UNIVERSITAT POLITÈCNICA DE VALÈNCIA
DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS
INDUSTRIAL DOCTORATE MENTION

Encapsulation of Underexploited Bioactives using high-throughput Electrospray Assisted by Pressurized Gas with Application Interest in Functional Foods and Nutraceuticals

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CERTIFY:

That the work ***"Encapsulation of Underexploited Bioactives using high-throughput Electrospray Assisted by Pressurized Gas with Application Interest in Functional Foods and Nutraceuticals"*** has been developed by Juan David Escobar García under their supervision in ***Bioinicia S.L. and IATA***, as an Industrial Thesis Project in order to obtain the degree of PhD in Food Technology at the ***Universitat Politècnica de València.***

Valencia, December 2024.

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ACKNOWLEDGMENTS

A DIOS Y A MI FAMILIA

A Dios, por ser mi refugio, mi guía y mi fortaleza. Gracias por darme la capacidad de soñar, la fuerza para perseverar y la luz que me ha sostenido incluso en los momentos más desafiantes. Sin tu presencia, este logro no habría sido posible.

A mi esposa. Gracias por tu apoyo incondicional, tu paciencia y tu fe en mí, incluso cuando yo dudaba de mis propias fuerzas. Este logro es tan tuyo como mío.

A nuestros tres hijos, la mayor inspiración y fuerza diaria. Sus sonrisas, su alegría y su amor puro son el motor que me impulsa a seguir creciendo y a construir un futuro mejor. Espero que encuentren en este logro un ejemplo de vida: que, con fe, trabajo arduo y perseverancia, los sueños pueden hacerse realidad.

A mis padres, que me enseñaron el valor del esfuerzo, la integridad y la humildad. A mi hermana y hermano, por ser compañeros de vida y recordarme siempre lo que realmente importa. A toda mi familia, cuya fe y apoyo incondicional han sido el cimiento de cada uno de mis logros.

Victoria Para Quienes Perseveran

Iniciar una obra es cosa relativamente fácil, basta con avivar un poco la lumbre del entusiasmo.

Perseverar en ella hasta el éxito, es cosa diferente; eso ya es algo que requiere continuidad y esfuerzo.

Comenzar está al alcance de los demás; continuar, distingue a los hombres de carácter.

Por eso la médula de toda obra grande, desde el punto de vista de su realización práctica, es la perseverancia, virtud que consiste en llevar las cosas hasta el final.

Es preciso, pues, ser perseverante, formarse un carácter no sólo intrépido, sino persistente, paciente, inquebrantable.

Sólo eso es un carácter.

El verdadero carácter no conoce más que un lema: la victoria.

Y sufre con valor, con serenidad y sin desaliento, la más grande de las pruebas: la derrota.

La lucha tonifica el espíritu, pero cuando falta carácter, la derrota lo reprime y desalienta.

Hemos nacido para luchar.

Las más grandes victorias corresponden siempre a quienes se preparan, a quienes luchan y a quienes perseveran.

Anónimo

ACKNOWLEDGMENTS

A DIOS Y A MI FAMILIA

Salmo 138

Dios está en todas partes y lo ve todo

Señor, tú me sondeas y me conoces; me conoces cuando me siento o me levanto, de lejos penetras mis pensamientos; distingues mi camino y mi descanso, todas mis sendas te son familiares.

No ha llegado la palabra a mi lengua, y ya, Señor, te la sabes toda. Me estrechas detrás y delante, me cubres con tu palma. Tanto saber me sobrepasa, es sublime, y no lo abarco.

¿Adónde iré lejos de tu aliento, adónde escaparé de tu mirada? Si escalo el cielo, allí estás tú; si me acuesto en el abismo, allí te encuentro; si vuelo hasta el margen de la aurora, si emigro hasta el confín del mar, allí me alcanzará tu izquierda, me agarrará tu derecha.

Si digo: "que al menos la tiniebla me encubra, que la luz se haga noche en torno a mí", ni la tiniebla es oscura para ti, la noche es clara como el día.

Tú has creado mis entrañas, me has tejido en el seno materno. Te doy gracias, porque me has escogido portentosamente, porque son admirables tus obras; conocías hasta el fondo de mi alma, no desconocías mis huesos.

Cuando, en lo oculto, me iba formando, y entretejiendo en lo profundo de la tierra, tus ojos veían mis acciones, se escribían todas en tu libro; calculados estaban mis días antes que llegase el primero.

¡Qué incomparables encuentro tus designios, Dios mío, qué inmenso es su conjunto! Si me pongo a contarlos, son más que arena; si los doy por terminados, aún me quedas tú.

Señor, sondéame y conoce mi corazón, ponme a prueba y conoce mis sentimientos, mira si mi camino se desvía, guíame por el camino eterno.

ABSTRACT

The demand for functional foods, dietary supplements, nutraceuticals and functional ingredients has significantly increased due to the growing prevalence of chronic diseases and a societal shift toward preventive health solutions. However, incorporating bioactive compounds into end-consumer products presents several challenges, including their vulnerability to degradation from environmental stressors and their limited bioavailability. Encapsulation is a promising technological solution to address these challenges, enhancing stability and preserving bioactivity.

This doctoral research delves into the transformative potential of Electrospraying Assisted by Pressurized Gas (EAPG) technology for the encapsulation of underutilized bioactive compounds. The study highlights three particularly bioactives concretely eicosapentaenoic acid (EPA), an omega-3 fatty

acid; Dragon's blood sap (DBS), a natural resin known for its diverse bioactive properties; and camu camu extract (CCX), a nutrient-rich fruit extract known for its high vitamin C and polyphenol content.

In the first chapter, the encapsulation of EPA using EAPG technology emerged as a promising strategy to mitigate oxidative degradation and organoleptic impact, which are the main challenges associated with omega-3 oils. Integrating emulsions with the EAPG process for encapsulating EPA within whey protein concentrate (WPC) resulted in nanostructured microparticles that showed a marked improvement in oxidative stability, as evidenced by an accelerated oxidative stability test conducted under UV light. Furthermore, an organoleptic evaluation using reconstituted powdered milk confirmed a notable reduction in undesirable off-flavors, even more significant when combined with DHA encapsulates.

The second chapter deals with the characterization, drying, and encapsulation of Dragon's Blood Sap (DBS). The storage stability and photo-oxidation of DBS were initially assessed since environmental factors can degrade its functional components and significantly reduce its therapeutic potential. Notable degradation was observed with exposure to UV light and high humidity, underscoring the importance of implementing protective strategies to preserve its bioactive properties. To enhance stability, as a first approximation, EAPG and freeze-drying were compared to dry DBS. Both techniques preserved functionality; however, EAPG sample provided better morphology and stability. Secondly, DBS was encapsulated within WPC and zein (ZN) using the EAPG technique. The EAPG encapsulation method resulted in spherical microparticles, offering superior protection against UV light and environmental stressors in comparison with the neat bioactive, being the stability enhanced for the ratio 2:1, and more specially when ZN was used as encapsulating matrix.

The third chapter dealt with the drying and encapsulation and characterization of camu camu extract (CCX). The drying of CCX via EAPG produced irregular, non-uniform, and agglomerated structures with the

antioxidant activity and total soluble polyphenols content preserved in comparison to the neat CCX. The encapsulation of CCX within WPC and ZN resulted in the formation of spherical microparticles that exhibited enhanced stability under the studied storage conditions compared to non-encapsulated CCX, particularly at a 2:1 ratio of encapsulant to bioactive compounds. demonstrated remarkable protection against the studied storage conditions, including high humidity and UV radiation.

The findings illustrate the remarkable versatility and wide-ranging industrial applications of EAPG technology. This innovative approach enables the production of uniform microparticles at room temperature, ensuring that the bioactivity of sensitive, heat-labile compounds is preserved throughout the process. Through this research, the essential role of the studied encapsulation materials, such as whey protein concentrate (WPC) and zein (ZN), has been highlighted as crucial for developing customized stabilization solutions tailored to different bioactive substances. Beyond its technological advancements, this doctoral research introduces a versatile, scalable, and cost-effective methodology to the field of encapsulation science, laying the groundwork for the development of functional foods and dietary supplements with extended shelf life, enhanced bioavailability, and improved consumer acceptance. These advancements align with the growing demand for clean-label products and cater to a health-conscious consumer base, paving the way for new product formulation and development opportunities.

RESUMEN

La demanda de alimentos funcionales, suplementos dietéticos, nutracéuticos e ingredientes funcionales ha aumentado significativamente debido a la creciente prevalencia de enfermedades crónicas y a un cambio social hacia soluciones de salud preventiva. Sin embargo, la incorporación de compuestos bioactivos en productos de consumo presenta diversos desafíos, como su vulnerabilidad a la degradación por factores ambientales y su limitada biodisponibilidad. La encapsulación se presenta como una solución tecnológica prometedora para abordar estos desafíos, mejorando la estabilidad y preservando la bioactividad.

Esta investigación doctoral explora el potencial transformador de la tecnología de Atomización Electro hidrodinámica Asistida por Gas Presurizado (EAPG) para la encapsulación de compuestos bioactivos infrautilizados. El estudio se centra en tres compuestos bioactivos específicos: el ácido eicosapentaenoico

(EPA), un ácido graso omega-3; la savia de sangre de drago (DBS), una resina natural conocida por sus diversas propiedades bioactivas; y el extracto de camu camu (CCX), un extracto de fruta rico en nutrientes, con alto contenido de vitamina C y polifenoles.

En el primer capítulo, la encapsulación del EPA mediante la tecnología EAPG surgió como una estrategia potencial para mitigar la degradación oxidativa y el impacto organoléptico, principales desafíos asociados con los aceites omega-3. La integración de emulsiones con el proceso EAPG para encapsular EPA en concentrado de proteína de suero (WPC) resultó en micropartículas nanoestructuradas que mostraron una mejora notable en la estabilidad oxidativa, como lo demostró un ensayo acelerado de estabilidad oxidativa realizado bajo luz ultravioleta. Además, una evaluación organoléptica utilizando leche en polvo reconstituida confirmó una reducción significativa de sabores indeseables, aún más pronunciada cuando se combinó con encapsulados de DHA.

El segundo capítulo aborda la caracterización, secado y encapsulación de la savia de sangre de drago (DBS). Inicialmente, se evaluó la estabilidad de almacenamiento y la foto-oxidación de DBS, ya que los factores ambientales pueden degradar sus componentes funcionales y reducir significativamente su potencial terapéutico. Se observó una degradación notable con la exposición a luz ultravioleta y alta humedad, subrayando la importancia de implementar estrategias de protección para preservar sus propiedades bioactivas. Para mejorar su estabilidad, como primera aproximación, se compararon el EAPG y la liofilización para secar DBS. Ambas técnicas preservaron la funcionalidad; sin embargo, las muestras procesadas por EAPG presentaron mejor morfología y estabilidad. Posteriormente, DBS fue encapsulado en WPC y zeína (ZN) mediante la técnica EAPG. En comparación con el compuesto bioactivo sin encapsular, éste método produjo micropartículas esféricas que ofrecieron una protección superior contra la radiación ultravioleta y el estrés ambiental, destacando la estabilidad mejorada para la proporción 2:1, especialmente cuando se utilizó ZN como matriz de encapsulación.

El tercer capítulo aborda el secado, encapsulación y caracterización del extracto de camu camu (CCX). El secado del CCX mediante EAPG produjo estructuras irregulares, no uniformes y aglomeradas, preservando la actividad antioxidante y el contenido de polifenoles solubles totales en comparación con el CCX sin procesar. La encapsulación del CCX en WPC y ZN resultó en la formación de micropartículas esféricas que demostraron una mayor estabilidad bajo las condiciones de almacenamiento estudiadas en comparación con el CCX no encapsulado, particularmente en una proporción 2:1 de encapsulante a compuesto bioactivo. Estas micropartículas ofrecieron una notable protección contra las condiciones de almacenamiento, incluida alta humedad y radiación ultravioleta.

Los hallazgos ilustran la notable versatilidad y las amplias aplicaciones industriales de la tecnología EAPG. Este enfoque innovador permite la producción de micropartículas uniformes a temperatura ambiente, asegurando que la bioactividad de compuestos sensibles al calor se preserve a lo largo del proceso. A través de esta investigación, se ha destacado el papel esencial de los materiales de encapsulación estudiados, como el WPC y la ZN, como elementos cruciales para el desarrollo de soluciones de estabilización personalizadas, adaptadas a diferentes sustancias bioactivas. Más allá de los avances tecnológicos, esta investigación doctoral introduce una metodología versátil, escalable y rentable al campo de la ciencia de la encapsulación, sentando las bases para el desarrollo de alimentos funcionales y suplementos dietéticos con una vida útil extendida, mayor biodisponibilidad y mejor aceptación por parte del consumidor. Estos avances están alineados con la creciente demanda de productos con etiquetas limpias y responden a un mercado de consumidores conscientes de la salud, abriendo el camino a nuevas oportunidades en la formulación y desarrollo de nuevos productos.

RESUM

La demanda d'aliments funcionals, suplements dietètics, nutracèutics i ingredients funcionals ha augmentat significativament a causa de la creixent prevalença de malalties cròniques i un canvi social cap a solucions de salut preventiva. No obstant això, la incorporació de compostos bioactius en productes de consum final presenta diversos desafiaments, com ara la seua vulnerabilitat a la degradació per factors ambientals i la seua limitada biodisponibilitat. L'encapsulació es presenta com una solució tecnològica prometedora per abordar aquests desafiaments, millorant l'estabilitat i preservant la bioactivitat.

Aquesta investigació doctoral explora el potencial transformador de la tecnologia de Electrospray Assistit per Gas Pressuritzat (EAPG) per a l'encapsulació de compostos bioactius infrautilitzats. L'estudi se centra en tres compostos bioactius específics: l'àcid eicosapentaenoic (EPA), un àcid gras

omega-3; la saba de sang de drago (DBS), una resina natural coneguda per les seues diverses propietats bioactives; i l'extracte de camu camu (CCX), un extracte de fruit ric en nutrients, amb alt contingut en vitamina C i polifenols.

En el primer capítol, l'encapsulació de l'EPA mitjançant la tecnologia EAPG va sorgir com una estratègia potencial per a mitigar la degradació oxidativa i l'impacte organolèptic, principals desafiaments associats amb els olis omega-3. La integració d'emulsions amb el procés EAPG per encapsular EPA en concentrat de proteïna de sèrum (WPC) va resultar en micropartícules nanostructurades que van mostrar una millora notable en l'estabilitat oxidativa, com ho va demostrar un assaig accelerat d'estabilitat oxidativa realitzat sota llum UV. A més, una avaluació organolèptica utilitzant llet en pols reconstituïda va confirmar una reducció significativa de sabors indesitjables, encara més pronunciada quan es va combinar amb encapsulats de DHA.

El segon capítol tracta sobre la caracterització, l'assecat i l'encapsulació de la saba de sang de drago (DBS). Inicialment, es va avaluar l'estabilitat d'emmagatzematge i la foto-oxidació de la DBS, ja que els factors ambientals poden degradar els seus components funcionals i reduir significativament el seu potencial terapèutic. Es va observar una degradació notable amb l'exposició a llum UV i alta humitat, subratllant la importància d'implementar estratègies de protecció per preservar les seues propietats bioactives. Per a millorar la seua estabilitat, com a primera aproximació, es van comparar l'EAPG i la liofilització per assecar DBS. Ambdues tècniques van preservar la funcionalitat; tanmateix, les mostres processades per EAPG van presentar millor morfologia i estabilitat. Posteriorment, DBS es va encapsular en WPC i zeïna (ZN) mitjançant la tècnica EAPG. Aquest mètode va resultar en micropartícules esfèriques que van oferir una protecció superior contra la llum UV i l'estrès ambiental en comparació amb el compost bioactiu sense encapsular, destacant l'estabilitat millorada per a la proporció 2:1, especialment quan es va utilitzar ZN com a matriu d'encapsulació.

El tercer capítol tracta sobre l'assecat, encapsulació i caracterització de l'extracte de camu camu (CCX). L'assecat del CCX mitjançant EAPG va produir

estructures irregulars, no uniformes i aglomerades, preservant l'activitat antioxidant i el contingut de polifenols solubles totals en comparació amb el CCX sense processar. L'encapsulació del CCX en WPC i ZN va resultar en la formació de micropartícules esfèriques que van demostrar una major estabilitat sota les condicions d'emmagatzematge estudiades en comparació amb el CCX no encapsulat, particularment en una proporció 2:1 d'encapsulant a compost bioactiu. Aquestes micropartícules van oferir una notable protecció contra les condicions d'emmagatzematge, incloent alta humitat i radiació UV.

Els resultats il·lustren la notable versatilitat i les àmplies aplicacions industrials de la tecnologia EAPG. Aquest enfocament innovador permet la producció de micropartícules uniformes a temperatura ambient, assegurant que la bioactivitat de compostos sensibles a la calor es preserve al llarg del procés. A través d'aquesta investigació, s'ha destacat el paper essencial dels materials d'encapsulació estudiats, com el WPC i la ZN, com a elements crucials per al desenvolupament de solucions de estabilització personalitzades, adaptades a diferents substàncies bioactives. Més enllà dels avenços tecnològics, aquesta investigació doctoral introdueix una metodologia versàtil, escalable i rendible al camp de la ciència de l'encapsulació, establint les bases per al desenvolupament d'aliments funcionals i suplements dietètics amb una vida útil prolongada, major biodisponibilitat i millor acceptació per part del consumidor. Aquests avenços s'alineen amb la creixent demanda de productes amb etiquetes netes i responen a un mercat de consumidors conscients de la salut, obrint el camí a noves oportunitats en la formulació i desenvolupament de nous productes.

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1. PREAMBLE

The research performed in this PhD thesis involved four key projects, each one contributing to advancements in encapsulation technology, with specific objectives aligned to address social needs and food industry requirements.

The **FODIAC** project (H2020-MSCA-RISE-2017-778388), funded by the European Union, aimed to develop dietary solutions for managing diabetes and supporting cognitive function in the elderly. This project focused on leveraging bioactive ingredients, functional foods, and specialized diets to address these health concerns effectively.

The **BIOENCAP** project (INNCAD00-18-31), funded by the Valencian Innovation Agency (AVI), focused on the development and up -scaling of a novel electrohydrodynamic atomization technology for nano- and microencapsulation of high-value products, and more concretely eicosapentaenoic acid (EPA) rich oils to target the food and pharmaceutical markets, addressing the stability issues of polyunsaturated fatty acids.

The **CAPSULTEK** project (H2020-EIC-SMEinst-2018-2020-SME instrument-873827), supported by the European Union, was dedicated to scaling up the electrospray microencapsulation technology to effectively dry and encapsulate thermolabile active ingredients, with an annual production capacity of 10 tons. This was based on the patented electrospraying assisted by pressurized gas (EAPG) technology.

Lastly, the **BIOPAPERTECH** project (PID2021-128749OB-C31), funded by MCIN/AEI and co-financed by the European Regional Development Fund (ERDF), focuses on creating recyclable, sustainable, and safe flexible hybrid paper bioplastic packaging. The objective is to enhance functionality and biodegradability and reduce the environmental footprint of food packaging within a Circular Bioeconomy framework.

In this context, this doctoral thesis focused on developing and applying the EAPG technology for encapsulating and stabilizing thermolabile and oxidation-sensitive bioactive compounds such as EPA-rich oils and phenolic-rich botanical

extracts such as dragon's blood sap (DBS) and camu camu (CCX), to improve their stability, functionality, and sensory properties for the development of functional ingredients with well-known health benefits with potential use in the development of commercial bioactive ingredients for their potential use in the context of food, pharmaceutical, and nutraceutical industries.

2. GENERAL INTRODUCTION

1. Functional Foods, Dietary Supplements and Nutraceuticals Importance

Throughout history, food has served roles beyond basic nutrition, contributing to cultural practices, religious beliefs, and social structures. However, in today's world, chronic diseases, such as cardiovascular disease, diabetes, obesity, and age-related illnesses, have increased its incidence due to factors including an aging population, sedentary lifestyles, poor dietary habits, and environmental pollution (Tiwari A & Balasundaram P., 2023). Consequently, a shift toward preventive health solutions has prompted (WHO, 2020). Thus, the expectations of food have expanded further, with a growing social demand for products that not only meet nutritional needs but also provide health benefits and support well-being (Damián et al., 2022). This social demand is aligned with global objectives such as the United Nations Sustainable Development Goals (SDGs), particularly SDG 3, to ensure healthy lives and promote well-being for all at all ages (United Nations, 2024).

The previous objective is related also to the concept of "One Health", which emphasizes the interdependence of human health, animal health, and environmental sustainability, advocating for comprehensive solutions that address the needs of these three domains (Zinsstag et al., 2011). In line with this approach, sustainable packaging has emerged as a crucial element in reducing the environmental impact of food systems (Kirchherr et al., 2017). Sustainable packaging solutions are designed to minimize resource use and waste generation. By incorporating active properties, such as antimicrobial and antioxidant agents or oxygen scavengers, these packaging solutions can enhance food safety and extend the shelf life of food products, thereby reducing food waste and enhancing supply chain efficiency (Alves et al., 2023).

This global situation is reflected in the current societal demands in the food market, such as more natural, additive-free, convenient, and sustainable products (Hassoun et al., 2022; Mintel, 2024). These demands align with consumer interest in health benefits and address the global shift toward sustainable and preventive

health solutions. As highlighted in recent studies, including FAO research (FAO, 2022), these preferences emphasize food safety, transparency, and their role in achieving health and sustainability goals. Consequently, there is a growing interest in novel food products that provide specific health benefits. Within this category, functional foods, dietary supplements, nutraceuticals, and bioactive ingredients have gained prominence, aiming to promote overall well-being, prevent chronic diseases, and address specific health conditions (Fanzo et al., 2023; A. Vignesh et al., 2024).

2. Market Overview and Segmentation Analysis

The market for functional foods, nutraceuticals, dietary supplements, and bioactive ingredients is experiencing substantial growth, driving the expansion of the market. This expansion is noticeable across various regions, with significant market potential in Europe and Spain. Table 1 outlines key market projections for each segment, including the expected market size by 2030 and the compound annual growth rate (CAGR) for the Global, European, and Spanish markets. This growth is fueled by the rising consumer interest in products that enhance overall health and well-being, particularly considering the heightened awareness of the role of nutrition in preventing and managing health conditions, especially during the COVID-19 pandemic.

Table 1. Segment Market Data Summary by 2023. Size (U$D Millions) and Compound Annual Growth Rate -CAGR- 2023–2030 (%).

Market Segment	Global		Europe		Spain	
	Size	CAGR	Size	CAGR	Size	CAGR
Functional Foods	586.069.2	8.6	168.553.5	8.6	12.793.2	7.3
Dietary Supplements	327.420.1	9.1	65.435.0	7.0	3.880.3	7.8
Nutraceuticals	599.706.1	9.5	129.536.5	9.5	8.031.2	9.6
Bioactive Ingredients	317.906.1	7.9	66.161.8			

(Fortune Business Insights, 2024a, 2024b, 2024c, 2024d, 2024e; Grand View Research, 2024c, 2024d, 2024a, 2024b, 2024e, 2023a, 2023b; Markets and Markets, 2023, 2024a, 2024b; Wunsch, 2024a, 2024b)

To better understand the characteristics of these market segments, the Overview of Products and Applications, illustrated in Table 2, provides a detailed breakdown of common products and applications that define each category. Combining these insights provides a comprehensive view of how the market is evolving and imparts a deeper understanding of how these trends translate into tangible product offerings. Together, these analyses emphasize the potential for innovation and highlight the demand for high-quality ingredients that deliver targeted health benefits, such as immune support, cardiovascular health, and healthy aging.

Table 2. Overview of Common Products and Applications of Bioactive Ingredients by Market Segments.

Market Segment	Common Products	Applications
Functional Foods	• Cereals • Dairy Products • Fortified Oils • Meat & Fish Products • Soy Products	• Digestive Health • Immune Support • Sports Nutrition • Weight Management
Nutraceuticals	• Dietary Supplements • Functional Beverages (such as Energy Drinks, Fortified Juices)	• Cardiovascular Health • Cognitive Function • Healthy Aging • Immune System Support
Dietary Supplements	• Capsules • Liquids • Powders • Soft Gels • Tablets	• Bone & Joint Health • Energy Management • General Health • Immune Health • Prenatal Support

(Fortune Business Insights, 2024a, 2024b, 2024c, 2024d, 2024e; Grand View Research, 2024c, 2024d, 2024a, 2024b, 2024e, 2023a, 2023b; Markets and Markets, 2023, 2024a, 2024b; Wunsch, 2024a, 2024b)

However, these categories' definitions vary widely across regions and regulatory bodies. Over the past few decades, evolving consumer awareness and scientific advancements have shaped the definitions and regulatory frameworks for functional foods, dietary supplements, and nutraceuticals (Food and Drug Administration, 2023). Table 3 illustrates the development of global definitions and regulatory bases for functional foods, dietary supplements, and

nutraceuticals, outlining key definitions and showcasing the variations in interpretations regarding health claims, scope, and regulatory status across different markets. By delineating these terms, the table also emphasizes the growing importance of these products in modern healthcare and nutrition strategies, representing a shift from treating diseases to preventing them through diet and supplementation. Understanding these definitions is important for placing functional foods, dietary supplements, and nutraceuticals within the wider context of food science, health policy, and consumer trends.

Table 3. Evolution of Global Definitions and Regulatory Frameworks for Functional Foods, Dietary Supplements and Nutraceuticals in chronological order.

Year, Region & Organism	Definition
1990 **Australia & New Zealand** **Australian Government**	Dietary supplements and nutraceuticals are regulated under the Therapeutic Goods Act of 1989 (Australian Government, 1990). Functional foods are not defined as a separate category under Australian law. Instead, foods that make health-related claims are regulated through Standard 1.2.7, which governs general and high-level health claims. These claims must be scientifically substantiated and approved by the relevant regulatory authority (Australian Government, 2016).
1991 **Japan** **Ministry of Health, Labour, and Welfare**	Foods for Specified Health Use (FOSHU) are foods that contain ingredients with specific health benefits and are approved by the Japanese government to claim their health-promoting benefits. These foods are consumed as part of a normal diet (Ministry of Health, 1991).
1994 **USA** **Office of Dietary Supplements - National Institutes of Health**	According to the Dietary Supplement Health and Education Act, Dietary supplements are products intended to supplement the diet, containing one or more ingredients such as vitamins, minerals, herbs, amino acids, or other substances to increase dietary intake (ODS - NIH, 1994).
1995 **China** **State Food and Drug Administration**	Health foods is a term used in China for foods with special health functions or vitamins and minerals or for special targeted populations to adjust body functions. These foods are not for the treatment of diseases and should not cause any acute, subacute or chronic hazards to human body (Zawistowski, 2011).
1998 **Canada** **Health Canada**	Functional foods are similar in appearance to conventional foods, consumed as part of the usual diet, and provide health benefits and/or reduce the risk of chronic diseases beyond basic nutrition. Nutraceuticals are products isolated or purified from foods, sold in medicinal forms not usually associated with food, that provide a physiological benefit or protection against chronic disease (Health Canada, 1998).

2002 **Europe** **European Parliament**	Food supplements are concentrated sources of nutrients with a nutritional or physiological effect, marketed in dose forms like capsules, tablets, powders, and liquids, designed to supplement the diet (European Parliament, 2002).
2002 **Korea** **Ministry of Food and Drug Safety**	Health functional food refers to food manufactured or processed in tablet, capsule, powder, or liquid forms, containing ingredients with functionality useful for the human body (Health Functional Food Code, 2021).
2006 **India** **Ministry of Law and Justice**	Foods for specific dietary uses, functional foods, or nutraceuticals are designed for particular dietary requirements due to specific conditions or disorders, containing ingredients like vitamins, minerals, and botanicals (Food Safety and Standards Act, 2006).
2006 **Europe** **European Food Safety Authority**	Functional foods are not officially categorized as a separate group under EU law, but health claims on food, including functional foods, are regulated by the EFSA under the EU Health Claims Regulation. (Regulation EC 1924, 2006).

3. Functional Foods

The concept of functional foods was born in Japan. In the 1980s, health authorities in Japan recognized that an improved quality of life must accompany increasing life expectancy for the expanding number of elderly people if healthcare costs were to be controlled (Day et al., 2009). The term "functional food" refers to foods or food ingredients that go beyond basic nutrition to provide additional physiological benefits that may help maintain normal health, improve physical or cognitive performance, or support weight management. According to the European Food Safety Authority (EFSA), functional foods are those for which health claims suggest that specific nutrients or ingredients contribute to maintaining normal health or performance or assist in weight control (EFSA, n.d.). Similarly, organizations such as the International Food Information Council define functional foods as offering health-promoting benefits beyond traditional nutritional value (International Food Information Council, 2020). Globally, the definition of functional foods lacks a standardized legal framework, and they are generally defined as foods integrated into the regular diet that extend beyond

fundamental nutrition by promoting health (Dj Nevena et al., 2022; Mellentin, 2020).

Functional foods are divided into three general categories based on how these are prepared:

- **Conventional foods** are whole, unmodified foods, such as vegetables and fruits, fish, dairy, and grains, naturally containing bioactive components that confer health benefits (Crowe & Francis, 2013).
- **Modified foods** are normal foods that have been enhanced, enriched, or fortified with functional food components, such as fruit juices with calcium, folate-enriched breads, and beverages with plant extracts.
- **Functional food ingredients** range from macronutrients, essential micronutrients needed at higher levels, or non-nutrient components like phytochemicals, which are derived from plants, microorganisms, marine organisms, and other inorganic raw materials (Crowe & Francis, 2013; Hasler & Brown, 2009).

4. Nutraceuticals and Dietary Supplements

In contrast, nutraceuticals and dietary supplements offer concentrated bioactive components and nutrients, typically administered in pill or powder form; they provide targeted health benefits that surpass those derived from regular dietary intake (Adefegha, 2018; Dickinson, 2011; Dudeja & Gupta, 2017; M. Pandey et al., 2014; Pastrana et al., 2017). The term "nutraceutical," derived from the terms nutrition and pharmaceutical, was created in 1989 by Dr. Stephen De Felice, founder and chairman of the Foundation for Innovation in Medicine (FIM) (Dudeja & Gupta, 2017). Hence, a "nutraceutical" is any substance that may be considered a food or part of a food that provides medical or health benefits, encompassing prevention and treatment of diseases (Cencic & Chingwaru, 2010).

Nutraceuticals have recently garnered significant interest due to their potential nutritional, safety, and therapeutic benefits. They can enhance health,

prevent chronic diseases, slow aging, or maintain overall bodily functions and integrity (A. Vignesh et al., 2024). They are valuable resources for preventing life-threatening conditions such as diabetes, renal and gastrointestinal disorders, and infections. Additionally, they provide indications for managing diseases related to oxidative stress, including allergies, Alzheimer's disease, cardiovascular diseases, cancer, eye conditions, Parkinson's disease, and obesity (M. Pandey et al., 2014).

5. Bioactive Ingredients

Bioactive ingredients, also known as bioactive compounds, are a family of compounds, including proteins, minerals, polyunsaturated fatty acids, vitamins, polyphenols, probiotics, and prebiotics, widely recognized for their significant direct and indirect impacts on human health and their extensive therapeutic benefits (Al-Dujaili & Al-Dujaili, 2015; Montserrat-De la Paz, 2021). They are also responsible for the health-promoting properties of the food products.

