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"VALORIZATION OF ECUADORIAN MANGO PEEL (*Mangifera indica L.*) AS A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS, TO OBTAIN AN ENCAPSULATED EXTRACT WITH FUNCTIONAL PROPERTIES BY SPRAY-DRYING"

DOCTORAL THESIS PREVIOUS THE AWARD OF THE POSTGRADUATE DOCTORAL DEGREE (DOCTORAL OF SCIENCE - PH.D.) IN FOOD SCIENCE AND TECHNOLOGY

VERÓNICA ELIZABETH MARCILLO PARRA
Chemical Engineer
verito_marcillo@hotmail.com

DIRECTOR: JENNY RUALES Ph.D.
jenny.ruales@epn.edu.ec

QUITO – ECUADOR

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CERTIFICATION

Certify that Verónica Elizabeth Marcillo Parra developed this work under my supervision.

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Jenny Ruales Ph.D.
THESIS DIRECTOR

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DEDICATION

To Alfonso,
Juan Diego and Alfonsito,
my three loves…

CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the work contained in the following papers, which are referred to in the text by their Roman numerals:

I. **Characterization and quantification of bioactive compounds and antioxidant activity in three different varieties of mango (*Mangifera indica* L.) peel from the Ecuadorian region using HPLC-UV/VIS and UPLC-PDA.**

Verónica Marcillo-Parra, Mayra Anaguano, Maritza Molina, Diego Santiago Tupuna-Yerovi, Jenny Ruales. (2021). *NFS Journal* 23, 1-7. DOI: 10.1016/j.nfs.2021.02.001.

II. **Phenolic compounds profile in mango peels (*Mangifera indica* L. cv. Tommy Atkins) by UHPLC-PDA obtained using different solvents and ultrasound-assisted extraction.**

Verónica Marcillo-Parra, Diego Santiago Tupuna-Yerovi, Maritza Molina and Jenny Ruales

Submitted

III. **Encapsulation of carotenoids from mango (*Mangifera indica* L.) peel with different encapsulating agents to enhance its encapsulation efficiency and stability.**

Verónica Marcillo-Parra, Diego Santiago Tupuna-Yerovi, Edwin Vera, Alessandro de Oliveira Rios, Filip Van Bockstaele, Paul Van Der Meeren, John Van Camp, Koen Dewettinck and Jenny Ruales

To be submitted

IV. **Encapsulation of bioactive compounds from fruit and vegetable by-products for food application – A review.**

Verónica Marcillo-Parra, Diego Santiago Tupuna-Yerovi, Zulay González and Jenny Ruales. (2021). *Trends in Food Science & Technology.* 116, 11-23. DOI: 10.1016/j.tifs.2021.07.009.

ABSTRACT

Peels are the most important by-product of mango processing and may become a source of environmental pollution. Peel bioactive compounds are of significant importance because of their functional values. Nevertheless, they migth be affected by sunlight, oxygen, temperature and others. Therefore, it is necessary to look for some stabilization techniques. Encapsulation approach is widely used as one food processing alternatives since it helps to improve the solubility and stability of bioactive compounds as carotenoids.

This research aimed to study the encapsulation process of mango (*Mangifera indica* L.) peel extracts by atomization to develop a functional ingredient with antioxidant properties. For this purpose, mango peels of different varieties were first characterized (composition, bioactive compound contents and antioxidant activity), then the extraction of phenolic compounds from mango peels with various solvents (50% methanol, 50% ethanol and 70% acetone in aqueous media) and ultrasound-assisted extraction (UAE) were evaluated. Carotenoid extracts from mango peels were also obtained by UAE and these extracts were encapsulated by spray drying, using different blends of maltodextrin (MD) and gum arabic (GA) as wall materials and different inlet air drying temperatures (IT) with the addition of fructooligosaccharides (FOS) and inulin, to evaluate the effect on encapsulation efficiency (EE) and characterize the resulting microcapsules. Stability measurements of the encapsulated carotenoids was carried out at different storage temperatures to determine the thermal degradation kinetics and define an estimated shelf-life of the encapsulated product.

The chemical composition of mango peel samples showed an interesting dietary fiber content, in which soluble dietary fiber constituted more than 50% of the total fiber. β-carotene was the primary carotenoid in all samples, with an average contribution of 65% of the total carotenoid content. The extracts obtained with 50% methanol and ultrasound-assisted extraction presented the highest phenolic compound contents, highlighting mangiferin as the predominant

component (222.34 mg/100 g DW), and had a considerable antioxidant activity (337 µmol Trolox/100 g DW). The encapsulation process of carotenoid extract using MD:GA (50:50, w/w) at 140 °C showed a high EE (67.91%) and was chosen to evaluate the effect of the addition of inulin and FOS. There was no statistical difference ($p < 0.05$) in EE (~65%) and water solubility index (>86%) between samples. The microencapsulated carotenoid extract (MCE) showed a microparticle diameter (<4.0 µm) and low moisture content (<3.7%). Thermal degradation kinetics followed a first-order kinetic reaction and encapsulated carotenoids showed a higher half-life than non-encapsulated carotenoids. The encapsulation by spray-drying improved the thermal stability of carotenoid extracts; thus, the MCE can be considered a antioxidant additive for use as a functional ingredient in the food.

RESUMEN

Las cáscaras son el subproducto más importante del procesamiento del mango y pueden convertirse en una fuente de contaminación ambiental. Los compuestos bioactivos de las cáscaras son de gran importancia por sus valores funcionales. Sin embargo, pueden verse afectados por la luz solar, el oxígeno, la temperatura y otros factores. Por lo tanto, es necesario buscar alguna técnica de estabilización. La encapsulación se utiliza ampliamente como una de las alternativas de procesamiento de alimentos, ya que ayuda a mejorar la solubilidad y la estabilidad de los compuestos bioactivos como los carotenoides.

El objetivo de esta investigación fue estudiar el proceso de encapsulación de los extractos de cáscara de mango (*Mangifera indica* L.) por atomización, con el fin de obtener un ingrediente con propiedades antioxidantes. Para este propósito, primero se caracterizaron las cáscaras de mango de diferentes variedades (composición, contenido de compuestos bioactivos y actividad antioxidante), y luego se evaluó la extracción de compuestos fenólicos de la cáscara de mango con diferentes disolventes (metanol 50%, etanol 50% y acetona 70% en medio acuoso) y la extracción asistida por ultrasonido. Además, también se obtuvieron los extractos de carotenoides de las cáscaras de mango por ultrasonido y estos extractos fueron encapsulados mediante secado por atomización, utilizando diferentes mezclas de maltodextrina (MD) y goma arábiga (GA) como materiales de pared y diferentes temperaturas de secado del aire entrada, con la adición de fructooligosacáridos (FOS) e inulina, para evaluar el efecto sobre la eficiencia de encapsulación (EE) y caracterizar las microcápsulas resultantes. La estabilidad de los carotenoides encapsulados se llevó a cabo a diferentes temperaturas de almacenamiento, para determinar la cinética de degradación térmica y definir una vida útil estimada del producto encapsulado.

La composición química de las cáscaras de mango mostraron un interesante contenido de fibra dietética, en el cual la fibra dietética soluble constituyó más del 50% de la fibra total. El β-caroteno fue el principal carotenoide

en todas las muestras, con una contribución media del 65% del contenido total de carotenoides. Los extractos obtenidos con metanol al 50% y ultrasonido presentaron el mayor contenido de compuestos fenólicos, siendo la mangiferina el componente predominante (222.34 mg/100 g), y una gran actividad antioxidante (337 µmol Trolox/100 g). El proceso de encapsulación del extracto de carotenoides realizado con MD:GA (50:50, w/w) a 140 °C mostró una alta EE (67.91%) y fue elegido para evaluar el efecto de la adición de inulina y FOS. No hubo diferencias estadísticas ($p < 0.05$) en la EE (~65%), ni en el índice de solubilidad en agua (>86%) entre las muestras con diferentes prebióticos. El extracto de carotenoides microencapsulado (ECM) mostró un diámetro de micropartículas (<4.0 µm) y un bajo contenido de humedad (<3.7%). La cinética de degradación térmica siguió una reacción cinética de primer orden y los carotenoides encapsulados mostraron un tiempo de vida media mayor que los carotenoides libres. La encapsulación mediante secado por atomización mejoró la estabilidad térmica de los extractos de carotenoides; por tanto, el ECM puede considerarse un aditivo antioxidante para su uso como ingrediente funcional en los alimentos.

ABBREVIATIONS

AA	Antioxidant activity
CE	Catechin equivalents
CSE	Conventional solvent extraction
DE	Dextrose equivalent
EE	Encapsulation efficiency
EY	Encapsulation yield
GA	Gum arabic
GAE	Gallic acid equivalents
IT	Inlet air drying temperature
MCE	Microencapsulated carotenoid extract
MD	Maltodextrin
TSS	Total soluble solids
TC	Total carotenoids
UAE	Ultrasound-assisted extraction
WPI	Whey protein isolate

INTRODUCTION

In the last two decades, agro-industrial research has focused on reduce waste generated from agricultural by-products, mainly for environmental reasons. These wastes (peels, pulp remains, and seeds) from tropical fruits, as it is in mango, are potential sources of various bioactive compounds (carotenoids, polyphenols, flavonoids, and others), which are of great interest for their application as nutraceuticals or innovation of food products (Marçal & Pintado, 2021).

It is essential to study alternative processes to the conventional ones to recover, extract, and stabilize the bioactive compounds since the matrices containing them are mixed, which difficult their easy availability. Extraction is the first step in any bioactive compound recovery study in plant samples and plays a vital role in the final product (González & González, 2010).

Ultrasound-assisted extraction is an unconventional method that produces acoustic cavitations in the solvent, which helps the bioactive diffuse rapidly from the plant material into the solvent. It is beneficial to obtain high-quality antioxidant extracts from different plant matrices. This extraction technique is environmentally friendly because it allows to reduce extraction time, energy, and use of solvent and improve yields and quality of the extracts (Azmir et al., 2013).

However, these extracts of bioactive substances are not stable in their extraction environment. The antioxidant activities of bioactive compounds as carotenoids can be reduced due to their degradation caused by light, oxygen, temperature, moisture, and the existence of unsaturated bonds in the molecular structures. Therefore, it is necessary to look for stabilization techniques. In this line, encapsulation processes use biopolymers to entrap the component of interest, to prevent undesirable interactions with the matrix and improve the storage and environmental stability of bioactive compounds (Oskan et al., 2019).

Spray drying is the most widely applied technique to encapsulate bioactive compounds. It is a simple, rapid, and relatively low-cost process. The method involves atomization of emulsions in a drying chamber at high temperature, which causes very rapid water evaporation, resulting in the formation of a capsule and entrapment of the ingredient within a coating material (Shishir et al., 2018).

One of the main influencing factors in the stability of encapsulated compounds is the nature of the wall material. The ideal encapsulating agent should show emulsifying properties, film-forming capacity, biodegradability, low viscosity, low hygroscopicity, and low cost. A single encapsulating agent can rarely have all of the above properties, so it is common to use a combination of two or more components (Dordević et al., 2014).

Gum arabic is the most commonly used encapsulating agent in spray drying. It has an excellent emulsifying property, a high solubility in water, and a low viscosity in solution. In turn, maltodextrin has a low emulsifying capacity and density at very high concentrations. It has been studied as a possible substitute for gum arabic in atomized emulsions or blended with gum arabic to satisfy the required properties by the wall material. Fructooligosaccharides as inulin may also be attractive encapsulating agents due to their prebiotic properties (Labuschagne, 2018).

The encapsulated products maintain good biological activity and physical characteristics over time, which has increased the possibilities to different industries, can develop new and attractive products with human health benefits (e.g., application of encapsulated β-carotene to enrich a food matrix as yogurt and pudding) (Donhowe et al., 2014).

1. OBJECTIVES

1.1. MAIN OBJECTIVE

The main objective of this thesis was to study encapsulation process of Ecuadorian mango (*Mangifera indica* L.) peel extract by spray-drying to develop a functional ingredient with antioxidant properties.

1.2. SPECIFIC OBJECTIVES

1. To analyze mango (*Mangifera indica* L.) peel composition, bioactive compound content, antioxidant activity, and carotenoid and phenolic profiles for the three most commercial varieties of the Ecuadorian region.
2. To evaluate the effect of the solid-liquid extraction using different solvents assisted by ultrasound on the phenolic compound content and antioxidant activity in Tommy Atkins mango peel extracts.
3. To obtain mango peel carotenoid extracts to perform the encapsulation of these extracts by spray drying using different blends of maltodextrin and gum arabic as wall materials and different inlet air drying temperatures with the addition of fructooligosaccharides and inulin, and evaluate the effect on encapsulation efficiency and characterize (moisture, solubility, morphology, color, and antioxidant activity) the resulting microcapsules.
4. To assess the stability of the encapsulated carotenoids to different storage temperatures, to determinate the thermal degradation kinetics, and define an estimated shelf-life of the encapsulated product.

2. LITERATURE REVIEW

2.1. MANGO (*Mangiferina indica* Linn.)

Mango (*Mangifera indica* L.) is one of the most popular tropical fruits in the world. It is highly appreciated due to its sensorial features (color, aroma, and flavor) and nutritional value (vitamins, micronutrients, and other phytochemicals), (Burton-Freeman et al., 2017). In 2018, mango fruit crop ranked sixth and its world production was 52.08 million metric tons, increasing 1.5 times in the last decade. India is the leading mango producer, 20.01 million metric tons, whereas Mexico and the United States of America are the top countries mango exporter and importers, respectively (FAO, 2018).

With a production of 75,800 metric tons, Ecuador is the second mango exporter to American markets (FAO, 2018). The varieties that mainly grow in Ecuador are Tommy Atkins (69%), Kent (15%), Haden (2.5%), and Keitt (2.5%) (Figure 2.1), which are highly demanded in the USA for their quality attributes demanded by consumers (Mango Ecuador Foundation, 2018).

FIGURE 2.1.- Main mango varieties (*Mangifera indica* L.) in Ecuador. Adapted: Mango Ecuador Foundation (2018)

Besides the fresh fruit, mango can also be processed into a puree, slices in syrup, juices, nectars, concentrates, jams, jelly powders, and dried fruit (Berardini et al., 2005). The significant by-products from mango processing are

peels and seeds, representing 35% to 60% of the total fruit weight, which are typically discarded and may become a source of environmental pollution (Dorta et al., 2014). In developed countries, 39% of food waste is produced by food manufacturing industries (Mirabella et al., 2014). Nowadays, food industry seeks to implement sustainable development through sustainability strategies such as waste valorization (e.g., extraction of high-value functional compounds) (Otles & Kartal, 2018).

2.2. MANGO PEEL AS AN AGRO-INDUSTRIAL BY-PRODUCT

The use of by-products to produce new products has been proposed as an alternative to reduce contamination problems and economic losses. Peels are the most important by-product of mango processing and represent 15% to 20% of the total fruit weight. Mango peels have a high potential to be reused as a source of nutritional compounds and antioxidant phytochemicals (Serna-Cock et al., 2016).

2.2.1. CHEMICAL CHARACTERIZATION AND BIOACTIVE COMPOUNDS

The quality and quantity of nutrients and bioactive compounds in mango peel depend on factors such as variety, maturity stage, soil conditions, geographic site of production, farming practices, processing conditions, among others (Alañón et al., 2019; Berardini et al., 2005; Rodriguez-Amaya & Kimura, 2004). Dorta et al. (2014) mentioned that characterization is essential for valorizing these mango by-products, whether for use in the food industry or other applications.

In general, the mango peels dry matter contain carbohydrates (>50%), protein (1.8% - 6.3%), fat (1.6% - 2.7%), ash (1.2% - 4.2%) and energy (Table 2.1) (Ajila et al., 2007; Baddi et al., 2015; Garcia-Amezquita et al., 2018).

TABLE 2.1.- Chemical composition* of mango peels.

Variety	Carbohydrate	Protein	Fat	Ash	Dietary fiber		Energy (kcal/100g)	Reference
					Insoluble	Soluble		
Raspuri	-	2.10	2.20	1.20	40.0	23.8	-	Ajila et al. (2007)
Badami	-	1.80	2.70	1.30	50.3	28.0	-	
Alphonso	86.40	3.80	2.60	3.30	-	-	384	Baddi et al. (2015)
Sugar	52.30	6.30	1.60	4.20	23.5	12.1	-	Sánchez-Camargo et al. (2019)
Tommy Atkins	-	4.28	2.35	2.83	13.8	14.3	-	Vergara-Valencia et al. (2007)

*Values g/100g on a dry weight basis (D.W.), except for energy.

In addition, mango peels are a sustainable source of dietary fiber (28.1% - 78.3%), in which soluble dietary fiber constitutes more than 30% of the total dietary fiber (Table 2.1). Dietary fiber is essential in the human diet, with numerous beneficial physiological functions (Serna-Cock et al., 2016). According to Ajila et al. (2007), proportions between 30 - 50% soluble dietary fiber and 50 - 70% insoluble dietary fiber can be considered a well-balanced nutritional fiber source.

On the other hand, mango peels have significant amounts of bioactive compounds, mainly carotenoids (Ajila et al., 2010; Ranganath et al., 2018), phenolic compounds (Barreto et al., 2008; López-Cobo et al., 2017), and flavonoids (Alañón et al., 2019; Berardini et al., 2005). These compounds are attractive for their excellent antioxidant (Dorta et al., 2012; Sogi et al., 2013) and functional properties (Asif et al., 2016; Masibo & He, 2008; Serna-Cock et al., 2016).

Carotenoids are lipophilic pigments that impart lighter (yellow to red) colors to plant foods (e.g., mango peel) (Karanjalker et al., 2018). Ajila et al. (2010) and Ranganath et al. (2018) reported that carotenoids usually present in mango peels include β-carotene, cis β-carotene, lutein, and violaxanthin, where β-carotene was among the most abundant carotenoid in green, yellow and red peels. These carotenoids are an essential component of the human diet with positive effects

on antioxidation and preventing vitamin A deficiency. In addition, carotenoids provide dietary sources for reducing the incidence of age-related eye diseases (e.g., macular degeneration or cataracts), cancer, and cardiovascular disease, whereby play a vital role in human health and nutrition (Álvarez-Henao et al., 2018; Liang et al., 2020).

On the other side, an essential group of secondary metabolites in mango peel are phenolic compounds. Major phenolics found in mango peel include gallates and gallotannins (gallic acid derivates), flavonol glycosides (quercetin derivates and rhamnetin hexoside), xanthones glycosides (mangiferin derivates), benzophenone derivates (maclurin and iriflophenone glycosides), and ellagic acid derivates (Alañón et al., 2019; Barreto et al., 2008; Dorta et al., 2014). These bioactive compounds have excellent attention for promoting health benefits due to their prominent antioxidant activity (Asif et al., 2016; Ulla et al., 2017). For example, a xanthone very predominant in mango peel is mangiferin (Luo et al., 2012; Ruales et al., 2018), which has shown promising chemotherapeutic and chemopreventative agent against oxidative-stress-related liver disorders (Gold-Smith et al., 2016; Ulla et al., 2017).

Phenolic compounds, in contrast to other bioactive compounds such as most carotenoids, are not chemically synthesized and need to be extracted from plant material (Schieber et al., 2001) to obtain a natural ingredient with potential use for the bioproducts design (e.g., antioxidant additive for an edible oil) (Sánchez-Camargo et al., 2019).

2.2.2. CHALLENGES FOR EXTRACTION OF BIOACTIVE COMPOUNDS

Extraction is the first and most important step in recovering bioactive compounds from plant biowastes. Due to the nature of these compounds, they are usually present in the form of complexes within the vegetal matrix (González & González, 2010). Influencing factors in the extraction processes are matrix properties, solvent type, temperature, pressure, and time. A wide range of extraction methods are used to isolate and purify the bioactive compounds from

biowastes, some of them based on non-conventional methods (Azmir et al., 2013).

For the majority of bioactive compounds in mango peel, the recovery step typically involves direct extraction using a range of solvents (i.e., conventional solvent extraction - CSE) with different polarities (e.g., methanol, ethanol, water, acetone, ethyl-acetate, hexane), in various concentrations or their blends to obtain extracts with antioxidant activity (Table 2.2). However, there are non-conventional methods such as microwave-assisted extraction (Dorta et al., 2013), supercritical fluids extraction (Souza et al., 2019), and ultrasound-assisted extraction (Martínez-Ramos et al., 2020; Morales et al., 2020), among others, which are more environmentally friendly. These methods allow a reduced solvent consumption, extraction time as well as a better yield and quality of extracts (Azmir et al., 2013).

TABLE 2.2.- List of extraction studies of bioactive compounds on varieties of mango peels.

Variety	Bioactive	Solvent	Extraction Method	Main Findings	Reference
Raspuri Badami	Polyphenols	Sodium phosphate buffer Ethyl alcohol Acetone	CSE	Acetone extracted maximun amount of polyphenols and it was higher in raw peels in both varieties.	Ajila et al. (2007)
Keitt	Flavonoids Tannins Proanthocyanidins	Methanol Ethanol Acetone Water	CSE	Extraction solvent was the most important factor. Ethanol or ethanol:water were the most suitable solvents to obtain mango peel extracts with high antioxidant activity and high bioactive content.	Dorta et al. (2012)
Keitt	Tannins Proanthocyanidins	Ethanol:Water	CSE MAE	Extraction method and the water content in the extractant were the factors with the greatest effect. MAE obtained the highest antioxidant activity and phytochemical content.	Dorta et al. (2013)

-	Phenolics Flavonoids Carotenoids	Ethanol	CSE SFE HPE	A sequential two stage extraction process (SFE and HPE) allowed the efficient recovery of bioactives compounds with important antioxidant activity.	García-Mendoza et al. (2015)
Tommy Atkins	Phenolics	Ethanol:Water	UAE	The best extraction of the total phenolics (yield 67%) was obtained with ethanol:water (50:50) without ultrasound application.	Guandalini et al. (2019)
-	Phenolic compounds	Ethanol Acetone Hexane	CSE UAE	Higher phenolic content was obtained with ethanol:acetone (60:40) and an increase in phenolics (avg. 630%) was observed when UAE was applied.	Martínez-Ramos et al. (2020)
Criollo	Phenols Mangiferin	Ethanol:Water	UAE	Maximun extraction of total phenols and mangiferin was found at 54 °C and 10 min in ultrasonic extraction process.	Morales et al. (2020)
Ataulfo Autochthonous	Mangiferin Lupeol	Ethanol:Water	MAC SOX UAE MAE HPE	Mangiferin and lupeol were found in higher concentrations in Ataulfo mango peels (maturity stage). UAE method was considered to obtain the best yield at room temperature and short time.	Ruiz-Montañez et al. (2014)
Tommy Atkins	Phenolic compounds	Ethanol Hexane Ethyl acetate	SOX MAC UAE SFE	SOX method with ethanol provided the highest extraction yield and the highest antioxidant activity. SFE extracts presented low yields.	Souza et al. (2019)

CSE: Conventional solvent extraction; HPE: High pressure extraction; MAC: Maceration extraction; MAE: Microwave-assisted extraction; SOX: Soxhlet extraction; SFE: Supercritical fluid extraction; UAE: Ultrasound-assisted extraction.

Ultrasound-assisted extraction (UAE) is beneficial to obtain high-quality antioxidant extracts from different plant matrices (Vilkhu et al., 2008). UAE produces acoustic cavitations in the solvent, which helps solvent penetration into the plant sample. It increases the contact surface area between the solvent and compound of interest, thus improving mass transfer processes (i.e., the bioactive diffuses rapidly from the plant material to the solvent) (Azmir et al., 2013). For

example, Martínez-Ramos et al. (2020) determined an increase (6.3 times on average) in total phenolic compounds from mango peels when the ultrasound was applied in comparison to the conventional solvent extraction method.

Several studies have also reported an essential recovery of carotenoids and more polar phenolic compounds in mango peels using supercritical fluid extraction alone or combined with high-pressure extraction and microwave-assisted extraction (Dorta et al., 2013; Garcia-Mendoza et al., 2015; Sánchez-Camargo et al., 2019).

Thus, discrepancies are found mainly between the extraction yield (bioactive content) in mango peels when different extraction processes were evaluated (Table 2.2). For example, Ruiz-Montañez et al. (2014) indicated that the UAE method allowed the best yield of bioactive compounds, while Guandalini et al. (2019) reported that the ultrasound did not affect the extraction yield of the total phenolics. These differences confirm that the role of each factor in the extraction process is not always evident. To obtain antioxidant-rich extracts, the conditions must be evaluated since each biowaste-solvent system behaves differently and in an unpredictable manner (González & González, 2010).

A problem in obtaining bioactive extracts from natural sources is that bioactive compounds such as carotenoids are extremely susceptible to oxidative reactions. These bioactives have a conjugated double bond system that is responsible for their coloring power. This also causes an instability of carotenoids due to the high degree of unsaturation of the bonds that subject the molecule to several degradation reactions (Rodriguez-Amaya, 2019).

The carotenoids present in biowaste are found naturally protected by the complex structure of plant tissue. However, extractants readily undergo *trans-cis* isomerization and oxidation catalyzed by light, acids, bases, oxygen, heat, traces of metal ions, etc. (González & González, 2010).

In addition to efficient extraction, it is crucial to avoid degradation reactions and to maintain the stability of the compounds. Current stabilization efforts have

been centered on microencapsulation and nanoencapsulation, which can prevent or reduce the degradation of bioactives present in the extracts (Fabela Morón, 2017; Rodriguez-Amaya, 2019).

2.3. ENCAPSULATION

Encapsulation technology has been widely used in food industry to provide components covered with an effective barrier against environmental and chemical interactions. It also improves stability at temperature, humidity, oxidation, and light exposure, and it helps to extend their shelf life (Dias et al., 2017).

2.3.1. DEFINITION AND GENERAL ASPECTS

Encapsulation is defined as a process in which the core material (liquid droplet, solid particle, or gas bubbles) is trapped (coated or embedded) in a food-grade material to obtain an encapsulated product with valuable properties. The core material, consisting of one or more ingredients, represents the coated, active, or encapsulated material. On the other hand, the outer material of the capsule may be in individual fractions or mixtures of several components and is known as the encapsulation agent, coating material, or matrix (Zuidam & Shimoni, 2010).

Some authors classify the encapsulation systems into two forms: reservoir and matrix system (Figure 2.2). In the reservoir system (capsule), the core is concentrated in the central region, surrounded by a defined and continuous film of wall material. Whereas the matrix system (sphere), the core is uniformly dispersed in the encapsulation agent or matrix and can also be present on the surface. These systems can combine and provide another encapsulate system called the coated matrix (Figure 2.2). The encapsulate systems can also be cylindrical, oval, or irregular-shaped (Shishir et al., 2018).

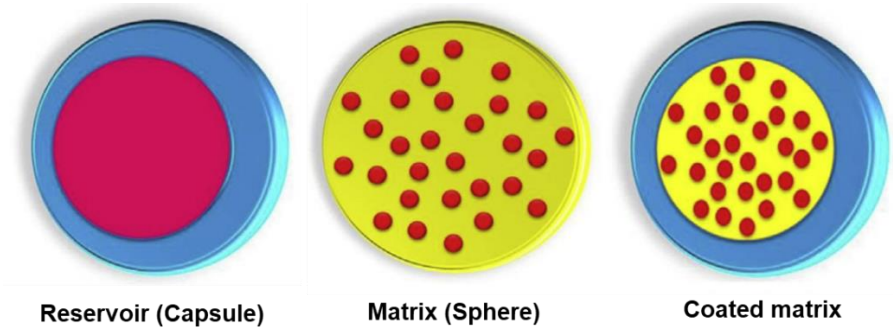

FIGURE 2.2.- Forms of encapsulate system: Reservoir and matrix. Adapted: Shishir et al. (2018)

In addition, the encapsulation might also be classified by the particle size of the outcome, e.g., nanoencapsulation (1 to 100 nm, nanoparticles), microencapsulation (100 to 1000 nm, microparticles), etc. (Sobel et al., 2014).

Food components that can benefit from microencapsulation include bioactive compounds (e.g., carotenoids), vitamins (e.g., vitamins A and D) and minerals (e.g., calcium), natural colors (e.g., anthocyanins), nutrients (e.g., peptides), among other (McClements, 2012).

Microencapsulation of food components mainly improves their stability under adverse storage conditions and thereby increases their shelf life. It also aids its solubilization and thus facilitates its incorporation into foods. It allows for the controlled delivery of the active components to the target site, thereby improving their bioavailability (Zuidam & Shimoni, 2010).

Several techniques are effective in the microencapsulation of food ingredients. The best process is selected for each specific type of core material, encapsulation agent, and intended final application. The main difference between each methodology depends on the active component entrapment method and its combination with the wall material; it can be a solution, an emulsion, or dispersion. Thus, microencapsulation techniques can thus be classified into (i) physical methods (mechanical) such as spray-drying, freeze-drying, extrusion, fluid-bead coating, and processes using supercritical fluids; (ii) physicochemical methods including spray cooling, ionic gelation, solvent evaporation, liposome

entrapment, and coacervation; and (iii) chemical methods such as interfacial polymerization, and molecular inclusion cross-linking (Ozkan et al., 2019; Zuidam & Shimoni, 2010).

2.3.2. MICROENCAPSULATION BY SPRAY-DRYING

Spray-drying is the most widely used microencapsulation technique in the food industry. It efficiently transforms liquid solutions into homogeneous powders with high productivity and an excellent cost-benefit ratio. The equipment is highly available as compared to other techniques (Anandharamakrishnan & Padma Ishwarya, 2015). Approximately 90% of microencapsulated products are obtained by spray-drying (Dordević et al., 2014).

Spray drying is the transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium. The spray-drying process consists of three important stages: (i) emulsification of core material into the wall solution; (ii) atomization of the emulsion into spray chamber; and (iii) drying of the atomized emulsion droplets on hot gas contact (Figure 2.3) (Anandharamakrishnan & Padma Ishwarya, 2015).

The first stage includes dispersing the core compound in an aqueous carrier solution and forming emulsion droplets with a diameter of 1 – 100 µm (to obtain a stable emulsion). Then, droplets are sprayed by the atomizer to the drying chamber, where they contact hot air (130 – 220 °C), creating a mist. The solvent is evaporated, and the microparticles (10 to 300 µm) are collected in a separator. The drying step only takes a short time (5 to 100 s); therefore, even heat-sensitive compounds can be spray-dried without a significant loss (Dordević et al., 2014; Shishir & Chen, 2017).

