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Inhibición del Crecimiento de Células Cancerosas Caco-2 por Extractos de Prunus Serotina, Obtenidos Mediante Sonicación

Inhibition of Caco-2 Cancer Cell Growth by *Prunus Serotina* Extracts Obtained by Sonication

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Resumen
El fruto y la semilla del cerezo negro o Capulín (*Prunus serotina*) son consumidos en el centro y sur de México. Ambos contienen altos niveles de antioxidantes y se le atribuyen propiedades anti-inflamatorias, anti-cancerígenas y anti-parasitarias. Métodos de extracción como la sonicación pueden ser utilizados para extraer la mayor cantidad de estos compuestos antioxidantes. Se hicieron extractos metanólicos de pulpa sola y pulpa con semilla, y se sometieron a ultrasonicación ó sin ultrasonicación. Con los extractos obtenidos, se evaluó la muerte de células de cáncer de colon Caco-2, el perfil de antioxidantes por DPPH, Azúcares y Fenoles Totales y el perfil de compuestos por cromatografía en capa fina (TLC). La pulpa molida con semilla, sin el proceso de sonicación, presenta 1.2-1.3 más antioxidantes que pulpa y semilla sonicadas. La muestra que contiene sólo pulpa tiene comportamiento similar, donde se observan 1.5 veces más de fenoles totales que la pulpa no sonicada. En los ensayos de citotoxicidad, corroboran lo observado en antioxidantes, donde hay más muerte de las células Caco-2 con los extractos de pulpa con semilla y sin sonicación. La identificación de compuestos por TLC, indica mayor cantidad de compuestos fenólicos en los extractos con semillas. Al moler el hueso con la pulpa, aumenta la cantidad de compuestos fenólicos. La sonicación en baño, no es un método muy eficiente para extraer más compuestos que la extracción por agitación de los frutos *P. serotina*.
Palabras clave: Capulín, cáncer de colon, sonicación, compuestos fenólicos.

Abstract
The fruit and seed of the black cherry or Capulin (*Prunus serotina*) are traditionally consumed in central and southern Mexico. Black cherries contain high antioxidants with anti-inflammatory, anti-cancer, and anti-parasitic properties. Sonication can be used to extract higher amounts of these antioxidant compounds compared to classical methods. Methanolic extracts were made from pulp alone and with seed with or without sonication. The antioxidant capacity of the extract by DPPH, total sugars, and phenols, as well as the compound profile by thin layer chromatography (TLC), was characterized. The cytotoxic activity of the extracts was evaluated in colon cancer cells Caco-2. Without the sonication process, the ground pulp with seed presents a 1.2-1.3 fold increase in antioxidants than the sonicated pulp and seed. The sample containing only pulp exhibits similar behavior, increasing 1.5 times more the content of total phenols. The increase in antioxidant capacity and total phenolic contents positively correlated with their antiproliferative effect in Caco-2 cells, where pulp extracts with seed and without sonication showed a marked decrease in cell viability. Identifying compounds by TLC indicates more phenolic compounds in the extracts with seeds. Grinding the seed with the pulp increases the content of phenolic compounds, while sonication in a bath is not a very efficient method for extracting more phenolic compounds than extraction by agitation from *P. serotina* fruits.
Keywords: Capulin, colon cancer, sonication, phenolic compounds

INTRODUCTION

The black cherry or Capulín (Prunus serotina) is a fruit tree belonging to the Rosaceae family that grows in cool, temperate, and humid climates, preferably in the presence of optimal and distributed rainfall. We can find 5 subspecies distributed throughout the American continent within its species. Prunus serotina is characteristic of regions such as Mexico, Ecuador, Colombia, Guatemala, and the Ecuadorian Andes (Pathania et al., 2022, Rios-Corripio & Guerrero-Beltrán, 2020). Capulín is consumed in Mexico by separating the pulp from the kernel (seed), consuming them together, or consuming the kernel alone or toasted. This fruit makes jams, sweetened waters, tamales, syrups, and alcoholic beverages. Its consumption has significant health benefits due to its high protein (37.95%) and fat content (40.37%), which includes oleic acid (35%) and linoleic acid (27%) (Pathania et al., 2022, Guzmán et al., 2018).

Capulín is rich in antioxidants, some of which are phenolic compounds (flavonoids, tannins, and terpenoids), which provide significant antioxidant activity (Rios-Corripio & Guerrero-Beltrán, 2020; Brozdowski et al, 2021). The plant also has antidepressant, antiparasitic, and antimicrobial properties; furthermore, it is rich in α-eleostearic acid, which has antiproliferative effects on tumor cells (Brozdowski et al., 2021; Gallardo-Rivera et al, 2021).

Oxygen reactive species (ROS) within the cell form highly reactive hydroxyl radicals species that damage DNA and produce mutations that initiate tumors while maintaining their progression (Brown & Bicknell, 2001). Several studies aim to prevent this oxidative damage using the antioxidant capacity of various phytochemical compounds (carotenoids, phenolics, alkaloids, and compounds with nitrogen, sulfur, and selenium), which contribute to preventing damage caused by reactive oxygen species (García-Solis et al., 2009). Colon cancer is one of the leading causes of death worldwide as well as nationally, ranking third and fourth, respectively. The increase in its incidence is associated with various factors (environmental, genetic, and dietary, among others), and the majority of cases occur after the age of 50 (Campo-Sánchez et al., 2018; Quezada-Gutiérrez et al., 2020). Caco-2 cells are widely used in colon cancer research and intestinal absorption studies (Schulz, 2021).

There are different results regarding the best solvent combination for extracting phenolic compounds from some Prunus species. Hernandez and colleagues (Hernández-Rodríguez, 2016) compared different combinations for extracting phenolic compounds from Prunus serotina Erhr, such as acetone/water, methanol/water, or ethanol/water; the acetone/water combination yielded the highest amount of phenolic compounds. Gallardo-Rivera et al. (2021) reported that the highest yield of phenolic compounds from the seed is obtained with methanol. Vasco et al. (2008) obtained 331±56 mg GAE/100 g of phenolic compounds from the pulp with acetone: water mixture (70:30 v/v). Meanwhile, Brozdowski et al. (2021) reported that up to 11,394 mg per kg of the sample was obtained from fresh fruit of Prunus serotina.

Using ultrasound for extracting bioactive compounds has been indicated as a Green Extraction technique because it reduces the amount of solvent used while reducing contamination and extraction times (Yusoff et al., 2022; Chaves et al., 2024). However, there are no reports on using such technologies to extract phenolic compounds from Prunus serotina.

In this study, the extraction of phenolic compounds from Prunus serotina fruit was carried out employing a factorial experimental design (2k) using organic solvents in two different samples (pulp with and without seeds), and with sonication or simple agitation. The extract's antioxidant activity, total reducing sugars, and phenolic contents were characterized. Cytotoxicity assays were conducted on Caco-2 cells to evaluate the effect antiproliferative effects of the extracts.

MATERIALS AND METHODS

Preparation of plant material and extraction

Two kilograms of moist P. serotina material with a red/purple hue were collected in June 2023 in Tlaxcala, Mexico (19.3610360, -98.1037970). The collected fruits were separated by ripeness, discarding overripe fruits (see Figure 1A) and green fruits (Figure 1C and 1D). The

selected fruits (Figure 1B) were washed, dried, and subsequently ground in a blender to generate two types of samples: pulp with and without seeds. The samples with seeds were passed through a sieve to remove seed residues. All samples were lyophilized (LABCONCO) until dryness. They were stored protected from light and refrigerated at 4°C until use. The extracts were prepared with 60% methanol (J.T. Baker, Xalostoc, State of Mexico) with 3% formic acid (J.T. Baker, Xalostoc, State of Mexico) using the method described by Brozdowski et al., 2021; with the following modifications: 2 g of lyophilized sample and 10 ml of extraction solution, placing duplicates of each condition in sonication (Branson 5510) for 45 minutes; and duplicates without sonication, only agitation (IKA KS 3000i control) at 250 rpm and 30°C for 45 minutes.

The extracts were centrifuged at 4400 rpm at 4°C for 10 minutes in three cycles (Centrifuge 5702 R Eppendorf); recovering the supernatants and centrifuging again for another 2 cycles under the same conditions, recovering the supernatants. The solutions were placed in the extraction hood (LABCONCO, Protector Laboratory Hood) for 24 h and then in the incubator (Memmert IFP 500) at 41.7°C for one week, obtaining a semi-solid solution from each sample, which was weighed to determine the yield obtained. The samples were resuspended in 2 ml of extraction solution and stored in the refrigerator at 4°C until use—the abbreviation to indicate the conditions is shown in the following Table.

Table 1. Conditions are used to extract compounds from the pulp and/or seed of Prunus serotina.

Sample	Process	Keyword/ abbreviation
Pulp + seed	with sonication bath	+Seed/+Sonication
Pulp + seed	without sonication bath	+ Seed /- Sonication
Pulp	with sonication bath	- Seed /+ Sonication
Pulp	without sonication bath	-Seed/- Sonication

Figure 1. Fruits of *Prunus serotina* at different stages of ripeness. Overripe fruits (1a) and still green fruits (1C and 1D) were discarded. Only ripe fruits (1B) were used for subsequent tests.

Characterization of the extracts

The total content of reducing sugars, total phenols, antioxidant capacity, and presence of phenolic compounds was analyzed using colorimetric methods and Thin Layer Chromatography (TLC).

Reducing Sugars

To measure reducing sugars (RS), the method described by Miller 1959 was employed, with modifications. Briefly, 300 µl of diluted extracts in Milli-Q water (1:50) were mixed with 300 µl of DNS reagent, heated at 100°C for 5 minutes using a thermomixer (Eppendorf Thermomixer compact), and then cooled in an ice bath for 5 minutes. Finally, a 1:6 dilution with Milli-Q water was performed, triplicates of each sample were placed in a 96-well plate, and absorbance was read at 540 nm using a plate reader (Biotek CYTATION|^5 imaging reader). RS were calculated as equivalents of D-glucose and fructose using standard curves (25-800 µg/ml) of the sugar used, with the following corresponding equations ($y = 0.0031x + 0.0453$ $R^2 = 0.988$) and ($y = 0.0033x + 0.0622$ $R^2 = 0.9913$). Results were presented in milligrams of D-glucose and fructose per gram of lyophilized extract (mg G/g LE and mg F/g LE).

Total Phenolic Content

The content of total phenolic compounds (TPC) was determined using the colorimetric method described by Ainsworth & Gillespie, 2007, with some modifications. 100 µl of extract (1:50 in Milli-Q water) was mixed with 200 µl of 10% (v/v) Folin-Ciocalteu reagent. After a 3-minute reaction, 800 µl of Na_2CO_3 (700 mM) was added, and the mixture was kept in the dark for two hours at room

temperature. Absorbance was measured at 765 nm using a plate reader (Biotek CYTATION|5 imaging reader). TPC was calculated as gallic acid equivalents (GAE) using a standard curve (25-200 µM) of gallic acid (y = 0.0055x + 0.1778 R^2 = 0.9487). Results were expressed in milligrams of gallic acid equivalents per gram of lyophilized extract (mg GAE/g LE).

DPPH Assay

The antioxidant capacity was assessed with the DPPH assay, following the method described by Brand-Williams et al., 1995, with some modifications. A stock solution of DPPH (150 µmol) was prepared using 80% ethanol and sonicated for 20 minutes. 92 µl of diluted samples (1:10 in Milli-Q water) were mixed with 908 µl of the DPPH stock solution and allowed to react for 30 minutes at room temperature. Absorbance was measured at 517 nm using a plate reader (Biotek CYTATION|5 imaging reader), and DPPH activity was reported as the percentage of inhibition calculated using the following equation:

$$\%\, Inhition = \frac{(Abs\ control) - (Abs\ Sample)}{(Abs\ control)} * 100$$

The IC50 was calculated using the regression equation (y = 1.547x + 2.2912 R^2 = 0.9885) from the Trolox standard curve (5-60 µM). Expressing the results in micromoles of Trolox equivalents (TE) per gram of lyophilized extract (µM TE/g LE).

Thin-Layer Chromatography (TLC)

The presence of phenolic compounds, terpenes, and alkaloids was analyzed using the TLC colorimetric method, with a mobile phase of methanol: chloroform (9:1) and silica gel plates (Master leav) as the stationary phase. 3 µl of undiluted extracts were placed on the stationary phase, which was placed inside a 250 ml beaker containing the mobile phase (10 ml), covered with a watch glass to prevent contamination and excessive evaporation of the stationary phase. After the capillary migration process, the silica gel plate was placed on aluminum foil to remove the excess mobile phase at room temperature. Once the stationary phase was dry, it was sprayed with 2-aminoethyl diphenylborinate using an atomizer and allowed to dry again at room temperature before visualization of migration using UV light on the transilluminator (MultiDoc.It Digital Imaging System UVP).

Cell Viability Assay

The human colon cancer cell line Caco-2 was cultured in DMEM medium (Sigma-Aldrich India) with 5% fetal bovine serum (Gibco, USA) and 1% Antibiotic-Antimycotic (Gibco, USA) and incubated at 37°C with 95% relative humidity and 5% CO_2. Caco-2 cells were seeded in 96-well plates at a density of $2.5x10^5$ cells/well. The plates were incubated for 24 hours prior to treatment. The extracts were diluted in PBS (Gibco, USA) to obtain a homogeneous concentration (600 µg/ml) of each sample and then filtered (0.22 µm Labfil filter) individually. Dilutions were in DMEM medium (1:10) to obtain a working concentration (300 µg/ml). Cells without cytotoxic compounds were used as control, representing 100%, while Cisplatin (100 µg/ml and 10 µg/ml) was used as a positive control. Caco-2 cells were incubated with the extracts for 48 hours, and cell viability was measured using the CellTiter 96® Aqueous One solution (Promega, Madison WI, USA) proliferation assay following the manufacturer's instructions. Absorbance was measured using a microplate reader (Biotek CYTATION|5 imaging reader) at 490 nm, and cell viability was calculated relative to control cells. The assay was performed in triplicates with three independent experiments on different days.

Statistical Analysis

Statistical analysis of the results obtained from the sample characterization and cytotoxicity assays was done using factorial regression modeling and Pareto charts in Minitab 21 Statistical Software (State College, PA, USA).

RESULTS AND DISCUSSIONS

Characterization of the extracts

The results of the characterization of the extracts are shown in Table 2. The highest antioxidant activity was obtained with the sample containing seeds and was not treated with sonication (5.46 ± 0.15 µM TE/g LE). Total phenols (55.47 ± 14.83 mg GAE/g LE) and reducing sugars (367.40 ± 30.57 mg glucose/g LE and 345.15 ± 28.76 mg fructose/g LE) showed a positive correlation

with antioxidant activity. The same sample had the highest values in all characterization assays.

Table 2. General data of the different assays used to characterize the obtained samples. Factorial regression analysis and Pareto diagram of standardized effects

Condition	DPPH µM TE/g LE	D-glucose mg G/g LE	Fructose mg F/g LE	Total phenols mg GAE/g LE
+Seed/-Sonication	5.46 ±0.15**	367.40 ±30.57**	345.15 ±28.76**	55.47 ±14.83**
+Seed/+Sonication	4.53 ±0.66	231.13 ±127.99	217.11 ±120.24	40.83 ±9.75
-Seed/-Sonication	5.38 ±0.14*	331.23 ±48.67*	311.14 ±45.71*	53.92 ±8.90*
-Seed/+Sonication	4.86 ±0.74	216.04 ±86.55	202.93 ±81.30	36.74 ±14.68

* $p < 0.000$-0.001, **Highest value obtained in the assay.

According to the statistical analysis, sonication did not increase the antioxidant capacity, total reducing sugar and phenolic contents. Although the seed is not a significant factor in the results obtained, the highest value among all the samples contains seeds. According to Sharma et al. (2013) and Babaoğlu et al. (2022), implementing a sonication process to extract bioactive compounds does not lead to a decrease in them. However, a comparison with the methodology applied in this project reveals that strict control over operating conditions such as temperature, time, wavelength amplitude, solvent concentration, and equipment is necessary to obtain favorable results. According to Aznar-Ramos et al. (2022), sonication is commonly performed using ultrasonic bath and probe equipment. The ultrasonic bath is more commonly used because it is more economical and readily available, but it has the limitation of intensity attenuation caused by the water bath. The probe equipment is more powerful as it directly applies sonication, resulting in less energy loss. Therefore, a higher yield of bioactive compounds is observed when using the probe equipment (Aznar-Ramos et al., 2022).

Regarding the yield of the extracts, this parameter could not be adequately measured due to gelatinization in the samples. Using a temperature of 41°C to evaporate the solvent may have affected the sugars present in the sample, leading to gelatinization. For fruits with sugar content, an additional step to remove sugars is recommended, as mentioned by Brozdowski et al. (2021).

Thin Layer Chromatography (TLC)

The results of the thin-layer chromatography can be seen in Figure 2, where letter A indicates a characteristic yellow color of terpenes, found in greater proportion in the samples without seeds, while letter B indicates a blue color attributed to phenolic compounds, which are present in the samples containing the seed.

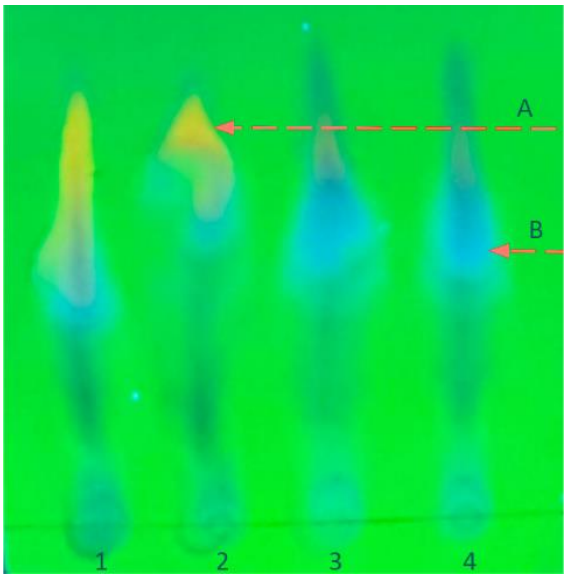

Figure 2. Thin-layer chromatography for the extracts. Numbers below the image 1) Seed/-Sonication 2) Seed/+Sonication 3) +Seed/-Sonication 4) +Seed/+Sonication A) Arrow indicating terpenes in yellow B) Arrow indicating phenolic compound in blue.

Similarly to what was observed in Table 2, thin-layer chromatography confirms that the sample with seeds without sonication has a higher content of phenolic compounds. Comparing our result with those obtained by Brozdowski et al., 2021 (11.399 mg GAE/g) and Andrea et al., 2020 (2.43 mg GAE/g MF), who used the same species as in this study, we obtained 4 times their reported amount of phenolic compounds. Similarly, phenolic

compounds have been found in the seeds of *other Prunus species*

(Gomaa, E. 2013, and Abraão et al., 2023), with a concentration of 0.0838 mg/g extract and 162.29 mg/g dry product. This suggests a higher yield of phenolic compounds in *Prunus serotina* than in *Prunus armeniaca L.* but lower than in *Prunus lusitanica L*.

Cytotoxicity assay on Caco-2 cell line

The cytotoxicity assay shown in Figure 3 depicts the percentage of viability of colon cancer cells (Caco-2) exposed to hydroalcoholic extracts and cisplatin controls of 100 and 10 µg/ml. Samples with seeds had a greater antiproliferative effect than those containing seeds, especially the one treated with sonication, reducing viability by 32.7% ($p \leq 0.003$). This data supports the findings of thin-layer chromatography, where an increase of phenolic compounds results in the death of colon cancer cells.

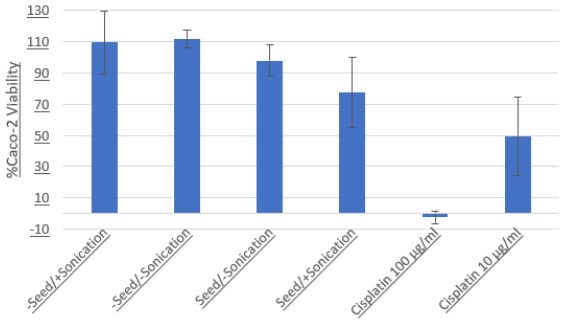

Figure 3. Representation of the percentage of cell viability of each of the extracts (300 µg/ml) and cisplatin controls against the Caco-2 cell line. *p <0.003

Sonication is a widely used technique for releasing nutraceutical compounds in plant samples. However, we found that sonication in a bath does not increase the release of antioxidant compounds. Furthermore, the seed contains more antioxidant compounds than the pulp, and the antioxidant compounds can decrease the viability of Caco-2 cells by approximately 12%. Extracts of *Prunus armeniaca L*. can decrease cell viability by approximately 90.9% in liver cancer (HepG2), 46% in breast cancer (MCF-7), and 54.3% in colon cancer (HCT-116), using a concentration of 50 µg/ml of the obtained extracts (Gomma, 2013); while *Prunus lusitanica L*. can decrease cell viability by approximately 75% in liver cancer (HepG2), 55% in transformed macrophages cancer (RAW 264.7), and 30% in colon cancer (Caco-2), using a concentration of 750 µg/ml of the obtained extracts (Abraão et al, 2023). Compared to extracts from other variants of *Prunus.* The viability obtained in this study is acceptable because the concentrations used in the different studies and the cell lines vary, placing our concentration in the acceptable range for conducting a cytotoxic assay.