Bioactive compounds are sourced from a variety of natural origins. Proteins, essential amino acids, and polyunsaturated fatty acids can be derived from fish, nuts, and seeds, while minerals and vitamins are abundant in fruits, vegetables, and dairy products (Dolganyuk et al., 2023). Polyphenols, known for their antioxidant properties, are commonly found in berries, tea, and certain spices (K. B. Pandey & Rizvi, 2009). Probiotics and prebiotics, beneficial for gut health, are typically derived from fermented foods and specific dietary fibers, respectively (Dahiya & Nigam, 2022).

In the following lines, special attention is paid to polyphenols and polyunsaturated fatty acids due to their antioxidant and anti-inflammatory action and their preventive lifestyle preventive diseases:

5.1. Polyphenols

Polyphenols are natural antioxidants with various biological attributes, such as anti-aging, anti-inflammatory, anti-viral, anti-microbial, and anti-cancer properties (Fernandes et al., 2018; Scalbert et al., 2005). They are crucial for health

because they neutralize free radicals responsible for many degenerative diseases. For that reason, a great number of research activities in the field of health-related dietary aspects have demonstrated a significant link between the regular intake of phytochemicals (e.g., polyphenols, carotenoids, phytosterols) and the prevention of lifestyle-related diseases, such as cancer, obesity, diabetes, and cardiovascular complications (Fernandes et al., 2018; Gresele et al., 2011).

Additionally, these compounds arouse the interest of the food industry as food preservatives since they decrease product deterioration caused by oxidation, reduce the nutritional loss of the food, and improve the conservation of their organoleptic characteristics, pigment, color, and odor (Martysiak-Żurowska & Wenta, 2012).

Plants and, more concretely, medicinal plants are considered readily available sources of polyphenols (Bhatt et al., 2013). In recent years, certain underutilized plants with high levels of bioactive compounds have gained scientific and commercial interest due to their potential health benefits and applications in the food industry. ***Myrciaria dubia***, commonly known as camu camu, is an Amazonian fruit with one of the highest recorded levels of natural vitamin C, along with significant amounts of polyphenols, flavonoids, and other phytochemicals (García-Chacón et al., 2023). These compounds exhibit strong antioxidant, anti-inflammatory, and immunomodulatory properties, which could support the prevention of oxidative stress-related diseases and enhance overall health (Langley et al., 2015). Due to its high antioxidant potential, camu camu shows promise as a functional ingredient in foods and dietary supplements aimed at health-conscious consumers.

Another notable example is Dragon's blood sap, extracted from trees of the Croton genus, particularly ***Croton lechleri***, native to the Amazon rainforest. This reddish resin is rich in bioactive compounds such as taspine, proanthocyanidins, and diterpenoids, which possess remarkable anti-inflammatory, antimicrobial, wound healing, and antioxidant properties (Jones, 2004). Traditionally used in indigenous medicine, Dragon's Blood sap is now being studied for its potential

applications in pharmaceuticals and functional foods, where its bioactive components could act as natural preservatives and health enhancers (Peres et al., 2023). Despite their promising properties, these plants remain underutilized in mainstream food and nutraceutical industries, presenting opportunities for further exploration and sustainable development in functional food innovation.

Polyphenols suffer from chemical instability, susceptibility to environmental factors like heat, light, and oxygen, and limited bioavailability, often compromising their therapeutic and functional efficacy (Bartosz & Irene, 2016).

5.2. Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) are essential fats critical in maintaining human health, particularly in cardiovascular, cognitive, anti-inflammatory, eye health, and immune system functions, as well as their benefits for prenatal and maternal wellness (GOED, 2024). These fatty acids, which include omega-3 and omega-6 families, cannot be synthesized by the human body and must be obtained through diet (Calder, 2015). Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are predominantly found in marine sources like fish oils and algae. In contrast, plant sources, including flaxseeds and walnuts, are rich in alpha-linolenic acid (ALA) (Simopoulos, 2002). Omega-3 PUFAs possess anti-inflammatory, anti-thrombotic, and anti-arrhythmic properties, which contribute to preventing cardiovascular diseases such as atherosclerosis and hypertension (Simonetto et al. (2019). Omega-6 PUFAs, such as linoleic acid (LA) and arachidonic acid (AA), are essential for cell membrane integrity, skin health, and immune response modulation (Calder, 2015). However, an imbalanced dietary ratio of omega-6 to omega-3 fatty acids has been associated with increased risks of chronic inflammatory conditions. Current evidence suggests that a balanced intake of these fatty acids is crucial for reducing the risk of metabolic and inflammatory diseases (Simopoulos, 2002).

Despite these proven health advantages, global consumption levels of omega-3 fatty acids remain below recommended levels, highlighting a pressing

public health issue (GOED, 2019). Marine fish and certain algae remain primary sources, though dietary supplements and fortified or functional foods offer accessible alternatives. However, incorporating these bioactive fats into functional foods and nutraceuticals poses challenges, particularly due to their vulnerability to degradation by environmental factors like light, heat, and oxygen, which can reduce their stability and effectiveness (McClements, 2012).

6. End-Consumer Healthy Food Products Challenges

The increasing interest in these novel health-promoting food products offers a promising opportunity for the food industry to innovate and diversify its products. However, incorporating bioactive compounds into food products involves several challenges.

A key issue is that bioactive ingredients are highly sensitive to environmental factors, such as light, heat, oxygen, enzyme activity, and pH, which can compromise their stability, effectiveness, and bioavailability. This instability often results in reduced efficacy when these compounds are integrated into food matrices, particularly in cases where water-insoluble bioactives, like polyunsaturated fatty acids, must be combined with hydrophilic food systems (Gómez-Mascaraque & López-Rubio, 2016). Additionally, many bioactive compounds impart undesired tastes or textures, further complicating their use in consumer products; for example, certain bioactive peptides are known to introduce bitter flavors (Lafarga & Hayes, 2017; Mohan et al., 2015).

Therefore, encapsulation technologies have emerged as a viable solution to these formulation and regulatory obstacles.

7. Microencapsulation as Technological Alternative

Microencapsulation is a versatile technology used to protect and stabilize bioactive ingredients for their incorporation into functional foods, nutraceuticals,

and dietary supplements (Villaño et al., 2021; Ye et al., 2018). This technique involves surrounding the bioactive compound—whether in the form of liquid droplets, solid particles, or gases—with a food-grade encapsulating agent, which forms a protective barrier that shields it from environmental factors and potential reactions with the food matrix. This protective layer preserves the stability and effectiveness of the bioactives during processing and storage, ensuring optimal final product performance (Oxley, 2014; Vasisht, 2014). Moreover, microencapsulation may enable the controlled and targeted release of these compounds, enhancing the bioavailability and efficacy of these active ingredients (Corrêa-Filho et al., 2019).

To ensure bioavailability and efficacy, several factors must be considered when designing an encapsulating system:

- **Bioactive compound**: biological functionality, physical and chemical properties, and stability
- **Coating material**: physical and chemical properties and stability
- **Encapsulation process**: selection of the most adequate process to achieve the requested protection
- **Formulation**: composition, shape, structure, size, triggers for controlled release

In terms of shape, microencapsulated particles can be mostly spheres, spheroids, or irregular particles. Needles, fibers or films are less common in the food industry.

Regarding the size, microencapsulated particle sizes generally range from a few nanometers to several millimeters in diameter. Nanoparticles smaller than 100 nm represent an advanced option within this technology (Schoonjans et al., 2023). However, their classification as "novel foods" requires rigorous regulatory approval before use in food applications (European Commission, n.d.). Particles exceeding 20 µm, on the other hand, may impact the texture of the food, potentially altering consumer acceptance.

According to their structure, microencapsulated particles can be homogeneous, mononuclear (single core), also known as core-shell, polynuclear (multiple cores), multishell, and matrix types (Kalkumbe et al., 2022).

7.1. Encapsulating Matrixes

Selecting an appropriate encapsulating-coating- material is crucial for the success and performance of the encapsulation process (Alu'datt et al., 2022; S. Gupta et al., 2016). The coating material choice significantly impacts the microcapsule's size, shape, and stability and the encapsulated core's release kinetics, bioavailability, and efficacy. The type and concentration of the coating material directly influence the encapsulation efficiency, protection, and functional performance of bioactive compounds (Espinoza-Espinoza et al., 2024; Misra et al., 2022). Coating materials must possess sufficient mechanical strength to withstand processing and storage while maintaining film-forming capabilities to create a uniform protective layer around the core material (C. Wang et al., 2024; Ye et al., 2018). Ideally, these materials should exhibit favorable rheological properties, facilitate emulsification and stabilization of active ingredients, and remain chemically non-reactive with the bioactive core throughout processing and shelf life.

In selecting coating materials, factors such as edibility, biodegradability, cost-effectiveness, and compliance with food-grade safety regulations must also be considered (Rojas et al., 2024). Sustainable, clean-label formulations that minimize synthetic additives are preferred following consumer demands. Table 4 provides an overview of commonly used coating materials for microencapsulation, highlighting their key properties and associated encapsulation techniques.

Table 4. Encapsulation Coating Materials: Properties and Techniques.

Coating Material	Properties	Techniques	Reference
Polysaccharides			
Chitosan	• Antimicrobial • Biocompatible • Mucoadhesive	• Coacervation • Nanoprecipitation • Spray drying	(Rojas et al., 2024; A. Vignesh et al., 2024)
Cyclodextrin	• Inclusion complex formation • Solubility • Volatile adsorbent	• Coacervation • Electrohydrodynamic encapsulation • Spray drying	(Saha et al., 2023; V. Vignesh et al., 2024)
Gums	• Biocompatible • Biodegradable • Elastic gels forming • Hydrocolloidal • Stabilizers	• Coacervation • Extrusion • Phase separation • Spray drying	(Castro-López et al., 2021; Espinoza-Espinoza et al., 2024)
Sodium alginate	• Biocompatible • Biodegradable • Film-forming • Water-soluble	• Coacervation • Spray drying	(Rojas et al., 2024; Sawant et al., 2024)
Starch	• Biocompatible • Biodegradable • Film-forming	• Extrusion • Fluidized bed coating • Spray drying	(Galanakis, 2024; V. Vignesh et al., 2024)
Lipids			
Lipids	• Plasticizing properties • Gas - water vapor barrier	• Fluidized bed • Spray chilling/cooling • Extrusion	(Karami et al., 2023; Wandrey et al., 2010)
Liposomes (phospholipids)	• Hydrophilic and hydrophobic biocompatibility	• Extrusion • Liposome encapsulation	(Hosseini et al., 2021; Wandrey et al., 2010)
Proteins			
Albumin **Gelatin** **Whey Protein** **Zein**	• Biodegradable • Edible • Enhanced bioavailability • Excellent film-forming properties • Gas, moisture and light barrier • High encapsulation efficiency • Structural stability	• Coacervation • Electrohydrodynamic atomization • Electrospraying • Extrusion • Spray drying	(Rojas et al., 2024; V. Vignesh et al., 2024)

Proteins are advantageous as encapsulating agents because they can form matrices that support and protect the core bioactive material. They offer solubility, emulsification, viscosity-building, gelation, and film formation (Su et al., 2018). Among protein-based materials, whey protein (WP) and zein (ZN) are particularly effective for encapsulating bioactive compounds and enhancing their bioavailability and stability.

Whey proteins, a mix of globular proteins derived from milk whey, are widely utilized in encapsulation systems due to their capacity to form hydrogels and nanoparticles, which enable the controlled release of bioactive compounds. Whey proteins protect and stabilize active substances, maintaining their integrity until targeted release (Livney, 2010). As safe and nutritionally beneficial biopolymers, whey proteins have a structure rich in amino acids, making them suitable for encapsulating various bioactives, including fat-soluble vitamins, antioxidants, fatty acids, phytosterols, lycopene, lutein, polyphenols, flavor compounds, minerals, and live probiotic cells (López-Rubio & Lagaron, 2012; Tavares et al., 2014).

Zein, a prolamin protein derived from corn endosperm, makes up about 45-65% of corn's total protein content and is classified as an alcohol-soluble material (Parris et al., 2005; Patel et al., 2010; Zhong & Jin, 2009). Zein's ability to form a stable, water-insoluble matrix makes it effective for encapsulating hydrophobic bioactive compounds, enhancing their protection under various environmental conditions (Lan et al., 2023). Recognized as a biodegradable and environmentally friendly material, zein is approved for food applications. Beyond the food industry, zein is also used in pharmaceuticals for controlled drug-release systems, as a protective layer for encapsulated drugs, and as a tablet coating (Abuzeineh et al., 2021). This highlights the versatility and effectiveness of zein in encapsulating bioactive compounds.

7.2. Microencapsulation Techniques

A wide range of encapsulating techniques is available. Selecting the most effective method remains challenging due to the diversity of encapsulation processes, each with advantages and disadvantages, highlighting the need for tailored approaches to achieve the desired product qualities.

7.2.1. Spray Drying

Spray drying is one of the most used industrial techniques for encapsulating bioactive compounds, particularly in the food industry. It is highly valued for its operational flexibility, scalability, and, most importantly, its cost-effectiveness (Pérez-Masiá et al., 2014). This method involves atomizing a feed solution consisting of the bioactive compound and the encapsulating wall material into a fine mist within a drying chamber. In the chamber, the droplets encounter a current of hot air, rapidly removing the solvent and consequently forming particles. This technique is also widely used because it is highly adaptable to different formulations and encapsulation agents (Gharsallaoui et al., 2007).

Spray drying has demonstrated its potential to stabilize bioactive compounds like polyunsaturated fatty acids (PUFAs) and polyphenols by reducing oxidative degradation, improving storage stability, and enabling controlled release (Akbarbaglu et al., 2021; Kolanowski et al., 2006). Table 5 summarizes key findings on the encapsulation matrices, particle sizes, and efficiencies achieved for polyphenols and PUFAs using spray drying.

Table 5. Encapsulation of Bioactive Compounds via Spray Drying: Matrices, Particle Size, and Efficiency.

Bioactive Compound	Coating Material	Particle Size (µm)	Encapsulation Efficiency (%)	Reference
Polyphenols				
Green Tea	• Maltodextrin • Gum Arabic	5–20	80–90	(Massounga Bora et al., 2018)
Tamarillo	• Gum Arabic • Modified Starch	2–10	76–88	(Ramakrishnan et al., 2018)
Carotenoids (Beta-carotene)	• Gum Arabic • Whey Protein Isolate	2–7	87–92	(Akbarbaglu et al., 2021)
PUFA				
Algae Oil	• Sodium Caseinate • Maltodextrin • Lecithin	1.5–5	85–95	(Kaushik et al., 2015)
Chia Oil	• Chia seed gum	18.4	67.3	(Geranpour et al., 2020)
Chia Oil	• Chia seed protein Isolate	21.6	74.7	(Timilsena et al., 2017)
Chia Oil	• Chia seed gum • Chia seed protein Isolate	9.2	93.9	(Timilsena et al., 2017)
Chia Oil	• Whey Protein	3.5–4.2	85–91	(Timilsena et al., 2017)
Fish Oil	• Maltodextrin • Gum Arabic	2–6	70–95	(Kolanowski et al., 2006)

Despite its widespread use, spray drying has certain limitations; the process faces notable challenges, including oxidative stress, uneven encapsulation for PUFAs, thermal degradation, and polyphenols' particle morphology inconsistencies. Particularly when encapsulating thermosensitive compounds, the high temperatures during the drying process can lead to degradation, thus limiting their application (Pérez-Masiá et al., 2014). Consequently, alternative encapsulation techniques may be necessary for such sensitive compounds to preserve their bioactivity and functional properties.

7.2.2. Spray Chilling and Spray Cooling

Spray chilling, also known as spray cooling, is a technique in which the mixture of the core material and the encapsulating wall is atomized into a cooled or chilled environment, causing the wall material to solidify rapidly around the core (Madene et al., 2006; Zuidam & Nedović, 2010). This process is one of the most cost-effective encapsulation techniques at a commercial scale. It is widely used to encapsulate aroma compounds, enhance their heat stability, delay their release in moist environments, and convert liquid flavors into free-flowing powders (Gouin, 2004).

Spray chilling has been successfully applied for the microencapsulation of various sensitive bioactives, including tocopherols, ascorbic acid, vitamin D, and probiotics, using triglycerides and other lipids as wall materials (Gamboa et al., 2011; Karami et al., 2023; Olivares-Tenorio et al., 2024). Table 6 summarizes key findings on the encapsulation matrices, particle sizes, and efficiencies achieved for polyphenols and PUFAs using the spray chilling / cooling technique.

Table 6. Encapsulation of Bioactive Compounds via Spray Chilling / Spray Cooling: Matrices, Particle Size, and Efficiency.

Bioactive Compound	Coating Material	Particle Size (μm)	Encapsulation Efficiency (%)	Reference
Polyphenols				
Anthocyanins (Elderberry Extract)	• Palm oil • Stearic acid	50–120	60–75	(Ribeiro et al., 2020)
Garlic Acid	• Soybean Oil • Hydrogenated soybean	24 - 36	54 – 101	(Consoli et al., 2016)
Proanthocyanidins (Cinnamon Extract)	• Fully hydrogenated palm oil	80–150	70 – 85	(Tulini et al., 2017)
Green Tea Extract	• Hydrogenated Palm Oil • Vegetable fat fully hydrogenated and interesterified	81 – 86	15 – 190	(Cutrim et al., 2019)
PUFA				
Docosahexaenoic Acid (DHA)	• Dodecenyl succinylated agarose	100 – 400	65 – 85	(Xiao et al., 2020)
Fish Oil (EPA And DHA)	• Vegetable fat • Hydrogenated palm oil	100 – 250	71 – 98	(Fadini et al., 2019)
Sacha Inchi Oil	• Acacia gum • Hydrogenated palm oil	150 – 300	68 – 93	(Fadini et al., 2018)

Despite its advantages, spray chilling has some limitations. The technique typically generates solid particles, which may restrict its application in products requiring low-fat or fat-free formulations (Okuro et al., 2013). Additionally, there are concerns about the potential loss of core bioactive ingredients through diffusion from the lipid matrix during storage, as well as the degradation of the extractable oil over time, which can limit the shelf life and efficacy of the encapsulated ingredient (Gouin, 2004; Okuro et al., 2013) highlighting the need for careful formulation and storage considerations when applying spray chilling.

7.2.3. Fluidized-Bed Coating

The fluidized-bed coating technique is widely utilized for various purposes, including drying wet products, agglomerating particles, improving flow properties, and producing encapsulated or coated particles for controlled release or taste masking applications (Frey, 2014). This method involves suspending particles in a fluidizing gas stream while a solubilized or melted coating material is sprayed onto the particles. The liquid evaporates upon contact with the warm gas stream, forming a solid, uniform coating around the particles. One of the primary advantages of fluidized-bed coating is its ability to operate at relatively low temperatures, making it suitable for encapsulating heat-sensitive bioactive compounds. This contrasts with higher-temperature processes such as spray-drying, allowing for the protection of sensitive materials during processing (Đorđević et al., 2015; Meiners, 2012). Moreover, the fluidized bed technique has a lower overall cost than other encapsulation methods, especially when operating at larger industrial scales.

The fluidized bed method has been studied to protect different bioactive compounds. Schmidt et al., (2015) used it to granulate sodium benzoate solutions, creating particles between 100 and 700 µm. Additionally, Bellumori et al. (2021) used fluidized bed coating to encapsulate phenolic compounds, like tyrosol and hydroxytyrosol, from olive oil by-products using modified starch, maltodextrin, and sunflower lecithin as coating materials. This method resulted in

stable encapsulation, keeping around 84% of the phenolic compounds after 75 days of storage. The particles produced measured between 150 and 300 μm.

On the other hand, fluidized-bed processing encounters challenges when handling fine particles due to poor fluidization behavior. Fine particles exhibit strong cohesive forces, leading to aggregation, channeling, and elutriation—where particles are expelled from the fluidization zone. These problems can compromise the quality and uniformity of the coating (Timsina et al., 2019). Furthermore, the fluidized-bed coating is most effective with preformed spherical particles with a narrow size distribution and good flow properties, limiting its application to specific particle types (Đorđević et al., 2015). Mixing different particle sizes can result in smaller particles rising while larger ones settle, leading to uneven coatings and inconsistent products. Additionally, clumping during processing may further degrade the quality. To enhance the potential of this dry coating system for industrial applications, improvements are needed, particularly in reducing or suppressing the moisture content in the fluidizing and atomizing air.

7.2.4. Coacervation

It has been extensively studied for encapsulating reactive, sensitive, or volatile additives and nutrients, with the primary objectives of enhancing shelf life, enabling alternative food processing techniques, masking unpleasant flavors, and controlling the release of bioactive compounds (Ye et al., 2018). The coacervation process consists of three basic steps: emulsification, coacervation, and shell formation and/or hardening. This method involves the interaction of oppositely charged biopolymers, typically a protein (e.g., gelatin or whey protein) and a polysaccharide (e.g., gum arabic), to form a stable, protective coating around the core material (Yan & Zhang, 2014). Coacervation is a widely utilized microencapsulation technique in food, pharmaceuticals, and agriculture, owing to its high encapsulation efficiency (up to 99%) and cost-effective processing. Coacervation is categorized into two main types: simple coacervation, driven by changes in pH, temperature, or ionic strength, and complex coacervation, which

occurs due to the attraction between oppositely charged polyelectrolytes, such as proteins and polysaccharides. These properties make coacervation an ideal method for encapsulating bioactive compounds, enhancing their stability, controlled release, and protection against adverse conditions, such as extreme pH or oxidation (Timilsena et al., 2019; J. Wang, 2020).

Coacervation has been effectively utilized for polyphenols. Timilsena et al., (2020) demonstrated that using gelatin and gum arabic coacervates for encapsulating polyphenols enhanced the retention of antioxidant properties and prolonged shelf life under storage conditions. (Ahammed et al., 2023) created a gelatin-zein composite film using complex coacervation to protect green tea polyphenols (TP). Transglutaminase (TG) crosslinked the film, achieving over 30% TP retention immediately and about 80% after storage, enhancing TP release. On the other hand, a notable application of coacervation is encapsulating polyunsaturated fatty acids like palm oil. Marfil et al. (2015) encapsulated palm oil using a complex coacervation system of gelatin and gum arabic, producing spherical microcapsules with an average particle diameter of 284.74 ± 207.26 μm, ensuring a stable and controlled release profile.

Despite its versatility, coacervation is inefficient for encapsulating hydrophilic compounds. Furthermore, its application in the food industry is limited due to the high costs and complexity associated with the process (Đorđević et al., 2015).

7.2.5. Coextrusion

Coextrusion is an encapsulation technique that utilizes a concentric nozzle capable of processing two materials simultaneously. The nozzle has a central hole for the active compound and a ring-shaped indentation surrounding the central hole for the encapsulating materials (Silva et al., 2018). The encapsulated material resulting from this method is relatively larger in size than that formed using any other method, and this technology is useful with limited wall materials (Choudhury et al., 2021).

Coextrusion has emerged as an effective encapsulation technique for protecting sensitive bioactive compounds, including polyunsaturated fatty acids (PUFAs) and polyphenols. Alginate is one of the most used coating materials in coextrusion due to its biocompatibility and ability to form robust hydrogels. Studies have demonstrated its efficacy in encapsulating canola, kenaf seed, and linseed oils, achieving up to 90% encapsulation efficiencies and significantly reducing oxidation during storage (Bennacef et al., 2023; Chew & Nyam, 2016). While direct applications for polyphenols are limited, this technique shows potential when combined with biopolymers or antioxidants, such as quercetin, to enhance oxidative stability and extend shelf life.

However, the microcapsules formed by this method tend to have high moisture content, which can compromise their stability by making them more susceptible to microbial contamination. This increased moisture can reduce shelf life, often shorter than 30 days, particularly if proper storage conditions are not maintained (Niño-Vásquez et al., 2022). Therefore, while coextrusion offers enhanced protection for certain applications, it may not be suitable for products requiring longer shelf lives without additional processing or drying steps.

7.2.6. Electrohydrodynamic processing

Electrohydrodynamic processes, particularly electrospinning and electrospraying, have emerged as scalable and cost-effective technologies to produce high-performance fibers or capsules in the micro or nanoscale (Jacobsen et al., 2018; Niu et al., 2020).

Electrospinning generates fibers by subjecting a polymer solution to a high-voltage electric field, resulting in ultrathin structures that, once dried, form stable micro or nanofibers. This technology is advantageous for encapsulating heat-sensitive substances, as it avoids high temperatures, making it ideal for applications such as food packaging, edible films, and agricultural materials (Castro Coelho et al., 2021; Jaworek, 2016; V. Vignesh et al., 2024; Zhang et al., 2020).

Conversely, electrospraying involves the application of a high electric field to a low-viscosity polymer solution, producing ultrafine droplets that solidify into micro- or nano-sized particles or capsules (Jayaprakash et al., 2023; Tapia-Hernández et al., 2017). Compared to other common encapsulation techniques, the electrospraying technique presents several key advantages: (i) it enables easy incorporation of bioactive compounds into the polymer matrix, (iii) encapsulation efficiencies exceeding 80% can be achieved, (iv) the particle size and particle morphology can be adjusted by modifying process parameters, (vi) aqueous solutions can be used to minimize toxicity concerns, (viii) the process does not require high temperatures (Paximada et al., 2017; V. Vignesh et al., 2024; Wen et al., 2017; Zhang et al., 2020). All these advantages make this technique ideal for applications such as bioactive ingredients, drug delivery systems and encapsulation of agricultural products.

Previous studies have highlighted the potential of electrohydrodynamic processes for protecting and enhancing bioactive compounds such as polyphenols and polyunsaturated fatty acids (PUFAs). Table 7 summarizes key findings on encapsulating bioactive compounds via Electrospray Electrohydrodynamic Processing. González-Cruz et al., (2023) employed electrospraying to encapsulate polyphenol-rich extracts from Biloxi blueberries in zein, resulting in nanocapsules with notable photoprotective and thermoprotective properties. Similarly, Beşir & Kahyaoglu (2020) encapsulated bitter melon extract in zein nanofibers, achieving higher polyphenol content and antioxidant activity than particles made with other polymers like gelatin and maltodextrin.

Table 7. Encapsulation of Bioactive Compounds via Electrospray Electrohydrodynamic Processing: Matrices, Particle Size, and Efficiency.

Bioactive Compound	Coating Material	Particle Size (μm)	Encapsulation Efficiency (%)	Reference
Polyphenol				
Anthocyanin-rich Black carrot extract	• Chitosan • Gelatin	–	72.4 – 79.5	(Atay et al., 2018)
Broccoli extract	• Zein	–	86.9 – 99.3	(Radünz et al., 2020)
Catechin (EGCG)	• Bacterial cellulose • Whey protein isolate	0.18 – 0.24	> 98	(Paximada et al., 2021)
Catechin (EGCG)	• Chitosan	–	77.2 – 82	(Gómez-Mascaraque et al., 2016)
Catechin (EGCG)	• Gelatin	0.05 – 2.12	~ 100	(Gómez-Mascaraque et al., 2015)
Catechin (EGCG)	• Gelatin • Zein	24 - 76	~ 90	(Gómez-Mascaraque et al., 2017)
Curcumin	• Gelatin	0.20 - 0.40	96.5 – 97.5	(Gómez-Estaca et al., 2017)
Curcumin	• Gelatin	0.5 - 1.2	100	(Gómez-Estaca et al., 2015)
Curcumin	• Zein	0.17 - 0.90	85 – 90	(Gomez-Estaca et al., 2012)
Green tea catechins	• Zein	0.12 – 0.19	89 – 98	(Bhushani et al., 2017)
Meletin	• Gliadin	50	–	(Yang et al., 2018)
Olive leaf extracts	• Whey protein concentrate	0.23 – 0.66	36. 6 – 83.6	(Soleimanifar et al., 2020)
Resveratrol	• Zein	0.23 – 0.33	39 – 68	(Jayan et al., 2019)

Bioactive Compound	Coating Material	Particle Size (μm)	Encapsulation Efficiency (%)	Reference
PUFA				
Cod liver oil	• Dextran • Glucose syrup (GS) • Pullulan • Whey protein concentrate (WPC)	1.0 – 10.0	74.9 – 88.4	(García-Moreno et al., 2018)
Fish Oil	• Dextran • Pullulan	0.3 – 0.6	~ 68	(García-Moreno et al., 2017)
Orange Essential Oil	• Zein • β-cyclodextrin	1.5 – 1.8	~ 35	(Kringel et al., 2020)
α-linolenic acid	• Gelatin • Soy protein isolate (SPI) • Whey protein concentrate (WPC)	0.5 – 4.0	11 – 72	(Gómez-Mascaraque & López-Rubio, 2016)

In recent research on polyunsaturated fatty acids (PUFAs), Rahmani-Manglano et al., (2024) discovered that emulsion-based methods, such as spray drying and monoaxial electrohydrodynamic atomization (EAPG), achieved high encapsulation efficiency (EE > 83%) for PUFAs while also reducing oxidation levels. This discovery helped to maintain the stability of fish oil capsules, making them suitable for fortified products like salad dressings. Furthermore, Prieto et al. (2021) and Prieto & Lagaron (2020) effectively encapsulated docosahexaenoic acid (DHA) using whey protein concentrate and wheat gluten extract. Table 8 summarizes the key findings on encapsulating bioactive compounds via Electrospray Assisted by Pressurized Gas Electrohydrodynamic Processing.

Table 8. Encapsulation of Bioactive Compounds via Electrospray Assisted by Pressurized Gas Electrohydrodynamic Processing: Matrices, Particle Size, and Efficiency.

Bioactive Compound	Coating Material	Particle Size (μm)	Encapsulation Efficiency (%)	Reference
Algae oil	• Wheat gluten extract	1.5 – 5.1	69 – 74	(Prieto et al., 2021)
Algae oil	• Whey protein concentrate • Maltodextrin	2.0 – 8.2	60 – 86	(Prieto & Lagaron, 2020)
Algae oil	• Whey protein concentrate	0.9 – 5.4	61 – 85	(Prieto et al., 2022)
Cod liver oil	• Zein	1.7 – 3.1	82 – 84	(Miguel et al., 2019)
Docosahexaenoic acid (DHA)	• Zein	0.6 – 2.2	83 – 85	(Busolo et al., 2019)
Fish Oil	• Glucose syrup • Whey protein concentrate	1.6 – 7.3	50 – 99	(Rahmani-Manglano et al., 2024)

However, the traditional electrospraying process is limited by its low production yield. This challenge was solved by Bioinicia S.L. in collaboration with the research group of Dr. Lagaron by the development of the already patented Electrospraying Assisted by Pressurized Gas (EAPG) technology. EAPG utilizes a pneumatic injector to atomize polymer solutions under controlled environmental conditions, achieving higher production efficiency while maintaining particle quality. This novel technology resolves the key limitation of low production yield inherent to conventional electrospraying, enabling industrial-scale production of more than 1 kg/h of free-flowing powder under controlled ambient conditions (Busolo et al., 2019). Moreover, EAPG reduces the denaturation of bioactives because the process operates at room temperature and ensures particle morphology and size uniformity (Prieto & Lagaron, 2020). The process is highly versatile since it accommodates a wide range of encapsulating matrices, including WPC, ZN, maltodextrin, wheat gluten extract, polyvinylpyrrolidone, and

hydroxypropyl methylcellulose (Miguel et al., 2019; Prieto et al., 2021; Torres-Giner et al., 2020).