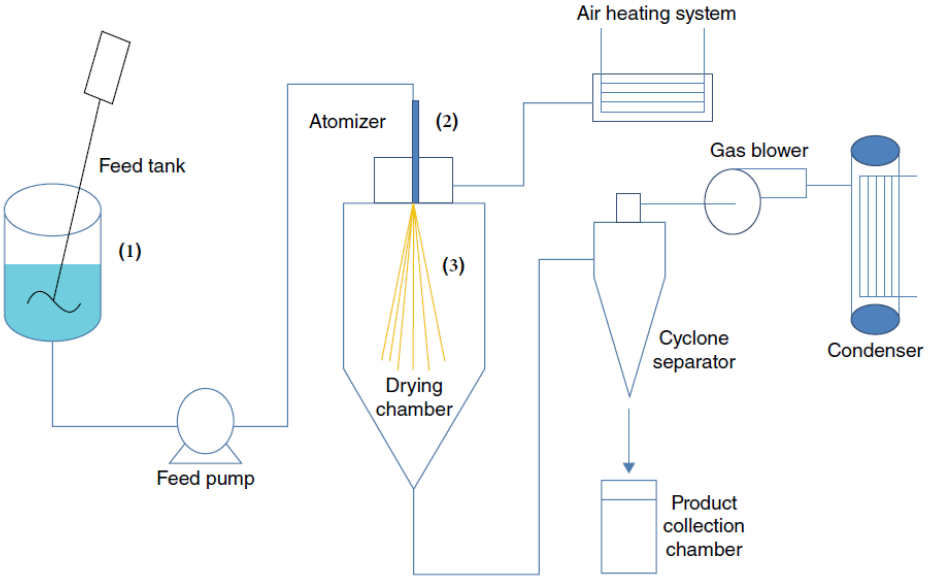

FIGURE 2.3.- Schematic diagram of the spray-drying process. (1) Emulsification. (2) Atomization. (3) Drying.
Adapted: Anandharamakrishnan & Padma Ishwarya (2015)

The powder product quality obtained by the spray-drying process depends on some factors such as the characteristics of the atomized liquid (solid content, type of carrier agent, droplet size, viscosity), spray dryer design (airflow, atomizer type), and the operation conditions (inlet and outlet temperature, feed flow rate, airflow rate, atomizer speed) (Shishir & Chen, 2017).

A significant advantage of spray drying is its general applicability to both hydrophilic and hydrophobic solutions (Özkan & Bilek, 2014). Among other benefits of this technology are the gradual release of the encapsulated compound, facilitating transportation and handling, and the change of specific characteristics (humidity, solubility, density, and hygroscopicity) to improve its distribution at the moment of incorporation in food matrices (Anandharamakrishnan & Padma Ishwarya, 2015).

Despite the described advantages, spray drying has a limited number of wall materials available. Another significant disadvantage of this technique is the problem of product stickiness through the drying chamber walls, resulting in low yields and the possibility of degradation of sensitive compounds at high drying temperatures (Özkan & Bilek, 2014).

2.3.3. ENCAPSULATING AGENTS

The nature of the encapsulating material is a crucial factor that depends on the core material or ingredient to be coated and the desired characteristics of the final encapsulated products. It is difficult to find an encapsulating agent that can meet all the ideal properties, have good emulsification, be film-forming, biodegradable, have low viscosity (even at high concentrations) and low hygroscopicity, as well as must be food grade and low-cost (Shishir et al., 2018).

Encapsulation agents can be selected from various natural or synthetic polymers. Materials used mainly in the food industry as encapsulating material include carbohydrates (maltodextrins, modified starch, saccharose), proteins (whey protein, gelatin, sodium caseinate, lecithin), lipids (fatty acids, phospholipids, waxes), and gums (gum arabic, sodium alginate), of which carbohydrate polymers are the most widely used (Anandharamakrishnan & Padma Ishwarya, 2015).

Maltodextrins (MD) are hydrolyzed starch products with different molecular weights (depends on dextrose equivalence DE), highly water-soluble (~ 70%), and low viscosity (even with concentrated solutions). They are odorless, colorless, and do not mask the original flavor. Due to their physicochemical properties and low cost, they are extensively used as wall material for encapsulation (mainly by spray drying). Both hydrophilic and hydrophobic materials can be microencapsulated with maltodextrin (Wandrey et al., 2010). Although maltodextrin exhibits poor emulsification capacity, this limitation can be overcome by combining maltodextrin with other wall materials with good emulsifying capacity as gums, proteins, or modified starches to obtain the best results after the encapsulation process (Dordević et al., 2014).

Hydrocolloids, commonly known as gums, are often explored in view of their encapsulation capacities. These compounds are long-chain polymers that dissolve or disperse in water to achieve a thickening or viscosity-enhancing effect. Gums are generally used as texturizing agents, but their secondary properties include encapsulation capabilities, emulsion stabilization,

crystallization control, and the inhibition of syneresis. In addition, some of the gums are also capable of forming gels (Labuschagne, 2018).

Gum arabic (GA) has been an encapsulating agent used for many years in spray drying, for encapsulation of lipophilic core compounds, due to its excellent emulsifying property. It is non-toxic, odorless, tasteless, and has low viscosity in aqueous solution (Wandrey et al., 2010). Despite all its features, the price of gum arabic varies greatly as function of market availability and quality. About barrier properties, GA shows limited protection against oxidative reactions since it acts as a semi-permeable membrane, which may compromise the shelf life of the encapsulated product (Anandharamakrishnan & Padma Ishwarya, 2015).

An interesting alternative as an encapsulating agent, although less explored, is inulin. It is a fructooligosaccharide obtained commercially from chicory. This polysaccharide can improve powder functionality through its known prebiotic activity. Inulin has excellent viscoelastic properties that could allow smoother particles' formation, while its lower water absorption may provide better shelf-stability to encapsulated bioactive compounds (Labuschagne, 2018).

Several authors mention that using a single agent does not have all the characteristics required to carry out correct microencapsulation and subsequent drying (Dordević et al., 2014; Labuschagne, 2018). Therefore, the use of different agents in the same matrix (e.g., a mixture of maltodextrin with gum arabic) could result in more resistant and uniform microspheres, among other aspects that ensure the quality of the powders. This quality will depend on different morphological characteristics (size, structure, density), yield, active material content, release, physical state, and polymer-active material interaction (Anandharamakrishnan & Padma Ishwarya, 2015; Shishir et al., 2018).

2.3.4. ENCAPSULATION BY SPRAY-DRYING OF BIOACTIVE COMPOUNDS

Bioactive compounds provide significant health benefits, but they are chemically unstable and susceptible to oxidative degradation. Encapsulation can preserve the bioactive compounds from environmental stresses, improve their stability, solubility, bioactivity, and controlled release in possible applications as functional food ingredients (Shishir et al., 2018).

Among the bioactive compounds that have been encapsulated by spray-drying are carotenoids, anthocyanins, phenolic compounds, vitamins, etc. Table 2.3 presents some studies on microencapsulation of these compounds, the wall materials and necessary encapsulation conditions used, and the main findings.

TABLE 2.3.- Encapsulated bioactive compounds by a spray-drying process.

Encapsulated Bioactive Compounds	Encapsulating Agents	Encapsulation Conditions	Main Findings	References
Astaxanthin	Whey protein isolate Sodium caseinate	30% TSS 160-180 °C IT	ME of astaxanthin was reasonably high and comparable for both encapsulating systems (~95%) at 160 °C IT. The powders had good properties including water activity (<0.3), surface morphology and oxidative stability.	Shen & Quek (2014)
Annatto seed bixin	Maltodextrin DE20 Gum arabic Sucrose Tween 80	40% TSS 180 °C IT	The encapsulated systems with GA or MD + Tween 80 showed the highest ME (86% and 75%, respectively). Bixin microcapsules with GA was 3 to 4 times more stable under illumination than that encapsulated with maltodextrin.	Barbosa et al. (2005)
Annatto seed norbixin	Maltodextrin DE20 Gum arabic	20-40% TSS 150 °C IT	Formulation 0:100 (MD:GA) and 40% TSS showed the highest ME (74%) and a good PY (61%). Norbixin microcapsules had uniform particle diameter, homogeneity, high solubility (> 90%), low moisture content (<3%) and AA. Improved the thermal stability of norbixin.	Tupuna et al. (2018)

β-carotene	Maltodextrin DE15 Gum arabic Modified starch Whey protein	20% TSS 190 °C IT	The emulsions stability had a significant effect on ME but had no effect on β-carotene retention during 60 days of storage with access to daylight. The highest retention of biactive was obtained with mixture of GA + MD.	Dluzewska et al. (2020)
Blackberry pomace (peel and seeds) anthocyanins	Maltodextrin DE10	500 mg/mL 170 °C IT	There was a reduction of anthocyanins in the samples at higher pH (5.0 and 6.5), and an overall reduction of AA was observed. At lower pHs higher stability of the microcapsules was obtained. The encapsulated anthocyanins half-life time was higher than non-encapsulated extract.	Santos et al. (2019)
Brazil nut (cake extract) phenolic compounds	OSA (octenyl succinic anhydride modified starch) Inulin	9-12 % TSS 180 °C IT	Microcapsules with formulation OSA-starch and inulin (1:1) showed the best properties, and stability of phenolic compounds and antioxidant activity during 120 days of storage.	Gomes et al. (2019)
Cactus pear phenolic compounds and betalains extract	Maltodextrin DE10 Inulin	3-30% TSS 120-160 °C IT	The optimal conditions for bioactive compounds extract were 3:1 and 5:1 ratio of core/coating material, and 140 and 120 °C IT, for systems with MD and inulin, respectively. During storage at 60 °C, all systems increased phenolic content and also showed a slow degradation of betalains (indicaxanthins).	Sáenz et al. (2009)
Chilli peppers carotenoids	Maltodextrin DE20 Gum arabic	Biopolymers:extract -solution ratio of 4:1 (w/w) 160 °C IT	The encapsulation of non-aqueous extracts allowed for the protection of carotenoids against oxidative and deterioration processes. Microcapsules did not present cracks, and preserved about 85% of carotenoids and 80% of the AA after storage for 60 days.	Guadarrama-Lezama et al. (2012)
Espresso spent coffee phenolic compounds and caffeine	Whey protein isolate Maltodextrin Gum arabic Inulin	20 g/100 mL 160 °C IT	WPI alone showed greater efficiency in the maintenance of the AA measured by different methods. The combination of WPI and inulin (proportion 1:1) demonstrated high protection and stability of encapsulated bioactive compounds after 42 days of storage at two different temperatures (25 °C and 35°C).	Abrahão et al. (2019)

Jabuticaba pomace anthocyanins	Maltodextrin Gum arabic Modified starch	30% TSS 140-180 °C IT	The use of MD alone (30%) as wall material with the higher IT (180 °C) permited to obtain microcapsules with the higher anthocyanin retention and lower overall color difference, moisture content and higroscopicity. The formation of more homogeneous particles was observed when MD and GA were used.	Silva et al. (2013)
Lettuce and cabbage lutein extract	Maltodextrin Gum arabic Modified starch	10% TSS 185 °C IT	Formulation with Gum arabic (100%) had the highest ME (92%), but the formulation (MD:GA:MS) (33.3:33.3:33.3) was that best protected the lutein extract until days 20 in the storage conditions (45 °C and 75% relative humidity).	Álvarez-Henao et al. (2018)
Lutein	Gelatin Porous starch	10-30 % TSS 170-190 °C IT	The optimal process of lutein encapsulation was 1/30 ratio of core/wall material and inlet gas temperature of 190 °C, which allowed to obtain a good ME (94%) and a high PY (93%). Microcapsules showed stability against heat, pH, light and oxygen and their retention were improved about 15-50% than that of free lutein.	Wang et al. (2012)
Lutein crude powder	Maltodextrin Sucrose Dihydrate trehalose Inulin Modified starch	20 % TSS 180 °C IT	Lutein microencapsulated with inulin, trehalose and modified starch showed the higher ME (>70%). The best PY (~92%) were obtained with modified starch and MD. Inulin exhibited the greatest protective capacity during storage at 25 °C for 20 days.	Ding et al. (2020)
Mango seed kernels phenolic compounds	Maltodextrin Gum arabic Gelatin Sodium alginate	Different % TSS 180 °C IT	The formulation 5.95% GA, 23.9% MD and 0.11% alginate presented the best stability and encapsulation efficiency (~90%) phenolic compunds in the required region of viscosity (<300 cPs) of the multiple emulsion.	Maisuthisakul & Gordon (2012)
Peach palm residues β-carotene	Process 1: Maltodextrin DE16 Gum arabic Process 2: Gelatin Sugar Lecithin	25% TSS 160 °C IT 6% TSS 100 °C IT	The microcapsules of β-carotene obtained with process 1 encapsulation process showed the highest levels of stability against temperature, light, pH and oxygen. The retention rates were improved about 15-25% than that of free total carotenoids.	Ordoñez-Santos et al. (2018)

Pineapple peel phenolic compounds	Maltodextrin Gum Arabic Inulin	20% TSS 150-190 °C IT	The microcapsules with differents wall materials presented differences in their morphology, however the AA of the extract was preserved after encapsulation in all cases. The particles with inulin showed a large degree of agglomeration. The encapsulated phenolic compounds using MD and GA at 150 ºC had good stability and their AA did not change significantly during six months of storage at 5 ºC.	Lourenço et al. (2020)
Pequi carotenoids	Maltodextrin DE10 Gum arabic	30% TSS 150-190 °C IT	The highest carotenoids content (25 µg/g) and solubility (>96%), and the lowest moisture content (<3%) were obtained at 190 °C. Microspheres presented an average size of 20 µm. No morphological and color differences for all particles.	Alves et al. (2017)
Red pepper seeds, skin leftovers and stems vitamins (A and E)	Gum arabic Tween 80	40% TSS 185 °C IT	High ME for vitamin E (73%) and provitamin A (77%). The stability of encapsulated provitamin A was higher than free provitamin A over storage time (35 days) at temperature room. The microcapsules showed a thermal stability up to 200 ºC.	Romo-Hualde et al. (2012)

TSS: Total soluble solids; IT: Inlet temperature; GA: Gum arabic; MD: Maltodextrin; DE: Dextrose equivalent; WPI: Whey protein isolate; ME: Microencapsulation efficiency; PY: Process yield; AA: Antioxidant activity.

The use of different encapsulating agents or their blends can provide distinct effects on the product obtained and the operating conditions that affect the properties of the powders (Table 2.3). In general, the microcapsules of carotenoids and phenolic compounds showed the most outstanding results with the addition of maltodextrin alone or blended with other encapsulating materials as gum arabic, whey protein, modified starch, etc. (Abrahão et al., 2019; Alves et al., 2017; Dluzewska et al., 2020; Lourenço et al., 2020; Maisuthisakul & Gordon, 2012; Ordoñez-Santos et al., 2018; Saénz et al., 2009; Silva et al., 2013). It demonstrates the remarkable efficacy of this carbohydrate for encapsulation.

The highest values of microencapsulation efficiency (ME > 74%) and process yield (PY > 60%) were achieved when several operating parameters as inlet temperature, type and wall material concentration, core/wall material ratio,

among others, were optimized (Álvarez-Henao et al., 2018; Barbosa et al., 2005; Maisuthisakul & Gordon, 2012; Romo-Hualde et al., 2012; Shen & Quek, 2014; Tupuna et al., 2018; Wang et al., 2012).

Some studies demonstrate that it was possible to obtain microcapsules with good surface morphology (e.g., smooth surface without cracks and more homogeneous particles) (Guadarrama-Lezama et al., 2012; Shen & Quek, 2014; Silva et al., 2013), thereby facilitating sufficient entrapment of core.

Therefore, encapsulation of bioactive compounds with suitable encapsulating agents and appropriate spray drying process conditions can preserve antioxidant activity (Gomes et al., 2019; Guadarrama-Lezama et al., 2012; Lourenço et al., 2020) and improve the stability of these encapsulated against heat (Abrahão et al., 2019; Ding et al., 2020; Tupuna et al., 2018), pH (Santos et al., 2019), light (Barbosa et al., 2005) and oxygen (Ordoñez-Santos et al., 2018; Wang et al., 2012).

2.3.5. FOOD APPLICATION OF ENCAPSULATED BIOACTIVE COMPOUNDS

Functional components are encapsulated for later incorporation into processed foods. Some examples of food applications of encapsulated bioactive compounds are shown in Table 2.4.

TABLE 2.4.- Food applications of encapsulated bioactive compounds.

Encapsulated Bioactive Compounds	Matrix	Purpose	References
β-carotene	Yogurt Pudding	Incorporation *In vitro* release and bioavailability	Donhowe et al. (2014)
Blueberry anthocyanins and phenolic compounds	Ice cream Cakes	Enrichment	Tatar Turan et al. (2015)
Brewers spent grain flavonoids	Fish burgers	Enrichment	Spinelli et al. (2016)

Grape pomace anthocyanins	Biodegradable Film	Food packaging Stability improvement against oxidation	Stoll et al. (2017)
Grape skin anthocyanins	Apple puree	Fortification Stability improvement against oxidation	Lavelli et al. (2016)
Lycopene	Cake	Colouring	Aguiar Rocha et al. (2012)
Natural β-carotene	Yogurt Bread	Incorporation *In vitro* release	Rutz et al. (2017)
Pomegranate peels phenolic compunds	Ice cream Hazelnut paste	Enrichment Stability improvement against oxidation.	Çam et al. (2014) Kaderides et al. (2015)

Encapsulated phenolic compounds are the most commonly used to improve stability and functional properties in various foods. Tatar Turan et al. (2015) encapsulated anthocyanins and phenolic compounds of blueberry, which were added to ice cream and cakes, and obtained enriched products with these bioactive compounds. Similar results of incorporation, good stability, and fortification of food products with microcapsules of phenolic compounds were found by Spinelli et al. (2016) (fish burgers), Lavelli et al. (2016) (apple puree), Çam et al. (2014) (ice cream), and Kaderides et al. (2015) (hazelnut paste). Likewise, Stoll et al. (2017) demonstrated the efficacy of an active biodegradable packaging endowed with encapsulated anthocyanins on extra-virgin olive oil.

In some studies incorporated encapsulated carotenoids as an ingredient in different food matrixes and observed the effect of *in* vitro release of these compounds and their bioavailability, concluding that carotenoids microparticles added in foods had a higher retention inside the capsule (Rutz et al., 2017) which was further evidenced in retained bioavailability (Donhowe et al., 2014). Aguiar Rocha et al. (2012) used encapsulated lycopene in cakes and determinated that the microcapsules could release pigment and color homogenously the studied food matrix.

3. PAPER I

Characterization and quantification of bioactive compounds and antioxidant activity in three different varieties of mango (*Mangifera indica* L.) peel from the Ecuadorian region using HPLC-UV/VIS and UPLC-PDA.

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Characterization and quantification of bioactive compounds and antioxidant activity in three different varieties of mango (*Mangifera indica* L.) peel from the Ecuadorian region using HPLC-UV/VIS and UPLC-PDA

Verónica Marcillo-Parra [a,b], Mayra Anaguano [a], Maritza Molina [a], Diego Santiago Tupuna-Yerovi [c], Jenny Ruales [a,*]

[a] Department of Food Science and Biotechnology (DECAB), Escuela Politécnica Nacional (EPN), 17012759 Quito, Ecuador
[b] Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armadas ESPE, 171-5-231B Sangolquí, Ecuador
[c] Agroindustrial Engineering Department, Pontifícia Universidad Católica del Ecuador – Sede Manabí (PUCEM), Campus Chone, 130301 Portoviejo, Manabí, Ecuador

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ABSTRACT

Mango peel is gaining recognition for its nutritional and functional value. This by-product shows variable composition depending on different factors such as variety, stage maturity, and geographic site of production. This work sought to evaluate mango peel composition, bioactive compound content, antioxidant activity, and carotenoid and phenolic profiles for the three most commercial varieties of the equatorial region using UV–Vis spectroscopy, HPLC, and UPLC. Significant differences ($p < 0.05$) were found between all varieties regarding the content of bioactive compounds. Total phenolic, flavonoid, and carotenoid content ranged from 2930 to 6624 mg GAE/100 g, 502–795 mg CE/100 g, and 3.7–5.7 mg/100 g, respectively. A high positive correlation ($r = 0.961$) between the phenolic content and ABTS radical-scavenging activity was found for all samples. β-Carotene and lutein were identified and quantified, the samples of cvs. Haden and Kent showed the highest β-carotene content (8 mg/100 g). Gallic acid and rutin were identified in all samples, whereas mangiferin had the highest amount (314 mg/100 g) in cv. Tommy Atkins. The results suggest that mango peel has bioactive compounds with significant antioxidant properties, which can be used as functional ingredients in different industrial products.

1. Introduction

Mango (*Mangifera indica* L.) is one of the world's most popular tropical fruits. It is highly appreciated for both its sensorial features and its nutritional value [1]. World mango production was 52.08 million metric tons in 2018, with a 150% increase from the previous decade. At 20.01 million metric tons, India is the leading producer of mango, while Mexico and the United States of America are the leading exporters and importers of mangos, respectively [2]. With a production of 75,800 metric tons, Ecuador is the second-largest exporter of mango to American markets [2], which prize the mango varieties Tommy Atkins (69%), Kent (15%), and Haden (2.5%) for having the quality attributes demanded by consumers [3].

Besides fresh fruit, mango can also be processed into pulp, juices, nectars, concentrates, jams, jelly powders, and dried fruit [4]. Mango peels and seeds represent 35% to 60% of the total fruit weight and create a significant amount of waste following processing [5]. In developed countries, 39% of food waste is produced by the food manufacturing industries [6]. In recent years, the food industry has moved towards sustainable development via sustainable strategies such as waste valorization (e.g., extraction of high-value functional compounds) [7].

Mango peels provide energy, dietary fiber, carbohydrates, protein, and fats [8–10] and are rich in phytochemicals such as phenolic compounds [11,12], flavonoids [13,14], and carotenoids [15,16]. These bioactive compounds are of interest thanks to their high antioxidant activity [17,18] and their therapeutic properties [19–21]. However, the quality and quantity of bioactive compounds in mango peel depend on factors such as variety, maturity stage, soil conditions, geographic site of production, farming practices, and processing conditions, among others [4,22,23]. Dorta et al. [5] mention that characterization is an essential

* Corresponding author at: Department of Food Science and Biotechnology, Faculty of Chemical and Agroindustrial Engineering, Escuela Politécnica Nacional, Ecuador.
E-mail addresses: vemarcillo@espe.edu.ec (V. Marcillo-Parra), mayra.anaguano@epn.edu.ec (M. Anaguano), mayra.molina@epn.edu.ec (M. Molina), santiagotupuna@hotmail.com (D.S. Tupuna-Yerovi), jenny.ruales@epn.edu.ec (J. Ruales).

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step for valorizing these mango by-products, whether for use in the food industry or other applications.

In this context, this study sought to characterize the Ecuadorian region's three most commercial varieties of mango peel by evaluating bioactive compounds' contents, assessing its antioxidant activity, and identifying and quantifying their carotenoid and polyphenol profiles.

2. Materials and methods

2.1. Reagents and standards

Methyl *tert*-butyl ether (MTBE), potassium peroxodisulfate, 2,2′-azonobis3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$), Folin-Ciocalteu reagent (2.0 N), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Company Ltd. (St. Louis, USA). Aluminum chloride hexahydrate and sodium nitrite were obtained from Fisher Chemical (Waltham, USA). A sodium carbonate monohydrate was provided by JT Baker (Dagenham, England). Methanol, acetonitrile, and formic acid were obtained from Merck (Darmstadt, Germany). Carotenoids (β-carotene and lutein ≥97%) and polyphenols ((+)-catechin hydrate, ferulic acid, gallic acid, (−)- epicatechin and mangiferin ≥98%; chlorogenic acid and quercetin ≥95% and rutin ≥94%) standards were acquired from Sigma-Aldrich Company Ltd. (St. Louis, USA). All reactants were of analytical grade and suitable for High-Pressure Liquid Chromatography (HPLC).

2.2. Samples

A total of three different varieties of mango fruits — Tommy Atkins, Haden, and Kent — from two provinces of Ecuador were used. They were collected and provided by the Carbell farm located in Guayas (1° 55′05"S, 80° 03'29" W, and 12 m above sea level) (Tommy Atkins - G, Haden, and Kent) and Asoprovalle located in Imbabura (0° 26′39"N, 77° 58'28" W, and 1654 m above sea level) (Tommy Atkins - I). Tommy Atkins mango peel (by-product) from agroindustrial processing (Tommy Atkins - BP - I) was provided by ENVAGRIF C.A. (Pichincha, Ecuador), the fruits were also cultivated in Imbabura. Ripe fruits without defects and physiological disorders were selected, sanitized, peeled, and then hand-separated into pulp, peel, and seed kernel. The sliced fresh mango peels (including sanitized Tommy Atkins – BP - I) were frozen into liquid nitrogen and lyophilized (Lyovac GT2, Leybold-Heraeus, Cologne, Germany) at 50 mPa and − 40 °C. Freeze-dried samples were ground and sifted into powder (≤ 0.4 mm) using a sieve shaker (Ro-Tap RX-29, W.S. TYLER, Cleveland, USA) and stored in airtight aluminized bags at −20 °C. These freeze-dried mango peel samples were used for all analyses.

2.3. Proximate composition and dietary fiber of mango peel

The analysis of moisture, protein, lipid, and ash content was carried out following the official AOAC methods [24]. The samples' carbohydrate content was obtained using the difference between the total percentage of moisture, protein, lipids, and ash. The energy value was expressed in kcal/100 g and was calculated using the conversion factors of nutrients into energy. The determination of total dietary fiber (soluble and insoluble) by the enzymatic-gravimetric method was carried out according to Asp et al. [25]. All analyses were performed in triplicate.

2.4. Bioactive compounds analysis and antioxidant activity (AA) by UV–Vis spectroscopy

2.4.1. Preparation of extracts

According to the procedure described by Georgé et al. [26], the extracts were prepared with some modifications. Freeze-dried samples (0.2 g) were homogenized in 10 mL of acetone/water solution (70:30, *v*/v) under agitation using magnetic stirring for 30 min. The supernatant was filtered through a Whatman filter paper, and 5 mL of extraction solution was added. Then, the supernatant was filtered again and transferred to amber bottles. This procedure was performed in triplicate. These extracts were used for the phenolic, flavonoid, and antioxidant activity assessments.

2.4.2. Total phenolic content

The phenolic compounds were extracted and quantified following the modified Folin-Ciocalteu assay [26] using a UV/Vis spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). A five-point analytical curve was built using concentrations of gallic acid anhydrous standard solutions (from 10 to 100 mg/L). The curves showed satisfactory linearity within the absorbance obtained for each concentration (R^2 = 0.999). The results are expressed as mg of gallic acid equivalents (GAE)/100 g on a dry weight (DW) basis.

2.4.3. Total flavonoid content

The flavonoids were assessed using a colorimetric method [27], based on the formation of a complex flavonoid-aluminum, with the maximum absorbance at 510 nm. Catechin hydrate standard at concentrations between 50 and 300 mg/L was used to calculate the calibration curve (R^2 = 0.999). Results are expressed as mg of catechin equivalents (CE)/100 g DW.

2.4.4. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS) radical scavenging activity

The antioxidant activity of mango peel extracts was evaluated following the ABTS assay described by Re et al. [28], with modifications. A five-point calibration curve (2.5 mM Trolox standard stock solution) was prepared for the analysis at 734 nm. The antioxidant activity of extracts is expressed as mM Trolox/100 g DW.

2.4.5. Total carotenoid content

Carotenoid extraction was performed following a methodology adapted from Rodriguez-Amaya & Kimura [23]. Freeze-dried samples (2.5 g) were ground with 10 mL of acetone in a pre-chilled pestle and mortar, and the procedure was repeated five times until getting a clear extract. Carotenoids were recovered in a separating funnel with hexane/petroleum ether (50:50 *v*/v). The extract was subsequently washed with distilled water several times to remove residual acetone. The upper phase was collected in a 50 mL volumetric flask and made up to volume with hexane/petroleum ether. The carotenoids were quantified using a UV/Vis spectrophotometer at 450 nm. The total carotenoid content was calculated using the absorption coefficient ($A^{1\%}_{1cm} = 2592$) of β-Carotene and is expressed as mg/100 g DW.

2.4.6. Analysis of carotenoids by HPLC-UV/VIS

The carotenoid extracts were evaporated until they were dry in a rotary evaporator ($T = 35$ °C), according to Rodriguez-Amaya & Kimura [23]. The dried residues were dissolved in 5 mL of mobile phase (methanol/methyl-*tert*-butyl ether, 50:50, *v*/v), filtered through a 0.45 μm PTFE syringe filter (Millipore, Milford, USA) directly into sample vials, and 20 μL were injected into the HPLC. The carotenoids were separated in an HPLC system (HP 1050 multi-solvent-delivery, Hewlett-Packard Company, Palo Alto, USA) equipped with HPLC software (Agilent ChemStation, Agilent, Santa Clara, USA) to process the chromatographic data. A carotenoid column C30 (250 × 4.6 mm i.d.; 5 μm particle size) (YMC Inc., Wilmington, USA) adjusted at 30 °C was used. Carotenoids were eluted at a flow rate of 1 mL/min. Each carotenoid was identified using a UV–visible detector at 450 nm. The mobile phases were (A) methanol, (B) methyl-*tert*-butyl ether, and (C) Milli-Q water. The separation was carried out during 60 min, using the following mobile phase gradient: 0 min, (A:B:C, 90:5:5); 12 min (A:B:C, 95:5:0); 25 min (A:B:C, 89:11:0); 40 min, (A:B:C, 75:25:0); 60 min, (A:B:C, 50:50:0). The identification was based on comparing retention times with authentic carotenoid standards, such as β-carotene and lutein. The

carotenoids were quantified using five-point analytical curves ($R^2 \geq 0.999$) of lutein and β-carotene (2–30 μg/mL). The concentration of each carotenoid is expressed in mg/100 g DW. All analyses were performed in triplicate.

