences the characteristics of the 3D prints, primarily due to material saturation, which limits gelatinization. When examining the microstructure of 3D prints, lower starch concentrations result in less densely distributed cells but offer greater organization and better printing precision. Conversely, higher starch concentrations lead to material agglomeration in certain areas, leading to decreased printing accuracy. In

terms of textural properties, all samples exhibit great resistance and resilience to deformation, significantly impacting the preservation of the physical structure of the food products produced through this technology. These samples consistently display viscoelastic behavior, with elastic properties predominating, making them comparable to 3D materials obtained by using commercial starches. Further research should focus on assessing the interaction of these starches with other components, particularly with those that can be included in the production of customized foods or enhancing the value of the printed products. The results obtained in this work demonstrate the potential of both ulluco and cubio starches as a material for the 3D food printing industry. Additionally, they consolidate this technology as a viable alternative for adding value to the chain of these crops. Ultimately, the good performance of these starches encourages further exploration and research to expand our understanding of their applications in 3D printing.

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Statements and Declarations

Competing Interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution. Luis Daniel Daza Ramírez: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Writing—original draft. Cristina Reche: Formal analysis, Writing – original draft. Angélica Sandoval-Aldana: Methodology; Project administration; Resources; Supervision; Writing—original

draft. Henry Alexander Váquiro: Conceptualization; Methodology; Project administration; Resources; Supervision; Writing—original draft. Valeria Eim: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision. All authors reviewed the manuscript.

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Effect of the addition of Chachafruto flour on the stability of oil-in-water emulsions and the physicochemical properties of spray-drying microcapsules	
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Abstract:	This study aimed to assess the stability of Chachafruto flour (CHF) as a stabilizing agent for an oil-in-water emulsion and its impact on the physicochemical properties of the emulsion after spray drying. Emulsions with varying CHF concentrations (0%, 1%, 3%, and 5%) were prepared and compared to a control. The results from the emulsion stability test (centrifugation) and storage stability test (4 weeks) showed that the highest emulsion stability was achieved with 5% CHF, attributed to its protein content (30.1%). The emulsions exhibited enhanced stability, both in terms of homogeneity and sedimentation, in the buffer. The emulsion coated with CHF and water activity (aw) 0.95 was associated with water stability. The emulsions coated with CHF showed good encapsulation properties. FTIR confirmed the presence of characteristic peaks during the encapsulation process, according to the quality. Furthermore, the addition of CHF improved the thermal stability of the emulsions. This study represents the first investigation on the emulsifying properties of Chachafruto flour.

CHAPTER 8

Effect of the addition of Chachafruto flour on the stability of oil-in-water emulsions and the physicochemical properties of spray-drying microcapsules

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Effect of the addition of Chachafruto flour on the stability of oil-in-water emulsions and the physicochemical properties of spray-drying microcapsules

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Abstract

This study aimed to assess the suitability of Chachafruto (*Erythrina edulis* Triana ex Micheli) flour (CHF) as a stabilizing agent for an oil-in-water (O/W) emulsion and its impact on the physicochemical properties of the emulsion after spray drying. Emulsions with varying CHF concentrations (2%, 3%, and 4%) were prepared and compared to a control. The results from the creaming index and particle size analyses indicated that the highest emulsion stability, even after 168 h, was achieved with 4% CHF, attributed to its high protein content (20.5%) and Z-potential. The resulting encapsulates exhibited spherical and rough surface morphologies but without holes on the surface. Additionally, low moisture content (MC <5%) and water activity (a_w <0.2) were associated with excellent powder stability. The encapsulates added with CHF showed good reconstitution properties, mainly characterized by preserving the initial particle size of the emulsions. Fourier-transform infrared spectroscopy confirmed the absence of chemical interactions during the encapsulation process, contributing to the high stability. Furthermore, the addition of CHF improved the thermal stability of the encapsulates. This study demonstrated that CHF represents an industrial alternative for emulsion stabilization, mainly at high concentrations (4%) due to its excellent protein and starch content, without the need for purification of the protein fraction. Likewise, this work presents an additional use alternative for chachafruto cultivation.

Keywords: Erythrina, emulsion stability, encapsulation, dry emulsions

1. Introduction

The encapsulation of oil and lipophilic compounds has been used as an alternative to extend the shelf life of the core ingredients. In the food and pharmaceutical industry, encapsulation is achieved through the implementation of chemical (coacervation, molecular inclusion, and co-crystallization) or physical methods (spray-drying, extrusion, freeze-drying, vacuum drying, spray-cooling or chilling, and fluidized bed coating) (Sonawane, Bhanvase, Sivakumar, & Potdar, 2020). Among these methods, spray drying is considered one of the most suitable techniques for encapsulation purposes. The encapsulation of lipophilic compounds by spray drying comprises a two-stage process (Esparza, Ngo, & Boluk, 2020). In the first stage, a stable oil-in-water (O/W) emulsion is prepared with suitable viscosity characteristics for the drying equipment (Umaña, Turchiuli, Rosselló, & Simal, 2021b). Meanwhile, in the drying stage, the O/W emulsion is fed through an atomizer and is put in contact with either a co-current or counter-current hot gas flow responsible for the dehydration of the sample until it becomes a powder (dry emulsion) (Daza et al., 2016). However, different drawbacks can negatively impact the stability of the emulsions before and during the drying stage, such as coalescence, creaming, sedimentation, flocculation, oiling off, and Ostwald ripening (Esparza et al., 2020; Yan et al., 2020). Therefore, emulsion stabilization is an essential step in producing dry emulsions.

Emulsifiers are ingredients that can be adsorbed to droplet surfaces and reduce the interfacial tension, thereby facilitating droplet breakup and favoring emulsion stability (Yan et al., 2020). At the industrial level, synthetic emulsifiers such as glycerin fatty acid esters, polysorbates, sorbitol anhydride fatty acid esters, sucrose fatty acid esters, and soybean ester phospholipids and others are commonly used (Nagtode et al., 2023; Soni et al., 2023). However, using these additives in different foods or processed products can negatively impact the overall quality of the products, such as food taste. More recently, the adverse impact of these additives on consumer health has been proven (Gao et al., 2016; Partridge et al., 2019). In this context, the search for new natural, renewable, clean-label, and environmentally

friendly sources of emulsifiers has become a challenge for researchers (Burgos-Díaz et al., 2018; Francisco et al., 2020; Umaña et al., 2021b). The emulsifying properties of plant proteins, including soybeans, peas (Francisco, de Oliveira Júnior, Marin, Alvim, & Hubinger, 2020; Ribeiro, Morell, Nicoletti, Quiles, & Hernando, 2021), and cereals (Rosenberg, Rosenberg, & Zhang, 2018) have been widely studied as have shown good emulsifying properties mainly due to their surface activity and fast interfacial adsorption kinetics.

Chachafruto (*Erythrina edulis* Triana ex Micheli) is a legume native to South America cultivated mainly at altitudes between 1,600 and 2,000 m above sea level. This plant is characterized by the production of elongated pods that contain seeds used for human nutrition due to their high protein content (18–25%) (Intiquilla et al., 2019; Vilcanqui-Pérez, Chaquilla-Quilca, Sarmiento-Casavilca, Céspedes-Orosco, & Ventura-Saldivar, 2022). Nonetheless, to date, studies on Chachafruto have focused on obtaining peptides with biological activity (Guerra-Almonacid, Torruco-Uco, Murillo-Arango, Méndez-Arteaga, & Rodríguez-Miranda, 2019; Intiquilla et al., 2019). Palma-Albino et al. (2021) prepared hydrolysates from various Chachafruto flour protein fractions using a sequential process with digestive and microbial enzymes. These hydrolysates exhibited significant antiradical activity and inhibition of the angiotensin-converting enzyme (ACE), as well as good anti-diabetic properties by inhibiting the enzymes α -amylase, α -glucosidase, and dipeptidyl peptidase 4 (DPP-IV). Furthermore, a significant neuroprotective effect was reported. On the other hand, the use of Chachafruto flour as a substitute for wheat flour in bread preparation was evaluated, observing that the product elaborated with Chachafruto showed better nutritional properties, such as an increase in protein and crude fiber content, without affecting the physical and sensory properties (Vilcanqui-Pérez et al., 2022). These findings demonstrate the great potential of this legume flour to be used as raw material in food processes. However, there is a notable lack of research specifically addressing the technological aspects of Chachafruto, including its emulsifying properties and the potential for application in food colloidal systems.