EAPG provides a cutting-edge solution by enabling precise encapsulation of hydrophilic and lipophilic compounds, creating stable particles shielding bioactives from adverse environmental conditions such as heat, oxygen, and UV radiation. Given these advantages, this technology could have the potential to encapsulate innovative and underexploited bioactives with significant nutritional and functional value, such as camu camu (***Myrciaria dubia***), Dragon's blood sap (***Croton lechleri***), and eicosapentaenoic Acid (EPA) for the creation of functional foods and nutraceutical products that leverage the full potential of challenging bioactive compounds.

References

ABUZEINEH, H., ABDEL RAHIM, S., CESPI, M., BISHARAT, L., & BERARDI, A. (2021). Time-controlled release by the incorporation of superdisintegrants within the coat of zein dry coated tablets. Journal of Drug Delivery Science and Technology, 65, 102716. https://doi.org/10.1016/J.JDDST.2021.102716

ADEFEGHA, S. A. (2018). Functional Foods and Nutraceuticals as Dietary Intervention in Chronic Diseases; Novel Perspectives for Health Promotion and Disease Prevention. Journal of Dietary Supplements, 15(6), 977–1009. https://doi.org/10.1080/19390211.2017.1401573

AHAMMED, S., EASDANI, M., LIU, F., & ZHONG, F. (2023). Encapsulation of Tea Polyphenol in Zein through Complex Coacervation Technique to Control the Release of the Phenolic Compound from Gelatin–Zein Composite Film. Polymers, 15(13), 2882. https://doi.org/10.3390/polym15132882

AKBARBAGLU, Z., PEIGHAMBARDOUST, S. H., SARABANDI, K., & JAFARI, S. M. (2021). Spray drying encapsulation of bioactive compounds within protein-based carriers; different options and applications. In Food Chemistry (Vol. 359). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2021.129965

AL-DUJAILI, E., & AL-DUJAILI, E. A. (2015). Cronicon NUTRITION Natural Polyphenols: Potential for Disease Prevention. In Review Article, EC Nutrition (Vol. 2). https://www.researchgate.net/publication/288182361

ALU'DATT, M. H., ALROSAN, M., GAMMOH, S., TRANCHANT, C. C., ALHAMAD, M. N., RABABAH, T., ZGHOUL, R., ALZOUBI, H., GHATASHEH, S., GHOZLAN, K., & TAN, T. C. (2022). Encapsulation-based technologies for bioactive compounds and their application in the food industry: A roadmap for food-derived functional and health-promoting ingredients. In Food Bioscience (Vol. 50). https://doi.org/10.1016/j.fbio.2022.101971

ALVES, J., GASPAR, P. D., LIMA, T. M., & SILVA, P. D. (2023). What is the role of active packaging in the future of food sustainability? A systematic review. Journal of the Science of Food and Agriculture, 103(3), 1004–1020. https://doi.org/10.1002/jsfa.11880

ATAY, E., FABRA, M. J., MARTÍNEZ-SANZ, M., GOMEZ-MASCARAQUE, L. G., ALTAN, A., & LOPEZ-RUBIO, A. (2018). Development and characterization of chitosan/gelatin electrosprayed microparticles as food grade delivery vehicles for anthocyanin extracts. Food Hydrocolloids, 77, 699–710. https://doi.org/10.1016/J.FOODHYD.2017.11.011

AUSTRALIAN GOVERNMENT. (1990). Therapeutic Goods Act 1989. www.legislation.gov.au

AUSTRALIAN GOVERNMENT. (2016). Australia New Zealand Food Standards Code - Standard 1.2.7 - Nutrition, Health and Related Claims.

BARTOSZ, T., & IRENE, T. (2016). Polyphenols encapsulation – application of innovation technologies to improve stability of natural products. Physical Sciences Reviews, 1(2). https://doi.org/10.1515/psr-2015-0005

BELLUMORI, M., DE MARCHI, L., MAINENTE, F., ZANONI, F., CECCHI, L., INNOCENTI, M., MULINACCI, N., & ZOCCATELLI, G. (2021). A by-product from virgin olive oil production (pâté) encapsulated by fluid bed coating: evaluation of the phenolic profile after shelf-life test and in vitro gastrointestinal digestion. International Journal of Food Science & Technology, 56(8), 3773–3783. https://doi.org/10.1111/ijfs.15068

BENNACEF, C., DESOBRY, S., PROBST, L., & DESOBRY-BANON, S. (2023). Alginate Based Core–Shell Capsules Production through Coextrusion Methods: Recent Applications. Foods, 12(9), 1788. https://doi.org/10.3390/foods12091788

BEŞIR, A., & KAHYAOGLU, T. (2020). Development and characterization of functional electrohydrodynamic particles and fibers using bitter melon (Momordica charantia L.) extract. Journal of Food Measurement and Characterization, 14(4), 2333–2342. https://doi.org/10.1007/s11694-020-00480-7

BHATT, I. D., RAWAT, S., & RAWAL, R. S. (2013). Antioxidants in medicinal plants. In Biotechnology for Medicinal Plants: Micropropagation and Improvement (Vol. 9783642299742, pp. 295–326). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-642-29974-2_13

BHUSHANI, J. A., KURREY, N. K., & ANANDHARAMAKRISHNAN, C. (2017). Nanoencapsulation of green tea catechins by electrospraying technique and its effect on controlled release and in-vitro permeability. Journal of Food Engineering, 199, 82–92. https://doi.org/10.1016/J.JFOODENG.2016.12.010

BUSOLO, M. A., TORRES-GINER, S., PRIETO, C., & LAGARON, J. M. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. Innovative Food Science and Emerging Technologies, 51, 12–19. https://doi.org/10.1016/j.ifset.2018.04.007

CALDER, P. C. (2015). Functional Roles of Fatty Acids and Their Effects on Human Health. Journal of Parenteral and Enteral Nutrition, 39(1S). https://doi.org/10.1177/0148607115595980

CASTRO COELHO, S., NOGUEIRO ESTEVINHO, B., & ROCHA, F. (2021). Encapsulation in food industry with emerging electrohydrodynamic techniques: Electrospinning and electrospraying – A review. Food Chemistry, 339(December 2019), 127850. https://doi.org/10.1016/j.foodchem.2020.127850

CASTRO-LÓPEZ, C., ESPINOZA-GONZÁLEZ, C., RAMOS-GONZÁLEZ, R., BOONE-VILLA, V. D., AGUILAR-GONZÁLEZ, M. A., MARTÍNEZ-ÁVILA, G. C. G., AGUILAR, C. N., & VENTURA-SOBREVILLA, J. M. (2021). Spray-drying encapsulation of microwave-assisted extracted polyphenols from Moringa oleifera: Influence of tragacanth, locust bean, and carboxymethyl-cellulose formulations. Food Research International, 144, 110291. https://doi.org/10.1016/j.foodres.2021.110291

CENCIC, A., & CHINGWARU, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. In Nutrients (Vol. 2, Issue 6, pp. 611–625). MDPI AG. https://doi.org/10.3390/nu2060611

CHEW, S.-C., & NYAM, K.-L. (2016). Microencapsulation of kenaf seed oil by co-extrusion technology. Journal of Food Engineering, 175, 43–50. https://doi.org/10.1016/j.jfoodeng.2015.12.002

CHOUDHURY, N., MEGHWAL, M., & DAS, K. (2021). Microencapsulation: An overview on concepts, methods, properties and applications in foods. In Food Frontiers (Vol. 2, Issue 4, pp. 426–442). John Wiley and Sons Inc. https://doi.org/10.1002/fft2.94

CLYDESDALE, F. (2004). Functional Foods: Opportunities & Challenges. FoodTechnology, 58(12). https://www.ift.org/news-and-publications/food-technology-magazine/issues/2004/december/features/functional-foods-opportunities-and-challenges

CONSOLI, L., GRIMALDI, R., SARTORI, T., MENEGALLI, F. C., & HUBINGER, M. D. (2016). Gallic acid microparticles produced by spray chilling technique: Production and characterization. LWT, 65, 79–87. https://doi.org/10.1016/j.lwt.2015.07.052

CORRÊA-FILHO, L. C., MOLDÃO-MARTINS, M., & ALVES, V. D. (2019). Advances in the application of microcapsules as carriers of functional compounds for food products. In Applied Sciences (Switzerland) (Vol. 9, Issue 3). MDPI AG. https://doi.org/10.3390/app9030571

CROWE, K. M., & FRANCIS, C. (2013). Position of the Academy of Nutrition and Dietetics: Functional Foods. Journal of the Academy of Nutrition and Dietetics, 113(8), 1096–1103. https://doi.org/10.1016/j.jand.2013.06.002

CUTRIM, C. S., ALVIM, I. D., & CORTEZ, M. A. S. (2019). Microencapsulation of green tea polyphenols by ionic gelation and spray chilling methods. Journal of Food Science and Technology, 56(8), 3561–3570. https://doi.org/10.1007/s13197-019-03908-1

DAHIYA, D., & NIGAM, P. S. (2022). Probiotics, Prebiotics, Synbiotics, and Fermented Foods as Potential Biotics in Nutrition Improving Health via Microbiome-Gut-Brain Axis. Fermentation, 8(7), 303. https://doi.org/10.3390/fermentation8070303

DAMIÁN, M. R., CORTES-PEREZ, N. G., QUINTANA, E. T., ORTIZ-MORENO, A., GARFIAS NOGUEZ, C., CRUCEÑO-CASARRUBIAS, C. E., SÁNCHEZ PARDO, M. E., & BERMÚDEZ-HUMARÁN, L. G. (2022). Functional Foods, Nutraceuticals and Probiotics: A Focus on Human Health. Microorganisms, 10(5), 1065. https://doi.org/10.3390/microorganisms10051065

DAY, L., SEYMOUR, R. B., PITTS, K. F., KONCZAK, I., & LUNDIN, L. (2009). Incorporation of functional ingredients into foods. Trends in Food Science and Technology, 20(9), 388–395. https://doi.org/10.1016/j.tifs.2008.05.002

DICKINSON, A. (2011). History and overview of DSHEA. Fitoterapia, 82(1), 5–10. https://doi.org/10.1016/J.FITOTE.2010.09.001

DJ NEVENA, I., HIPPE, B., LILJA, S., & HASLBERGER, A. G. (2022). Precise Nutrition and Functional Foods. In Advances in Precision Nutrition, Personalization and Healthy Aging (pp. 231–267). Springer International Publishing. https://doi.org/10.1007/978-3-031-10153-3_10

DOLGANYUK, V., SUKHIKH, S., KALASHNIKOVA, O., IVANOVA, S., KASHIRSKIKH, E., PROSEKOV, A., MICHAUD, P., & BABICH, O. (2023). Food Proteins: Potential Resources. Sustainability, 15(7), 5863. https://doi.org/10.3390/su15075863

ĐORĐEVIĆ, V., BALANČ, B., BELŠČAK-CVITANOVIĆ, A., LEVIĆ, S., TRIFKOVIĆ, K., KALUŠEVIĆ, A., KOSTIĆ, I., KOMES, D., BUGARSKI, B., & NEDOVIĆ, V. (2015). Trends in Encapsulation Technologies for Delivery of Food Bioactive Compounds. In Food Engineering Reviews (Vol. 7, Issue 4). https://doi.org/10.1007/s12393-014-9106-7

DUDEJA, P., & GUPTA, R. K. (2017). Nutraceuticals. In Food Safety in the 21st Century: Public Health Perspective (pp. 491–496). Elsevier Inc. https://doi.org/10.1016/B978-0-12-801773-9.00040-6

EFSA. (n.d.). General function claim. Retrieved November 7, 2024, from https://www.efsa.europa.eu/en/glossary/general-function-claim

ESPINOZA-ESPINOZA, L. A., MUÑOZ-MORE, H. D., NOLE-JARAMILLO, J. M., RUIZ-FLORES, L. A., ARANA-TORRES, N. M., MORENO-QUISPE, L. A., & VALDIVIEZO-MARCELO, J. (2024). Microencapsulation of vitamins: A review and meta-analysis of coating materials, release and food fortification. In Food Research International (Vol. 187). Elsevier Ltd. https://doi.org/10.1016/j.foodres.2024.114420

EUROPEAN COMMISSION. (n.d.). What is the current Novel Food legislation? Retrieved October 2, 2024, from https://food.ec.europa.eu/food-safety/novel-food/legislation_en

EUROPEAN PARLIAMENT. (2002). DIRECTIVE 2002/46/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002L0046

FADINI, A. L., ALVIM, I. D., RIBEIRO, I. P., RUZENE, L. G., SILVA, L. B. DA, QUEIROZ, M. B., MIGUEL, A. M. R. DE O., CHAVES, F. C. M., & RODRIGUES, R. A. F. (2018). Innovative strategy based on combined microencapsulation technologies for food application and the influence of wall material composition. LWT, 91, 345–352. https://doi.org/10.1016/j.lwt.2018.01.071

FADINI, A. L., DUTRA ALVIM, I., PAGANOTTI, K. B. DE F., BATAGLIA DA SILVA, L., BONIFÁCIO QUEIROZ, M., MIGUEL, A. M. R. DE O., & RODRIGUES, R. A. F. (2019). Optimization of the production of double-shell microparticles containing fish oil. Food Science and Technology International, 25(5), 359–369. https://doi.org/10.1177/1082013219825890

FANZO, J., MCLAREN, R., BELLOWS, A., & CARDUCCI, B. (2023). Challenges and opportunities for increasing the effectiveness of food reformulation and fortification to improve dietary and nutrition outcomes. In Food Policy (Vol. 119). Elsevier Ltd. https://doi.org/10.1016/j.foodpol.2023.102515

FAO. (2022). Changing consumer preferences and food consumption patterns. In Thinking about the future of food safety. FAO. https://doi.org/10.4060/cb8667en

FERNANDES, S. S., COELHO, M. S., & SALAS-MELLADO, M. DE LAS M. (2018). Bioactive Compounds as Ingredients of Functional Foods: Polyphenols, Carotenoids, Peptides From Animal and Plant Sources New. In Bioactive Compounds: Health Benefits and Potential Applications. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814774-0.00007-4

FOOD AND DRUG ADMINISTRATION. (2023, September 2). New Dietary Ingredients in Dietary Supplements - Background for Industry | FDA. https://www.fda.gov/food/new-dietary-ingredient-ndi-notification-process/new-dietary-ingredients-dietary-supplements-background-industry

FOOD SAFETY AND STANDARDS ACT (2006). ∫https://fssai.gov.in/upload/uploadfiles/files/FOOD-ACT.pdf

FORTUNE BUSINESS INSIGHTS. (2024a). Functional Food Market Size, Trends, Growth, Analysis, 2032. https://www.fortunebusinessinsights.com/functional-foods-market-102269

FORTUNE BUSINESS INSIGHTS. (2024b). Nutraceutical Ingredients Market Size, Share | Trends [2032]. https://www.fortunebusinessinsights.com/nutraceutical-ingredients-market-102282

FORTUNE BUSINESS INSIGHTS. (2024c). Nutraceuticals Market Size, Trends & Growth Report [2032]. https://www.fortunebusinessinsights.com/nutraceuticals-market-102530

FORTUNE BUSINESS INSIGHTS. (2024d, October). Food Supplement Ingredients Market Size, Share | Growth [2032]. https://www.fortunebusinessinsights.com/food-supplement-ingredients-market-110415

FORTUNE BUSINESS INSIGHTS. (2024e, November). Dietary Supplements Market Size, Share & Trends | Report 2032. https://www.fortunebusinessinsights.com/dietary-supplements-market-102082

FREY, C. (2014). Fluid Bed Coating-Based Microencapsulation. In Microencapsulation in the Food Industry (pp. 65–79). Elsevier. https://doi.org/10.1016/b978-0-12-404568-2.00007-8

GALANAKIS, C. M. (2024). The Future of Food. In Foods (Vol. 13, Issue 4). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods13040506

GAMBOA, O. D., GONÇALVES, L. G., & GROSSO, C. F. (2011). Microencapsulation of tocopherols in lipid matrix by spray chilling method. Procedia Food Science, 1, 1732–1739. https://doi.org/10.1016/j.profoo.2011.09.255

GARCÍA-CHACÓN, J. M., MARÍN-LOAIZA, J. C., & OSORIO, C. (2023). Camu Camu (Myrciaria dubia (Kunth) McVaugh): An Amazonian Fruit with Biofunctional Properties–A Review. ACS Omega, 8(6), 5169–5183. https://doi.org/10.1021/acsomega.2c07245

GARCÍA-MORENO, P. J., ÖZDEMIR, N., STEPHANSEN, K., MATEIU, R. V., ECHEGOYEN, Y., LAGARON, J. M., CHRONAKIS, I. S., & JACOBSEN, C. (2017). Development of carbohydrate-based nano-microstructures loaded with fish oil by using electrohydrodynamic processing. Food Hydrocolloids, 69, 273–285. https://doi.org/10.1016/j.foodhyd.2017.02.013

GARCÍA-MORENO, P. J., PELAYO, A., YU, S., BUSOLO, M., LAGARON, J. M., CHRONAKIS, I. S., & JACOBSEN, C. (2018). Physicochemical characterization and oxidative stability of fish oil-loaded electrosprayed capsules: Combined use of whey protein and carbohydrates as wall materials. Journal of Food Engineering, 231, 42–53. https://doi.org/10.1016/j.jfoodeng.2018.03.005

GERANPOUR, M., ASSADPOUR, E., & JAFARI, S. M. (2020). Recent advances in the spray drying encapsulation of essential fatty acids and functional oils. In Trends in Food Science and Technology (Vol. 102, pp. 71–90). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.05.028

GHARSALLAOUI, A., ROUDAUT, G., CHAMBIN, O., VOILLEY, A., & SAUREL, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. In Food Research International (Vol. 40, Issue 9, pp. 1107–1121). https://doi.org/10.1016/j.foodres.2007.07.004

GOED. (2019). About EPA and DHA.

GOED. (2024). Annual Report 2023. https://goedomega3.com/storage/app/media/annual%20reports/GOED%20Annual%20Report%202023-%20v3.pdf

GOMEZ-ESTACA, J., BALAGUER, M. P., GAVARA, R., & HERNANDEZ-MUNOZ, P. (2012). Formation of zein nanoparticles by electrohydrodynamic atomization: Effect of the main processing variables and suitability for encapsulating the

food coloring and active ingredient curcumin. Food Hydrocolloids, 28(1), 82–91. https://doi.org/10.1016/J.FOODHYD.2011.11.013

GÓMEZ-ESTACA, J., BALAGUER, M. P., LÓPEZ-CARBALLO, G., GAVARA, R., & HERNÁNDEZ-MUÑOZ, P. (2017). Improving antioxidant and antimicrobial properties of curcumin by means of encapsulation in gelatin through electrohydrodynamic atomization. Food Hydrocolloids, 70, 313–320. https://doi.org/10.1016/j.foodhyd.2017.04.019

GÓMEZ-ESTACA, J., GAVARA, R., & HERNÁNDEZ-MUÑOZ, P. (2015). Encapsulation of curcumin in electrosprayed gelatin microspheres enhances its bioaccessibility and widens its uses in food applications. Innovative Food Science and Emerging Technologies, 29, 302–307. https://doi.org/10.1016/j.ifset.2015.03.004

GÓMEZ-MASCARAQUE, L. G., HERNÁNDEZ-ROJAS, M., TARANCÓN, P., TENON, M., FEUILLÈRE, N., VÉLEZ RUIZ, J. F., FISZMAN, S., & LÓPEZ-RUBIO, A. (2017). Impact of microencapsulation within electrosprayed proteins on the formulation of green tea extract-enriched biscuits. LWT - Food Science and Technology, 81, 77–86. https://doi.org/10.1016/J.LWT.2017.03.041

GÓMEZ-MASCARAQUE, L. G., LAGARÓN, J. M., & LÓPEZ-RUBIO, A. (2015). Electrosprayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. Food Hydrocolloids, 49, 42–52. https://doi.org/10.1016/j.foodhyd.2015.03.006

GÓMEZ-MASCARAQUE, L. G., & LÓPEZ-RUBIO, A. (2016). Protein-based emulsion electrosprayed micro- and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. Journal of Colloid and Interface Science, 465, 259–270. https://doi.org/10.1016/j.jcis.2015.11.061

GÓMEZ-MASCARAQUE, L. G., SANCHEZ, G., & LÓPEZ-RUBIO, A. (2016). Impact of molecular weight on the formation of electrosprayed chitosan microcapsules as delivery vehicles for bioactive compounds. Carbohydrate Polymers, 150, 121–130. https://doi.org/10.1016/J.CARBPOL.2016.05.012

GONZÁLEZ-CRUZ, E. M., ISAAC, A. G., CRISTINA, P., LAGARÓN, J. M., MONTSERRAT, C. S., & RAGAZZO-SÁNCHEZ, J. A. (2023). Nanoencapsulation of Polyphenolic-Rich Extract from Biloxi Blueberries (Vaccinium Corymbosum L.) by Electrospraying using Zein as Encapsulating Material. Biointerface Research in Applied Chemistry, 13(1). https://doi.org/10.33263/BRIAC131.078

GOUIN, S. (2004). Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology, 15(7–8), 330–347. https://doi.org/10.1016/J.TIFS.2003.10.005

GRAND VIEW RESEARCH. (2024a). Health And Wellness Foods Market Size, Share Report, 2030. https://www.grandviewresearch.com/industry-analysis/health-wellness-foods-market-report

GRAND VIEW RESEARCH. (2024b). Healthy Foods Market Size, Share. Industry Report, 2030. https://www.grandviewresearch.com/industry-analysis/healthy-foods-market-report

GRAND VIEW RESEARCH. (2024c). Nutraceuticals Market Size, Share And Growth Report, 2030. https://www.grandviewresearch.com/industry-analysis/nutraceuticals-market

GRAND VIEW RESEARCH. (2024d). Nutraceuticals Market Size, Share And Growth Report, 2030. https://www.grandviewresearch.com/industry-analysis/nutraceuticals-market

GRAND VIEW RESEARCH. (2024e, August). Nutraceuticals Market Size & Outlook, 2030. https://www.grandviewresearch.com/horizon/outlook/nutraceuticals-market-size/global

GRAND VIEW RESEARCH. (2023a). Europe Functional Foods Market Size & Outlook, 2030. https://www.grandviewresearch.com/horizon/outlook/functional-foods-market/europe

GRAND VIEW RESEARCH. (2023b). Functional Foods Market Size & Outlook, 2030. https://www.grandviewresearch.com/horizon/outlook/functional-foods-market-size/global

GRESELE, P., CERLETTI, C., GUGLIELMINI, G., PIGNATELLI, P., DE GAETANO, G., & VIOLI, F. (2011). Effects of resveratrol and other wine polyphenols on vascular function: An update. In Journal of Nutritional Biochemistry (Vol. 22, Issue 3, pp. 201–211). https://doi.org/10.1016/j.jnutbio.2010.07.004

GUPTA, D., BLEAKLEY, B., & GUPTA, R. K. (2007). Dragon's blood: Botany, chemistry and therapeutic uses. Journal of Ethnopharmacology, 115(3), 361–380. https://doi.org/10.1016/j.jep.2007.10.018

GUPTA, S., KHAN, S., MUZAFAR, M., KUSHWAHA, M., YADAV, A. K., & GUPTA, A. P. (2016). Encapsulation: entrapping essential oil/flavors/aromas in food. In Encapsulations. Elsevier Inc. https://doi.org/10.1016/b978-0-12-804307-3.00006-5

HASLER, C. M. (2002). Functional Foods: Benefits, Concerns and Challenges—A Position Paper from the American Council on Science and Health. The Journal of Nutrition, 132(12), 3772–3781. https://doi.org/10.1093/jn/132.12.3772

HASLER, C. M., & BROWN, A. C. (2009). Position of the American Dietetic Association: functional foods. Journal of the American Dietetic Association, 109(4), 735–746. https://doi.org/10.1016/j.jada.2009.02.023

HASSOUN, A., CROPOTOVA, J., TRIF, M., RUSU, A. V., BOBIŞ, O., NAYIK, G. A., JAGDALE, Y. D., SAEED, F., AFZAAL, M., MOSTASHARI, P., KHANEGHAH, A. M., & REGENSTEIN, J. M. (2022). Consumer acceptance of new food trends resulting from the fourth industrial revolution technologies: A narrative review of literature and future perspectives. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.972154

HEALTH CANADA. (1998). NUTRACEUTICALS/FUNCTIONAL FOODS AND HEALTH CLAIMS ON FOODS. Standard 1.2.7.

HEALTH FUNCTIONAL FOOD CODE (2021). www.mfds.go.kr

HOSSEINI, S. F., RAMEZANZADE, L., & MCCLEMENTS, D. J. (2021). Recent advances in nanoencapsulation of hydrophobic marine bioactives: Bioavailability, safety, and sensory attributes of nano-fortified functional foods. Trends in Food Science & Technology, 109, 322–339. https://doi.org/10.1016/j.tifs.2021.01.045

INTERNATIONAL FOOD INFORMATION COUNCIL. (2020). 2020 Food & Health Survey. https://foodinsight.org/wp-content/uploads/2020/06/IFIC-Food-and-Health-Survey-2020.pdf

JACOBSEN, C., GARCÍA-MORENO, P. J., MENDES, A. C., MATEIU, R. V, & CHRONAKIS, I. S. (2018). Use of Electrohydrodynamic Processing for Encapsulation of Sensitive Bioactive Compounds and Applications in Food Supplemental Material. https://doi.org/10.1146/annurev-food-030117

JAWOREK, A. (2016). Electrohydrodynamic microencapsulation technology. In Encapsulations. Elsevier Inc. https://doi.org/10.1016/b978-0-12-804307-3.00001-6

JAYAN, H., MARIA LEENA, M., SIVAKAMA SUNDARI, S. K., MOSES, J. A., & ANANDHARAMAKRISHNAN, C. (2019). Improvement of bioavailability for resveratrol through encapsulation in zein using electrospraying technique. Journal of Functional Foods, 57, 417–424. https://doi.org/10.1016/J.JFF.2019.04.007

JAYAPRAKASH, P., MAUDHUIT, A., GAIANI, C., & DESOBRY, S. (2023). Encapsulation of bioactive compounds using competitive emerging techniques: Electrospraying, nano spray drying, and electrostatic spray drying. Journal of Food Engineering, 339, 111260. https://doi.org/10.1016/J.JFOODENG.2022.111260

JONES, K. (2004). Review of Sangre de Drago (Croton lechleri) - A South American Tree Sap in the Treatment of Diarrhea, Inflammation, Insect Bites, Viral Infections, and Wounds: Traditional Uses to Clinical Research. Https://Home.Liebertpub.Com/Acm, 9(6), 877–896. https://doi.org/10.1089/107555303771952235

KALKUMBE, A., WAGHMARE, S., & KAMBLE, H. (2022). MICROENCAPSULATION: A REVIEW. International Research Journal of Modernization in Engineering Technology and Science. https://doi.org/10.56726/IRJMETS26546

KARAMI, A., GHOLAMI, A. ABROUMAND, BABALOO, H., & FARHADIAN, N. (2023). Encapsulation: Spray chilling and cooling. In Principles of Biomaterials Encapsulation : Volume One (pp. 109–130). Elsevier. https://doi.org/10.1016/B978-0-323-85947-9.00003-0

KAUSHIK, P., DOWLING, K., BARROW, C. J., & ADHIKARI, B. (2015). Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. Journal of Functional Foods, 19, 868–881. https://doi.org/10.1016/j.jff.2014.06.029

KIRCHHERR, J., REIKE, D., & HEKKERT, M. (2017). Conceptualizing the circular economy: An analysis of 114 definitions. Resources, Conservation and Recycling, 127, 221–232. https://doi.org/10.1016/j.resconrec.2017.09.005

KOLANOWSKI, W., ZIOLKOWSKI, M., WEIßBRODT, J., KUNZ, B., & LAUFENBERG, G. (2006). Microencapsulation of fish oil by spray drying - Impact on oxidative stability. Part 1. European Food Research and Technology, 222(3–4), 336–342. https://doi.org/10.1007/s00217-005-0111-1

KRINGEL, D. H., SILVA, W. M. F., BIDUSKI, B., WALLER, S. B., LIM, L., DIAS, A. R. G., & ZAVAREZE, E. DA R. (2020). Free and encapsulated orange essential oil into a β-cyclodextrin inclusion complex and zein to delay fungal spoilage in cakes. Journal of Food Processing and Preservation, 44(5). https://doi.org/10.1111/jfpp.14411

LAFARGA, T., & HAYES, M. (2017). Bioactive protein hydrolysates in the functional food ingredient industry: Overcoming current challenges. In Food Reviews International (Vol. 33, Issue 3, pp. 217–246). Taylor and Francis Inc. https://doi.org/10.1080/87559129.2016.1175013

LAN, X., ZHANG, X., WANG, L., WANG, H., HU, Z., JU, X., & YUAN, Y. (2023). A review of food preservation based on zein: The perspective from application types of coating and film. Food Chemistry, 424, 136403. https://doi.org/10.1016/J.FOODCHEM.2023.136403

LANGLEY, P. C., PERGOLIZZI, J. V., TAYLOR, R., & RIDGWAY, C. (2015). Antioxidant and associated capacities of camu camu (Myrciaria dubia): A systematic review. Journal of Alternative and Complementary Medicine, 21(1), 8–14. https://doi.org/10.1089/ACM.2014.0130/ASSET/IMAGES/LARGE/FIGURE1.JPEG

LIVNEY, Y. D. (2010). Milk proteins as vehicles for bioactives. Current Opinion in Colloid & Interface Science, 15(1–2), 73–83. https://doi.org/10.1016/j.cocis.2009.11.002

LÓPEZ-RUBIO, A., & LAGARON, J. M. (2012). Whey protein capsules obtained through electrospraying for the encapsulation of bioactives. Innovative Food Science and Emerging Technologies, 13(JANUARY), 200–206. https://doi.org/10.1016/j.ifset.2011.10.012

MADENE, A., JACQUOT, M., SCHER, J., & DESOBRY, S. (2006). Flavour encapsulation and controlled release - A review. International Journal of Food Science and Technology, 41(1), 1–21. https://doi.org/10.1111/j.1365-2621.2005.00980.x

MARFIL, P. H. M., VASCONCELOS, F. H. T., PONTIERI, M. H., & TELIS, V. R. N. (2015). Development and validation of analytical method for palm oil determination in microcapsules produced by complex coacervation. Química Nova. https://doi.org/10.5935/0100-4042.20150164

MARKETS AND MARKETS. (2023). Bulk Food Ingredlents Market - Global Forecast to 2028. www.marketsandmarkets.com

MARKETS AND MARKETS. (2024a). Functional Food Ingredients Market - 2023 |. https://www.marketsandmarkets.com/Market-Reports/functional-food-ingredients-market-9242020.html

MARKETS AND MARKETS. (2024b). Nutraceutical Ingredients Market by Type. Global Forecast to 2029. https://www.marketsandmarkets.com/Market-Reports/nutraceutical-ingredient-market-1319.html

MARTYSIAK-ŻUROWSKA, D., & WENTA, W. (2012). A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci. Pol, 1(11), 83–89. http://www.food.actapol.net/pub/9_1_2012.pdf

MASSOUNGA BORA, A. F., MA, S., LI, X., & LIU, L. (2018). Application of microencapsulation for the safe delivery of green tea polyphenols in food systems: Review and recent advances. In Food Research International (Vol. 105, pp. 241–249). Elsevier Ltd. https://doi.org/10.1016/j.foodres.2017.11.047

MCCLEMENTS, D. J. (2012). Requirements for food ingredient and nutraceutical delivery systems. In Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals (pp. 3–18). Elsevier. https://doi.org/10.1533/9780857095909.1.3

MEINERS, J. A. (2012). Fluid bed microencapsulation and other coating methods for food ingredient and nutraceutical bioactive compounds. Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals, 151–176. https://doi.org/10.1533/9780857095909.2.151

MELLENTIN, J. (2020). Key Trends in Functional Foods & Beverages for. factor 1, 1–8. https://www.nutraceuticalsworld.com/issues/2020-11/view_features/key-trends-in-functional-foods-beverages-for-2021/?widget=listSection

MIGUEL, G. A., JACOBSEN, C., PRIETO, C., KEMPEN, P. J., LAGARON, J. M., CHRONAKIS, I. S., & GARCÍA-MORENO, P. J. (2019). Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil. Journal of Food Engineering, 263, 348–358. https://doi.org/10.1016/j.jfoodeng.2019.07.019

MINISTRY OF HEALTH, L. AND W. (1991). Food with Health Claims, Food for Special Dietary Uses, and Nutrition Labeling. https://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html

MINTEL. (2024). Global Food and Drink Trends | 2025. https://www.mintel.com/insights/food-and-drink/global-food-and-drink-trends/#download

MISRA, S., PANDEY, P., DALBHAGAT, C. G., & MISHRA, H. N. (2022). Emerging Technologies and Coating Materials for Improved Probiotication in Food Products: a Review. In Food and Bioprocess Technology (Vol. 15, Issue 5, pp. 998–1039). Springer. https://doi.org/10.1007/s11947-021-02753-5

MOHAN, A., RAJENDRAN, S. R. C. K., HE, Q. S., BAZINET, L., & UDENIGWE, C. C. (2015). Encapsulation of food protein hydrolysates and peptides: A review. In RSC Advances (Vol. 5, Issue 97, pp. 79270–79278). Royal Society of Chemistry. https://doi.org/10.1039/c5ra13419f

MOHANTY, S., & SINGHAL, K. (2018). Functional Foods As Personalised Nutrition: Definitions and Genomic Insights. In Functional Food and Human Health (pp. 513–535). Springer Singapore. https://doi.org/10.1007/978-981-13-1123-9_22

MONTSERRAT-DE LA PAZ, S. (2021). Active ingredients from foods: Biochemical and processing aspects. Foods, 10(6). https://doi.org/10.3390/FOODS10061240

NIÑO-VÁSQUEZ, I. A., MUÑIZ-MÁRQUEZ, D., ASCACIO-VALDÉS, J. A., CONTRERAS-ESQUIVEL, J. C., AGUILAR, C. N., RODRÍGUEZ-HERRERA, R., & FLORES-GALLEGOS, A. C. (2022). Co-microencapsulation: a promising multi-approach technique for enhancement of functional properties. In Bioengineered (Vol. 13, Issue 3, pp. 5168–5189). Taylor and Francis Ltd. https://doi.org/10.1080/21655979.2022.2037363

NIU, B., SHAO, P., LUO, Y., & SUN, P. (2020). Recent advances of electrosprayed particles as encapsulation systems of bioactives for food application. In Food Hydrocolloids (Vol. 99). Elsevier B.V. https://doi.org/10.1016/j.foodhyd.2019.105376

ODS - NIH. (1994). Dietary Supplement Health and Education Act of 1994. https://ods.od.nih.gov/About/DSHEA_Wording.aspx#sec1

OKURO, P. K., EUSTÁQUIO DE MATOS JUNIOR, F., & SÍLVIA FAVARO-TRINDADE, C. (2013). Technological Challenges for Spray Chilling Encapsulation of Functional Food Ingredients. Food Technology and Biotechnology, 51, 171–182.