CONCLUSIONS

The obtained extracts from *P. serotina* pulp were evaluated with and without sonication for their effect on the viability of Caco-2 colon cancer cells, where the viability of these cells was reduced by 12% with the pulp+seed condition without sonication. Additionally, the antioxidant activity through DPPH, total sugars, and total phenols assays was characterized, revealing that the sample with the highest amount of these compounds corresponded to pulp+seed without sonication. Thin-layer chromatography showed that pulp+seed samples contained the highest amount of phenolic compounds. Sonication in a bath may not be ideal for obtaining antioxidant compounds because the sonication conditions are not fully controlled. On the other hand, *P. serotina* seed contains a high amount of antioxidants, which promote the death of Caco-2 cells in greater quantity than pulp alone.

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Usos y Aplicaciones de Prebióticos en la Industria Alimentaria

Uses and Applications of Prebiotics in the Food Industry

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Resumen

Los prebióticos son ingredientes funcionales los cuales otorgan beneficios a la salud del consumidor. Hoy en día, se han encontrado diferentes tipos de prebióticos, con estructuras y propiedades que los caracterizan particularmente, los más aplicados son los FOS, GOS y la inulina. Los prebióticos pueden otorgar diferentes tipos de beneficios, entre ellos aliviar la inflamación intestinal, bajar los niveles de colesterol, además de prevenir enfermedades como la diabetes, el cáncer y desordenes gastrointestinales. Por ello, han sido utilizados en la industria de los alimentos como un ingrediente funcional en distintos tipos de productos, ya que además de los beneficios a la salud del consumidor, éstos también son capaces de modificar positivamente las características fisicoquímicas del alimento, por ello han surgido como una estrategia prometedora en la industria alimentaria que al ser introducidos en la dieta humana por medio de verduras, frutas crudas, o añadidos directamente en alimentos pueden ser favorables para la microbiota intestinal de los consumidores.

Palabras clave: Prebióticos, beneficios, microbiota intestinal, industria alimentaria, alimentos funcionales.

Abstract

Prebiotics are functional ingredients that provide health benefits to the consumer. Nowadays, different types of prebiotics have been found, with structures and properties that characterize them particularly. The most applied are FOS, GOS, and inulin. Prebiotics can provide different benefits, including alleviating intestinal inflammation, lowering cholesterol levels, and preventing diseases such as diabetes, cancer, and gastrointestinal disorders. Therefore, they have been used in the food industry as a functional ingredient in different types of products, since in addition to the health benefits to the consumer, they are also able to modify the physicochemical characteristics of the food positively, so they have emerged as a promising strategy in the food industry that when introduced into the human diet through vegetables, raw fruits, or added directly to food can be favorable for the intestinal microbiota of consumers.

Keywords: Prebiotics, benefits, gut microbiota, food industry, functional food.

INTRODUCCIÓN

En los últimos años se ha incrementado la demanda social de alimentos saludables, con ellos, el incremento en la búsqueda de componentes funcionales que agreguen un beneficio a la salud mediante la dieta. Entre estos componentes se encuentran los prebióticos, los cuales se definen como ingredientes que no pueden ser hidrolizados por enzimas gastrointestinales y que al mismo tiempo tienen un efecto beneficioso en la salud del consumidor, al incrementar selectivamente el crecimiento de bacterias en el tracto gastrointestinal (de la rosa *et al*., 2019). Los prebióticos se encuentran de forma natural en algunos alimentos, como las frutas, verduras, los cereales y otras plantas comestibles que se han reportado con contenido de hidratos de carbono que constituyen prebióticos potenciales (Markowiak & Ślizewska, 2017), algunos ejemplos a mencionar son los tomates, los espárragos, las cebollas, la avena, el trigo, entre otros (Yuan Kun

Lee & Seppo Salminen, 2008), aunque también pueden ser sintetizados por enzimas o microorganismos (Sampaio Paulo *et al*., 2021), que pertenecen en su mayoría a los siguientes géneros: *Lactobacillus, Bifidobacterium, Lactococcus*, *Streptococcus* y *Enterococcus* (Simón, 2005), y tienen el objetivo de promover su proliferación en el tracto intestinal y así proporcionar un beneficio para la salud del huésped (Bhola y Bhadekar, 2024).

Por su gran impacto se evalúa que el mercado mundial de prebióticos en 2018 fue de 3.400 millones de dólares y se espera que alcance 8.340 millones de dólares en 2026 (Colombo Pimentel *et al*., 2022). Es importante mencionar que los efectos del prebiótico en su adición a alimentos dependen del tipo y la dosis implementada, al igual que la matriz alimentaria y el tipo de consumidor. En los últimos años han sido ampliamente utilizados para la elaboración de productos alimenticios con valor funcional, como sustitutos de comidas, bebidas, lácteos y té (Wang *et al*., 2022). Este articulo presenta una revisión sobre los usos y aplicaciones de los prebióticos porque es importante analizar la información sobre sus posibles aplicaciones en la industria alimentaria.

PREBIÓTICOS

La definición de prebiótico partió del concepto de probiótico, que en 2001 la definición más amplia fue aceptada y se reafirmó en 2014 (Depeint et al., 2008; FAO & WHO, 2001). El principal objetivo de los prebióticos es mejorar la salud de las personas y son sustratos no viables utilizados como nutrientes por los microorganismos benéficos que se albergan en el huésped (Gibson et al., 2017). En un huésped saludable, la microbiota intestinal tiene la capacidad de metabolizar polisacáridos que el organismo no puede degradar, disminuyendo la inflamación, modulando las respuestas inmunes e inhibiendo la proliferación de microorganismos patógenos (Liu *et al.*, 2024).

Los prebióticos son definidos como sustratos utilizados selectivamente por los microorganismos del huésped (probióticos) que al ser consumidos confieren un beneficio a la salud (Gibson *et al*., 2017). Existen varios tipos de prebióticos como por ejemplo; los fructooligosacáridos (FOS), inulina, galactooligosacáridos (GOS), polisacáridos, maltodextrina, estaquiosa, entre otros y en los últimos años ha existido una gran interés hacia los polifenoles como posibles prebióticos, que junto con los ya mencionados pueden tener distintas aplicaciones por sus diversas propiedades fisicoquímicas a los cuales se les puede aplicar diferentes tipos de aplicaciones, ya que cada uno cuenta con diferentes propiedades (Alves-Santos *et al*., 2020).

BENEFICIOS DE LOS PREBIÓTICOS

Los prebióticos presentan diferentes actividades biológicas como la capacidad de bajar los niveles de triglicéridos, fosfolípidos y colesterol y que contribuyen a la prevención de enfermedades crónico-degenerativas (Muñiz Márquez *et al*., 2019). A través de la fermentación de los prebióticos se producen ácidos grasos volátiles de cadena corta (AGV) como el ácido butírico, acético y propiónico que tienen como función la disminución del pH de la zona intestinal, limitando el crecimiento de microorganismos patógenos, mantiene la homeostasis de la mucosa intestinal y ejercen propiedades antinflamatorias (Nabizadeh *et al.,* 2023). Por lo tanto, los prebióticos actúan en la prevención de enfermedades tanto digestivas e intestinales, así como en padecimientos asociados a la alimentación tales como la obesidad, la diabetes, osteoporosis, enfermedades cardiovasculares y algunos tipos de cáncer (Cáceres R., 2020). Existen distintos tipos de prebióticos que aportan un beneficio al huésped de manera concreta al ser ingeridos y así ayudar en la proliferación de las bacterias benéficas denominas probióticas y por ende generar beneficios en la salud de las personas (Figura 1).

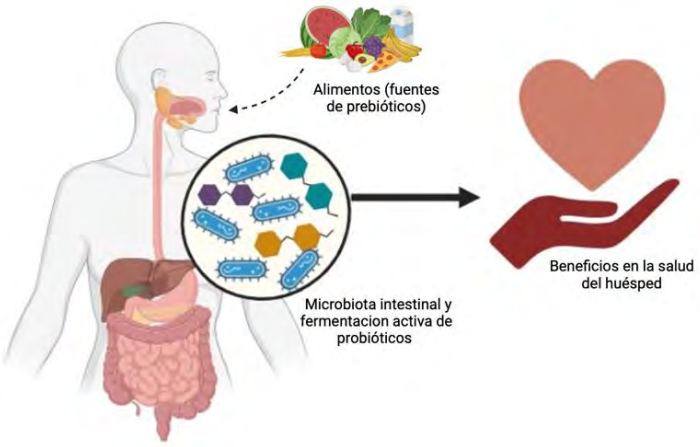

Figura 1. La ingesta de prebióticos promueve la proliferación de probióticos generando beneficio a la salud del huésped

MECANISMOS DE ACCIÓN

Los prebióticos proporcionan la fuente de energía, modulan su composición y la función de la microbiota intestinal (Flint *et al*., 2007). En su mayoría los prebióticos están en productos naturales, pero una tendencia de la industria alimentaria en los últimos años es la adición de estos en los alimentos con la finalidad de mejorar su valor nutricional y que sean saludables (Markowiak & Ślizewska, 2017). Los prebióticos pueden servir de medio para los probióticos y no son digeridos por las enzimas del huésped y llegan al colon donde son fermentados por bacterias (por ejemplo, del género *lactobacillus* o *bifidobacterium*), su consumo impacta en la composición de la microbiota intestinal y a su actividad metabólica (Loo *et al*., 2005). La estructura de los prebióticos es importante porque determina los efectos fisiológicos que presentara y los microorganismos que puedan utilizarlos como fuente de energía en el intestino (Lee & Salminen, 2009). El mecanismo de acción beneficioso de los prebióticos sigue sin estar claro sin embargo se han propuesto varios modelos posibles (figura 2).

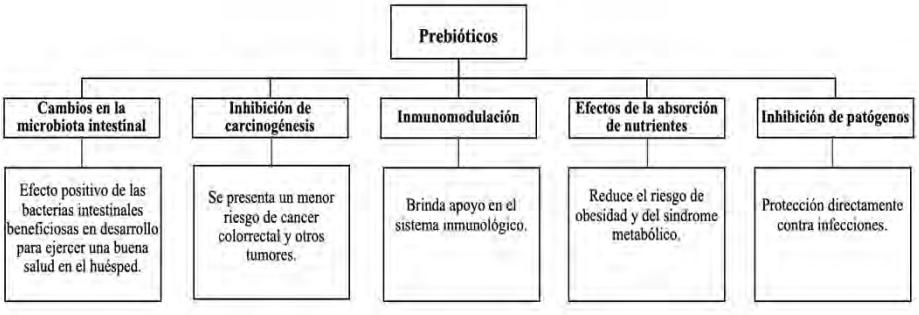

Figura 2. Mecanismos de acción de los prebióticos y sus efectos (Markowiak & Ślizewska, 2017)

TIPOS DE PREBIÓTICOS

Distintos tipos de prebióticos ayudan en estimular el crecimiento de diferentes bacterias intestinales (Markowiak & Ślizewska, 2017) Por razones de eficiencia en la seguridad de los prebióticos, su desarrollo con destino a beneficiar a salud de los consumidores debe tener presente los perfiles individuales de las especies que podrían resultar (Chung *et al.,* 2016). Las verduras, frutas, cereales y otras plantas comestibles son fuentes de carbohidratos (por ejemplo; verduras verdes, legumbres, ajo, cebollas, tomates, plátanos, etc.), algunos son producidos artificialmente (por ejemplo; galactooligosacáridos, fructooligosacáridos, lactulosa, etc.) y se cree que los fructanos, como la inulina y la oligofructosa, son de los más utilizados y eficaces en la relación con muchas especies de prebióticos (Markowiak & Ślizewska, 2017).

Maltodextrina (RMD)

La maltodextrina se considera un almidón resistente de tipo V, que es elaborado por reordenación intencionalmente de las moléculas del almidón, se compone de D-glucosa conectada en cadenas de longitud variable (Astina & Sapwarobol, 2019). Es un prebiótico generalmente reconocido como seguro por sus siglas en inglés (GRAS) (Oluwatosin *et al*., 2022). Este prebiótico cuenta con propiedades que lo hacen soluble en agua, promueve una baja viscosidad, además de soportar altas temperaturas y bajos niveles de pH además de modular los niveles de glucosa en sangre, reducir los niveles de colesterol, mejora la retención y absorción de minerales (Trithavisup *et al*., 2022).

Estaquiosa (STA)

La STA es un polímero tetrasacárido dietético conformado por dos residuos alfa-D-galactosil unidos (1 → 6) al residuo D-glucosil de la sacarosa (He *et al*., 2024). Este prebiótico se encuentra de forma natural en legumbres y se caracteriza por tener propiedades que otros oligosacáridos no poseen, como prevenir el daño hepático, mejora los síntomas de la diabetes y es capaz de reducir los niveles de azúcar en sangre, además de mitigar la colitis, la inflamación intestinal y la diarrea (Zhang *et al.*, 2023). Además, la STA puede mejorar el crecimiento y la vitalidad de bacterias beneficiosas (*Bifidobacterium* y *Lactobacillus acidophilus*) que realizan la función crucial en la regulación de la microbiota intestinal y proporcionar resistencia a los patógenos (Zhong *et al*., 2018).

Fructanos

Esta clasificación está formada por la inulina (INU) y el fructooligosacárido (FOS) u oligofructosa, su estructura base es una cadena alineada de fructuosa con enlace β (2→1), suelen tener terminales con enlace β (2→1) de glucosa (Davani-Davari *et al*., 2019). La INU tiene un grado de polimerización de hasta 60, es un polisacárido que se encuentra de forma natural en raíces y rizomas en diferentes tipos de plantas, entre ellas *Achicoria, Dalia* y *Alcachofa de Jerusalén (Akram et al*., 2024). Actualmente la INU es utilizada como un ingrediente alimentario funcional actuando como agente estabilizador de espuma, como sustituto de grasa o azúcar y como un modificador de textura (Lin *et al*., 2024). Por otro lado, los FOS presentan grado de polimerización inferior a 10, son considerados prebióticos bien establecidos, ya que están enlazados con una alta variedad de efectos beneficiosos sobre varias actividades fisiológicas tanto en animales como en humanos. Entre estos efectos beneficiosos se encuentra el incremento de la absorción de calcio, magnesio y hierro; además de reducir la ansiedad y el colesterol (Braga *et al*., 2022).

Lactulosa

Se caracteriza por ser un disacárido semisintético que se conforma por galactosa y fructosa(4-O-β-d-galactopiranosil-d-fructosa), por sus características puede recrearse mediante la isomerización de la lactosa (galactosa y glucosa), es fermentado principalmente por bacterias que son benéficas como los *lactobacillus* (Karim & Aider, 2022; Masanetz *et al*., 2011). Es considerado un carbohidrato prebiótico que tiene la capacidad de impulsar la actividad de bacterias que fomentan la salud gastrointestinal, así como inhibir el crecimiento de bacterias patógenas como *Salmonella,* además es empleado en el tratamiento del estreñimiento, encefalopatía hepática, en mantener el nivel de glucosa e insulina adecuado en la sangre y la prevención de tumores (Karim y Aider, 2022).

Galactooligosacáridos (GOS)

Los galactooligosacáridos (GOS) son producto de la extensión de la lactosa, se pueden presentar en dos grupos: I) Exceso de lactosa en C_3, C_4 o C_6 y II) Elaborados a partir de la lactosa por medio de transglucosilación enzimática (Gibson *et al.,* 2010). Son considerados como prebióticos bien establecidos, los cuales se encuentran disponibles como suplemento alimenticio. Tienen la capacidad de disminuir los síntomas gastro intestinales inflamatorios, modular la actividad

de las citoquinas, así como impulsar la función de la barrera intestinal (Holmes *et al*., 2022).

Otros Oligosacáridos

Se caracterizan por ser carbohidratos de bajo peso molecular, estos pueden ser aislados mediante procesos fisicoquímicos, síntesis química o reacción enzimática de productos naturales. Además de sus características prebióticas estos son capaces de ejecutar actividades antitumorales, antiinflamatorias, hipolipemiantes y antioxidantes (Chen *et al*., 2024). Están constituidos por carbohidratos que poseen una baja cantidad de monosacáridos, los cuales están unidos por enlaces glicosídicos (Bhola y Bhadekar, 2024).

Entre los oligosacáridos destacan los oligosacáridos de leche humana (HMO) que contienen entre 5 – 15g de oligosacáridos por litro y se han identificado distintos tipos sin embargo el más abundante es un trisacárido conformado por glucosa, galactosa y fucosa y se denominan los componentes más importantes de la leche materna, ya que son capaces de promover el desarrollo del sistema intestinal, nervioso e inmunológico neonatal (Okburan y Kızıler, 2023). Se desconoce la capacidad del tracto gastrointestinal de los adultos para emplear los HMO de acuerdo con las limitadas investigaciones clínicas a la fecha *(Jackson et al*., 2023).

Otro oligosacárido de gran interés en los últimos años es el Xilooligosacáridos (XOS) porque aportan beneficios para la salud de los consumidores y pueden incorporarse en alimentos, se pueden producir mediante hidrolisis química o enzimática (Santibáñez et al., 2021), realizan diferentes tipos de actividades bilógicas, entre ellas, prebióticas, antioxidantes, antiinflamatorias, anticancerígenas y antimicrobianas (Yan *et al*., 2023) y por ultimo los Isomaltooligosacáridos (IMOS) son polisacáridos de bajo grado con enlaces glucosídicos α-(1→6) entre los residuos de la glucosa y monosacáridos de 2-5, se encuentran de forma natural en alimentos fermentados (X. Chen *et al.,* 2022). Son utilizados principalmente en el industria alimentaria, cosmética y farmacéutica. Siendo su principal atributo su efecto prebiótico, además de poseer bajo valor calórico, resistencia a la cristalización y un bajo índice glucémico (Rengarajan y Palanivel, 2020).

Posibles prebióticos

Se han realizado investigaciones que presentan a los polifenoles como candidatos a posibles prebióticos debido a su capacidad de proliferar microorganismos probióticos (Liu *et al*., 2024). Los polifenoles son sustancias químicas, metabolitos secundarios y los primeros dadores de bioactividad antioxidante de las plantas y son ricos en muchos alimentos vegetales como frutas, verduras, cereales, café y té (J. Chen *et al*., 2024; Mithul Aravind *et al*., 2021; Sayers *et al.,* 2021). Cuentan con propiedades beneficiosas para salud como capacidad antioxidante y antiinflamatorias (Bhola y Bhadekar, 2024).

ALIMENTOS PREBIÓTICOS

En los últimos años la adición de los prebióticos en la formulación de alimentos funcionales ha resultado prometedores para contrarrestar problemas relacionados con la salud de los consumidores (Singla & Chakkaravarthi, 2017). Los criterios a tomar en cuenta para su aceptabilidad son el sabor, la textura y la palatabilidad general (Maina, 2018). Por ello, en los estudios recientes se han centrado en mejorar todos estos aspectos para asegurar la calidad que el consumidor requiere pero que al mismo tiempo sea beneficioso para su salud.

Entre los alimentos enriquecidos con prebióticos son en su mayoría complementados con fibras, desde pan y pasta, hasta bebidas, esto permite a los consumidores una mayor aceptabilidad y que puedan incorporarlos en su dieta diaria (Lazou, 2024). Por mencionar algunos de estos alimentos enriquecidos con prebióticos, como por ejemplo Montemurro et al., (2021) reportaron que el pan sin gluten con adición de psilio mejoraba su textura y presentaba efectos beneficios en la microbiota intestinal de personas con enfermedad celíaca. En otro estudio Koleva *et al*., (2012), informaron que el consumo de FOS e inulina reducen los marcadores de inflamación en el colón, estos se añadieron a un alimento semi-solido llamado yogurt (Brennan & Tudorica, 2008) y en alimentos a base de granos de cereal como el pan (Miolla *et al*., 2023), haciendo que sean más accesibles y puedan ser aceptados por los consumidores. Estos son algunos de los alimentos funcionales enriquecidos con prebióticos, sin duda que es un gran campo de investigación para la industria alimentaria en donde se pueden implementar técnicas, formulaciones nuevas, etc. Para cumplir exigencias de con los consumidores ofreciendo alimentos que tengan un beneficio para su salud.

APLICACIÓN EN LA INDUSTRIA ALIMENTARIA

La aplicación de los prebióticos en la industria alimentaria les da un gran potencial funcional para poder dar un beneficio en la salud de los consumidores y así mismo cumplir con las exigencias de calidad e inocuidad, por ello es un tema de gran interés en los últimos años reportando muchas aplicaciones en alimentos con fuentes de obtención de manera natural y comercial dando un beneficio en su mayoría en la microbiota intestinal con crecimiento de bacterias benéficas como se muestra algunos ejemplos en la tabla 1.

Tabla 1. Alimentos de la industria con potencial prebiótico

Prebiótico	**Fuente de obtención**	**Aplicación en alimento**	**Beneficio a la salud**	**Referencia**
Polisacárido	Fracción derivada del mejillón azul	Alimento nutraceútico	Potencial prebiótico de *Bifidobacterium animalis subsp. Lactis,* mejoras en la microbiota intestinal	(Adler et al., 2024)
FOS	Comercial	Helado de nieve	Mejora la salud digestiva, estimula la producción de SCFA´S y mejora la estabilidad del alimento	(Soukoulis et al., 2010)
Compuestos fenólicos (prebióticos naturales)	Extracción de plátano verde, moringa y soya	Adición en pan con harinas enriquecidas	Aumenta el número de probióticos en intestino y mejora la salud intestinal	(Bonik et al., 2024)
Inulina y maltodextrina	Frutafit HD (adquiridos en Hardline Nutrition)	Aplicación en queso Lor por secado al vacío	Produce *Lactobacillus acidophilus*, beneficiando la microbiota intestinal	(Kaan et al., 2024)
INU, XOS, GOS, STA	Comerciales proporcionados por Zhejiang Yano Biotech Company	Bebida fermentada Suancai	Efectos positivos en la microbiota para mejorar la salud	(Zhao et al., 2024)
Ácido ferúlico combinado con arabinoxilano	Obtenidos de manera comercial	Aplicación en cereales integrales	Aumentó la abundancia de Bifidobacterium, *Faecalibaculum* y *Akkermansia*	(Fang et al., 2024)
β-glucano	A partir de *Aureobasidium thailandense* NRRL 58543	Aditivo alimentario funcional en gominolas	Crecimiento de *L. casei* y *L. Brevis* y efecto benéfico en la microbiota intestinal	(Kayanna et al., 2022)

CONCLUSIÓN

El estudio de los prebióticos proporciona información adecuada para poder implementarlos en la elaboración de alimentos funcionales en la industria alimentaria debido a la variedad de beneficios a la salud que aportan al huésped, son relevantes por sus propiedades preventivas sobre enfermedades como la diabetes, cáncer, diarrea, inflamación, niveles de colesterol y azúcar en sangre; así mismo por sus propiedades fisicoquímicas que son beneficiosas para la estabilidad del alimento. Por ello es importante continuar con el estudio de compuestos prebióticos y posibles candidatos para ampliar sus aplicaciones en la industria alimentaria.