Considering the limited number of studies on the emulsifying potential of Chachafruto flour and its high protein content, this work hypothesizes that the flour obtained from Chachafruto can be used successfully to stabilize an O/W emulsion and its subsequent spray drying. Accordingly, the aim of this study is to evaluate the stability of the emulsion prepared using Chachafruto flour and the physicochemical characteristics of the powders obtained after spray drying.

2. Material and methods

2.1 Raw material and characterization

Chachafruto pods were harvested on the farm "El Mirador," located in the village La India, Coello Cocora, Ibagué, Colombia (4°22'14.2"N, 75°19'16.4"W). Samples were selected and washed, and those exhibiting any mechanical damage were discarded. The samples were sliced and dried at 40°C in a forced-air oven for 72 h, ground (ultra centrifugal mill ZM 200– Retsch®, Germany), and sieved to a particle size <0.05 mm. The Chachafruto flour (CHF) was stored under refrigeration conditions until its use. Commercial sunflower oil ($\rho = 0.9095 \text{ g}\cdot\text{mL}^{-1}$) was purchased from a local store (Palma de Mallorca, Spain).

The CHF was analyzed to determine its protein (method 920.87), lipids (Method 920.85), moisture (Method 925.10), and ash (Method 923.03) contents (AOAC, 2000). The total carbohydrate content was obtained by subtracting the sum of the protein, lipid, moisture, and ash contents from 100. Color was evaluated using a Konica Minolta colorimeter (Cr 410, Minolta Co., Japan). Results were recorded according to the CIELAB color system (L^* : darkness to lightness, a^* : greenness to redness, and b^* : yellowness to blueness). The morphology of the CHF was evaluated using scanning electron microscopy (S-3400N, HITACHI, Germany) at an accelerating voltage of 15 kV under vacuum pressure of 40 Pa and different magnifications (500× and 1000×). For the analysis, samples were placed on stubs using double-sided carbon tape and covered with a gold layer. The total phenolic compounds (TPC) and the antioxidant capacity were determined using the Folin Ciocalteu, DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging, and ferric reducing antioxidant power (FRAP) assays (Daza, Fujita, Granato, Fávoro-Trindade, & Genovese, 2017).

2.2 O/W emulsion preparation

Maltodextrin ($11 \leq \text{DE} \leq 14$) (Glucidex® 12, Roquette, France), was dissolved in distilled water using a magnetic stirrer (200 rpm and 40°C) to prepare the emulsions.

The CHF at different concentrations (Table 1) was added to the MD/water solution and mixed for 30 min to hydrate the protein. The resulting mixture was homogenized for 10 min at 16000 rpm using an Ultra-Turrax® digital disperser (T25 Digital, IKA, Germany). Then, the sunflower oil was added, and the mixture was homogenized for 15 min at 16000 rpm. The resulting emulsions were labeled as CHE1 (2% CHF), CHE2 (3% CHF), and CHE3 (4% CHF). The control emulsion (CE) was prepared similarly but with the addition of Tween®20 (Panreac AppliChem, Barcelona, Spain) instead of flour. To maintain the temperature of the samples below 40°C, an ice bath was used in the homogenization stage.

Table 1. Composition of emulsions prepared using chachafruto flour and control emulsion (CE)

Component (% w/w)	CE	CHE1	CHE2	CHE3
Water	60.00	60.00	60.00	60.00
Maltodextrin	34.86	33.00	32.00	31.00
CHF	-	2.00	3.00	4.00
Tween® 20	0.14	-	-	-
Sunflower oil	5.00	5.00	5.00	5.00

CHF: Chachafruto flour; CE: control emulsion; CHE1: emulsion with 2% of CHF; CHE2: emulsion with 3% of CHF; CHE3: emulsion with 4% of CHF

2.3 Emulsion characterization

The apparent viscosity (η) of emulsions was measured using a rotational viscometer (ST-DIGIT R, J. P Selecta, Spain) equipped with an R3 spindle (up to 1000 cPs at 100 rpm) and in a speed range from 6 to 100 rpm. The pH and conductivity of the emulsions were measured directly on the samples using a pH meter (Crison, pH 25, Spain) and a conductivity meter (HI 9033, Hanna Instruments, USA) at $25 \pm 1^\circ\text{C}$. The Z-potential was measured using a Nano Zetasizer (Malvern, Nano ZS90, UK); for this measurement, the emulsions were diluted (1/500), and the pH was adjusted to the original value when necessary.

2.4 Emulsion stability

The samples to determine the creaming index (CI) were poured into a 10 mL graduated cylinder and left to stand at room temperature. The total height (HT) and the height of the serum (HS) layer at the bottom of the cylinder were measured at different times with a Vernier caliper. Results were obtained using Eq. (1):

$$CI = (H_S/H_T) \times 100 \quad \text{Eq. (1)}$$

The droplet size distribution of the emulsions was determined by laser diffraction analysis at different time intervals (0, 2, 4, 6, 8, 24, 48, and 168 h) using a laser particle analyzer (Sync Microtrac MRB, wet operation Flowsync, Montgomeryville, PA, USA). Samples were stored in sealed vials at room temperature ($25 \pm 2^\circ\text{C}$).

Before the analysis, the samples were manually homogenized by gently rotating the containers. The refractive index values used for the dispersing water and the sunflower oil droplets were 1.330 and 1.475, respectively. Results were reported as diameters at the 10th percentile (d_{10}), median percentile (d_{50}), and 90th percentile (d_{90}). In addition, the span factor was calculated using Eq. (2).

$$\text{Span factor} = (d_{90} - d_{10})/d_{50} \quad \text{Eq. (2)}$$

2.5 Spray drying of emulsions

The prepared emulsions were spray dried at 170°C (inlet temperature) using a Buchi B-290 spray dryer (Buchi, Switzerland). The spray drying conditions were adjusted as follows: rate of aspiration of 32 m³·h⁻¹ (80%), pumping at 8.3 mL·min⁻¹ (25%), and an atomization air flow rate of ~0.83 m³·h. Sample atomization was carried out using a two-fluid nozzle atomizer with an opening of 0.7 mm, and the nozzle cleaner was set to 2 strikes per min. The outlet temperature was 100 ± 3°C. The samples were mixed continuously, utilizing a magnetic stirrer during the entire process. Each treatment was obtained in triplicate. For identification purposes, the following notation was employed: encapsulation control (EC), encapsulation with 2% CHF (E1), encapsulation with 3% CHF (E2), and encapsulation with 4% CHF (E3).

2.6 Microcapsules characterization

2.6.1 Encapsulation efficiency and physicochemical characterization

The encapsulation efficiency was carried out according to the methodology described by Umaña et al. (2021b). The moisture content (MC) of the samples was determined using a moisture analyzer (MB90 Halogen, Ohaus, Switzerland). The water activity (a_w) was measured employing a dew point hygrometer (Pawkit®, Aqualab, USA). The morphology of the powders was evaluated through scanning electron microscopy (SEM) according to the methodology described in section 2.1. The particle size distribution of the dry emulsions was determined by laser diffraction granulometry (Sync Microtrac MRB, dry operation TurboSync, Montgomeryville, PA, USA). The hygroscopicity and solubility of powders were established according to Daza et al. (2016). The color characteristics were measured as described in section 2.1. The total color change (ΔE) was calculated to compare each powder containing CHF with the EC using Eq. (3).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \text{Eq. (3)}$$

2.6.2 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples were obtained using a Fourier-transform infrared spectrometer (Nicolet 380, Thermo Scientific Inc., USA). The measurements were

recorded over a 4000 to 500 cm^{-1} range, with a resolution of 4 cm^{-1} and 40 scans at room temperature ($\sim 20.4^\circ\text{C}$). For the analysis, pellets were prepared by mixing and homogenizing the sample and potassium bromide (KBr).