OLIVARES-TENORIO, M. L., CORTES-PRIETO, C. M., LONDOÑO-UNIVIO, N. C., ROJAS-DÍAZ, D. A., QUINTANILLA-CARVAJAL, M. X., TIBAQUIRA-PEREZ, L. N., RICAURTE-PUENTES, L. Y., RADA-BULA, A. I., ROMERO, H. M., & GARCIA-NUÑEZ, J. A. (2024). Bioactive compounds in palm oil: A comprehensive review of recent advances in physicochemical characteristics, health-promoting properties and technologies for extraction, concentration, fractionation, encapsulation and functional food applications. Journal of Food Composition and Analysis, 132, 106306. https://doi.org/10.1016/J.JFCA.2024.106306

OXLEY, J. (2014). Overview of Microencapsulation Process Technologies. In Microencapsulation in the Food Industry (pp. 35–46). Elsevier. https://doi.org/10.1016/B978-0-12-404568-2.00004-2

PANDEY, K. B., & RIZVI, S. I. (2009). Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. Oxidative Medicine and Cellular Longevity, 2(5), 270–278. https://doi.org/10.4161/oxim.2.5.9498

PANDEY, M., VERMA, R. K., & SARAF, S. A. (2014). Nutraceuticals: New Era of Medicine and Health. Asian Journal of Pharmaceutical and Clinical Research. https://www.researchgate.net/publication/240719276

PARRIS, N., COOKE, P. H., & HICKS, K. B. (2005). Encapsulation of essential oils in zein nanospherical particles. Journal of Agricultural and Food Chemistry, 53(12), 4788–4792. https://doi.org/10.1021/jf040492p

PASTRANA, L., GONZÁLEZ, R., ESTÉVEZ, N., PEREIRA, L., RODRÍGUEZ AMADO, I., FUCIÑOS, P., FUCIÑOS, C., RÚA, M. L., ALONSO, E., & TRONCOSO, R. (2017). Functional Foods. In Current Developments in Biotechnology and Bioengineering: Food and Beverages Industry (pp. 165–200). Elsevier Inc. https://doi.org/10.1016/B978-0-444-63666-9.00007-8

PATEL, A., HU, Y., TIWARI, J. K., & VELIKOV, K. P. (2010). Synthesis and characterisation of zein-curcumin colloidal particles. Soft Matter, 6(24), 6192–6199. https://doi.org/10.1039/c0sm00800a

PAXIMADA, P., ECHEGOYEN, Y., KOUTINAS, A. A., MANDALA, I. G., & LAGARON, J. M. (2017). Encapsulation of hydrophilic and lipophilized catechin into nanoparticles through emulsion electrospraying. Food Hydrocolloids, 64, 123–132. https://doi.org/10.1016/j.foodhyd.2016.11.003

PAXIMADA, P., KANAVOU, E., APOSTOLIDIS, E., & MANDALA, IOANNA. G. (2021). Encapsulation of catechin in electrosprayed food-grade particles. Food Hydrocolloids for Health, 1, 100021. https://doi.org/10.1016/j.fhfh.2021.100021

PERES, I. S. A., CONCEIÇÃO, K. A. O., SILVA, L. A. F., KHOURI, N. G., YOSHIDA, C. M. P., CONCHA, V. O. C., LUCARINI, M., DURAZZO, A., SANTINI, A., SOUTO, E. B., & SEVERINO, P. (2023). Dragon's Blood: antioxidant properties for nutraceuticals and pharmaceuticals. Rendiconti Lincei. Scienze Fisiche e Naturali, 34(1), 131–142. https://doi.org/10.1007/s12210-022-01122-4

PÉREZ-MASIÁ, R., LAGARON, J. M., & LÓPEZ-RUBIO, A. (2014). Development and Optimization of Novel Encapsulation Structures of Interest in Functional Foods Through Electrospraying. Food and Bioprocess Technology, 7(11), 3236–3245. https://doi.org/10.1007/s11947-014-1304-z

PRIETO, C., & LAGARON, J. M. (2020). Nanodroplets of docosahexaenoic acid-enriched algae oil encapsulated within microparticles of hydrocolloids by emulsion electrospraying assisted by pressurized gas. Nanomaterials, 10(2). https://doi.org/10.3390/nano10020270

PRIETO, C., TALÓN, E., & LAGARON, J. M. (2021). Room temperature encapsulation of algae oil in water insoluble gluten extract. Food Hydrocolloids for Health, 1, 100022. https://doi.org/10.1016/j.fhfh.2021.100022

PRIETO, C., TALÓN, E., NOREÑA, C. Z., & LAGARON, J. M. (2022). Effect of Whey Protein Purity on the Characteristics of Algae Oil-Loaded Encapsulates Obtained by Electrospraying Assisted by Pressurized Gas. Nanomaterials, 12(18). https://doi.org/10.3390/nano12183096

RADÜNZ, M., HACKBART, H. C. D. S., BONA, N. P., PEDRA, N. S., HOFFMANN, J. F., STEFANELLO, F. M., & DA ROSA ZAVAREZE, E. (2020). Glucosinolates and phenolic compounds rich broccoli extract: Encapsulation by electrospraying and antitumor activity against glial tumor cells. Colloids and Surfaces B: Biointerfaces, 192, 111020. https://doi.org/10.1016/J.COLSURFB.2020.111020

RAHMANI-MANGLANO, N. E., GUADIX, E. M., YESILTAS, B., PRIETO, C., LAGARON, J. M., JACOBSEN, C., & GARCÍA-MORENO, P. J. (2024). Non-emulsion-based encapsulation of fish oil by coaxial electrospraying assisted by pressurized gas enhances the oxidative stability of a capsule-fortified salad dressing. Food Chemistry, 431, 137157. https://doi.org/10.1016/J.FOODCHEM.2023.137157

RAMAKRISHNAN, Y., ADZAHAN, N. M., YUSOF, Y. A., & MUHAMMAD, K. (2018). Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation. Powder Technology, 328, 406–414.

https://doi.org/10.1016/j.powtec.2017.12.018

REGULATION EC 1924. (2006). European Community (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union, L 404, 9–25.

RIBEIRO, A. M., ESTEVINHO, B. N., & ROCHA, F. (2020). Microencapsulation of polyphenols - The specific case of the microencapsulation of Sambucus Nigra L. extracts - A review. Trends in Food Science & Technology, 105, 454–467. https://doi.org/10.1016/j.tifs.2019.03.011

ROJAS, K., VERDUGO-MOLINARES, M. G., & VALLEJO-CARDONA, A. A. (2024). Use of encapsulating polymers of active compounds in the pharmaceutical and food industry. In Food Chemistry Advances (Vol. 4). Elsevier Ltd. https://doi.org/10.1016/j.focha.2024.100619

SAHA, D., KHATANIAR, A., SINGH, A. K., & JHA, A. N. (2023). Review of methods for encapsulation of nutraceutical compounds. In Nutraceuticals: Sources, Processing Methods, Properties, and Applications (pp. 127–156). Elsevier. https://doi.org/10.1016/B978-0-443-19193-0.00010-1

SAWANT, R. B., NIKAM, S. P., ROY, A., KUMAR, A., MOHAMMED, O. A., SHARMA, K., RAI, A. K., ROY, A., GAUR, A., & VERMA, R. (2024). Nanocarriers for nutraceutical delivery: A miniaturized revolution in health. In Nano-Structures and Nano-Objects (Vol. 39). Elsevier B.V. https://doi.org/10.1016/j.nanoso.2024.101321

SCALBERT, A., JOHNSON, I. T., & SALTMARSH, M. (2005). Polyphenols: antioxidants and beyond. In The American journal of clinical nutrition (Vol. 81, Issue 1 Suppl). https://doi.org/10.1093/ajcn/81.1.215s

SCHMIDT, M., HOFFMANN, T., BÜCK, A., & TSOTSAS, E. (2015). Experimental investigation of continuous fluidized bed spray granulation with internal classification. Procedia Engineering, 102, 565–574. https://doi.org/10.1016/j.proeng.2015.01.127

SCHOONJANS, R., CASTENMILLER, J., CHAUDHRY, Q., CUBADDA, F., DASKALEROS, T., FRANZ, R., GOTT, D., MAST, J., MORTENSEN, A., OOMEN, A. G., RAUSCHER, H., WEIGEL, S., ASTUTO, M. C., CATTANEO, I., BARTHELEMY, E., RINCON, A., & TARAZONA, J. (2023). Regulatory safety assessment of nanoparticles for the food chain in Europe. Trends in Food Science & Technology, 134, 98–111. https://doi.org/10.1016/j.tifs.2023.01.017

SILVA, M. P., TULINI, F. L., MARTINS, E., PENNING, M., FÁVARO-TRINDADE, C. S., & PONCELET, D. (2018). Comparison of extrusion and co-extrusion encapsulation techniques to protect Lactobacillus acidophilus LA3 in simulated

gastrointestinal fluids. LWT, 89, 392–399. https://doi.org/10.1016/j.lwt.2017.11.008

SIMONETTO, M., INFANTE, M., SACCO, R. L., RUNDEK, T., & DELLA-MORTE, D. (2019). A Novel Anti-Inflammatory Role of Omega-3 PUFAs in Prevention and Treatment of Atherosclerosis and Vascular Cognitive Impairment and Dementia. Nutrients, 11(10), 2279. https://doi.org/10.3390/nu11102279

SIMOPOULOS, A. P. (2002). Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. Journal of the American College of Nutrition, 21(6), 495–505. https://doi.org/10.1080/07315724.2002.10719248

SOLEIMANIFAR, M., JAFARI, S. M., & ASSADPOUR, E. (2020). Encapsulation of olive leaf phenolics within electrosprayed whey protein nanoparticles; production and characterization. Food Hydrocolloids, 101, 105572. https://doi.org/10.1016/J.FOODHYD.2019.105572

SU, J., WANG, X., LI, W., CHEN, L., ZENG, X., HUANG, Q., & HU, B. (2018). Enhancing the Viability of Lactobacillus plantarum as Probiotics through Encapsulation with High Internal Phase Emulsions Stabilized with Whey Protein Isolate Microgels. Journal of Agricultural and Food Chemistry, 66(46), 12335–12343. https://doi.org/10.1021/acs.jafc.8b03807

TAPIA-HERNÁNDEZ, J. A., RODRÍGUEZ-FÉLIX, F., & KATOUZIAN, I. (2017). Nanocapsule formation by electrospraying. In Nanoencapsulation Technologies for the Food and Nutraceutical Industries. Elsevier Inc. https://doi.org/10.1016/B978-0-12-809436-5.00009-4

TAVARES, G. M., CROGUENNEC, T., CARVALHO, A. F., & BOUHALLAB, S. (2014). Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. In Trends in Food Science and Technology (Vol. 37, Issue 1, pp. 5–20). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2014.02.008

TIMILSENA, Y. P., ADHIKARI, R., BARROW, C. J., & ADHIKARI, B. (2017). Digestion behaviour of chia seed oil encapsulated in chia seed protein-gum complex coacervates. Food Hydrocolloids, 66, 71–81. https://doi.org/10.1016/j.foodhyd.2016.12.017

TIMILSENA, Y. P., AKANBI, T. O., KHALID, N., ADHIKARI, B., & BARROW, C. J. (2019). Complex coacervation: Principles, mechanisms and applications in microencapsulation. International Journal of Biological Macromolecules, 121, 1276–1286. https://doi.org/10.1016/J.IJBIOMAC.2018.10.144

TIMILSENA, Y. P., HAQUE, MD. A., & ADHIKARI, B. (2020). Encapsulation in the Food Industry: A Brief Historical Overview to Recent Developments. Food and Nutrition Sciences, 11(06), 481–508. https://doi.org/10.4236/fns.2020.116035

TIMSINA, R., THAPA, R. K., MOLDESTAD, B. M. E., & EIKELAND, M. S. (2019). Effect of particle size on flow behavior in fluidized beds. International Journal of Energy Production and Management, 4(4), 273–286. https://doi.org/10.2495/EQ-V4-N4-287-297

TIWARI A, & BALASUNDARAM P. (2023). Public Health Considerations Regarding Obesity. StatPearls Publishing.

TULINI, F. L., SOUZA, V. B., THOMAZINI, M., SILVA, M. P., MASSARIOLI, A. P., ALENCAR, S. M., PALLONE, E. M. J. A., GENOVESE, M. I., & FAVARO-TRINDADE, C. S. (2017). Evaluation of the release profile, stability and antioxidant activity of a proanthocyanidin-rich cinnamon (Cinnamomum zeylanicum) extract co-encapsulated with α-tocopherol by spray chilling. Food Research International, 95, 117–124. https://doi.org/10.1016/j.foodres.2017.03.010

UNITED NATIONS. (2024). Goal 3 | Department of Economic and Social Affairs. https://sdgs.un.org/goals/goal3#progress_and_info

VASISHT, N. (2014). Factors and Mechanisms in Microencapsulation. In Microencapsulation in the Food Industry. Elsevier Inc. https://doi.org/10.1016/b978-0-12-404568-2.00002-9

VIGNESH, A., AMAL, T. C., SARVALINGAM, A., & VASANTH, K. (2024). A review on the influence of nutraceuticals and functional foods on health. Food Chemistry Advances, 5, 100749. https://doi.org/10.1016/J.FOCHA.2024.100749

VIGNESH, V., SETH, D., SINGH, C. N., DWIVEDI, M., SINGH, S. K., & KUMARI, S. (2024). Electrohydrodynamic encapsulation: A novel technique to enhance the stability of bioactive compounds in food. In Trends in Food Science and Technology (Vol. 151). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2024.104626

VILLAÑO, D., GIRONÉS-VILAPANA, A., GARCÍA-VIGUERA, C., & MORENO, D. A. (2021). Development of functional foods. In Innovation Strategies in the Food Industry: Tools for Implementation, Second Edition (pp. 193–207). Elsevier. https://doi.org/10.1016/B978-0-323-85203-6.00017-7

WANDREY, C., BARTKOWIAK, A., & HARDING, S. E. (2010). Materials for encapsulation. In Encapsulation Technologies for Active Food Ingredients and Food Processing. https://doi.org/10.1007/978-1-4419-1008-0_3

WANG, C., TU, Q., YE, Z., SHI, Y., XIAO, M., FANG, Y., LU, Y., & YOU, R. (2024). Active constituents, encapsulation technology, bioactivities and applications in food industry by essential oils of Litsea cubeba (Lour) Pers: A review. Trends in Food Science & Technology, 153, 104728. https://doi.org/10.1016/j.tifs.2024.104728

WANG, J. (2020). Complex coacervation between proteins and polysaccharides for the encapsulation of active molecules [Université de Lyon]. https://theses.hal.science/tel-03840266v1

WEN, P., ZONG, M. H., LINHARDT, R. J., FENG, K., & WU, H. (2017). Electrospinning: A novel nano-encapsulation approach for bioactive compounds. In Trends in Food Science and Technology (Vol. 70, pp. 56–68). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2017.10.009

WHO. (2020, April 29). Healthy diet. https://www.who.int/news-room/fact-sheets/detail/healthy-diet

WUNSCH, N.-G. (2024a, February 6). Health and wellness food trends in Europe - statistics and facts | Statista. https://www.statista.com/topics/3731/health-and-wellness-food-trends-in-europe/

WUNSCH, N.-G. (2024b, April 29). Global dietary supplements and functional foods market - statistics & facts | Statista. https://www.statista.com/topics/8402/global-dietary-supplements-and-functional-food-market/#editorsPicks

XIAO, Q., CHEN, G., ZHANG, Y., WENG, H., CAI, M., & XIAO, A. (2020). Evaluation of a novel self-emulsifiable dodecenyl succinylated agarose in microencapsulation of docosahexaenoic acid (DHA) through spray-chilling process. International Journal of Biological Macromolecules, 163, 2314–2324. https://doi.org/10.1016/j.ijbiomac.2020.09.108

YAN, C., & ZHANG, W. (2014). Coacervation Processes. In Microencapsulation in the Food Industry (pp. 125–137). Elsevier. https://doi.org/10.1016/b978-0-12-404568-2.00012-1

YANG, Y.-Y., ZHANG, M., LIU, Z.-P., WANG, K., & YU, D.-G. (2018). Meletin sustained-release gliadin nanoparticles prepared via solvent surface modification on blending electrospraying. Applied Surface Science, 434, 1040–1047. https://doi.org/10.1016/j.apsusc.2017.11.024

YE, Q., GEORGES, N., & SELOMULYA, C. (2018). Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. In Trends in Food Science and Technology (Vol. 78, pp. 167–179). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2018.05.025

ZAWISTOWSKI, J. (2011). Legislation of functional foods in Asia. In Functional Foods: Concept to Product: Second Edition (pp. 73–108). Elsevier Ltd. https://doi.org/10.1533/9780857092557.1.73

ZHANG, C., LI, Y., WANG, P., & ZHANG, H. (2020). Electrospinning of nanofibers: Potentials and perspectives for active food packaging. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 2, pp. 479–502). Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12536

ZHONG, Q., & JIN, M. (2009). Zein nanoparticles produced by liquid-liquid dispersion. Food Hydrocolloids, 23(8), 2380–2387. https://doi.org/10.1016/j.foodhyd.2009.06.015

ZINSSTAG, J., SCHELLING, E., WALTNER-TOEWS, D., & TANNER, M. (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. Preventive Veterinary Medicine, 101(3–4), 148–156. https://doi.org/10.1016/j.prevetmed.2010.07.003

ZUIDAM, N. J., & NEDOVIĆ, V. A. (2010). Encapsulation technologies for active food ingredients and food processing. Encapsulation Technologies for Active Food Ingredients and Food Processing, 1–400. https://doi.org/10.1007/978-1-4419-1008-0

3. OBJECTIVES

The main objective of this PhD thesis is to evaluate the potential of the Electrospraying Assisted by Pressurized Gas (EAPG) technology to develop microencapsulation formulations of underexploited bioactive ingredients, their characterization, and the evaluation of their stability under different storage conditions for their potential application in end-consumer health-enhancing products.

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

- **Study of bioactive compounds from underexploited natural sources**: Identify and characterize bioactive compounds in non-conventional plant and fruit sources with recognized health benefits, ensuring their suitability for incorporation into food matrices. This will involve assessing their functional properties, as highlighted in previous research on plant-based bioactives.
- **Study the potential of EAPG technology to encapsulate challenging bioactives.** Investigate the potential applications of EAPG technology in the encapsulation of complex bioactive compounds, focusing on its ability.
- **Design and development of encapsulation formulations to guarantee the bioactive compound stability under storage conditions**: Develop encapsulation matrices with optimal structural and functional characteristics for protecting bioactive compounds. This includes investigating various combinations and formulations of whey protein concentrate (WPC) and zein (ZN) to maximize stability under storage conditions.
- **Validation of microencapsulated bioactives in agri-food matrices**: Validate the application of the microencapsulated bioactives in model agri-food matrices to assess their potential in functional food products. This involves evaluating the effectiveness and release profile of the encapsulated compounds within food systems, as explored in our practical applications of encapsulated plant bioactives

4. CHAPTER 1

Eicosapentaenoic Acid - EPA

Room Temperature Nanoencapsulation of Bioactive Eicosapentaenoic Acid Rich Oil within Whey Protein Microparticles

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Nanomaterials 2021, 11, 575
https://doi.org/10.3390/nano11030575
Impact Factor (2021): 5.719
Current Impact Factor: 4.2
Citescore:8.5
Article citations: 13

Abstract

In this study, emulsion electrospraying assisted by pressurized gas (EAPG) has been performed for the first time to entrap ca. 760 nm droplets of the bioactive eicosapentaenoic acid (EPA)-rich oil into whey protein concentrate (WPC) at room temperature. The submicron droplets of EPA oil were encapsulated within WPC spherical microparticles, with sizes around 5 μm. The EPA oil did not oxidize in the course of the encapsulation performed at 25 °C and in the presence of air, as corroborated by the peroxide value measurements. Attenuated Total Reflection—Fourier Transform Infrared spectroscopy and oxygen consumption tests confirmed that the encapsulated EPA-rich oil showed increased oxidative stability in comparison with the free oil during an accelerated oxidation test under ultraviolet light. Moreover, the encapsulated EPA-rich oil showed increased thermal stability in comparison with the free oil, as measured by oxidative thermogravimetric analysis. The encapsulated EPA-rich oil showed a somewhat reduced organoleptic impact in contrast with the neat EPA oil using rehydrated powdered milk as a reference. Finally, the oxidative stability by thermogravimetric analysis and organoleptic impact of mixtures of EPA and docosahexaenoic acid (DHA)-loaded microparticles was also studied, suggesting an overall reduced organoleptic impact compared to pure EPA. The results here suggest that it is possible to encapsulate 80% polyunsaturated fatty acids (PUFAs)-enriched oils by emulsion EAPG technology at room temperature, which could be used to produce personalized nutraceuticals or pharmaceuticals alone or in combination with other microparticles encapsulating different PUFAs to obtain different targeted health and organoleptic benefits.

Keywords: encapsulation; WPC microparticles; EPA-rich oil; electrospraying; oxidative and thermal stability; personalized nutrition

1. Introduction

Understanding the relationship between nutrition and health has resulted in the development of new products; most of them aimed at preventing nutrition-related diseases and improve physical and mental well-being (Panse & Phalke, 2016). Polyunsaturated fatty acids (PUFAs) are one of the most studied compounds in healthcare, according to the Global Organization for EPA and DHA Omega-3 (GOED & Council for Responsible Nutrition, 2015). Hence, there are more than 36,000 published studies on PUFAs, including more than 4000 human clinical trials. Numerous investigations have demonstrated that PUFAs are fundamental for growth and development as well as due to their positive effects on behavior and on the health of the brain, eyes, heart, joints, and skin (Kaushik et al., 2015). Therefore, there is a great interest from the industry in incorporating PUFAs into the active ingredient market. Currently, the PUFAs market is based, especially on alpha-linolenic acid (ALA) C18:3, eicosapentaenoic acid (EPA) C20:5, and docosahexaenoic acid (DHA) C22:6. However, DHA and EPA are claimed to have the most potent health benefits of the PUFAs (European Food Safety Authority -EFSA-, 2011). Differential biochemical and physiological responses to EPA and DHA have been observed (Russell & Bürgin-Maunder, 2012). In particular, EPA is a precursor of multiple metabolites performing essential functions in biological membranes and serves as a precursor for numerous lipid regulators in cellular metabolism (Wen & Chen, 2010). Moreover, it is involved in the therapy of certain illnesses, such as protective effect in liver steatosis (Albracht-Schulte et al., 2019), correction of postprandial hypertriglyceridemia, hyperglycemia and insulin secretion ability (Sawada et al., 2016), reduction of the occurrence of major coronary event (MCE) (Sasaki et al., 2012; Yokoyama et al., 2007), antiatherosclerotic effects (Nelson et al., 2017), anti-inflammatory effects, depression, hyperactivity disorders (Chang et al., 2019), and joint health, among others. However, the poor solubility, low stability and limited bioavailability of this sensitive bioactive compound highly reduced its intended health effect, reduced the food product lifetime, and produced consumerist reluctance due to bad

organoleptic properties, which greatly limited their application in the health-enhancing products industry.

The main challenge to overcome in the production of functional foods based on omega-3 PUFAs is their oxidation, which produces mainly peroxides, alcohols and aldehydes (Eratte et al., 2015), reducing their nutritional value and increasing their organoleptic impact (GOED & Council for Responsible Nutrition, 2015). Several approaches have been developed to minimize oxidative deterioration and producing stable and easy-to-handle forms (Kaushik et al., 2015), improving dispersibility, masking off-flavors and increasing bioavailability (Augustin & Hemar, 2009). Several strategies employ antioxidants, limit the exposure to ambient air, refine and blanket storage containers with inert gases or make use of microencapsulation. Among them, microencapsulation is one of the most promising approaches, which consists of the entrapment of the bioactive compound within a protective matrix, which restricts the interaction of the functional ingredient with the environment (Gómez-Mascaraque & López-Rubio, 2016). Moreover, microencapsulation increases bioavailability, reduces organoleptic impact and favors the controlled delivery of these functional ingredients. Microencapsulation processes are classified into physical and chemical methods, being spray-drying, freeze-drying, coacervation and fluid bed coating, the most common methods used until now for the encapsulation of bioactives (Sobel et al., 2014; Tavares et al., 2014). However, these methods involve temperatures over 100 °C or non-food grade chemicals that could impact the bioactivity of the functional ingredient or prevent its use in food.

Electrospraying is an innovative technology based on electrically charged polymer solution, which after drying, leads to micro- or nanoparticles (Gómez-Mascaraque & López-Rubio, 2016). Electrospraying is a simple and adaptive technology performed at ambient temperature, which prevents bioactivity loss, and does not need a posterior phase to obtain dry powder (Gómez-Mascaraque & López-Rubio, 2016). The use of high voltage results in high encapsulation efficiency and controlled particle size distribution. This process presents

additional strong points as the large variety of potential carrier matrices, such as carbohydrates, gums, lipids, proteins and waxes, are used either alone or in combination (Lu et al., 2019; Martins et al., 2014). However, the principal disadvantage of the electrospraying technology is its low yield (Torres-Giner et al., 2010), which has restricted the technology transfer to industry. In this sense, Busolo et al., (2019) set up a novel high-yield technology based on the integration of electrospraying and gas-driven nebulization, called electrospraying assisted by pressurized gas (EAPG). In this process, a polymer solution is atomized by means of a pneumatic injector to produce nebulized droplets, which are further size reduced to form encapsulates within a high electric field at the ambient temperature inside a drying compartment, being recovered as a non-agglomerated powder. The advantages of this technique have been previously proven for the encapsulation of fish oil into zein (Busolo et al., 2019; Miguel et al., 2019) and into the combination of whey protein concentrate and carbohydrates (García-Moreno et al., 2018), and the encapsulation of algae oil into whey protein concentrate and maltodextrin (Prieto & Lagaron, 2020). These works have demonstrated the potential of this technology to stabilize DHA-enriched algae and fish oils. However, as far as we know, no scientific studies have been published dealing with the encapsulation of EPA-rich oils by electrospraying or EAPG.

The main objective of the present study is to investigate the submicron encapsulation of an EPA-rich oil via EAPG technology to produce EPA encapsulates, which could be used in personalized nutrition alone or in combination with other DHA encapsulates. Whey protein concentrate was selected as the wall material because of its amphiphilic, gelation and protective qualities (Augustin & Oliver, 2014). Produced particles were characterized in relation to their morphologic attributes, oil entrapment efficiency, stability against oxidation, and organoleptic characteristics into a food reference. Finally, thermal stability and organoleptic impact were also studied in combination with DHA encapsulates.