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Impact of Oatmeal Consumption: A Pilot Study to Evaluate Cardiovascular Disease Indicators in Overweight and Obese Men

Impacto del Consumo de Avena: Estudio Piloto para Evaluar Indicadores de Enfermedades Cardiovasculares en Hombres Obesos y con Sobrepeso

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Resumen
Las enfermedades cardiovasculares son una de las diez principales causas de muerte en todo el mundo. El uso de productos naturales con potenciales efectos terapéuticos, como Avena sativa L. (comúnmente conocida como avena), ha demostrado ser eficaz en el tratamiento de enfermedades cardiovasculares de una manera asequible (de manera económica). El objetivo del presente estudio piloto fue determinar el efecto del consumo de bebidas de avena en el perfil lipídico sérico de hombres jóvenes con sobrepeso u obesidad durante un periodo de 6 semanas. Se realizó un estudio aleatorizado en el que 26 sujetos recibieron una bebida de avena (55 g) durante el periodo de estudio. Las variables medidas fueron el peso, el índice de masa corporal (IMC) y el perfil lipídico sérico, al inicio y al final del estudio. Los resultados del análisis estadístico (T-test) mostraron que el grupo experimental presentó una reducción significativa de los niveles séricos de colesterol total, triglicéridos y VLDL (p= 0,020, p= 0,006 y p= 0,007, respectivamente), así como una reducción de ratios clave como colesterol total o triglicéridos respecto a HDL. En conclusión, el consumo de bebidas de avena durante un periodo de 6 semanas demostró tener un efecto cardioprotector en hombres obesos con sobrepeso.
Palabras clave: *Avena sativa* L., Colesterol, Triglicéridos, VLDL, sobrepeso.

Abstract
Cardiovascular diseases are one of the top ten causes of death worldwide. Natural products with potential therapeutic effects, such as Avena sativa L. (commonly known as oats), effectively treat cardiovascular diseases in an affordable (cost-effective) manner. The present pilot study aimed to determine the effect of oatmeal beverage consumption on the serum lipid profile of overweight or obese young men over 6 weeks. In a randomized study, 26 participants were given an oatmeal beverage (55 g) for 6 weeks. The variables measured were weight, body mass index (BMI), and serum lipid profile at baseline and the end of the study. The results of the statistical analysis (T-test) showed that the experimental group had a significant reduction in serum levels of total cholesterol, triglycerides, and VLDL (p= 0.020, p= 0.006, and p= 0.007, respectively), as well as a reduction in key ratios such as total cholesterol or triglycerides to HDL. In conclusion, the consumption of oatmeal beverages for 6 weeks was shown to have a cardioprotective effect in overweight, obese men.
Keywords: *Avena sativa* L., Cholesterol, Triglycerides, VLDL, overweight.

INTRODUCTION

In recent years, industrialization, globalization, and economic changes have considerably modified lifestyle habits in our country, which has significantly impacted the cardiovascular health of the population. Latin America, in particular, has the highest levels of sedentary lifestyles in the world, accompanied by an intake of highly processed and caloric 'junk food' (high in sugars and saturated fats) (Montalvo-Martínez et al., 2018; Melo G et al., 2023). On the other hand, these lifestyles have also shifted the culture towards increased stress (Lopes-Cortes et al., 2021). Therefore, sedentary lifestyles, junk food, and increased stress have been associated with an increase in the prevalence of overweight and obesity (Cárdenas-Pérez et al., 2018; Montalvo-Martínez et al., 2018). Therefore, there has been a significant increase in mortality from heart-related diseases (Fernández, 2011; World Health Statistics, 2011).

Cardiovascular diseases (CVD) are disorders of the heart and blood vessels, such as coronary heart disease, stroke, and rheumatic heart disease (WHO, 2024). CVD is often related to the metabolic environment, physical inactivity (sedentary lifestyle), and dietary intake (highly processed and caloric foods) (Alhabib et al., 2020; Munawir-Alhejely et al., 2020; Murillo-Zamora et al., 2016). Mortality and morbidity from CVD are elevated in overweight individuals, particularly those with abdominal obesity. Because of this, public health organizations worldwide have focused on reducing risk factors associated with obesity, such as hypertension (HTN), dyslipidemias, diabetes, or insulin resistance (AlQuaiz et al., 2019; Hedayatnia et al., 2020).

In particular, dyslipidemia is an important comorbidity caused by obesity. It can be defined as an increase in serum levels of total cholesterol (TC >200 mg/dL), low-density lipoprotein cholesterol (LDL >100 mg/dL) and/or triglycerides (TG >150 mg/dL), or a decreased serum high-density lipoprotein cholesterol (HDL <40 mg/dL) (Hedayatnia et al., 2020). According to the Mayo Clinic, the two main risk parameters used are TC: HDL and TG: HDL ratios, which, under normal (non-obese) conditions, should be less than four (values for TC, TG, and HDL values are expressed in mg/dL) (Mayo Clinic, 2024). However, it is important to emphasize that considering only these ratios does not represent a single descriptive factor and that in each patient, other physiological and biochemical considerations should be determined, such as BMI, glucose, insulin levels, and others (Cardenas-Perez et al., 2018; Ramos-Tovar & Muriel 2017; Yancy et al., 2013).

Functional foods are regarded as having potentially beneficial effects on health beyond their basic nutritional value, and they are commonly known as "nutraceuticals". They offer a unique approach to various disorders, including CVD, as their absorption enhances lipid and carbohydrate metabolism (Ahmad, et al., 2012; López-Méndez et al., 2016; Essa MM et al., 2023). Therefore, given today's stressful and sedentary lifestyle and the lack of available options, a beverage would be ideal for consuming functional food. A functional or activated beverage could be useful between a busy and healthy lifestyle (Granato et al., 2010). In particular, oatmeal, as a model functional food, is unique amongst all cereal crops because it contains biologically active components that go beyond their nutritional value and have value in health care and cosmetics, among others (Bao et al., 2014; Schuster et al., 2015; Paudel et al., 2021). β-glucans found in cereals are recognized for their numerous properties on the body due to their ability to lower cholesterol and glucose (EFSA). A meta-analysis of twenty-eight randomized controlled trials showed that consumption of βG in doses ≥ 3 g (at a molecular weight (MW) of at least 100 kDa) significantly reduced LDL and TC levels (Bao et al., 2014; Tiwari & Cummins, 2011). In addition, other meta-analyses have shown that oatmeals reduce both TC and LDL (0.30 mmol/L and 0.27 mmol/L, respectively) (Abumweis et al., 2010).

In developing countries, the availability and cost of many functional foods make them an impractical option for the population (Granato et al., 2010; López-Méndez et al., 2016). However, the biologically active components, nutrient composition, and, above all, the low cost of a raw oatmeal beverage make it an ideal candidate for the treatment of obesity-related morbidities (Ahmad et al., 2012; Biörklund et al., 2005; Granato et al., 2010; Rondanelli et al., 2011). Therefore, the present pilot study aimed to evaluate the initial effects of standardized oatmeal beverage consumption on lipid profile and other risk factors in overweight/obese men over 6 weeks.

MATERIALS Y METHODS

Study design

A randomized controlled pilot study was carried out on the effect of oatmeal for 6 weeks of consumption. The Institutional Research Board (16-FaSPyN-SA-13) approved the study in the Faculty of Public Health and Nutrition (UANL).

Twenty-six participants were recruited in the metropolitan area of Monterrey, N.L. Mexico through social media and flyers posted throughout the Universidad Autónoma de Nuevo León. Participants who met the following inclusion criteria were recruited: age between 20 and 30 years, not currently taking any medication, and/or a specialized diet to treat serum cholesterol levels. In addition, a family history interview was conducted to determine any underlying pathology. Furthermore, recruited participants had to have a BMI indicative of overweight or obesity grade I (BMI 25-35 kg/m2), with either high cholesterol (≥ 200 mg/dL) or high triglycerides (≥ 150 mg/dL), with glucose (≤ 126 mg/dL), and no other co-existing pathologies. Exclusion criteria for the study were non-adherence to the intervention, lack of data collection points, undeclared physical activity,

initiation of a dietary program, and/or pharmacological treatment for cholesterol levels. Participants who met the criteria were provided with an informed consent form.

Participants were randomly assigned to two groups: Group 1 (Control group) received no intervention and did not modify their daily nutritional regimen, and Group 2 (Experimental group) received individualized weekly bags (day 0 and week 3) containing the oatmeal mixture (55 g), to be diluted in water and taken daily. Weekly forms were used to assess intakes and compliance with the protocol. At the initial time and the end of the 6 weeks, the participants' anthropometric and biochemical parameters described above were assessed.

Participants were asked to record and weigh their daily food ration in both groups. Macronutrient composition (g) and total energy intake (kcal) were estimated using food composition tables that included nutrient compositions of Mexican foods (Sistema Mexicano de Alimentos Equivalentes) (Pérez-Lizaur et al., 2014).

Anthropometric measures

Anthropometric measurements included body weight and body composition, including parameters such as total body water, minerals, proteins, free fat mass, fat mass, BMI, waist circumference, hip circumference, visceral body fat (In Body A120, Seoul, Korea), and height (SECA 274, Hamburg, Germany).

Biochemical parameters

Blood specimens were collected after an overnight fast (12-14 h) following standard procedures by a trained phlebotomist within the Clinical Pathology Service of the Hospital Universitario Dr. José Eleuterio González. Serum samples were collected by venipuncture using vacutainers without anticoagulants. The samples were kept for 15 minutes at room temperature and then centrifuged at 3500 rpm for 15 minutes at room temperature. The serum supernatant was immediately prepared for analysis using the UniCel DxI 800 immunoassay system (Brea, California). Glucose (mg/dL), insulin (mU/mL), cholesterol (mg/dL), triglycerides (mg/dL), HDL (mg/dL), LDL (mg/dL) and VLDL (mg/dL) levels were reported at time 0 and 6 weeks. HOMA-IR was calculated by the formula = [glucose (nmol/L) * insulin (mU/mL) / 22.5], using fasting values.

Dietetic parameters

Oatmeal beverages consisted of 55 g of oatmeal in 250 ml of water. Beverage contained 1.6 g of βG per serving according to Food and Drug Administration (FDA) recommendations, corresponding to 50 % of the required βG per day (López-Mendez et al., 2016). A simplified compliance questionnaire was used to assess weekly compliance. Weekly bags containing oatmeal mixture were provided on day zero and in the third week of the study. Weekly compliance was measured using a simplified compliance questionnaire (Knobel et al., 2002).

For the experimental group, the percentage of adherence to the study was evaluated according to the number of bags of oatmeal consumed per week, according to the following criteria: very good adherence ≥ 133 bags of oatmeal (91-100%), good adherence 119-132 (81-90%), regular adherence 104-118 (71-80%), and poor adherence ≤ 103 bags of oatmeal (≤ 70%). During the study, participants completed questionnaires about food consumption and physical activity frequency. Other individual foods were grouped with beverage consumption, according to the Mexican Equivalence System (Pérez-Lizaur et al., 2014), and the physical activity should not be modified in participants of each group.

Statistical analysis

Statistical analyses were performed using the t-test. Results were expressed as mean ± standard deviation. Covariance was analyzed with a 95% confidence interval for between-group comparison. A p-value of < 0.05 was considered statistically significant in all comparisons.

Elevated risk of myocardial infarction and/or stroke was determined using the Castelli index and high-density lipoprotein, including triglycerides, in relation to total cholesterol (TC: TG and TC: HDL) (Millán et al., 2009). In addition, the relative variation of LDL (groups ≤ 100mg/dL, 100-150mg/dL, and ≥ 150mg/dL) and HDL, as well as glucose and insulin levels were analyzed. All statistical analyses were performed with SPSS 18.0.

RESULTS AND DISCUSSION

Dyslipidaemia is one of the most harmful consequences of obesity, which cannot only be costly to treat but also life-threatening. Because of this, new and effective therapies must be developed to prevent and treat dyslipidemias (Almeda-Valdés et al., 2017; Fernández, 2011; Fonseca & De Oliveira Izar, 2015; Thomas, 2016). The present study evaluated the effect of raw oatmeal consumption on markers of cardiovascular disease in an overweight/obese male population over 6 weeks.

Fifty-eight male participants were recruited, and only twenty-six met the inclusion criteria. Five participants were randomly assigned to the control group and twenty-one to the experimental group. The results related to food frequency, except oatmeal consumption, showed slightly significant changes. No adverse effects were reported. Food composition was converted to nutrient composition and total energy, and the experimental group showed a significant reduction in caloric intake, a possible satiating effect of adding the oatmeal beverage. In addition, the macronutrient composition of the experimental group showed a slight (carbohydrate) to significant (lipid and protein) reduction. Regarding compliance, the percentage of adherence of the participants in the experimental group was lower during the first week of the intervention (82%), increasing to 97% at the end of the study. The comparison of food frequency of the control and experimental group at 0 and 6 weeks is shown in Table 1.

Table 1. Comparison of food frequency of the control and experimental group at 0 weeks and at 6 weeks with t-student test (95% confidence, n=26).

VARIABLE	CONTROL GROUP 0 weeks (n=5)	CONTROL GROUP 6 weeks (n=5)	α	EXPERIMENTAL GROUP 0 weeks (n=21)	EXPERIMENTAL GROUP 6 weeks (n=21)	α
Animal-source food	6.4 ± 0.89	6.2 ± 0.83	0.374	5.9 ± 1.21	6.0 ± 1.02	0.358
Fats	6.4 ± 0.89	6.0 ± 1.73	0.648	6.1 ± 1.07	6.1 ± 0.91	0.680
Fatty grains	3.8 ± 2.28	4.0 ± 2.23	0.704	3.7 ± 2.23	3.7 ± 2.39	1.000
Non-fatty grains	4.8 ± 1.48	5.8 ± 1.09	0.189	5.6 ± 1.44	5.7 ± 1.14	0.452
Sugars	6.2 ± 1.30	6.0 ± 1.22	0.374	5.2 ± 1.88	5.1 ± 2.04	0.214
Fruits	3.2 ± 2.58	3.0 ± 2.73	0.374	3.1 ± 2.04	3.2 ± 1.89	0.789
Vegetables	3.8 ±1.48	4.0 ± 1.58	0.374	3.6 ± 1.95	3.7 ± 2.02	0.526
Legumes	4.2 ± 1.92	4.4 ± 1.94	0.621	3.9 ± 2.18	4.1 ± 2.32	0.348
Dairy	3.4 ± 2.40	3.4 ± 2.60	1.000	4.2 ± 2.38	4.2 ± 2.50	0.407
Oat	0.6 ± 0.89	0.2 ± 0.44	0.477	0.5 ± 0.85	7.0 ± 0.0	<0.001

Data shown as mean ± SD

Moreover, results showed that consuming oatmeal beverages for 6 weeks decreased the participants' total cholesterol, triglycerides, and VLDL levels. VLDL lipoproteins showed a 23% decrease, which correlates with a decrease in triglycerides, due to VLDL lipoproteins transport triglycerides in plasma (Fonseca & De Oliveira Izar, 2015; Schuster et al., 2015). Furthermore, this correlates with the ratio of triglycerides to HDL, because this ratio is key to predicting the risk of myocardial infarction. Although the final proportion was greater than three, that means high-risk (recommended values <3), the observed decrease was greater than 38% in just 6 weeks without other changes in the diet (Hegab, 2018; Prasad et al., 2019). The lack of change in LDL could be attributed to the short treatment duration in this pilot study, as previous studies have shown that longer treatment durations (8 to 12 weeks) have been able to significantly reduce LDL concentration (Biörklund et al., 2005; Maki et al., 2010). Descriptive analysis of anthropometric and biochemical values of the control and experimental group at 0 and 6 weeks are shown in Table 2. Particularly for the experimental group, biochemical values showed a significant decrease, in particular TC (p= 0.009, 7.70%), TG (p= 0.006, 23.19%), and VLDL (p= 0.007, 22. 3%), as shown in Table 2. As expected, these results validated the TG:HDL ratio since a significant decrease was demonstrated (p= 0.199, 38%). Furthermore, no significant changes were observed in the TG:HDL ratio. The correlation of participants with risk parameters between the control and experimental groups at 0 weeks and 6 weeks is shown in Table 3. Likewise, no significant changes were observed in waist circumference (p= 0.98), total body fat (p= 0.92), and visceral body fat (p= 0.56) (data not shown).

Previous research studies have shown significant reductions in total and LDL cholesterol due to the consumption of β-glucans (βG) derived from oats, which are one of these biologically active components that significantly decrease TC and have been incorporated into other nutritional matrices such as bread and cookies (Ahmad et al., 2012; Maki et al., 2010; Paudel et al., 2021). These studies reported a high-calorie diet that provided 3 g of βG compared to controls on a low-calorie/fiber-free diet. The low-calorie diet rich in fiber decreased waist circumference in the experimental group, which allows consideration that the diet of the present study, together with βG oats, is essential to observe differences in weight and body composition (Karmally et al., 2005; Rondanelli et al., 2011). Wolever et al. demonstrate a significant decrease in LDL cholesterol due to oatmeal consumption, namely, medium PM (530,000 g/mol) or high PM (2,210,000 g/mol) (Wolever et al., 2010).

Table 2. Descriptive analysis of anthropometric and biochemical values of the control and experimental group at 0 and 6 weeks with t-student test (95% confidence, n= 26).

VARIABLE	CONTROL GROUP 0 weeks (n=5)	CONTROL GROUP 6 weeks (n=5)	α	EXPERIMENTAL GROUP 0 weeks (n=21)	EXPERIMENTAL GROUP 6 weeks (n=21)	α
Weight (kg)	85.81 ± 0.73	86.01 ± 0.73	0.059	85.74 ± 0.36	85.89 ± 0.36	0.053
BMI (kg/m^2)	29.29 ± 0.55	29.25 ± 0.55	0.057	29.97 ± 0.26	29.77 ± 0.26	0.053
Glucose (mg/dL)	89.20 ± 5.38	92.00 ± 8.88	0.291	89.24 ± 10.41	90.05 ± 8.00	0.076
Insulin (mU/mL)	11.86 ± 5.02	12.86 ± 3.85	0.099	12.42 ± 10.79	10.76 ± 6.58	0.345
HOMA-IR	2.60 ± 1.11	2.94 ± 1.02	0.197	2.884 ± 2.980	2.474 ± 1.554	0.298
Cholesterol (mg/dL)	201.20 ± 36.5	217.00 ± 53.0	0.345	200.33 ± 27.7	184.9 ± 28.7	0.009
Triglycerides (mg/dL)	188.20 ± 75.7	232.20 ± 52.7	0.271	198.71 ± 107.1	152.61 ± 86.5	0.006
HDL (mg/dL)	40.46 ± 2.6	44.02 ± 6.5	0.211	43.13 ± 9.2	40.78 ± 11.3	0.308
LDL (mg/dL)	123.10 ± 24.1	126.54 ± 50.8	0.806	116.32 ± 26.5	112.35 ± 24.5	0.507
VLDL (mg/dL)	37.64 ± 15.1	46.44 ± 10.5	0.271	39.31 ± 21.5	30.52 ± 17.3	0.007

kg/m^2= kilogram(s) per square meter,
mg/dL= milligrams per deciliter
Data shown as mean ± SD

Table 3. Ratio of participants with risk parameters between the control and experimental groups at 0 weeks and at 6 weeks (n = 26).

RATIO	CONTROL GROUP 0 weeks (n=5)	CONTROL GROUP 6 weeks (n=5)	EXPERIMENTAL GROUP 0 weeks (n=21)	EXPERIMENTAL GROUP 6 weeks (n=21)
TC: HDL < 4	0	1*	3	7*
LDL				
< 100 mg/dL	1	2*	6	8*
100–150 mg/dL	3	2	13	11**
> 150 mg/dL	2	1	2	2
HDL < 40 mg/dL	3	2**	7	9
> 40 mg/dL	2	3*	14	12

* Value increase

Data shown as mean ±SD

** Value decrease

On the other hand, some studies have not shown the beneficial effects of consuming oatmeal. Chen et al. demonstrated that consumption of 8 g of oat-based bread for 90 days showed no significant results on BMI, TC, or LDL cholesterol in participants with hypercholesterolemia (Chen et al., 2006). Another study examining the effect of oat-based bread consumption for six weeks showed no significant changes in total cholesterol and triglyceride concentrations (Momenizadeh et al., 2014).

It is important to note that these studies involved oat-based bread prepared by baking, and the properties and effects of raw oats could be affected by temperature, especially βG. A meta-analysis by Bao et al. showed significant reductions in subgroups consuming oats (βG) at high doses (≥ 5 g/day) and low doses (≤ 5 g/day). A more significant reduction in serum insulin levels was reported. However, the reason is unknown. (Bao et al., 2014).