2.5. Analysis of the phenolic compounds by UPLC-PDA

The extraction of phenolics was carried out as described by Dorta et al. [17], with modifications. A 1 g freeze-dried sample was suspended in methanol 50% (1:25, *w/v*). The suspensions were homogenized with an ultra-turrax (T25 digital, IKA, Staufen, Germany) at 3000 rpm for 1 min. They were then placed in hermetically sealed tubes to avoid solvent loss and kept in a water bath at 25 °C for 60 min to perform the phenolic extraction. Extracts were centrifuged using a centrifuge (IEC CL31R Multispeed, Thermo Scientific, USA) at 3000 xg for 20 min. Aliquots of 2 mL of the extract were evaporated in an evaporator system ($T = 35$ °C) with nitrogen until dry. The dried residues were dissolved in 1 mL of methanol and filtrated through a 0.22 μm PVDF syringe filter (Millipore, Milford, USA) directly into sample vials for injection in chromatography equipment for further analysis. The identification of phenolic compounds was performed using Ultra Performance Liquid Chromatography (UPLC) equipment (Acquity UPLC H-Class System, Waters, Milford, USA), coupled with a quaternary solvent manager and a PDA detector. Chromatography data software (Empower 3, Waters, Milford, USA) was used for data processing. The polyphenols column (2.1 × 100 mm i.d.; 1.8 μm particle size) (Acquity UPLC HSS T3, Waters, Milford, USA) was used at 30 °C. The mobile phase was composed of a mixture of formic acid and Milli-Q water (1:1000, *v*/v) (A) and acetonitrile (B). The gradient was programmed as follows: 0 min, 0% B; 5 min, 15% B; 8 min, 25% B; 12 min, 40% B; 18 min, 70% B; 22 min, 100% B; 26 min, 40% B; 28 min, 20% B and 30–35 min, 0% B [29,30]. The flow rate was 0.4 mL/min, and the injection volume was 1.5 μL. Simultaneous monitoring was performed at 280 nm (hydroxybenzoic acids and flavan-3-ols), 320 nm (hydroxycinnamic acids and xanthones), and 360 nm (flavonols). The phenolic compounds were quantified using calibration curves ($R^2 \geq 0.999$) in the 5–200 μg/mL range for all standards. Phenolic concentration is expressed in mg/100 g DW. All analyses were performed in triplicate.

2.6. Statistical analysis

The results are expressed as mean ± standard deviation. A unidirectional analysis of variance (ANOVA) and a Fisher LSD test (multiple-range) was carried out to correlate the data and to establish statistically-significant differences ($p < 0.05$) between samples with a 95% confidence interval. Statistical software (Centurion XVI·I, Statgraphics, Virginia, USA) was used for all calculations.

3. Results and discussion

3.1. Chemical characterization of mango peel

The chemical composition of freeze-dried samples is shown in Table 1. In general, the proximate analysis showed significant differences ($p < 0.05$) between varieties. The moisture content (4.9–5.8%) was lower than the range reported (7.4 to 9.8%) by Kaur and Srivastav [31], where the mango peel was dried in an oven and ground using cryogenic grinding as an emerging technology. Freeze-drying thus allows us to obtain powders with lower moisture content, which extends shelf-life by reducing microbial growth during storage.

Total protein ranged from 2.6 to 4.0%, with the lowest value for Haden and highest in Kent and Tommy Atkins - G (Table 1). Several studies using mango fruits from other regions, carried out by Ajila et al. [8] (cvs.: Raspuri and Badami), Baddi et al. [9], and Vergara-Valencia et al. [32] (cv. Tommy Atkins), reported similar protein content in mango peel: i.e., 2.1, 3.8, and 4.2%, respectively. The number of lipids was low (Table 1) when compared to other components, and these findings match the results reported by Kaur and Srivastav [31] in mango peel samples from six varieties (2.35–2.74%). The ash content in samples ranged from 2.18% (Haden) to 3.05% (Tommy Atkins - G) (Table 1). These results are higher than the findings (1.38–1.57%) of Onuh et al. [33] for three different mango peel varieties. They are similar to the data (2.8%) reported by Vergara-Valencia et al. [32] for Tommy Atkins mango peel. For carbohydrate content, the results for each variety ranged from 91.13 to 93.16%, as expected. This value demonstrates that mango peel can be an excellent energy source: the level was calculated using the conversion factors. The average value for all samples was 397 kcal/100 g DW, which is higher than the value reported by Garcia-Amezquita et al. [10] for mango peel.

Table 1
Chemical composition of freeze-dried mango peel samples.

Component (%)	Tommy Atkins - G	Haden	Kent	Tommy Atkins - I	Tommy Atkins - BP - I
Moisture	5.60 ± 0.11^{b}	5.40 ± 0.13^{c}	5.80 ± 0.03^{a}	4.90 ± 0.07^{d}	5.30 ± 0.10^{c}
Protein	4.03 ± 0.05^{a}	2.65 ± 0.05^{c}	4.04 ± 0.14^{a}	3.37 ± 0.06^{b}	3.27 ± 0.11^{b}
Lipid	1.80 ± 0.07^{b}	2.01 ± 0.03^{a}	1.48 ± 0.05^{c,d}	1.58 ± 0.09^{c}	1.39 ± 0.02^{d}
Ash	3.05 ± 0.07^{a}	2.18 ± 0.04^{d}	2.82 ± 0.03^{b,c}	2.84 ± 0.02^{b}	2.75 ± 0.05^{c}
Carbohydrate	91.1 ± 0.16^{e}	93.2 ± 0.22^{a}	91.7 ± 0.14^{d}	92.2 ± 0.07^{c}	92.6 ± 0.17^{b}
Soluble dietary fiber	22.7 ± 0.17^{a}	15.4 ± 0.19^{d}	18.9 ± 0.32^{c}	21.1 ± 0.39^{b}	18.6 ± 0.21^{c}
Insoluble dietary fiber	16.9 ± 0.16^{b}	12.7 ± 0.13^{e}	14.1 ± 0.30^{d}	19.3 ± 0.29^{a}	16.4 ± 0.33^{c}
Total dietary fiber	39.6 ± 0.32^{a}	28.1 ± 0.29^{d}	33.1 ± 0.63^{c}	40.3 ± 0.26^{a}	35 ± 0.55^{b}
Energy [kcal/100 g]	396 ± 1.09^{b}	401 ± 0.48^{a}	396 ± 0.40^{b}	396 ± 0.72^{b}	395 ± 0.45^{b}

Values on a dry weight basis (DW).
Mean ± SD ($n = 3$). Values with different letters in the same row are significantly different (p ˂ 0.05).
G: Guayas, I: Imbabura, BP: By-products.

The differences in chemical composition between mango peel samples of different varieties grown in the same zone (Tommy Atkins - G, Haden and Kent) and between samples of the Tommy Atkins variety from different regions (Guayas and Imbabura) (Table 1) may be due to the inherent characteristics of each variety, geographical conditions, and agronomic practices [34].

Significant differences ($p < 0.05$) were observed among the varieties in terms of insoluble dietary fiber, soluble dietary fiber, and total dietary fiber (Table 1). The total dietary fiber content in the mango peel varieties ranged from 28 to 40%, in which soluble dietary fiber constitutes more than 50% of the total dietary fiber. Tommy Atkins - I mango peel had the highest dietary fiber content. Dietary fiber is vital in the human diet. According to the results reported by Ajila et al. [8], proportions between 30 and 50% of soluble dietary fiber and 50–70% of insoluble dietary fiber can be considered a source of well-balanced dietary fiber.

3.2. Bioactive compounds and antioxidant activity

Total phenolic, flavonoid and carotenoid contents, and antioxidant activity of the extracts obtained from freeze-dried samples of different varieties are shown in Table 2. The results showed that polyphenols were the major bioactive compounds in mango peel. The total phenolic content was significantly different ($p < 0.05$) among the varieties. The highest total phenolic content was observed in the Haden (6624 mg GAE/100 g DW), whereas the lowest total phenolics value was observed in the Tommy Atkins - G (2930 mg GAE/100 g DW). The total phenolic content was different from that reported by other authors for Haden (not

Table 2
Total phenolic, flavonoid, carotenoid contents, and antioxidant activity of the extracts were obtained from freeze-dried mango peel samples.

Phytochemical characterization	Tommy Atkins - G	Haden	Kent	Tommy Atkins - I	Tommy Atkins - BP - I
Total Phenolics (mg GAE/100 g DW)	2931 ± 175^{d}	6624 ± 193^{a}	5149 ± 154^{b}	4146 ± 72.2^{c}	3920 ± 32.7^{c}
Total Flavonoids (mg CE/100 g DW)	502 ± 23.2^{d}	682 ± 14.5^{b}	600 ± 11.8^{c}	779 ± 11.9^{a}	795 ± 9.87^{a}
Total Carotenoids (mg/100 g DW)	4.65 ± 0.18^{b}	5.69 ± 0.20^{a}	4.53 ± 0.03^{b}	3.69 ± 0.09^{d}	4.14 ± 0.11^{c}
Antioxidant Activity (mM Trolox/100 g DW)	23 ± 2.85^{c}	53.9 ± 4.2^{a}	49.9 ± 1.24^{a}	34.3 ± 2.5^{b}	34.6 ± 1.55^{b}

Values on a dry weight basis (DW).
Mean ± SD (n = 3). Values with the different letters in the same row are significantly different ($p \leq 0.05$).
G: Guayas, I: Imbabura, BP: By-products.

detected - 293 mg/100 g DW) [11] and Kent (234–9121 mg/100 g DW) mango peel [14]. However, the TPC of Tommy Atkins mango peel was similar to the values reported by Barreto et al. [11] (2513 mg GAE/100 g DW) and Hung et al. [35] (4858 mg GAE/100 g DW). Differences in total phenolic content could be attributed to different factors such as the genetic basis, agronomic practices, harvest stage, and environmental conditions, among others [36,37].

Regarding the total flavonoid content, in freeze-dried samples, these compounds varied significantly between varieties (Table 2), ranging from 502 to 795 mg CE/100 g DW. These results were within the range of total flavonoid content reported by Ocampo et al. [38] (320–2160 mg CE/100 g DW) for twelve different Philippine genotype mango samples of the species *Mangifera indica*. Our results were also similar to the values reported by Dorta et al. [17] in Keitt mango peel extracts obtained using different extraction conditions (170–700 mg CE/100 g DW). Flavonoids are a subcategory of phenolic compounds, and the difference in total flavonoid content may be influenced by the factors mentioned above and the extraction method [17].

As for total carotenoid content, the results also presented significant differences ($p < 0.05$) between varieties (Table 2). The highest total carotenoid content was observed in Haden mango peel (5.69 mg/100 g DW) (yellow-red coloration), while the lowest total carotenoid content was found in Tommy Atkins - I (3.69 mg/100 g DW) (red coloration). Similar results for mango peels were also reported by Ranganath et al. [16], who found that total carotenoid content was higher in yellow-colored cultivars, followed by green cultivars. The lowest value was observed for red-colored cultivars at the fully-ripe stage. Likewise, the average total carotenoid content (4.17 mg/100 g DW) for the Tommy Atkins - I samples analyzed matches the total carotenoid value reported by Sogi et al. [18] for cv. Tommy Atkins from India (4.05 mg/100 DW). According to other studies, the carotenoid levels in mango fruit peel depend on several factors, such as genetics, maturity stage, and agronomic practices, among others [23,39].

The antioxidant activity varied significantly ($p < 0.05$) between varieties (Table 2). Haden and Kent samples showed the highest antioxidant activity. There were no significant differences ($p > 0.05$) between them. They were followed by Tommy Atkins - I and Tommy Atkins – BP - I samples, which showed no statistical variation ($p > 0.05$). The lowest value was observed in Tommy Atkins - G samples. In our study, the antioxidant activity results on a fresh weight (FW) basis ranged from 5.42 to 12.75 mM Trolox/100 g FW. These values are in the same range as that reported by Liu et al. [40] in eight different Chinese mango cultivars (5.8–18.4 mM Trolox/100 g FW). Furthermore, the average antioxidant activity value of the Tommy Atkins - I samples (6.79 mM Trolox/100 g FW) was similar to the result observed by Castro-Vargas et al. [41] in the Colombian Tommy Atkins variety (6.28 mM Trolox/100 g FW). On the other hand, certain literature has mentioned that mango peel has a high antioxidant activity, similar to other tropical fruit by-products [42,43]. These results suggest a clear opportunity to use the Ecuadorian mango peel from different varieties as a natural ingredient rich in phytochemicals with antioxidant properties.

Finally, a high positive Pearson's correlation coefficient ($r = 0.961$) between the total phenolic content and ABTS radical-scavenging activity was found in all samples. This value indicates a strong correlation between total phenolic content and antioxidant activity. The same effect was found in other studies on the characterization of extracts from mango by-products (peel and kernel) using different in vitro methods to assess the antioxidant activity [41,42].

3.3. Identification and quantification of carotenoids

HPLC was used to identify the carotenoid profile. Two major peaks were identified using the authentic standard carotenoids (β-carotene and lutein) in all samples. The amount of each carotenoid in the mango peel samples of different varieties is shown in Table 3. The analytical HPLC chromatograms of all mango peel samples are shown in Fig. 1.

According to the chromatograms, all samples showed the same carotenoid profile. The β-Carotene was both the principal provitamin A and the primary pigment. The concentration of this compound varied significantly ($p < 0.05$) between samples (Table 3), with an average β-carotene contribution of 65% of the total carotenoid content. The second primary pigment was lutein, which also presented significant differences ($p < 0.05$) between varieties (Table 3). In general, the β-carotene contents of the samples analyzed were higher than the results reported by Ocampo et al. [38] for mango peel from thirteen different genotypes.

Moreover, Ranganath et al. [16] reported β-carotene in mango peel from twelve different varieties ranging from 24.52 to 98.06% of the total

Table 3
Characterization of carotenoids by HPLC-UV/VIS and phenolic compounds by UPLC-PDA of freeze-dried mango peel samples.

Compounds (mg/100 g DW)	Tommy Atkins - G	Haden	Kent	Tommy Atkins - I	Tommy Atkins - BP - I
Carotenoids					
Lutein	1.25 ± 0.02^{d}	0.93 ± 0.01^{e}	2.71 ± 0.03^{a}	1.64 ± 0.02^{b}	1.60 ± 0.01^{c}
β-carotene	4.86 ± 0.10^{c}	8.03 ± 0.13^{a}	7.84 ± 0.19^{a}	2.91 ± 0.08^{d}	5.60 ± 0.13^{b}
Phenolics					
Gallic acid	32.3 ± 1.77^{b}	43.1 ± 0.13^{a}	ND	32.7 ± 2.82^{b}	28.6 ± 2.22^{c}
Chlorogenic acid	ND	ND	ND	ND	ND
Catechin	ND	ND	ND	ND	ND
Mangiferin	89.1 ± 1.12^{c}	5.72 ± 0.47^{d}	ND	314 ± 9.74^{a}	194 ± 10.3^{b}
Epicatechin	9.24 ± 0.72^{a}	9.01 ± 0.81^{a}	ND	ND	ND
Rutin	27 ± 0.88^{b}	28.7 ± 2.04^{b}	26.6 ± 2.17^{b}	46.5 ± 2.80^{a}	45.7 ± 3.23^{a}
Ferulic acid	ND	ND	ND	ND	ND
Quercetin	2.7 ± 0.15^{b}	2.48 ± 0.17^{b}	2.05 ± 0.05^{c}	3.32 ± 0.18^{a}	ND

Values on a dry weight basis (DW).
Mean ± SD (n = 3). Values with the different letters in the same row are significantly different ($p \leq 0.05$). ND: not detected.
G: Guayas, I: Imbabura, BP: By-products.

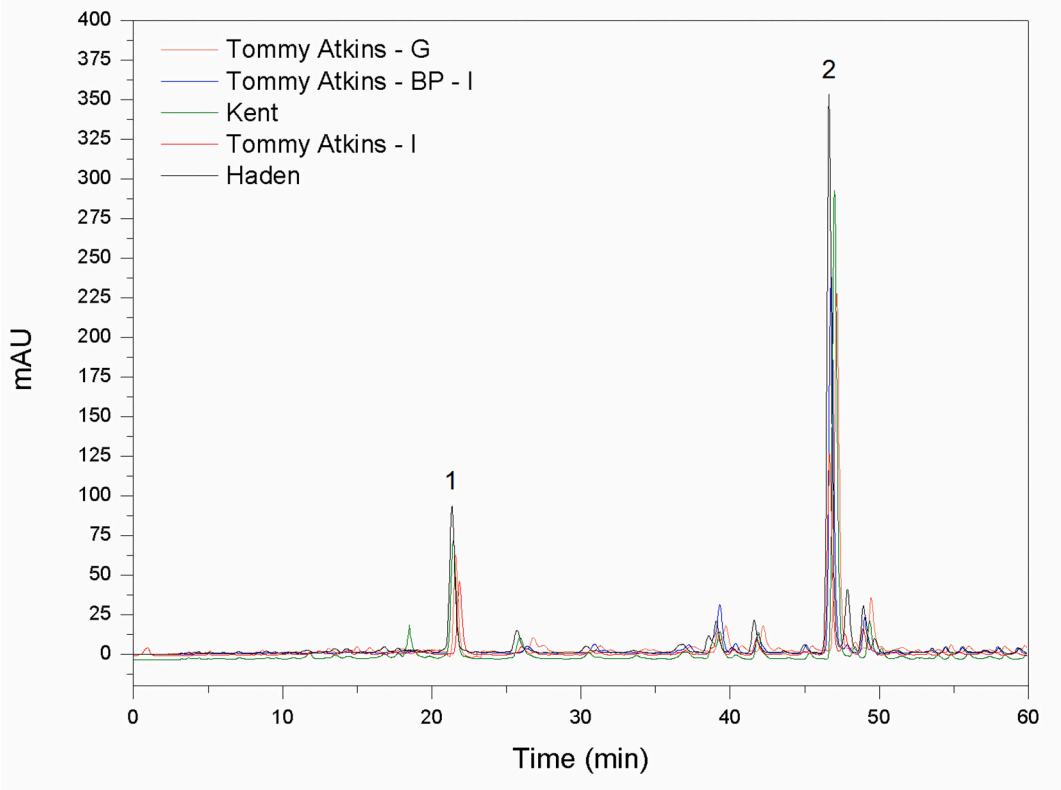

Fig. 1. HPLC chromatogram of extracts from freeze-dried mango peel samples. Peaks: 1, Lutein; 2, β-Carotene.

carotenoid content. On the other hand, other authors have observed lutein to be the significant carotenoid, followed by β-carotene and low amounts of other carotenoids in Badami [15] and Tommy Atkins [44] mango peels. These differences could be due to the variety, agricultural conditions, maturity, and carotenoid analysis using saponification, which might cause degradation and loss of some carotenoids [23]. Mango peel could thus be considered a source of carotenoids, whether β-carotene or lutein, which can be used as functional ingredients in the

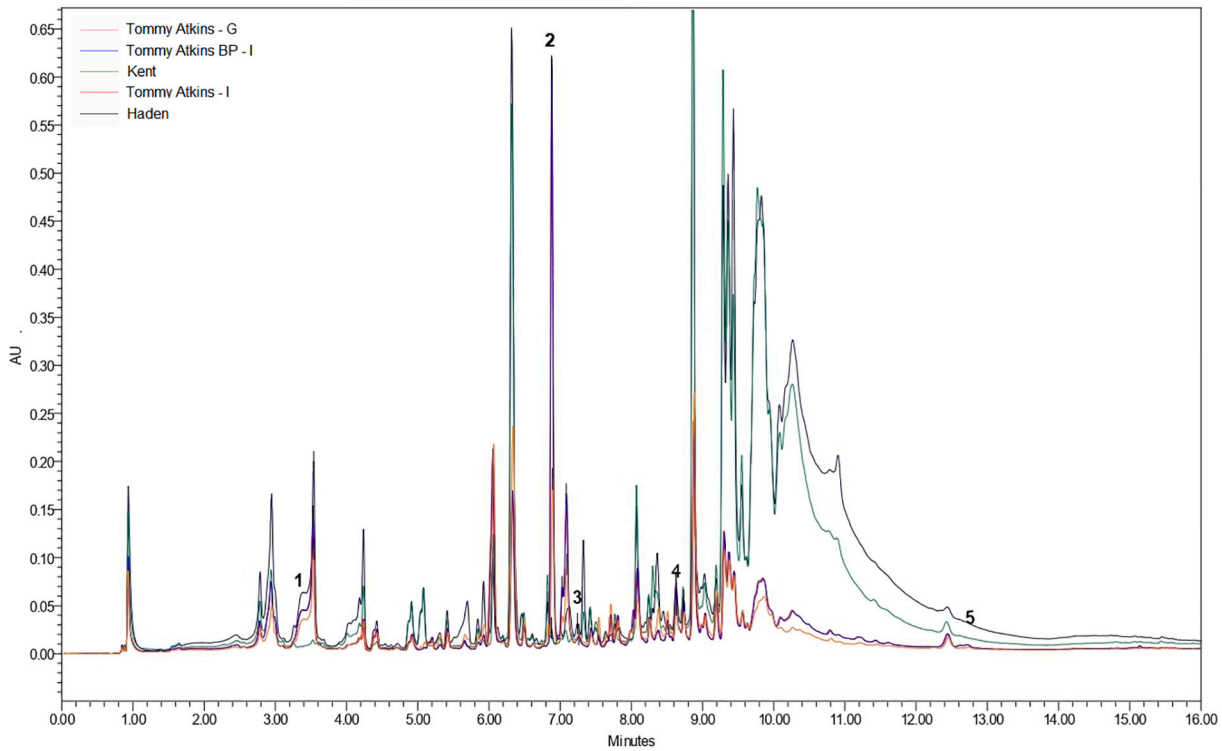

Fig. 2. UPLC-PDA chromatogram of extracts from freeze-dried mango peel samples. Peaks: 1, Gallic acid; 2, Mangiferin; 3, Unidentified; 4, rutin; 5, Quercetin.

food and pharmaceutical industry.

3.4. Identification and quantification of phenolic compounds

UPLC was used to determine the phenolic compound profiles of the mango peel samples. The chromatographic separation of phenolic compounds in methanol extract from all mango peel samples is shown in Fig. 2. Some phenolic compounds were identified by their retention times and UV/vis spectra using the corresponding standard compound in all samples. The content of each phenolic compound was calculated using the corresponding calibration curve, and the results are shown in Table 3.

Some phenolic compounds showed significant differences ($p < 0.05$) between varieties, while catechin, chlorogenic, and ferulic acid were not detected in all samples. Likewise, no chlorogenic acid was detected in twelve mango peel genotypes from the Philippines [38]. In contrast, catechin and ferulic acid amounts were reported by Alañón et al. [22] and Singh et al. [43] in Keitt mango peel from Spain and mango peel from India, respectively. Gallic acid was present in all samples (except Kent), with an amount varying from 28.6 to 43.2 mg/100 g DW. This compound has been reported as the common phenolic acid found in certain mango peel varieties from Brazil [11], China [45], India [43], and mango cv. Ataulfo by-product (peel and paste) from Mexico [46]. Mangiferin was the most predominant xanthone in Tommy Atkins - I mango peel (313.9 mg/100 g DW), while this compound was not detected in the Kent sample. Berardini et al. [14] determined that there was a large amount of mangiferin in Tommy Atkins (126.3 mg/100 g DW) and smaller amounts in Haden (1.1 mg/100 g DW) and Kent (1.4 mg/100 g DW) mango peels. Mangiferin was also quantified in eleven mango peel varieties from China, with amounts ranging from 4 to 749 mg/100 g DW [47], while the xanthone was not detectable in Keitt mango peel [22]. The flavonoids found in the samples include epicatechin (only in Tommy Atkins - G and Haden), rutin, and quercetin (Table 3). Among these compounds, quercetin was reported by Berardini et al. [4] and Singh et al. [43] to be an essential mango peel flavonoid in Tommy Atkins from Brazil (6.53 mg/100 g DW) and Indian mango (29.1 mg/100 g DW), respectively. Rutin was not detected in mango peel from Vietnam [42], but this flavonoid was identified in Keitt, Sensation, and Gomera mango peels from Spain [5]. These differences in the presence or absence of phenolic compounds can be firmly attributed to cultivar variability, maturity stage, and the extraction method [5,22]. Finally, several studies indicated mango peel's high bioactivity, mainly due to the presence of phenolic compounds such as mangiferin, which is a potent antioxidant [4] and a heat-stable phytochemical [46], with multiple functional effects [20] benefitting human health. Thus, our results also showed that mango peel could be a suitable raw material for the recovery of phenolic compounds, such as mangiferin, rutin, quercetin, or it could be used directly in the formulation of food, cosmetic and nutraceutical products.

4. Conclusions

The results showed that mango peel is an exciting by-product of mango processing, which can serve as a source of energy, is low in fat, and contains a fair amount of well-balanced dietary fiber. Freeze-dried peel samples of different mango varieties showed significant differences ($p < 0.05$) in the content of bioactive compounds (such as polyphenols, flavonoids, carotenoids) and antioxidant activity. The mango fruit variety type and growing location are likely responsible for the differences in the amounts of these bioactive compounds and their antioxidant activity. It was possible to identify and quantify the amounts of β-carotene, lutein, gallic acid, mangiferin, rutin, and quercetin in mango peel. The results presented in this research suggest that Ecuadorian mango peels of different varieties represent a valuable by-product with high antioxidant properties that could be useful in the food, cosmetology, and pharmaceutical industries.

Declaration of Competing Interest

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

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4. PAPER II

Phenolic compounds profile in mango peels (*Mangifera indica* L. cv. Tommy Atkins) by UHPLC-PDA obtained using different solvents and ultrasound-assisted extraction.

Verónica Marcillo-Parra, Diego Santiago Tupuna-Yerovi, Maritza Molina and Jenny Ruales

Submitted

Phenolic compound profile of mango peel (*Mangifera indica* L. cv. Tommy Atkins) by UHPLC-PDA obtained using different solvents and ultrasound-assisted extraction

Verónica Marcillo-Parra[1,2], Diego Santiago Tupuna-Yerovi[3,4], Maritza Molina[1] and Jenny Ruales[1]*

[1] Department of Food Science and Biotechnology (DECAB), Escuela Politécnica Nacional (EPN), Zip Code: 17012759, Quito, Ecuador. (jenny.ruales@epn.edu.ec)

[2] Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armadas ESPE, Zip Code: 171-5-231B, Sangolquí, Ecuador. (vemarcillo@espe.edu.ec)

[3] Institute of Food Science and Technology (ICTA), Universidade Federal do Rio Grande do Sul (UFRGS), Campus do Vale, Zip Code: 91501-970, Porto Alegre, RS, Brazil. (santiagotupuna@hotmail.com)

[4] Agroindustrial Engineering Department, Pontifícia Universidad Católica del Ecuador – Sede Manabí (PUCEM), Campus Chone, Zip Code: 130301, Portoviejo, Manabí, Ecuador.

* Corresponding author: Department of Food Science and Biotechnology, Faculty of Chemical and Agroindustrial Engineering, Escuela Politécnica Nacional, E-mail: jenny.ruales@epn.edu.ec, Tel/fax: +593 999228983.

Abstract

The performance of two techniques, conventional solvent extraction (CSE) and ultrasound-assisted extraction (UAE) using different solvents (50% methanol, 50% ethanol, 70% acetone in aqueous media), for the extraction of phenolics from mango peel, were evaluated. For each mango peel extract (MPE), a further purification step was performed on an OASIS HLB 6cc VAC packed column. Subsequently, the phenolic compound profile was quantified using Ultra-high Performance Liquid Chromatography-Photodiode Array (UHPLC-PDA) and for antioxidant activity using ABTS assay. The results indicated that using 50% methanol as an extraction solvent allowed to recover greater amounts of phenolics, such as mangiferin, gallic acid, quercetin, and rutin. Additionally, the extracts obtained by UAE showed a higher phenolic compound content, with mangiferin being the most predominant (222.34 mg/100 g DW) and higher antioxidant activity (approximately 311 µmol

Trolox/100 g DW) than extracts obtained by CSE. Overall, the results indicate the potential use of these mango by-products as an ingredient in the development of functional food.

Keywords: Mango (*Mangifera indica L*); extraction; mangiferin; antioxidant activity; UHPLC-PDA.

1. Introduction

Mango (*Mangifera* indica L.) is a tropical fruit that is highly appealing due to its nutritional content and sensory properties (Burton-Freeman et al., 2017). Besides fresh fruit, mango can also be consumed in the form of pulp, juices, nectars, squash, jams, pickles, jelly powders, and dried fruit (Ravani & Joshi, 2013). The most significant by-products of mango processing are peels and seeds (35-60% of the total fruit weight) (Dorta et al., 2012). These represent essential biowastes that must be treated using sustainability strategies such as waste valorization, e.g., extraction of bioactive compounds (Otles & Kartal, 2018).

Mango peel is an exciting source of phenolic compounds such as gallates and gallotannins (gallic acid derivates), flavonol glycosides (quercetin derivates and rhamnetin hexoside), xanthone glycosides (mangiferin derivates), benzophenone derivates (maclurin and iriflophenone glycosides), and ellagic acid derivates (Alañón et al., 2019; Barreto et al., 2008; Dorta et al., 2014). Thanks to exceptional levels of antioxidant activity, these compounds have garnered attention for their health benefits (Asif et al., 2016; Ulla et al., 2017). One xanthone of particular interest in mango peel is mangiferin (Luo et al., 2012; Ruales et al., 2018), which has shown promising chemotherapeutic and chemopreventative properties against oxidative-stress-related liver disorders (Gold-Smith et al., 2016; Ulla et al., 2017). In contrast to other bioactive compounds, such as most carotenoids, these phenolic compounds are not chemically synthesized and need to be extracted from plant material (Schieber et al., 2001), thereby obtaining a natural ingredient with potential use for the design of bioproducts (e.g., an antioxidant additive for an edible oil) (Sánchez-Camargo et al., 2019).

For most phenolic compounds found in mango peel, the recovery step typically involves direct extraction using a range of solvents (i.e., conventional solvent extraction - CSE) with different compositions or blends to obtain extracts with antioxidant activity (Dorta et al., 2012). However, there

are also non-conventional methods, such as microwave-assisted extraction (Dorta et al., 2013), supercritical fluids extraction (Souza et al., 2019), and ultrasound-assisted extraction (UAE) (Martínez-Ramos et al., 2020; Morales et al., 2020). By reducing solvent consumption and extraction time and producing better yield and extract quality, these methods lessen environmental impact (Azmir, 2013).

UAE is beneficial in obtaining high-quality antioxidant extracts from different plant matrices (Vilkhu et al., 2008). UAE produces acoustic cavitation in the solvent, which aids solvent penetration into the plant sample and increases the contact surface area between the solvent and the compound of interest, thereby improving mass transfer processes (i.e., the bioactive compound diffuses rapidly from the plant material to the solvent) (Azmir et al., 2013). Martínez-Ramos et al. (2020) determined an increase (630% approximately) in total phenolic compounds from mango peel when the ultrasound was applied, compared to the conventional solvent extraction method.