2.6.3 Thermal analysis

The weight loss (WL) and thermal degradation temperature (TD) of the samples were assessed using a thermogravimetric analyzer (SDT 2960, TA instruments, Japan) according to the method described by Daza et al. (2023). A temperature range between 30°C and 600°C was used, with a heating rate of $20^\circ\text{C min}^{-1}$ under a nitrogen stream.

2.7 Statistical analysis

The results were reported as mean and standard deviation for at least three replicates. An analysis of variance (ANOVA) at a 95% confidence level was used to determine statistically significant differences. Multiple range tests were carried out using the Tukey-Kramer range test to determine which mean results differed significantly from others.

3. Results and discussion

3.1 Flour characterization

The physicochemical properties of CHF are detailed in Table 2. This flour is primarily composed of carbohydrates and proteins. The carbohydrate content can be associated with the high content of compounds such as starch. A high efficiency of starch extraction in Chachafruto has previously been reported (Daza et al., 2023). Further, comparable protein levels (18-25%) have also been reported beforehand for this material (Intiquilla et al., 2019). Additionally, CHF exhibits low lipid content and moderate ash content. The observed ash content aligns with findings from other legume-derived flours, such as pea flour (Sridharan, Meinders, Bitter, & Nikiforidis, 2020). Moreover, the presence of phenolic compounds with antioxidant capacity was registered. This attribute could positively influence the preservation of the encapsulated oil and contribute to its functional characteristics.

Table 2. Physicochemical composition and antioxidant capacity of Chachafruto flour (CHF)

Parameter	Value
Chemical composition	
Moisture content (%)	11.86 ± 0.05
Lipids (% db)	0.78 ± 0.01
Protein (% db)	16.38 ± 0.15
Ashes (% db)	4.53 ± 0.40
Carbohydrates (% db)	78.31 ± 0.34
Antioxidant capacity	

TPC (mgGAE/g)	5.15 ± 0.29
DPPH (mmol Trolox/g)	7.60 ± 0.65
FRAP (μmol Trolox/g)	8.28 ± 0.59
CIELab* color coordinates	
<i>L</i>	62.69 ± 0.57
<i>a</i> *	7.33 ± 0.07
<i>b</i> *	12.24 ± 0.18
Other parameters	
Solubility (%)	33.1 ± 3.3

TPC: total phenolic content; GAE: gallic acid equivalent; *L*: CIELab* coordinates (Luminosity); *a**: CIELab* coordinates (redness/greenness); *b**: CIELab* coordinates (yellowness/blueness). Values are expressed as means ± standard deviation of at least three repetitions.

3.2 Emulsion characterization

The η of the emulsions (25°C, $\gamma=3.3 \text{ s}^{-1}$) was $95 \pm 1 \text{ mPa}\cdot\text{s}$, $120 \pm 20 \text{ mPa}\cdot\text{s}$, $173 \pm 23 \text{ mPa}\cdot\text{s}$, and $237 \pm 6 \text{ mPa}\cdot\text{s}$ for CE, CHE1, CHE2, and CHE3, respectively ($p<0.05$). The higher viscosity observed in the emulsions containing CHF was expected as this material is rich in polymers such as polysaccharides and proteins (Santamaria, Garzon, Moreira, & Rosell, 2021; Umaña, Wawrzyniak, Rosselló, Llavata, & Simal, 2021c). The liquid feed viscosity is a property that can influence different characteristics of the final product, such as particle size, morphology, and homogeneity. The higher the viscosity, the larger the particle dimensions and the lower the homogeneity (O'Sullivan, Norwood, O'Mahony, & Kelly, 2019). Besides, high viscosities can cause atomizer clogging, preventing an adequate evaporation process and thus reducing the encapsulation performance (Esposito et al., 2020). However, the higher viscosity of these emulsions is interesting since it could positively affect their stability by hindering the mobility of the droplets.

Fig. 1. shows the pH, Z-potential, conductivity, and CI of the prepared emulsions. The pH values ranged from 5.1 to 5.9. The CE registered the lowest value with significant differences with respect to the emulsions containing CHF ($p<0.05$). However, no statistical differences were observed between the CHE1, CHE2, and CHE3 samples ($p>0.05$). The results observed in this work were lower than those observed for O/W emulsions stabilized using mushroom by-products at different concentrations (1.5–7.5% w/w), which recorded a pH range between 6.83 and 6.89 (Umaña, Turchiuli, Eim, Rosselló, & Simal, 2021a). As the authors mentioned, the differences between the pH values may be due to variations in the amino acids composition in the protein of the flours used to stabilize the emulsions.

All samples presented Z-potential values lower than -30 mV, indicating that the surface of the particles is negatively charged. The CHE2 and CHE3 emulsions

showed the highest Z-potential values (absolute value) with significant differences compared to CE and CHE1 ($p < 0.05$). The Z-potential is a physical property of suspended particles and can be related to the physical stability of the emulsion. High Z-potential values (absolute values) are associated with greater emulsion stability. According to Sridharan et al. (2020), values below -30 mV provide strong electrostatic repulsion, reducing the probability of coalescence in the emulsion prepared. The differences in Z-potential may be linked to the conductivity of the emulsions, as higher concentrations of CHF lead to increased levels of dissolved minerals and ions in the medium. Previously, it has been reported that including NaCl, which dissociates into Na^+ and Cl^- ions in an aqueous medium, in emulsions stabilized with carboxymethyl starch/xanthan gum can increase the conductivity of these emulsions. This increase in conductivity enhances electrostatic repulsion, reduces the size of droplet particles, and raises the Z-potential (Cai, Du, Zhu, & Cao, 2020). The results of this work agree with those previously reported by Umaña et al. (2021a), who observed a rise in the conductivity of an O/W emulsion with an increase in the concentration of flour obtained from mushroom by-products, associating this behavior with the increase of ions in the system.

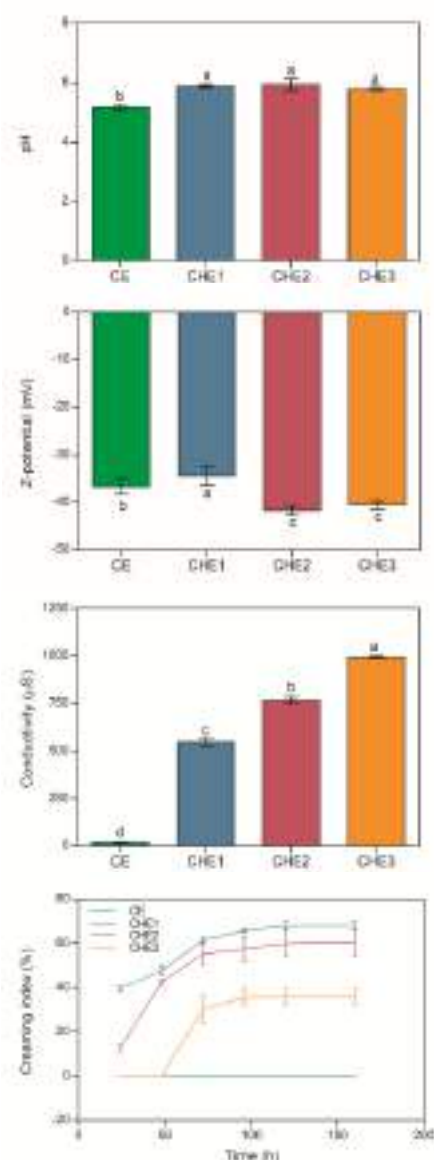


Fig. 1. pH, Z-potential, conductivity, and Creaming index (CI) of control emulsion (CE) and emulsions prepared with 2% (CHE1), 3% (CHE2), and (CHE3) of Chachafruto flour (CHF).