Regarding the results of insulin resistance evaluated by the HOMA-IR index, a decrease in insulin resistance was observed in the experimental group, compared to the control group, which presented an increase in the HOMA-IR index values. In the present pilot study, approximately 1.6 g of βG was administered per serving, compared to the study by Biorklund et

al., in which doses of 5 g of oat-derived βG were administered to hypercholesterolemic participants, significantly decreased postprandial glucose and insulin concentrations (Biörklund et al., 2005). It is important to mention that there are no previous reports on the effect of oatmeal beverages on this population, so this pilot study is relevant. However, it is proposed that the number of participants and the evaluation period of oatmeal beverage consumption be extended to evaluate significant differences in the results.

CONCLUSIONS

In this pilot study, consuming 55 g of raw oatmeal as a beverage for six significantly reduced total cholesterol, triglyceride, and VLDL cholesterol levels in overweight or obese male participants. This effect and slight decreases in HDL values led to a 38% reduction in the TC: HDL ratio, a high-risk parameter related to myocardial infarction. Therefore, this study represents a new perspective in evaluating oatmeal's effects on cardiovascular risk patients.

From this perspective, extending the time and number of participants is proposed to evaluate the significant long-term effect. Furthermore, it is proposed to include the determination of C-reactive protein (CRP) and inflammatory factors to correlate them with the degree of insulin resistance and the effect of oatmeal on this type of pro-inflammatory complication in overweight and obese patients.

DECLARATIONS

The authors declare no conflict of interest.

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Effect of Particle Size, NaOH Concentration, and S/L Ratio on the Extraction of Humic Acid from Leonardite

Efecto Del Tamaño De Partícula, Concentración De NaOH Y Relación S/L En La Extracción De Ácido Húmico A Partir De Leonardita

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Resumen
Los ácidos húmicos son componentes cruciales para la calidad del suelo, generando un creciente interés en la investigación agrícola y medioambiental. Este estudio se centra en evaluar estadísticamente el efecto del tamaño de partícula, porcentaje de sólidos y concentración de NaOH en la extracción y producción de ácido húmico a partir de leonardita, empleando un diseño experimental 2^3. El ANOVA mostró que todos los factores individuales tienen un impacto significativo en la extracción de ácido húmico ($p<0.05$). Además, el factor que tiene la mayor significancia de acuerdo con el valor F es la concentración de NaOH. El mayor rendimiento de ácido húmico fue de 875.16 g AH/kg, este se logró empleando un tamaño de partícula de 375 µm, 0.5 M NaOH y 4% de sólidos. Los resultados de este estudio proporcionan una base para mejorar los procesos industriales de extracción de ácidos húmicos.

Palabras clave: fertilizante, leonardita, rendimiento, sustancias húmicas.

Abstract
Humic acids are crucial for soil quality, generating growing agricultural and environmental research interest. This study focuses on statistically evaluating the effect of particle size, solid percentage, and NaOH concentration on the extraction and production of humic acid from leonardite, employing a 2^3 experimental design. ANOVA showed that all individual factors significantly impact humic acid extraction ($p < 0.05$). Furthermore, the factor with the greatest significance according to the F-value is the NaOH concentration. The highest humic acid yield was 875.16 g HA/kg, achieved using a particle size of 375 µm, 0.5 M NaOH, and 4% solids. The results of this study provide a foundation for improving industrial processes for humic acid extraction.

Keywords: fertilizer, humic substances, leonardite, performance.

INTRODUCCIÓN

Los ácidos húmicos son macromoléculas polielectrolíticas que juegan un papel crucial en los ciclos globales de carbono y nitrógeno, así como en la regulación de la movilidad de nutrientes y contaminantes ambientales (Christi et al., 2000; Xu et al., 2022). Estos se encuentran presentes en carbón marrón como el lignito, este tipo de carbón es de bajo rango con bajo grado de carbonificación (Peña et al., 2005; Giannoulli et al., 2009). Asimismo, se ha reportado que los ácidos húmicos pueden actuar como fitohormonas, ya que contienen sustancias que estimulan el crecimiento celular y su bioactividad está asociada a un mayor contenido de grupos nitrogenados en su estructura, similar a la actividad promotora de crecimiento del ácido indolacético (Nardi et al., 2002; Campitelli et al., 2006; Pasqualoto et al., 2009).

La importancia del estudio y del manejo de las sustancias húmicas radica en la gran influencia de la industria, sobre todo en la agricultura ayudando al crecimiento y desarrollo de los cultivos (Bezuglova & Klimenko, 2022; Nsengumuremyi et al., 2022). Los impactos secundarios abordan el papel de las sustancias húmicas en la mejora de la fertilidad del suelo, centrándose particularmente en sus efectos sobre los aspectos físicos, químicos y biológicos del mismo (Nardi et al., 2021; Veobides-Amado et al., 2018). No obstante, la agricultura no es el único ámbito de aplicación de las sustancias húmicas (Ramos-Ruíz, 2000), sino que cuenta con otros usos entre los que destacan la medicina y productos farmacéuticos, dispersante de polvos cerámicos, síntesis de materiales, así como en la industria cosmética como protector solar (Hricikova et al., 2023; Kapil et al., 2023). Algunos estudios han demostrado que la presencia de materia orgánica y, por tanto, de los ácidos húmicos, tiene una fuerte influencia en la dispersión de las arcillas (Phelps, 1982; De Souza & Bragança, 2018; Qin et al. 2018). Sin embargo, existen pocos estudios específicos sobre la extracción de AH y su aplicación como dispersante cerámico (De Souza & Bragança, 2018). El presente estudio se centra en investigar la obtención de ácido húmico a partir de leonardita utilizando un diseño experimental 23. Con este enfoque experimental se evaluó el efecto de tres factores en dos niveles. Los factores considerados están relacionados con la extracción, la composición de la leonardita y las condiciones de reacción.

MATERIALES Y METODOS

Caracterización del material

Distribución granulométrica

Para el desarrollo de los experimentos se utilizó leonardita procedente de Colombia, Nuevo León, México. Se obtuvo una muestra representativa empleando el método de cuarteo. Para realizar la distribución de tamaños se utilizó un RO-TAP, modelo RX-29. El material fue tamizado en lotes de 200 g durante 20 minutos, para lo cual se utilizaron 8 tamices de la serie Tyler.

Contenido de Humedad

La determinación de la humedad se realizó empleando el procedimiento estándar de la normativa ASTM D3173-03. Para ello, se secó 1 g de leonardita en una estufa de laboratorio a 100°C (+/- 10°C) durante 60 minutos. Posteriormente, las muestras fueron retiradas y almacenadas en un desecador durante 10 minutos con la finalidad de que el peso se estabilizara, evitando en lo posible la adsorción de humedad del ambiente. Una vez obtenido el peso final, se realizaron los cálculos correspondientes en cada una de las fracciones de interés (Ec. 1).

$$Humedad, \% = \frac{(A-B)}{A} * 100 \quad \text{(Ec 1)}$$

Donde: A es el peso de la muestra (g), y B es el peso de la muestra después del calentado (g).

Contenido de ceniza

El contenido de ceniza se determinó utilizando como referencia la norma ASTM D3174. Se utilizó 1 g de material, el cual fue sometido a un proceso de calentamiento controlado en una mufla. Durante la primera hora se alcanzó una temperatura de 500 °C, posteriormente durante la segunda hora se incrementó hasta 750 °C, para finalmente mantenerla constante durante dos horas más. El contenido de ceniza fue calculado utilizando la Ecuación 2.

$$Ceniza, \% = \frac{(C-B)}{A} * 100 \quad \text{(Ec 2)}$$

Donde: A es el peso de muestra; B es peso del crisol y C corresponde al peso del crisol más el peso del residuo.

Densidad

Para determinar la densidad se empleó el método del picnómetro (basado en la ASTM D2320-98). El método consiste en llenar con alcohol etílico (grado reactivo; ρ= 0.79 g/mL) y registrar el peso (M1). Después añadir 0.25 g de muestra en el picnómetro (M2). Finalmente, se introduce la tapa del picnómetro y se registra el peso nuevamente (M3). La densidad se obtiene mediante la ecuación 3. Es importante mencionar que previo a cada medición el picnómetro se lavó con agua destilada, así como con acetona para eliminar completamente el agua.

$$d_s = \frac{M_2}{\frac{(M_1+M_2)-M_3}{d_l}} \quad \text{(Ec 3)}$$

Donde: d_s es la densidad de la leonardita y d_l es la densidad del líquido.

Diseño experimental 2^3

Los experimentos se diseñaron con el objetivo de evaluar el efecto del tamaño de partícula (µm), la concentración de NaOH (M) y el porcentaje de sólidos (%). Se evaluaron dos niveles para cada factor. Para el tamaño de partícula se

seleccionaron las partículas de 90.5 y 375 µm para generalizar el comportamiento en partículas finas y gruesas, respectivamente. Los niveles de la concentración de NaOH y del porcentaje de sólidos se determinaron de acuerdo con lo reportado en la literatura (Asing et. al., 2009; De Souza y Bragança, 2018; Nazarbek et al. 2022; Niewes et al., 2023 Yang et al., 2024).

La Tabla 1 presenta los niveles utilizados para cada factor, resultando en 8 combinaciones. La extracción de ácido húmico se evaluó después de 8 horas de proceso, tiempo fijado a partir de pruebas preliminares.

Tabla 1. Diseño experimental factorial 2^3

	90.5µm		375µm	
	0.1 M NaOH	0.5 M NaOH	0.1 M NaOH	0.5 M NaOH
4 % sólidos	1	2	3	4
8 % sólidos	5	6	7	8

El análisis de varianza (ANOVA) y las gráficas de efectos individuales y de interacción se realizaron con el software Minitab 18, codificando los factores y sus niveles según la Tabla 2.

Tabla 2. Codificación del diseño experimental 23 de acuerdo con el nivel de cada factor.

	Factor	Nivel	Codificación
A	Tamaño de partícula, µm	90.5	-1
		375	+1
B	Porcentaje de sólidos, %	4	-1
		8	+1
C	Concentración NaOH, M	0.1	-1
		0.5	+1

Extracción de ácido húmico

La metodología utilizada para la obtención de sustancias húmicas a partir de leonardita se diseñó tomando como base la reportada por Lamar et al., 2014 y Asing et al., 2009. Sin embargo, esta fue adaptada considerando la infraestructura disponible.

La obtención de sustancias húmicas es un proceso conformado por varias etapas, las cuales se presentan en la Figura 1. Una vez seleccionadas las condiciones experimentales (i.e., tamaño de partícula, porcentaje de sólidos y concentración de NaOH), los sólidos se pusieron en contacto con 80 mL de la solución extractante y se mantuvieron en agitación a 150 rpm durante 8 h, con ayuda de un agitador orbital.

Al concluir este periodo de agitación, las soluciones fueron filtradas y posteriormente acidificadas hasta pH 1, para lo cual se adiciono la cantidad necesaria de HCl 6 M. Este último paso tiene como objetivo lograr la precipitación del ácido húmico (el cual es insoluble a pH ácidos); logrando la separación del ácido fúlvico y la humina, los cuales permanecen en la solución. Posteriormente, la solución acidificada se dejó coagulando durante 24 horas. Al término del cual la suspensión fue transferida a tubos de polipropileno de 15 mL y centrifugada durante 45 minutos a una velocidad de 24000xg. Finalmente, la solución fue decantada y el sólido obtenido (i.e., ácido húmico) fue secado en una estufa de laboratorio a 90°C, hasta obtener un peso constante.

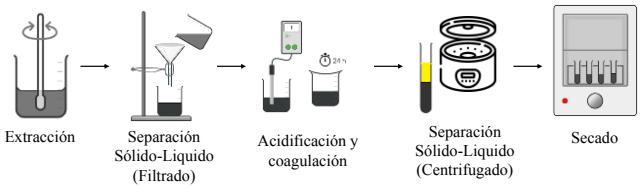

Figura 1. Proceso generalizado para la obtención de sustancias húmicas

RESULTADOS

Caracterización del material

En la Tabla 3 se presenta el peso retenido y bajo tamaño acumulativo para cada una de las fracciones de los tamaños de partícula de la leonardita. Se observa que la muestra empleada tiene una distribución de tamaño de partícula amplia, en donde las partículas de 605 µm tienen mayor presencia (38.79%).

En la Tabla 4 se presenta el contenido de cenizas en los dos niveles de tamaño seleccionados, así como la desviación estándar (σ). Se observa que, las partículas finas presentan un mayor contenido de cenizas en comparación con las más gruesas. Esto indica que existe una mayor presencia de compuestos inorgánicos (e.g., FeO y arcillas) asociadas a las partículas finas. Resulta importante considerar el contenido de ceniza en la materia prima para la obtención de AH. De acuerdo con el estudio realizado por Asing et al., (2009) quienes evaluaron diferentes tipos de materia prima con contenidos de ceniza diversos (2-13%), está variable tuvo un impacto importante en el rendimiento del proceso.

Tabla 3. Distribución de tamaños de la leonardita.

Tamaño de partícula, µm	Peso retenido, %	Bajotamaño acumulativo, %
701	0	100
605	38.79	61.21
375	22.91	38.3
215	9.61	28.69
165	6.16	22.54
128	7.76	14.78
90.5	6.03	8.74
64	3.45	5.3
26.5	5.3	0

Tabla 4. Contenido de ceniza (%) en función del tamaño de partícula

Tamaño de partícula, µm	Ceniza, %	σ (*n=2*)
90.5	28.78	0.13
375	24.52	0.10

El contenido de humedad se presenta en la Tabla 5. De acuerdo con los resultados, las partículas gruesas tienen un mayor contenido de humedad que las partículas finas. El contenido de humedad se asocia a los compuestos orgánicos, por lo que un mayor contenido indica mayor materia orgánica.

Tabla 5. Contenido de humedad (%) en función del tamaño de partícula

Tamaño de partícula, µm	Humedad, %	σ (*n=2*)
90.5	15.43	0.27
375	16.91	0.19

En la Tabla 6 se presenta la densidad en cada tamaño de partícula. Se observa que las partículas finas presentan una mayor densidad en comparación con las partículas gruesas. Considerando que el mayor contenido de materia inorgánica (ceniza) se asocia a las partículas finas (Tabla 4), se puede asociar a que se presente una mayor densidad. De acuerdo con Yan et al. (2021) el rendimiento de AH depende más del método de extracción que de la proporción de AH presente en la materia prima. Sin embargo, considerar la densidad resulta importante en el proceso ya que se puede utilizar para correlacionar el contenido de materia inorgánica y orgánica presente.

Tabla 6. Densidad (g/mL) en función del tamaño de partícula

Tamaño de partícula, µm	Densidad, g/mL	σ (*n=2*)
90.5	1.72	0.13
375	1.44	0.06

Extracción de ácido húmico

La Tabla 7 presenta los resultados obtenidos en cada tratamiento y sus réplicas. Los factores A, B y C representan el tamaño de partícula, porcentaje de sólidos y concentración de NaOH, respectivamente. Adicionalmente se incluye la desviación estándar (σ).

Tabla 7.- Rendimiento de la extracción de los ácidos húmicos en diferentes condiciones experimentales

A	B	C	Yield, gAH/kg	σ
-1	-1	-1	248.16	0.86
-1	-1	1	705.85	16.05
-1	1	-1	125.82	2.43
-1	1	1	657.49	12.32
1	-1	-1	232.88	7.01
1	-1	1	875.16	1.90
1	1	-1	88.92	1.13
1	1	1	699.02	17.71

Conociendo el valor de la variable de respuesta, se realizó el análisis de varianza para determinar si los efectos individuales y de interacción de los diferentes factores estudiados tienen una influencia significativa.

ANOVA para la extracción de ácido húmico

El ANOVA presentado en la Tabla 8, mostró que todos los factores individuales, así como sus s interacciones (i.e., A×B y A×C) tienen un impacto significativo en la extracción de ácido húmico ($p < 0.05$). De acuerdo con el valor F, la concentración de NaOH (C) presenta el mayor efecto, seguido por el porcentaje de sólidos (B).

Para complementar el ANOVA y comprender mejor el efecto de los factores estudiados, se presentan las gráficas de los efectos individuales y su interacción. Las cuales relacionan la extracción de ácido húmico y el nivel al que se fijan los factores.

Tabla 8. ANOVA para el diseño experimental factorial 23 para la extracción de ácido húmico (R2 = 99.94%).

Factor	G.L.	S.C.	M.C.	F	P
A	1	6,297	6,297	64.26	0
B	1	60,214	60,214	614.44	0
C	1	1,256,394	1,256,394	12,820.55	0
A × B	1	5,583	5,583	56.97	0
A × C	1	17,288	17,288	176.41	0
B × C	1	436	436	4.45	0.068
A × B × C	1	2,815	2,815	28.73	0.001

En la Figura 2 se presentan las gráficas de efectos individuales para los tres factores de estudio establecidos como A, B y C. En la Figura 2A se observa que el rendimiento de ácido húmico es mayor al utilizar un tamaño de partícula de 375 µm en comparación con 90.5 µm. El rendimiento se incrementa hasta 473.99 g de AH/kg de leonardita con partículas de 375 µm, en comparación con 434.31 g de AH/kg con partículas de 90.5 µm. Esto puede estar relacionado con el mayor contenido de materia orgánica en las partículas más grandes, que facilita la extracción de ácidos húmicos. De acuerdo con estudios publicados generalmente se trabaja con un tamaño de partícula heterogénea (bulk), por lo que es importante considerar el efecto que tiene el uso de partículas finas o gruesas, ya que esto está directamente relacionado con el requerimiento de etapas de trituración y molienda, así como con la calidad de la materia prima (Sharafi et al. 2024; Sarlaki et al. 2024; Yang et al., 2024).

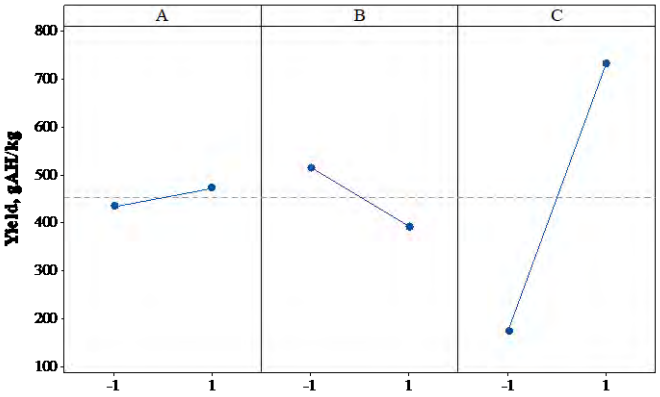

Figura 2. Efectos principales en la extracción de ácido húmico. A) tamaño de partícula, B) porcentaje de sólidos y C) concentración de NaOH.

Por su parte en la Figura 2B, se observa claramente que al aumentar el porcentaje de sólidos de 4% a 8%, el rendimiento del proceso disminuye. Con un 8% de sólidos, se obtiene un rendimiento de 392.81 g de AH/kg, mientras que con un 4% de sólidos, el rendimiento aumenta a 515.50 g de AH/kg.

Por último, en la Figura 2C se aprecia al aumentar la concentración de NaOH de 0.1 M a 0.5 M, el rendimiento se incrementa significativamente, de 173.93 g a 734.38 g de AH por kg. Este aumento indica que, en concentraciones más bajas de NaOH, el proceso de extracción está limitado por la cantidad de reactivo disponible para reaccionar con la leonardita. Al incrementar su concentración, hay más NaOH disponible para promover la disolución y extracción de los ácidos húmicos, lo que resulta en un mayor rendimiento. Por lo tanto, el NaOH actúa como el factor clave que limita la eficiencia del proceso, ya que su concentración determina la cantidad máxima de ácidos húmicos que se pueden extraer en las condiciones establecidas (Brigante et al., 2007).

En las siguientes Figuras se presentan las gráficas de los efectos de interacción entre los 3 factores estudiados, así como su representación tridimensional

En la Figura 3 se analiza la interacción entre el tamaño de partícula y el porcentaje de sólidos (A×B). En la gráfica de la izquierda (Fig. 3A) se observa que el mayor rendimiento (554.02 gAH/kg) se obtiene utilizando un tamaño de partícula de 375 µm (+1) y 4% de sólidos (-1). La combinación de un tamaño de partícula de 90.5 µm (-1) y 4% de sólidos (-1) genera un rendimiento de 467.98 gAH/kg. Al aumentar el porcentaje de sólidos a 8% (+1) y mantener el tamaño de partícula en 375 µm (+1), el rendimiento disminuye a 393.97 gAH/kg. Estos resultados muestran que la reducción del tamaño de partícula y el aumento del porcentaje de sólidos impactan negativamente el rendimiento de extracción.

Por otro lado, se observa que al evaluar la combinación -1/-1 (i.e., tamaño de partícula de 90.5 □m y 4% de sólidos) se obtuvo un rendimiento de 467.98 gAH/kg. Adicionalmente, al evaluar la interacción en los niveles +1/-1, el rendimiento presenta el mismo valor que el obtenido de la combinación (+1/+1). Es decir, el rendimiento se ve afectado al disminuir el tamaño de partícula y aumentar el porcentaje de sólidos. El mayor rendimiento se obtuvo al utilizar 4% de sólidos y un tamaño de partícula de 375 µm. Este comportamiento se puede relacionar con el contenido de impurezas en las partículas finas (i.e., aluminosilicatos, carbonatos y óxidos metálicos)). Estás especies pueden recubrir o encapsular las sustancias húmicas en la matriz de la leonardita, dificultando su solubilización durante la extracción alcalina. Emplear una etapa de descalcificación (pretratamiento ácido) mejora la exposición de los ácidos húmicos, facilitando su extracción. (Nieweś et al., 2023)

La Figura 3B muestra que, independientemente del porcentaje de sólidos, si no se usa una concentración adecuada de NaOH, el rendimiento del tratamiento será bajo.