While studies exist on UAE from mango peel, factors such as solvent composition and ultrasound should be carefully selected to analyze extracts' qualitative and quantitative profiles. This work is thus aimed at examining the effect of the solvent type (50% methanol, 50% ethanol, and 70% acetone) and the extraction method (conventional or ultrasound-assisted extraction) on the phenolic profile of mango peel extracts and their antioxidant activity.

2. Materials and methods

2.1. Reagents and standards

HPLC-grade methanol, ethanol, acetone, acetonitrile, and formic acid were obtained from Merck (Darmstadt, Germany). Polyphenol standards ((+)-catechin hydrate, ferulic acid, gallic acid, (-)-epicatechin and mangiferin ≥ 98%; chlorogenic acid and quercetin ≥ 95% and rutin ≥ 94%), 2,2'-azonobis3-etheylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and potassium peroxodisulfate were purchased from the Sigma-Aldrich Company, Ltd. (St Louis, USA). Sodium carbonate monohydrate was obtained from JT Baker (Dagenham, England). The other chemicals and reagents were all analytical grade.

2.2. Raw material

Mangos (*Mangifera indica* L. cv. Tommy Atkins) were obtained from the Carbell farm in Guayas (1°55'05''S, 80°03'29''W, and 12 m above sea level), Ecuador. Ripe fruits without defects and physiological disorders were selected, sanitized, peeled, and separated into pulp, peel, and seed kernel by hand. The sliced fresh mango peels were frozen and freeze-dried using a Leybold-Heraeus Lyovac GT2 (Cologne, FRG) at 50 mPa and -40 °C. The dried sample was ground to powder (≤ 0.4 mm) using a W.S. TYLER Ro-Tap® RX-29 (USA) and stored in airtight aluminized bags at -20 °C until analysis. This mango peel powder sample was used for all analyses.

2.3. Preparation of extracts

2.3.1. Conventional solvent extraction (CSE)

CSE of phenolic compounds from mango peel powder was carried out as described by Dorta et al. (2012) with slight modifications. 1 g of mango peel sample was suspended in different solvents: 50% methanol, 50% ethanol, and 70% acetone in aqueous media (solid:solvent ratio 1:25, *w/v*). The mixtures were homogenized using an Ultra Turrax (IKA T25 Digital, Staufen, Germany) at 3000 rpm for 1 min. The extractions were carried out in a shaken water bath at 25 °C for 60 min in hermetically-sealed tubes. Extracts were centrifuged (Thermo Scientific IEC CL31R Multispeed Centrifuge, USA) at 3000 x *g* for 20 min at 4 °C. The supernatants were filtered using Whatman filter paper No. 1 and were stored at -20 °C until the analysis was carried out.

2.3.2. Ultrasound-assisted extraction (UAE)

UAE was conducted in an ultrasonic cleaner bath (Branson 3210, Danbury, USA) at 25 °C for 30 min with a power of 120 W and a frequency of 40 kHz (Souza et al., 2019). The extraction process (solid:solvent ratio and solvent types) was the same for CSE; the extracts were centrifuged, filtered, and stored under the previously described conditions (section *2.3.1*).

2.3.3. Sample purification

The purification of mango peel extract (MPE) was performed according to Dorta et al. (2014) with some modifications and using Oasis HLB Waters extraction cartridges (Milford, USA) (200 mg, 6 mL). The cartridges were equilibrated with 3 mL of methanol followed by 3 mL of deionized water.

The extracts (2 mL) were loaded on Oasis HLB cartridges. After loading the sample, the cartridges were washed with 4 mL of deionized water to remove sugars. Phenolic compounds were recovered from the cartridges by eluting with 4 mL of methanol:formic acid (99:1, v/v). The solvent was evaporated to dryness under a nitrogen stream at 35 °C. The dried residues were dissolved in formic acid 0.1% and filtrated through a 0.22 μm PVDF Millipore syringe filter (Milford, USA) directly into sample vials to injection in UPLC-PDA for further analysis.

2.4. Phenolic compounds profile by UHPLC-PDA analysis

The phenolic compounds of MPE were analyzed using UHPLC-PDA. Sample vials were injected automatically into a Waters Acquity UPLC H-Class System chromatograph (Milford, USA), equipped with a quaternary solvent manager, sample manager FTN, column manager, and PDA detector. The Empower 3 software (Milford, USA) was used for data processing. The Acquity UPLC ® HSS T3 column (Milford, USA) (1.8 μm, 2.1 x 100 mm) was used at 30 ˚C. Separation was carried out in 35 min under the same mobile phases, gradient, flow rate, and injection volume described by Marcillo-Parra et al. (2021). The phenolic compounds were identified and quantified using calibration curves ($R^2 \geq 0.999$) in the 5-200 μg/mL range for (+)-catechin, chlorogenic acid, ferulic acid, gallic acid, (–)-epicatechin, mangiferin, rutin, and quercetin. The phenolic concentration was expressed in mg/100 g on a dry weight (DW) basis. All analyses were performed in triplicate.

2.5. Antioxidant activity assay

The ABTS radical cation decolorization assay was performed, following the procedure described by Re et al. Campo (1999). A calibration curve for five sequential points (2.5 mM Trolox standard stock solution) was prepared for the analysis at 734 nm. The antioxidant activity of purified MPE was expressed as μmol Trolox/g DW.

2.6. Statistical Analysis

The experimental design was completely randomized, with three replicates. The results were expressed as mean ± standard deviation. STATGRAPHICS Centurion XVI.I (Virginia, USA) software

was used for statistical analyses. Unidirectional variance analysis (ANOVA) was carried out using the Fisher LSD test ($p < 0.05$) to test significant differences between samples.

3. Results and Discussion

3.1. Effect of solvent type on MPE phenolic compound content and antioxidant activity

The effect of conventional and ultrasound-assisted extraction using 50% methanol, 50% ethanol, and 70% acetone on MPE phenolic compound content and antioxidant activity were analyzed. Notably, one-step purification was performed using the Oasis HLB extraction cartridge for all extract samples. This removed sugars, resulting in better chromatographic separation by UHPLC-PDA (Figure 1). Some phenolic compounds, such as gallic acid, (–)- epicatechin, mangiferin, rutin, and quercetin, were identified (Figure 1) and quantified (Table 1), but others, such as catechin, chlorogenic acid, and ferulic acid, were not detected in all extract samples (Table 1).

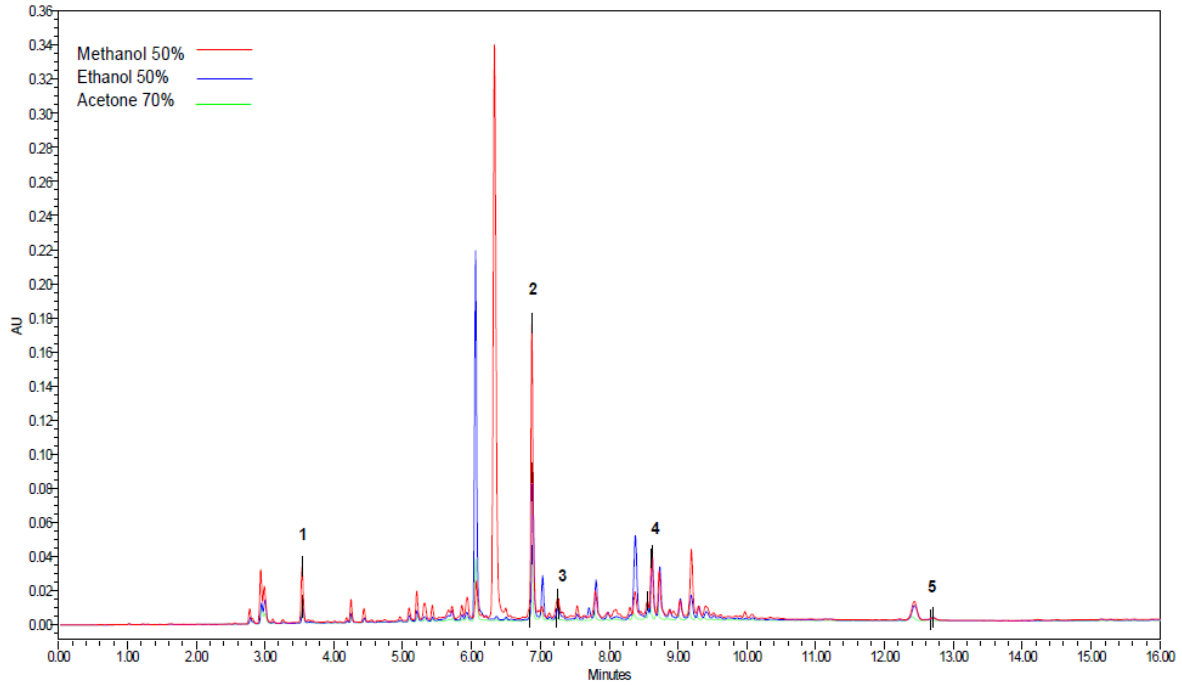

Fig 1. UHPLC-PDA chromatograms of mango peel extracts (Tommy Atkins) obtained by extraction with different solvents and were recorded at 280 nm. Peaks: 1, galic acid; 2, mangiferin; 3, epicatechin; 4, rutin; 5, quercetin.

3.1.1. Conventional solvent extraction (CSE)

The results presented in Table 1 indicate significant differences ($p < 0.05$) in the content of phenolic compounds in MPE depending on the solvent type. In general, extraction with 50% methanol showed the highest phenolic quantitative composition, and the predominant phenolic was mangiferin (74.76 mg/100 g DW). Other important phenolics identified and quantified were gallic acid (9.10 mg/100 g DW), rutin (28.77 mg/100 g DW), and quercetin (2.29 mg/100 g DW). Meanwhile, the phenolic content obtained with ethanol and acetone were not as high as the obtained with methanol (Table 1). González & González (2010) indicated that the polarity of the solvent and the different características of the phenolic compounds affect extraction efficiency. Solvents thus have an affinity with the bioactive compounds from the solid sample, making the solvent system selective during extraction. In a study carried out by Dorta et al. (2012), significant extraction of phenolics (flavonoids, tannins, and proanthocyanidins) was obtained with pure methanol followed by blends of ethanol:water or acetone:water. Martínez-Ramos et al. (2020) reported that the most remarkable total phenolic content was obtained by conventional extraction using an ethanol:acetone (60:40) blend. Other studies of MPE using 80% acetone showed the presence of large quantities of phenolic acids, flavonols, and xanthone glycosides (Ajila et al., 2010; Ribeiro et al., 2008). Overall, no single solvent allows optimum recovery for all phenols or even a limited range of phenols (González & González, 2010). In our study, the bioactive compound of interest in Tommy Atkins MPE was mangiferin, a molecule with potential pharmacologic properties, which showed better extraction with ethanol and methanol (2.6 to 4.6 times more) than with acetone. Barreto et al. (2008) also found notable mangiferin content (between 337 to 2153 mg/100g DW) in methanol extracts of mango peel (16 cultivars of mango were studied). In contrast, mangiferin was not detected in acetone extracts of mango peel cv. Tommy Atkins (Ribeiro et al., 2008).

Table 1. Phenolic compounds by UHPLC-PDA in mango peel extracts obtained by different solvents and extraction methods.

Compounds (mg/100 g DW)	Conventional solvent extraction (CSE)			Ultrasound-assisted extraction (UAE)		
	Methanol 50%	Ethanol 50%	Acetone 70%	Methanol 50%	Ethanol 50%	Acetone 70%
Gallic acid	9.10 ± 0.38$^{a/B}$	4.09 ± 0.34$^{b/B}$	2.17 ± 0.11$^{c/B}$	15.24 ± 0.13$^{a/A}$	5.89 ± 0.09$^{b/A}$	2.85 ± 0.13$^{c/A}$
Chlorogenic acid	ND	ND	ND	ND	ND	ND
Catechin	ND	ND	ND	ND	ND	ND
Mangiferin	74.76 ± 4.42$^{a/B}$	42.73 ± 2.35$^{b/B}$	16.04 ± 0.01$^{c/B}$	222.34 ± 8.12$^{a/A}$	57.23 ± 3.21$^{b/A}$	18.26 ± 1.34$^{c/A}$
Epicatechin	ND	ND	ND	11.18 ± 0.75$^{a/A}$	4.44 ± 0.23$^{b/A}$	1.50 ± 0.04$^{c/A}$
Rutin	28.77 ± 2.78$^{b/A}$	31.99 ± 0.98$^{a/B}$	7.62 ± 0.68$^{c/B}$	30.05 ± 1.81$^{b/A}$	37.77 ± 0.85$^{a/A}$	10.82 ± 0.73$^{c/A}$
Ferulic acid	ND	ND	ND	ND	ND	ND
Quercetin	2.29 ± 0.14$^{a/B}$	2.46 ± 0.20$^{a/B}$	1.51 ± 0.05$^{b/B}$	2.69 ± 0.04$^{b/A}$	2.85 ± 0.06$^{a/A}$	1.68 ± 0.05$^{c/A}$

Mean ± SD (n = 3). Different lower- or upper-case letters denote significant differences ($p \leq 0.05$) between extraction solvents or methods used to obtain mango peel extract. ND: not detected.

The antioxidant activity of MPE obtained by CSE with different solvents was further evaluated with the ABTS assay, the results are shown in Table 2. The extracts obtained with 70% acetone and 50% methanol had significantly ($p < 0.05$) higher capacity to scavenge ABTS radicals (average value: 257.33 µmol Trolox /g DW) than the ethanol extracts. Dorta et al. (2012) reported similar results for MPE, high ABTS radical scavenging activity was observed in 50% acetone and 50% methanol extracts (599.30 µmol Trolox /g DW approximately). Barreto et al. 2008 mentioned that the antioxidant potential of methanolic extracts of mango by-products could be related to the high content of mangiferin, which, when assayed individually, had extraordinarily high activity in all antioxidant assays in comparison to the other substances. Furthermore, Ajila et al. (2007) reported that acetone MPE (rich in polyphenols, anthocyanins, and carotenoids) showed good antioxidant activity in different trials, which can be attributed to the synergistic interactions of the bioactive compounds present.

3.1.2. Ultrasound-assisted extraction (UAE)

When UAE was performed, the behavior observed for the phenolic compound profile (Table 1) and antioxidant activity (Table 2) was similar to CSE with different solvents. Altogether, the best

extraction was obtained when 50% methanol was used, where the contents of gallic acid, epicatechin, and mangiferin were 2.5 to 12 times higher than 50% ethanol and 70% acetone MPE. While rutin and quercetin (flavonols) were found in all extracts, the content was highest in ethanolic extracts (e.g., rutin was 1.25 to 3.5 times more than methanolic and ketonic extracts, respectively). Souza et al. (2019) mentioned that these flavonols were also enriched in ethanolic extracts, especially rutin, the primary compound found in all MPE. In addition, Martínez-Ramos et al. (2020) have described a direct relationship between the phenolics extracted using UAE and the solvent polarity. Thus, they determined a maximum value of phenolic compounds in mango peels during UAE with the ethanol-acetone blend and indicated that ethanol has a unique characteristic to extract glycosidic and non-glycosidic phenolic compounds. In contrast, acetone can usually only extract non-glycosidic phenolics.

Table 2. ABTS values of mango peel extract obtained by different solvents and extraction methods.

Solvent	ABTS (µmol Trolox/g DW)	
	Conventional solvent extraction	**Ultrasound assisted extraction**
Acetone 70%	$264.41 \pm 9.51^{a/B}$	$364.00 \pm 13.00^{a/A}$
Methanol 50%	$250.25 \pm 12.35^{a/B}$	$337.00 \pm 16.59^{a/A}$
Ethanol 50%	$189.78 \pm 13.36^{b/B}$	$231.53 \pm 16.30^{b/A}$

(Mean ± SD, n = 3). Different lower- or upper-case letters denote significant differences ($p \leq 0.05$) of ABTS values between extraction solvents or extraction methods used to obtain mango peel extract.

Regarding antioxidant activity in MPE obtained by UAE using different solvents, no significant difference ($p < 0.05$) was observed between 50% methanol and 70% acetone extracts (average value: 350.5 µmol Trolox /g DW). The lowest antioxidant activity value (231.53 µmol Trolox /g DW) was noted in 50% ethanol extract (Table 2). Nevertheless, these results suggested that the MPE obtained using organic solvents of high (methanol or ethanol) or intermediate (acetone) polarity in composition with water reached high antioxidant activity values evaluated with ABTS assay. Similar

results were confirmed when the analysis of antioxidant activity was quantified in MPE obtained by UAE using the solvents mentioned above, in either pure or mixed form (Martínez-Ramos et al., 2020; Morales et al., 2020; Ruales et al., 2018; Souza et al., 2019). In general, the characteristics of these aqueous solvents (methanol, ethanol, and acetone; between 50 – 95%) allow antioxidant compounds such as phenolics to be extracted from plant material more efficiently (González & González, 2010).

3.2. Effect of UAE on the phenolic compound content

Unidirectional analysis of variance (ANOVA) and the experimental results of the MPE phenolic compound profile and antioxidant activity, obtained through CSE and UAE, are shown in Table 1 and Table 2, respectively. The results indicated that regardless of solvent type, the extraction process with UAE had higher phenolic contents than with CSE (Table 1). Mangiferin content increased from 1.14 to 2.97 times when the UAE was applied. In addition, epicatechin was detected in all extracts obtained by UAE, while this flavanol was not present in CSE extracts. These results match the values reported by Ruiz-Montañez et al. (2014), these authors observed that UAE of mangiferin in mango peel cv. Ataulfo had an increase of 1.86 times on average compared to different conventional extraction methods. Safdar et al. (2017) reported that UAE was a more efficient method and allowed for the extraction of a comparatively higher phenolic compound content from mango peel than that obtained using traditional extraction by maceration. Both works indicated that emerging extraction techniques, such as UAE, improve extraction efficiency with shortened extraction time. This could be attributed to ultrasound aiding in the breakdown of cell walls and the reduction of particle size through acoustic cavitation (violent collapse and implosion of gas bubbles in the liquid medium), which in turn improves the mass transfer of the cell content (target compounds from vegetal tissues) to the solvent (González & González, 2010). According to many studies, UAE has been demonstrated to effectively increase the extraction yield of phenolic compounds in many plant materials (He et al., 2016; Ma et al., 2009; Martínez-Ramos et al., 2020). In contrast, other works found that UAE did not affect the total phenolic content of extracts (Aspé & Fernández, 2011;

Da Porto et al., 2013; Guandalini et al., 2019), but mentioned that using ultrasound resulted in lower solvent consumption and shorter extraction time.

On the other hand, the antioxidant activity values (Table 2) showed a similar behavior to that of phenolic content. For all solvents, the MPE obtained by UAE had significantly ($p < 0.05$) higher antioxidant activity than CSE (average of 31%). A previous study carried out by Marcillo-Parra et al. (2021) showed a high positive correlation between phenolic content and antioxidant activity in MPE; however, since the ABTS radical-scavenging test measures the antioxidant activity of hydrophilic (phenolics) and lipophilic (carotenoids) compounds, the carotenoids present in the extract were also responsible for the antioxidant activity levels. These results agree with Galvan D'Alessandro et al. (2012), which found that UAE extracts presented higher antioxidant activity than CSE extracts. As such, UAE was a rapid and straightforward method for extracting antioxidant compounds from black chokeberry. However, Souza et al. (2019) reported that similar antioxidant activity values were quantified in ethanolic MPE obtained using Soxhlet, maceration, and UAE from air-dried mango peel samples, which could be related to the correct combination of different factors (e.g., temperature, solvent, time, etc.) during bioactive compound extraction.

Overall, the impact of the solvent type and the extraction method on the phenolic compound content of extracts was crucial. Hence, polar solvents in an aqueous medium thus extracted a higher phenolic content, and more specifically, the use of 50% ethanol (organic extraction solvent) could serve as a suitable alternative in terms of food safety. In addition, extraction with UAE resulted in MPE with higher phenolic content, and better antioxidant activity than in MPE obtained using a conventional process. This could be directly used to formulate novel food, cosmetic, or nutraceutical products with functional properties.

4. Conclusions

The results of this work demonstrated that 50% methanol significantly increased the recovery of phenolic acids (gallic acid), xanthones (mangiferin), and flavonoids (rutin and quercetin) from mango peels during CSE and UAE. Furthermore, UAE extracts had significantly ($p < 0.05$) higher antioxidant

activity by ABTS assay than CSE extracts, confirming the suitability of UAE for the preparation of antioxidant-rich mango peel extracts. These results also showed that mango peels could be a suitable raw material for recovering antioxidant compounds, such as polyphenols, which could be used for fortification/enrichment in various bioproducts.

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5. PAPER III

Encapsulation of Carotenoids from Mango (*Mangifera indica* L.) Peel with Different Encapsulating Agents to Enhance its Encapsulation Efficiency and Stability.

Verónica Marcillo-Parra, Diego Santiago Tupuna-Yerovi, Edwin Vera, Alessandro de Oliveira Rios, Filip Van Bockstaele, Paul Van Der Meeren, John Van Camp, Koen Dewettinck and Jenny Ruales

To be submitted

Encapsulation of Carotenoids from Mango (*Mangifera Indica* L.) Peel with Different Encapsulating Agents to Enhance its Encapsulation Efficiency and Stability

Verónica MARCILLO-PARRA[a,b], Diego Santiago TUPUNA-YEROVI[c,e], Edwin VERA[a], Alessandro de Oliveira RIOS[c], Filip VAN BOCKSTAELE[d], Paul VAN DER MEEREN[d], John VAN CAMP[d], Koen DEWETTINCK[d] and Jenny RUALES[a]*

[a] Department of Food Science and Biotechnology (DECAB), Escuela Politécnica Nacional (EPN), Zip Code: 17012759, Quito, Ecuador.
(jenny.ruales@epn.edu.ec; edwin.vera@epn.edu.ec)

[b] Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armadas ESPE, Zip Code: 171-5-231B, Sangolquí, Ecuador.
(vemarcillo@espe.edu.ec)

[c] Institute of Food Science and Technology (ICTA), Universidade Federal do Rio Grande do Sul (UFRGS), Campus do Vale, Zip Code: 91501-970, Porto Alegre, RS, Brazil.
(santiagotupuna@hotmail.com; alessandro.rios@ufrgs.br)

[d] Ghent University, Faculty of Bioscience Engineering, Coupure Links 653, 9000 Ghent, Belgium.
(filip.vanbockstaele@ugent.be; paul.vandermeeren@ugent.be; john.vancamp@ugent.be)

[e] Agroindustrial Engineering Department, Pontifícia Universidad Católica del Ecuador – Sede Manabí (PUCEM), Campus Chone, Zip Code: 130301, Portoviejo, Manabí, Ecuador.
(dtupuna@pucem.edu.ec)

* Corresponding author: Department of Food Science and Biotechnology, Faculty of Chemical and Agroindustrial Engineering, Escuela Politécnica Nacional, E-mail: jenny.ruales@epn.edu.ec, Tel/fax: +593 999228983.

Abstract

Mango peel is rich in antioxidant carotenoids. Though it is commonly discarded as a by-product of industrial processing, these wastes can be turned into ingredients with potential applications in functional products. Encapsulation techniques are an alternative used for the improvement of carotenoid stability and physical properties. Encapsulating agents and operating conditions influenced the encapsulation efficiency. Different proportions of encapsulating agents (gum arabic and maltodextrin) at different inlet air drying temperatures (IT) were used to assess the effect on encapsulation efficiency (EE). The T140MD50GA50 formulation showed high encapsulation efficiency (67.91%) and was chosen to evaluate the effect of adding inulin and fructooligosaccharides (FOS). EE (~65%) and the water solubility index (>85%) did not show a statistical difference between samples with prebiotics. The microencapsulated carotenoid extract (MCE) showed a microparticle diameter (<4.0 um), bimodal particle size distribution, and low moisture content (<3.7%). The stability assessment was carried out at 5, 25, and 45 °C for 45 days. MCE showed a first-order thermal kinetic behavior and required more activation energy (*Ea*) (3.41 kcal/mol) than free carotenoid extract (1.33 kcal/mol) for degradation. The MCE showed considerable antioxidant activity (7286.67 ± 256.97 µmol TE/100g), which remained in high percentages after storage at different temperatures during thermal stability assessment. Encapsulation improved the thermal stability of carotenoids; thus, the MCE can be considered a natural additive with functional properties for use in food, beverages, and nutritional supplements.

Keywords: Mango by-products; microencapsulation, carotenoids stability, bioactive compounds

Chemical compounds studied in this article.

Gum Arabic (PubChem CID: 405237855), Maltodextrin (PubChem CID:62698), Fructooligosaccharides (FOS) PubChem CID: 56312194, Inulin (PubChem CID:24763), β-carotene (PubChem CID:5280489, Sodium Bicarbonate (PubChem CID:516892), N-heptane (PubChem CID:386618061), Ethanol (PubChem CID:702), Methanol (PubChem CID:887), Hexane (PubChem CID:8058), Acetone (PubChem CID:180), ABTS (PubChem CID:5360881), Potassium peroxodisulfate (PubChem CID: 24412), Trolox (PubChem CID: 40634).

1 Introduction

Mango (*Mangifera indica* L.) is a tropical fruit that is widely consumed throughout the world thanks to its sweet, exotic taste and succulence. Thanks to its bioactive compound content, it is considered healthy, including phenols, carotenoids, and vitamin C [1]. Mango by-products (peel and seed) represent 35% to 60% of the total fruit weight. Depending on the variety, the peel may account for 15 to 18% of the whole fruit weight. It represents a rich source of bioactive compounds, including carotenes with antioxidant activity [2–4]. The bioactive components present in mangos could help reduce blood cholesterol levels, regulate glucose levels, and have anti-carcinogenic effects [5].

Carotenoids are natural pigments with structural diversity and multiple functions. Humans cannot synthesize them, so they must be provided in the diet. A lack of carotenoids promotes a vitamin A deficiency responsible for blindness in children, known as a serious problem in developing countries [6]. Carotenoids also offer health benefits related to anti-inflammatory effects, chronic diseases protection, and immunity improvement. Also, they have potential use in treatment for some types of cancer due to their antioxidant activity [7].

Mango is an essential source of β-carotene; however, research has often been focused on the pulp. The total carotenoids in the mango peel increase significantly with the ripening of the fruit [8]. Studies on the carotenoid profile of mango peels from different varieties showed that the main identified compounds were β-carotene, violaxanthin, and lutein [4,9–11]. Nevertheless, these compounds are unstable under food processing and storage conditions such as pH, temperature, light, and oxygen [12].

Fortunately, encapsulation techniques such as spray-drying have demonstrated the encapsulation effectiveness of carotenoids by forming a coating that protects them against undesirable degradation conditions and improving their physicochemical properties [13]. Microencapsulation is a technological process for conserving different materials inside capsules to permit a controlled release under specific requirements [14]. Using biopolymers with different functional properties (emulsifying and film-forming ability and rheological behavior) as encapsulating agents considerably affects microcapsules' encapsulation efficiency (EE) and storage stability. Polysaccharides, such as gum

arabic (GA), maltodextrin (MD), and some proteins, are frequently used as encapsulating materials [15].

Adding fructooligosaccharides (FOS) or inulin to the encapsulating matrix can contribute to its functional properties and positively affect human health. The main difference between this prebiotic is structural; FOS shows a linear molecular structure with shorter chain molecules, whereas inulin has a slightly more extended design with more cross-links [16]. Recently, they have attracted more attention from various industries, mainly because of their prebiotic nature. They are degraded by gut bacteria (such as bifidobacteria), and they are beneficial for human health [17].

Regarding the encapsulation, thanks to its low hydrolysis capacity, inulin results in microcapsules that are more resistant to variations in pH [18]. This protects bioactive compounds that are susceptible to degradation in the human digestive tract until they can be released and absorbed in the intestine [19]. Several studies have been conducted on the encapsulation of different core materials, such as ginger essential oil [20], corn oil [21], oregano essential oil [19], astaxanthin oleoresin [22], and phenolics and betalains [23], among others. They have used inulin as secondary wall material for stability improvement of these compounds and assessing the effect on EE, and other biological benefits such as dietary fiber availability, calcium bioavailability improvements, and immunomodulatory properties.

The use of soybean lecithin as an emulsifying agent not only provides stability to the emulsion; in pharmaceuticals, it is widely used due to its ability to form thin films on the bioactive compound, which, due to the liposomes present in the lecithin, protects the stomach digestion. This improves compound bioaccessibility and bioavailability, whose absorption in the intestinal tract increases [24].

This study aimed to perform the encapsulation of carotenoids from mango peel by spray-drying using different blends of wall materials (MD:GA) and different inlet air drying temperatures (IT) with the addition of FOS and inulin. The objective was to evaluate the effect on EE, characterization of encapsulated carotenoids, and the thermal stability assessment during storage to estimate a shelf-life for this future application as an additive in food matrices.

2 Materials and Methods

2.1 Materials

Mango (*Mangifera indica* L. Var. Tommy Atkins) fruits were provided by Asoprovalle, located in Imbabura (0°26'39''N, 77°58'28''W, and 1654 m above sea level), Ecuador. They were washed, and the peel was removed and stored at -20 ºC. The edible polysaccharides —Gum Arabic (GA) (Alpha Trading Gmbh, Hamburg, Germany), and Maltodextrin (MD) (Norbright Industry Co., Tianjin, China)—, and the prebiotics —FOS (fructooligosaccharides) (Sigma-Aldrich, St. Louis, MO, USA) and Inulin (Sigma, St. Louis, MO, USA)—, were used as encapsulating agents. All reactants were of analytical grade and suitable for High-Pressure Liquid Chromatography.

2.2 Extraction of Carotenoids from Mango Peel

The mango peel was dried by freeze-drying and pulverized. The carotenoids were then extracted from the powder obtained, following the procedure described by Calderón & Vera (2018), using n-heptane and ultrasound-assisted extraction. The extract in n-heptane was dried in a rotary evaporator (Büchi EL 131, Flawil, Switzerland). Finally, ethanol was used to recover the carotenoids and concentrated until a total carotenoid content (TCC) of 250 µg eq. β-carotene/mL extract was achieved. This was then stored at -20°C. The (TCC) was quantified by a spectrophotometer (Shimadzu UV-160A, Kyoto, Japan) using a wavelength of 450 nm, according to Rodriguez-Amaya & Kimura (2004).