3.3. Emulsion stability

The concentration of CHF in the emulsion significantly influenced the CI values; the higher the flour concentration, the greater the stability of the emulsion during the storage time (lower CI values) ($p < 0.05$). The emulsion with the lowest CHF (CHE1) concentration showed a low stability value with a CI close to 40% in a short storage period (24 h). In contrast, CHE2 presented a low CI with a value about 2.5 times lower than CHE1 (about 15%). Conversely, CE and CHE3 did not show phase separation in the first 48 h, indicating emulsion stability. At the end of the storage period (168 h), CHE1 and CHE2 presented CI values above 50%, while CHE3 showed greater stability than CHE1 and CHE2, with a CI lower than 40%. However,

the control emulsion sample was the most stable since it did not exhibit the formation of a serum layer and maintained stability throughout the storage period. It is possible to correlate the viscosity of emulsions prepared with CHF with their stability. As mentioned earlier, the higher the concentration of the added material, the more it hinders the mobility of fat droplets, thereby preventing emulsion coalescence. Similar observations were made for emulsions stabilized by adding oat and eggplant flours (Rawal, Annamalai, Bhandari, & Prakash, 2023; Zhu et al., 2020). In both studies, the authors noted that higher flour concentrations led to reduced phase separation, associating it with an increase in viscosity due to the increase in solids in the formulation, as well as the increase in the effective density of the oil droplets due to the adsorption of amphiphilic molecules on its surface (Zhu et al., 2020).

The particle size distributions of the emulsions at different storage times are shown in Fig. 2. To evaluate the stability of these emulsions and avoid the influence of insoluble particles present in the CHF, the results were analyzed by subtracting the particle size distribution corresponding to a CHF suspension prepared using the concentration of each treatment (2%, 3%, and 4%) from the particle size distribution of each emulsion. Percentiles d10, d50, and d90, as well as the span factor of the samples at the beginning (0 h) and at the end (168 h) of the storage period, are presented in Fig. 3. At time 0 h, all samples showed a unimodal distribution with similar d10 ($>1.0 \mu\text{m}$) and d90 ($>5 \mu\text{m}$) values ($p>0.05$) and with differences in d50 ($>2 \mu\text{m}$) only in the CHE2 sample ($p>0.05$). Additionally, samples CHE2 and CHE3 exhibited a narrower distribution, with significantly lower span values than CE and CHE1. During the first 48 h of storage, a noticeable shift to the right and a broadening of the CE and CHE1 particle size distributions were evident. A slight shift was also observed in CHE2, whereas CHE3 showed no changes in the initial hours of storage. The most significant increase in particle size occurred at 168 h in samples CE and CHE1. The CE sample exhibited a transition from a unimodal to a bimodal distribution, while the CHE1 sample maintained a unimodal distribution but experienced a considerable widening. This widening could be linked to the instability noted in CI of CHE1. The d50 value at 168 h increased approximately 1.4 and 3.5 times compared to the initial value at 0 h for CE and CHE1, respectively ($p<0.05$). However, no significant increases were observed for CHE2 and CHE3. A similar trend was observed for the d90 and span values, with d90 diameter increases of 2.8 and 6.6 times for CE and CHE1, respectively. For its part, the span value increased by more than 50% only for CE and CHE1.

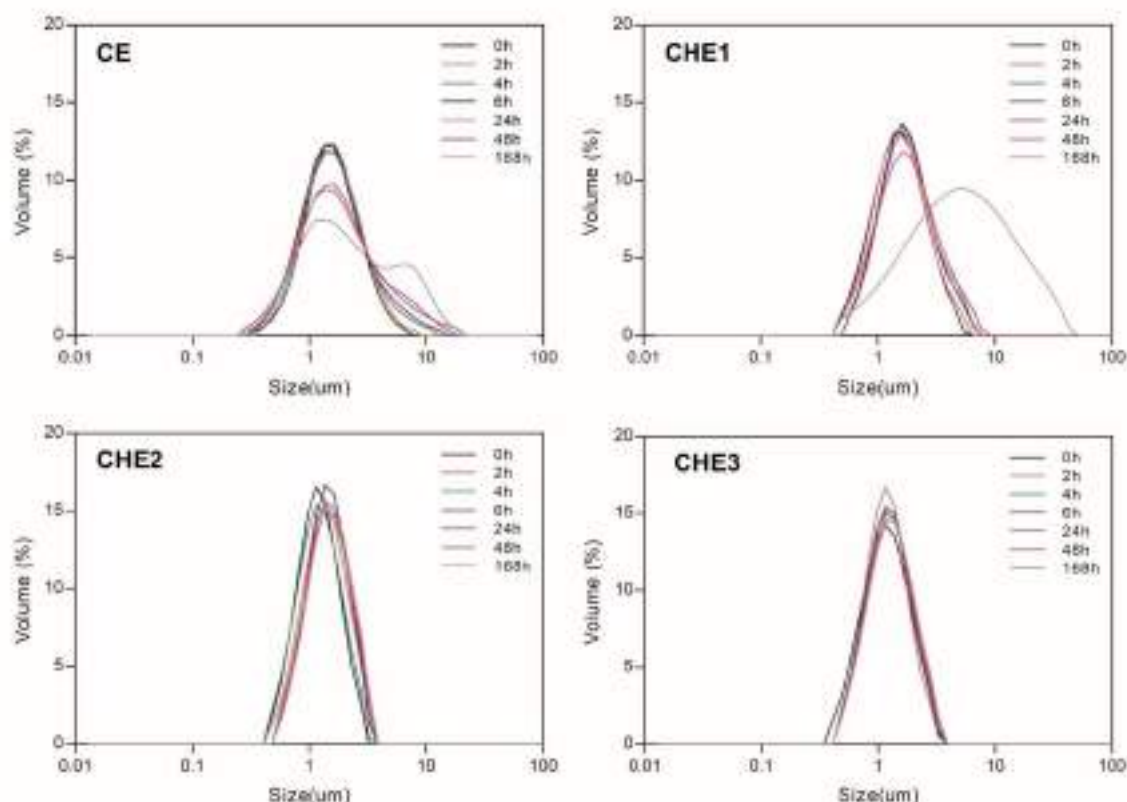


Fig. 2. Particle size distribution of control emulsion (CE) and emulsions prepared with 2% (CHE1), 3% (CHE2), and (CHE3) of Chachafruto flour (CHF).

Overall, an increase in CHF concentration increased particle size stability, with maximum stability obtained using 3 or 4% of the flour. The stability of the emulsions evaluated can be associated with the lower d50 at time 0 h for CHE2 (1.15 μm) and CHE3 (1.21 μm) compared to the d50 values observed for CE (1.35 μm) and CHE1 (1.38 μm), since, as described by Stokes' law, a decrease in the particle size prevents gravitational separation and aggregation of the oil droplets (Hong, Kim, & Lee, 2018b). Using a higher amount of CHF can reduce the adsorption time of emulsifier compounds, such as proteins or starch, on oil droplet surfaces (Hong et al., 2018b). Moreover, as previously discussed, a high emulsifier concentration can increase the emulsion interface and generate a higher packing density. This prevented the flocculation and coalescence of droplets, resulting in the long-term stabilization of dispersed droplets (Zhu et al., 2020). Furthermore, the increase in the viscosity of the continuous phase resulting from high flour contents decreases the fluidity between the droplets, thus delaying or preventing oil aggregation, coalescence, or sedimentation in droplets, producing more stable emulsions (Hong, Cheng, Gan, Lee, & Peh, 2018a; Zhang & Li, 2022; Zhu et al., 2020).