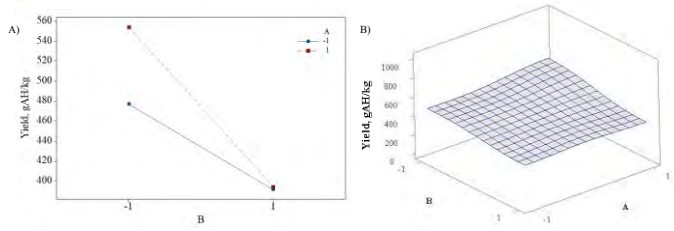

Figura 3.- Efecto de la interacción del tamaño de partícula y el porcentaje de sólidos ($E_{A\times B}$) A) grafica de interacción B) grafica de superficie.

En la Figura 4 se presenta la interacción entre el tamaño de partícula y la concentración de NaOH (A×C). La Fig. 4A muestra que el mayor rendimiento (787.09 gAH/kg) es al utilizar un tamaño de partícula de 375 µm y una concentración de NaOH de 0.5 M (+1/+1). Además, se aprecia que con una concentración de NaOH de 0.1 M (+1/-1), el rendimiento disminuye a 160.9 gAH/kg. Se ha estudiado el uso de concentraciones de NaOH en el rango de 0.15-0.35 M (Sharafi et al. 2024; Yang et al., 2024). Sin embargo, se reportan rendimientos por debajo de 689 gAH/kg. Esto muestra la influencia de la concentración de NaOH en el proceso.

Adicionalmente, en la Fig. 4B se observa que los mayores rendimientos se obtienen con 0.5 M de NaOH, mientras que los menores rendimientos se logran con la concentración más baja de NaOH, independientemente del tamaño de partícula. De acuerdo con Yang et al. (2024) la selección de la concentración adecuada de NaOH permitirá evitar el uso excesivo de extractante durante el proceso de extracción de AH.

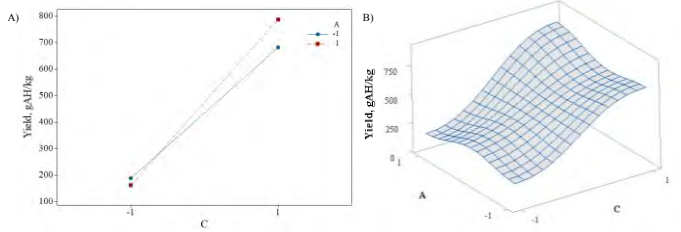

Figura 4.- Efecto de la interacción de los factores tamaño de partícula y concentración de NaOH ($E_{A\times C}$). A) grafica de interacción B) grafica de superficie.

En la Figura 5 se presenta la interacción entre los factores concentración de NaOH y el porcentaje de sólidos.

En la Figura 5A se observa como el rendimiento es mayor cuando el porcentaje de sólidos se mantiene en su nivel más bajo (4%), el rendimiento aumenta significativamente al incrementar la concentración del agente lixiviante (-1 a +1), el rendimiento obtenido fue de 240.5 gAH/kg, mientras que al utilizar el mismo % de sólidos y una concentración de 0.5 M de NaOH (-1/+1) se logró un rendimiento de 790.5 gAH/kg.

Por su parte, cuando el porcentaje de sólidos es de 8% (nivel +1), el rendimiento también aumenta al incrementarse la concentración de NaOH. Sin embargo, este efecto es menos significativo, ya el que rendimiento obtenido es de 107.37 y 678.25 gAH/Kg al utilizar 0.1 y 0.5 M de NaOH, respectivamente. Estos resultados reafirman que el factor con mayor significancia en el proceso es la concentración de NaOH.

En la Figura 5B se presenta una superficie tridimensional que muestra la relación conjunta entre los factores B y C (porcentaje de sólidos y concentración de NaOH). La curvatura corrobora que existe una relación no lineal de estos factores sobre el rendimiento. Estos resultados sugieren que, aunque ambas variables tienen un efecto positivo en el rendimiento, el efecto de C es más pronunciado cuando B se encuentra en su nivel inferior (-1), y muestran una interacción compleja entre B y C que afecta el rendimiento del proceso de obtención de AH a partir de leonardita.

Los OH^- son esenciales para la solubilización de los compuestos húmicos de la leonardita. Una vez que los OH^- reaccionan y se consumen, el proceso de extracción se detiene, independientemente de la cantidad de sólido presente. En este caso, utilizar un mayor porcentaje de sólidos resulta en una extracción menos eficiente.

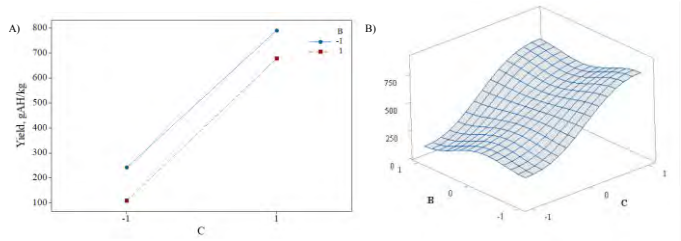

Figura 5. Efecto de la interacción de los factores porcentaje de sólidos y concentración de NaOH (EB×C) A) grafica de interacción B) grafica de superficie.

Por último, la Figura 6 presenta el diagrama de Pareto, que resume los efectos de cada factor y sus interacciones en el rendimiento de extracción de AH. Las barras representan los factores estudiados y se muestra una línea de referencia para indicar su significancia estadística ($\alpha = 0.05$). Se concluye que todos los factores individuales, así como las interacciones A×B y A×C, tienen un impacto significativo en la extracción de ácidos húmicos. Sin embargo, la concentración de NaOH encabeza el diagrama con el mayor efecto estandarizado.

El análisis de estos resultados evalúa el efecto de los factores individuales y sus interacciones en la extracción de ácidos húmicos a partir de leonardita. Los resultados indican que la concentración de NaOH es el factor más crítico, seguido por el tamaño de partícula y el porcentaje de sólidos. Las interacciones entre estos factores también juegan un papel importante, especialmente en combinaciones específicas que maximizan el rendimiento de extracción.

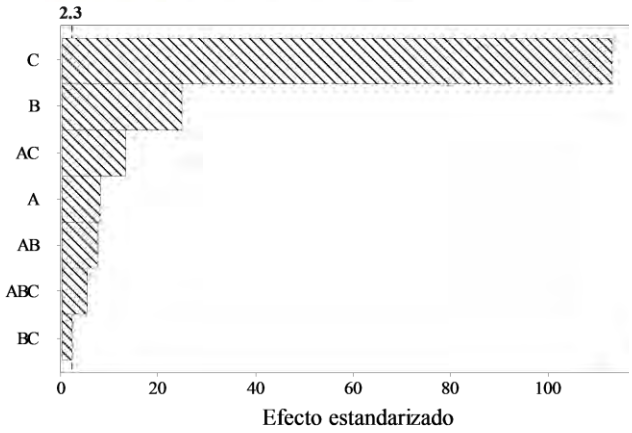

Figura 6.- Diagrama de Pareto de los efectos estandarizados en la extracción de ácido húmico de leonardita. A) tamaño de partícula, B) porcentaje de sólidos y C) concentración de NaOH.

CONCLUSIONES

La concentración de NaOH se identificó como el factor más crítico en la extracción de ácidos húmicos. Una concentración de 0.5 M de NaOH resultó en el mayor rendimiento, alcanzando hasta 734.38 g de AH/kg de leonardita. Esto resalta la importancia de utilizar una concentración adecuada de agente lixiviante para maximizar la eficiencia del proceso de extracción

Las interacciones entre los factores, especialmente entre el tamaño de partícula y la concentración de NaOH (A×C), y entre el tamaño de partícula y el porcentaje de sólidos (A×B), mostraron ser significativas. Estas interacciones deben ser consideradas en el diseño de procesos para aumentar la eficiencia la extracción de ácidos húmicos, ya que combinaciones específicas de estos factores pueden mejorar notablemente el rendimiento.

El tamaño de partícula y el porcentaje de sólidos tuvieron impactos significativos en el rendimiento de extracción. Las partículas más grandes (375 µm) proporcionaron un mayor rendimiento en comparación con las más pequeñas (90.5 µm). Además, un menor porcentaje de sólidos (4%) favoreció la extracción de ácidos húmicos, obteniendo un rendimiento significativamente mayor que con un 8% de sólidos. Estos resultados sugieren que un mayor tamaño de partícula y un menor porcentaje de sólidos facilitan la disponibilidad de materia orgánica y mejoran la interacción con el NaOH.

Los resultados de este estudio proporcionan una base para la mejorar los procesos industriales de extracción de ácidos húmicos, lo que puede tener aplicaciones significativas en la agricultura y otras industrias. Al incrementar la eficiencia de la extracción, se pueden producir ácidos húmicos de alta calidad de manera rentable, beneficiando tanto a los productores como a los usuarios finales en la mejora de la fertilidad del suelo y el rendimiento de los cultivos.

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Encapsulation of lipase from *Aspergillus niger* GH1 and evaluation of their disintegration *In Vitro*

Encapsulación se lipasas de *Aspergillus niger* GH1 y evaluación de su digestión I*n Vitro*

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Abstract

Lipases are enzymes that hydrolyze triglycerides to fatty acids and glycerol. These enzymes are naturally present in the human body and are essential to digest fats. In the present study, we focused on the encapsulation of lipases produced by Aspergillus niger GH1 fungus in an alginate matrix, using three concentrations of sodium alginate (2, 2.5, and 3.0 % w/v) and two concentrations of CaCl2 were evaluated (0.1 and 0.2M) such as crosslinking agent. The results show that alginate provides a diffusion barrier which enables resist gastric conditions, so they can be released into the intestine in 28 minutes, where the enzymes hydrolysis triglycerides. The results showed that with 3.0 % alginate and 0.2 M CaCl2 concentration, the morphological characteristics and enzymatic activity are the best results, thus being effective for encapsulating and releasing lipases.

Keywords: encapsulation; enzyme; *Aspergillus niger*.

Resumen

Las lipasas son enzimas que hidrolizan los triglicéridos en ácidos grasos y glicerol. Estas enzimas están presentes de forma natural en el cuerpo humano y son esenciales para la digestión de las grasas. En el presente estudio nos centramos en la encapsulación de lipasas producidas por el hongo Aspergillus niger GH1 en matriz de alginato, utilizando tres concentraciones de alginato sódico (2, 2,5 y 3,0% p/v) y se evaluaron dos concentraciones de CaCl2 (0,1 y 0,2M) como agente gelificante. Los resultados muestran que el alginato proporciona una barrera de difusión que permite resistir las condiciones gástricas, por lo que pueden ser liberados en el intestino en 28 minutos, el lugar donde las enzimas realizan la hidrólisis de los triglicéridos. Los resultados mostraron que con un 3,0 % de alginato y una concentración de CaCl2 0,2 M, las características morfológicas y la actividad enzimática son las que mejores resultados presentan, resultandos eficaces para encapsular y liberar lipasas.

Palabras clave: Encapsulación; enzima; *Aspergillus niger*.

INTRODUCTION

Lipases (EC3.1.1.3) are a kind of enzyme with considerable physiological significance and industrial potential. (Sirisha et al., 2016). This enzyme catalyzes triacylglycerol hydrolysis at the oil-water interface to release glycerol and free fatty acids (Dheeman et al. 2010).

The interest in lipases has increased in recent years due to their diverse catalytic properties (Zuin et al., 2022). They have a big field of industrial applications, such as additives in detergent formulations, in the food industry for the manufacture of products with low dietary fat and cholesterol, in the paper industry with the objective removing the wax from the paper pulp, in the pharmaceutical industry in obtaining bioactive molecules, chemical synthesis processes for obtaining optically pure compounds (Alves et al., 2024), modification of fats and other lipids by hydrolysis and esterification(Lipases as Biocatalysts for Enzymatic Interesterification | SpringerLink, n.d.) to name a few.

With advances in immobilization techniques, the use of enzymes and immobilized cells is increasing (Di Cosimo et al., 2013), due to the fragile nature of the enzyme protein, which has limited stability in the structure and functionality (Mulinari et al., 2020). It is considered that an enzyme is suitable for a commercial application if its stability is sufficient for the application and the method of encapsulation allows it to do so. Encapsulation is a process where a continuous thin coating is formed around solid particles, liquid droplets, or gas cells that are fully contained within the capsule wall (Kok et al., 2018). This technology has been used in the food industry for more than 60 years as a way to provide liquid and solid ingredients as an effective barrier for environmental and/or chemical interactions until release is desired (Sheldon et al., 2021).

Enzyme encapsulation is an attractive method among the different immobilization strategies to improve the reusability and stability of enzymes because it can separate enzymes from a hazardous external environment (Di Cosimo et al., 2013).

The active substance is covered with a porous polymer film(Liu et al., 2022); this membrane barrier or film is usually made of components with strings to create a net with a hydrophobic and/or hydrophilic property(Basso & Serban, 2019).

The encapsulation technique allows food packaging and other materials such as oils, probiotic bacteria, enzymes, whey, vegetable pigments, minerals, vitamins, and food additives. Nearly any material that needs to be protected, isolated, slowly released over time, or released at a certain time can be encapsulated (Zou et al., 2023). The principal coating materials typically used in food industries are carbohydrates, cellulose, gums, lipids, and proteins. Encapsulation is carried out through various processes chemicals such as conservation, interfacial polymerization, ionic gelification, polymer incompatibility, entrapping in liposomes, and mechanical processes like techniques of co-crystallization, freeze drying/cooling, extrusion, and finally, there is the spray drying technique, this being the most important and used in the food industry. (Gupta et al., 2024)

In this study, we used the ionic gelification method for the lipase immobilization to fabricate calcium alginate beads. Alginate has the following advantages: a) Alginate, the major shell material, is extracted from algae, which is cheap and has been used in food and drugs for many years. It is full of human-necessary microelements and is used as a health food in many countries. Moreover, with anti-cancer activity, alginate can absorb heavy metal ions and free radicals from the human body. The capsule is very stable. The capsule's shell comprises at most 15% of the capsule weight, which can improve the loading capacity of the bioactive agent within the capsule. The purpose of this work was to encapsulate lipases obtained from the fermentation of the fungus Aspergillus niger GH1 to provide protection and stability to digestive conditions when being incorporated into a food product.

MATERIALS Y METHODS

Obtention of lipase extract by fermentation with *Aspergillus niger* GH1

The enzyme extract was obtained by fermentation in a liquid medium of Czapek-dox, this contained the following composition: 2 g/L of NaNO3; 1 g/L K2HPO4; 0.5 g/L MgSO4.7H2O; 0.5 g/L KCl; 0.01 g/L FeSO4.7H2O, also a carbon source supplemented with 1% olive oil w/w. The initial pH was adjusted to 6.0 and sterilized at 15 Lb of pressure at 121°C for 15 min.

Conditions of fermentation were inoculum; 1 X 107 spores/ml of A. niger GH1 (conidial suspension in Tween 80 0.01%), incubation time; for eight days at 130 rpm, and temperature; at 30 °C. After, the fungal cultures were vacuum filtered and then centrifuged at 12,000 rpm for 5 min (Coca and Harrison, 2001). The supernatant was collected, and enzyme activity of lipase was determined by measuring the increase in absorbance at 348 nm produced by the release of p-nitrophenol as a result of hydrolysis of 50 mM p-nitrophenyl ester propionate (p-NPP) in 25 mM phosphate buffer pH 7.0 and 37 °C (Bastida et al., 1998). The enzyme unit is required to

hydrolyze one micromole of p-NPP per minute under the described conditions.

Selection of conditions for encapsulation of lipases

The ionic gelification technique encapsulated lipases. An experimental block design was obtained, which is shown in Table 1; three repetitions were performed for each treatment. Sodium alginate was dispersed in distilled water at a temperature of 60 °C at constant agitation during 30 minutes of mixing to immobilize the enzyme subsequently 30 mL of distilled water was added to 120 mL of sodium alginate solution and mixed at room temperature at constant stirring for 10 minutes. The suspension was passed through a 20 mL syringe and was dropped into the solution of CaCl2 in continuous agitation to keep the capsules from sticking between them. It was kept for 30 minutes at room temperature in the solution (Figure 1).

Table 1. Design of the experimental matrix for each lipase encapsulation treatment

Treatment	Alginate solution (%)	$CaCl_2$ (mol/L)
A	2	0.1
B		0.2
C	2.5	0.1
D		0.2
E	3.0	0.1
F		0.2

After each treatment, the capsules were collected by filtration and washed with distilled water to remove excess Ca2+ on the surface of the capsule. The capsules were dried for 24 h at 28 ° C.

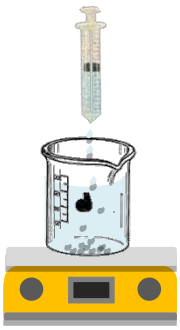

Figure 1. Schematic representation of the method used to encapsulate lipases: an alginate solution with lipases extract was dripped into a stirred calcium chloride solution.

Characterization of alginate capsules

Morphology and particle size

The particle size of the alginate beads was measured by hand using a caliper, which was determined by the average of 30 samples. Morphology was determined using an optical microscope adapted with Dino-lite AM3111 0.3MP Digital microscope.

Moisture content

The moisture contents of the capsules were determined automatically in moisture analysis equipment (Ohaus), using 0.5 g of capsules; the test was conducted at a constant temperature of 110 °C (Gangawane et al., 2024).

Colorimetry

The capsules were used to determine CIELAB color coordinates using a chromameter. CIELAB colorimetric system was interpreted as follows: L* indicates lightness read from 0 (black) to 100 (white). The positive a* value indicates the red color, while the negative a* value represents the green color. Similarly, positive and negative b* values indicate yellow and blue, respectively. Chroma values denote the saturation or purity of color. In a color wheel, values close to the center, at the same L* value, indicate dull or gray colors, whereas values near the circumference represent vivid or bright colors (Moreira et al., 2017).

Hygroscopicity

Hygroscopicity was determined according to the method proposed by Cai and Corke (2000), with some modifications. Samples of about 1g were placed in desiccators with a saturated solution of NaCl (relative humidity of 75.3%). After one week, samples were weighed, and hygroscopicity was expressed as adsorbed moisture per 100g of sample (g/100g).

Water activity

Water activity was measured using 1 g of lipase capsules in Aqualab equipment (DECAGON DEVICES INC.).

Enzymatic activity

Enzyme activity was determined to alginate beads by measuring the increase in absorbance at 348 nm produced by the release of p-nitrophenol as a result of hydrolysis of 50 mM p-nitrophenyl ester propionate (p-NPP) in 25 mM phosphate buffer pH 7.0 and 37 ° C (Bastida et al., 1998). The enzyme unit

is the enzyme required to hydrolyze 1 micromole of p-NPP per minute under the described conditions.

Disintegration in vitro of lipase encapsulated

The disintegration of capsules was determined according to disintegration tests from FEUM (FEUM, 2011) with some modifications. An ELECSA disintegrator weighing 250 mg of the capsule was used and placed in the basket tubes. A simulated gastric fluid (2.0 g dissolved sodium chloride and 3.2 g pepsin in 7.0 mL of hydrochloric acid was completed to 1000 mL of water. The solution pH 1.2) and placed in motion team for 2 h at 37 °C ±2. Elapsed capsules were changed to a simulated intestinal (dissolved 6.8 g of potassium phosphate monobasic in 250 mL of water and added 190 mL of 0.2N sodium hydroxide and was completed to 1000 mL of water, the pH of the solution was 7.5) at 37 °C ± 2, time was measured until complete disintegration of capsules.

RESULTS

To define the concentrations of the ingredients and conditions for the production of the lipase beads, three alginate concentrations (2.0 %, 2.5 % and 3.0 %) and two concentrations of the crosslinking agent (0.1 and 0.2 M of CaCl2) were tested. Capsules were obtained with all the formulations tested, as shown in Figure 2. The best morphological appearance was 3.0% of alginate, while 2.5 % and 2.0 % showed a semispherical appearance.

The size of the capsules was measured for each concentration (Table 2), and the different sizes obtained are shown below, where the capsules were in a range of 1.49 to 1.71 mm; Flores et al 2011 measured the size of the alginate capsules of three different concentrations of sodium alginate (1.0%, 1.5%, and 2.0%) the capsule diameter was from 1.86 to 2.03 mm.

The best results were obtained with the concentration of 3.0% alginate and 0.2M CaCl2; the capsules had a spherical and regular appearance, while the other concentrations showed irregularly shaped; this polysaccharide forms strong gels with Ca+2, giving spheres with good strength and flexibility (Anandharamakrishnan et al., 2010; Birchal et al., 2006). The size and morphology can be influenced by different parameters such as speed stirring, the encapsulating matrix type, the technique used, the substance to be encapsulated, and some other parameters that can be considered (Gharsallaoui et al., 2007; Jin & Chen, 2009; Langrish & Fletcher, 2001).

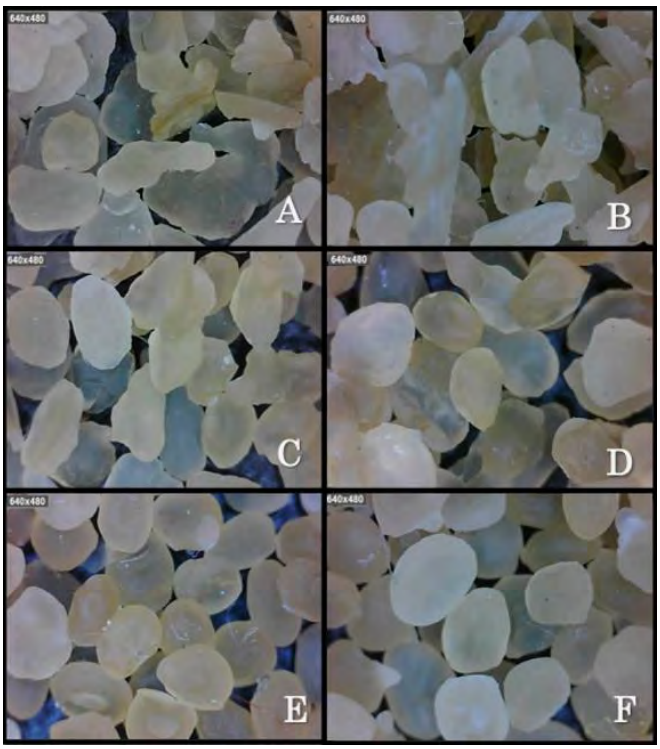

Figure 2. Morphological appearance of lipase capsules. A) 2.0%, 0.1 M, B) 2.0%, 0.2 M, C) 2.5%, 0.1 M, D)2.5%, 0.2 M, E) 3.0%, 0.1M and F) 3.0%, 0.2M

Table 2. Size of capsules with extract of lipase.