2.3 Encapsulation of Carotenoids

2.3.1 Experimental Design

The experimental process was performed in two stages. In the first stage, a 2^2 factorial rotatable central composite design was used to choose the best conditions to optimize the encapsulation process. The variables assessed were inlet air drying temperature (IT) and polysaccharide ratio (MD:GA). For MD:GA, the levels were 75:25 and 25:75, and for IT were 180 ºC and 150 ºC, with a triplicate of treatment at the center point. As response variables, encapsulation yield (EY) and encapsulation efficiency (EE) were used. In the second stage, the treatment that showed the most significant EE and EY was chosen to assess the use of inulin instead of FOS on EE and EY. An

experiment using a formulation without prebiotics was carried out as the control sample. The experimental design is shown in Tables S1 and S2 as supplementary material.

2.3.2 Preparation of Emulsions

The procedure was carried out according to previous encapsulation studies of carotenoids using polysaccharides and prebiotics as encapsulating agents [20,21,26] to prepare 100 mL of emulsion achieving 30% (w/w) of total soluble solids (TSS). For the first stage, to prepare each formulation according to each experimental design process, 27 g of the polysaccharides (MD:GA) and 1.5 g of FOS were solubilized in 30 mL of distilled water under agitation for 12 h. Separately, 1.5 g of soy lecithin was used as an emulsifier. This was homogenized with 20 mL of distilled water for 3 min using an Ultra Turrax (IKA T25, Taiwan, China) at 13000 rpm. Then, 20 mL of the carotenoid extract (250 µg eq. β-carotene/mL) was slowly added to hydrated soy lecithin at a core (carotenoids)/encapsulating agent of 1:6000 (w/w). To obtain the pre-emulsion was performed the homogenization using an ultra-túrrax (IKA T50, Taiwan, China) for 3 min at 5000 rpm. Following this, it was placed in an ultrasonic bath with cold water at 20 °C for 20 min, and finally, it was homogenized using an ultra-túrrax at 10000 rpm and 20 °C for 6 min. Magnetic stirring at 200 rpm was applied to the final emulsion during the drying process.

2.3.3 Encapsulation Process

The encapsulation was conducted using a mini spray dryer (Büchi B-290, Flawil, Switzerland) with 0.7 mm of spray nozzle diameter. The spray nozzle diameter was 0.7 mm. The operating conditions used were: 0.27 L h^{-1} of emulsion feed flow and 600 N L h^{-1} of airflow rate. According to each IT used, the outlet air drying temperature ranged from 90 ºC to 120 ºC.

2.4 Characterization of Microcapsules

2.4.1 Encapsulation Yield (EY)

A gravimetric method determined the encapsulation yield (EY). According to dry matter content, it was considered as the ratio of the mass of microencapsulated carotenoid extract (MCE), weighted at the end of the encapsulation process, to the mass of solids (wall materials) before the encapsulation process [24], following the Eq. (1):

$$EY(\%) = (\text{Mass of MCE (g)}/\text{Mass of initial solids (g)}) \times 100 \quad (1)$$

2.4.2 Encapsulation Efficiency (EE)

Following the procedure of Rodríguez-Huezo et al. (2004), the extraction and quantification were performed using spectrophotometry, with minimal modifications. The number of total carotenoids (TCA) and surface carotenoids (SCA) of MCE were used to calculate the EE using the Eq. (2):

$$EE\ (\%) = (TCA\text{-}SCA/TCA) \times 100 \quad (2)$$

2.4.3 Moisture Content

The moisture content of the MCE was analyzed using a thermal scale (RADWAG PMR 50/1, Toruńska, Poland) immediately following each experiment. The sample's water was evaporated throughout the heating with halogen light for 3 min, and the moisture content was reported as a percentage.

2.4.4 Water Solubility Index (WSI)

The WSI of the MCE was measured following the procedure reported by Stoll et al. (2016) in an aqueous medium. To each 2.5 g MCE, 30 mL water was added and mixing using a digital Ultra Turrax T25 for 2 min. The mixture was placed in a water bath at 25 ˚C for 30 min, centrifuged (3500 xg, 15 min, 4 ˚C), and the supernatant was collected. It was dried in an oven at 105 ˚C overnight. The total solids content in the supernatant was expressed in percentage.

2.4.5 Colorimetric Assessment

Color attributes were assessed by a colorimeter (Konica Minolta CR-200, Tokyo, Japan), according to the CIELab parameters (*L*, a*, b**). To indicate the tonality and vividness, the *Hue angle* and *Chroma* values, respectively. The Eq. (3) and (4) were used [13]:

$$Hue = \tan^{-1}(b^*/a^*) \quad (3)$$

$$Chroma = [(a^*)^2 + (b^*)^2]^{1/2} \quad (4)$$

In addition, total color difference between samples during thermal stability assessment was calculated by the Eq. (5) as follows [28]:

$$\Delta E = [(L_o^* - L^*) + (a_o^* - a^*)^2 + (b_o^* - b^*)^2]^{1/2.} \quad (5)$$

Where L_o*, a_o* and b_o* are the value of the MCE samples, and L*, a* and b* are the measured values of each sample after the storage period (45 days).

2.4.6 Cryo-scanning Electron Microscopy (Cryo-SEM)

A small MCE sample was placed on a sticky carbon surface mounted on an aluminum stub. Then it was vitrified in nitrogen slush and transferred under vacuum conditions to an attached cryo-preparation chamber (Quorum Technologies PP3010T Cryo-SEM, Laughton, UK) conditioned at -140 °C. Subsequently, the sample was sublimated for 15 min at -70 °C to remove frost artifacts, sputter-coated with platinum using argon gas, transferred into the cold stage, maintained at -140 °C, and imaged using a scanning electron microscope (Jeol JSM-7100F, Tokyo, Japan) at 3 kV to different magnifications (1000 – 10000x).

2.4.7 Particle Size Distribution (PSD)

PSD determination of MCE was performed by static light scattering using a mastersizer (Malvern Instruments Mastersizer 3000, Worcestershire, UK) attached with a Hydro MV wet dispersion unit. The MCE samples were first dispersed in distilled water (0.15 g/10 mL) to moisten the particles and avoid agglomeration in the dispersion unit during the analysis. The $D_{[4,3]}$ value expressed the weighted average volume diameter, assuming spherical particles with the same volume as the actual particles. To evaluate the PSD, the *Span* value was calculated. All measures were performed in triplicate.

2.5 Thermal Stability of the MCE

Based on the previous results, the carotenoid extract was encapsulated using a formulation with MD:GA at a 50:50 ratio, inulin, and 140 ºC as IT for the drying process. The obtained MCE was used to perform the experiments under different storage temperatures. 3 g MCE was placed in amber glass bottles with hermetic screw caps, and the bottles were then stored at 5, 25, and 45 °C. A sample was periodically taken at 0, 5, 10, 15, 30, and 45 days of exposure to heat in storage, and HPLC measured the β-carotene retention. The non-encapsulated carotenoid extract (Free-CE) was used as a control sample. According to the β-carotene content, the kinetic parameters were

calculated using the Origin 8.0 software (Origin Lab Co., MA, USA). Also, the half-life time ($t_{1/2}$) was evaluated using the Eq. (6) [22]:

$$t_{1/2} = \ln 2/kt \quad (6)$$

Where: $t_{1/2}$ = Half-time value; kt = Kinetic constant

2.5.1 High-performance liquid chromatography (HPLC)

First, the extraction of total carotenoids from each sample was developed following the procedure described in section 2.4.2. The identification and quantification of carotenoids were made following the method reported by Marcillo-Parra et al. (2021) using an HPLC (Hewlett-Packard HPLC HP 1050, Palo Alto, USA) equipped with a carotenoid column (YMC C30, Wilmington, USA) (250 x 4.6 mm i.d.; 5 µm particle size), at 1 mL/min flow rate and detection at 450 nm. 20 µL were used as injection volume, and the column temperature was 30 °C. The chromatographic data were processed using the ChemStation software (Agilent, Santa Clara, USA). All samples were prefiltered before injection using a modified 0.45 µm syringe membrane (Millipore PTFE, São Paulo, Brazil). The β-carotene was identified as the predominant carotenoid in the extract according to the retention times of β-carotene standards. The β-carotene was quantified using a calibration curve ($R^2 \geq 0.999$), and the retention was expressed in percentage. All analyses were performed in triplicate.

2.5.2 Antioxidant Activity

Antioxidant activity was measured at the outset and following 45 days of storage using the ABTS radical cation decolorization assay, according to Re et al. (1999). The extract's antioxidant activity was expressed as µmol Trolox equivalent (TE)/100 g encapsulated.

2.6 Statistical Analysis

Results were expressed as mean value ± standard deviation. One-way variance analyses (ANOVA) and Tukey's test at a significance level of 5% were performed to evaluate the data correlation and identify significant statistical differences among the samples using the Statistica 12.0 software (StatSoft, Inc., Tulsa, USA).

3 Results and Discussion

3.1 Encapsulation Efficiency (EE) and Encapsulation Yield (EY)

One of the main goals of the encapsulation process is to get a high amount of the studied compound inside the capsule avoiding the presence of this compound on the surface. The results showed that EE was more influenced by the inlet drying temperature (IT) than the encapsulating agent ratio MD:GA. EE also depends on the combinations of these variables. The EE values showed statistically significant differences ($p<0.05$) between the experiments carried out using different formulations and operating conditions (Fig. 1.).

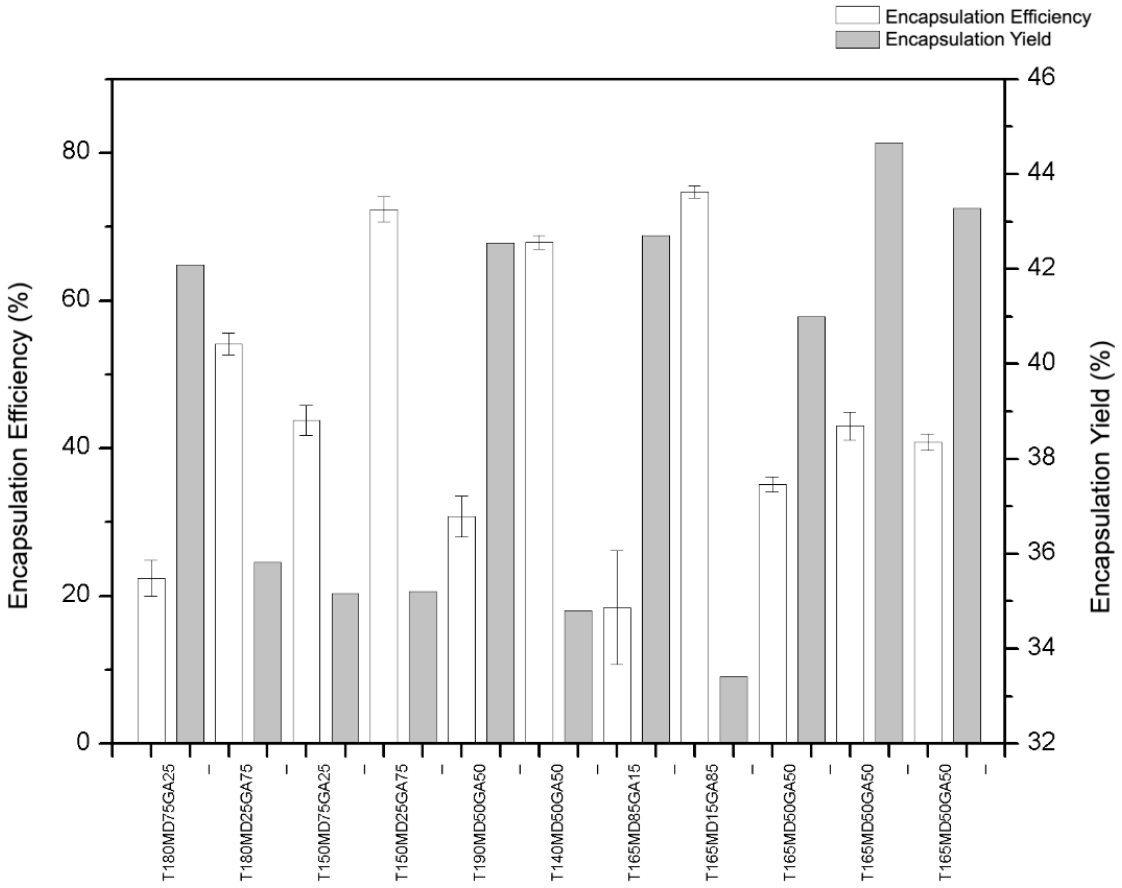

Fig 1. Encapsulation efficiency and encapsulation yield of the microencapsulated carotenoid extract using different formulations (MD:GA) and inlet air drying temperatures.

The increase in IT resulted in EE reduction, a behavior that can be directly linked to the bioactive compound content in the MCE. The degradation of carotenoids and the polymerization of phenolic compounds at higher temperatures may explain this phenomenon. The balance between the rate of water evaporation and film-formation may be due to high IT; the microcapsule wall, therefore, breaks

down, leading to a low EE. The increasing temperatures led to higher PY, which can be attributed to the greater efficiency of the higher IT heat and mass transfer processes. Another reason may be insufficient water evaporation in microcapsules at lower temperatures [13,30,31].

In the T180MD25GA75 and T150MD25GA75, the same MD:GA ratio was used (higher proportion of GA than MD), and EE increased from 54.12% to 72.41%, respectively; i.e., the EE was higher when low IT was used (Fig. 1.). It was also observed that the addition of GA influenced the EE: when GA was used in higher (85%), or lower (15%) proportions using the same IT (165 ºC), the EE showed the higher (74.71%) and lower (18.67%) values, respectively (Fig. 1.). The experiments performed with formulations that have an equal proportion of encapsulating agents (MD:GA = 50:50) reached the highest EE (67.91%) when the lowest IT (140 ºC) was used (Fig. 1.). The increase of EE indicates that inside the capsules, a high quantity of bioactive compounds was retained. The intrinsic properties of encapsulating agents (emulsification, viscosity, particle size) and operating conditions such as drying temperatures, feed flow, airflow, among others, are factors that significantly affect EE during the encapsulation process [13,23,32–34].

The high EE was reached when GA was used higher than other encapsulating agents. This effect also was reported in other studies about the encapsulation of bioactive compounds [13,27,35–38]. These results confirm the significant impact of GA on EE obtained in this study when the encapsulation process was carried out using spray drying.

The chemical structure of encapsulating agents is responsible for their specific properties. GA has glycoproteins linked to this chain structure by covalent bonds. In this way, the interaction of GA with both hydrophilic and hydrophobic sections of the molecule is possible and promotes its emulsifying properties. It has high compatibility with many polymers and a tremendous film-forming ability, which helps retain compounds inside the capsule [33,39–41]. MD has a hydrolyzed starch structure that has a good oxygen barrier. However, it has low emulsification and film-forming ability, which are intrinsic properties of this polymer [42].

Microcapsule bioaccessibility is one of the essential objectives for application in food as a functional ingredient. During the digestion process, the encapsulating agents of the microcapsules are dissolved, and the core compounds are delivered slowly. The encapsulating agent viscosity

influences this process; therefore, the expected bioaccessibility is not reached. The GA is more viscous than MD: i.e., it would be better to develop microcapsules to avoid using high amounts of GA. The highest EE was reached with the experiment T165MD15GA85 (Fig. 1). According to the results of the statistical analysis, when the difference between MD and GA was decreased and lower IT was used, the EE values did not show significant differences ($p>0.05$) (between T165MD15GA85 and T150MD25GA75, and between T150MD25GA75 and T140MD50GA50) (Fig.1).

Encapsulation yield is defined as the recovering efficiency of the solid particles (microcapsules) from the spray dryer over the TSS content presented in each formulation at the beginning of the drying process. Based on the EY is possible to establish a cost-benefit relation and evaluate the viability of obtaining encapsulated carotenoids using the spray drying technology with an optimal encapsulating agent ratio. The EY showed a variation ranging from 33.41% to 44.66%, considerably affected by the MD:GA ratio and IT (Fig.1). Rajabi et al. (2015) and Bakowska-Barczak & Kolodziejczyk (2011) reported that the encapsulation of bioactive compounds obtained a high encapsulation yield in the complete absence, or the presence in minimal quantities, of GA. The experiments that achieved the highest EY were T165MD50GA50 (central point); however, the EE was less than 50%.

In contrast, the experiments with a lower MD:GA ratio (especially when GA is lower than or equal to MD) showed high EY values when temperatures from 165 ºC were used. For 140 and 150 °C, the EY was less than 40%. This loss was mainly caused by many semi-moist drops addition into the chamber, commitment of dry microparticles inside of cyclone filter, and is almost impossible to recover them from the chamber and very difficult in the case of cyclone filter. In other studies, the authors reported that when a low IT (140 - 150˚C) was used, moist microparticles were obtained with a high tendency of addition in the inner walls of the chamber and cyclone filter [45,46]. The core:encapsulating agent is another factor that significantly influences EY: when the relationship between them is lower, higher EY was achieved [23,34]. In this study, the core:encapsulating agent ratio used was 1:6000.

Based on these results, the EE was considered the main factor in choosing the conditions to perform the second stage of encapsulation to evaluate the use of inulin and FOS. Therefore, the experiment

T140MD50GA50 (MD:GA ratio = 50:50 and IT = 140) was chosen. The EE and EY values for experiments with different prebiotics as additives are shown in Table 1.

The EE was almost 65%, and the EY was less than 50% for all experiments; in the case of EE, significant differences ($p>0.05$) were not founded between them, i.e., the addition of prebiotics did not affect the efficiency and yield of the drying process using the chosen encapsulating agent blend (MD:GA, 50:50). Inulin and FOS are polysaccharides of interest: they exhibit prebiotic effects, but their sticky and hygroscopic behavior limits their application as encapsulating matrices [47]. Other studies mentioned that inulin showed less effectiveness in encapsulating phenolic compounds [23,44]. When inulin and FOS were combined with whey protein, a higher EE of lutein and probiotic bacteria was exhibited, respectively [47,48]. Gomes et al. (2019) reported that inulin in combination with modified starch allowed an efficient prebiotic encapsulant matrix for encapsulating bioactive compounds to be obtained.

3.2 Moisture content

The results of moisture content and particle size distribution of MCE obtained with different formulations (MD:GA) and IT are shown as supplementary material (Table S3).

The moisture content of MCE varied between 2.85 and 4.30%, and there were significant differences ($p>0.05$) between experiments. The specific hygroscopicity of each encapsulating agent, IT, and %TSS is the most responsible parameters influencing the final moisture content [50]. In our study, the results did not show a tendency for the IT effect. Thus, the different MD:GA ratios and the environmental factors may have been responsible for moisture content variations since during the collection of microparticles from the cyclone for all experiments, the relative humidity was not constant in the laboratory. The results of the characterization of MCE produced under optimal conditions (T140MD50GA50) and with different prebiotics are shown in Table 1.

Table 1. Encapsulation efficiency, encapsulation yield, and characterization of microcapsules obtained in experiments using the optimal MD:GA ratio and inlet air drying temperature with the addition of different prebiotics.

Samples	%EY	% EE	Moisture (%)	Particle size distribution		WSI (%)
				$D_{4,3}$ (µm)	SPAN	
MC-FOS	42.57	66.59 ± 1.41 [a]	3.50 ± 0.10 [ab]	3.78 ± 0.23 [a]	3.49 ± 0.28 [b]	86.85 ± 0.60 [a]
MC-INU	38.99	65.53 ± 0.95 [a]	3.61 ± 0.08 [bc]	3.02 ± 0.29 [a]	2.49 ± 0.01 [a]	88.82 ± 0.66 [a]
MC-WP	42.66	64.42 ± 0.19 [a]	3.38 ± 0.08 [a]	2.73 ± 0.09 [a]	2.58 ± 0.02 [a]	89.19 ± 1.88 [a]

Colorimetry

Samples	*L**	*a**	*b**	Chroma	Hue
MC-FOS	94.67 ± 0.84 [a]	-7.30 ± 0.16 [a]	27.23 ± 1.11 [a]	28.19 ± 1.11 [a]	74.99 ± 0.23 [a]
MC-INU	94.76 ± 0.32 [a]	-7.26 ± 0.30 [a]	26.59 ± 1.03 [a]	27.56 ± 1.07 [a]	74.73 ± 0.15 [a]
MC-WP	95.14 ± 0.56 [a]	-6.83 ± 0.12 [a]	25.17 ± 0.80 [a]	26.08 ± 0.81 [a]	74.82 ± 0.20 [a]

Mean ± SD (n = 3). Different letters in the same row mean a significant difference ($p<0.05$)
MC-FOS: Formulation with fructooligosaccharides; MC-INU: Formulation with inulin; MC-WP: Formulation without prebiotic; WSI: Water solubility index.

Moisture content values showed significant differences ($p<0.05$); however, a low average value (3.55%) was achieved and followed the same tendency for all experiments. This behavior is considered an essential factor that could affect the quality of microcapsules throughout the prevention of deterioration, especially during their storage.

3.3. Water solubility index (WSI)

The WSI was *>85%* for all formulations with prebiotics addition (Table 1), i.e., the encapsulation improved microcapsule solubility [52,53] and did not show significant differences between experiments ($p>0.05$); i.e., the addition of prebiotics did not influence the WSI. However, the lowest WSI was achieved when the formulation with FOS was used. The specific WSI of each encapsulating agent, especially the prebiotics, could be responsible for this behavior, and sometimes heat is needed to achieve better solubilization. On the other hand, hydrophobic interactions could happen between MD, GA, and prebiotics due to this chemical structure.

Loksuwan (2007) and Tupuna et al. (2018) reported the same tendency for encapsulated β-carotene and norbixin, respectively. In this way, the WSI does not depend on the hydrophilic or hydrophobic properties of the core material (bioactive compound of interest). The WSI mainly depends on the encapsulating agents' specific properties and their ratio. It is exciting to note that the formulations with prebiotics did not show statistically significant differences in EE and WSI that are considered

among the main parameters for future applications of MCE. Therefore, the prebiotics did not affect the properties of MCE, but they did offer functionality to MCE when added as encapsulating agents. This is very important when developing functional supplements with good technological characteristics using low concentrations of prebiotics.

3.4 Colorimetry of MCE

The MCE obtained after each experiment did not show significant differences ($p>0.05$) for the parameters *L**, *a**, *b**, and the Chroma and Hue values (Table 1). According to the CIELab color chart (second quadrant), the MCE showed a slight yellow tonality (the characteristic coloration of carotenoids) for all experiments, which means that small amounts of pigment are still on the surface of MCE according to the EE values (~65%) (Table 1). This result suggests that the color evaluated largely corresponds to the characteristic color of the encapsulating agents [22]. The a* and b* value parameters define the coloration, while the *L** indicates a higher brightness. The *L** of each encapsulating agent is responsible for the luminosity of the MCE due to the core material being in very low proportion in all formulations. According to the results, the EE and the specific color of the wall materials have a dominant effect on microcapsule coloration.

The Chroma and Hue angle values allow the saturation, vividness, and tonality of MCE to be established. These parameters are essential in evaluating the EE and the possible coloration that will be reached in the future application of microcapsules as additives in food matrices. The MCE obtained from different formulations did not show significant differences ($p>0.05$) in Chroma and Hue angle values. These values indicate a light yellow tone and a low intensity for all MCEs (Table 1). The color of microcapsules is directly correlated with EE, MD:GA ratio, and the specific color of encapsulating agents. Nevertheless, a yellow tonality within the typical coloration was observed for all MCEs. The same effect was reported for the encapsulation of norbixin [13] and β-carotene [53] when MD and GA were used as encapsulating agents.

3.5 Particle size distribution (PSD)

The MCE obtained with different formulations and operating conditions showed significant differences ($p<0.05$) in particle size ($D_{[4,3]}$) when different wall material ratios (MD:GA) were used.

The $D_{[4,3]}$ value was less than 4.00 µm for all experiments, i.e., all microcapsules were within the size range of microparticles [54,55] (Table S3), and its PSD is shown in Fig. 2a.

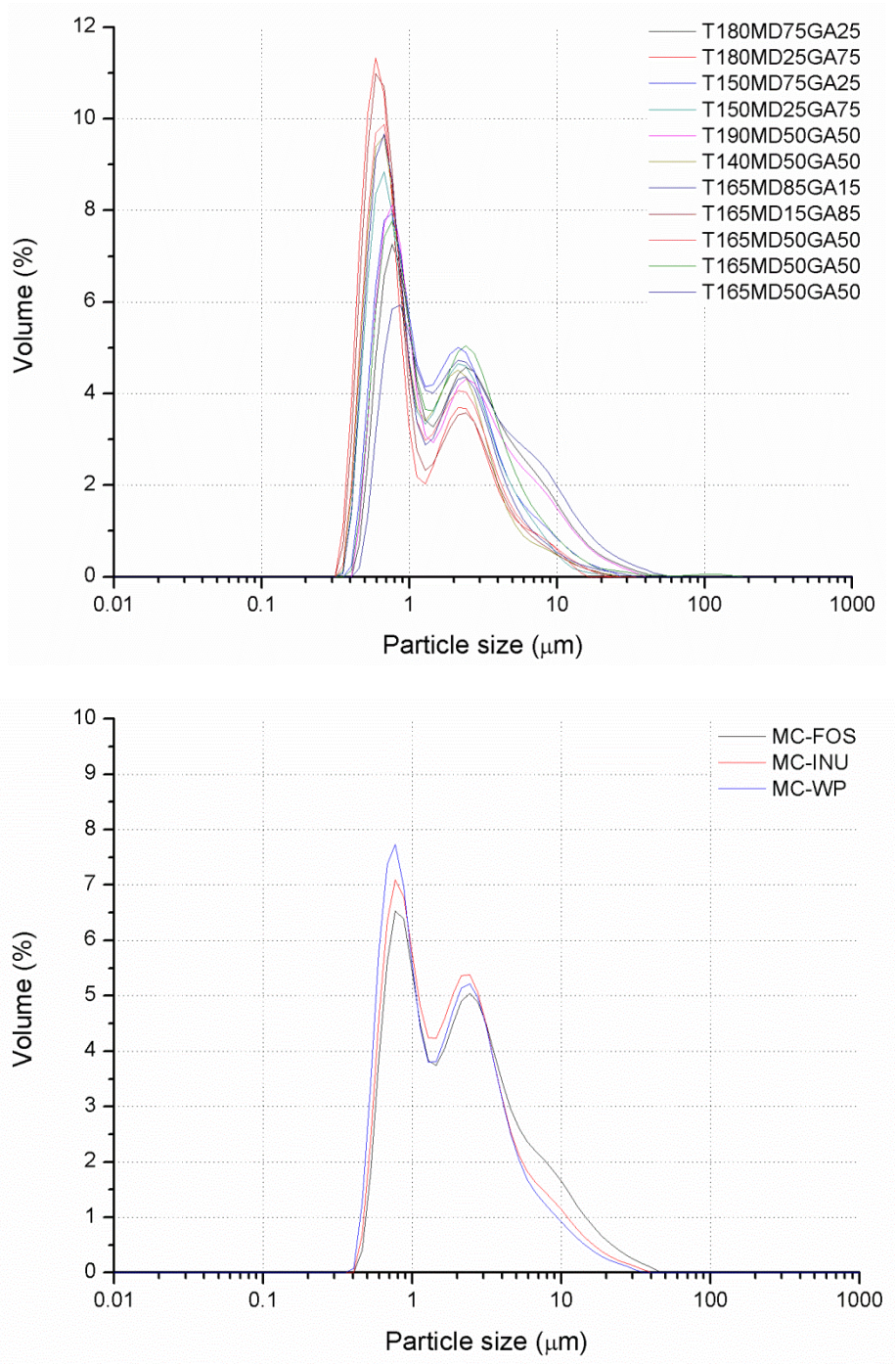

Fig. 2. Particle size distribution: a) Microcapsules obtained in experiments with different wall material ratios and inlet air drying temperatures; b) Microcapsules obtained with an optimal MD:GA ratio and inlet air drying temperature with the addition of different prebiotics.

The physical characteristics of encapsulating agents, the rheology of the formulation, the %TSS, and the drying operating conditions, influence the significant differences in microcapsule particle size

[57,58]. When MD is used at a higher IT, the heat transfer coefficient is more elevated, causing faster water evaporation; consequently, a higher particle diameter was obtained [59]. In our study, MCE obtained using IT up to 165 °C and higher levels of MD in the encapsulating agent ratio (MD:GA) showed a larger particle size. This value ranged from 1.76 μm (T180MD25GA75) to 4.24 μm (T165MD85GA15), with the highest MD level being responsible for the greatest increased particle size: i.e., when MD was used in a higher proportion, larger particle size was obtained. This behavior could be related to the viscosity and other specific physical properties of GA (Table S3). When evaluating the diameter of MCE developed under optimal conditions IT=140 °C and MD:GA = 50:50, and with the addition of prebiotics, the value of $D_{[4,3]}$ did not show significant differences ($p>0.05$), i.e.; the prebiotic did not influence the microcapsule particle size (Table 1). Nonetheless, the %TSS and specific viscosity of each wall material directly affect the size of the particle and could be responsible for the different $D_{[4,3]}$ values; high density produces particles with larger diameters [13,60].

The PSD was bimodal with two populations: i.e., the drying process allows a heterogeneous-sized particle to be obtained, and the MCE samples in all experiments showed the same behavior (Fig. 2). The *Span* value showed significant differences ($p<0.05$) between all investigations according to the MD:GA ratio and IT (Table S3). When the PSD is bimodal, there are large and small particles. This can be attributed to the agglomeration process when the interaction between minuscule particles forms large particles, and different IT produces particles with other structures and sizes. It mainly occurs at a low feed flow rate [61]. Ferrari et al. (2012) reported the same PSD when different formulations of MD and GA were used. In the case of MCE with prebiotics, the PSD also was bimodal (Fig. 2b), and the *Span* value of MC-FOS was statistically higher than for MC-INU ($p>0.05$) (Table 1).

One of the main advantages of obtaining microparticles with a *Span* <2 is their WSI. A low *Span* value means high homogeneity in particle size because there are no significant changes in the physical properties of the microcapsules during the encapsulation process [63]. Therefore, when the PSD is more homogeneous, the microcapsules are more soluble in water [13]. In our study, when MCE was obtained with the addition of FOS and inulin and without prebiotics, all values were >2 and matched the WSI results <90% for MCE with prebiotics. In addition, the MCE obtained with FOS

added was less homogeneous than microcapsules with inulin. In conclusion, the specific physical properties of encapsulating agents (polysaccharides, inulin, and FOS), the MD:GA ratio, and spray drying operation parameters were responsible for the heterogeneous particle size.