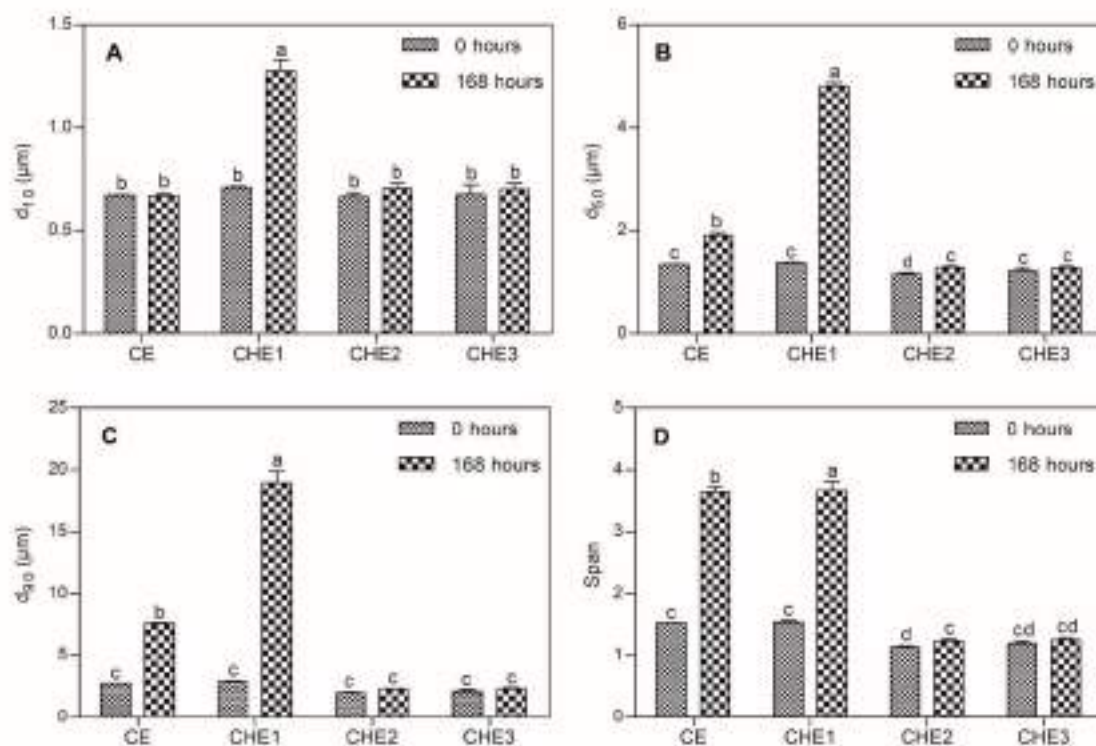


Fig. 3. Particle size distribution parameters of control emulsion (CE) and emulsions prepared with 2% (CHE1), 3% (CHE2), and 3% (CHE3) of Chachafruto flour (CHF).

The findings of this study align with those previously reported for emulsions stabilized with pea flour (Sridharan et al., 2020). The authors noted that emulsions prepared with a higher concentration of flour (protein) resulted in droplets with smaller particle sizes (2.5 μm) compared to emulsions prepared with lower concentrations of proteins (10 μm). The authors explain that a low concentration of proteins is inadequate for covering the interface formed during emulsification, and thus, the droplet size is determined by the number of proteins rather than the energy input from homogenization. Conversely, at a protein concentration that adequately covers the interface (>0.2% of protein), the droplet size becomes independent of the protein amount.

3.4. Microcapsules characterization

3.4.1 Physicochemical properties of microcapsules

The SEM micrographs of CHF, CE, and the encapsulated powders are presented in Fig. 4. Starch granules (indicated by green arrows) and potential protein aggregates were observed in CHF (red arrows), which can be associated with the high protein and carbohydrate contents of CHF. Similar observations were made by Sridharan et al. (2020) in pea flour samples where the presence of starch and protein granules

was evidenced. Overall, the encapsulates exhibited heterogeneous, irregular, and spherical morphologies, with some particles showing dented and jagged surfaces while others appeared smooth. The morphologies observed in this study are likely influenced by the drying temperatures employed in spray drying, which typically exceed 100°C. This high temperature leads to rapid evaporation, creating a hard crust that preserves the initial spherical shape of the particles. Previous research indicates that lower (~150°C) or intermediate (~170°C) drying temperatures result in particles with jagged surfaces, whereas higher temperatures (>190°C) can produce smooth particles with surface holes, potentially compromising nucleus stability. In addition, particle surface irregularities enhance the dispersibility and rehydration of the encapsulates (Bajac et al., 2022).

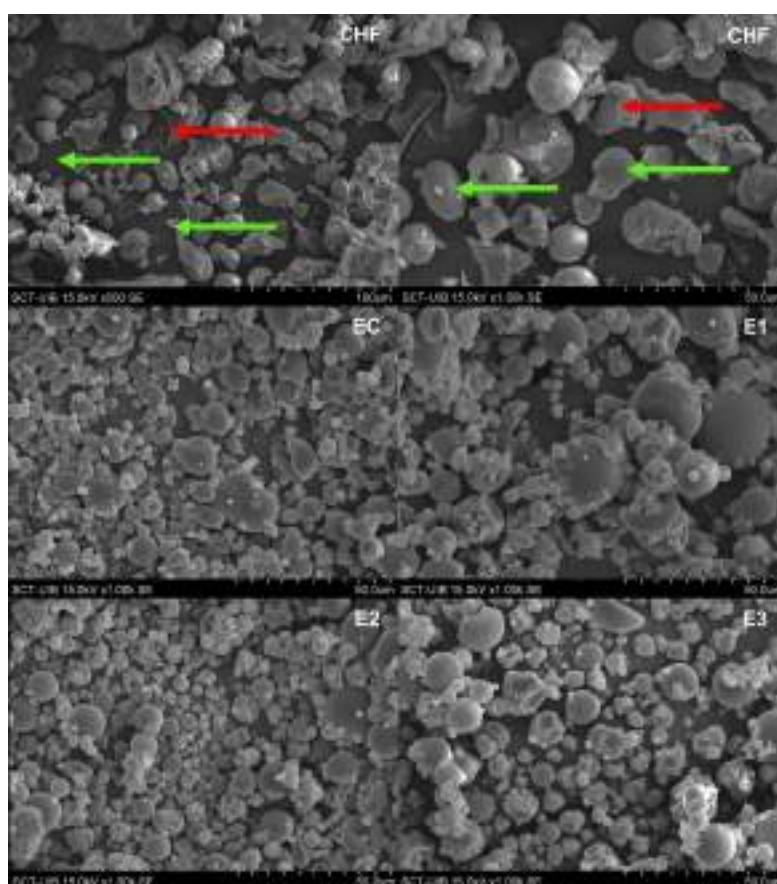


Fig. 4. Scanning electronic microscope images of Chachafruto flour (CHF), encapsulated control (EC) and encapsulated prepared with 2% (E1), 3% (E2) and 4% (E3) CHF.

The MC, a_w , solubility, and hygroscopicity of the capsules evaluated in this study are shown in Table 3. The MC was significantly higher in E2 and E3 compared to EC and E1 ($p < 0.05$). This behavior may result from a higher CHF content, which can increase the concentration of compounds with high water-holding capacity, such as starch and proteins (Daza et al., 2023; Premi & Sharma, 2017). However, all samples

exhibited MC values lower than 5%, consistent with previous reports of microcapsules obtained by spray drying (Umaña et al., 2021c). The a_w of the encapsulates was lower than 0.2 without significant differences between the samples ($p>0.05$). A recent study established that an a_w value below 0.6 results in minimal or no growth of microorganisms (Sukri, Putri, Mahani, & Nurhadi, 2023). Despite the low solubility of CHF (Solubility <35%), its inclusion in the emulsions had no impact on the hygroscopicity or solubility of the resulting encapsulates ($p>0.05$). Based on the established classification for powder hygroscopicity [non-hygroscopic (<10%), slightly hygroscopic (10-15%), and hygroscopic (15-20%)], the tested samples can be categorized as non-hygroscopic (Jović et al., 2023). Previous studies have demonstrated the low hygroscopicity of powders encapsulated with MD, establishing a relation between hygroscopicity and the dextrose equivalent (DE) of the wall material (Jović et al., 2023; Zhu et al., 2022). However, no reports of using CHF as an adjuvant in oil encapsulation exist. The high solubility of the samples indicates the excellent reconstitution capacity of the powders, making them suitable for inclusion in food systems (Daza et al., 2016).

Table 3. Characteristics of powders (dried emulsions).

Parameter	EC	E1	E2	E3
Efficiency (%)	91.7±0.2 ^a	91.4±0.3 ^a	91.6±0.2 ^a	92.9±0.1 ^a
Moisture content (%)	2.46±0.31 ^b	2.63±0.1 ^b	3.19±0.13 ^a	3.30±0.31 ^a
a_w	0.10±0.05 ^a	0.13±0.04 ^a	0.10±0.01 ^a	0.12±0.02 ^a
Hygroscopicity (g H ₂ O/100g)	8.10±0.69 ^a	7.65±0.48 ^a	8.00±0.23 ^a	7.40±0.92 ^a
Solubility (%)	97.4±8.7 ^a	97.6±3.9 ^a	96.4±3.7 ^a	93.9±1.7 ^a
L	94.3±0.1 ^a	93.3±0.70 ^a	93.2±0.1 ^a	93.2±0.20 ^a
a^*	-0.42±0.01 ^b	-0.25±0.02 ^a	-0.21±0.02 ^a	-0.20±0.02 ^a
b^*	0.57±0.02 ^b	1.35±0.04 ^a	1.42±0.07 ^a	1.52±0.02 ^a
ΔE	-	1.18±0.60 ^a	1.24±0.11 ^a	1.31±0.15 ^a

a_w : water activity; L: CIELab* coordinates (Luminosity); a^* : CIELab* coordinates (redness/greenness); b^* : CIELab* coordinates (yellowness/blueness); EC: encapsulated control; E1: encapsulated with 2% of Chachafruto flour (CHF); E2: encapsulated with 3% of CHF; E3: encapsulated with 4% of CHF. Values are expressed as means ± standard deviation of at least three repetitions. Different letters in the same row indicate significant differences between samples ($p<0.05$).