2. Materials and Methods

2.1. Materials

EPA-rich oil was provided by Solutex GC S.L. (Madrid, Spain) as Omegatex 8000TGD. According to the producer, the EPA content is > 800 mg/g as triglycerides. The oil was maintained blanketed with nitrogen, in darkness, at −20 °C. Whey protein concentrate (WPC) 80% was purchased from Beurrespa (Madrid, Spain). According to the distributor, WPC contained 81.6% protein (on a dry basis), 7.5% fat and 4.3% moisture. Algae oil was provided by Q'omer Bioactive Ingredients (Valencia, Spain) with a DHA content of 40 wt %, according to the manufacturer. DHA-loaded WPC microparticles were obtained similarly to the work of Prieto & Lagaron, (2020). Span 20 and hydrochloric acid 37 vol % were purchased at Sigma-Aldrich (Saint Louis, MO, USA). Ammonium thiocyanate (99%) and isopropanol (99.5%) were supplied by Acros Organics (Geel, Belgium). Barium chloride dihydrate (reagent grade), iron (III) chloride hexahydrate (PRS), chloroform (99%) and methanol (reagent grade) were obtained from Panreac Química SLU (Barcelona, Spain). Iron (II) sulfate heptahydrate (analytical grade) was supplied by Labkem–Labbox (Mataró, Spain). Isooctane (≥99.0%) was purchased from Honeywell (Morristown, NJ, USA). Bottled mineral water was supplied by Agua de Broncales (Teruel, Spain). Ethanol 96 vol % was obtained from Guinama (Valencia, Spain). Skimmed powdered milk was purchased at Pirinea S.L. (Getafe, Spain). Deionized water was employed during the investigation.

2.2. Emulsion Preparation Procedure

An oil in water emulsion was used to encapsulate the EPA-rich oil into WPC. The continuous aqueous phase was elaborated by WPC dissolution into the water at 20% w/w concentration. Span 20, which was used as a surfactant, was added to the aqueous solution at 1% w/w concentration. EPA-rich oil, which constituted the organic dispersed phase, was gently incorporated to the continuous phase into a 2:1 WPC to EPA ratio. The emulsion was blended with an UltraTurrax T-25

homogenizer (IKA, Staufen, Germany) at 17,000 rpm for 5 min, succeeded by 5 min of ultrasounds (90%) (Bandelin Sonopuls, Berlin, Germany). The emulsion was placed in chilled water to prevent the temperature from rising, and constant nitrogen bubbling was maintained to avoid EPA oxidation during homogenization. Particles obtained from a WPC solution without EPA oil were used as a control sample.

2.3. Emulsion Droplet Size

Laser diffraction analysis was performed in a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK) to measure the emulsion droplet size. A small volume of the emulsion was introduced in recirculating water at 3000 rpm to obtain an obscuration of 12%. The refractive indices for the dispersed phase and the continuous phase were that of sunflower oil (1.469) and water (1.330), respectively. Results were expressed in terms of the Sauter mean diameter (D3,2). All measurements were performed three times.

2.4. Encapsulation Process

EPA encapsulation was performed by EAPG by means of the proprietary CapsultekTM pilot plant from Bioinicia S.L. (Valencia, Spain). This pilot device is composed of a nebulizer, the droplets product of which are subjected to an electric field, an evaporating compartment, and a cyclone as described by Busolo et al., (2019). All tests were developed at controlled ambient conditions, 25 °C and 30% relative humidity (RH). EPA-rich oil emulsion was exposed to a continuous bubbling of nitrogen, and it was pumped at a flow rate of 30 mL/h to the injection unit, working with an airflow rate of 10 L/min and a voltage of 10 kV. EPA-loaded WPC capsules were recovered from the cyclonic unit and kept in airtight containers, in the dark, at −20 °C to prevent oxidation.

2.5. Particle Morphology Analysis

Scanning electron microscopy (SEM) was used to study the morphology of the particles in a Hitachi S-4800 FE-SEM (Hitachi High Technologies Corp., Tokyo, Japan). An acceleration of 5 kV for the electron beam was selected. Around 5 mg

of each sample was sputtered with gold/palladium before SEM observation. Average diameters were obtained via the software Image J Launcher v1.41 (National Institutes of Health, Bethesda, MD, USA).

Transmission electron microscopy (TEM) was employed to examine the internal morphology of the particles in a JEOL JEM 1010 (JEOL Ltd., Tokyo, Japan). Particles were subjected to an inclusion process in LR-white resin and subsequent polymerization. Ultrathin sections were cut by means of an ultramicrotome and placed on the TEM grid (Ayache et al., 2010).

2.6 Evaluation of the Extractable Oil

Extractable EPA-rich oil (EO) from the particles was quantified using UV-vis spectrophotometry. 25 mg of EPA–loaded WPC capsules were thoroughly washed with 5 mL of isooctane, and the mixture was filtrated. The absorbance of the filtrated liquid phase was determined at 318 nm using a UV4000 spectrophotometer (Dinko Instruments, Barcelona, Spain). Standard curve of EPA-rich oil in isooctane was built from 0.0 to 1.0 mg/mL ($y = 0.0217x + 0.0015$; $R2 = 0.98$) to calculate the amount of EPA-rich oil solubilized in the isooctane. The extractable oil was calculated as the percentage of the extracted EPA-rich oil with respect to the total mass of EPA-rich oil in the particle. The procedure was performed three times.

2.7. Accelerated Stability Test

A 300 W Ultra-Vitalux lamp from OSRAM (Garching, Germany) was employed to irradiate samples with ultraviolet (UV) light and accelerate the oxidation of EPA-rich oil. This lamp made up of a quartz discharge tube and a tungsten filament, generates radiation analogous to that of daylight. The bulb, made of special glass, filters radiation and allows only wavelengths similar to natural sunlight to pass through (315–400 nm, 13.6 W after 1 h of exposure; 280–315 nm, 3 W after 1 h of exposure) (Fernandez et al., 2009; OSRAM, n.d.).

The accelerated oxidative stability test was performed at room conditions under UV light for 10 days. Approximately, Petri dishes with 10 g of particles were

located under a UV lamp. Daily, aliquots were sampled for analysis. The distance between the lamp and the samples were 20 cm. The evolution of the oxidation was measured by attenuated total reflection- Fourier-transform infrared spectroscopy and peroxide value.

2.8. Peroxide Value (PV) Evaluation

The peroxide value determination was performed according to the methodology described elsewhere (Prieto & Lagaron, 2020). The oil was extracted from the particles according to the Bligh and Dyer method (Bligh & Dyer, 1959), and it was analyzed according to the PV method of Shantha and Decker (Shantha & Decker, 1994). PV represented in milliequivalents of peroxides per kilogram of oil was calculated according to Equation (1):

$$PV = \left[\frac{As-Ab}{m}\right] \cdot \left[\frac{V}{(2 \cdot 55.84 \cdot m_0 \cdot S)}\right] \qquad (1)$$

where ***As*** is the absorbance of the test sample and ***Ab*** is absorbance of blank; m is the slope of the standard curve, $\boldsymbol{m_0}$ is the mass of EPA oil, 55.84 g/mol is the iron atomic weight, ***S*** is the volume of the oil solution sample, ***V*** is the volume of solvent employed to solubilize the EPA oil. ***PV*** determination was performed three times.

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

A Bruker Tensor 37 FT-IR spectrometer (Bruker, Ettlingen, Germany) with the low-temperature Golden Gate ATR sampling accessory (Specac Ltd., Orpington, UK) was used to obtain the ATR-FTIR spectra of approximately 50 mg EPA-loaded WPC capsules. Spectra were recorded between 4000 and 600 cm^{-1}, with a 4 cm^{-1} resolution, averaging 10 scans. Results were normalized to the intensity of the band 1455 cm^{-1} for a better comparison among the different capsules. The OPUS 4.0 software (Bruker, Ettlingen, Germany) was used to perform the analysis of the spectra. Measurements were performed in triplicate.

2.10. Headspace Oxygen Volume Depletion

A comparison of the headspace oxygen volume depletion between the free EPA-rich oil and the obtained EPA-loaded WPC capsules was performed using a multichannel oxygen meter OXY-4 mini (PreSens Precision Sensing GmbH, Regensburg, Germany). The analysis over time was carried out via the fluorescence decay method using 2.5 g of EPA-loaded WPC capsules inserted in 100 mL Schleck flasks with spot sensors, at 25 °C and 0% RH. An equivalent amount of EPA-rich oil was used in the case of free EPA-rich oil. Values were taken for 165 h and normalized to the initial headspace oxygen volume. The measurements were performed in duplicate.

2.11. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis of encapsulates was performed on a 550-TA Instruments thermogravimetric analyzer (New Castle, DE, USA). Approximately 5 mg of samples were placed on the platinum pan and kept under air atmosphere with a flow rate of 50 mL/min and heated in the temperature range of 25–700 °C, at a heating rate of 10 °C/min.

2.12. Organoleptic Test

The organoleptic impact of free EPA-rich oil was compared to the impact of EPA-rich oil-loaded WPC capsules when added to the rehydrated powdered milk, used as a reference. 37.5 mg of EPA-rich oil was added to 25 g of skim milk powder and 130 mL of bottled mineral water. In the case of EPA-rich oil-loaded WPC capsules, 75 mg were added using the mentioned preparation conditions. Eight trained panelists from the IATA-CSIC compared the general fishiness characteristics, including color, odor, taste and appearance, with the model food product (rehydrated milk powder free of oil or capsules). Samples were scored according to a five-point hedonic scale: (0) no difference; (1) little difference; (3) clear difference; and (5) big difference. A similar analysis was performed comparing the organoleptic impact of the free oil with the encapsulates, but in this case, mixing EPA and DHA enriched oils in a ratio of 50:50 in weight.

2.13. Statistical Analysis

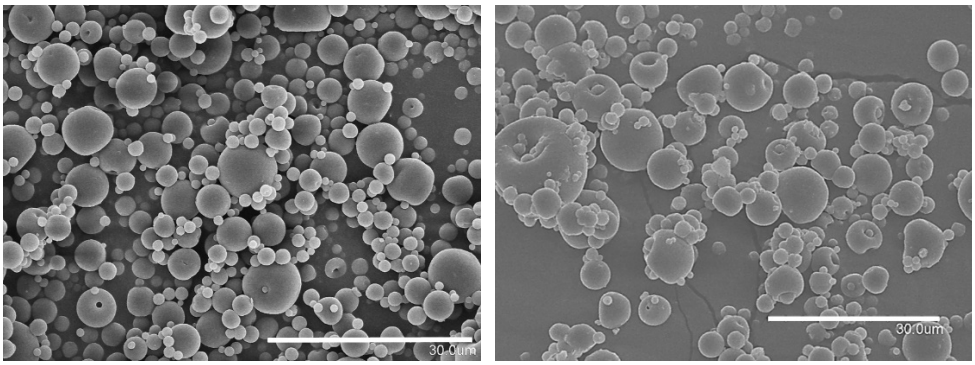
Statgraphics Centurion Version 17.2.04 (Statistical Graphics Corp., Warrenton, VA, USA) was used for statistical analysis. Data were represented as mean ± standard deviation. Initially, data were subjected to analysis of variance (ANOVA) to evaluate the effects of the examined parameters on the different samples. Kruskal–Wallis test by ranks was developed to contrast the action of the medians instead of the median values for organoleptic analyses. Differences between means and medians were contemplated as significant at p-value < 0.05.

3. Results and Discussion

The aim of this research was to study the encapsulation of nanodroplets of EPA-rich oil into WPC microparticles via the emulsion EAPG process. Obtained microcapsules were characterized in terms of morphology, extractable oil, oxidative and thermal stability and organoleptic impact.

3.1. Morphology

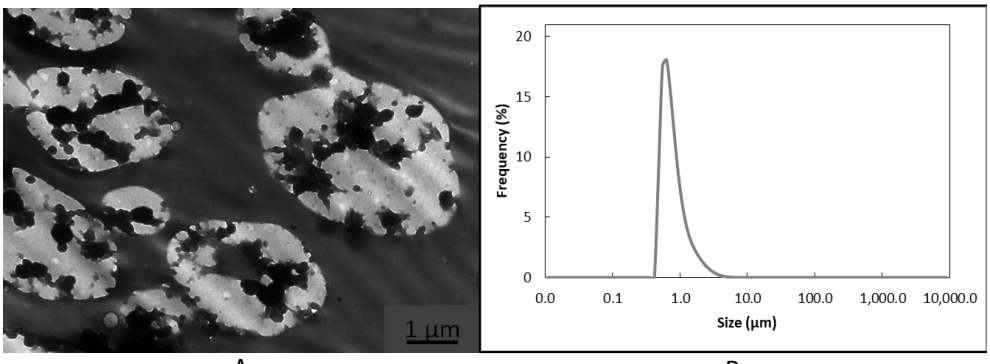
Neat WPC capsules with an average particle size of 5.11 ± 2.25 μm, spherical structure, with unwrinkled surface and without fissures or dents were obtained, as shown in Figure 1A. The encapsulation of EPA-rich oil into WPC at ratio 2:1 led to a similar microcapsule morphology of neat WPC but with an average particle size of 5.59 ± 2.68 μm (Figure 1B). These kind of morphological attributes, spherical shape and smooth surface, are suitable for food applications due to a better dispersibility into the final formulation and a minimum texture impact (de Boer et al., 2017). Particles with similar morphologies were obtained by Prieto and Lagaron when encapsulating algae oil into WPC via EAPG (Prieto & Lagaron, 2020) and by García-Moreno et al. when encapsulating fish oil into WPC and carbohydrates by using the same technology (García-Moreno et al., 2018). A standard deviation of approximately 2 μm was observed for neat and EPA-loaded microparticles; however, this polydispersion should not affect the organoleptic properties of the final product since the size is far below the tongue detection limit of 20 μm (Weidner, 2009).



A B

Figure 1. SEM micrographs: (**A**) whey protein concentrate (WPC) microparticles; (**B**) eicosapentaenoic acid (EPA)-rich oil-loaded WPC capsules. Scale bar corresponds to 30 μm.

Figure 2A shows the manner in which EPA-rich oil was entrapped within the WPC matrix. It consisted of a spongelike structure, which is known to enhance oil preservation (Joye et al., 2019) and favor the oil absorption in the gastrointestinal tract (Dima et al., 2020). The internal morphology of the microcapsules coincides with the measured size, i.e., 0.762 ± 0.001 μm, of the droplets in the emulsion (Figure 2B). The polydispersion observed in the internal nanostructure of the produced microparticles could be due to the lack of stability of the emulsion, which could be optimized to increase the oil stability.



A B

Figure 2. A) TEM image of the EPA-rich oil-loaded WPC capsules. **B**) Emulsion droplet size distribution obtained by laser diffraction analysis.

3.2. Extractable Oil

UV-vis spectrophotometry was used to measure the extractable EPA-rich oil in the capsules using a thorough oil extraction method. The extractable oil of the here-prepared EPA-rich oil-loaded WPC capsules was 30.0 ± 1.1%. Previous studies of encapsulation of omega-3-rich oils via electrospraying-based technologies reported similar results. Prieto & Lagaron, (2020) reported an extractable oil in organic solvent of 35 ± 5% for DHA-enriched algae oil encapsulated within WPC using EAPG as encapsulating technology. Gómez-Mascaraque & López-Rubio, (2016) prepared different protein particles for the encapsulation of α-linolenic acid (ALA), finding that the higher oil retention capacity was achieved using WPC and soy protein isolate complex with values of 67 ± 5% and 61 ± 4%, respectively via electrospraying. The increased oil retention obtained for EPA could be due to the inherent characteristics of oil, such as increased lipophilicity or viscosity. Regarding other technologies, Olloqui et al. encapsulated EPA and DHA into calcium alginate, obtaining oil retentions up to 93.5 ± 2.2%. However, this result was obtained with an EPA oil loading of 1% in the alginate beads (Olloqui et al., 2018), whereas in this work, the oil loading in the microparticle was 33%.

3.3. Accelerated Stability Test

The stability against oxidation of EPA-loaded WPC microparticles versus the free EPA oil was compared by irradiating the samples with UV light for 10 days. PV and ATR-FTIR spectroscopy were used to quantify the EPA-rich oil oxidation.

The PV quantifies the formation of primary fatty acid oxidation products, specifically hydroperoxides. The initial PV for non-encapsulated and encapsulated EPA-rich oil reached 12.7 ± 2.0 meq/kg and 13.8 ± 1.4 meq/kg, respectively. According to the provider, this EPA-rich oil was deodorized, and consequently, it does not surprise the high initial PV value. Nevertheless, the slight difference between the free EPA-rich oil and the encapsulated in WPC could be due to the emulsion preparation and also to the contact of the non-encapsulated oil with oxygen in the course of the EAPG process.

Regarding the evolution of the PV with the exposure of the particles to UV light, the PV of the free EPA-rich oil increased rapidly during the first day due to the formation of hydroperoxides, as shown in Figure 3. During the second day, the concentration of hydroperoxides slightly diminished as a consequence of their secondary oxidative conversion into aldehydes, ketones, and alcohols responsible for the off-flavors and consumer reluctance. After the second day, the peroxide value was arrested for the free EPA oil. However, in the case of encapsulated EPA-rich oil into WPC, a slow monotonic increase was obtained. Both curves showed a sigmoid behavior, characteristic of autocatalytic oxidation processes that occur as a chain reaction of free radicals (Aragao et al., 2008). Lipid oxidation reactions are characterized by accelerating with time, with the possibility of showing an induction period. However, the height of the peroxide value, the general curve shape, and the induction period depends on the oxidation kinetic parameters, as well as the composition of the sample (Choe & Min, 2006). Thus, the WPC-EPA curve showed a longer induction time. This last behavior could be a consequence of the inherent antioxidant properties of the WPC (Tong et al., 2000), which could delay the generation of free radicals.

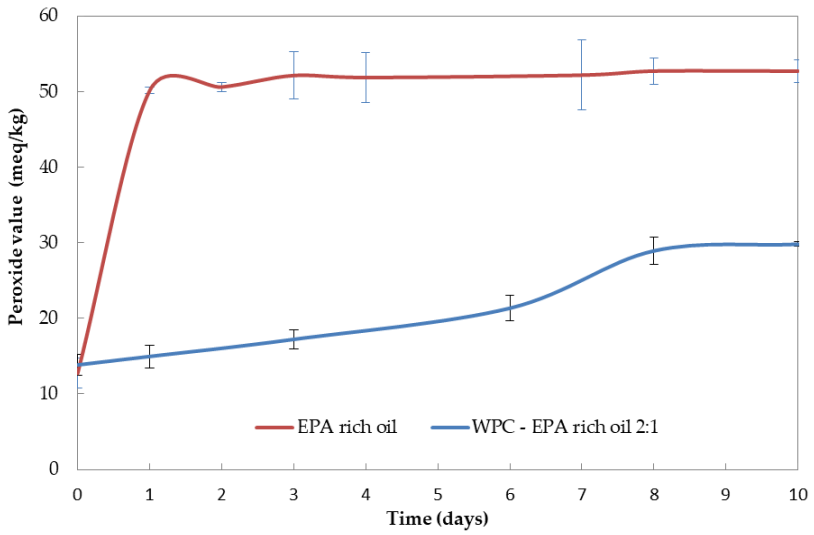


Figure 3. Evolution of the peroxide value of non-encapsulated EPA-rich oil compared with the EPA-loaded WPC microparticles.

ATR-FTIR spectroscopy has been demonstrated to be a good methodology to evaluate the extension of secondary oil oxidation reactions (Prieto & Lagaron, 2020). Initially, the ATR-FTIR spectra of the neat EPA-rich oil were studied in the course of the accelerated oxidative reactions under UV light. The ATR-FTIR spectrum of the fresh non-encapsulated EPA oil is compared in Figure 4A with the ATR-FTIR spectrum of non-encapsulated oxidized EPA oil subjected to 10 days of accelerated oxidation. Spectra were normalized in intensity to ease the comparison. The peak at ca. 1455 cm^{-1} was used as an internal standard for normalization, similarly to the previous study (Prieto & Lagaron, 2020). This band is assigned to the bending vibrations of CH_2, CH_3 aliphatic groups (Guillén & Cabo, 1999), which did not show any variation during oxidation. Figure 4A shows the ATR-FTIR spectra of the EPA-rich oil, where main bands were easily identified. The peak at 3012 cm^{-1} corresponds to the stretching vibrations of alkenes in PUFAs. A reduction in the intensity of this band was observed over UV exposure time as a result of the oxidation reactions, in which the desaturations disappear (Guillén & Cabo, 1997; Moomand & Lim, 2014). Further overlapping stretching modes from EPA-rich oil appear at 2964, 2919 and 2872 cm^{-1}, corresponding to the asymmetric CH_3, antisymmetric CH_2 and the symmetric CH_2 stretching, respectively (Kiefer et al., 2010; Mizutani et al., 2003). The higher intensity of the band at 3012 cm^{-1} with respect to the bands at 2964, 2919 and 2872 cm^{-1} confirms the high omega-3 PUFAs content of 80 (Karunathilaka et al., 2019), %, according to the producer. It is also remarkable the peak at ca. 1748 cm^{-1} attributed to the ester and acid groups stretching vibrations of triacylglycerols (Moomand & Lim, 2014; Safar et al., 1994). The intensity of this band was reduced at the same time that the band expanded towards lower wavenumbers as a consequence of the formation of primary and secondary oxidation products (Guillén & Cabo, 1999). The band at 1650 cm^{-1} was assigned to stretching vibrations of C=C bonds, and alterations in intensity suggested the generation of trans double bonds due to radiation (Petzold et al., 2008). The band at ca. 1145 cm^{-1} has been associated with the number of saturated acyl groups in the oil. The location of this band moved towards larger wavenumbers as a consequence of the oxidation reactions, in

which smaller saturated acyl molecules are formed (Guillén & Cabo, 1999). The peak at ca. 705 cm^{-1}, assigned to the convergence of the methylene rocking vibration and the out-of-plane bending vibration of disubstituted cis-alkenes (Guillén & Cabo, 1999), presented a decrease in intensity as oxidative reactions occurred (Petzold et al., 2008).

Regarding WPC, the characteristic bands of proteins at 1650 cm^{-1} and 1550 cm^{-1} attributed to amide I and amide II (Andrade et al., 2019) were easily discerned in Figure 4B. However, these spectra did not show significant alterations as a result of the exposure to UV light for 10 days, only a slight decrease in the intensity of the bands attributed to water, probably due to the warming provoked by the UV light bulb.

The evolution of the characteristic bands of the EPA-loaded WPC microparticles over UV light exposure time is shown in Figure 4C. The most significant changes were a decrease in the intensity of the band at 3012 cm^{-1} and an apparent increase in the relative intensity of the band at 1741 cm^{-1} in addition to a band broadening of the same band. Via UV-vis spectrophotometry, it was also observed the disappearance of the band at 318 nm due to the oxidation of the oil (results not shown). However, as demonstrated in previous work (Prieto & Lagaron, 2020), the widening of the peak at 1741 cm^{-1} is the most suitable to quantify the generation of secondary oxidation products. Therefore, Figure 5 shows the evolution of the 1741 cm^{-1} broadband at half maximum over UV light exposure time. The band broadening of the free EPA-rich oil was maintained stable until the third day, after which it experienced a significant and strong rise in slope. However, the encapsulated EPA-rich oil into WPC showed a moderate and non-significant broadening during the first day, after which it was arrested. EPA-rich oil seemed to be more stable to UV light when compared to DHA enriched oil, which showed a broadening of approximately 40 cm^{-1} after being subjected to 10 days under UV light (Prieto & Lagaron, 2020). The different behavior could be due to the different compositions of both oils. Regarding the encapsulates, DHA-loaded WPC microparticles also showed a non-significant

broadening of approximately 6 cm^{-1} after being subjected to 10 days of UV light (Prieto & Lagaron, 2020). The obtained results for the encapsulates indicated that the encapsulation within the WPC protein seems to block UV radiation, decreasing the generation of secondary oxidation products (Ha et al., 2019).

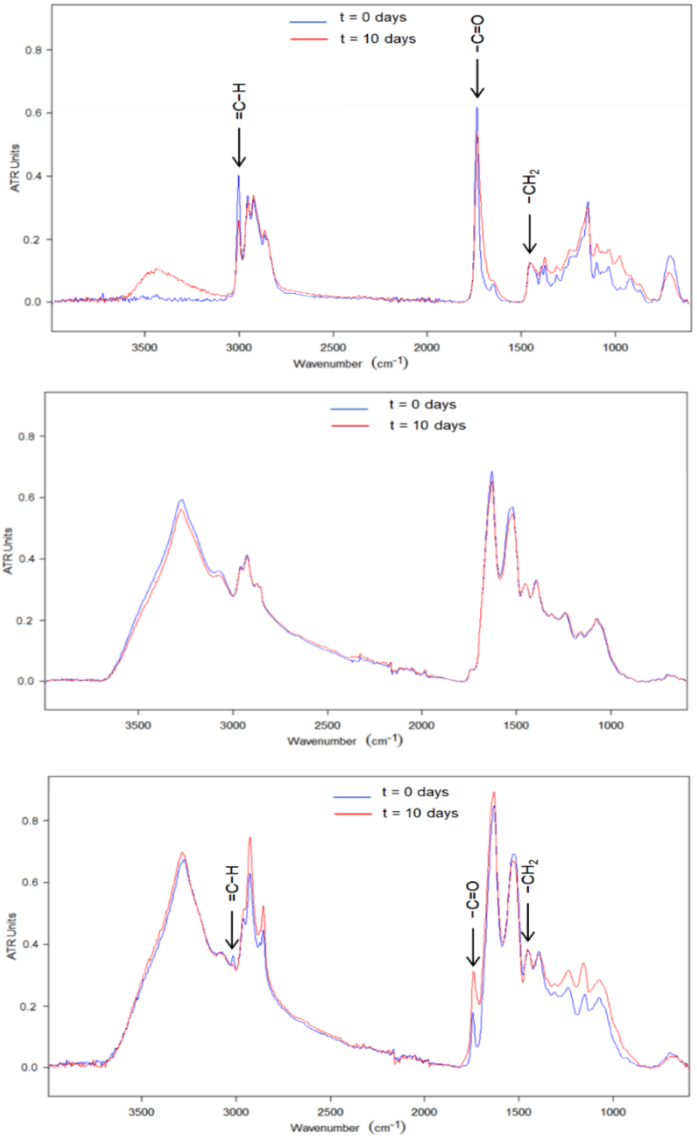


Figure 4. Evolution of the attenuated total reflection—Fourier transform infrared spectroscopy (ATR-FTIR) spectra during the accelerated oxidation test: A) EPA-rich oil, B) WPC and C) WPC-EPA-rich oil microparticles.

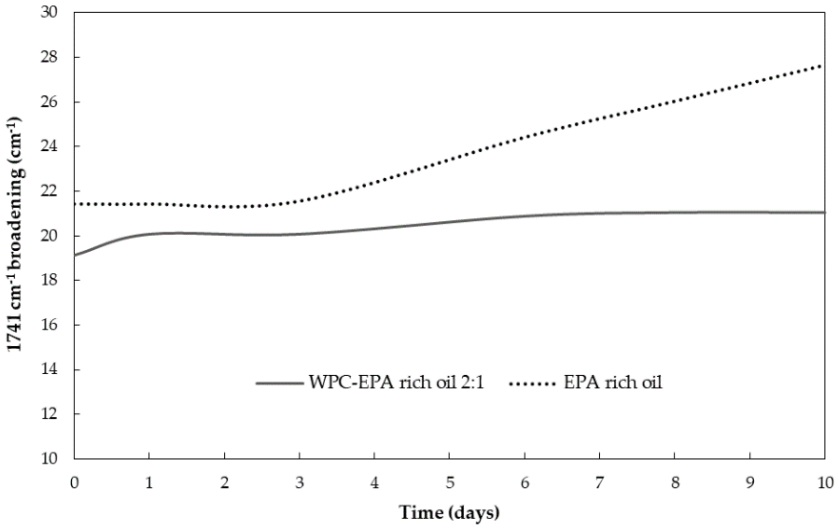


Figure 5. Peak widening of the free EPA-rich oil and the encapsulated EPA-rich oil into WPC, obtained as the 1741 cm^{-1} broadband at half maximum, over UV-light exposure time.

3.4. Headspace Oxygen Volume Depletion Test

The headspace oxygen volume depletion test was performed to study the oxygen barrier capacity of the WPC to protect the EPA-rich oil by the fluorescence decay method at room temperature and 0% RH for an equivalent amount of EPA-rich oil. In the result of the EPA-loaded microparticles, the signal of the neat WPC was subtracted to evaluate only the oxygen depletion due to the oil. As shown in Figure 6, free EPA-rich oil showed a higher oxygen consumption than the encapsulated oil, which implied a higher oxidation rate. The fast oxygen consumption observed at the beginning maybe due to the oxidative reactions of the non-encapsulated oil in the microparticles. After 165 h of experiment, the neat oil showed approximately 28% of oxygen consumption, while the microparticles consumed around 5%. Prieto and Lagaron obtained a 7% of oxygen consumption for the encapsulation of algae oil into WPC using the EAPG technology, whereas free algae oil consumed around 20% (Prieto & Lagaron, 2020), and Busolo et al.

obtained an 8% of oxygen consumption when encapsulating fish oil into zein using the same technology, whereas the free fish oil consumed around 28% (Busolo et al., 2019). The obtained results are probably due to the exceptional oxygen-barrier properties of the whey protein concentrate at low relative humidity (Hong & Krochta, 2003).

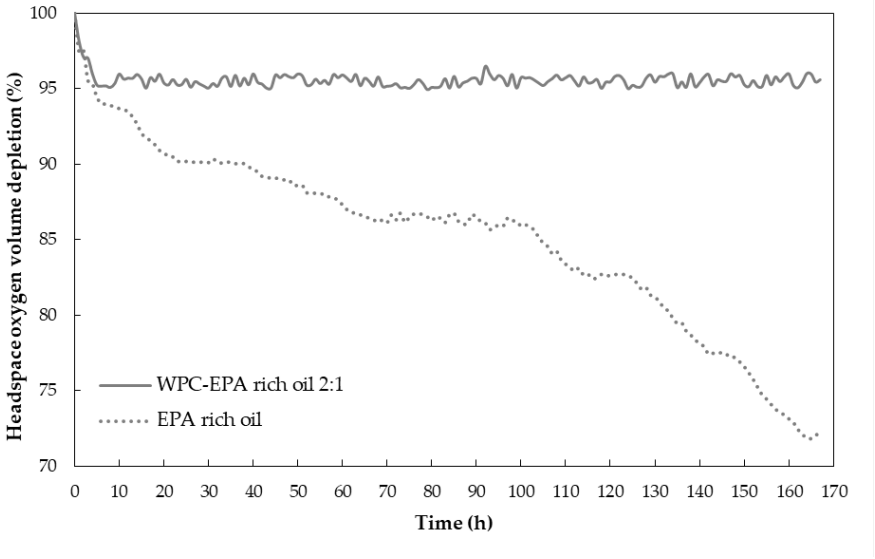


Figure 6. Comparison of the averaged headspace oxygen volume depletion between the neat oil and EPA-loaded WPC microparticles.

3.5. Thermogravimetric Analysis

Thermogravimetric analysis was used to study the thermal stability in the presence of oxygen. This analysis was performed to compare the stability of the free EPA-rich oil and the encapsulated EPA-rich oil. Results are shown in Figure 7A,B. EPA-rich oil demonstrated to be stable until 150 °C, showing a first weight loss of 38% between 150 and 300 °C, followed by a second weight loss of 62% until 490 °C. However, the EPA-loaded WPC microparticles showed increased thermal stability. The microparticles showed a small event under 100 °C attributed

to the evaporation of sorbed water on the microparticles, being stable until 200 °C, where microparticles showed a weight loss of 52% until 350 °C, a second weight loss of 27% until 500 °C, and a final weight loss of 14% until 585 °C. This delay in the TGA curve is ascribed to the protection of the WPC.