Treatment	Size (mm)
A	1.51 ± 0.15
B	1.66 ± 0.16
C	1.59 ± 0.14
D	1.52 ± 0.12
E	1.49 ± 0.13
F	1.71 ± 0.10

A) 2.0% sodium alginate and 0.1 M $CaCl_2$, B) 2.0% sodium alginate and 0.2 M $CaCl_2$, C) 2.5% sodium alginate and 0.1 M $CaCl_2$, D) 2.5% sodium alginate and 0.2 M $CaCl_2$, E) 3.0% sodium alginate and 0.1M $CaCl_2$ and F) 3.0% sodium alginate and 0.2M $CaCl_2$. Data are mean (±standard deviation) of three replicates.

The results of the moisture content determined for each of the samples can be seen in Figure 3.

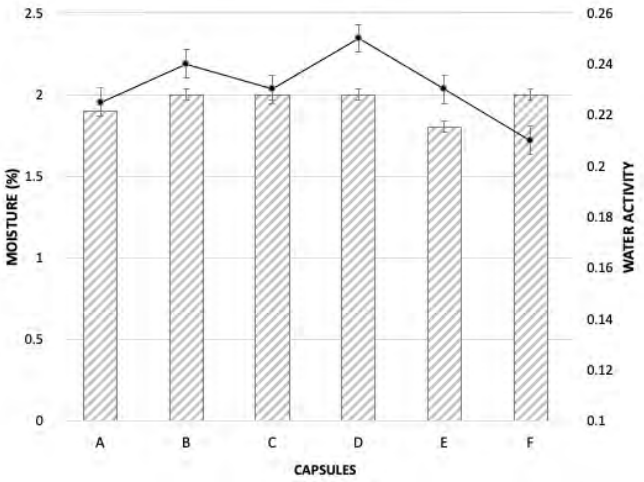

Figure 3. Moisture content (y-axis primary) and water activity (y-axis secondary) of alginate capsules at different concentrations. A) 2.0 % sodium alginate and 0.1 M $CaCl_2$, B) 2.0 % sodium alginate and 0.2 M $CaCl_2$, C) 2.5 % sodium alginate and 0.1 M $CaCl_2$, D) 2.5 % sodium alginate and 0.2 M $CaCl_2$, E) 3.0 % sodium alginate and 0.1M $CaCl_2$ and F) 3.0 % sodium alginate and 0.2M $CaCl_2$. Data are mean (±standard deviation) of three replicates.

The moisture content of the capsules was in the range of 1.8 and 2%, (Delgado et al., 2022a), showing moisture content values between 96% and 95% in alginate capsules. The difference with this work was that the capsules were pre-dried so that results were expected, and these values were not significantly different; the moisture content and the water activity are relatively low; these results indicate the stability of the capsules and prevent the growth of microorganisms that may affect its functionality.

The hygroscopicity results can be seen in Table 3; this was evaluated in a desiccator with a saturated solution of NaCl and was weighed; the capsules lost weight, indicating that they cannot absorb atmospheric moisture. (Delgado et al., 2022). Demonstrated that alginate capsules are more stable when they have a low hygroscopicity.

Figure 4 shows the color results obtained in the capsules. The results show that the color of the capsules is in the brown-yellow color range; all capsules 2.0 %, 2.5 %, and 3.0 % are in the same place, so the evaluated concentration of sodium alginate does not affect the color of the capsule.

Table 3. Hygroscopicity of alginate capsules. Initial weight and final weight of the alginate capsules after 7 days of the experiment

Capsules	Initial weight	Final weight
A	1.007	0.971 ± 0.005
B	1.003	0.970 ± 0.001
C	1.006	0.948 ± 0.005
D	1.004	0.958 ± 0.010
E	1.004	0.951 ± 0.004
F	1.003	0.968 ± 0.007

A) 2.0 % sodium alginate and 0.1 M $CaCl_2$, B) 2.0 % sodium alginate and 0.2 M $CaCl_2$, C) 2.5 % sodium alginate and 0.1 M $CaCl_2$, D) 2.5 % sodium alginate and 0.2 M $CaCl_2$, E) 3.0 % sodium alginate and 0.1M $CaCl_2$ and F) 3.0 % sodium alginate and 0.2M $CaCl_2$. Data are mean (±standard deviation) of three replicates.

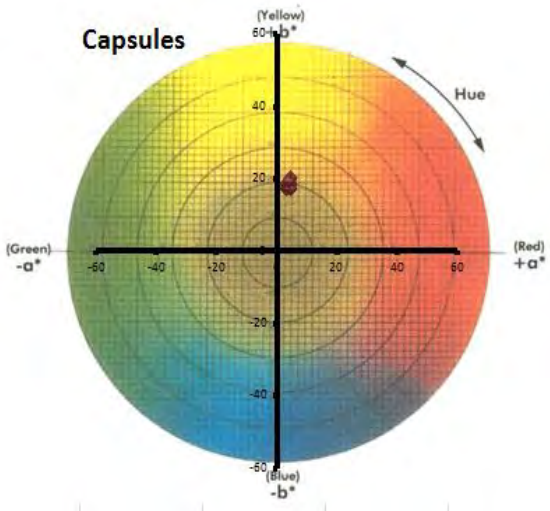

Figure 4. Polar scatter plot of color of capsules A) 2.0 % sodium alginate and 0.1 M $CaCl_2$, B) 2.0 % sodium alginate and 0.2 M $CaCl_2$, C) 2.5 % sodium alginate and 0.1 M $CaCl_2$, D) 2.5 % sodium alginate and 0.2 M $CaCl_2$, E) 3.0 % sodium alginate and 0.1M $CaCl_2$ and F) 3.0 % sodium alginate and 0.2 M $CaCl_2$. Data are mean (±standard deviation) of three replicates

The enzymatic activity was evaluated; the results are shown in Figure 5, where the enzymatic extract obtained from a previous fermentation with Aspergullus niger GH1 was 20.02

UE/mL, the highest enzyme encapsulated was with 2.0 % of alginate sodium and 0.2 M, this capsules shows an activity of 17.86 UE/mL, following by the capsules of 2.0 % 0.2 M, 2.5 % 0.1 M, 2.5 % 0.2 M, 3.0 % 0.1 M, with an activity of 15.77 UE/mL, 17.20 UE/mL, 14.44 UE/mL and 15.88 UE/mL respectively, and the and concentration which encapsulated the lowest amount of enzyme was 2.0 % and 0.1 M with and activity of 11.73 UE/mL. Flores et al. (2011) encapsulated proteolytic enzymes, and the best results were with the low alginate concentration of 70.8 % of enzymatic encapsulation, while the present work with the highest alginate concentration achieved encapsulation 87.6 %, which indicates that our method is a better combination for encapsulating enzymes.

For the disintegration of the capsules, the concentration of 3.0% sodium alginate and 0.2 M $CaCl_2$ was chosen as it showed the highest enzymatic activity. The disintegration time of the alginate capsules was determined in a tablet disintegrator (ELECSA) to see if they could resist simulated stomach and intestinal fluid conditions (Figure 6).

The results show that the capsules are resistant to acid pH since after being in contact with simulated gastric fluid at pH 1.2, they did not show any change in their morphology after the capsules were evaluated in an intestinal medium to measure the disintegration time after 5 minutes lipase capsules began to diminish in size and 28 minutes after the capsules were completely disintegrated so that they can resist gastric conditions and under intestinal conditions are completely disintegrated, alginate capsules can be designed for an active pharmaceutical ingredient with gastric solubility, enteric solubility or colon solubility easily. Moreover, it can be used in food and industry due to its perfect safety, stability, and low cost. Calcium alginate gels are widely used in food, pharmaceutical, and medical applications (Ta et al., 2021). It has been reported that calcium alginate beads can protect bioactive molecules from the stomach's harsh, acidic environment Gopal, 2022).

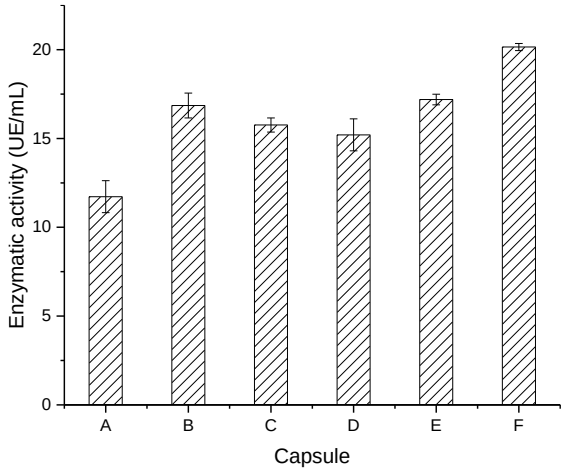

Figure 5. Lipolytic enzyme activity of capsules at different concentrations. A) 2.0% sodium alginate and 0.1 M CaCl2, B) 2.0% sodium alginate and 0.2 M CaCl2, C) 2.5% sodium alginate and 0.1 M CaCl2, D) 2.5% sodium alginate and 0.2 M CaCl2, E) 3.0% sodium alginate and 0.1M CaCl2 and F) 3.0% sodium alginate and 0.2M CaCl2. Data are mean (±standard deviation) of three replicates.

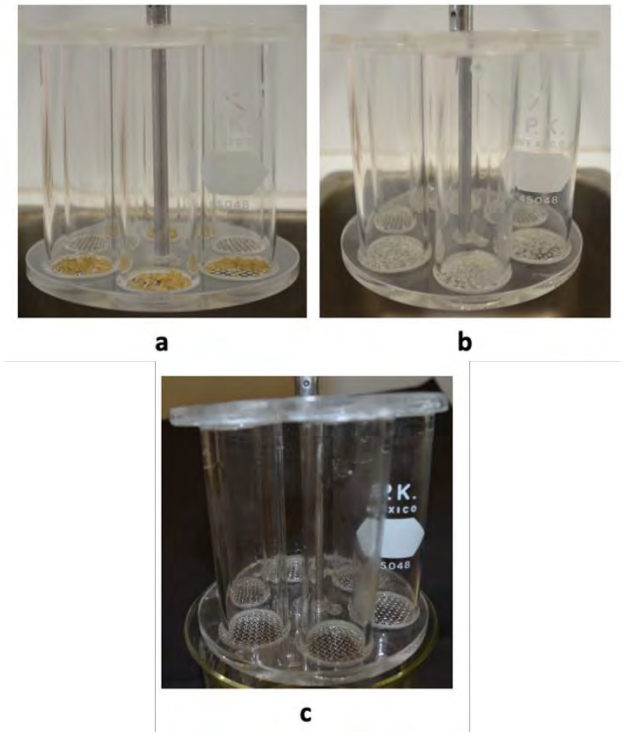

Figure 6. Disintegration of lipases capsules. a) Capsules before being in contact with the medium simulated gastric. b) Capsules after two hours in the middle gastric. c) Total Disintegration of capsules.

CONCLUSIONS

Lipase encapsulation has great potential for the food industry because it can be the vehicle for incorporating these enzymes in many food products that may benefit humans. Designing an encapsulation technique that allows for preserving the enzyme and resisting digestive conditions greatly contributes to the industry and facilitates better-quality manufacturing processes and products. In this work, the best combination to obtain capsules with desirable characteristics is 3% sodium alginate crosslinking agent CaCl2 solution 0. 2 M, which is resistant to digestive conditions. The technique used in this study for forming the capsules was an ionic gelification reaction between a polysaccharide and a counter ion, also known as the alginate drip method. The process is performed quickly and can encapsulate any food, either hydrophobic, hydrophilic thermosensitive liquid, or solid.

This research highlights that the method used to encapsulate lipases has a practical opportunity in the food industry thanks to its technological advantages since it can protect the active material from environmental degradation (heat, air, light, and humidity, among others). Encapsulated compounds are released gradually. Their physical properties can be modified according to their needs. Characteristics such as taste and odor of the material can be masked due to encapsulation.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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Evaluación y Estudio de Especies de Hongos Comestibles Silvestres Aislados en el Área Forestal de la Universidad Autónoma Agraria Antonio Narro (UAAAN)

Evaluation and Study of Wild Edible Fungi Species Isolated in the Forestry Area of the Universidad Autónoma Agraria Antonio Narro (UAAAN)

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Resumen

En México, existe una gran diversidad de especies forestales, como los géneros *Quercus, Pinus, Abies* y *Acacia,* que representan un valioso patrimonio ecológico y cultural. Sin embargo, factores como el sobrepastoreo, el cambio climático y los incendios forestales han degradado significativamente los ecosistemas, afectando la biodiversidad de bosques y selvas. Los hongos silvestres son esenciales para la riqueza biocultural del sureste de México, aunque esta región también se ha visto impactada por estas problemáticas. En el norte del país, la cultura de consumo de hongos silvestres es limitada debido a las condiciones climáticas extremas, con temperaturas superiores a 30 °C y precipitaciones anuales de 350 a 400 mm. A pesar de estas condiciones, diversas especies de hongos silvestres logran desarrollarse. Este estudio se realizó en el Departamento de Forestal de la Universidad Autónoma Agraria Antonio Narro, en Saltillo, Coahuila, para recolectar e identificar hongos silvestres de la región. Durante el muestreo, se encontraron ejemplares de los géneros *Agaricus, Amanita* y *Pleurotus*. El trabajo busca contribuir al conocimiento y conservación de hongos silvestres comestibles, resaltando su importancia ecológica y cultural en los ecosistemas mexicanos.

Palabras clave: Biodiversidad, Biomasa, Conservación ambiental, Ecología forestal, Hongos silvestres.

Abstract

Mexico hosts a remarkable diversity of forest species, including the genera *Quercus, Pinus, Abies*, and *Acacia*, which constitute a significant ecological and cultural heritage. However, factors such as overgrazing, climate change, and forest fires have severely degraded ecosystems, impacting the biodiversity of forests and jungles. Wild mushrooms represent a vital component of the bio-cultural richness in southeastern Mexico, although this region has been significantly affected by these issues. In northern Mexico, the culture of wild mushroom consumption is limited due to extreme climatic conditions, characterized by temperatures exceeding 30 °C and annual precipitation ranging from 350 to 400 mm. Despite these environmental challenges, several wild mushroom species thrive. This study was conducted in the Forestry Department of the Autonomous Agrarian University Antonio Narro, in Saltillo, Coahuila, to collect and identify wild mushrooms present in the region. During sampling, specimens of varying sizes belonging to the genera *Agaricus, Amanita*, and *Pleurotus* were documented.
This research aims to contribute to the knowledge and conservation of edible wild mushrooms, emphasizing their ecological and cultural significance within Mexican ecosystems.

Keywords: Biodiversity, Biomass, Environmental conservation, Forest ecology, Wild fungi.

INTRODUCIÓN

En el sur y sureste de México se encuentra una notable diversidad genética de especies forestales, favorecida por un régimen climático caracterizado por precipitaciones anuales superiores a 800 mm y temperaturas que oscilan por encima de los 25 °C. Estas condiciones propician el desarrollo de una amplia variedad de hongos, los cuales pueden encontrarse en tres principales formas de vida: saprófitos, simbióticos y parásitos (Torres-Gómez et al., 2022).

Los hongos macroscópicos, también denominados hongos superiores o basidiomicetos, están constituidos por estructuras filamentosas denominadas hifas, las cuales se agrupan para formar el micelio. Este último da origen al cuerpo fructífero, que presenta diversas características morfológicas dependiendo del género y la especie. Entre estas estructuras destacan el anillo, la volva y el sombrero, el cual alberga en su interior las laminillas portadoras de las esporas (F. Atila et al., 2021; Funda Atila et al., 2018)

Las hifas se desarrollan a partir de las esporas, también conocidas como propágulos o estructuras reproductivas. Estas esporas se generan en los poros situados en la parte inferior del sombrero del hongo, desde donde son liberadas al medio ambiente. Al depositarse en el suelo, las esporas pueden formar estructuras de resistencia denominadas esclerocios, las cuales se adhieren a la materia orgánica circundante. Bajo condiciones óptimas de humedad y temperatura, los esclerocios germinan y dan lugar al micelio (Piña-Guzmán et al., 2016).

El micelio, tras completar su etapa inicial de desarrollo, entra en una fase fenológica en la que se forma el cuerpo fructífero, el cual emerge desde el suelo. Durante esta etapa se observa la presencia de la volva, una estructura membranosa que origina el hongo. Dependiendo del género y la especie, las estructuras morfológicas del hongo, como el tallo, el anillo y el sombrero, pueden variar significativamente. Además, el sombrero suele estar recubierto por una membrana denominada velo (Guzmán Luna et al., 2025).

El cultivo de hongos comestibles, como las setas, puede realizarse utilizando diversos sustratos. Durante la temporada de lluvias, estos organismos proliferan de manera natural en sustratos como la corteza de árboles, aprovechando las condiciones de alta humedad. Fuera de este periodo, es posible cultivarlos en sustratos alternativos que incluyen residuos agroindustriales como bagazo de caña de azúcar, paja de arroz, paja de avena, rastrojo y olotes de maíz, sorgo, fibra de coco y otros materiales ricos en compuestos orgánicos, como azúcares, lignina y lignocelulosa (Pérez-Chávez et al., 2022). Estos componentes sirven como fuentes de energía para los hongos, que los degradan mediante procesos enzimáticos, transformándolos en biomasa fúngica. El producto final incluye cuerpos fructíferos de alto valor nutricional, ricos en proteínas, vitaminas, minerales y compuestos bioactivos de interés para la alimentación humana (Funda Atila, 2019; González et al., 2021).

En México, diversas especies forestales proporcionan hábitats adecuados para el desarrollo de múltiples especies de hongos, que incluyen tanto hongos comestibles como tóxicos y aquellos con propiedades beneficiosas para la salud. Entre estas especies destaca el árbol de cazahuate (Ipomoea murucoides), presente en los estados de Morelos, Puebla y otras regiones. De manera natural, este árbol facilita el crecimiento del hongo *Pleurotus ostreatus*, una especie de gran interés biológico y económico debido a su alto contenido nutricional, su capacidad para degradar compuestos lignocelulósicos y su potencial en la biotecnología aplicada (Oliveira do Carmo et al., 2021).

La recolección de hongos silvestres en México es una práctica poco conocida, en parte debido al desconocimiento sobre sus patrones de desarrollo, estructuras biológicas, procesos ecológicos y las variables socioeconómicas asociadas (Alanis-Pavón et al., 2024). Según (Martinez-Medina et al., 2021), la falta de gestión forestal sostenible ha propiciado actividades de extracción no reguladas que impactan negativamente el hábitat, generan compactación del suelo, alteran los equilibrios ambientales y dificultan el ciclo biológico de ciertas especies de hongos. Esto, a su vez, reduce su producción, incrementa la erosión genética y afecta el desempeño ecológico que tienen en los ecosistemas forestales (Franco-Maass et al., 2016; Guo et al., 2024)

La importancia de la gestión forestal sostenible radica en la administración y uso de los bosques de manera que se preserve la biodiversidad genética, la productividad, la capacidad de regeneración, la vitalidad y el potencial ecológico, económico y social de los ecosistemas, tanto a nivel nacional como global, sin causar deterioro en otros sistemas (Chizmar et al., 2024). En este contexto, los hongos silvestres comestibles desempeñan un papel esencial en los ecosistemas forestales. Su contribución incluye el reciclaje de nutrientes mediante la descomposición de residuos lignocelulósicos y excrementos de animales, lo que resulta en la incorporación de fertilizantes al suelo (Brunialti et al., 2020; Mann et al., 2024). Además, los hongos poseen un gran potencial biotecnológico y ecológico. Pueden ser empleados como micorrizas, estableciendo relaciones simbióticas con las raíces de las plantas, lo que mejora la absorción de nutrientes esenciales como nitrógeno, fósforo, cobre y zinc, elementos clave en la cadena trófica. Estas interacciones benefician la flora y fauna, contribuyendo al

equilibrio del sistema forestal y a su resiliencia (Esquivel-Mendiola et al., 2024).

México es un país caracterizado por una amplia diversidad de especies forestales, particularmente en las regiones del sur y sureste, donde las condiciones climáticas son favorables para el desarrollo de hongos silvestres. Estas áreas registran niveles de humedad superiores a 800 mm anuales y temperaturas promedio de entre 24-28 °C, lo que crea un entorno óptimo para el crecimiento de estas especies.

Los hongos silvestres desempeñan un papel ecológico fundamental al descomponer la materia orgánica de las especies forestales, transformándola en biomasa de alto valor biológico. Su composición nutricional destaca por un contenido proteico del 18 al 20 %, superior al de muchas hortalizas, así como por su bajo contenido calórico y graso (Cheng & Brewer, 2021; Hassan, 2011; Kaprasob et al., 2022). Además, los hongos son una fuente significativa de vitaminas esenciales como niacina, tiamina y riboflavina, nutrientes indispensables para mejorar la calidad de vida y el bienestar humano.