3.6 Cryo-scanning Electron Microscopy (Cryo-SEM)

The MCE was obtained with different formulations (MD:GA), and IT showed a rounded outer surface with differences in size and shape. The same behavior was observed in MCE obtained using prebiotics and without a core (MC-empty) formulation. The photographs of scanning electron microscopy (SEM) analyses of MCE samples obtained from the experiments: T165MD15GA85 (highest EE = 74.71 ± 0.83%), T165MD50GA50 (central point) (EE = 43.01 ± 1.03%), and T165MD85GA15 (lowest EE = 18.68 ± 0.66) are shown in Figure 3. In the case of SEM analyses of MCE samples obtained using the experiment T140MD50GA50, the photographs are shown in Figure 4, with the addition of different prebiotics (Fig. 4a) and the respective microcapsules without a core (empty) (Fig. 4b) for each formulation.

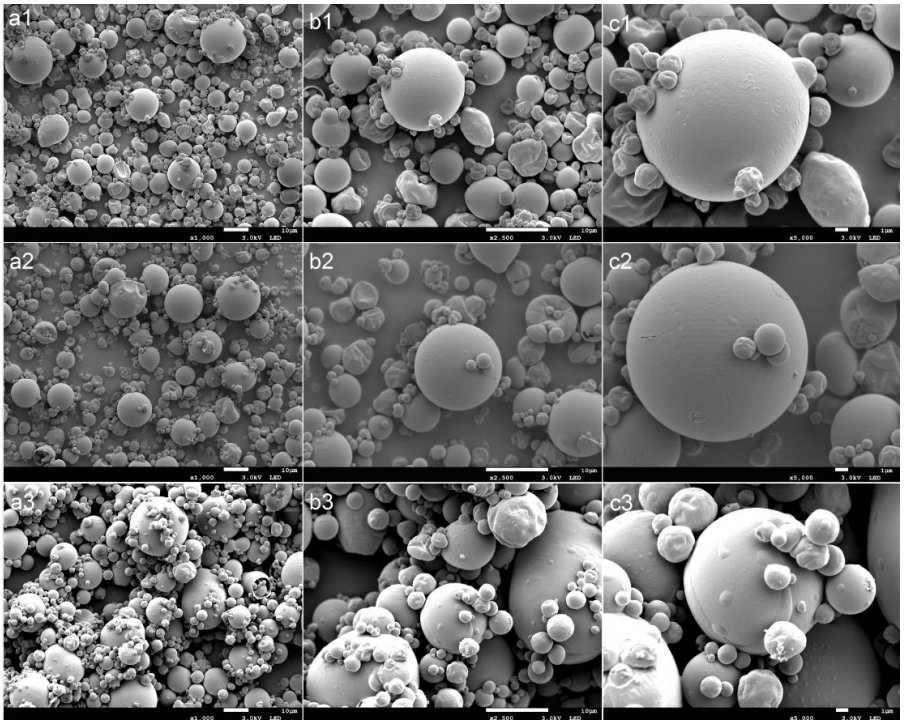

Fig. 3. Cryo-SEM photographs of the MCE samples obtained with the same IT (165 ºC) and a different MD:GA ratio: 1) 85:15; 2) 50:50 and 3) 15:85 at three magnifications: a) 1000x; b); 5000x and c) 10000x.

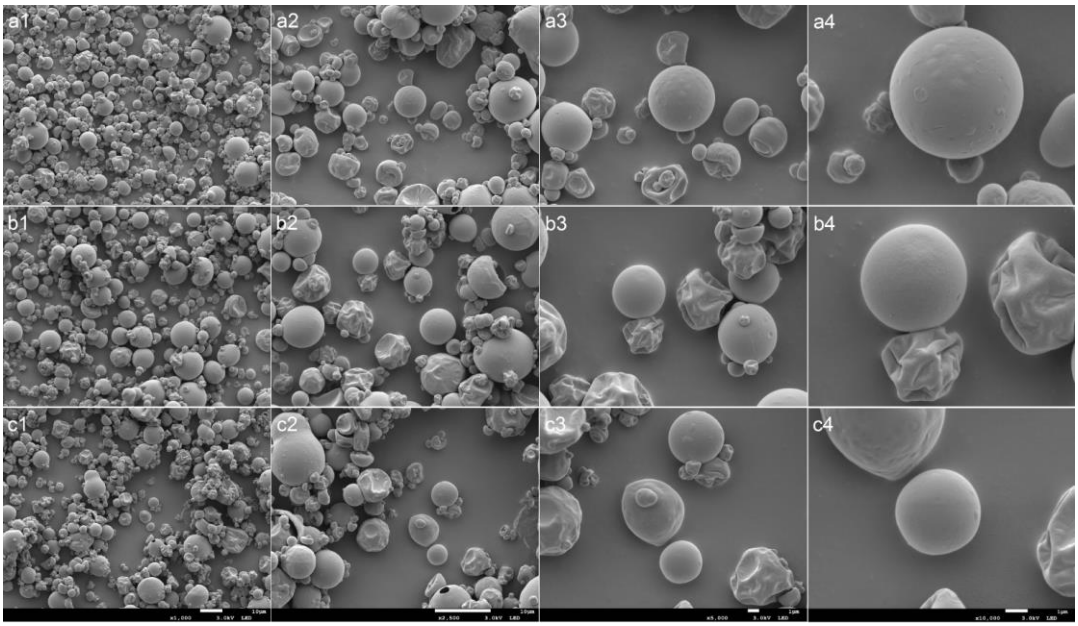

Fig. 4a. Cryo-SEM photographs of the MCE samples obtained with the addition of different prebiotics: a) FOS, b) inulin, and c) without prebiotics at four magnifications: 1) 1000x; 2); 2500x; 3) 5000x and 4) 10000x.

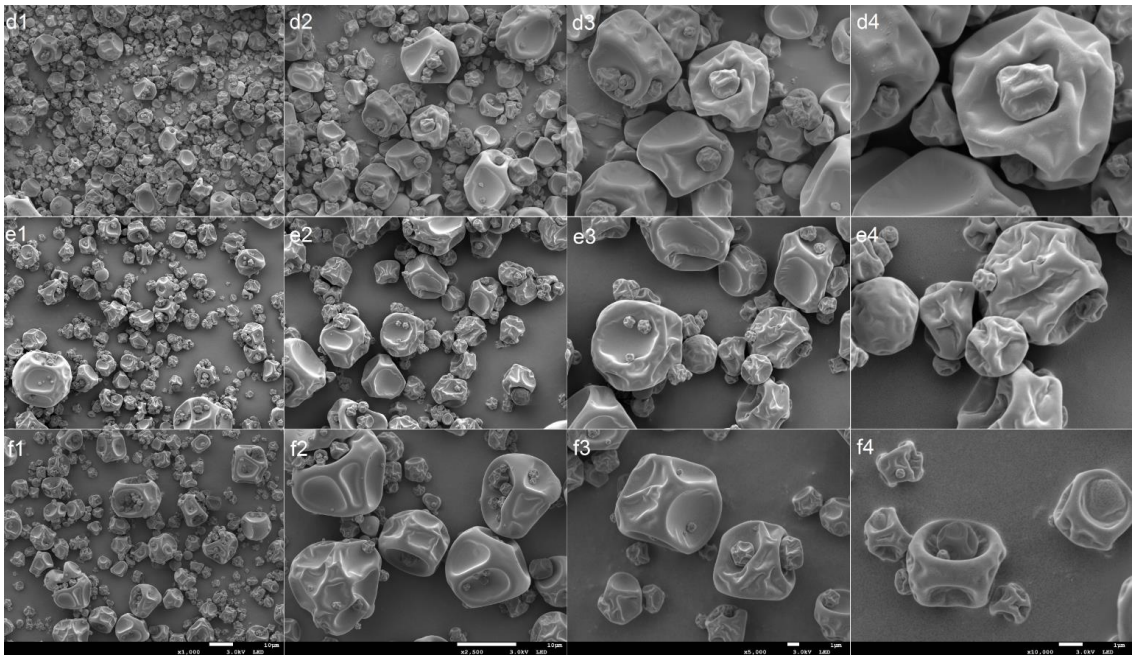

Fig. 4b. Cryo-SEM photographs of the microcapsules without a core (empty) obtained with the addition of different prebiotics: d) FOS, e) inulin, and f) without prebiotics at four magnifications: 1) 1000x; 2); 2500x; 3) 5000x and 4) 10000x.

The MCE showed a spherical form, typical of microparticles obtained by spray drying (Fig. 4a). The surface of MCE showed a morphology irregularity caused by the fast evaporation of liquid droplets, which promotes a wrinkling of microparticles due to the releasing or minimal amount of air that remained within de MCE after encapsulation [13]. Therefore, in the MC-empty photographs, a no spherical structure with an irregular surface (Fig. 4b). The morphology of MC-FOS and MC-INU was more spherical than MC-WP (Fig. 4a), with fewer irregularities. Other authors reported similar photographs in various studies about the encapsulation of bioactive compounds using MD and GA as encapsulating agents [13,22,63,64].

3.7 Thermal stability of MCE

The MCE obtained by treatment T140MD50GA50 with inulin was used. Bioactive compound stability depends on the matrix and the environmental storage conditions, including the pH, temperature, light, and water activity. β-carotene was identified as the predominant carotenoid among the extracts. It was quantified to express the carotenoid concentration during thermal stability assessment. Then, based on β-carotene retention, the degradation curves were developed for each temperature (Fig. 5) and the rate degradation constant (*kt*) was calculated. According to the Arrhenius equation, the activation energy (*Ea*) was determined (Table 2). The total color difference (*ΔE*) and antioxidant activity after the stability test were assessed for day 0 and day 45 at the three storage temperatures; the results are shown in Table 3.

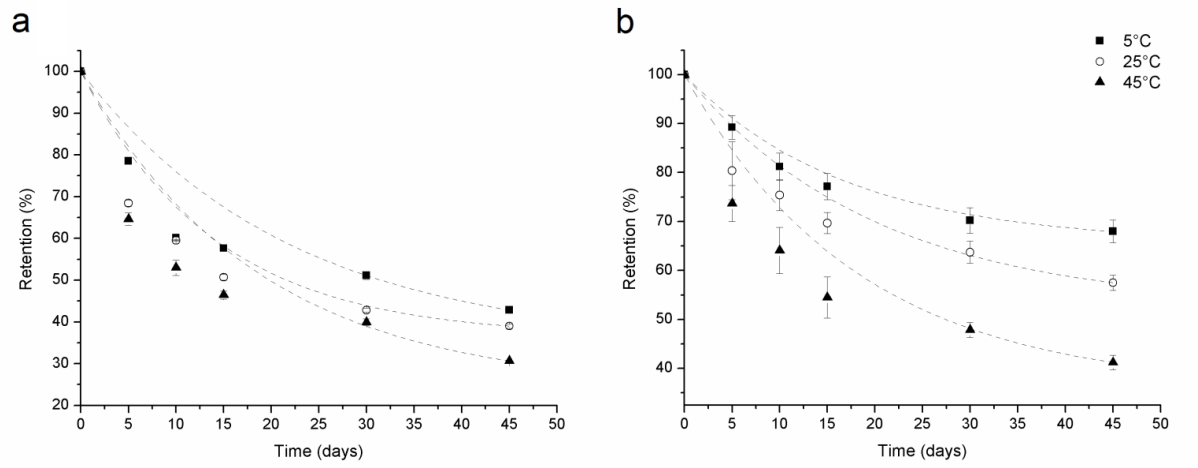

Fig. 5. β-Carotene retention (%) during the thermal assessment a) Non-encapsulated β-Carotene (Free-CE), b) Encapsulated β-Carotene (MCE) at 5 °C (■), 25 °C (○), and 45 °C (▲), respectively.

Table 2. Kinetic degradation constant (*kt)*, half-life time ($t_{1/2}$), and activation energy (*Ea*), for β-Carotene retention.

Sample	Extract			MCE		
Temperature	5 °C	25 °C	45 °C	5 °C	25 °C	45 °C
Rate parameters						
k_t x 10^{-3} (min^{-1})	1.63	1.82	2.21	0.79	1.05	1.73
$t_{1/2}$ (days)	42.45	38.05	31.27	87.37	65.99	41.79
E_a (kcal/mol)		1.33			3.41	

MCE: Microencapsulated carotenoid extract

Table 3. Total color difference and antioxidant activity assessment of MCE after thermal stability test

Temperature	5 °C	25 °C	45 °C
Color			
ΔE	0.92 ± 0.32[a]	1.48 ± 0.29[b]	2.63 ± 0.26[c]
Antioxidant activity (µmol TE/100g)			
Day 0	-	7286.67 ± 256.97[a]	-
Day 45	6706.65 ± 178.78[a]	5334.45 ± 199.88[b]	3432.15 ± 272.66[c]

Mean ± SD (n = 3). Different letters in the same line means significant differences ($p < 0.05$)

As expected, the β-carotene concentration decreased when temperature and time increased, and both MCE and Free-CE showed the same effect (Fig. 5). The β-carotene losses were higher for Free-CE, verifying that the encapsulation process offers more protection against heat (Fig. 5). According to the *Ea* value, the degradation reactions follow the first order for MCE and Free-CE. According to β-carotene retention, our study demonstrated that the encapsulated carotenoids are more stable than free carotenoids when both were exposed to heat. Several studies proved that higher bioactive compound losses through degradation occur following exposure to high temperatures in the non-encapsulated form, along with reporting a first-order kinetic behavior [13,22,23,26,49,65,66].

Based on the *kt* values, the half-life time ($t_{1/2}$) was determined for each temperature; it indicates the time necessary to lose the 50 % of carotenoids concentration measured at the beginning of the

thermal assay. As expected, $t_{1/2}$ depended on the temperature, and according to β-carotene retention, it was higher for MCE than Free-CE (Table 2). When temperature increases the degradation of carotenoids is faster and as a consequence, the $t_{1/2}$ decreases. When both were exposed to lower and room temperatures, the $t_{1/2}$ of encapsulated β-carotene was approximately two times greater than the $t_{1/2}$ of non-encapsulated β-carotene. This behavior indicates that encapsulated carotenoids achieved a higher $t_{1/2}$ than free carotenoids. We can predict that MCE will be able to maintain their quality for an extended time when food storage temperatures (4 – 25 °C) be used. Therefore, the encapsulation is an excellent technology to increase the shelf life of bioactive compounds. The *Ea* measures the sensitivity of carotenoids against heat: i.e., when *Ea* is lower, the encapsulated compound is more sensitive to degradation. Loss occurs from Free-CE before MCE, indicating that the encapsulated compound has greater resistance to heat. β-carotene requires more than double *Ea* for thermal degradation than free β-carotene. In hot temperatures (45 °C), the $t_{1/2}$ of MCE was longer (10 more days) (Table 2). Therefore, the fast decrease of β-carotene retention in Free-CE and MCE (Fig. 5) at the first days of the experiment is due to the free compounds on the surface microcapsules and were consequently exposed directly to heat.

The use of GA as encapsulating agent to create encapsulated bioactive compounds increases $t_{1/2}$ and significantly influences the improvement of the shelf-life of them compared to other polymers. Their emulsifying properties and its film-forming ability around the compound of interest provides a plasticity effect that protects the encapsulated matrix [13]. The chemical composition of the wall material influences the oxidative stability of bioactive compounds. According to Castellani et al. (2010), the arabinogalactan protein in the GA structure offers good properties for this polymer. They have the ability to form molecular complexes with carotenoids through hydrophobic interactions, helping in carotenoids protection from degradation. Various studies have reported on the efficient antioxidant properties of GA and its ability to safeguard carotenoids from oxidation [67,68].

MD showed good encapsulation properties by forming an amorphous glassy matrix, a solid network whose structure is stable thanks to the hydrogen bonds between carbonate chains. In this way, the matrix efficiently protects the encapsulated compounds from external conditions [69]. Blends of MD

with GA as encapsulating agents can provide efficient barrier protection for β-carotene after the encapsulation process [54].

Blends of different polysaccharides may have better functional properties than a single polysaccharide. The mixture of these blends with prebiotics (oligosaccharides) offers more functionality to the bioactive compound without decreasing its stability [50]. In the study carried out by Ding et al. (2020) on the encapsulation of lutein, the authors reported that the results might indicate that inulin had better strength and impermeability than other carbohydrates.

The *ΔE* value indicates a color difference between two samples: 0 to 0.5 (imperceptible difference); 0.5 to 1.5 (slight difference); 1.5 to 3.0 (just-noticeable difference); 3.0 to 6.0 (notable difference); 6.0 to 12.0 (extremely notable difference); and above 12.0 (entirely different shade). Thus, the difference becomes evident when the *ΔE* value is greater than 3 [70]. According to the results for *ΔE* (Table 3), the hot temperature showed a high *ΔE* value as was expected. At 5 ºC and 25 ºC, there was a slight difference, and at 45 ºC, the MCE showed a just-noticeable difference. During storage, the parameter L* decrease, and the parameters a* and b* increase, which could be due to the degradation of carotenoids on the microcapsule surface (non-encapsulated area), according to the values of β-carotene retention. The loss could be related to oxidation, and it was higher at high storage temperatures. However, all *ΔE* values are less than 3, i.e., the color difference is not evident [54,71].

According to the antioxidant activity values for the carotenoid extract (9224.77 ± 211.42 µmol TE/100g) and the MCE on day 0, following the drying process, it was possible to maintain almost 80% of the antioxidant activity of the carotenoid extract, i.e., the high temperatures did not cause high carotenoid degradation and a decrease in antioxidant activity. As expected, the ABTS assay results showed a decrease in the antioxidant activity of MCE samples as compared to the initial value (day 0): 7.96%, 26.79%, and 52.90% when stored at 5, 25 y 45 °C, respectively, during 45 days (Table 3). However, it was possible to maintain up to 70% of antioxidant activity at room temperature. Other studies reported losses of 7.2% and 16% of antioxidant activity during 60 and 75 days of storage, respectively, at 25 ºC, for encapsulated phenolic compounds [31,50]. This reduction could be because carotenoids are highly sensitive to degradation by heat (high temperatures)

compared to other bioactive compounds [30]. Likewise, the different blend of wall materials and spray-drying parameters leads to a different EE. In our study, the EE was less than 65%, which means that many carotenoids that remained on the microcapsule surface were degraded and responsible for the antioxidant activity loss. Therefore, it is important to encapsulate the compound of interest and achieve a high EE. Based on these results is evident the importance of using different assays for a determination of antioxidant activity more exactly and representative since each method has its specificity and a particular site of action [72].

4 Conclusion

Carotenoid extracts obtained from mango peel were encapsulated by spray drying. For the purpose, different formulations (MD:GA) with FOS and Inulin as encapsulating agents were tested. The use of GA in amounts greater than or equal to MD led to a high EE (≈ 75%) but a low EY (<45%). According to EE and PSD, the MCE showed a good WSI (>85%) and the yellow tonality characteristic of carotenoids, making them an effective water-soluble natural coloring. The kinetic rate parameters of thermal degradation of MCE were assessed. Based on β-carotene retention, MCE required activation energy much higher (almost 2.5 times) for degradation than for Free-CE when both were stored using the same temperatures. The $t_{1/2}$ required for degradation of MCE (41.79 days) at 45 °C was almost the same as the Free-NC (42.45 days) at 5 °C. The antioxidant activity of carotenoid extract decreases by a small percentage (≈20%) after the drying process, and the encapsulation shows efficient protection for carotenoids at high storage temperatures. Therefore, it was proven that spray drying microencapsulation using MD, GA, and prebiotics as encapsulating agents is an excellent technology in enhancing the thermal stability of carotenoids, thereby offering a longer shelf-life during storage and greater resistance following digestion after consumption. These findings turn the encapsulated carotenoids from mango peel into a promising and attractive natural additive with functional properties for application in food matrices and supplements.

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Suplemmentary Material

Table S1. Values for each process variable according to the experimental design.

Variables	Axial point	Low level	Central Point	High level	Axial point
	-1.414	-1	0	1	1.414
Temperature	140	150	165	180	190
MD:GA	3:17	1:3	1:1	3:1	17:3
MD	15	25	50	75	85
GA	85	75	50	25	15

MD: Maltodextrin, GA: Gum Arabic.

Table S2. Codification of encapsulation experiments according to the values of each variable.

Experiment	TEMPERATURE	MD:GA
T180MD75GA25	180 (1)	3:1 (1)
T180MD25GA75	180 (1)	1:3 (-1)
T150MD75GA25	150 (-1)	3:1 (1)
T150MD25GA75	150 (-1)	1:3 (-1)
T190MD50GA50	190 (1.414)	1:1 (0)
T140MD50GA50	140 (-1.414)	1:1 (0)
T165MD85GA15	165 (0)	17:3 (1.414)
T165MD15GA85	165 (0)	3:17 (-1.414)
T165MD50GA50	165 (0)	1:1 (0)
T165MD50GA50	165 (0)	1:1 (0)
T165MD50GA50	165 (0)	1:1 (0)

Table S3. Characterization of microcapsules obtained in experiments with different proportions of wall materials and inlet air drying temperature.

Experiments	Moisture (%)	Particle size distribution	
		$D_{[4,3]}$ (µm)	SPAN
T180MD75GA25	3.41 ± 0.17 ^{bcd}	3.57 ± 0.03 ^{d}	2.95 ± 0.03 ^{abc}
T180MD25GA75	3.62 ± 0.09 ^{de}	1.76 ± 0.04 ^{a}	3.63 ± 0.02 ^{e}
T150MD75GA25	4.30 ± 0.07 ^{f}	2.48 ± 0.05 ^{c}	2.64 ± 0.02 ^{a}
T150MD25GA75	4.05 ± 0.11 ^{f}	2.11 ± 0.23 ^{abc}	3.06 ± 0.18 ^{abcd}
T190MD50GA50	3.74 ± 0.07 ^{e}	3.30 ± 0.07 ^{d}	3.44 ± 0.04 ^{cde}
T140MD50GA50	3.69 ± 0.02 ^{e}	1.92 ± 0.13 ^{a}	2.94 ± 0.08 ^{abc}
T165MD85GA15	3.52 ± 0.04 ^{cde}	4.24 ± 0.44 ^{e}	2.87 ± 0.13 ^{ab}
T165MD15GA85	2.85 ± 0.04 ^{a}	1.83 ± 0.07 ^{a}	3.59 ± 0.08 ^{de}
T165MD50GA50	3.22 ± 0.09 ^{b}	1.98 ± 0.07 ^{ab}	3.26 ± 0.12 ^{bcde}
T165MD50GA50	3.40 ± 0.14 ^{bcd}	2.39 ± 0.10 ^{bc}	3.37 ± 0.69 ^{bcde}
T165MD50GA50	3.30 ± 0.10 ^{bc}	2.14 ± 0.23 ^{abc}	3.39 ± 0.01 ^{bcde}

6. PAPER IV

Encapsulation of bioactive compounds from fruit and vegetable by-products for food application – A review.

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Encapsulation of bioactive compounds from fruit and vegetable by-products for food application – A review

Verónica Marcillo-Parra [a,b], Diego Santiago Tupuna-Yerovi [c,d], Zulay González [b], Jenny Ruales [a,*]

[a] Department of Food Science and Biotechnology (DECAB), Escuela Politécnica Nacional (EPN), Zip Code: 17012759, Quito, Ecuador
[b] Department of Life Sciences and Agriculture, Universidad de las Fuerzas Armadas ESPE, Zip Code: 171-5-231B, Sangolquí, Ecuador
[c] Institute of Food Science and Technology (ICTA), Universidade Federal do Rio Grande do Sul (UFRGS), Campus do Vale, Zip Code: 91501-970, Porto Alegre, RS, Brazil
[d] Agroindustrial Engineering Department, Pontifícia Universidad Católica del Ecuador – Sede Manabí (PUCEM), Campus Chone, Zip Code: 130301, Portoviejo, Manabí, Ecuador

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ABSTRACT

Background: Fruit and vegetable by-products (FVBP) from the food industry and post-harvest represent one of the world's most common environmental pollution problems. However, these wastes are now being recognized for their functional value. Encapsulation is among the most popular food processing alternatives since it helps to improve the stability and bioavailability of bioactive compounds. Encapsulated products provide excellent health benefits, and therefore can be used as functional ingredients in food.

Scope and approach: This review describes the creation of encapsulated bioactive compounds (EBC) using microencapsulation techniques and their application in food. The work aimed to identify the various previously studied bioactive compounds from FVBP related to their extraction, characterization, encapsulation, and application. Encapsulation protects bioactive compounds against the adverse conditions inherent in food processing, and its use allows for functional food to be obtained.

Key findings and conclusions: Encapsulation technologies, such as spray-drying, freeze-drying, and coacervation, are those most used to produce EBC from FVBP. The effectiveness of these technologies in producing EBC with high encapsulation efficiency and excellent functional properties has been proven, and the stability and solubility of bioactive compounds were thereby improved. EBC from FVBP were added to food with various purposes, including enrichment, fortification, coloring, and stability improvement against oxidation and microbial proliferation. Several studies regarding the application of EBC from FVBP in food have been performed thus; EBC is a promising alternative in the treatment of agricultural and agro-industrial by-products and one that merits further investigation.

1. Introduction

At a global scale, food industry by-products represent a critical source of pollution: food losses and waste occur throughout the food supply chain, accounting for around 1.3 billion tons per year, or 16% of the total food supply (FAO, 2011; Kummu et al., 2012). In case of fruits and vegetables, food losses of 20%–40% begin in initial agricultural production and continue throughout the production phases, all the way to the final consumer (FAO, 2011). A wide range of by-products come from fruit and vegetable processing —particularly from the juice industry, including leaves, peels, unusable pulp, seeds, cull fruits and stones— and a large amount of this material is ultimately discarded (Amaya-Cruz et al., 2015; Fernández et al., 2018). The conversion of these wastes into high-value food products could mitigate losses by increasing demand for post-harvest and agro-industrial by-products that can be used as sources of bioactive compounds with antioxidant properties and applied in the development of functional products (Socaci, 2017).

Several studies on fruit and vegetable by-products (FVBP) —in the form of peels, seeds, flower, leaf, stem, pomace, bagasse, and extracts— have found a wide variety of bioactive compounds, such as phenolic

* Corresponding author. Department of Food Science and Biotechnology, Faculty of Chemical and Agroindustrial Engineering, Escuela Politécnica Nacional, Ecuador.
E-mail addresses: vemarcillo@espe.edu.ec (V. Marcillo-Parra), santiagotupuna@hotmail.com (D.S. Tupuna-Yerovi), zulayestefaniagonzalez@gmail.com (Z. González), jenny.ruales@epn.edu.ec (J. Ruales).

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Abbreviations

EBC	Encapsulated Bioactive Compounds
EPC	Encapsulated Phenolic Compounds
FVBP	Fruit and Vegetable By-Products
TPC	Total Phenolic Compounds
TAC	Total Anthocyanin Content
TFC	Total Flavonoid Content
TCC	Total Carotenoid Content
EY	Encapsulation Yield
EE	Encapsulation Efficiency
MD	Maltodextrin
GA	Gum Arabic
DE	Dextrose Equivalent
WPC	Whey Protein Concentrate
WPI	Whey Protein Isolate
C/W	Core/wall material

acids, flavonoids, anthocyanins, carotenoids, and vitamin C (Barros et al., 2012; Kabir et al., 2015; Silva et al., 2014). FVPB extracts and isolated compounds with antioxidant properties have been used as ingredients to develop new functional food products with high rates of consumer interest and acceptance (Socaci, 2017). These compounds could also be used as nutraceuticals in medicinal and pharmaceutical products. Due to their bioactive compound content and biological activity in humans, a number of FVBP may have applications as natural additives in the food, biotechnology, and pharmaceutical industries (Coman et al., 2020). However, their instability under normal food processing and storage conditions, such as pH variations, temperature, light, oxygen, and ions, limits their use. Fortunately, the encapsulation effectiveness of specific bioactive compounds has been demonstrated using encapsulation techniques to decrease sensitivity to environmental conditions while improving stability and water solubility (Gomes et al., 2019; Souza et al., 2017).

The possibility of producing food with added natural ingredients that can provide health benefits has drawn more attention from food researchers in recent years. The use of encapsulated bioactive compounds (EBC) in foods is a growing industry trend, resulting in the expansion of well-known functional foods (Lavelli et al., 2016; Oancea et al., 2018; Tupuna-Yerovi et al., 2020).

This review article's primary goal is to demonstrate the encapsulation process's potential in improving the stability, solubility, and bioaccessibility of bioactive compounds extracted from FVBP, and the application of encapsulated products as functional food ingredients (Fig. 1).

2. Bioactive compounds in FVBP

Due the increasing amounts of agro-industrial wastes, several studies have focused on the characterization of bioactive compounds, mainly in FVBP. These organic residues can provide a cheap source of bioactive compounds (Table 1), which can be used to develop new ingredients or additives for functional food products.