The particle size distribution, including the d10, d50, and d90 percentiles and the span of the encapsulates, are shown in Figs. 5A and 5D-G. The EC sample displayed a bimodal distribution. In contrast, the other samples exhibited a unimodal distribution with a minor shoulder on the right side. The behavior of the control sample may be attributed to particle agglomeration or aggregation. Surprisingly, samples with higher viscosities yielded smaller particle sizes ($p<0.05$). Additionally, samples containing CHF showed a narrower size distribution (span values <2.5),

indicating greater homogeneity in particle sizes (de Oliveira et al., 2020). The Span and the d50 for the encapsulates containing CHF were comparable to those previously reported for pomegranate seed oil encapsulated using the atomization method with various wall materials individually or in combination (Comunian, da Silva Anthero, Bezerra, Moraes, & Hubinger, 2020).

The reconstituted emulsions generally exhibited a bimodal particle size distribution (Fig. 5B). The EC-reconstituted sample maintained a distribution similar to the encapsulates obtained after drying. However, in the samples containing CHF, a separation of the particle population was observed, with one group ranging from 0.5 to 10 μm and another group ranging from 10 μm to over 100 μm . This distribution differs from the initial distribution observed in the emulsions and is likely due to material separation in the spray dryer. Larger particles may have been trapped in the drying chamber, while smaller particles were collected in the cyclone. The particle size distribution of CHF was subtracted from the distributions of the resuspended encapsulates to compare the distributions of the samples before and after the drying process (Fig. 5C). This analysis revealed an increase in the size of the resuspended droplets of 6.7, 1.8, 1.8, and 1.6 times for EC, E1, E2, and E3, respectively, compared to the droplets of the initial emulsions (CE, CHE1, CHE2, and CHE3, respectively). The increase in particle size in reconstituted emulsions has been previously observed and reported for emulsions dried by spray drying using inulin, soy protein isolate, and octenyl succinic anhydride (OSA) starch as stabilizing and wall materials (Guo, Fan, Zhou, & Li, 2023). The authors attributed this increase in particle size to the mechanical damage produced during the drying process, which can lead to particle agglomeration. Additionally, they linked thermal stress to the loss of conformation and, consequently, the emulsifying capacity of the wall materials, which could contribute to an increase in aggregation. Despite the above, adding CHF in high concentrations seems to maintain the integrity and stability of the reconstituted emulsions effectively.

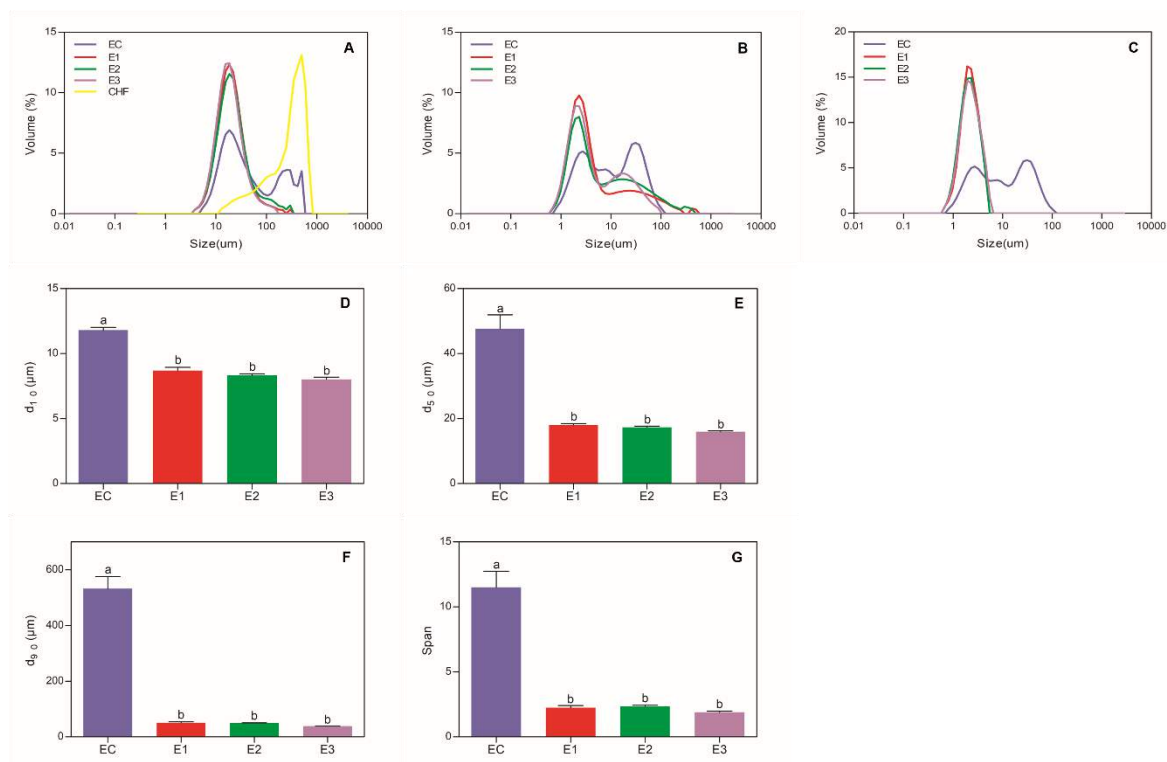


Fig. 5. Particle size distribution and particle size parameters of encapsulated control (EC) and encapsulated prepared with 2% (E1), 3% (E2) and 4% (E3) CHF.

The color parameters of the encapsulate are detailed in Table 3. Incorporating CHF into the emulsions did not alter the colorimetric properties of the powders significantly ($p > 0.05$). The L^* values of all samples closely resembled those of MD. However, differences in the a^* and b^* parameters of the encapsulate were observed with the addition of CHF compared to CE and MD ($p < 0.05$), possibly due to Maillard reactions triggered by high drying temperatures. Additionally, the ΔE of the powders with CHF was less than 1.5, a change considered imperceptible to the human eye (Gundlach et al., 2018; Torres-González, Valença, Santos, Silva, & Mendes, 2023).

The FTIR spectra of the oil, microcapsules, and wall materials are depicted in Fig. 6A. Except for oil, all samples exhibit a broad band at 3400 cm^{-1} , attributed to the stretching of hydroxyl groups (--O--H) (Karaaslan et al., 2021). The oil sample exhibited its primary bands at 2920 cm^{-1} and 2853 cm^{-1} , corresponding to the stretching vibration of the --C--H bond, with an additional band at 1742 cm^{-1} attributable to the carbonyl ester groups (--C=O) (Lavanya, Kathiravan, Moses, & Anandharamakrishnan, 2020). The CHF and MD displayed absorption bands at 2933 cm^{-1} , corresponding to the stretching vibration of the C--H bond in carboxylic groups, as well as bands at 1647 cm^{-1} (C=O stretching), 1420 cm^{-1} (bending of CH_2), 1387 cm^{-1} (O--H bending), and 1158 cm^{-1} , 1084 cm^{-1} , and 1024 cm^{-1} (C--O stretching and C--O--H bending); results for MD agree with those previously reported (Kang, Lee, Kim, & Chang, 2019; Karrar et al., 2021). Due to the presence of carbohydrates

and proteins, in the case of CHF, the absorption bands observed at 1647 cm^{-1} , 1420 cm^{-1} , and 1387 cm^{-1} may also be associated with type I amide, type II amide and C-N stretching, respectively (Karrar et al., 2021). All microcapsules exhibited typical bands corresponding to the wall materials and the oil, indicating that the components retained their structure throughout the drying process. Similar findings were observed in the microencapsulation of pepper seed oil using MD and gum Arabic as encapsulating materials (Karaaslan et al., 2021). FTIR analysis confirmed that the spray drying process was conducted without any chemical interactions between the components, highlighting the stability of the samples. This finding is consistent with previous observations made during the encapsulation of *Nigella sativa* (Mohammed, Alhelli, & Meor Hussin, 2021), flaxseed (Mohseni & Goli, 2019), and pepper seed oils (Karaaslan et al., 2021).