La recolección de hongos silvestres en México está estrechamente vinculada a las prácticas culturales de las comunidades rurales, las cuales son influenciadas por factores como el género, el origen, la ocupación y el grupo étnico al que pertenecen. Estas comunidades poseen un amplio conocimiento tradicional sobre el consumo de hongos, sus propiedades medicinales y su uso en rituales, transmitido de manera vertical, de padres a hijos, y de forma horizontal entre individuos de la misma generación. Este conocimiento, parte integral de su identidad cultural, continúa vigente en la actualidad (Molina-Castillo et al., 2023a; Torres-Gómez et al., 2022).

MATERIALES Y MÉTODOS

El estudio se llevó a cabo en el Departamento de Ciencias Forestales de la Universidad Autónoma Agraria Antonio Narro, localizado en Buenavista, Saltillo, Coahuila, México. El área de investigación se encuentra en las coordenadas 25°21'8.87" N y 101°1'58.97" W, a una altitud de 1786 metros sobre el nivel del mar (msnm). El sitio incluye una zona reforestada con especies de *Pinus* y *Cupressus,* proporcionando un entorno propicio para la colecta de hongos silvestres.

La colecta de ejemplares se realizó en dos áreas específicas: 1) En el área interna del Departamento de Ciencias Forestales, específicamente bajo árboles de *Pinus spp*. (ocote), se realizó la prospección mediante la remoción controlada de la materia orgánica acumulada en el suelo, utilizando un rastrillo para garantizar la mínima perturbación del entorno. 2) En la zona externa adyacente a los invernaderos forestales, se efectuó la búsqueda en áreas con características ambientales complementarias, favoreciendo la identificación de hongos en condiciones diferenciadas. Durante la colecta, se fotografiaron los hongos encontrados para documentar su morfología y condiciones de desarrollo. Posteriormente, se tomaron muestras representativas de cada hongo identificado. Las colectas se llevaron a cabo por la mañana, registrándose datos precisos de cada ejemplar, como la fecha, ubicación, morfología (sombrero, tallo o estípite, presencia de anillo y vulva) y especies encontradas. Los hongos se limpiaron con tela suave para eliminar tierra y residuos orgánicos, descartando ejemplares con evidencia de plagas. Las muestras se almacenaron en bolsas de papel etiquetadas y se colocaron en cestas para su transporte.

La identificación de las especies se realizó mediante análisis morfológico, apoyándose en referencias especializadas como (Munir et al., 2023; Yahia et al., 2017) (Figura 2). Este enfoque permitió documentar las características taxonómicas de los hongos recolectados y sus condiciones de desarrollo en el ecosistema estudiado.

Figura 1. Hongos recolectados, Universidad Autonoma Agraria Antonio Narro

RESULTADOS Y DISCUSIÓN

Las diversas etnias que habitan las distintas áreas ecológicas de México practican la recolección de hongos silvestres comestibles como una estrategia de supervivencia. Su conocimiento empírico sobre este recurso ha permitido no solo satisfacer necesidades alimenticias, sino también generar ingresos económicos, mejorar el bienestar social y contribuir a la conservación del medio ambiente (Molina-Castillo et al., 2023b). A nivel global, el consumo de hongos silvestres también es común en varios países. Estos hongos, recolectados de

manera natural, se encuentran en una diversidad de hábitats que incluyen bosques, prados y parcelas agrícolas. Su identificación y clasificación han permitido documentar una amplia variedad de especies, adaptadas a diferentes condiciones ecológicas (Tabla 1).

Tabla 1. Países productores de hongos comestibles silvestres

País	Cantidad (Ton)
Japón	150,000
U.R.S.S.	23,000
Chile	3,500
Francia	3,000
Italia	3,000
México	14,200

Los hongos fueron recolectados en el Departamento de Ciencias Forestales de la Universidad Autónoma Agraria Antonio Narro, ubicado en un área reforestada con especies de *Pinus spp., Cupressus spp*. y *Arecaceae* (palmas), que proporcionan un ambiente favorable para su desarrollo, con temperaturas y humedad relativa superiores al 80 %. Durante la temporada otoñal, se observa una defoliación natural, o inducida por factores climáticos, que contribuye a la incorporación de materia orgánica al suelo, favoreciendo el crecimiento de diversas especies de hongos (Tabla 2).

Es relevante destacar que los hongos recolectados en Buenavista cumplen diversas funciones ecológicas, como la descomposición de materia orgánica y el reciclaje de nutrientes, lo que contribuye al equilibrio del ecosistema forestal. Estos procesos son fundamentales para la conservación de los bosques y la mejora de la calidad del suelo. De acuerdo con estudios previos (Almeida et al., 2024; Vega et al., 2022), estos hongos desempeñan un papel crucial en la preservación de la biodiversidad y deben ser considerados en estrategias de manejo y conservación forestal.

Tabla 2. Géneros de hongos recolectados en Buenavista, Coahuila, 2023

Genero	Especies asociadas
Amanita	Pasto de zacate (*Cynodon nlemfuensis*)
Agaricus	Pasto de zacate (*Cynodon nlemfuensis*)
Bolethus	Corteza de coníferas (*Pinophyta*)
Lactarius	Bosque de Quercus, bosque de *Pinus*
Laetiporus	Corteza de *Pinus pinea*
Schizophilum	Tallo de palma (*Phoenix dactylifera*)
Pleurotus	Tallo de palma datilera (*Phoenix dactylifera*)

En Coahuila, la cultura de consumo de hongos silvestres es limitada, debido a las condiciones ambientales extremas, con temperaturas superiores a los 38 °C y una precipitación pluvial de 300 a 350 mm anuales, factores que dificultan el desarrollo de hongos en la región. Sin embargo, la recolección y estudio de estos hongos en la región pueden proporcionar valiosa información sobre sus roles ecológicos y potenciales aplicaciones, como en la domesticación de especies comestibles, una práctica común en diversas regiones tropicales de México (Molina-Castillo et al., 2023a).

Los hongos recolectados en el Departamento de Ciencias Forestales desempeñan un papel importante en la descomposición de hojas y hojarasca de especies forestales, contribuyendo a la incorporación de nutrientes al suelo y mejorando su estructura y textura. Esto resalta la necesidad de un manejo forestal adecuado para preservar los hábitats de estas especies y promover la sostenibilidad a largo plazo.

Lactarius scrobiculatus (Tabla 3) se desarrolla en bosques de *Pinus* y *Quercus*. Es una especie ectomicorrizógena, con un píleo ancho y convexo, de color blanco o amarillento, y es comestible. Por otro lado, el género *Amanita,* que se encuentra en bosques y jardines, es un degradador de materia orgánica y contiene compuestos tóxicos como las falotoxinas, triptaminas y alcaloides, que pueden causar efectos adversos graves como vómitos y diarreas (Sawangwong et al., 2024).

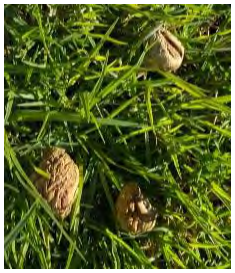
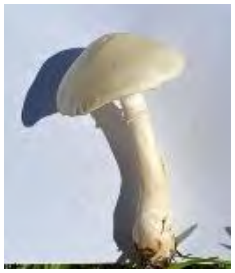
Pleurotus (Tabla 3), un hongo lignocelulósico, tiene la capacidad de descomponer una amplia variedad de sustratos, incluidos residuos agrícolas e industriales (Jin et al., 2021).

Además, presenta un alto valor nutricional, con proteínas superiores al 18 % (Stoffel et al., 2019). Este género se encuentra principalmente en tallos de diversas especies vegetales, especialmente en épocas de alta temperatura (23-30 °C) y humedad relativa del 80 %. En la colecta realizada en agosto, los hongos mostraron signos de estrés hídrico debido a las altas temperaturas.

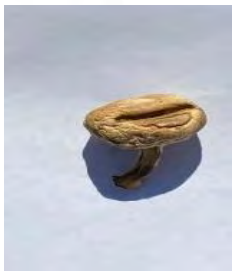
Agaricus, conocido por su cultivo en la región de París desde 1650, es otro hongo importante, ampliamente utilizado como fuente alimentaria. En el Departamento de Ciencias Forestales, se encontró *Agaricus campestris*, que contribuye significativamente a la descomposición de materia orgánica y mejora la estructura del suelo, además de proporcionar nutrientes a las especies forestales y proteger el ecosistema (Nesse et al., 2024). Este hongo también posee propiedades nutracéuticas, lo que resalta la importancia de conservar los hongos silvestres en su hábitat natural.

Finalmente, *Laetiporus* se recolectó en tallos de *Pinus* y es considerado un hongo comestible. Su desarrollo se asocia con la descomposición de materia lignocelulósica, y su presencia en el ecosistema indica la necesidad de un manejo forestal adecuado para mantener la biodiversidad productiva. Estudios similares (Song et al., 2018) han demostrado que los hongos como *Pleurotus* no solo mejoran la calidad del suelo, sino que también son fundamentales en las cadenas tróficas, interactuando con la flora y fauna, y contribuyendo al equilibrio ecológico en los bosques.

Tabla 3. Evidencia fotográfica de género de hongos en el contexto de estudio

Género	Evidencia fotográfica
Lactarius	
Lactarius	
Laetiporus	
Agaricus	
Amanita	
Amanita,	

Agaricus



Pleurotus

CONCLUSIONES

Los hongos recolectados de los géneros *Amanita, Pleurotus* y *Agaricus* poseen un alto valor alimenticio y nutricional, lo que los convierte en un recurso importante para la sociedad. Estos géneros tienen un significativo potencial en diversas aplicaciones, especialmente en el ámbito de la biotecnología y la nutrición. Dada su relevancia, es fundamental continuar con investigaciones interdisciplinarias que profundicen en el estudio de sus propiedades y roles ecológicos. Es crucial considerar todos los componentes involucrados en la recolección de estos hongos, así como la importancia de mantener la integridad de los ecosistemas forestales. La conservación de los bosques y los recursos forestales es esencial, ya que contribuye a mejorar la calidad del ambiente y favorece la sostenibilidad de los recursos naturales.

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Bioactive Packaging as a Sustainable Solution For Food Preservation: A Review

Empaques Bioactivos Como Una Solución Sostenible Para La Conservación De Alimentos: Una Revisión

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Resumen

Los empaques convencionales son líderes en la industria alimentaria, pero presentan desventajas como su escasa biodegradabilidad y el aumento de la contaminación y las emisiones de gases de efecto invernadero. Los empaques bioactivos son una tecnología en tendencia que está ganando popularidad gracias a la incorporación de compuestos antimicrobianos y antioxidantes naturales procedentes de residuos agroindustriales y plantas infravaloradas. El desarrollo de envases bioactivos ofrece varias ventajas, como la prolongación de la vida útil, la reducción del deterioro de los alimentos, la mejora de la seguridad alimentaria y el uso de fuentes sostenibles. Este artículo de revisión explora el área en expansión de los empaques bioactivos. Describe cómo se incorporan los compuestos bioactivos a matrices de envasado hechas de polímeros naturales biodegradables como la celulosa, el quitosano, el ácido poliláctico, los lípidos y las proteínas. Además, se repasan las principales sustancias antimicrobianas y antioxidantes naturales extraídos de plantas como aceites esenciales y polifenoles en materiales de envasado de alimentos. Por último, se describieron las principales técnicas, como la encapsulación y la nanoemulsión, para incorporar estos compuestos bioactivos.

Palabras clave: Aceites esenciales, Antioxidantes, Empaques bioactivos, Polifenoles, Polisacáridos, Vida útil.

Abstract

Conventional packaging is a leader in the food industry but presents disadvantages such as poor biodegradability and increased pollution and greenhouse gas emissions. Bioactive packaging is a rising technology that is gaining popularity due to incorporating naturally occurring antimicrobial and antioxidant compounds sourced from agro-industrial waste and undervalued plants. The development of bioactive packaging offers several advantages such as extended shelf life, reduced food spoilage, improved food security, and use of sustainable sources. This review article explores the expanding area of bioactive packaging. Describe how bioactive compounds are incorporated into packaging matrices made of natural biodegradable polymers such as cellulose, chitosan, polylactic acid, lipids, and proteins. Additionally, review the main antimicrobial substances and natural antioxidants extracted from plants like essential oils and polyphenols into food packaging materials. Finally, the main techniques such as encapsulation and nanoemulsion were described to incorporate these bioactive compounds.

Keywords: Antioxidants, Bioactive packaging, Essential Oils, Polyphenols, Polysaccharides, Shelf life.

INTRODUCTION

Conventional packaging refers to systems primarily employing non-renewable materials, valued for their convenience, affordability, and superior moisture barrier, mechanical, and handling properties (Donkor et al., 2023). However, conventional packaging, which functions as a static, physical barrier, provides passive protection against microorganisms, oxygen, odors, and light (Fuciños et al., 2016).

The production and disposal of plastics derived from fossil fuels contributes to various environmental issues, including greenhouse gas emissions, environmental persistence, and pollution (Atiwesh et al., 2021). To address these challenges, recent advancements in packaging technology have transformed it into an interactive system. While plastics (42%), paperboard (31%), metals (15%), glass (7%), and other materials (5%) are commonly used as a packaging material (Jeevahan & Chandrasekaran, 2019), plastic remains as the dominant material in food packaging. Despite its desirable barrier properties, the non-biodegradable nature of plastic, coupled with low recycling rates, contributes to environmental pollution (Nogueira et al., 2020).

To mitigate these environmental concerns and enhance food quality, researchers have focused on developing active and biodegradable packaging solutions (Bhargava et al., 2020). Microbial spoilage remains a significant challenge in the food industry. To combat this, functional packaging with antimicrobial and/or antioxidant properties is being explored (Sofi et al., 2018). The development of bioactive packaging is one of the options currently being studied to delay food spoilage. There are different external and internal factors that cause food spoilage as shown in Figure 1. Bioactive packaging, which incorporates integrated components like antimicrobials, antioxidants, and phytochemicals, offers a promising approach to extend shelf life and improve food quality by actively modifying the food's environment through controlled release or absorption of bioactive substances (Pérez-Santaescolástica et al., 2022). Naturally occurring bioactive compounds from plant extracts have proven to be an effective alternative because they also often possess antimicrobial and antifungal properties (Jafarzadeh et al., 2020).

Food packages are necessary to protect food from environmental conditions such as humidity, oxidation, temperature, and deterioration by microorganisms, and facilitates the transport of food since it acts as a barrier that withstands mechanical damage (Versino et al., 2023).

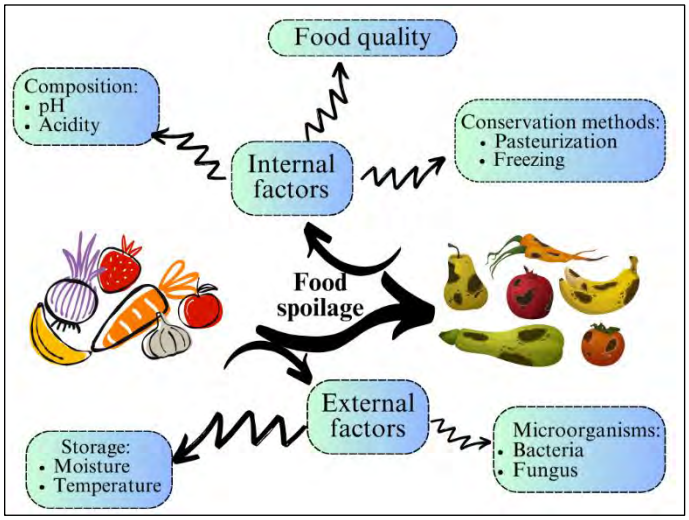

Figure 1. Internal and external factors that can affect the shelf life of food products.

BIOACTIVE PACKAGING

Bioactive packaging is an emerging technology that incorporates naturally occurring antimicrobial and antioxidant compounds into the food packaging matrix to preserve the quality of food products (Baghi et al., 2022). This innovative approach to packaging involves the development of materials that interact with the contents they hold to offer added benefits, such as improved quality, safety, and extended shelf life of the product. These materials often incorporate bioactive compounds, which are substances capable of exerting biological effects (Chandrasekar et al., 2023). Figure 2 shows an advantage and disadvantage comparison between conventional and bioactive packaging.

Figure 2. Main advantages and disadvantages between conventional and bioactive packaging

Films and Coatings

Films and coating technologies are employed for several crucial functions. Primarily, they act as barriers, offering protection against mechanical, chemical, and biological threats to food integrity. Notably, these technologies also minimize the migration of aroma, flavor compounds, and valuable products such as antimicrobials and antioxidants (Gupta et al., 2022). Edible films and coatings are synonymous terms describing edible packaging materials. But they differ in their mode of application: films are solidified prior to application, whereas coatings are applied in liquid form and subsequently dried. (Mohamed et al., 2020). Antioxidant, antimicrobial, antifungal, and even flavoring compounds can be added to these films and coatings to improve the quality and extend the shelf life of the food (Arroyo et al., 2019). On another hand, Betancur-D'Ambrosio et al., (2024) developed edible films using cassava starch, beeswax, and ethanolic propolis extract. Characterization of the edible films revealed that they exhibited antimicrobial activity against Aspergillus niger, reducing its growth by 51%. This makes these films suitable for use as coatings for fruits and vegetables. Aparicio-Fernández et al., (2018) developed edible films composed of carboxymethyl cellulose, prickly pear peel powder, and aqueous extracts from red prickly pear. To characterize these films, the researchers employed various techniques, including DPPH radical scavenging activity, total phenolic content determination, moisture content analysis, tensile strength testing, and puncture resistance measurement. The study demonstrated that the incorporation of prickly pear components significantly enhanced the antioxidant.

MATERIALS USED AS A MATRIX FOR EDIBLE FILMS AND COATING

Biopolymers derived from plants, animals, and microbial synthesis can be utilized to create eco-friendly food packaging materials with potential applications as carriers for functional compounds (Abdullah et al., 2022). These biopolymers can be polysaccharides, are abundant, affordable, and easy to process, but their hydrophilicity limits their water vapor barrier. Conversely, lipids offer better water vapor barrier due to their hydrophobicity. However, the lack of stretch in these materials relegates them mainly to coating applications (Chen et al., 2019). Other compounds can be integrated as antimicrobial agents to effectively inhibit microbial growth in packaging systems. This not only improves food quality but also contributes to environmental sustainability by reducing the dependence on fossil fuel-based plastics (Hu et al., 2022).

Biobased polymers

Biopolymers can be obtained from living organisms such as plants and microorganisms (Asgher et al., 2020). Proteins like ovalbumin, and beta-lactoglobulin can also be used as film-forming materials to thermal stability and interfacial wettability. On the other hand, carbohydrates like chitosan, and starch aldehyde along with other molecules, are used for enhanced antioxidant and antibacterial activity (Sahraeian et al., 2024).

Cellulose

Cellulose can be used as a filler or reinforcement agent in biodegradable/compostable polymers processing, including food packaging applications like formulation of biofilms, coatings, gels, or even hard packages (Alhanish & Ghalia, 2022). Ramesh & Radhakrishnan, (2019) created a biodegradable and environmentally friendly food packaging film by combining polyvinyl alcohol (PVA) with cellulose nanoparticles (CNP) extracted from potato peels and fennel seed oil. Analyses confirmed that the CNP-PVA film showed significant improvements in the mechanical properties. Moreover, it displayed enhanced antibacterial properties, antioxidant activity, and a reduced rate of oxygen transfer, indicating better food preservation potential. Das et al., (2022) developed an edible coating based on carboxymethyl cellulose nanoemulsion incorporated with cardamom essential oil. When applied to tomatoes, this coating effectively inhibited the growth of Escherichia coli and Listeria monocytogenes, extending the fruit's shelf life by mitigating weight loss, firmness decline, and microbial spoilage. Notably, the application of the edible coating did not adversely affect the organoleptic properties of the tomatoes, such as weight loss, firmness, and color. Liu et al., (2021a) created hydroxyethyl cellulose with sodium alginate based edible coatings incorporating asparagus waste extracts to enhance strawberry quality and shelf life. These coatings demonstrated significant antifungal activity against Penicillium italicum, delayed color change, reduced weight loss, and preserved polyphenolic and flavonoid content of the fruit. This study confirmed the effectiveness of these coatings in maintaining postharvest quality and extending shelf life of strawberries.

Microcrystalline cellulose is a partially degraded form of natural cellulose, or a synthetic fiber composed of crystalline regions organized into rod-like structures. These crystalline regions coexist with amorphous regions, preserving certain properties of native cellulose. Microcrystalline cellulose-based active films, leveraging these properties, offer innovative solutions for extending the shelf life of food products by providing antimicrobial protection against microbes and

preventing lipid oxidation (Bangar et al., 2023). Cheng et al. (2021) developed active antibacterial films by combining microcrystalline cellulose and yam starch. Low concentrations of microcrystalline cellulose (5-25%) improved film properties, including reduced weight loss and increased thermal stability. Additionally, the incorporation of essential oils such as α-terpineol, eugenol, and carvacrol significantly enhanced the antimicrobial activity against S. aureus and E. coli. These films proved effective in extending the shelf life of pork by inhibiting bacterial growth and delaying spoilage.

Kowalczyk et al. (2021) developed edible films using methylcellulose and corn starch incorporated with fireweed (Chamaenerion angustifolium L.) extract to investigate their physicochemical and antioxidant properties. The results demonstrated that methylcellulose-based films were mechanically stronger than corn starch-based films.

Chitosan

Chitosan, a versatile biopolymer derived from chitin, has gained prominence in the food industry due to its unique properties, including emulsifying, antimicrobial, antioxidant, and gelling abilities (Tamer & Çopur, 2010). Popescu et al., (2022) developed chitosan-based coatings using medium and high molecular weight chitosan, combined with ascorbic or acetic acid and essential oils from sea buckthorn or grape seed. These coatings effectively preserved the postharvest quality of organic strawberries and apple slices during cold storage. The coatings reduced microbial load particularly yeast and molds, maintained higher levels of antioxidants and polyphenols, and lowered water activity, thereby inhibiting spoilage. These findings highlight the potential of chitosan-based coatings as a promising strategy for extending the shelf life and improving the quality of fresh fruits.