2.1. Fruit by-products

The type of by-products primarily depends on the fruit type. Fruits can be classified according to the physical characteristics of peel and seeds, and said classification also serves to define the necessary processing method and steps. Pomes (e.g., apple and pears) have a central seed-containing core surrounded by a thick layer of pulp and a thin layer of peel, while berries are small and juicy, with thin skins that are an attractive source of bioactive compounds. Usually, the entire fruit is pressed to extract the juice producing by-products in the form of pomace (peel, seeds, and leftover pulp), which is discarded following the processing of juices, jam, jelly, fruit bars, and marmalades. Apple pomace (approx. 25% of fresh fruit weight) is rich in phenolic compounds —phloridzin and chlorogenic acid being the most abundant— which can act as radical scavenger agents, showing antioxidant properties (Rana et al., 2015). The amount of total phenolic compounds (TPC) is higher in seeds than in peel (Rabetafika et al., 2014), is higher in the peel than in

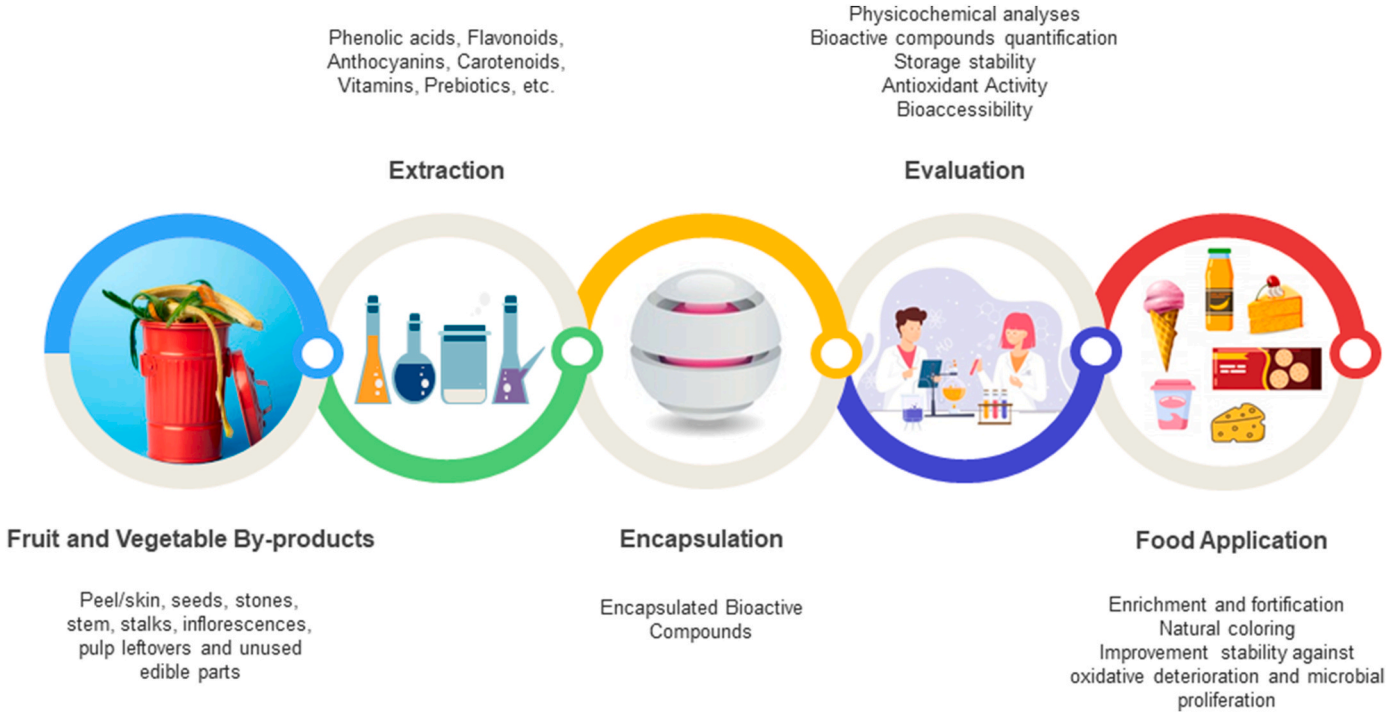

Fig. 1. Food application of encapsulated bioactive compounds extracted from fruit and vegetable by-products.

Table 1
Bioactive compounds from fruit and vegetable by-products.

Food by-product	Bioactive compounds	References
Fruits		
Acerola pomace	Anthocyanins, carotenoids, vitamin C, and yellow flavonoids	Rezende et al. (2017); Silva et al. (2014)
Apple pomace Blackberry pomace Blackcurrant pomace Black chokeberry press cake residue Cranberry skin Pear pomace Sour cherry peel	Phenolic acids, flavonoids, and anthocyanins	Rana et al. (2015) Santos et al. (2019) Kapasakalidis et al. (2006) Kitrytė et al. (2017) Oszmiański et al. (2016) Fernández et al. (2018) Yılmaz et al. (2015)
Cactus peel and mucilaginous part with seeds	Phenolic acids, flavonoids, betalains, and tocopherols (vitamin E)	Melgar et al. (2017)
Cashew apple peel and pulp's leftovers Mango peel, pulp's leftovers, and seed kernel Peach peel and pulp's leftovers	Phenolic acids, carotenoids, anthocyanins, and yellow flavonoids, vitamin C, and tannins	Silva et al. (2014) Marcillo-Parra et al. (2021); Ruales et al. (2018) Amaya-Cruz et al. (2015); Gil et al. (2002)
Clementine peel Grapefruit peel Kumquat peel	Carotenoids	Agócs et al. (2007)
Grape pomace	Phenolic acids, flavonoids, anthocyanins, tannins, and stilbenes	Goula et al. (2016); Ky and Teissedre (2015); Montibeller et al. (2019)
Guava pomace Papaya pomace Passion fruit seeds Surinam cherry pulp's leftovers	Carotenoids, anthocyanins, and yellow flavonoids	Silva et al. (2014)
Jaboticaba pomace	Phenolics, anthocyanins, and tocopherols (vitamin E)	Albuquerque et al. (2020); Souza et al. (2017)
Lemon peel Lima peel Mandarin peel Orange peel and seeds	Phenolics, vitamin C, carotenoids, and flavonoids	Agócs et al. (2007) Barros et al. (2012) González-Molina et al. (2010) Chen et al. (2017)
Persimmon peel Pomegranate peel Quince peel and seeds	Phenolic compounds	Kabir et al. (2015) Kaderides et al. (2015)
Pineapple peel and pulp's leftovers Sapodilla pomace	Anthocyanins and carotenoids	Silva et al. (2014)
Plum press cake residue	Phenolic acids, vitamin C, anthocyanins, and flavonoids	Gil et al. (2002); Sójka et al. (2015)
Raspberry and strawberry extrudate	Phenolic acids and anthocyanins	Vázquez-González et al. (2020)
Star fruit peel and seeds	Phenolic acids, flavonoids, and vitamin C	Saikia et al. (2015)
Vegetables		
Artichoke stalk, leaf, receptacles, and outer bract	Phenolic acids, flavonoids, and inositols	Ruiz-Cano et al. (2014)
Asparagus leaf and spears	Phenolics, flavonoids, and saponins	Fuentes-Alventosa et al. (2013)
Black carrot peel Sweet potato (Shinzami) peel	Phenolic acids and anthocyanins	Kamiloglu et al. (2016) Kim et al. (2012)
Broccoli stalk, inflorescence, and leaves Cauliflower leaves and stem Chicory leaves Lettuce leaves Yarrow leaves dust	Phenolic acids and flavonoids	Ferreira et al. (2018) Llorach et al. (2003) Llorach et al. (2004) Kabir et al. (2015) Vladić et al. (2016)
Cabbage leaves	Phenolic compounds	Kabir et al. (2015)
Olive press cake residue Onion scape, umbel, surpluses, and root	Phenolic acids, flavonoids, and tyrosol	Fernández et al. (2018) Roldán et al. (2008)
Potato peel	Phenolic acids	Akyol et al. (2016)
Red pepper peel and seeds	Carotenoids and tocopherols (vitamin E)	Silva et al., (2013)
Tomato peel and seeds	Carotenoids, phenolic acids, and flavonoids	Fernández et al. (2018); Stajčić et al. (2015)

pulp (Vieira et al., 2011).

The TPC content and antioxidant activity of berry by-products is generally represented by anthocyanins and depends on the solvent and operating conditions used for extraction (Kapasakalidis et al., 2006; Kitrytė et al., 2017; Santos et al., 2019). Oszmiański et al. (2016) reported that the TPC in cranberry juices was lower than in fruit and pomace, and the TPC in pomace was higher than in fruit. In studies performed with blueberry, red raspberry, and cranberry pomace, the extracts showed remarkable antioxidant activity. The pleasant flavor and high bioactive compound content of tropical berries —such as acerola and jaboticaba— have led to increased consumption. However, their processed by-products contain higher amounts of bioactive compounds —with antioxidant activity and moderate anti-inflammatory, anti-proliferative, and antimicrobial activities— than that found in the fruit's edible fleshy part. Neither was any hepatotoxicity shown, meaning that there are no toxicity issues with application (Albuquerque et al., 2020; Rezende et al., 2017).

The wine and juice industry rejects large amounts of grape by-products in the form of pressed grape pomace. Phenolic compounds are not fully extracted during the winemaking process, and various studies have identified numerous phenolic compounds left behind in grape pomace from different extraction methods (Goula et al., 2016; Montibeller et al., 2019). The seeds showed more TPC and antioxidant activity than skin (Ky & Teissedre, 2015). The phenolic compounds showed potential benefits for cardiovascular and metabolic health, cancer prevention, and antioxidant, anti-inflammatory, and antimicrobial activity (Ferri et al., 2017; Thimothe et al., 2007).

Drupes (e.g., mangos, peaches, plums, and olives) have an outer peel covering a soft fleshy fruit, which surrounds a single hard stone or pit containing the seed. They must be peeled prior to processing, and the seed/kernel is often removed, leaving behind only the edible fleshy part for use. Mango by-products (35%–60% of fresh fruit weight) have a high concentration of phenolic acids and carotenoids and lower amounts of flavonoids and phytosterols (Marcillo-Parra et al., 2021). The total carotenoid content (TCC) is higher in the peel than in the seed kernel, while mangiferin is the predominant phenolic compound in both (Ruales et al., 2018). Extracts from mango, peach, and plum by-products showed antioxidant activity, antimicrobial activity, inhibitory activity against α-amylase, and the prevention of degenerative liver diseases (Amaya-Cruz et al., 2015; Sójka et al., 2015). Citrus fruits have a thick outer rind and a membrane that separates the pulp into segments. They are generally consumed in the form of fresh-squeezed, concentrated, or pasteurized juice, producing high amounts of discarded by-products. Orange, lemon, lime, mandarin, clementine, kumquat, and grapefruit peels are rich in bioactive compounds. Carotenoids and flavonoids —hesperidin the predominant— are more abundant than phenolic acids, flavones, and limonoids (Agócs et al., 2007; González-Molina et al., 2010), and the peel contains higher amounts of phenolic compounds, vitamin C, and minerals than the pulp (Barros et al., 2012). The antioxidant and anti-inflammatory activities are positively correlated with bioactive compound content (Chen et al., 2017).

Juicy fruits such as pomegranate and cactus fruit have thick skins and numerous seeds, generating many by-products. Pomegranate by-

products (up to 40% of the whole) have a higher amount of phenolic compounds, which showed antioxidant, antibacterial, anti-allergic, and anti-inflammatory properties (Panichayupakaranant et al., 2010; Çam et al., 2014). For cactus fruit by-products, a high bioactive compound content was reported by Melgar et al. (2017), with the amount in peel being higher than in seeds. The correlation factors demonstrated a synergetic effect between the functional properties (antioxidant and antimicrobial) and the bioactive compound content. Likewise, the extracts showed no toxicity, making cactus fruit by-products a potential functional ingredient in the food industry.

Silva et al. (2014) evaluated the bioactive compounds in by-products from several Brazilian tropical fruits. The authors reported that in pineapple, acerola, cashew, guava, papaya, mango, and surinam cherry, the level of at least one of the four compounds analyzed (β-carotene, lycopene, anthocyanins, and yellow flavonoids) was higher in by-products than in pulp. Passion fruit showed no statistical difference between values, while the number of bioactive compounds was undetectable in monbin, soursop, and tamarind by-products. These findings support the potential commoditization of traditional and non-traditional fruit by-products rich in bioactive compounds.

2.2. Vegetable by-products

Vegetable handling, commercialization, and processing produces a significant amount of by-products. Vegetables are processed for different ends, including canning, juice, concentrate, jam, and fermented beverages. As with fruits, the by-products depends on the vegetables type. Vegetables can be classified according to which edible part of the plant is processed for human consumption. When different parts of the same plant are edible, they may fall into more than one category, e.g., roots and leaves of beetroot can be consumed.

In the case of edible flowers of certain vegetables, such as artichoke, broccoli, and cauliflower, the unprocessed part is considered a by-product. The TPC, total flavonoid content (TFC), and antioxidant activity of artichoke by-products vary widely by the bract positions of the artichoke head and the thermal treatments used (Ruiz-Cano et al., 2014). The fractions of most interest for use as functional ingredients are situated closest to the artichoke heart. Llorach et al. (2003) reported a high TPC in cauliflower by-products. The amount was higher in by-products than in edible parts, showing potential antioxidant activity. Interestingly, cauliflower by-product TPC was higher than that of apple and grape pomaces.

When an edible stalk is the central part of a vegetable, e.g., asparagus, the remainder of the plant is considered a by-product. Asparagus by-products are rich in many bioactive compounds, found in the discarded edible part of the spears. Compound extraction was optimized, and enhanced TPC and TFC were obtained, along with increased antioxidant activity. Given the antioxidant properties, regular use could aid in the prevention of several diseases related to oxidative damage in humans (Fuentes-Alventosa et al., 2013; Kabir et al., 2015).

Bulbs are vegetables that usually grow just below the ground's surface and produce a fleshy, leafy shoot above ground, often consisting of layers clustered segments, e.g., onions. Onion by-products from handling and processing and industrial onion seed production showed an exciting amount of bioactive compounds and remarkable antioxidant activity, along with providing an efficient anti-browning effect; thereby highlighting their potential use as functional ingredients (Fernández et al., 2018; Roldán et al., 2008).

Vegetables whose edible portion is a long or round-shaped taproot are classified as roots (e.g., carrots); while when they grow underground on a plant root they are known as tubers (e.g., potatoes). They are processed in a variety of ways —chopped, frozen, and canned— and processing begins with peeling and removal of the top and bottom, producing by-products. Carrots peel is rich in phenolic compounds and carotenoids (Seregelj et al., 2021). Kamiloglu et al. (2016) reported a high TPC and total anthocyanin content (TAC) in by-products from black carrot processing. They mentioned that phenolic compounds are one of the main contributors to extract antioxidant activity. Recent years have seen an increase in processed potato consumption in the form of cakes, croquettes, snacks, mash, dehydrated powder, and even prepared modified starch. Many tons of peel are generated as a result of this processing. TPC is higher in the peel than in the edible fleshy part, and the phenolic extracts showed considerable antioxidant activity and other functional properties (Akyol et al., 2016). Other varieties of colored potato, such as the Korean purple-fleshed sweet potato ('Shinzami' cultivar), showed a singular anthocyanin content (Kim et al., 2012).

The packaging process produces large amounts of waste and residues. These by-products may account for high percentages of the harvested material, as in the processing of edible plant leaves. Waste from the fresh-cut salad industry currently presents a serious environmental issue, but since by-products are highly perishable, management is not always easy. Llorach et al. (2004) obtained enriched phenolic extracts from lettuce and chicory by-products. They reported the presence of flavonoids, and both samples showed a very similar phenolic acid profile. The purified extracts showed remarkable antioxidant activity, higher than that obtained in another study with extracts from cauliflower by-products.

In several plants, the "vegetable" is a fleshy, seed-containing fruit. In these cases, processing by-products may be in the form of a pomace (e.g., tomato) or seeds (e.g., pepper). Tomato by-products have been extensively studied to assess recovery and identify and quantify phenolic compounds and their health benefits. The most abundant carotenoid is lycopene, which comprises 70%–80% of the TCC, and tomato peel contains nearly five times more lycopene than tomato pulp (Saini et al., 2018). Tomato by-products are also rich in phenolic compounds (Fernández et al., 2018). According to the correlation coefficients reported by Stajčić et al. (2015), TCC contributes significantly to the remarkable antioxidant activity of by-product extracts. Red pepper (chili) is commonly used in food recipes and consumed fresh, dried, pickled, or powdered. It generates many by-products, including seeds from red pepper paste or powder processing, which contain fatty acids (over 80% of which are unsaturated) and capsaicinoids (responsible for sensory qualities, i.e., heat). Red pepper peel is a good source of carotenoids such as β-Carotene (peel), essential for human health, and vitamin D, which has protective health benefits (Romo-Hualde et al., 2012; Silva et al., 2013).

Most studies on FVBP have focused on phenolic compounds and carotenoids and their subtypes: they are the most abundant, are widely distributed across plants, and can offer health benefits. However, other bioactive compounds, such as terpenes and alkaloids, are also naturally occurring, and these findings indicate a need for future research on their characterization and identification.

3. Encapsulation

Encapsulation technology has been widely used in the food industry to provide components that create the main characteristics (texture, taste, and color) as part of new product development. It now shows potential applications in obtaining functional ingredients incorporated into food to provide health benefits. Encapsulation is a process in which a core material is packed into food-grade wall material. It can come either in individual fractions or in mixtures of various components to obtain capsules with different properties (Chranioti & Tzia, 2015).

Nanoencapsulation and microencapsulation refer to capsule particle size, which ranges from 10 to 1000 nm (nano) and from 3 to 800 μm (micro), respectively (Sobel et al., 2014). These techniques can coat bioactive core compounds with wall materials to create capsules, which form an effective barrier against environmental and chemical interactions. Encapsulation allows for the controlled delivery of functional compounds to the target site, thereby improving their bioavailability and water solubility and increasing their stability (Davidov-Pardo et al.,

2013; Lauro et al., 2015; Lourenço et al., 2020).

3.1. Microencapsulation

More specifically, microencapsulation is a technique whereby various food ingredients —in the form of solid particles, liquid droplets, or gas bubbles— are stored in a microscopic-sized shell for protection and later release at controlled rates under specific conditions (Sobel et al., 2014). The primary objective of encapsulation is to efficiently protect certain substances (core materials), such as bioactive compounds sensitive to adverse environmental conditions such as light, temperature, humidity, oxygen, and variations in pH. Promoting an increased shelf-life of the product and allows for controlled release of the substances in question. Commonly-used wall materials include hydrocolloids (gum arabic, alginate, chitosan, pectin), carbohydrates (modified starch, maltodextrin, cyclodextrins), cellulose, proteins (casein, whey protein, gelatin, soy protein), and lipids (hydrogenated vegetable oils, phospholipids, mono- and triglycerides) (Vila et al., 2015).

A range of techniques has been developed and used for encapsulation in the food and pharmaceutical industries. The excellent process is selected for each specific variation of core substance, coating material, and intended final application. The primary difference between each methodology depends on the bioactive compound entrapment method and its combination with the wall material: it can be a solution, an emulsion, or dispersion, depending. Microencapsulation techniques can thus be classified into (i) physical methods (mechanical) such as spray-drying, freeze-drying, extrusion, fluid-bead coating, and processes using supercritical fluids; (ii) physicochemical methods including spray cooling, ionic gelation, solvent evaporation, liposome entrapment, and coacervation; and (iii) chemical methods such as interfacial polymerization, and molecular inclusion cross-linking (Fang & Bhandari, 2010; Garti & McClements, 2012; Ozkan et al., 2019).

Irreversible aggregations and the migration of bioactive compounds are core issues with microcapsules, and drying technologies present an excellent alternative to improve compound stability. They are widely used in the food industry to deliver emulsified food ingredients, such as lipophilic bioactive compounds. In the food industry, spray-drying and freeze-drying are the most commonly-used techniques to achieve the controlled release of functional compounds (Garti & McClements, 2012) and recover by-products from agro-industrial processes. The coacervation technique can be considered as the best encapsulation method, thanks to its high loading capacity, low temperature, improved thermal stability, and ability to provide a controlled release of active materials. Furthermore, it requires no specific equipment, and the procedure is simple; it uses non-toxic solvents and low agitation. However, unlike more automated processes, coacervation entails more preparation and training, and a great deal of care must be taken in order to ensure a successful outcome (Ozkan et al., 2019).

3.2. Encapsulation of bioactive compounds from FVBP by-products

In contrast to the traditional linear economy, by-products generated from agricultural and agro-industrial processes should be used as a source of bioactive compounds to develop high-value food products and reduce environmental pollution. A review of the literature yields examples of FVBP used as sources of bioactive compounds. Several of these studies began with identifying and quantifying possible nutraceutical agents but only went as far as research into profile characterization. In contrast, other studies continued on to encapsulation (Table 2), with the intent of isolate compounds of interest and improving their stability, solubility, bioactivity, and controlled release in possible applications as functional food ingredients.

Several studies have been carried out to obtain encapsulated phenolic compounds (EPC) from FVBP extracts. In most, only TPC has been considered as the core microcapsule agent in calculating encapsulation efficiency (EE) and encapsulation yield (EY). An integrated process based on extraction and encapsulation of phenolic compounds was proposed to recover FVBP from the Brazil nut (press cake residue), olive pomace, mill wastewater and leaves, pineapple peel, pomegranate peel, mango seed kernel, and yarrow (herbal dust from the filter tea industry) using spray-drying and freeze-drying (Table 2). The microcapsules showed the best overall results for TPC and TFC, and the addition of maltodextrin (MD) as an encapsulating agent —alone or blended with other materials— improved the physical properties, thus demonstrating this carbohydrate's remarkable efficacy in encapsulation. The best EE and EY results were achieved when several operating parameters —inlet temperature, wall material concentration, core/wall material (C/W) ratio, feed flow rate, compressed air flow rate, and drying air flow rate— were optimized. In EY, values of $<50\%$ were found when higher inlet temperature and C/W ratio were applied. Even when the same bioactive compound was encapsulated using the same drying technique, the authors reported EY variations: this could be explained by the effect of some operating parameters, such as feed solids concentration.

Various studies also assessed the bioavailability of EPC: Davidov-Pardo et al. (2013) reported a high release of phenolic compounds from encapsulated commercial grape seed extract (95% TPC) in simulated gastric and intestinal fluid. Encapsulation likewise provided adequate thermal stability to phenolic compounds. Saikia et al. (2015) obtained EPC from star fruit pomace. The *in vitro* gastrointestinal simulation digestion showed a more significant release of phenolic compounds in simulated gastric fluid ($pH = 1.2$) than in simulated intestinal fluid ($pH = 6.8$). EPC from red orange pomace was stable in the gastric environment, demonstrating that the encapsulation process improved phenolic compounds stability and prolonged shelf-life and antioxidant properties. In addition it maintained the phenolic extract's inhibitory effects on metalloproteinases activity (Lauro et al., 2015).

These studies demonstrate that it was possible to obtain microcapsules with a high phenolic compound retention using different drying methods. Their antioxidant properties are already well-known; therefore, the microcapsules obtained showed exceptional antioxidant activity In addition, the microcapsules showed satisfactory stability under storage conditions. The release of phenolic compounds in the gut is influenced by pH, and depends on the characteristics of each wall material used in the drying process. Encapsulation could thus protect the phenolic compounds while encouraging their controlled delivery and release during the digestion process.

In addition to TPC, other studies assessed the extraction, quantification, identification, and encapsulation of phenolic acids, flavonoids, anthocyanins, carotenoids, ascorbic acid, and prebiotics. Rezende et al. (2018) encapsulated an extract obtained from acerola pomace. They reported that the EE of phenolic compounds, carotenoids, and ascorbic acid was higher for freeze-dried microcapsules due to the use of high temperatures during spray-drying.

Anthocyanins are natural water-soluble compounds that give blackberries their typical color and which have been considered as potential substitutes for synthetic dyes in the food industry. Their chromophoric groups are highly sensitive to pH variations, and changes in pH impact stability. Several studies have been carried out to obtain encapsulated anthocyanins from berry by-products, such as blackberry and jabuticaba pomaces, by spray-drying and freeze-drying with different wall materials (Table 2). The microcapsules showed high anthocyanin retention, and the optimal value was reached when MD alone was used: this carbohydrate was efficient in reducing anthocyanin degradation. The spray-dried samples showed better stability at a lower pH, and longer anthocyanin half-life than non-encapsulated extracts. The microcapsules likewise showed higher antioxidant activity, and this was influenced by pH: at a lower pH, no significant difference was observed during storage. MD was thus the best wall material for anthocyanin protection. According to the morphological analyses of spray-dried microcapsules, the use of MD and gum arabic (GA) allowed more homogeneous particles to form, improving water solubility, and

Table 2
Encapsulated bioactive compounds from fruit and vegetable by-products.

Food by-product	Encapsulated bioactive compound	Wall Materials	Encapsulation Method	Main Findings	References
Acerola pomace	Phenolic compounds Carotenoids Ascorbic acid	Maltodextrin Gum arabic	Spray-drying Freeze-drying	Encapsulation efficiency up to 50% was reached.	Rezende et al. (2018)
Blackberry pomace	Anthocyanins	Maltodextrin	Spray-drying	The encapsulated anthocyanins half-life time was higher than non-encapsulated extract.	Santos et al. (2019)
			Freeze-drying	Better anthocyanin retention in microcapsules was obtained using MD DE10 than MD DE20	Yamashita et al. (2017)
Brazil nut cake extract	Phenolic compounds	Octenyl succinic anhydride-modified starch Inulin	Spray-drying	Stability and antioxidant activity of EPC with wall materials ratio (1:1) were maintained after 120 days.	Gomes et al. (2019)
Espresso spent coffee	Phenolic compounds Caffeine	Maltodextrin Whey protein isolate Gum arabic Inulin	Spray-drying	The combination of WPI and inulin demonstrated protection and stability of encapsulated bioactive compounds after 42 days.	Abrahão et al. (2019)
Grape skin and seeds	Anthocyanins	Maltodextrin	Spray-drying Freeze-drying	Spray-dried samples showed higher anthocyanin retention values than freeze-dried samples.	de Souza et al. (2015)
Grape skin	Anthocyanins	Maltodextrin Gum arabic	Freeze-drying	High encapsulation efficiency and anthocyanin retention up to 89.6% and 91.5%, respectively.	Stoll et al. (2016)
	Anthocyanins	Gum Arabic Polydextrose Partially hydrolyzed guar gum	Spray-drying Freeze-drying	Spray-dried samples had better physical characteristics when compared to freeze-dried samples.	Kuck and Noreña (2016)
Grape seeds	Phenolic compounds	Maltodextrin Mesquite gum Zein	Spray-drying	The releases in simulated gastric and intestinal fluid were 71% and 63%, respectively. The EPC did not show degradation up to 180 °C.	Davidov-Pardo et al. (2013)
Jaboticaba pomace	Anthocyanins	Maltodextrin Gum Arabic Modified starch	Spray-drying	The use of MD alone (30%) at higher inlet temperature (180 °C) showed the higher anthocyanin retention.	Silva et al., (2013)
		Maltodextrin Pectin Soy protein isolate	Freeze-drying	The encapsulation process was able to stabilize and extend shelf-life of anthocyanins.	Souza et al. (2017)
Mango seed kernels	Phenolic compounds	Maltodextrin Gum Arabic Gelatin Sodium alginate	Spray-drying	The combination 5.95% GA, 23.9% MD and 0.11% sodium alginate showed the best stability and encapsulation efficiency of EPC.	Maisuthisakul and Gordon (2012)
Oat bran	Phenolic compounds Xylooligosaccharides	Maltodextrin Whey protein concentrate	Complex coacervation	The xylooligosaccharides showed prebiotic properties. The release percent of EPC was ranged from 70% to 83% after 2 h of digestion.	Bannikova et al. (2020)
Olive pomace	Phenolic compounds	Maltodextrin	Spray-drying	Optimal operation parameters showed high recovery of phenolic compounds, remarkable antioxidant activity and good stability under storage.	Aliakbarian et al. (2018)
Olive mill wastewater	Phenolic compounds	Maltodextrin Skimmed milk powder	Spray-drying	High encapsulation efficiency (93.7%) with optimal operation parameters was reached.	Goula and Lazarides (2015)
Olive leaf	Phenolic compounds	Maltodextrin Trehalose dehydrate	Freeze-drying	The wall materials and the C/W ratio had a significant effect on the encapsulation efficiency and the antioxidant, thermal, physical and microstructural properties of EPC.	González-Ortega et al. (2020)
Orange peel oil	Phenolic compounds	Maltodextrin Gelatin	Freeze-drying	High encapsulation efficiency (75.75%) and encapsulation yield (90.19%). All encapsulated oil samples showed antioxidant and antibacterial properties.	de Araújo et al. (2020)
Pepper seeds oil	Polyunsaturated fatty acids	Starch sodium octenyl succinate Soy protein isolated Gelatin Maltodextrin	Spray-drying	The loading efficiency of the microcapsules (up to 94.35%) was reached. The starch sodium octenylsuccinate and MD formulation displayed the best physicochemical results.	Wang et al. (2017)
		Maltodextrin Gum arabic		Encapsulated pepper seed oil showed high thermal stability and antimicrobial properties.	Karaaslan et al. (2021)
Pepper seeds, skin leftovers, and stems	Vitamins (A and E)	Gum Arabic Tween 80	Spray-drying	High encapsulation efficiency for vitamin E (73.4%) and provitamin A (77.1%). The stability of encapsulated provitamin A was higher than free provitamin A over storage time (35 days) at temperature room.	Romo-Hualde et al. (2012)
Pineapple peel	Phenolic compounds	Maltodextrin Inulin Gum Arabic	Spray-drying	The EPC showed good stability and antioxidant activity during six months of storage at 5 °C.	Lourenço et al. (2020)
Pomegranate peel	Phenolic compounds	Maltodextrin Skimmed milk powder Whey protein isolate Gum arabic	Spray-drying	The highest encapsulation efficiency (99.80%) was reached when MD/WPI (50:50), and optimal operating parameters were used.	Kaderides et al. (2015)

(*continued on next page*)

Table 2 (*continued*)

Food by-product	Encapsulated bioactive compound	Wall Materials	Encapsulation Method	Main Findings	References
Pomegranate seeds	Polyunsaturated fatty acids	Whey protein isolate Gum arabic	Complex Coacervation	Microcapsules produced with polymer concentration (5%) and C/W ratio of 2.75 showed highest punicic acid content and oil retention.	Costa et al. (2020)
Red orange pomace	Phenolic compounds Anthocyanins	Cellulose acetate phthalate Tween 60 Tween 80	Spray-drying	The non-encapsulated extracts showed an incomplete *in vitro* dissolution rate in simulated biological fluids, probably due to their low solubility.	Lauro et al. (2015)
Star fruit pomace	Phenolic compounds	Maltodextrin	Spray-drying Freeze-drying	Encapsulation efficiency was higher for freeze-dried (97.2%) than for spray-dried samples (79.07%).	Saikia et al. (2015)
Yarrow herbal dust	Phenolic compounds	Maltodextrin	Spray-drying	The formulation with the lowest amount of MD (5%) showed the high values for bioactive compounds content, and antioxidant activity.	Vladić et al. (2016)

EPC: Encapsulated phenolic compounds, MD: Maltodextrin, GA: Gum Arabic, DE: Dextrose equivalent, WPI: Whey protein isolate, C/W: Core/wall material.

paving the way for use in beverages as a natural colorant. On the other hand, freeze-dried microcapsules had irregular structures with different sizes and low water solubility. Regardless of drying method, it was possible to obtain natural colorants with antioxidant properties and a good shelf-life. The stability at lower pHs allows them to be used as a dye with antioxidant properties in various foods, such as yogurt, beverages, juices, soft drinks, jams, and jellies.