Since most of the published studies were developed with fish or algae oils rich in DHA, free and encapsulated DHA enriched oil from algae were also studied in order to establish a comparison. Free DHA-enriched oil showed increased thermal stability in comparison with EPA-rich oil since it was stable until 215 °C, as shown in Figure 7C. DHA enriched oil showed a total weight loss between 215 and 600 °C. However, the DHA-loaded microparticles showed (see Figure 7D) a small thermal event due to the humidity sorbed in the microparticles, but a first weight loss of 15% occurred at 110 °C. This was not seen in the pure oil and was probably due to the potential interaction of the oil with the polymer.

Finally, a mixture of EPA and DHA-rich oils was also studied; since both compounds have differential health benefits, EPA and DHA encapsulates could be combined in different ratios with different health effects for personalized medicine or nutrition purposes. Free EPA and DHA oils were combined in a ratio of 50:50, as well as their microparticles. Results are shown in Figure 7E,F, respectively. The combination of EPA and DHA resulted in increased thermal stability in comparison with the neat EPA oil, since the first thermal event occurred at 170 °C, showing a TGA curve similar to that of EPA-rich oil, but with a decreased first thermal event. Regarding the encapsulates, the mixture of microparticles also resulted in an earlier thermal event, showing a similar TGA curve but with a somewhat decreased thermal stability in comparison with the EPA encapsulates.

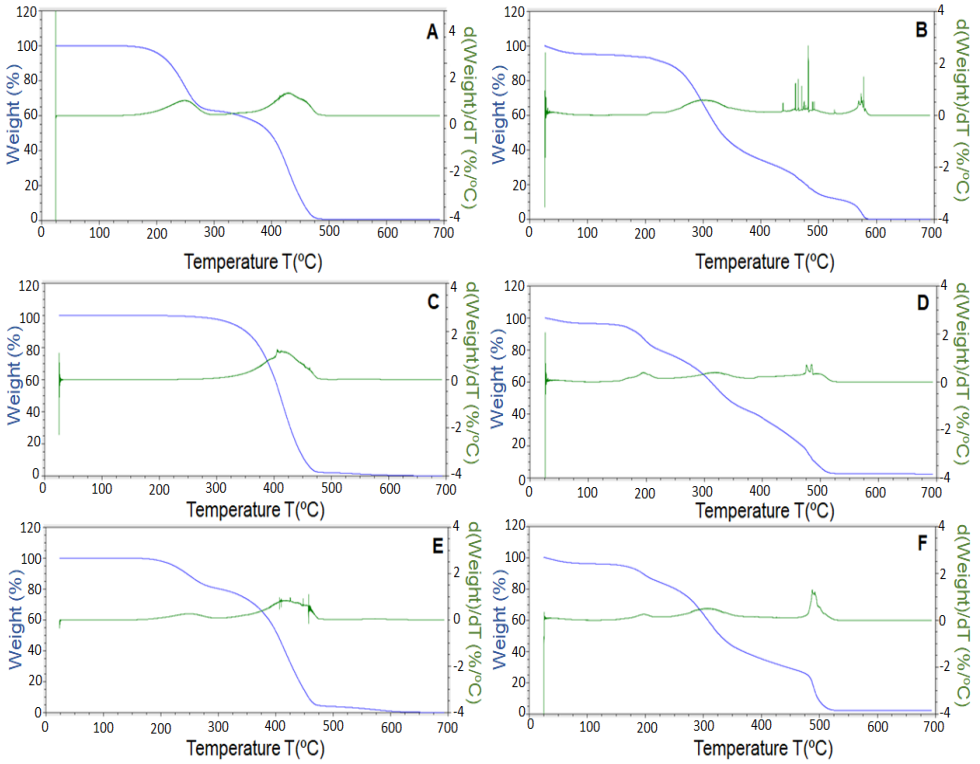


Figure 7. Thermogravimetric analysis curves in the presence of oxygen of (**A**) EPA-rich oil, (**B**) EPA-loaded WPC microparticles, (**C**) docosahexaenoic acid (DHA)-rich oil, (**D**) DHA-loaded WPC microparticles, (**E**) EPA–DHA oils mixture (50:50), (**F**) mixture of EPA-loaded WPC microparticles and DHA-loaded WPC microparticles in mass ratio 50:50.

3.6. Organoleptic Properties

The organoleptic impact of the oil-loaded microparticles was evaluated in comparison with the neat EPA-rich oil, employing rehydrated powdered milk as reference. The assay was carried out with fresh samples and the samples upon completion of the accelerated oxidation test. Qualified panelists detected a high organoleptic impact for the free EPA-rich oil, as shown in Figure 8. This result is consistent with the high initial peroxide value and with the fact that the oil suffered from a deodorization process, according to the manufacturer. Nevertheless, the capsules offered a somewhat reduced impact also after 10 days

of UV light exposure, according to the panelists, even if the effect was not significant.

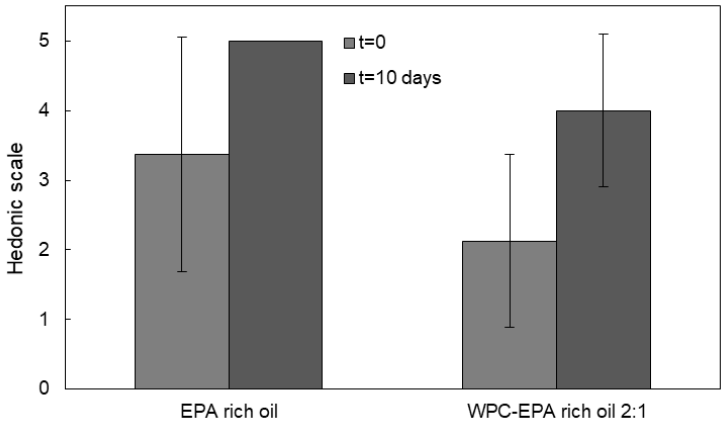


Figure 8. Comparison of the organoleptic impact of reconstituted powder milk containing free oil and EPA-rich oil-loaded microcapsules for fresh samples (t = 0) and upon the completion of the accelerated oxidation test. Data were expressed as mean value ± standard deviation.

EPA and DHA encapsulates are also combined in different ratios with different health or organoleptic purposes. In Figure 9, a comparison of the organoleptic impact of the combination of the free mixture of EPA and DHA in weight ratio 50:50 and a mixture of encapsulates of EPA and DHA in a weight ratio of 50:50 is shown. When combining EPA and DHA, the organoleptic impact of the free oil was reduced in comparison to Figure 8 at t = 0, being more similar to the organoleptic impact reported by Prieto & Lagaron, (2020). However, the organoleptic impact of the free oil mixture was seen to increase to its maximum after 10 days of UV exposure. The panelist did not report organoleptic impact for the fresh encapsulates, but they reported a reduced organoleptic impact after 10 days of UV exposure in comparison with the pure EPA encapsulates. This is likely related to the lower organoleptic impact of the algae-derived DHA oil. Therefore,

the combination of different encapsulated PUFAs can be used to tailor a new line of personalized products with targeted health and organoleptic benefits.

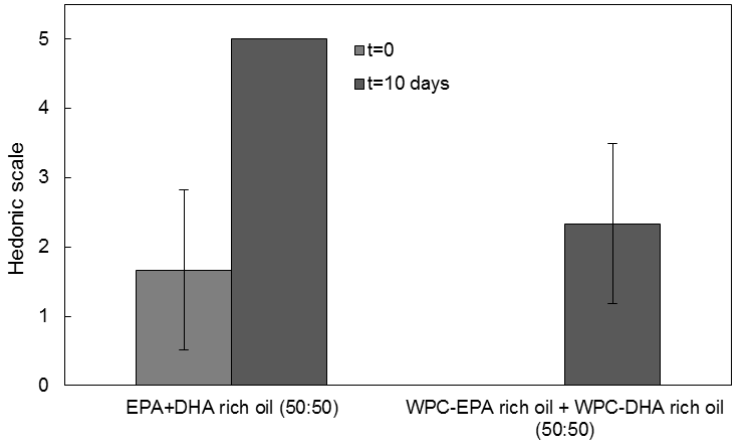


Figure 9. Comparison of the organoleptic impact of reconstituted powder milk containing EPA and DHA enriched free oil (50:50) and EPA and DHA-loaded microcapsules in ratio 50:50, for fresh samples (t = 0) and upon the completion of the accelerated oxidation test. Data were expressed as mean values ± standard deviation.

4. Conclusions

In this work, EPA-rich oil was first successfully encapsulated for the first time in submicron droplets within whey protein concentrate microparticles using the emulsion electrospraying assisted by the pressurized gas process. The obtained results suggest that it is possible to encapsulate 80% PUFAs-enriched oils by emulsion EAPG technology at room temperature without affecting the bioactivity of the EPA oil into nanostructured microparticles, which favor oil protection and gastric absorption, with an increased oxidative and thermal stability, and a reduced organoleptic impact. Another advantage of this encapsulation process is that it is available at an industrial scale. Furthermore, thanks to the differential health effects of the EPA, these encapsulates could be used in personalized

medicines or nutraceuticals, alone or in combination with other microparticles encapsulating PUFAs to obtain targeted health and organoleptic benefits.

Acknowledgements

J.D.E.-G. wants to thank the Spanish Ministry of Economy, Industry and Competitivity for his predoctoral grant (DI-6906). The authors would like to thank Alberto Rodríguez Escrivá for his technical assistance. This research was funded by the MICIU (RTI-2018-097249B-C1), EU H2020-MSCA-RISE-2017-778388-FODIAC and the EU H2020-EIC-SMEinst-2018-2020-SME instrument-873827-CAPSULTEK, CDTI-CIEN DANTIAN-IDI-20190954 and the CYTED thematic network (319RT0576).

References

ALBRACHT-SCHULTE, K., GONZALEZ, S., JACKSON, A., WILSON, S., RAMALINGAM, L., KALUPAHANA, N. S., & MOUSTAID-MOUSSA, N. (2019). Eicosapentaenoic acid improves hepatic metabolism and reduces inflammation independent of obesity in high-fat-fed mice and in HepG2 cells. Nutrients, 11 (3). https://doi.org/10.3390/nu11030599

ANDRADE, J., PEREIRA, C. G., ALMEIDA JUNIOR, J. C. DE, VIANA, C. C. R., NEVES, L. N. DE O., SILVA, P. H. F. DA, BELL, M. J. V., & ANJOS, V. DE C. DOS. (2019). FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. LWT, 99, 166–172. https://doi.org/10.1016/J.LWT.2018.09.079

ARAGAO, G. M. F., CORRADINI, M. G., & PELEG, M. (2008). A Phenomenological Model of the Peroxide Value's Rise and Fall During Lipid Oxidation. Journal of the American Oil Chemists' Society, 85 (12). https://doi.org/10.1007/s11746-008-1305-z

AUGUSTIN, M. A., & HEMAR, Y. (2009). Nano- and micro-structured assemblies for encapsulation of food ingredients. Chemical Society Reviews, 38, (4) 902–912. https://doi.org/10.1039/B801739P

AUGUSTIN, M. A., & OLIVER, C. M. (2014). Use of milk proteins for encapsulation of food ingredients. In Microencapsulation in the food industry (pp. 211–226). Elsevier. https://doi.org/10.1016/b978-0-12-404568-2.00019-4

AYACHE, J., BEAUNIER, L., BOUMENDIL, J., EHRET, G., & LAUB, D. (2010). Sample preparation handbook for transmission electron microscopy. In Sample Preparation Handbook for Transmission Electron Microscopy. Springer New York. https://doi.org/10.1007/978-1-4419-5975-1

BLIGH, E. G., & DYER, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911–917. https://doi.org/10.1139/o59-099

BUSOLO, M., TORRES-GINER, S., PRIETO, C., & LAGARON, J. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. Innovative Food Science & Emerging Technologies, 51, 12–19. https://doi.org/10.1016/j.ifset.2018.04.007

CHANG, J. P.-C., SU, K.-P., MONDELLI, V., SATYANARAYANAN, S. K., YANG, H.-T., CHIANG, Y.-J., CHEN, H.-T., & PARIANTE, C. M. (2019). High-dose eicosapentaenoic acid (EPA) improves attention and vigilance in children and adolescents with attention deficit hyperactivity disorder (ADHD) and low endogenous EPA levels. Translational Psychiatry, 9 (1), 303. https://doi.org/10.1038/s41398-019-0633-0

CHOE, E., & MIN, D. B. (2006). Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety, 5, 169–186. https://doi.org/10.1111/j.1541-4337.2006.00009.x

DE BOER, A. H., HAGEDOORN, P., HOPPENTOCHT, M., BUTTINI, F., GRASMEIJER, F., & FRIJLINK, H. W. (2017). Dry powder inhalation: Past, present and future. Expert Opinion on Drug Delivery, 14, 499–512. https://doi.org/10.1080/17425247.2016.1224846

DIMA, C., ASSADPOUR, E., DIMA, S., & JAFARI, S. M. (2020). Bioavailability of nutraceuticals: Role of the food matrix, processing conditions, the gastrointestinal tract, and nanodelivery systems. Comprehensive Reviews in Food Science and Food Safety, 19, 954–994. https://doi.org/10.1111/1541-4337.12547

EFSA PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES (NDA). (2011). Scientific Opinion on the substantiation of health claims related to docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and gamma-linolenic acid (GLA) and contribution to normal cognitive function (ID 532) and maintenance of normal bone (ID 642, 697, 1552) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9, 2224. https://doi.org/10.2903/j.efsa.2011.2224

ERATTE, D., MCKNIGHT, S., GENGENBACH, T. R., DOWLING, K., BARROW, C. J., & ADHIKARI, B. P. (2015). Co-encapsulation and characterisation of omega-3 fatty acids and probiotic bacteria in whey protein isolate–gum Arabic complex coacervates. Journal of Functional Foods. https://doi.org/10.1016/j.jff.2015.01.037

FERNANDEZ, A., TORRES-GINER, S., & LAGARON, J. M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. Food Hydrocolloids, 23, 1427–1432. https://doi.org/10.1016/j.foodhyd.2008.10.011

GARCÍA-MORENO, P. J., PELAYO, A., YU, S., BUSOLO, M., LAGARON, J. M., CHRONAKIS, I. S., & JACOBSEN, C. (2018). Physicochemical characterization and oxidative stability of fish oil-loaded electrosprayed capsules: Combined use of whey protein and carbohydrates as wall materials. Journal of Food Engineering, 231, 42–53. https://doi.org/10.1016/j.jfoodeng.2018.03.005

GOED OMEGA 3, COUNCIL FOR RESPONSIBLE NUTRITION. (2015). Oxidation in omega-3 oils: An overview. Retrieved from https://goedomega3.com/storage/app/media/scientific-reports/Oxidation%20in%20Omega-3%20Oils_%20An%20Overview.pdf

GÓMEZ-MASCARAQUE, L. G., & LÓPEZ-RUBIO, A. (2016). Protein-based emulsion electrosprayed micro- and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. Journal of Colloid and Interface Science, 465, 259–270. https://doi.org/10.1016/j.jcis.2015.11.061

GUILÉN, M. D., & CABO, N. (1997). Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. Journal of the American Oil Chemists' Society, 74, 1281–1286. https://doi.org/10.1007/s11746-997-0058-4

GUILÉN, M. D., & CABO, N. (1999). Usefulness of the frequency data of the fourier transform infrared spectra to evaluate the degree of oxidation of edible oils. Journal of Agricultural and Food Chemistry, 47, 709–719. https://doi.org/10.1021/jf9808123

HA, H.-K., RANKIN, S. A., LEE, M.-R., & LEE, W.-J. (2019). Development and characterization of whey protein-based nano-delivery systems: A review. Molecules, 24 (18), 3254. https://doi.org/10.3390/molecules24183254

HONG, S.-I., & KROCHTA, J. (2003). Oxygen barrier properties of whey protein isolate coatings on polypropylene films. Journal of Food Science, 68, 224–228. https://doi.org/10.1111/j.1365-2621.2003.tb14143.x

JOYE, I. J., CORRADINI, M. G., DUIZER, L., BOHRER, B., LAPOINTE, G., FARBER, J., SPAGNUOLO, P., & ROGERS, M. (2019). A comprehensive perspective of food nanomaterials. In Marine Enzymes Biotechnology: Production and Industrial Applications, Part III (pp. 1–45). Elsevier. https://doi.org/10.1016/bs.afnr.2019.01.001

KARUNATHILAKA, S. R., CHOI, S. H., MOSSOBA, M. M., YAKES, B. J., BRÜCKNER, L., & ELLSWORTH, Z. (2019). Rapid classification and quantification of marine oil omega-3 supplements using ATR-FTIR, FT-NIR and chemometrics. Journal of Food Composition and Analysis, 77, 9–19. https://doi.org/10.1016/j.jfca.2018.12.009

KAUSHIK, P., DOWLING, K., BARROW, C. J., & ADHIKARI, B. (2015). Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. Journal of Functional Foods, 19, 868–881. https://doi.org/10.1016/j.jff.2014.06.029

KIEFER, J., NOACK, K., BARTELMESS, J., WALTER, C., DÖRNENBURG, H., & LEIPERTZ, A. (2010). Vibrational structure of the polyunsaturated fatty acids eicosapentaenoic acid and arachidonic acid studied by infrared spectroscopy. Journal of Molecular Structure, 965 (1–3), 121–124. https://doi.org/10.1016/j.molstruc.2009.11.052

LU, X., CHEN, J., GUO, Z., ZHENG, Y., REA, M. C., SU, H., ZHENG, X., & MIAO, S. (2019). Using polysaccharides for the enhancement of functionality of foods: A review. Trends in Food Science & Technology, 86, 311–327. https://doi.org/10.1016/j.tifs.2019.02.024

MARTINS, I. M., BARREIRO, M. F., COELHO, M., & RODRIGUES, A. E. (2014). Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. Chemical Engineering Journal, 245, 191–200.

https://doi.org/10.1016/j.cej.2014.02.024

MIGUEL, G. A., JACOBSEN, C., PRIETO, C., KEMPEN, P. J., LAGARON, J. M., CHRONAKIS, I. S., & GARCÍA-MORENO, P. J. (2019). Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil. Journal of Food Engineering, 263, 348–358. https://doi.org/10.1016/j.jfoodeng.2019.07.019

MIZUTANI, Y., MATSUMURA, Y., IMAMURA, K., NAKANISHI, K., & MORI, T. (2003). Effects of water activity and lipid addition on secondary structure of zein in powder systems. Journal of Agricultural and Food Chemistry, 51, 229–235. https://doi.org/10.1021/jf0205007

MOOMAND, K., & LIM, L.-T. (2014). Oxidative stability of encapsulated fish oil in electrospun zein fibres. Food Research International, 62, 523–532. https://doi.org/10.1016/j.foodres.2014.03.054

NELSON, J., WANI, O., MAY, H., & BUDOFF, M. (2017). Potential benefits of eicosapentaenoic acid on atherosclerotic plaques. Vascular Pharmacology, 91, 1–9. https://doi.org/10.1016/j.vph.2017.02.004

OLLOQUI, E. J., CASTAÑEDA-OVANDO, A., CONTRERAS-LÓPEZ, E., HERNANDEZ-SÁNCHEZ, D., TAPIA-MARURI, D., PILONI-MARTINI, J., & AÑORVE-MORGA, J. (2018). Encapsulation of fish oil into low-cost alginate beads and EPA-DHA release in a rumino-intestinal in vitro digestion model. European Journal of Lipid Science and Technology, 120 (9), 1800036. https://doi.org/10.1002/ejlt.201800036

OSRAM. (n.d.). ULTRA VITALUX 300 W 230 V E27 | OSRAM PIA. Retrieved February 24, 2021, from https://www.osram.com/ecat/ULTRA-VITALUX%20UV-A-UV-A%20-%20Blacklight%20Lamps-Ultraviolet-Entertainment%20&%20Indtry/com/en/GPS01_265609/ZMP_60829/

PANSE, M. L., & PHALKE, S. D. (2016). World market of omega-3 fatty acids. In Omega-3 fatty acids (pp. 79–88). Springer.

PETZOLD, C., LYNGSTADAAS, S. P., RUBERT, M., & MONJO, M. (2008). UV-induced chemical coating of titanium surfaces with eicosapentaenoic acid. Journal of Materials Chemistry, 18 (45), 5502. https://doi.org/10.1039/b811932e

PRIETO, C., & LAGARON, J. M. (2020). Nanodroplets of docosahexaenoic acid-enriched algae oil encapsulated within microparticles of hydrocolloids by emulsion electrospraying assisted by pressurized gas. Nanomaterials, 10 (2). https://doi.org/10.3390/nano10020270

RUSSELL, F. D., & BÜRGIN-MAUNDER, C. S. (2012). Distinguishing health benefits of eicosapentaenoic and docosahexaenoic acids. Marine Drugs, 10, 2535–2559. https://doi.org/10.3390/md10112535

SASAKI, J., YOKOYAMA, M., MATSUZAKI, M., SAITO, Y., ORIGASA, H., ISHIKAWA, Y., OIKAWA, S., ITAKURA, H., & HISHIDA, H. (2012). Relationship between coronary artery disease and non-HDL-C, and effect of highly purified EPA on the risk of coronary artery disease in hypercholesterolemic patients treated with statins: Sub-analysis of the Japan EPA Lipid Intervention Study (JELIS). Journal of Atherosclerosis and Thrombosis, 19, 194–204. https://doi.org/10.5551/jat.8326

SAWADA, T., TSUBATA, H., HASHIMOTO, N., TAKABE, M., MIYATA, T., AOKI, K., YAMASHITA, S., OISHI, S., OSUE, T., & YOKOI, K. (2016). Effects of 6-month eicosapentaenoic acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. Cardiovascular Diabetology, 15, 1–14. https://doi.org/10.1186/s12933-016-0359-5

SHANTHA, N. C., & DECKER, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. Journal of AOAC International, 77 (2), 421–424. https://doi.org/10.1093/jaoac/77.2.421

SOBEL, R., VERSIC, R., & GAONKAR, A. G. (2014). Introduction to microencapsulation and controlled delivery in foods. In A. G. Gaonkar, N. Vasisht, A. R. Khare, & R. Sobel (Eds.), Microencapsulation in the food industry (pp. 3–12). Academic Press. https://doi.org/10.1016/b978-0-12-404568-2.00001-7

TAVARES, G. M., CROGUENNEC, T., CARVALHO, A. F., & BOUHALLAB, S. (2014). Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. Trends in Food Science & Technology, 37 (1), 5–20. https://doi.org/10.1016/j.tifs.2014.02.008

TONG, L. M., SASAKI, S., MCCLEMENTS, D. J., & DECKER, E. A. (2000). Mechanisms of the Antioxidant Activity of a High Molecular Weight Fraction of Whey. Journal of Agricultural and Food Chemistry, 48 (5), 1473–1478. https://doi.org/10.1021/jf991342v

TORRES-GINER, S., MARTINEZ-ABAD, A., OCIO, M. J., & LAGARON, J. M. (2010). Stabilization of a nutraceutical omega-3 fatty acid by encapsulation in ultrathin electrosprayed zein prolamine. Journal of Food Science, 75, N69–N79. https://doi.org/10.1111/j.1750-3841.2010.01678.x

WEIDNER, E. (2009). High pressure micronization for food applications. The Journal of Supercritical Fluids, 47(3), 556–565. https://doi.org/10.1016/J.SUPFLU.2008.11.009

WEN, Z., & CHEN, F. (2010). Production of eicosapentaenoic acid using heterotrophically grown microalgae. In Z. Cohen & C. Ratledge (Eds.), Single cell oils (2nd ed., pp. 151–177). AOCS Press. https://doi.org/10.1016/B978-1-893997-73-8.50012-8

YOKOYAMA, M., ORIGASA, H., MATSUZAKI, M., MATSUZAWA, Y., SAITO, Y., ISHIKAWA, Y., OIKAWA, S., SASAKI, J., HISHIDA, H., & ITAKURA, H. (2007). Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): A randomised open-label, blinded endpoint analysis. The Lancet, 369, 1090–1098. https://doi.org/10.1016/S0140-6736(07)60527-3

5. CHAPTER 2

DRAGON'S BLOOD SAP - DBS

Dragon's Blood Sap: Storage Stability and Antioxidant Activity

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Molecules 2018, 23, 264
doi:10.3390/molecules23102641
Impact Factor (2018): 3.060
Current Impact Factor: 4.2
Cite Score:7.4
Article citations: 17

Abstract

Currently, consumers are demanding additive-free, fresher and more-natural products. Dragon's Blood Sap (DBS), the deep red latex of the specie of tree Croton lechleri (Müll. Arg.) contains a high concentration of phenolic compounds of great interest for the food, pharmaceutical and cosmetic industry. These chemical compounds are highly susceptible to degradation. Therefore, the DBS storage stability and its photo-oxidation was studied by FT-IR and UV-vis spectrophotometry during 39 days at different temperatures (4-21°C) and relative humidities (0-56%), as well as under UV light exposure. It was observed that the degradation of phenolic compounds was reduced at 0%RH, not being significant the effect of temperature in the range studied. UV light irradiation degraded DBS in a 20%. DBS has an exceptional high and stable antioxidant content (≥93% inhibition percentage of DPPH) which makes it a unique property to consider the DBS as an antioxidant agent or ingredient for consumer products formulations.

Keywords: Dragon's Blood Sap, proanthocyanidins, storage stability, antioxidant activity, FT-IR, UV-Vis spectrophotometry.

1. Introduction

Phenolic compounds are one of the most widely occurring groups of phytochemicals. This group of bioactive compounds containing one or more aromatic rings with one or more hydroxyl groups attached to them is divided into the following classes: Phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, and proanthocyanidins), stilbenes, and lignans (Brglez Mojzer et al., 2016; Kopjar et al., 2014)]. These compounds are secondary plant metabolites involved in the defense against ultraviolet radiation or aggression by pathogens, contributors to plant pigmentations and antioxidants, as well as responsible for their organoleptic properties (Brglez Mojzer et al., 2016; Ignat et al., 2011). Additionally, these compounds have also demonstrated a huge number of potential benefits for human health, related to their presumable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular, and neurodegenerative diseases (Manach et al., 2004; Volf et al., 2014). Fruits, vegetables, leguminous plants, and cereals are good natural sources for polyphenols. The content of polyphenols varies significantly as a function of several parameters including genetic factors, environmental factors (climate, agronomic factors), manner of cultivation, and ripeness (Brglez Mojzer et al., 2016). In recent years, special attention has been paid to the isolation of phenolic compounds from different raw materials, especially from inexpensive or residual sources (medicinal plants, fruits, vegetables, industrial by products, and beverages).

Dragon's Blood Sap (DBS) is the common name of the deep red latex that oozes out upon cutting or slashing the bark of the specie of tree Croton lechleri (Müll. Arg.), also known as Croton draco var. cordatus (Müll. Arg.), and Oxydectes lechlen (Müll. Arg.) Kuntze (The United States Pharmacopeial Convention Herbal Medicines Compendium, n.d.). It is a native biodiversity species of tree from South America (Brazil, Bolivia, Colombia, Ecuador, and Peru) (Grandtner & Chevrette, 2013).

DBS, very well-known in traditional medicine, has been related to several therapeutic uses such as analgesic, anti-inflammatory, antibacterial, antifungal, antihemorrhagic, antimicrobial, antioxidant, antiseptic, antitumor and cytotoxic, antiulcer and antidiarrheal, antiviral, astringent, cicatrizing, immunomodulatory, muscular tissue regenerator, mutagenic and antimutagenic, purifier, skin conditioning, wound healing, among others (Z. Chen et al., 1994; Gupta et al., 2007; Pieters et al., 1995). DBS bioactivity is due to the high content of proanthocyanidins (>90% dry weight of the resin), but also to its content in taspine, catechin, epigallocatechin, epicatechin, and a small percentage of terpene compounds (Rossi et al., 2011, 2013).

The interest in DBS relies on the high content of polyphenols, especially proanthocyanidins, one of its main constituents, which, due to their low water solubility or poor bioavailability, constitute the only dietary group of antioxidants present in the colon, which is particularly exposed to oxidizing agents and may be affected by inflammation and numerous diseases such as cancer (Aizpurua-Olaizola et al., 2016; Alonso-Castro et al., 2012; Halliwell et al., 2000; Manach et al., 2004). However, DBS could be involved in other industrial applications. Currently, consumers are demanding additive-free, fresher, and more-natural products. In this sense, polyphenols may be used as natural antioxidants, natural colorants, or preservatives for foods. Moreover, they could be used as additives in the production of paints, paper, cosmetics, and pharmaceutical products, replacing current synthetic additives, some of which are being restricted due to their carcinogenicity (Volf et al., 2014). Taking into account the beneficial health effect offered by these compounds, their incorporation in food, cosmetics, pharmaceutical, or agricultural products will represent an added value.

Hitherto, special attention has been paid to the therapeutic potential of DBS (Alonso-Castro et al., 2012; Z. P. Chen et al., 1994; D'yakonov et al., 2017; Gupta et al., 2007; Pieters et al., 1995), its antimicrobial activity (Lopes et al., 2004; Ortiz et al., 2003), and its chemical composition (Cai et al., 1991, 1993b, 1993a; De Marino et al., 2008; Pieters et al., 1995; Rossi et al., 2011). However, little work was done

on the study of its stability. It is well known that these bioactive compounds are highly susceptible to degradation. During the different processing stages, storage, and consumption, polyphenols could be subjected to different stress conditions such as high temperatures, light, oxygen, solvents, presence of enzymes, proteins, metallic ions, or associations with other food constituents, which can damage their structures and thus reduce their beneficial activity (Volf et al., 2014).

Therefore, the aim of this work is to study the storage stability at different conditions of temperature and humidity and the photo-oxidation of DBS, as well as the effect of these stress conditions on its antioxidant activity.

2. Materials and Methods

Dragon's Blood Sap (DBS) was kindly provided by Q'omer BioActive Ingredients (Valencia, Spain). Natural DBS (total solids 25.95% ± 0.83%) was used as received without further purification. Samples of DBS were freeze-dried and sealed until used for storage stability and photo-oxidation tests. Saturated salt solutions of magnesium nitrate (56%RH), potassium carbonate (44%RH), potassium acetate (23%RH), silica gel, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (≥97.0%), and potassium bromide FT-IR grade (KBr) were purchased from Sigma-Aldrich (Tres Cantos, Spain).

2.1. Effect of the storage conditions and the photo-oxidation on DBS stability

The DBS storage stability was studied under different conditions of relative humidity (0, 23, 44 and 56%RH at 21°C), different storage temperatures (4 and 21°C at 0%RH) and under UV-light irradiation. This study was performed by ultraviolet-visible (UV-Vis) spectrophotometry and Fourier-Transform Infrared spectroscopy (FT-IR) comparing the spectra of freeze-dried DBS with the spectra of the samples at different conditions, which were collected every three days during a time span of 39 days.

For such study, samples of freeze-dried DBS were equilibrated in desiccators stored at different percentages of relative humidity, by using silica gel (0%RH) and oversaturated salt solutions of potassium acetate (23%RH), potassium carbonate (44%RH) and magnesium nitrate (56%RH) respectively; at cooling temperature (4°C) or ambient temperature (~21°C), accordingly.

An Ultra-Vitalux lamp from OSRAM Lighting S.L. (Madrid, Spain) was used to irradiate samples with ultraviolet (UV) light. This lamp operates with a power of 300 W that produces a blend of radiation very similar to that of natural sunlight, which is generated by a quartz discharge tube and a tungsten filament. The radiation of 315−400 nm after 1 h of exposure is of 13.6 W, and the radiation of 280−315 nm after 1 h of exposure is of 3.0 W (Torres-Giner et al., 2017).