Chitosan-based edible coatings and films have demonstrated significant potential for preserving the quality and extending the shelf life of various food products, including fruits, vegetables, and even complex foods like meat. For instance, Sutharsan et al. (2023) developed chitosan-based bioactive films incorporated with catechin, quercetin, and luteolin. These composite films exhibited enhanced antioxidant and antimicrobial properties, effectively inhibiting the growth of foodborne pathogens (Listeria monocytogenes, Salmonella typhimurium, Escherichia coli and Staphylococcus aureus). When applied to beef, these films maintained the product's quality during storage at 4°C for two weeks and extended its shelf life by preserving color and inhibiting microbial growth. These findings highlight the versatility of chitosan-based films as a sustainable and effective approach for improving food safety and quality.

Polylactic acid (PLA)

Polylactic acid (PLA) is one of the major biodegradable polymers that can be obtained from microbial lactic fermentation processes. There is a growing trend in the use of PLA to formulate rigid and flexible food packaging because it exhibits excellent physical characteristics, is biodegradable, and is industrially available as a raw material (Kaushalya et al., 2019). In addition, the incorporation of bioactive molecules in PLA composite films has been reported, PLA can be processed into films using techniques such as compression molding, and solvent casting, and the most common is extrusion (Rojas et al., 2021). Mohamad et al., (2020) formulated bioactive PLA films incorporating curry essential oils, thymol, and kesum extracts as bioactive agents. The objective was to evaluate their potential to extend the shelf life of foods. The study found favorable compatibility between thymol, kesum extracts, and PLA matrix, while curry showed limited interaction. FTIR analysis confirmed the successful integration of the active agents into the PLA film. Direct food contact analysis revealed that all PLA films with bioactive agents effectively preserved chicken meat for up to two weeks.

Lipids-base materials

Materials such as waxes, paraffin, and shellac resins are used in creating protective films and coatings for food applications. These substances exhibit a characteristic that inhibits moisture penetration owing to their hydrophobic nature (Yousuf et al., 2022). Aguirre-Joya et al., (2018) developed a bioactive coating with optimal water vapor permeability value (WVP) formulated with candelilla wax and Aloe vera mucilage to which crude extracts of Larrea tridentata were added, the edible coating showed antioxidant and fungistatic capabilities. The study revealed potent antioxidant activity in Larrea tridentata leaf extracts, verified by *In Vitro* assays (ABTS, DPPH, FRAP, LOI). In addition, key phenolic compounds identified in Larrea tridentata leaves extracts (NDGA, Quercetin, and Kaempferol) via HPLC-MS were associated with a significant antifungal effect against common fruit-damaging fungi (*Botrytis cinerea, Colletotrichum gloeosporioides, Fusarium oxysporum* and *Alternaria alternata*).

Protein-base materials

Proteins used in packaging formulation require a denaturation step of the molecule to break the peptide bonds; the

amino acids and peptides obtained can interact with other molecules through ionic, covalent or hydrogen bonds to strengthen the formula. (Soro et al., 2021). Pedro et al., (2023) optimized the formulation of whey protein concentrate films to enhance their overall mechanical properties. This involved incorporating essential oil derived from Foeniculum vulgare Mill. (fennel) into the films. The authors concluded that these materials hold promise for extending the shelf life of food products and potentially preventing foodborne illnesses caused by pathogenic microorganisms. In another study, eco-friendly packaging material based on black chickpea protein isolate, electrospun nanofibers, and citral-loaded nanoliposomes were designed. The authors concluded that the black chickpea protein isolate was successfully extracted and enhanced mechanical and thermal properties (Amjadi et al., 2024). On the other hand, protein mixture with bioactive compounds, and tea saponins extracted from Camellia oleifera residues were evaluated to prepare biodegradable films. The authors mentioned that the interaction of protein with chitosan, the obtained biopolymer films improved mechanical and physical properties (Nie et al., 2024).

Robledo et al. (2018) developed porous, heterogeneous quinoa protein-chitosan edible films incorporating 10% thymol nanoemulsions. These films were shown to inhibit fungal growth on inoculated cherry tomatoes within 7 days at 5°C. Yousuf & Srivastava (2019) investigated the efficacy of soy protein isolate-based coatings combined with honey for fresh-cut pineapple packaging. This combined treatment extended the shelf life of the fruit to 16 days at 4°C while significantly preserving phenolic compounds and inhibiting microbial growth. On another hand, Aitboulahsen et al. (2018) investigated a gelatin-based edible coating enriched with Mentha pulegium essential oil to extend the shelf life of strawberries. By incorporating two concentrations of Mentha pulegium essential oil (0.5% and 1%) into the gelatin coating, researchers found a significant reduction in microbial growth (count of total aerobic mesophilic flora, yeast, and molds) compared to uncoated strawberries.

POTENTIAL BIOACTIVE COMPOUNDS FOR FOOD PACKAGING

Essential oils

Bioactive agents, such as essential oils (EOs), have been incorporated into food packaging materials to enhance the shelf life and quality of the food. EOs are natural additives extracted from aromatic compounds and are known for their antimicrobial and antioxidant properties. By incorporating EOs into edible/biodegradable films and coatings, the packaging can help protect against oxidative and bacterial deterioration effects, thereby extending the shelf life of processed food (Akram et al., 2019). These secondary metabolites, abundant in aromatic and medicinal plants, play a crucial role in plant defense against pathogens. Monoterpenoids exhibit potent antibacterial activity by disrupting microbial growth and interfering with essential cellular processes (Siddiqui et al., 2024). While the precise mechanism of EOs against bacteria's remains unclear, research suggests that their lipophilic constituents disrupt the bacterial cell membrane. This disruption compromises membrane integrity, leading to increased permeability and altered ion transport processes in bacteria (Samrot et al., 2021).

Polyphenols

Polyphenols are a group of chemical compounds of phenolic nature, found in a wide variety of plants. They are considered secondary metabolites, i.e., substances that are not essential for plant growth or development, but have other functions, such as defense against oxidative stress, pests, and pathogens (Williamson, 2017). Polyphenols like ellagic acid from pomegranate (Punica granatum) extracts have been implemented in bioplastics and edible coatings for the development of food packaging which proved to extend the shelf life of food due to compounds with antioxidant and antimicrobial activity present in pomegranate (Ko et al., 2021). Polyphenols exhibit antibacterial activity by interacting with various microbial cell sites, leading to the loss of cellular components, disruption of the cytoplasmic membrane, and subsequent cell death. The principal mechanism of action are the modification of the cell membrane permeability, the formation of cytoplasmic granules and the rupture of cytoplasmatic membrane; and other mechanism consists in disturbing intracellular functions by the formation of hydrogen bonding among phenolic compounds and enzymes (Zamuz et al., 2021; Rempe et al., 2017).

Phenolic acids

Phenolic acids are among the main bioactive compounds present in various plant species, confer color, flavors, and astringency to foods, Phenolic acids have been tested as crosslinkers to improve the physical and mechanical properties of some edible protein-based films, and it has also been reported that by incorporating phenolic acids into coating matrices or edible films, they have acquired antioxidant and antimicrobial properties. (Ordoñez et al., 2022). Kaczmarek, (2020) developed a study of the incorporation of tannic acid into edible films based on sodium alginate. The films were characterized using in vitro methods including DPPH assay, water vapor permeation rate (WVPR), and Fourier-transform infrared spectroscopy (FTIR-

IR). The results demonstrated improvements in the physical properties of the films and the acquisition of antioxidant properties by the edible films.

These compounds exert their antimicrobial effects through multiple mechanisms, including inhibition of nucleic acid synthesis, inactivation of essential bacterial enzymes, and disruption of cytoplasmic membrane integrity (Yang et al., 2023). The number and arrangement of functional groups attached to the benzene ring, as well as the length of the saturated side chain, significantly impact their ability to inhibit microbial growth (Lobiuc et al., 2023). Phenolic acids possess antioxidant properties through multiple mechanisms, including hydrogen atom transfer, single-electron transfer-proton transfer, sequential proton loss electron transfer, and transition metal chelation (Zeb, 2020). Some examples of bioactive compounds reportedly used in the formulation of bioactive packaging are listed in Table 1.

MAIN METHODS FOR THE EXTRACTION OF BIOACTIVE COMPOUNDS

Traditionally, hydro-distillation, maceration and shaking water bath are some methods used to extract compounds of interest present in some plants. These methods, also known as conventional extraction techniques, although easy to perform are known to be slow, inefficient, and require large volumes of solvent (Pogorzelska-Nowicka et al., 2024). In response, recent advances offer alternative methods such as microwave-assisted extraction and ultrasound-assisted extraction for a more efficient and sustainable approach to the extraction of bioactive compounds (Estrada-Gil et al., 2022). Microwave assisted extraction is an emerging technology that offers several advantages over conventional techniques. By subjecting plant materials to microwave irradiation, microwave extraction can significantly accelerate the extraction process, resulting in higher yields and shorter extraction times. Moreover, typically requires smaller volumes of solvents, primarily water and ethanol, which reduces costs and environmental impact (Bagade & Patil, 2021). Ultrasound assisted extraction, on the other hand, relies on the application of ultrasonic waves to disrupt plant cell walls and plasma membranes of plant cells and release the compounds of interest present in the cells such as polyphenols, essential oils, etc. The cavitation phenomenon generated by ultrasonic waves facilitates the mass transfer of solutes from the plant matrix to the extraction solvent (Dzah et al.,2020).

Additionally, bio-based techniques such as solid-state fermentation and submerged fermentation provide sustainable and eco-friendly approaches to producing valuable bioactive compounds. Solid and submerged fermentation has demonstrated considerable potential over time as a viable system for the industrial-scale production of numerous valuable products such as antioxidants, polyphenols, enzymes, antibiotics, and organic acids, among others (El-Sayed et al., 2020; Dey et al., 2016). No single method of extracting bioactive compounds can be taken as the best, the choice of extraction technique depends on various factors, including the target compounds, desired yield, and environmental considerations.

METHODS OF INCORPORATING PLANT-DERIVED BIOACTIVE COMPOUNDS

For the integration of bioactive compounds in edible films and coatings, it´s crucial to consider the type of packaging to be used and the materials to be used, the nature and characteristics of the bioactive compound to be used, and the type of food on which these films will be applied. Various methods exist for incorporating bioactive compounds or plant extracts into coatings, films, or packaging. However, the choice of method depends on factors such as compound type, packaging type, and desired packaging properties. (Nogueira et al., 2020). Some of the most reported methods for the addition of bioactive agents in bioactive packaging are described in Table 2.

Film formation by casting method

The casting method offers several advantages for film formation. It is a simple and cost-effective technique, requiring minimal specialized equipment. The wet-based process promotes improved particle interactions, resulting in homogeneous structures with fewer defects (Suhag et al., 2020). The simplicity and minimal equipment requirements of this method make it a popular choice for film production at laboratory and pilot scales (Lisitsyn et al., 2021).

The casting method suffers from several drawbacks, including the possibility of solvent retention, which can result in the incorporation of harmful chemicals within the polymer; and the potential for denaturing delicate biomolecules, such as proteins, due to the use of solvents (Anbukarasu et al., 2015).

Encapsulation

Encapsulation is one of the most widely used methods to preserve the properties of bioactive compounds, this technique is widely used in the elaboration of edible coatings and films as it helps to improve the bioavailability and stability of the bioactive compound. Encapsulation can be subdivided into two main categories: nanoencapsulation and microencapsulation. Nanoencapsulation specifically pertains to encapsulation particles ranging in size from 10 to 1000 nanometers, whereas

microencapsulation encompasses particles with dimensions between 3 and 800 micrometers (Marcillo-Parra et al., 2021)

More specifically, microencapsulation protects the bioactive components within a homogeneous or heterogeneous matrix, resulting in the production of microcapsules endowed with numerous advantageous traits. This approach serves as a viable alternative for converting liquid, unstable substances into stable, free-flowing powders (Premi & Sharma, 2017).

The primary advantage of encapsulation technology in food packaging lies in its ability to enhance the performance and longevity of bioactive compounds. By encapsulating these compounds, they are shielded from degradation, volatilization, and undesirable interactions with packaging materials. This protection also improves compatibility between the bioactive compound and the packaging polymer, ensuring optimal performance (Becerril et al., 2020). Encapsulation technologies, while promising, face several disadvantages and limitations. Micro and nanoencapsulation processes often require specialized equipment and complex procedures, increasing costs. Additionally, the matrix material significantly influences the resulting micro and nanocapsules properties. Macro encapsulation, on the other hand, exhibits lower stability and fracture resistance (Huang et al., 2023).

Nanoemulsion technique

Emulsions are heterogeneous colloidal mixtures consisting of two immiscible liquids. This technique has generated interest in recent years to be applied in the elaboration of bioactive packaging in the food industry such as edible coatings, since through emulsification, edible coatings can be formulated with bioactive compounds that can protect and release sensitive bioactive compounds into the food (Katsouli et al., 2018).

Nanoemulsions differ from conventional emulsions because conventional emulsions are in most cases unstable and degrade over time. In addition, the particle size of nanoemulsions ranges from 10 to 100 nm, which makes them more stable, and they have a better release capacity from the encapsulated bioactive compounds. The main methods for the preparation of nano-emulsions consist of a one-step process in which all the components are combined in a suitable solution and homogenized to obtain nanodroplets. Alternatively, a two-step process can be used in which an aqueous solution of the components is first prepared and then combined with a separate solution of a biopolymer (Pandey et al., 2022).

The advantages of nanoemulsion include improving the targeting, adsorption, encapsulation, solubility, bioaccessibility, permeability, and bioavailability of weakly soluble ingredients due to the nanosized and large surface area of the droplets (Ahari et al., 2021). Nanoemulsion can also protect the loaded bioactive ingredients against hydrolytic and enzymatic degradation (Manzoor et al., 2023). The primary disadvantage of nanoemulsions is their extended emulsification time, which can lead to coalescence and a consequent increase in droplet size. The manufacturing process is associated with significant costs (Sneha & Kumar, 2022).

CHALLENGES AND PROSPECTS

Bioactive packaging represents a remarkable change in the field of food preservation; food packaging is no longer inherent in packaging that does not interact with the food. The use of sources of bioactive compounds derived from plants or agro-industrial wastes has been shown through various research studies to be viable and have the potential to be used in the formulation of bioactive packaging to extend the shelf life of food and even, why not, provide health benefits. However, although the development of bioactive packaging has great advantages, it still faces challenges because its use on an industrial scale is limited due to potential operating costs. Although bioactive compounds extracted from different plants and even agro-industrial wastes can impart potent biological activities to food packaging, it is important to consider and evaluate the possible negative effects that may occur, such as toxicity or alteration of the organoleptic characteristics of the packaged food, so it is important to evaluate the concentration and use the right amounts of extracts so that they do not pose a risk to the consumer and in turn ensure and maintain the quality of the product (Qian et al., 2021).

Edible coatings and films are the most studied forms of bioactive packaging at present because they are the easiest to implement, in addition to this, with the help of other techniques such as micro and nanoencapsulation are in trend because in most cases the bioactive compounds are very sensitive to temperature changes, so to be incorporated into a bioactive packaging matrix usually opt for encapsulated to be protected in a certain way within the polymeric matrix and in turn, the formed packages can be shaped by thermal methods such as thermo plasticization to confer more appropriate physical and mechanical properties (Majid et al., 2018).

CONCLUDING REMARKS

Bioactive packaging, integrating antimicrobial and antioxidant compounds from natural sources into biodegradable materials, offers a sustainable solution for food preservation. This emerging technology not only extends shelf life and prevents spoilage but also promotes the valorization of agro-industrial waste and undervalued plants. While challenges remain in process optimization and technological development,

continued research, exploring diverse sources of active compounds and expanding applications, will pave the way for a more sustainable and functional future in food preservation. This interdisciplinary approach, combining materials science, food technology, and environmental studies, presents an opportunity for synergy between functionality and sustainability, making bioactive packaging an exceptional option for forward-thinking companies.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

No data was used for the research described in the article.

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Table 1. Bioactive compounds used in the formulation of bioactive packaging.

Bioactive compound	Function	Packaging matrix	Type of packaging	Assay	Reference
Cloves/prickly ash/ fennel geranium/ cinnamon extracts	Antioxidant and antibacterial properties	Chitosan base films	Film	DPPH/Total variable count of bacteria (*S. aureus* and *E. coli*)	Liu et al., (2021b)
Rosemary Extract	Antioxidant properties	Furcellaran/gelatin hydrolysate/glycerol	Film	DPPH/FRAP	Jancikova et al., (2019)
Monascus red/phycocyanin/safflower yellow	Antioxidant and other biological activities	Porcine skin gelatin/chitosan/	Film	ABTS method/Water sensibility and barrier properties/Physical properties	Liu et al., (2024c)
Satureja khuzestanica essential oil	Antimicrobial properties	PEG 6000/ Hydroxyl propyl methyl cellulose	Film	Physical and mechanical properties/ *Escherichia coli* ATCC 25,922, *Shigella flexneri* PTCC 1234, *Salmonella typhi* PTCC 1609, and *Staphylococcus aureus* ATCC 25,923	Aghajani et al., (2024)
Ellagic acid	Antioxidant and antimicrobial properties	Chitosan base film	Film	DPPH/ CFU bacteria count (*S. aureus* and *P. aeruginosa*)	Vilela et al., (2017)
Phenylalanine	Antimicrobial and antioxidant properties	Carboxymethyl cellulose/Polyvinyl alcohol	Film	Moisture adsorption capacity, water solubility, oxygen permeability, and water vapor transmission rate	Kurabetta et al., (2024)

Rosemary essential oil	Physical properties and antimicrobial properties	Starch-carboxymethylcellulose	Film	Water vapor permeability, Film thickness, solubility/ Agar diffusion method	Mohsenabadi et al., (2018)
Neem leaf extract	Antimicrobial and antifungal properties	Chitosan/Pectin	Film	Moisture, water solubility, water vapor permeability, optical properties, *Staphylococcus aureus* and *Aspergillus niger*	Firdaus et al., (2024)
Spearmint (*Mentha spicata*)	Antimicrobial	Chitosan/ carboxymethyl cellulose	Coating	Total variable count of bacteria (*Listeria monocytogenes*)	Shahbazi, (2018)
Grape seed extract	Antimicrobial	Chitosan	Coating	Plate count agar for total mesophilic and Psychotropic microorganisms.	Hassanzadeh et al., (2018)
Pomegranate peel extract	Antifungal	Alginate/chitosan	Coating	Mycelial growth of *Colletotrichum gloeosporioides* by agar diffusion technique	Nair et al., (2018)
Orange peel powder	Antimicrobial and antioxidant properties	Cellulose	Film	Mechanical and structural properties. Water barrier, DPPH, *Staphylococcus aureus* and *E. coli*	Riaz et al., (2024)
Gallic acid present in Rosemary extract	Antioxidant	Whey protein concentrate/carboxymethyl cellulose/glycerol	Coating	DPPH	Hosseini et al., (2020)
Mango puree/pineapple pomace	Antioxidant and antimicrobial properties	Corn starch/gelatin	Film	DPPH/FRAP/inhibition of growth with diffusion method on Petri dishes	Susmitha et al., (2021)
Green tea and rosemary polyphenolic extracts	Antioxidant	PLA	Film	DPPH/β-carotene bleach assay/Folin-Ciocalteu	Andrade et al., (2023)

Table 2. Methods used to integrate bioactive compounds into bioactive packaging.

Bioactive compound	Incorporation method	Bioactive packaging matrix	Type of packaging	References
Vitamin E	Nanoencapsulation	Carboxymethyl cellulose	Film	Mirzaei-Mohkam et al., (2020)
Pink pepper essential oil	Emulsion	Protein/pectin coating applied in polyethylene terephthalate boxes for storage of cherry tomatoes	Coatings applied to polyethylene terephthalate boxes	Locali-Pereira et al., (2021)
Mango leaf extracts	Supercritical solvent impregnation	Nano fibrillated cellulose	Film	Bastante et al., (2021)
Avocado by-products	Emulsion	Ethyl cellulose/Paper	Film	Acquavia et al., (2023)
Thymol and Carvacrol	Microencapsulation	Maltodextrin/soy protein	Coatings	Ulloa et al., (2017)
Thymol	Emulsion (nanoencapsulation)/casting method	Quinoa protein / Chitosan	Film	Robledo et al., (2018)
Date fruit (Khalas variety) seeds	Emulsion	Carboxymethyl cellulose-poly(vinyl)	Film	Lawal et al., (2024)
Oregano, tea tree and peppermint	Nanoencapsulation	Cellulose nanocrystals/chitosan	Film	Hossain et al., (2019)

Pistachio hull Lavender oil	Emulsion Microencapsulation	Chitosan Gum acacia, sodium caseinate, gelatin, chitosan, β-cyclodextrin, and polyvinyl alcohol	Film Coatings	Kepekci et al., (2024) Zhang et al., (2020)
Undaria pinnatifida, *Sargassum pallidum*, *Ulva lactuca*	Ultrasonic homogenization	Cellulose nanocrystals	Film	Wang et al., (2024)
Mentha longifolia L. essential oils	Nanoencapsulation	Balangu seed gum	N.R	Rezaeinia et al., (2019)
Catechin	Nanoencapsulation	Chitosan-sodium tripolyphosphate	Coating	Shankar Kumar Mandal et al., (2019)
Carvacrol	Microencapsulation	Sodium Alginate	Film	Cheng et al., (2019)
Tea polyphenols nanoparticles	Emulsion	Pectin	Film	Yang et al., (2024)

*N.R: No Reported