The thermal stability of the microcapsules and wall materials are shown in Fig. 6B-C. The total WL of the analyzed samples was 91.2%, 87.9%, 85.1%, and 84.7% for EC, E1, E2, and E3, respectively ($p < 0.05$). The thermal decomposition of the samples occurred in three stages. The first stage occurred below 130°C , with weight losses between 3.51 and 4.86%. This initial stage is linked to the evaporation of water and volatile compounds present in the samples (Umaña et al., 2021c). These values may also correspond to the moisture content previously reported for the encapsulates ($>5\%$). The second stage occurred at temperatures ranging from 285 to 340°C for CE and between 256 and 360°C for E1, E2, and E3. The most significant WL in this stage was observed for CE (65.1%), followed by E1 (61.1%), E3 (60.7%) and E2 (60.3%). This WL can be attributed to the evaporation of surface oil, degradation of amino groups, or the depolymerization of long molecular chains of proteins, starch, MD, and other carbohydrates (Comunian et al., 2020; Galindez, Daza, Homez-Jara, Eim, & Vázquez, 2019; Silva, Azevedo, Cunha, Hubinger, & Meireles, 2016; Tavares & Zapata Noreña, 2019). Adding CHF and interacting with the other components influenced the variation in degradation stages among the samples. The third stage occurred at temperatures above 360°C , resulting in weight losses of 17.2%, 16.1%, 15.6%, and 15.2% for CE, E1, E2, and E3, respectively ($p < 0.05$). The degradation of this stage is associated with the complete degradation of the core (oil) and the carbonization of residual materials (Zhu et al., 2022). At this stage, an increase in the initial degradation temperature of the samples was observed with the addition of CHF compared to the control sample (CE) ($p < 0.05$). This indicates greater stability of the microencapsulates obtained with the addition of CHF. As expected, the addition of CHF produced encapsulates with a higher amount of residual mass at the end of the degradation process, which is associated with the presence of inorganic compounds (e.g., ash) (Umaña et al., 2021b).

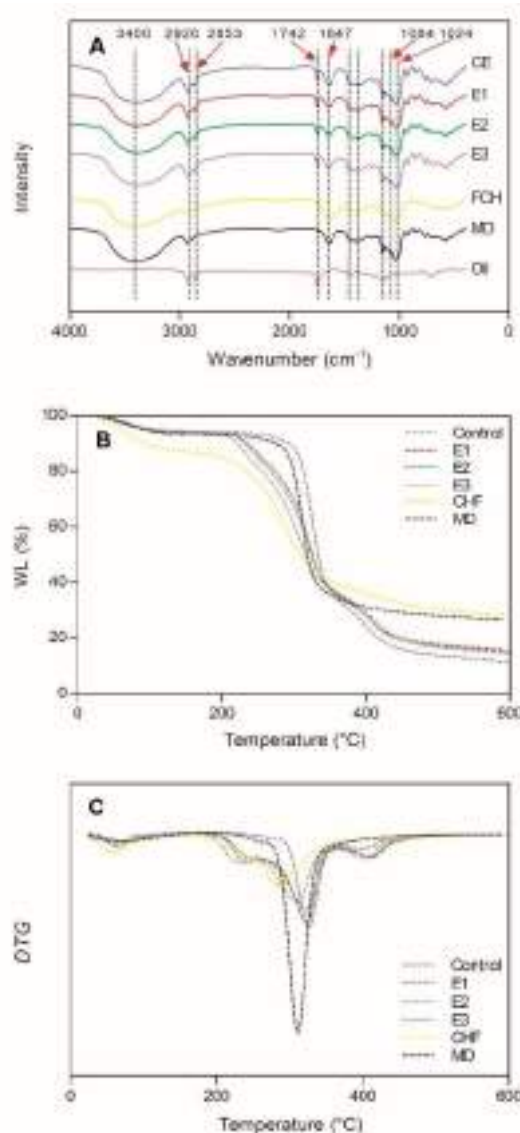


Fig. 6. FTIR spectrums, weight loss (WL) and DTG curves of encapsulated control (EC) and encapsulated prepared with 2% (E1), 3% (E2) and 4% (E3) CHF.

4. Conclusions

Incorporating CHF at concentrations of 2%, 3%, and 4% in O/W emulsions led to a notable increase in apparent viscosity, contributing to the stability of the emulsion against coalescence and flocculation. Creaming index and particle size analysis revealed that higher concentrations of CHF (3% and 4%) improved emulsion stability over 168 h at room temperature, likely due to its starch and protein contents (20.5%) and emulsifying capacity. Spray drying produced encapsulates with spherical and rough surface morphologies, with higher CHF concentrations yielding

smaller particle sizes. These morphological and particle characteristics are advantageous for flow and reconstitution properties, as evidenced by high solubility values and the ability to maintain small particle sizes after reconstitution. FTIR analysis confirmed the absence of chemical interactions during spray drying, highlighting sample stability. Additionally, the incorporation of CHF enhanced the thermal stability of the encapsulates, suggesting potential applications in thermally processed matrices. While this study represents the first application of CHF as an emulsion stabilizer, further research is necessary to explore its potential as a food ingredient fully.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Luis Daniel Daza: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Visualization. **Mónica Umaña:** Conceptualization, Writing – original draft. **Valeria Soledad Eim:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Project administration.

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CONCLUSION

4. CONCLUSIONS

According to the results obtained, the following conclusions have been established:

1. Starch characterization and modification

- 1.1. The ulluco starch showed a high dry basis extraction yield (43.2%), while the native starches of cubio (19.1%) and chachafruto (9.54%) showed intermediate or low extraction yields.
- 1.2. The native starches analyzed generally had low lipid, protein, and ash contents. Additionally, they showed high thermal stability and granule integrity observed under the microscope. These qualities suggest a high degree of purity and great industry application potential.
- 1.3. The native starches of cubio, ulluco, and chachafruto showed intermediate apparent amylose contents, which may be associated with their film-forming capacity. Furthermore, the color characteristics obtained by these materials make them attractive for the industry.
- 1.4. The particle size of the granule and the apparent amylose content of the native starches evaluated in this study influenced their technological properties, including texture, paste characteristics, and film-forming ability.
- 1.5. X-ray diffraction analysis revealed the presence of type B crystalline allomorphs in the starches, characteristic of other legumes and tubers. Furthermore, the low relative crystallinity of the materials is associated with low susceptibility to enzymatic degradation, indicating their potential as food ingredients with possible prebiotic effects.
- 1.6. The ANN treatment applied to ulluco starch proved to be an effective method for improving physicochemical parameters such as solubility, water-holding capacity, and gel texture, which are valuable properties for potential industrial applications.
- 1.7. The ANN treatment improved the pasting properties of ulluco starch, which are related to the starch's stability in thermal processes. Overall, moisture content was the most influential parameter in the ANN process.
- 1.8. Native starches and ANN-modified ulluco starch have properties comparable to commercial potato starch, highlighting their potential for use in food and non-food industries.