Ten grams of freeze-dried DBS were placed on Petri dishes at the correspondent condition and aliquots were taken each three days for analysis. For the FT-IR analysis, KBr pellets containing the freeze-dried DBS were subjected to the studied storage conditions.

2.2. Ultraviolet–Visible (UV–Vis) spectrophotometry

The UV–Vis spectral measurements were performed in a UV4000 spectrophotometer (Dinko Instruments). Aliquots of DBS subjected to the different study conditions were solubilized in methanol at a concentration of 0.5 mg/ml. Methanol was used as blank. Standard curves of DBS concentration were built using methanol solutions of known DBS concentration (0-0.8 mg/ml) at the different absorption bands.

2.3. Fourier-Transform Infrared spectroscopy FT-IR.

FT-IR spectra of DBS samples were collected using a FT-IR Tensor 37 equipment (Bruker, Rheinstetten, Germany) in the absorption mode using KBr pellets. All spectra were collected by averaging 10 scans in the range of 4.000 to 400 cm-1 with a 4 cm-1 resolution.

2.4. Antioxidant Capacity by DPPH Method

Spectrophotometric DPPH assay was used to study the inhibition percentage (IP) of DPPH by DBS. An aliquot of 0.1 ml of DBS methanol solutions (0.5 mg/ml) were added to 2.9 ml of DPPH (40 mg/l of DPPH in methanol), shaken in the vortex and kept in the dark for 30 min at room temperature. Sample absorbance was measured at 517 nm with UV–Vis spectrophotometer (UV4000, Dinko Instruments) using methanol as blank. The radical scavenging activity of each sample was calculated according to the Equation 1:

$$\text{IP (\%)} = \frac{A\ control - A\ \text{sample}}{A\ \text{control}} \text{ x } 100 \qquad \text{(Eq.1)}$$

Where IP (%) is the inhibition percentage of DPPH, A control is the absorbance of pure DPPH solution, A sample is the absorbance of the sample after reacting with DPPH. Antioxidant activity was also expressed as Trolox equivalent antioxidant activity (TEAC). Standard curve at different Trolox concentrations was build. Measurements were done in triplicate.

2.5. Statistical Analysis

Statgraphics Centurion XV (Statistical Graphics Corp.) was used for data analysis. Data were expressed as mean ± standard deviation. Firstly, analysis of variance (ANOVA) was carried out to investigate the effects of the studied parameters on the sample. Secondly, mean values were compared by using the Tukey's honestly significant difference (HSD). Differences between means were considered at p-value < 0.05.

3. Results and Discussion

DBS stability was studied considering several storage conditions, under different temperature and relative humidity (%RH) environments. Additionally, an accelerated ageing treatment was carried out subjecting the freeze-dried DBS to UV light irradiation. The damage of DBS was measured by UV-Vis

spectrophotometry and Fourier transform infrared spectroscopy technique (FT-IR). Moreover, the effect of these stress conditions on the DBS antioxidant activity was evaluated.

3.1. DBS stability by UV- vis

UV-Vis spectra of DBS samples were collected and analyzed by UV-Vis spectrophotometry. DBS presented four UV-Vis bands at a maximum absorption wavelength (λmax) of 331 nm, 347 nm, 464 nm, and 579 nm, as it is shown in Figure1. The complex nature of the DBS' spectrum makes the detailed theoretical analysis difficult (Cai et al., 1991). Nevertheless, some of the later λmax could be related to important bioactive materials, such as the quinoxaline, which has a UV absorption at λmax of 242 and 331 nm; and 6-thioguanine has UV absorption at a λmax of 258 and 347 nm (Armarego & Chai, 2009). The quinoxaline is a fused heterocycle of benzene and pyrazine rings with a wide array of pharmacological activities viz. anti-cancer, antimalarial, anti-inflammatory, antimicrobial, antiHIV, etc. (Armarego & Chai, 2009; Tariq et al., 2018). The 6-thioguanine is an antimetabolite of purine analogue type, used in the treatment of acute leukemias due to its inhibition of DNA synthesis in cancer cells (Papich, 2016; Rider, 2007).

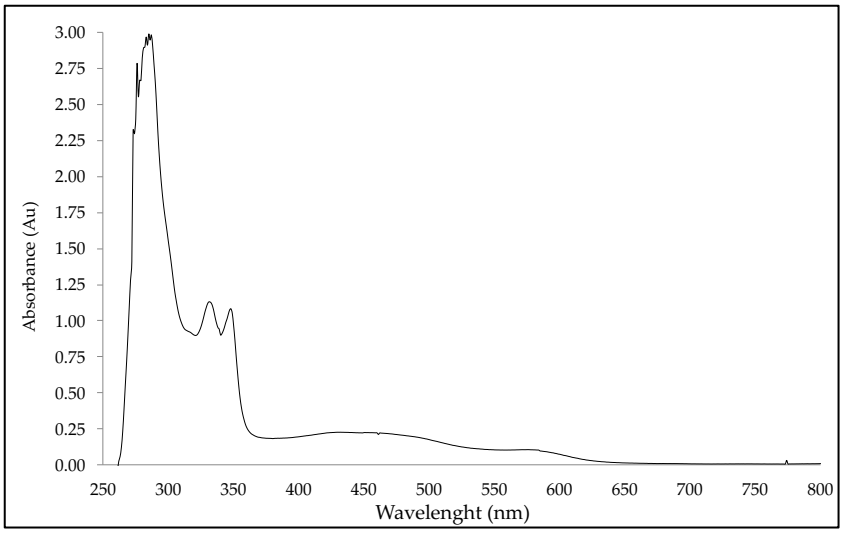

Figure 1. UV-vis spectrophotometry screening spectra of Dragon's Blood Sap at a concentration of 0.5 mg/ml.

3.1.1. DBS' stability according to relative humidity (%RH)

Figure 2 represents the variation of the concentration of the DBS compounds at various %RH and ambient temperature (~21°C) after 39 days of storage at the UV-Vis λmax identified in Figure 1. All the experimental conditions of %RH produced significant differences between the initial (0.52 ± 0.02 mg/ml) and final concentration of DBS samples. In order to clarify the effect of the %RH on DBS stability, a Tukey's honestly significant difference test (HSD) was performed and it is shown in Figure 3. Samples stored under 0%RH were more stable when compared with the other three %RH conditions (23%, 44% and 56%). Lavelli et al. found that the degradation kinetics of grape skin phenolics could be reduced by decreasing the water activity in the grape skin powder (Lavelli et al., 2017). They attributed this increased stability to a limited water mobility and diffusion rate of reagents, as well as to a decreased enzyme activity (Lavelli et al., 2017).

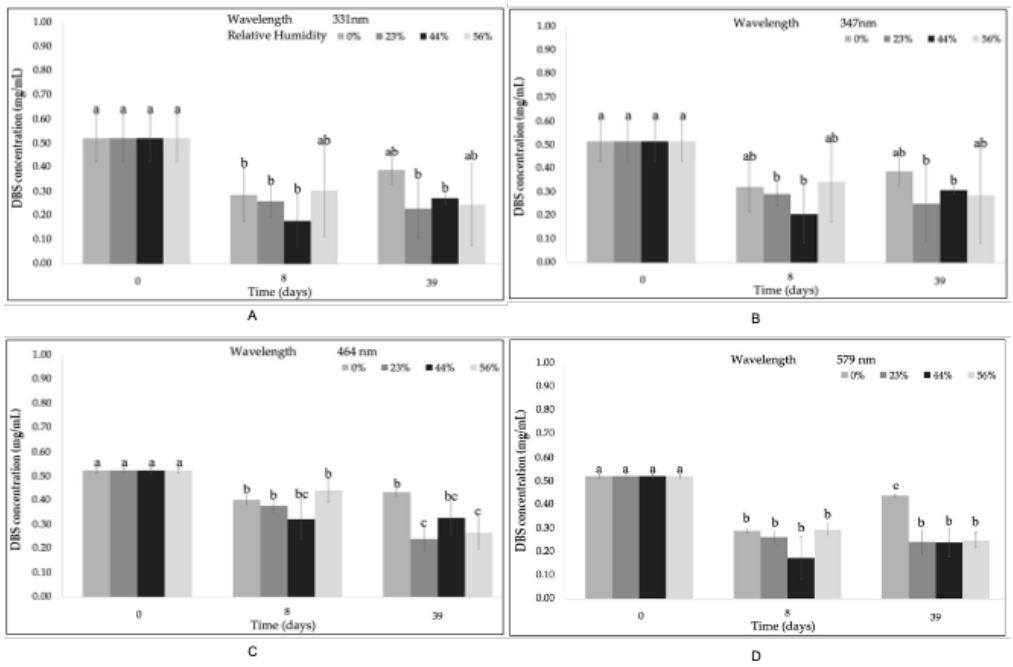

Figure 2. Evolution of the DBS concentration under various %RH and ambient temperature (~21°C) after 39 days of storage according to the different absorption bands: a) 331 nm; b) 347 nm; c) 464 nm; d) 579 nm. Different letters indicate significant differences among the samples ($p < 0.05$).

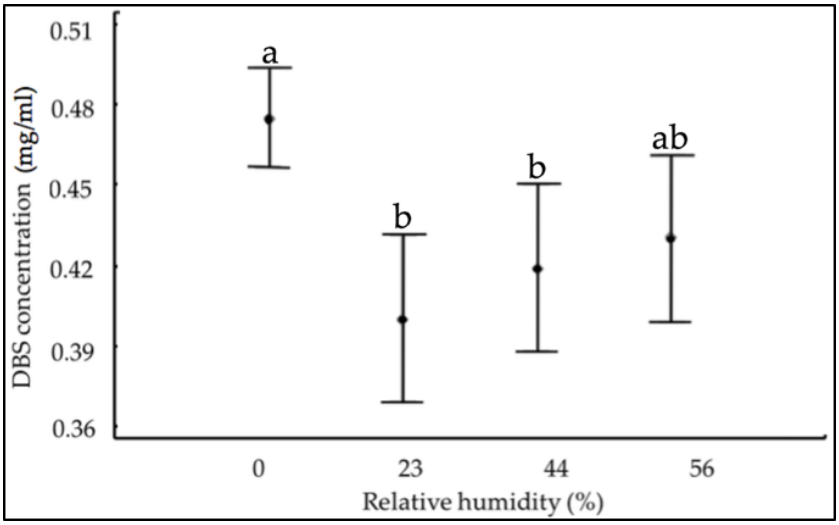

Figure 3. Tukey's HSD test of the evolution of Dragon's Blood Sap concentration after 39 days of storage at 21°C and different relative humidity. Different letters indicate significant differences among the samples ($p < 0.05$).

3.1.2. DBS' stability according to temperature

DBS stability considering the storage temperature (4°C and ~21°C) at the different wavelengths is represented in Figure 4. Similar to the previous analyses of DBS' stability according to relative humidity (%RH), certain differences between the initial (0.52±0.02 mg/ml) and final concentrations of all DBS samples were also identified in reference to both experimental temperatures. DBS components that absorbed at the lower wavelength region were not significantly changed. However, in the case of the DBS components that absorbed within the higher region, a reduction in DBS concentration was observed at 4°C when compared with ambient temperature. In addition, it was observed that after one week, the DBS concentration was reduced, but at the end of the experiment, the DBS concentration increased. This phenomenon could be due to the complex nature of DBS, which could interact at ambient temperature and produce other secondary metabolites absorbing in the long wavelength region.

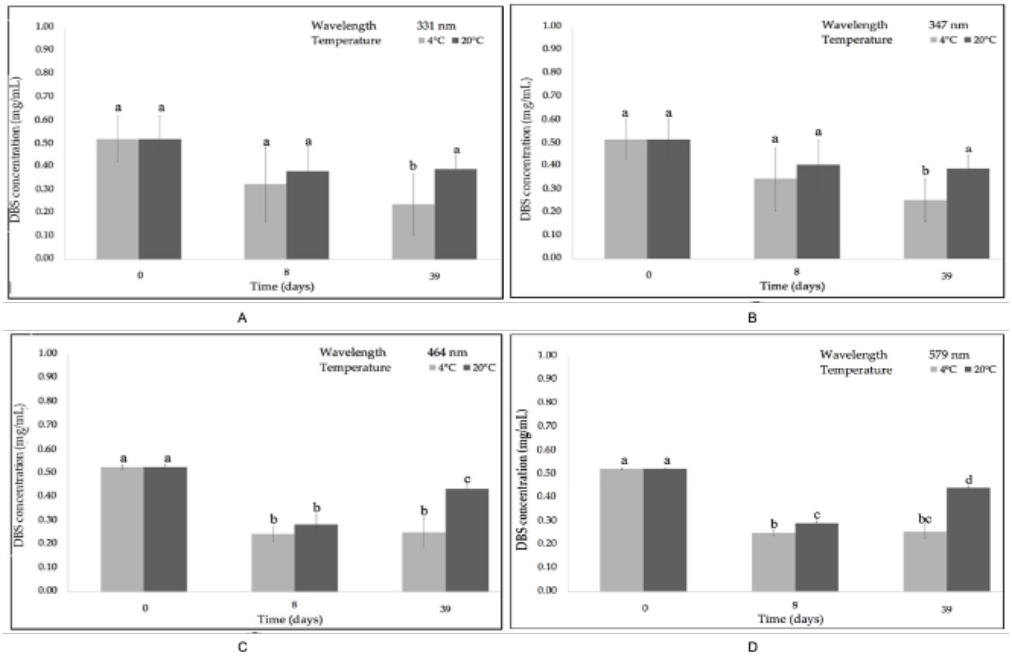

Figure 4. Evolution of DBS concentration after 39 days of storage under low (4°C) and ambient temperature (~21°C) and 0%RH, according to the different absorption bands: A) 331 nm; B) 347 nm; C) 464 nm; D) 579 nm. Different letters indicate significant differences among the samples ($p < 0.05$).

Figure 5 represents the Tukey's HSD test (honestly significant difference) in order to clarify the effect of the temperature on the concentration of DBS samples. With a level of 95.0% confidence, a statistically significant difference was observed between 4°C and 21°C, presenting a higher stability at ambient temperature. Kopjar et al., (2014) observed a higher stability of the polyphenols present in the sour cherry puree extracts at 4 ºC than at room temperature. Xu et al., (2015) found slower degradation kinetics for the proanthocyanidins dimers at temperatures lower than 25 ºC than at 40 ºC. Nóbrega et al., 2015) et al. studied the effect of hot air during a drying process, finding that temperatures higher than 60 ºC contributed to the degradation of phenolic compounds and consequently to a reduction in the antioxidant activity.

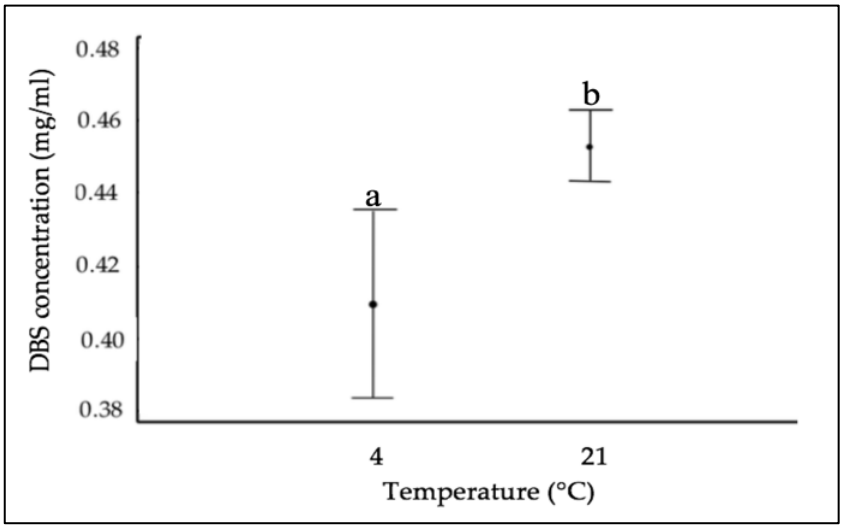

Figure 5. Tukey's test of the evolution of Dragon's Blood Sap concentration at different storage temperatures and 0%RH. Different letters indicate significant differences among the samples ($p<0.05$).

3.1.3. DBS' stability at ultraviolet (UV) Light Exposition

Figure 6 shows the effect of UV light on the DBS concentration. The radiation produced by UV light exposure produced a reduction into the DBS concentration, from an initial 0.52 ± 0.02 mg/ml to a final concentration of 0.32 ± 0.21 mg/ml for DBS compounds absorbed at lower UV-vis λmax region, and to a concentration of 0.48 ± 0.09 mg/ml in the case of DBS compounds absorbed at the higher UV-vis λmax region. Masek et al. (2015) observed that UV irradiation affected quercetin stability in a higher extent than temperature. Other authors have found that the effect of UV light caused a higher degradation than temperature in a copigment of anthocyanin and polyphenols complex (Bąkowska et al., 2003).

The complex nature of the DBS makes difficult the proper description of the chemical behavior of DBS under the different storage conditions. Therefore, previous results were compared with those obtained by FT-IR.

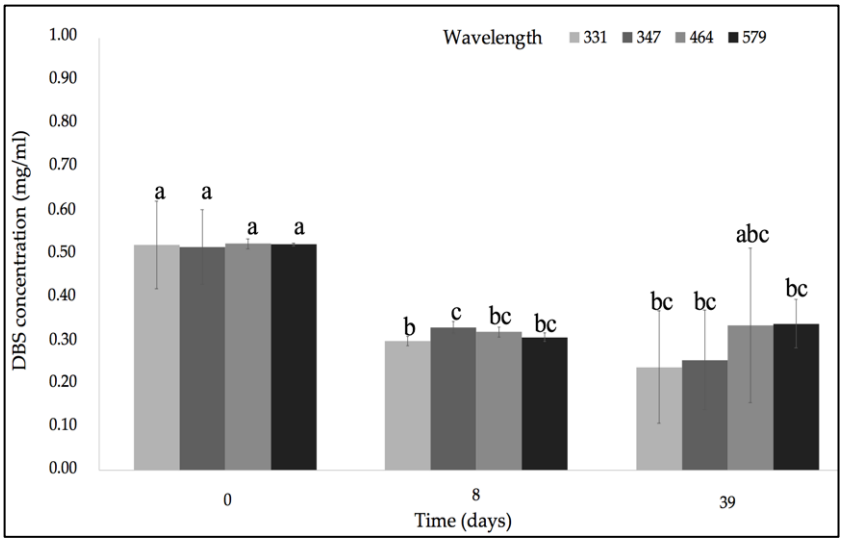

Figure 6. Evolution of the DBS concentration after 39 days of storage under UV-light according to the different absorption bands: 331 nm; 347 nm; 464 nm; 579 nm. Different letters indicate significant differences among the samples ($p<0.05$).

3.2. DBS stability by FT-IR

FT-IR spectroscopy was used to describe the changes of DBS composition exposed to certain storage conditions. Figure 7 represents the typical FT-IR spectrum of freeze-dried DBS samples. The spectrum of freeze-dried DBS was compared to the spectrum of natural DBS, which did not show significant differences in the chemical composition (results not shown). The DBS spectrum presented the characteristic bands of the proanthocyanidins, which are of its main constituents (Cai et al., 1991; Merlic, 2012; Namjoyan et al., 2016). Among the bands found in the spectrum, the intense O-H stretching band between the 3700 – 3000 cm^{-1} could be ascribed to the formation of the hydrogen bond between phenolic hydroxyl of proanthocyanidins. The absorption bands between 1100 and 1600 cm^{-1} were attributed to the polyflavonoids moiety, and the absorption bands between 700-1500 cm^{-1} were attributed to the presence of procyanidin structure (Biao et al., 2018).

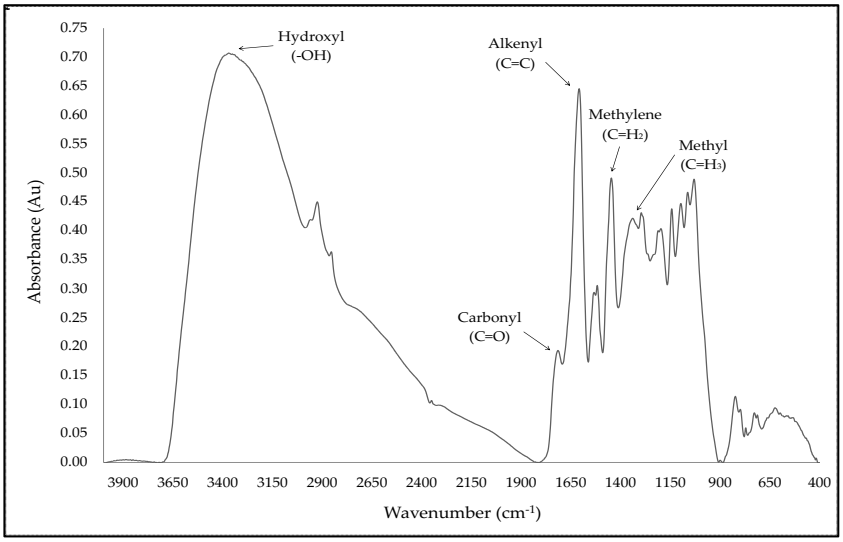

Figure 7. Typical FT-IR spectrum of freeze-dried DBS. Arrows indicates the Functional Groups bands discussed in the text.

The DBS spectrum (Figure 7) was characterized by the 3300 cm^{-1} band related to the hydroxyl groups (-OH), a high intense band appearing at 2920 cm^{-1}, and other one at 2854 cm^{-1}, corresponding to the alkyl C–H stretch (2950–2850 cm^{-1}) (Merlic, 2012). Another band was also assigned to the carbonyl group (~1700 cm^{-1}) and to the C–O bond with the absorption band around 1200 cm^{-1} (Merlic, 2012). The region around 1700–900 cm^{-1} was mostly related with phenolic compounds (de Souza et al., 2018). Since the absorption bands in the frame of 900 and 1100 cm^{-1} could be mainly assigned to carbohydrates (de Souza et al., 2018), the study was focused on the frame of 1700–1100 cm^{-1}. Within this region, there is a band at 1519 cm^{-1}, which is present in large amounts in aromatic C=C bending compounds with phenyl bonds similar to those in polyphenolic compounds, such as flavonoids (De Souza et al., 2015; Heneczkowski et al., 2001; Schulz & Baranska, 2007); as well as the band observed at 777 cm^{-1}, attributed to the skeletal stretching modes of the aromatic ring and CH out-of-plane deformation of the aromatic rings with two adjacent free hydrogen atoms, respectively, indicating

the prominent presence of procyanidin structure (Foo, 1981; Schulz & Baranska, 2007).

In Figure 8, the evolution of the bands with the time exposed under UV light irradiation is presented. The aromatic band at 1519 cm-1 was used as a reference to study the DBS stability with respect to the bands identified previously, since it remained unchanged under all conditions. Figure 8.a present the hydroxyl groups (-OH) behavior. In a similar fashion to the results obtained by UV-Vis spectrophotometry, DBS presented an interaction based on the humidity of the storage media. DBS concentration increased as the %RH was increased, which could be due to the absorption of water. At 0%RH, a slight reduction of the hydroxyl groups by effect of the relative humidity was noted. The UV light radiation produced a higher reduction, compared to the other storage conditions. This reduction could be due to the effect of the UV light but also to the light bulb temperature.

Figure 8.B. illustrates the effect of the storage conditions on the carbonyl group (1715 cm-1). An increment in the DBS absorption signal under UV light irradiation was observed. This phenomenon could be due to the biochemistry and oxidation of DBS, by which the hydroxyl groups were transformed into carbonyl groups (Masek & Chrzescijanska, 2015).

Finally, the evolution of the alkenyl, methylene and methyl groups is presented in Figures 8.C, 8.D and 8.E, respectively. At the storage conditions considered, no significant changes were observed into the chemical structure of the sample at these absorption bands. Nevertheless, UV light irradiation produced the biggest reduction in all the studied functional groups. According to the previous results, the best conditions for DBS storage should avoid high humidity and UV light irradiation.

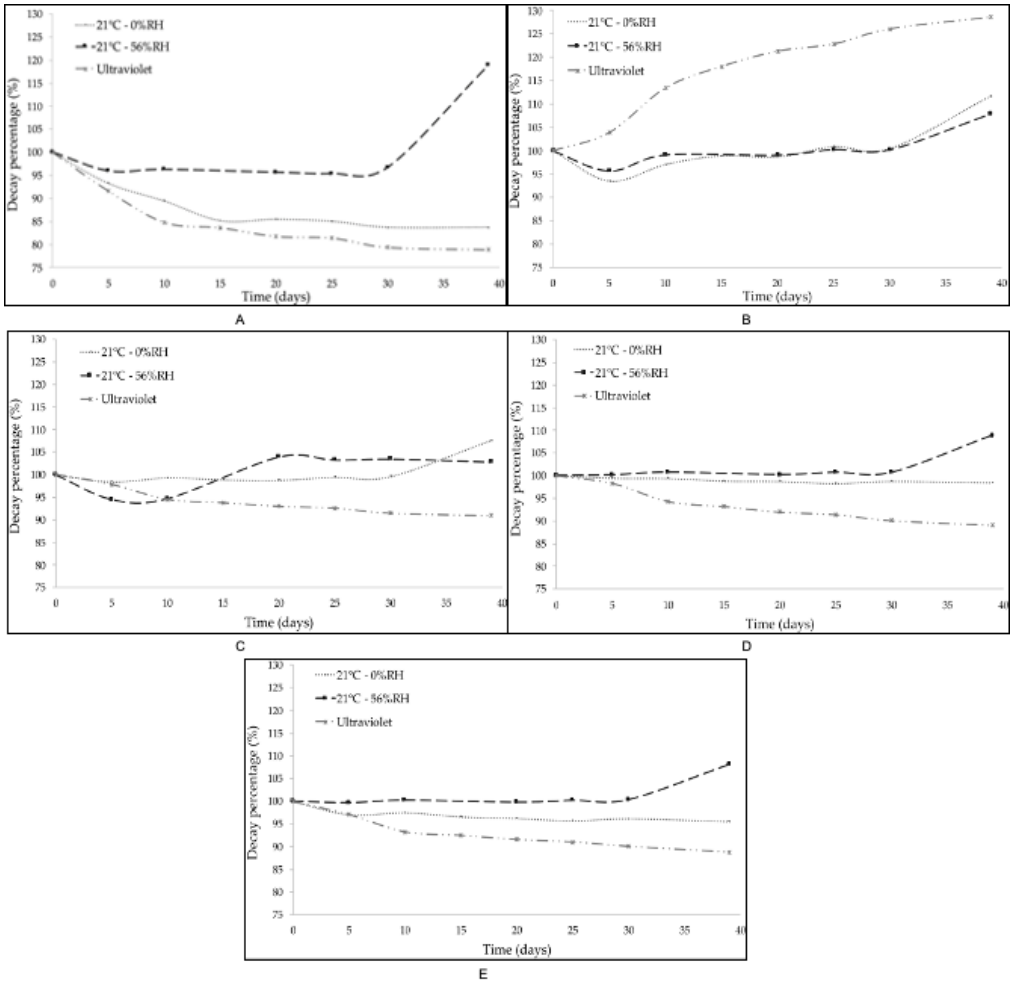

Figure 8. Evolution of the decay percentage of DBS characteristic functional groups with respect to the internal standard band at 1519 cm^{-1} after 39 days under different temperatures, %RH and UV-light irradiation: A) hydroxyl group (-OH) (3300 cm^{-1}); B) carbonyl group (C=O) (1715 cm^{-1}); C) alkenyl group (C=C) (1610 cm^{-1}); D) methylene group ($C-H_2$) (1440 cm^{-1}); and E) methyl group ($C-H_3$) (1342 cm^{-1})

3.3. Antioxidant Capacity Stability

The structural chemistry of proanthocyanidins suggests different antioxidant properties, such as (i) high reactivity as a hydrogen or electron donor, (ii) the ability to stabilize or delocalize an unpaired electron, and (iii) the ability to chelate transition metal ions (Plaza et al., 2014). In order to define the impact of different storage conditions on the DBS antioxidant activity, spectrophotometric DPPH assay was used to study the DPPH inhibition percentage (IP DPPH%) of DBS.

Table 1 presents the results of IP DPPH% of the DBS samples at the studied storage conditions. All samples had a high rate of inhibition of the DPPH free radical; with values higher than 90% and stable under the studied storage conditions. These results demonstrated that DBS had an exceptional high and stable antioxidant activity, which could be really interesting for multiple applications (Carlsen et al., 2010).

Table 1. Antioxidant capacity of DBS under different environmental conditions over a period time of 39 days. Data represent the mean ± SD of triplicate assay for each sample. The mean ± SD at each storage condition followed by the same letter are not significantly different at $P < 0.05$

Sample	Time (days)	IP DPPH (%)
Natural DBS	0	92.76 ± 0.29^{a}
4°C RH 0%	39	93.45 ± 0.35^{b}
21°C RH 0%	39	93.38 ± 0.28^{b}
21°C RH 23%	39	92.97 ± 0.50^{b}
21°C RH 44%	39	93.08 ± 0.19^{b}
21°C RH 56%	39	93.19 ± 0.19^{b}
UV	39	93.12 ± 0.13^{b}

The results obtained by UV-Vis spectrophotometry and FT-IR showed that the components of DBS were degraded under the studied storage conditions. However, its antioxidant activity was not affected. This phenomenon

demonstrated the synergic effect of the complex chemical profile of DBS, which could be used for multiple industrial applications.

4. Conclusions

The DBS stability was studied by UV-Vis spectrophotometry and FT-IR under several storage conditions, taking into consideration the effect on the antioxidant activity. Results demonstrated that changes were produced on the concentration of the DBS constituents at different storage conditions. For example, the presence of moisture contributed to the DBS degradation. In case of temperature, no significant effect was detected in the range studied. However, when UV light irradiation was applied, a significant reduction of the DBS concentration took place. Nevertheless, even when changes into its chemical structure were detected, the DBS antioxidant activity remained stable under the studied storage conditions.

The low degree of degradation of samples, just a 20% when DBS was subjected to UV-light during 39 days, could be due to the protective effect exhorted by proanthocyanidins, which are one of the main constituents of DBS. In addition, the synergic effect between constituents could contribute to that stability. Therefore, the high stability observed for DBS together with its natural origin, could confer interesting characteristics in multiple industrial added-value products, such as food, pharmaceuticals, nutraceuticals or cosmetics, paints or paper products, as an antioxidant agent or as an ingredient, opening an interesting opportunity to explore new applications for DBS.