Grape by-product encapsulation has been the subject of much study due to the high TPC and TAC. In a study performed by de Souza et al. (2015), the anthocyanin retention of spray-dried microcapsules was significantly influenced by inlet temperature and MD concentration; with higher temperatures, retention increased. Stoll et al. (2016) reported that malvidin-3-glucoside was the predominant anthocyanin, and the microcapsules obtained with GA showed the highest antioxidant activity. The EPC obtained using a combination of partially-hydrolyzed guar gum and polydextrose as wall material also showed high TPC and anthocyanins retention and notable antioxidant activity (Kuck & Noreña, 2016). In general, the microcapsules showed high EE and anthocyanin retention, independent of the type of wall material used, and the spray-drying technique has been shown to be superior: spray-dried samples had better results than freeze-dried samples, and favorable characteristics —such as low moisture content, water activity (aw), particle size, and hygroscopicity, along with high solubility and color stability— making this by-product useful as a natural food additive. These properties bode well for future technological application in food matrices.

Bannikova et al. (2020) encapsulated the TPC and xylooligosaccharides from oat bran using the complex coacervation technique. In the encapsulating agent, the use of a higher proportion of whey protein concentrate (WPC) than MD results in the highest EE. During the simulated digestion *in vitro*, full release of the core material was achieved in simulated intestinal conditions, further highlighting microcapsule structural stability in the gastric stage. The prebiotic activity of xylooligosaccharides was verified based on the growth dynamics of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*). The ability of encapsulation techniques to turn oat bran into functional ingredients was thus proven, opening up the possibilities for food products with bifidogenic properties. The presence of MD also improved WPC efficiency as a carrier of phenolic compounds. Among the advantages of using whey protein, we can highlight the ability to control the release rate of small molecules at different pH values. In the encapsulation of phenolic compounds from spent espresso processed by spray-drying, wall material type did not influence TPC, caffeine, and chlorogenic acid (5-CQA) retention. However, the whey protein isolate (WPI) alone effectively maintained the antioxidant activity compared with other wall material combinations (Abrahão et al., 2019). The encapsulation of phenolic compounds using whey protein thus preserves antioxidant activity while enabling incorporation into functional foods.

Polyunsaturated fatty acids are another bioactive compound with functional properties related to nerve function, blood clotting, brain health, and energy storage. They are thus considered “essential”: the body needs them to function, and they must be obtained as part of the diet. Unfortunately, the chemical structure of essential oils allows them to be easily oxidized. Wang et al. (2017) encapsulated red pepper seed oil —rich in polyunsaturated fatty acids and capsaicinoids— by spray-drying to protect them against oxidation. The use of a combination of MD and starch sodium octenyl succinate as wall material allows for a high EE. Costa et al. (2020) evaluated the encapsulation of pomegranate seed oil —rich in conjugated linolenic acids— by complex coacervation followed by spray-drying as a hardening step. An acceptable EE (>60%) was reached, positively influenced by the C/W ratio, and using WPI and GA as wall materials. Encapsulated polyunsaturated fatty acids were thus successfully obtained using different encapsulating agents.

Essential oils are known for their antimicrobial properties: De Araújo et al. (2020) reported the effective growth inhibition of *Staphylococcus aureus* and *Escherichia coli* using encapsulated essential oil extracted from orange peel. This inhibitory effect could be attributed to monoterpenes (ex., *D-limonene*) ordinarily found in essential oils. Meanwhile, Karaaslan et al. (2021) recently showed that pepper seed oil encapsulated with GA and MD was highly inhibitive against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. Encapsulation also served to protect pepper seed oil against oxidation during storage. These results confirmed that essential oils encapsulated from by-products can be used to produce functional ingredients with potential food product application.

Provitamin A and vitamin E were extracted from red pepper by-products and encapsulated by spray-drying using GA as wall material, and high values of EE (>70%) were achieved. Free vitamin E was stable during storage at room temperature, but high losses were observed in provitamin A. In contrast, neither of the encapsulated vitamins showed significant differences ($p > 0.05$) during storage. According to the thermogravimetric analysis results, no vitamin degradation was observed up to 200 °C. Encapsulation was thus proven to increase vitamin thermal stability, adding to the list of potential functional ingredients (Romo-Hualde et al., 2012).

Various studies have used complex coacervation followed by a drying technique as the second stage of the encapsulation process to facilitate microcapsule storage and future application (Costa et al., 2020; Oancea et al., 2018). However, it is not yet known if original coacervate structure is maintained during spray-drying: the high pressure and turbulent flow may break particle structure without altering microcapsule morphology (Costa et al., 2020). On the other hand, during freeze-drying, a possible double encapsulation, mediated by whey proteins and with a rounded outer surface, could allow for coacervates (Gheonea (Dima) et al., 2021).

Based on the correlation coefficient, bioactive compound content generally showed a positive correlation with encapsulated extract antioxidant activity, being higher for the TPC; thus, they are responsible for this functional property. The microcapsules showed high water

solubility, which would be essential for future application in aqueous food matrices, such as beverages. It was therefore possible to encapsulate the same bioactive compounds using different wall materials, such as gums, protein, and natural and modified polysaccharides, using different encapsulation methods. While FVBP currently have little or no commercial value, these findings clearly demonstrate them to be a potential source of valuable ingredients. The encapsulation process would allow for their incorporation into food, resulting in innovative products with functional properties.

4. Food application of EBC from FVBP: case studies/examples

In the food industry, functional components are encapsulated for later incorporation into processed foods. Various studies have been conducted on food application; however, other essential parameters have not been fulfilled, such as, for example, a complete delivery system analysis. Several examples of food applications of EBC from FVBP are shown in Table 3.

EPC from pomegranate peel extract is the by-product ingredient most commonly used to improve stability and functional properties in a variety of foods. Çam et al. (2014) enriched the formulation of ice cream with EPC. The ice cream's functional properties were improved, along with antioxidant and α-glucosidase inhibitory activities. During the sensory evaluation, the trained panelists detected no astringency in enriched ice creams: i.e., the product showed potential market acceptance. Kaderides et al. (2015) incorporated EPC into hazelnut paste, and the degree of oxidation was determined by measuring the peroxide value under accelerated storage conditions (60 °C) over 51 days. All the samples containing phenolic compounds (both encapsulated and non-encapsulated) had lower peroxide values than the control sample. The addition of EPC thus inhibited lipid oxidation, resulting in reduced peroxide formation and improved hazelnut paste shelf-life, despite the limited solubility of the non-encapsulated extract. The study performed by Hamid et al. (2020) demonstrated the antioxidant and antibacterial activities of EPC. Microcapsules were added in a ready-to-serve mango drink, and sensory acceptance was reported. Further enriched drinks showed significant improvement in phenolics, flavonoids, and antioxidant activity compared to the control sample.

Some fruit by-products can be used as wall materials to encapsulate bioactive compounds extracted from natural sources or industrial by-products. Kaderides et al. (2020) used orange juice by-product powder as a "green" wall material to encapsulate phenolic extract from pomegranate peel by spray-drying. The phenolic compounds (encapsulated and non-encapsulated) were then incorporated in cookies at a concentration of 0.5% (w/w) as functional supplements. The baking procedure caused a TPC loss of approximately 65% and 76% for the enriched and control samples, respectively, showing that in enriched cookies, many phenolic compounds were degraded, despite their coating. Nevertheless, the addition of phenolic compounds had a significant effect ($p < 0.05$) on the antioxidant activity retention of enriched cookies. The antioxidant activity level remained high and was significantly better than that of the control cookies during storage. In terms of oil oxidation, enriched cookies were highly stable. Results moreover showed that pomegranate peel EPC could be incorporated into cookies without negatively affecting sensory quality, at the same time as they provided food products with functional properties and storage stability.

Table 3
Food applications of encapsulated bioactive compounds from fruit or vegetal by-products.

Food by-product	Encapsulated bioactive compound	Wall materials	Encapsulation method	Food product	Bioactivity	References
Beetroot pomace	Phenolic compounds Betacyanins	Soy protein isolate	Freeze-drying	Pseudocereals-enriched einkorn wheat water biscuits	Antioxidant	Hidalgo et al. (2018)
Brewers spent grain	Flavonoids	Modified starch	Spray-drying	Fish burgers	Antioxidant	Spinelli et al. (2016)
Carrot waste	β-Carotene	Sodium alginate	Electrostatic extrusion	Yogurt	Antioxidant Antimicrobial	Šeregelj et al. (2021)
Cocoa hulls	Phenolic compounds	Maltodextrin Gum arabic	Spray-drying	Biscuits	Antioxidant	Papillo et al. (2019)
Eggplant peel	Phenolic compounds	Maltodextrin Gum arabic	Spray-drying	Gummy candies	Antioxidant	Sarabandi et al. (2019)
Grape skin	Phenolic compounds	Maltodextrin	Spray-drying	Apple puree	Antioxidant	Lavelli et al. (2016)
Grape skin	Anthocyanins	Maltodextrin	Freeze-drying	Biodegradable film for food packaging	Antioxidant	Stoll et al. (2017)
Grape seeds	Phenolic compounds Carvacrol	Chitosan	Ionic gelation	Biodegradable film for food packaging	Antioxidant Antimicrobial	Alves et al. (2018)
Grape seeds	Phenolic compounds	Whey protein concentrate Gum arabic	Spray-drying	Yogurt	Antioxidant	Yadav et al. (2018)
Jaboticaba peel and seeds	Phenolic compounds	Sodium alginate Chitosan	Ionic gelation	Cassava starch biscuits	Antioxidant	de Cássia Sousa Mendes et al. (2021)
Pomegranate peels	Phenolic compounds	Maltodextrin	Spray-drying	Ice cream	Antioxidant α-Glucosidase inhibition	Çam et al. (2014)
				Hazelnut paste	Antioxidant	Kaderides et al. (2015)
				Cookies	Antioxidant	Kaderides et al. (2020)
			Freeze-drying	Mango drink	Antioxidant Antibacterial	Hamid et al. (2020)
Sour cherry pomace	Phenolic compounds	Whey protein isolate Soy protein isolate	Freeze-drying	Cookies	Antioxidant	Šaponjac et al. (2016)
Sour cherry skin	Anthocyanins	Whey protein isolate Gum arabic	Coacervation and Freeze-drying	Fermented milk	Antioxidant Antibacterial Bioavailability	Oancea et al. (2018)
Tomato peel	Lycopene	Whey protein isolate Gum arabic	Coacervation and Freeze-drying	Salad dressing	Antioxidant α-Amylase and α-glucosidase inhibition	Gheonea (Dima) et al. (2021)

In the study performed by Tumbas Šaponjac et al. (2016), EPC from sour cherry pomace were also incorporated in cookies to replace 10% and 15% of flour content. According to the retention of bioactive compounds, TPC and anthocyanin baking stability was better when lower amounts of EPC were added. The enriched cookies showed higher TPC values than the control cookies during storage, and as expected, the antioxidant activity of control cookies was significantly ($p < 0.05$) lower than that of enriched cookies. The replacement of flour with EPC improved the functional value and color of the cookies and enhanced storage stability for these properties. The enriched cookies also received satisfactory acceptance following a sensorial analyses.

Beetroot pomace —a by-product of juice preparation— is rich in phenolic compounds and betalains. Betanin is concentrated in the red beetroot peel and is the only betalain approved for use in food. Red beetroot pomace extract (encapsulated and non-encapsulated) was added to einkorn water biscuits enriched with pseudocereal flour. The addition of antioxidant-rich microcapsules in pseudocereal-enriched blends improved TPC and antioxidant properties. Pseudocereal-enriched water biscuits thus showed the highest amounts of betanin, isobetanin, TPC, and antioxidant activity (Hidalgo et al., 2018).

In a similar fashion, cocoa hull EPC was incorporated into model biscuits. The enriched sample showed the best stability, maintaining high TPC and antioxidant activity following baking (Papillo et al., 2019). Recently, De Cássia Sousa Mendes et al. (2021) used jaboticaba pomace EPC in cassava starch biscuits. Following to the visual observations of expansion, the starch biscuit best suiting the experiment was chosen. Once the homogenous biscuit dough was obtained, 0.1 g of the microcapsules were added. When the dough reached the desired temperature (26 °C), the biscuits were shaped and baked in an electric oven at 180 °C for 20 min. The results show that encapsulation improves the heat stability of phenolic compounds, thereby mitigating the effects of baking, while highlighting the potential of by-products to enrich bakery products with new bioactive properties.

The spray-drying encapsulation of phenolic extracts from the brewery industry's spent grain residue was studied to determine optimal operating conditions. EPC were then added as an ingredient in fish burgers, which were prepared following a previously optimized recipe using minced fish mixed with 5% EPC. These were cooked in an electric convection oven at 180 °C for 15 min. The sensorial evaluation generally showed that in terms of quality (tenderness, juiciness, and taste), the cooked burgers were accepted. Spent brewery grain could be a potential functional ingredient because the analysis revealed a higher TPC and TFC than the control burger (without EPC). These bioactive compounds are responsible for the far higher antioxidant potential found in cooked burgers (48.49%) as compared to control samples (21.19%) (Spinelli et al., 2016).

The addition of phenolic compound-rich extracts to cooked foods, such as biscuits, cookies, and burgers, is impossible due to their easy thermal degradation and oxidation. Encapsulation techniques can coat extracts in an encapsulating agent, creating barriers that protect the compounds of interest from adverse environmental conditions. Incorporating EPC from FVBP left over from the manufacturing of juice and canned foods thus offers a novel alternative for food enrichment, and encapsulation improves the shelf-life stability of phenolic compounds and their functional properties.

Encapsulated anthocyanins from sour cherry skin were incorporated into fermented milk, and their prebiotic effect on the probiotic strain was tested. The encapsulating agent protected the anthocyanins from *in vitro* gastric digestion, facilitating their release into the intestine. The microcapsules also stimulated the growth of *Lactobacillus casei* during the storage period. The number of viable cells in the control sample decreased notably (78%) compared to the enriched fermented milk, where a much lower reduction (26%) was observed. As expected, the color parameters indicate a predominant reddish color of enriched fermented milk (Oancea et al., 2018). Aside from their prebiotic effect, these microcapsules could be used as a natural coloring for fermented beverages. Yadav et al. (2018) fortified a yogurt with encapsulated grape seed extract rich in phenolic compounds. The microcapsules were added to milk prior to fermentation, and the fortified yogurt showed similar sensory properties to the control sample (without phenolic extract). During storage, the physicochemical properties, TPC content, and antioxidant activity of the encapsulated-fortified yogurt were unaffected as compared to non-encapsulated-fortified yogurt. Furthermore, a notable increase in TPC and antioxidant activity was observed in encapsulated-fortified yogurt. The decrease in viable count of the yogurt starters (*Streptococcus thermophilus* and *Lactobacillus bulgaricus* subsp. *delbrueckii*) followed similar trends for all samples during storage at 4 °C, i.e., the addition of grape seed extract (encapsulated and non-encapsulated) had no effect on the viability of the yogurt starter cultures compared to the control samples. According to the CIELab color parameters values, the addition of encapsulated grape seed extract resulted in fortified yogurt color characteristics similar to the control. Recently, Šeregelj et al. (2021) evaluated yogurt fortification via the addition of encapsulated carotenoids from carrot waste in the traditional formulation. Two β-carotene rich concentrations were added after the fermentation stage. The fortified yogurt provides the recommended daily intake of β-carotene, and the microbiological and physicochemical properties remained stable during the storage period. In addition, the fortified yogurt samples showed a yellow coloration, which is correlated with the release of β-carotene from microcapsules over the storage period. According to the bioactivity analysis results, the yogurt fortified with β-carotene showed antioxidant, antihyperglycemic, and anti-inflammatory activity. In contrast, the control sample (commercial yogurt) showed no bioactivity.

These studies have found that fortification of yogurt with encapsulated natural antioxidants such as phenolics, anthocyanins, and carotenoids results in considerable improvement of its antioxidant and nutritional properties, and incorporation prior to or following the fermentation process had no effect on starter culture viability. In addition, the microcapsules conferred coloration to yogurt from bioactive compounds, acting as a natural additive. The functional value of fermented beverages can thus be enhanced by adding EBC, supporting their use as functional ingredients in food or nutraceuticals. This will aid in making the consumption of enriched fermented beverages more effective in reducing diseases associated with nutritional deficiencies, while exploiting food by-products rich in bioactive compounds.

Lavelli et al. (2016) incorporated EPC from grape skin into apple puree as a natural colorant with functional properties to substitute synthetic dyes and make use of the health benefits of bioactive compounds. The enriched samples were exposed to 95 °C to simulate a pasteurization/sterilization process and model the effectiveness of heat treatment, and the degradation of anthocyanins and non-colored phenolic compounds was assessed during heat processing and storage. The phloridzin, phloretin xyloglucoside, flavonols and proanthocyanidins remained stable in enriched apple puree, even following the most intensive treatment. However, extended exposure to high temperatures affected anthocyanin stability. Prior to application of the most intensive treatment, anthocyanins and antioxidant activity retention were 72% and 67%, respectively. During a 1-month storage period at 15–35 °C, the addition of EPC increased the content and stability of monomeric and dimeric flavanols and proanthocyanidins, and enriched apple puree continued to retain phenolic compounds, showing that encapsulation ensures processing and storage stability for bioactive compounds. We can conclude that encapsulated grape skin is suitable as a functional colorant and ingredient in food products, with added health benefits.

The encapsulation of eggplant peel extract was studied by Sarabandi et al. (2019). The microcapsules with the highest TPC and antioxidant activity were used to production gummy candy. The non-encapsulated and encapsulated eggplant peel phenolic extract were added at different levels at the final preparation step when the ingredient mixture dropped to 40 °C, and the sample without the extract was used as a control. Based on the sensorial analysis results, the use of EPC in fortified

gummy candy obtained the highest color score and resulted in an attractive red color in the product. Neither the non-encapsulated nor the encapsulated enriched samples affected the hardness, gumminess, and chewiness of candy. Panelists did not report any perception of astringency or unpleasant taste in the product prepared with 0–1.5% EPC, which was primarily attributable to the carbohydrates and gelatin used in the candy manufacturing process (these compounds serve to mask the astringent taste caused by the phenolic compounds of microcapsules). The addition of EPC from eggplant peel therefore improved color properties and overall product acceptability, meaning that it could be useful as a natural coloring with antioxidant properties in functional food formulation including sauces, chocolates, jelly, ice cream, candy products, and instant drink powders.

Recently, Gheonea (Dima) et al. (2021) added encapsulated lycopene into salad dressing preparations. It was added in different percentages during the homogenization phase (10 min) of dressing ingredients into sunflower oil. A sample without microcapsules was used as a control, and during 14 days of storage at 4 °C, all samples were analyzed for lycopene stability and antioxidant activity. The samples supplemented with 1% and 5% of microcapsules exhibited 3.5 and 14-times higher lycopene content than the control, respectively, meaning that antioxidant activity in the enriched samples was also higher than the control—thereby demonstrating the added value of the encapsulated lycopene via higher antioxidant activity. The rheological measurements carried out within 3 h after preparation showed the salad dressing samples to have typical solid-like behavior. Lycopene microcapsules could therefore be used as functional ingredients in food formulations.

Growing consumer concern for the environment has resulted in food packaging manufactured from natural sources, such as polysaccharides, proteins, and lipids. Natural and synthetic additives with antioxidant or antimicrobial properties can also be included in the biodegradable material matrix to prolong the shelf-life of packaged foods. Stoll et al. (2017) assessed the effect of an active biodegradable film, enhanced with encapsulated grape skin anthocyanins, on the quality of extra-virgin olive oil under accelerated storage conditions (heat and light). Compared to a commercial polypropylene, olive oil packaged in the enriched film pouches showed good oxidation stability under accelerated thermal and photo-oxidative conditions. According to the limits established by Codex Alimentarius, olive oil quality was maintained for over eight days, while oil packed in polypropylene pouches degraded prior to the 4th day of storage.

Alves et al. (2018) encapsulated grape seed extract and carvacrol using chitosan as an encapsulating agent. The microcapsules were incorporated into chitosan films, and their effect on physicochemical and microbiological properties was tested using salmon. The active chitosan film showed a lower water solubility value than the chitosan control film, which contributes to its application in food packaging. The salmon packed in active chitosan film and stored at 5 °C maintained luminosity values closer to fresh salmon and showed the lowest value of total volatile basic nitrogen and pH for a more extended storage period (up to seven days). The growth of the microorganisms in active chitosan films was slower than in the chitosan control film and (non-packaged) control samples. In general, microbial tests showed that salmon packed in active chitosan films showed lower bacterial count values and did not exceed the allowed maximum limit through 4–7 days of storage. The antimicrobial effect of the bioactive compounds in active chitosan films thus increases refrigerated salmon's shelf-life during storage.

Taken together, these studies demonstrated that EBC —such as phenolics, anthocyanins, and essential oils— could guarantee food products adequate protection against oxidative and microbial deterioration when incorporated in biodegradable films, and proved that their role in developing active packaging showed promise. Active biodegradable films could also be used in packaging for other types of food, such as bread, cheese, and meat. Active packaging could serve as a sustainable alternative to non-biodegradable materials, such as petroleum-based plastics, and it presents an attractive way to add value to food processing by-products while increasing food shelf-life.

5. Food application of EBC from FVBP: critical aspects

Recent studies have shown that FVBP contains considerable amounts of bioactive compounds. The main objective of the encapsulation technique is to increase their stability under the adverse temperature, light, humidity, and oxygen conditions present in food industry processing and storage. Unfortunately, the bioactive compounds extraction yield from FVBP is generally low, which may explain why research largely fails to include the application of EBC.

There is no encapsulation method that is technology-specific or recommended for each bioactive compound: several studies used two or three different technologies to encapsulate bioactive compounds and compare them (especially for the drying process). At times, there were significant differences in the encapsulation efficiency and loss of core material during the encapsulation processing (process yield) depending on the technology used. Although we can still recommend using encapsulation technology in these cases, the particular version used may not be the most appropriate. The differences did not depend only on the bioactive compound. Instead, they have more to do with the wall materials and operating parameters used: i.e., if other process conditions were used, the chosen technology might not be the most appropriate. For these reasons, studies at times include a second stage to optimize the encapsulation technology. Almost all studies have chosen encapsulation technology based on previous studies on similar compounds or equipment availability and costs. In specific cases, the methodology is chosen at random.

Certain studies, including toxicological assays of EBC from FVBP or novel food products, are necessary. These analyses must be performed to guarantee new additive safety. Likewise, the issue of relationships between food matrix structural components should be considered, along with the theoretical explanation of the release process. Future studies are thus necessary to investigate the interaction of bioactive compounds and encapsulating agents with other food product components.

Numerous works on the application of EBC from FVBP have been published in indexed journals or books. However, many studies examine the food matrix application of bioactive compounds that are non-encapsulated or are not obtained from by-products, for this reason, they are not included in this review. Montibeller et al. (2018) added anthocyanins extracted from grape by-products as a natural coloring in kefir and carbonated water and assessed their storage stability under light exposure. The results showed good anthocyanin retention and half-life time ($t_{1/2}$) of food products during storage. Utpott et al. (2020) extracted fiber from red pitaya by-products and applied used it strawberry ice cream as a fat substitute. The fiber addition improved the overrun and rheological properties of ice cream, showing a 73.5% reduction in fat. The ice cream also had a high overall acceptability, making it possible to develop an attractive alternative that reduced fat while increasing nutritional value.

The anthocyanin-rich barberry extract was encapsulated and used as a natural coloring in jelly powder, as an alternative to synthetic additives. The jelly sample tinted using encapsulated anthocyanins had sensory acceptability and physicochemical assessments that were higher, even, than the commercial jelly containing synthetic coloring. The wall materials tested in this study provided better protection and enhanced the storage stability of anthocyanins (Akhavan Mahdavi et al., 2016). Tupuna-Yerovi et al. (2020) incorporated encapsulated norbixin from annatto seeds into an isotonic tangerine soft drink as a natural colorant, without exceeding food additive regulations. According to color parameters, the beverage acquired the expected intense orange shade. The addition of encapsulated norbixin positively affected beverage stability during storage under accelerated heat and light conditions—and as a result, the $t_{1/2}$ was significantly higher (29.71 days) than beverage samples with non-encapsulated norbixin (393.39 min).

In the case of studies regarding the food application of bioactive

compounds extracted from FVBP without previous encapsulation (free), the results obtained could have been improved thanks to the protection conferred by wall materials as a barrier against adverse conditions. As far as the food application of EBC extracted from non by-products, the main difference is the number of bioactive compounds. As was detailed in section two of this review, many studies on the chemical characterization of fruit and vegetables reported a higher number of bioactive compounds in by-products than the edible part of the fruit or vegetable.

6. Conclusions

The encapsulation of bioactive compounds from FVBP is a promising alternative for the use of agro-industrial waste as an ingredient in the food industry. Several studies have demonstrated that bioactive compounds are extractable from by-products, and their stability and solubility can be improved with the use of appropriate encapsulation techniques and wall materials. The products obtained could offer health benefits, such as antioxidant, antimicrobial, anti-inflammatory properties and enzymatic inhibition. To date, more encapsulation and application studies have been carried out on bioactive compounds extracted from by-products from fruits than from vegetables. A wide variety of encapsulated bioactive compounds have been developed, characterized, and successfully applied in food. The use of encapsulation has increased in the food industry in recent years, but the industrial application of EBC is still far from being fully developed. Extraction process yield is low and various procedures are required to purify the compound of interest, and the technology is costly. R&D in food science and technology must merge food industry constraints and requirements to make by-product encapsulation viable—from the extraction of bioactive compounds through their application in the final product.

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Verónica Marcillo-Parra: Conceptualization, Investigation, Writing – original draft, Funding acquisition. **Diego Santiago Tupuna-Yerovi:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Zulay González:** Investigation, Writing – original draft. **Jenny Ruales:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

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7. DISCUSSION, GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

During this study, some results were achieved concerning the characterization of mango peels, extraction, and encapsulation of mango peel extracts, as well as evaluating the thermal stability of the encapsulates.

In the characterization from mango peels (cvs. Tommy Atkins, Haden, and Kent), as well as the determination of their antioxidant properties, it was found that all samples were a great source of dietary fiber (28 - 40%); phenolic compounds (2930 - 6624 mg GAE/100 g); flavonoids (502 - 795 mg CE/100 g); and carotenoids (3.7 - 5.7 mg TC/100 g). ABTS antioxidant activity ranged from 23 - 54 mM Trolox/100 g. The mango variety and growing location are likely responsible for the significant differences in the amounts of these bioactive compounds and their antioxidant activity. In addition, two essential carotenoids (β-carotene and lutein) were identified and quantified in all samples, whereas mangiferin was the important phenolic compound in cv. Tommy Atkins, and it was not detected in cv. Kent mango peels.

Of the methods (conventional solvent extraction and ultrasound-assisted extraction using 50% methanol, 50% ethanol, and 70% acetone as extraction solvents) evaluated to obtain extracts rich in phenolic compounds from mango peels (cv. Tommy Atkins), ultrasound-assisted extraction using 50% methanol presented a higher content of phenolics as gallic acid, epicatechin, rutin, quercetin and mangiferin (e.g., it was three-time more) compared to conventional extraction. In turn, the extracts also showed high antioxidant activity (337 µmol Trolox/100 g).

On the other hand, carotenoid extracts from mango peels were also obtained by ultrasound-assisted extraction. These extracts were encapsulated by spray drying. In the first stage, the encapsulation was carried out with different blends of maltodextrin (MD) and gum arabic (GA) as wall materials and different inlet air drying temperatures (IT), with the addition of fructooligosaccharides

(FOS), mainly to evaluate the effect on encapsulation efficiency (EE) and determinate encapsulation yield (EY). Thus, the formulation with a higher proportion of gum arabic than maltodextrin (MD:GA 15:85) at 165 °C (IT) showed the highest EE (74.71%), and in turn, the lowest EY (33%). However, a high EE (67.91% - 123.06 µg Total carotenoids/g of encapsulated) and better EY (35%) was also obtained with (MD:GA 50:50) at 140 °C (IT), which was chosen to evaluate the effect of the addition of prebiotics as inulin and FOS. There was no statistical difference ($p < 0.05$) in EE (~65%) and water solubility index (>86%) between samples. The microencapsulated carotenoid extract (MCE) showed a microparticle diameter (<4.0 µm), bimodal particle size distribution, and low moisture content (<3.7%). In addition, the majority of MCE showed a spherical form with a smooth surface and without fissures.

Finally, the formulation MD:GA (50:50) with inulin at 140 °C (IT) was selected to study thermal stability at 5, 25, and 45 °C for 45 days. Thermal degradation kinetics followed a first-order kinetic reaction. The activation energy (*Ea*) required for degradation of MCE (*Ea* = 3.41 kcal/mol) was twice as high as that needed for carotenoids non-encapsulated (*Ea* = 1.33 kcal/mol). Furthermore, the encapsulated did not show a total color difference ($\Delta E < 2.6$), and the antioxidant activity was preserved from 47% to 92% at different storage temperatures.

About the results obtained in this research, the main conclusions are mentioned below:

- Ecuadorian mango peels are interesting by-products of mango processing which contain a fair amount of well-balanced dietary fiber and can be considered as excellent sources of bioactive compounds as β-carotene and lutein (carotenoids), gallic acid (phenolic acids), mangiferin (xanthones), and rutin and quercetin (flavonoids).
- The method of ultrasound-assisted extraction (UAE) using 50% methanol (polar organic solvent) significantly increased the recovery of phenolic compounds from mango peels. This extract also presented a high antioxidant activity. It confirmed the suitability of the UAE for the preparation of antioxidant-rich mango peel extracts.

- It is possible to encapsulate carotenoids extract from mango peel using the spray drying technique and a combination of maltodextrin and gum arabic with inulin as an encapsulating agent. The process MD:GA (50:50) - inulin at 140 °C (inlet temperature) allowed high encapsulation efficiency. MCE powders had good solubility and significant morphological characteristics, and they also enhanced storage stability and extended the shelf life of this bioactive product.
- These microencapsulated carotenoid extracts showed good functionality. Thus, they can be used in foods, for example, to fortify dairy products, fruit and vegetable beverages, among others.

Their bioaccessibility and bioavailability condition the impact of encapsulated carotenoid extracts from mango peels on health, hence the importance and opportunities for future research, which also comprises investigation about the development of technological strategies to incorporate MCE powders in different food matrices without impairing their functional and sensory properties.

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