2. Preparation and characterization of biodegradable films

- 2.1. The biodegradable film obtained from chachafruto starch exhibited good mechanical, thermal, and barrier properties. These characteristics and water affinity suggest that the material could be used as packaging for foods with intermediate moisture levels. Additionally, the high film

- transparency demonstrated potential as packaging for products that need to be visually inspected by consumers.
- 2.2. Films based on cubio starch exhibited good barrier properties due to their homogeneous morphology. Additionally, they demonstrated high transparency, which is essential for industrial applications.
 - 2.3. Modified ulluco starch improved film characteristics such as solubility and swelling power, providing greater moisture resistance. Additionally, an increase in relative crystallinity was observed, which was associated with decreased film transparency. This could be related to less light passing through and greater protection of the food matrix.
 - 2.4. Although an increase in crystallinity was observed in the samples, there is no evidence of a relationship between this characteristic and the barrier properties of the materials, which could be influenced by the micro and macro cracks observed on the surface of the samples. This observation suggests that further tests should be conducted to determine the optimal concentration of modified starch and plasticizer in the preparation of biodegradable films and the starch gelatinization time to obtain continuous and more consistent morphologies.
 - 2.5. The physicochemical properties of biodegradable films based on low molecular weight chitosan were affected when lemongrass (LEO) and Tahiti lime (TLEO) essential oils were included in the formulation. However, these essential oils had a neutral effect on solubility and barrier properties.
 - 2.6. Reduced swelling power and crystallinity were observed, the latter associated with the loss of polymer chain ordering. This directly impacted mechanical properties by reducing tensile strength and Young's modulus and increasing material elasticity.
 - 2.7. No marked effect associated with the chitosan concentration in the evaluated system was observed. Considering the high fragility of films prepared with chitosan alone, the increase in elasticity is a desired characteristic for use in the food industry. Additionally, including LEO and TLEO demonstrated increased opacity and yellow index.
 - 2.8. The inclusion of TLEO showed a greater increase in this parameter compared to films prepared with LEO. Although these characteristics may be associated with a negative perception by consumers, they could be beneficial for preventing damage or alterations in photooxidation-sensitive foods.
 - 2.9. The addition of TLEO increased the total weight loss of the films, which may be associated with greater degradability.
 - 2.10. The use of TLEO and LEO as active components in chitosan films demonstrated the potential of these essential oils. It provided an

alternative to using waste from agricultural production, which aligns with the circular economy trend.

- 2.11. The increase in ulluco starch concentration in preparing starch-chitosan films produced materials with improved mechanical and thermal properties. However, barrier properties decreased, limiting applications for packaging with high ulluco starch concentrations.
- 2.12. The good thermal properties of ulluco starch and chitosan films are associated with their potential use over a wide range of temperatures. Ulluco starch has great technological potential for preparing environmentally friendly packaging for the food industry.

3. Food inks for 3D food printing

- 3.1. High concentrations (12%) of chachafruto starch in food inks resulted in low-quality 3D prints. However, food inks with high printing capacity were obtained at low and intermediate concentrations (8% and 10%), achieving 3D prints with excellent structural stability, texture, and morphology.
- 3.2. The unconventional starches of ulluco and cubio showed a great 3D printing capacity, which was associated with the pseudoplastic flow characteristics of the formed gels.
- 3.3. The concentration of ulluco and cubio starches significantly affected the characteristics of 3D prints. High concentrations (12%) led to material saturation, which limited gelatinization. This resulted in material agglomeration in certain areas, decreasing printing precision. On the other hand, lower starch concentrations (8%) produced less dense cell arrangements but with greater organization and printing precision.
- 3.4. All starches could produce 3D printed samples with high strength and recovery capacity against deformation, significantly impacting the preservation of the physical structure of foods produced using this technology.
- 3.5. All food inks exhibited viscoelastic behavior, with elastic properties predominant, making them comparable to 3D materials obtained using commercial starches.
- 3.6. The results demonstrate the potential of ulluco and cubio starches as materials for the 3D food printing industry. Furthermore, they consolidate this technology as a viable alternative to add value to the chain of these crops.

4. Natural emulsifier and encapsulation

- 4.1. The incorporation of chachafruto flour at concentrations of 2%, 3%, and 4% in O/W emulsions caused a noticeable increase in apparent viscosity, contributing to the emulsion's stability against coalescence and flocculation.

- 4.2. Analysis of the CI and particle size revealed that higher concentrations of CHF (3% and 4%) improved emulsion stability for 168 hours at room temperature, probably due to its protein content (16%) and emulsifying capacity.
- 4.3. Atomization produced encapsulates with spherical and rough surface morphologies, with higher CHF concentrations producing smaller particle sizes. These morphological and particle characteristics are advantageous for flow and reconstitution properties, as demonstrated by high solubility values and the ability to maintain small particle sizes after reconstitution.
- 4.4. FTIR analysis confirmed the absence of chemical interactions during atomization, highlighting sample stability.
- 4.5. The incorporation of CHF improved the thermal stability of the encapsulates, suggesting potential applications in thermally processed matrices.

5. General conclusions

- 5.1. The unconventional crops and agri-food by-product used in this study showed high industrial potential, mainly as a source of novel, biodegradable, and cost-effective compounds for packaging, 3D printing, and the stabilization and encapsulation of lipophilic components.
- 5.2. This work provided viable alternatives for adding value and revaluing the productive chains of tubers, oilseeds, and aromatic plants.

FUTURE WORK

5. FUTURE WORK

According to the results obtained in this Doctoral Thesis and to deepen and expand the knowledge related to the use of compounds obtained from unconventional crops and agri-food residues, the following aspects could be explored in future research:

1. To delve into the characterization of native starches using methods such as SAXS, NMR, and chromatography to understand their granule architecture better.
2. To implement other modification methods, such as enzymatic, chemical, and physical, to obtain starches with improved properties and optimized process parameters. Likewise, explore alternative uses for these modified starches.
3. To conduct further studies on the interaction of native and modified starches with other polymers and bioactive compounds to prepare biodegradable packaging with enhanced characteristics.
4. To evaluate the feasibility of native and modified starches in the preparation of packaging using the extrusion method.
5. Optimize essential oil concentration to produce biodegradable films with improved properties.
6. To prepare nano-particles of chachafruto, cubio, and ulluco starch and evaluate their application as reinforcement material for biodegradable packaging and as emulsifiers in food systems.
7. To model and optimize process parameters and formulation of food inks based on unconventional starches.
8. To evaluate the interaction of starches with other components or raw materials for 3D food printing and conduct sensory evaluations to determine their commercial viability.
9. To evaluate the effect of physical modifications on the properties of 3D-printed food items.
10. To model and optimize process parameters for obtaining stable emulsions using chachafruto flour.
11. To prepare a protein concentrate or isolate from chachafruto flour and evaluate its viability as a surfactant and encapsulating agent.

ANNEX

ANNEX

ANNEX I

Contributions to congresses

From the studies of this doctoral thesis, the following contributions to national and international congresses were carried out:

- 1. Title:** Use of ulluco starch edible coating to extend lima Tahití (*Citrus latifolia* Tanaka) shelf life

Authors: Angie Homez-Jara; Miguel Montealegre; Valeria Eim; Susana Simal; Luis Daniel Daza; Henry Alexander Váquiro

Event: V congreso internacional en investigación e innovación en Ingeniería, Ciencia y Tecnología de los Alimentos - IICTA 2021

Kind of event: Congress

Participation: Oral communication

City: Online

Date: 13-15 October 2021
- 2. Title:** Sorption isotherm of ulluco starch: A mathematical approach

Authors: Luis Daniel Daza, Susana Simal, Valeria Eim, Henry Váquiro

Event: Congreso Iberoamericano de Ingeniería de Alimentos – CIBIAXIII

Kind of event: Congress

Participation: Poster

City: Medellín, Colombia

Date: 15-18 March 2022
- 3. Title:** Almidón de chachafruto: caracterización parcial y evaluación de su potencial en la preparación de películas biodegradables

Authors: Valeria Eim, Luis Daniel Daza, Mónica Umaña, Carmen Roselló, Henry Váquiro

Event: XI Congreso Nacional CyTA - CESIA

Kind of event: Congress

Participation: Poster

City: Zaragoza, Spain

Date: 20-22 June 2022

4. **Title:** Efecto de la harina de chachafruto en la estabilización de emulsiones para obtención de encapsulados por el método desecado por atomización

Authors: Luis Daniel Daza, Angélica Sandoval-Aldana, Henry Alexander Váquiro, Mikhail Grachev, Valeria Eim

Event: XII Congreso Nacional CyTA CESIA

Kind of event: Congress

Participation: Poster

City: Barcelona, Spain

Date: 02-04 April 2024