Acknowledgements

J.D. Escobar thanks to the Spanish Ministry of Economy, Industry and Competitivity, for the pre-doctoral grant (DI-14-6906). Funding: "This research was funded by MINECO grant numbers [AGL2015-63855-C2-1-R and DI-14-6906]

References

AIZPURUA-OLAIZOLA, O., NAVARRO, P., VALLEJO, A., OLIVARES, M., ETXEBARRIA, N., & USOBIAGA, A. (2016). Microencapsulation and storage stability of polyphenols from Vitis vinifera grape wastes. Food Chemistry, 190, 614–621. https://doi.org/10.1016/j.foodchem.2015.05.117

ALONSO-CASTRO, A. J., ORTIZ-SÁNCHEZ, E., DOMÍNGUEZ, F., LÓPEZ-TOLEDO, G., CHÁVEZ, M., ORTIZ-TELLO, A. D. J., & GARCÍA-CARRANCÁ, A. (2012). Antitumor effect of Croton lechleri Mull. Arg. (Euphorbiaceae). Journal of Ethnopharmacology, 140 (2), 438–442. https://doi.org/10.1016/j.jep.2012.01.009

ARMAREGO, W. L. F., & CHAI, C. L. L. (2009). Purification of Organic Chemicals. In Purification of Laboratory Chemicals. https://doi.org/10.1016/b978-1-85617-567-8.50012-3

BĄKOWSKA, A., KUCHARSKA, A. Z., & OSZMIAŃSKI, J. (2003). The effects of heating, UV irradiation, and storage on stability of the anthocyanin–polyphenol copigment complex. Food Chemistry, 81 (3), 349–355. https://doi.org/10.1016/S0308-8146(02)00429-6

BIAO, L., TAN, S., MENG, Q., GAO, J., ZHANG, X., LIU, Z., & FU, Y. (2018). Green synthesis, characterization and application of proanthocyanidins-functionalized gold nanoparticles. Nanomaterials, 8 (1). https://doi.org/10.3390/nano8010053

BRGLEZ MOJZER, E., KNEZ HRNČIČ, M., ŠKERGET, M., KNEZ, Ž., & BREN, U. (2016). Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. In Molecules (Basel, Switzerland) (Vol. 21, Issue 7). https://doi.org/10.3390/molecules21070901

CAI, Y., CHEN, Z. P., & PHILLIPSON, J. D. (1993a). Clerodane diterpenoids from Croton lechleri. Phytochemistry, 34 (1), 265–268. https://doi.org/10.1016/S0031-9422(00)90816-1

CAI, Y., CHEN, Z. P., & PHILLIPSON, J. D. (1993b). Diterpenes from Croton lechleri. Phytochemistry, 32 (3), 755–760. https://doi.org/10.1016/S0031-9422(00)95166-5

CAI, Y., EVANS, F. J., ROBERTS, M. F., PHILLIPSON, J. D., ZENK, M. H., & GLEBA, Y. Y. (1991). Polyphenolic compounds from Croton lechleri. Phytochemistry, 30 (6), 2033–2040. https://doi.org/10.1016/0031-9422(91)85063-6

CARLSEN, M. H., HALVORSEN, B. L., HOLTE, K., BØHN, S. K., DRAGLAND, S., SAMPSON, L., WILLEY, C., SENOO, H., UMEZONO, Y., SANADA, C., BARIKMO, I., BERHE, N., WILLETT, W. C., PHILLIPS, K. M., JACOBS, D. R., & BLOMHOFF, R. (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition Journal, 9 (1). https://doi.org/10.1186/1475-2891-9-3

CHEN, Z. P., CAI, Y., & PHILLIPSON, J. D. (1994). Studies on the anti-tumour, anti-bacterial, and wound-healing properties of Dragon's blood. Planta Medica, 60 (6), 541–545. https://doi.org/10.1055/S-2006-959567

DE MARINO, S., GALA, F., ZOLLO, F., VITALINI, S., FICO, G., VISIOLI, F., & IORIZZI, M. (2008). Identification of Minor Secondary Metabolites from the Latex of Croton lechleri (Muell-Arg) and Evaluation of Their Antioxidant Activity. Molecules 2008, Vol. 13, Pages 1219-1229, 13 (6), 1219–1229. https://doi.org/10.3390/molecules13061219

DE SOUZA, V. B., THOMAZINI, M., BALIEIRO, J. C. D. C., & FÁVARO-TRINDADE, C. S. (2015). Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (Vitis labrusca). Food and Bioproducts Processing, 93 (October), 39–50. https://doi.org/10.1016/j.fbp.2013.11.001

DE SOUZA, V. B., THOMAZINI, M., ECHALAR BARRIENTOS, M. A., NALIN, C. M., FERRO-FURTADO, R., GENOVESE, M. I., & FAVARO-TRINDADE, C. S. (2018). Functional properties and encapsulation of a proanthocyanidin-rich cinnamon extract (Cinnamomum zeylanicum) by complex coacervation using gelatin and different polysaccharides. Food Hydrocolloids, 77, 297–306. https://doi.org/10.1016/j.foodhyd.2017.09.040

D'YAKONOV, V. A., DZHEMILEVA, L. U., & DZHEMILEV, U. M. (2017). Advances in the Chemistry of Natural and Semisynthetic Topoisomerase I/II Inhibitors (pp. 21–86). https://doi.org/10.1016/B978-0-444-63929-5.00002-4

FOO, L. Y. (1981). Proanthocyanidins: Gross chemical structures by infrared spectra. Phytochemistry, 20(6), 1397–1402. https://doi.org/10.1016/0031-9422(81)80047-7

GRANDTNER, M. M., & CHEVRETTE, J. (Eds.). (2013). C. In Dictionary of South American Trees (Vol. 2, pp. 87–191). Elsevier. https://doi.org/10.1016/B978-0-12-396490-8.00003-3

GUPTA, D., BLEAKLEY, B., & GUPTA, R. K. (2007). Dragon's blood: Botany, chemistry and therapeutic uses. Journal of Ethnopharmacology, 115 (3), 361–380. https://doi.org/10.1016/j.jep.2007.10.018

HALLIWELL, B., ZHAO, K., & WHITEMAN, M. (2000). The gastrointestinal tract: A major site of antioxidant action? Free Radical Research, 33 (6), 819–830. https://doi.org/10.1080/10715760000301341

HENECZKOWSKI, M., KOPACZ, M., NOWAK, D., & KUŹNIAR, A. (2001). Infrared spectrum analysis of some flavonoids. Acta Poloniae Pharmaceutica, 58 (6), 415–420.

IGNAT, I., VOLF, I., & POPA, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. In Food Chemistry (Vol. 126, Issue 4, pp. 1821–1835). https://doi.org/10.1016/j.foodchem.2010.12.026

KOPJAR, M., ORSOLIC, M., & PILIZOTA, V. (2014). Anthocyanins, phenols, and antioxidant activity of sour cherry puree extracts and their stability during storage. International Journal of Food Properties, 17 (6), 1393–1405. https://doi.org/10.1080/10942912.2012.714027

LAVELLI, V., SRI HARSHA, P. S. C., LAUREATI, M., & PAGLIARINI, E. (2017). Degradation kinetics of encapsulated grape skin phenolics and micronized grape skins in various water activity environments and criteria to develop wide-ranging and tailor-made food applications. Innovative Food Science & Emerging Technologies, 39, 156–164. https://doi.org/10.1016/J.IFSET.2016.12.006

LOPES, M. I. L. E., SAFFI, J., ECHEVERRIGARAY, S., HENRIQUES, J. A. P., & SALVADOR, M. (2004). Mutagenic and antioxidant activities of Croton lechleri sap in biological systems. Journal of Ethnopharmacology, 95 (2–3), 437–445.

https://doi.org/10.1016/j.jep.2004.08.025

MANACH, C., SCALBERT, A., MORAND, C., RÉMÉSY, C., & JIMÉNEZ, L. (2004). Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition, 79 (5), 727–747. https://doi.org/10.1093/ajcn/79.5.727

MASEK, A., & CHRZESCIJANSKA, E. (2015). Effect of UV-A Irradiation and Temperature on the Antioxidant Activity of Quercetin Studied Using ABTS, DPPH and Electrochemistry Methods. International Journal of Electrochemical Science, 10 (7), 5276–5290. https://doi.org/10.1016/S1452-3981(23)17254-3

MERLIC, C. A. (2012). IR Absorption Table. https://webspectra.chem.ucla.edu/irtable.html

NAMJOYAN, F., KIASHI, F., MOOSAVI, Z. B., SAFFARI, F., & MAKHMALZADEH, B. S. (2016). Efficacy of Dragon's blood cream on wound healing: A randomized, double-blind, placebo-controlled clinical trial. Journal of Traditional and Complementary Medicine, 6 (1), 37–40. https://doi.org/10.1016/j.jtcme.2014.11.029

NÓBREGA, E. M., OLIVEIRA, E. L., GENOVESE, M. I., & CORREIA, R. T. P. (2015). The Impact of Hot Air Drying on the Physical-Chemical Characteristics, Bioactive Compounds and Antioxidant Activity of Acerola (M alphigia emarginata) Residue. Journal of Food Processing and Preservation, 39 (2), 131–141. https://doi.org/10.1111/jfpp.12213

ORTIZ, T., HUMBERTO, J., MENDOZA, C., CADENAS, P., JULIO, E., & OLANO, A. (2003). Actividad antibacteriana de la Sangre de Grado (Croton lechleri) frente al Helicobacter pylori. In Rev Med Hered (Vol. 14, Issue 2).

PAPICH, M. G. (2016). Thioguanine. In Saunders Handbook of Veterinary Drugs. https://doi.org/10.1016/b978-0-323-24485-5.00548-9

PIETERS, L., DE BRUYNE, T., VAN POEL, B., VINGERHOETS, R., TOTTÉ, J., VANDEN BERGHE, D., & VLIETINCK, A. (1995). In vivo wound healing activity of Dragon's Blood (Croton spp.), a traditional South American drug, and its constituents. Phytomedicine, 2 (1), 17–22. https://doi.org/10.1016/S0944-7113(11)80043-7

PLAZA, M., POZZO, T., LIU, J., GULSHAN ARA, K. Z., TURNER, C., & NORDBERG KARLSSON, E. (2014). Substituent Effects on in Vitro Antioxidizing Properties, Stability, and Solubility in Flavonoids. Journal of Agricultural and Food Chemistry, 62 (15), 3321–3333. https://doi.org/10.1021/jf405570u

RIDER, B. J. (2007). Thioguanine. In xPharm: The Comprehensive Pharmacology Reference (pp. 1–4). Elsevier. https://doi.org/10.1016/B978-008055232-3.62746-1

ROSSI, D., GUERRINI, A., MAIETTI, S., BRUNI, R., PAGANETTO, G., POLI, F., SCALVENZI, L., RADICE, M., SARO, K., & SACCHETTI, G. (2011). Chemical fingerprinting and bioactivity of Amazonian Ecuador Croton lechleri Müll. Arg. (Euphorbiaceae) stem bark essential oil: A new functional food ingredient? Food Chemistry, 126 (3), 837–848. https://doi.org/10.1016/j.foodchem.2010.11.042

ROSSI, D., GUERRINI, A., PAGANETTO, G., BERNACCHIA, G., CONFORTI, F., STATTI, G., MAIETTI, S., POPPI, I., TACCHINI, M., & SACCHETTI, G. (2013). Croton lechleri Müll. Arg. (Euphorbiaceae) stem bark essential oil as possible mutagen-protective food ingredient against heterocyclic amines from cooked food. Food Chemistry, 139 (1–4), 439–447. https://doi.org/10.1016/j.foodchem.2013.01.076

SCHULZ, H., & BARANSKA, M. (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy, 43 (1), 13–25. https://doi.org/10.1016/J.VIBSPEC.2006.06.001

TARIQ, S., SOMAKALA, K., & AMIR, M. (2018). Quinoxaline: An insight into the recent pharmacological advances. In European Journal of Medicinal Chemistry (Vol. 143, pp. 542–557). Elsevier Masson SAS. https://doi.org/10.1016/j.ejmech.2017.11.064

THE UNITED STATES PHARMACOPEIAL CONVENTION HERBAL MEDICINES COMPENDIUM. (N.D.). Croton Lechleri Latex. Retrieved July 6, 2017, from http://hmc.usp.org/monographs/croton-lechleri-latex-0-1

TORRES-GINER, S., WILKANOWICZ, S., MELENDEZ-RODRIGUEZ, B., & LAGARON, J. M. (2017). Nanoencapsulation of Aloe vera in Synthetic and Naturally Occurring Polymers by Electrohydrodynamic Processing of Interest in Food Technology and Bioactive Packaging. Journal of Agricultural and Food Chemistry, 65 (22), 4439–4448. https://doi.org/10.1021/acs.jafc.7b01393

VOLF, I., IGNAT, I., NEAMTU, M., & POPA, V. I. (2014). Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. Chemical Papers, 68 (1), 121–129. https://doi.org/10.2478/s11696-013-0417-6

XU, Z., WEI, L., GE, Z., ZHU, W., & LI, C. (2015). Comparison of the degradation kinetics of A-type and B-type proanthocyanidins dimers as a function of pH and temperature. European Food Research and Technology, 240 (4), 707–717. https://doi.org/10.1007/s00217-014-2375-9

Dragon's Blood Sap Microencapsulation within Whey Protein Concentrate and Zein Using Electrospraying Assisted by Pressurized Gas Technology

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Molecules 2023, 28, 4137
https://doi.org/10.3390/molecules28104137
Impact Factor (2023): 4.2
Cite Score 7.4
Article Citations: 2

Abstract

Dragon's blood sap (DBS) obtained from the bark of Croton lechleri (Müll, Arg.) is a complex herbal remedy of pharmacological interest due to its high content in polyphenols, specifically proanthocyanidins. In this paper, electrospraying assisted by pressurized gas (EAPG) was first compared with freeze-drying to dry natural DBS. Secondly, EAPG was used for the first time to entrap natural DBS at room temperature into two different encapsulation matrices, i.e., whey protein concentrate (WPC) and zein (ZN), using different ratios of encapsulant material : bioactive compound, for instance 2:1 w/w and 1:1 w/w. The obtained particles were characterized in terms of morphology, total soluble polyphenolic content (TSP), antioxidant activity, and photo-oxidation stability during the 40 days of the experiment. Regarding the drying process, EAPG produced spherical particles with sizes of 11.38 ± 4.34 μm, whereas freeze-drying produced irregular particles with a broad particle size distribution. However, no significant differences were detected between DBS dried by EAPG or freeze-drying in TSP, antioxidant activity, and photo-oxidation stability, confirming that EAPG is a mild drying process suitable to dry sensitive bioactive compounds. Regarding the encapsulation process, the DBS encapsulated within the WPC produced smooth spherical microparticles, with average sizes of 11.28 ± 4.28 μm and 12.77 ± 4.54 μm for ratios 1:1 w/w and 2:1 w/w, respectively. The DBS was also encapsulated into ZN producing rough spherical microparticles, with average sizes of 6.37 ± 1.67 μm and 7.58 ± 2.54 μm for ratios 1:1 w/w and 2:1 w/w, respectively. The TSP was not affected during the encapsulation process. However, a slight reduction in antioxidant activity measured by DPPH was observed during encapsulation. An accelerated photo-oxidation test under ultraviolet light confirmed that the encapsulated DBS showed an increased oxidative stability in comparison with the non-encapsulated DBS, with the stability being enhanced for the ratio of 2:1 w/w. Among the encapsulating materials and according to the ATR-FTIR results, ZN showed increased protection against UV light. The obtained results demonstrate

the potential of EAPG technology in the drying or encapsulation of sensitive natural bioactive compounds in a continuous process available at an industrial scale, which could be an alternative to freeze-drying.

Keywords*:* Dragon's Blood Sap; encapsulation; microparticles; whey protein concentrate; zein; EAPG.

1. Introduction

Polyphenols are a group of natural compounds with phenolic structural features (Kesavan et al., 2018); which represent a diverse subclass of secondary metabolites present in plants (Siracusa & Ruberto, 2019). Polyphenols are divided into several sub-classes, such as phenolic acids, flavonoids (flavonols, flavones, flavanols, anthocyanidins, and isoflavones), stilbenes, and lignans, among others (Belščak-Cvitanović et al., 2018; Niaz & Khan, 2020). These compounds are well-known for their antioxidant properties (Thakur et al., 2020) and for their ability to protect the organism against reactive oxygen species (ROS) (Prakash & Gupta, 2009), which are responsible of several pathologies such as neurodegenerative, lung, and cardiovascular diseases, diabetes, and atherosclerosis (Pizzino et al., 2017; Rahman et al., 2012).

Due to their health benefits, the scientific community and the food, pharmaceutical, and cosmetic industries, among others, have paid special attention to the isolation of phenolic compounds from different raw materials, especially from inexpensive or residual sources such as medicinal plants, fruits, vegetables, industrial by-products, and beverages (Escobar et al., 2018). In this sense, dragon's blood sap (DBS) obtained from the bark of Croton lechleri (Müll, Arg.) trees is well-known due to its health benefits and as such, it is widely used in folk medicine due to its antioxidant, antimicrobial, anti-inflammatory, and cicatrizing properties, mainly due to its principal chemical constituents such as proanthocyanidins (>90% dry weight of the resin), taspin, catechin,

epigallocatechin, and epicatechin (Lock et al., 2016). Up to now, numerous studies have been published dealing with the characterization of its bioactive properties (Gupta & Gupta, 2011; Lopes et al., 2004). Escobar-García et al. demonstrated that the high antioxidant activity of DBS was susceptible to environmental factors such as the irradiation of UV light (Escobar et al., 2018), which may induce a reduction in its health benefits and consequently limit its commercial potential (Labuschagne, 2018).

To ensure the stability of natural bioactive compounds, a wide variety of delivery systems are available (McClements, 2012). Among them, one of the most appreciated approaches is the encapsulation of the bioactive compounds, which has garnered a growing interest during the last decade to protect natural bioactive compounds that are highly sensitive to the environmental conditions present during processing and storage, as well as in the upper gastrointestinal tract (Guo et al., 2021). Through this technology, the active agents are entrapped within an encapsulating matrix, which protects the bioactive compounds from environmental conditions (Nikkola, 2016). However, conventional encapsulation technologies such as spray drying, spray coating, freeze drying (FD), co-extrusion, and coacervation (Heck et al., 2021; Oxley, 2014; Rajan et al., 2018; Ribeiro & Veloso, 2021; Santos et al., 2021), among others, present multiple disadvantages. For example, spray drying, despite being available at an industrial scale, requires the use of high temperature, which may affect thermolabile bioactive compounds; freeze drying is an expensive process that produces irregular particles with a big particle size distribution, and it takes place in batch mode; and spray coating, co-extrusion, and coacervation, produce particles with a big particle size. All these factors affect the stability of the bioactive compound and the organoleptic properties of the final product and make the process difficult at an industrial level.

However, novel encapsulation technologies have been developed to overcome these challenges, such as encapsulation based on electrohydrodynamic processes (Gómez-Mascaraque & López-Rubio, 2016).

Concretely, the electrospraying process is based on the action of an external electric field over a polymer solution to produce ultrathin droplets, which after solvent evaporation at room temperature result in nano- or micro-sized particles (Prieto et al., 2021). Additionally, electrospraying is carried out at room temperature, which reduces the denaturation of bioactives, possesses high encapsulation efficiency, results in a dried powder with reduced particle size, and is versatile in terms of the encapsulating matrices that can be used (Prieto & Lagaron, 2020). Thus, this technology has been used for the encapsulation of polyphenols such as (−)-epigallocatechin gallate (EGCG), tannic acid, and green tea extract, among others, and has resulted in promising results (G. Gómez-Mascaraque et al., 2019; Gómez-Mascaraque & Lopez-Rubio, 2019). However, this technology presents a main disadvantage related to its poor yield, which is lower than 1 g per day in the majority of case studies (Basar et al., 2021).

Consequently, novel high-yield technology based on the integration of electrospraying, and gas-driven nebulization was developed to overcome the yield limit of electrospraying technology (Busolo et al., 2019). This novel high-throughput technology, termed electrospraying assisted by pressurized gas (EAPG), is based on the atomization of the polymer solution by a pneumatic injector using compressed air that nebulizes within a high electric field. By means of this technology, it is possible to obtain a throughput larger than 1 kg/h of dry powder on an industrial scale. During this process, the solvent is evaporated at room temperature in an evaporation chamber, and the encapsulated material is then collected as a free-flowing powder. The potential of this technology has been proven in sensitive materials such as omega-3-rich fish oil, algae oil, eicosapentaenoic-acid-rich oil, and pharmaceutical compounds such as carvedilol and valsartan, protected using diverse encapsulating materials, such as whey protein concentrate (WPC), zein (ZN), maltodextrin, wheat gluten extract, polyvinylpyrrolidone, and hydroxypropyl methyl cellulose (Escobar-García et al., 2021; Miguel et al., 2019; Prieto et al., 2021; Sharif et al., 2019; Torres-Giner et al., 2020). However, to the best of our knowledge, no scientific studies have been published dealing with the encapsulation of DBS by electrospraying or EAPG.

Therefore, in the present work, EAPG was first compared with freeze-drying to dry natural DBS to prove that it is a mild technology suitable to drying sensitive bioactive compounds. The obtained materials were compared in terms of morphology. Secondly, the EAPG process was used to study the encapsulation of DBS into two food-approved encapsulating matrices, i.e., WPC and ZN. These two proteins were selected as encapsulating matrices due to their effective microencapsulating properties as well as their antioxidant and photo-protective properties (Busolo et al., 2019; Prieto & Lagaron, 2020; Sharif et al., 2019). The obtained DBS microcapsules were characterized in terms of morphology, total soluble polyphenols (TSP), antioxidant activity, and accelerated oxidative stability under photo-oxidation conditions.

2. Material and Methods

The Dragon's Blood Sap (DBS) was provided by Q'omer BioActive Ingredients (Valencia, Spain). The natural DBS was used as received without any further purification. Whey protein concentrate (WPC) 80% was purchased from Beurrespa (Madrid, Spain). According to the distributor, the WPC was said to contain 81.6% protein (on a dry basis), 7.5% fat, and 4.3% moisture. The maize zein (ZN) protein, gallic acid, sodium carbonate, Folin–Ciocalteu reagent, and Span 20 were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The methanol (reagent grade) was purchased from Labkem-Labbox (Mataró, Spain). Ethanol 96% (v/v) was purchased from Panreac Química SLU (Barcelona, Spain). Deionized water was used throughout the study.

2.1. Determination of total solids

The total solids content is determined by measuring the mass of the sample of DBS before and after the water is removed. The evaporation process was performed at 60°C using a vacuum oven (Vaciotem-T, JP. Selecta, Barcelona, Spain) for 48h. The total solid content was calculated according to Equation (1):

$$\text{Total Solids (\%)} = ((\text{M dried} / \text{M initial}) \times 100) \quad (1)$$

where M dried is the final sample weight, and M initial is the initial sample weight.

2.2. Solution Preparation

First, a solution for the encapsulating materials was prepared. WPC was dissolved into water at 20% (w/v) concentration and ZN was dissolved into 85% (v/v) ethanol aqueous solution at 4% (w/v) concentration. Then, natural DBS resin was slowly added to the corresponding encapsulant solution into a 1:1 w/w and a 2:1 w/w encapsulant to DBS ratio. The 2:1 w/w ratio was selected according to our previous experience in the encapsulation of bioactive compounds [92]; however, since water in DBS evaporates during the EAPG process, an increased content of DBS in the particle was also considered, concretely the ratio 1:1 w/w. These ratios were calculated between the amount of encapsulating material and the solid content of DBS resin determined according to Section 3.1 (natural resin contains around 26% solids (wet basis)). The total solids content in the solution for the WPC formulations was 22% for the ratio of 1:1 and 21% for the ratio of 2:1, and for the ZN formulations, this was 7% for the ratio of 1:1 and 5% for the ratio of 2:1.

The solution was homogenized using a magnetic stirrer (H20 series, LBX Instruments, Premia de Dalt, Spain) at ambient temperature overnight until total DBS dissolution.

2.3. EAPG process

First, the natural DBS was dried by EAPG using the proprietary CapsultekTM pilot plant from Bioinicia S.L. (Valencia, Spain). This pilot installation comprises a nebulizer, the products of which are subjected to an electric field, a drying chamber, and a cyclonic collector, as described by Busolo et al. (2019). The experiments were developed at controlled ambient conditions, 25 °C and 30% relative humidity (RH). The DBS was pumped at a flow rate of 10 mL/h into the injection unit, with an air pressure of 10 L/min and a voltage of 15 kV. Particles

were collected from the cyclone, stored in flasks, under vacuum, at −20 °C, and protected from light to avoid oxidation until further analysis.

Secondly, the encapsulation of DBS in the selected encapsulation materials via EAPG was performed by processing previously prepared solutions using the same process parameters.

For these solutions and with this pilot installation, the efficiency of the process was around 50%; however, it can increase up to 90–100% at an industrial scale.

2.4. Freeze-drying

The natural DBS was also processed by FD to compare the EAPG drying process with conventional drying technology. This comparison allows comparing the obtained materials in terms of morphology, bioactive properties, and stability. Pure DBS was frozen at −80 °C in a static stage for two days and then dried on VirTis Genesis 35 EL freeze-drier (SP Scientifics, New York, NY, USA) equipment with a condenser temperature of −50 °C and a chamber pressure $P < 0.08$ mbar for 48 h. The FD samples were stored in flasks under vacuum at −20 °C and protected from light to avoid oxidation until further analysis.

2.5. Humidity

The humidity of the samples was calculated by drying the samples until obtaining a constant weight at 40 °C and by using a vacuum oven (Vaciotem-T, J.P. Selecta, Barcelona, Spain). The humidity content was determined from the difference in weight before and after drying.

The humidity contained in the dried samples was 4.7 ± 2.9% and 9.3 ± 1.5% for EAPG and FD, respectively.

2.6. Microscopy

Scanning electron microscopy (SEM) in a Hitachi S-4800 FE-SEM (Hitachi High Technologies Corp., Tokyo, Japan) was used to analyze the particles' morphology. The microscope was settled with an electron beam acceleration of 5 kV. Approximately 5 mg of capsules of each sample were coated with a

gold/palladium layer for SEM analysis. The average diameters were determined using Image J Launcher v1.41 (National Institutes of Health, Bethesda, MD, USA).

2.7. Photo-Oxidation Stability of DBS capsules

The particles encapsulating the DBS were exposed to ultraviolet (UV) radiation to accelerate DBS oxidation. An OSRAM Ultra-Vitalux lamp (OSRAM, Garching, Germany) irradiated the samples with UV light. A blend of radiation, similar to natural sunlight, is generated by a quartz discharge tube and a tungsten filament, operating the lamp with a power of 300 W. Only similar wavelengths to that of daylight pass through the special glass bulb. The radiation between 315–400 nm after 1 h of exposure is 13.6 W, and the radiation between 280 and 315 nm after 1 h of exposure is 3 W.

The photo-oxidation stability assay was developed at ambient temperature under UV light for 40 days. Approximately 20 g of the capsules was placed onto Petri dishes at 20 cm under a UV lamp. The thickness of the powder in the Petri dish was lower than 5 mm. To ensure homogenous treatment, the powder was stirred on a daily basis. Photo-oxidation was monitored by evaluating total soluble polyphenols, antioxidant capacity, and ATR-FTIR spectroscopy.

2.8. Total Soluble Polyphenols

The Folin–Ciocalteu method was used to analyze the total soluble polyphenols (TSP) of natural DBS, dried DBS, and DBS-loaded capsules. Approximately 10 mg of DBS and the equivalent amount of capsules were diluted in ethanol at 70% (v/v). After dissolution, 20 µL of the capsule's solution was mixed with 1.2 mL of water and 300 µL of sodium carbonate 7.5% (w/v) in Eppendorf tubes and homogenized for 1 min in a vortex shaker and left to stand for 5 min. Subsequently, it was mixed with 380 µL of water, and then 100 µL of the Folin reagent solution 10% (v/v) was added to react for 15 min in dark conditions and at room temperature. Absorbance was measured in triplicate at 765 nm using a UV/Vis spectrophotometer (UV4000 Dinko Instruments, Barcelona, Spain). The results were expressed in gallic acid equivalents (mg GAE/g of natural DBS).

2.9. Antioxidant activity

The DPPH free radical scavenging method was used to analyze the antioxidant capacity of the DBS capsules [2]. Approximately 10 mg of DBS and the equivalent amount of capsules were dissolved in 1 mL of methanol. Thereafter, 50 µL of the capsule's solution was dissolved in 950 µL of methanol. An aliquot of 0.1 mL of DBS methanol solution (0.5 mg/mL) was added to 1.9 mL of DPPH methanolic solution (0.094 mM), vortexed, and kept in dark conditions for 30 min at room temperature. The methanol solution was used as a blank, and absorbance was measured in triplicate at 517 nm using a UV/Vis spectrophotometer (UV4000 Dinko Instruments, Barcelona, Spain). The radical scavenging activity was calculated according to Equation (2):

$$\text{IP-DPPH (\%)} = ((Ac - As)/Ac) \times 100 \quad (1)$$

where IP-DPPH (%) is the inhibition percentage of DPPH, Ac is the absorbance of pure DPPH solution, and As is the absorbance of the incubated sample after reacting with DPPH.

2.10. Attenuated total reflection- Fourier transform infrared (ATR-FTIR)

A Bruker Tensor 37 FT-IR Spectrometer (Bruker, Ettlingen, Germany) was used to measure the ATR-FTIR spectra of DBS capsules. The low-temperature ATR sampling accessory Golden Gate (Specac Ltd., Orpington, UK) was used to guarantee proper contact between the diamond crystal and DBS encapsulated samples (50 mg approximately). All spectra were recorded within the wavenumber range 4000–600 cm^{-1} by averaging 10 scans, with a resolution of 4 cm^{-1}. The results were normalized to the intensity of the band at 1519 cm^{-1} for better comparison among the different capsules. Analysis of spectral data was carried out using the OPUS 4.0 data collection software program (Bruker, Ettlingen, Germany). The measurements were performed in triplicate.

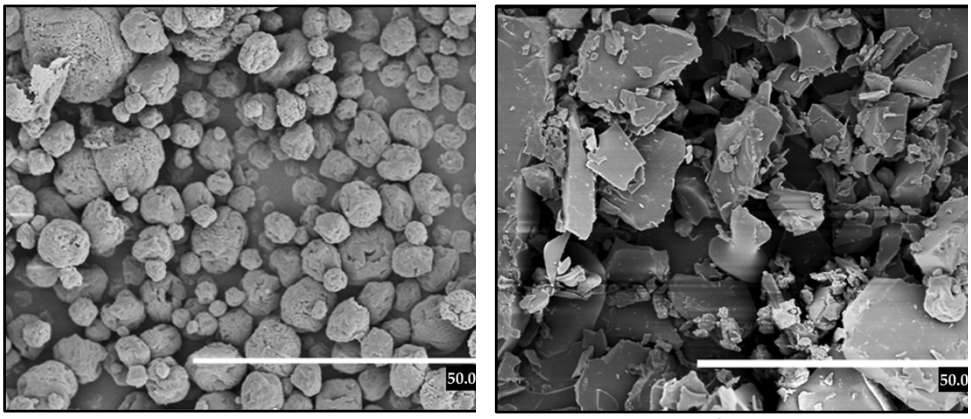
2.11. Statistical Analysis

Statgraphics Centurion Version 17.2.04 (Statistical Graphics Corp., Warrenton, Va, USA) was used for data analysis. Data were expressed as mean ± standard deviation. The significance of the results obtained for the different samples was studied by analysis of variance (ANOVA) followed by Tukey's test. Differences were considered significant at p-value < 0.05.

3. Results

3.1. Morphology

Firstly, the EAPG and the FD processes were used to dry natural DBS. Figure 1 makes a comparison of the morphologies of the obtained particles with both technologies. By means of EAPG (Figure 1a), the production of individual spherical particles with an average particle size of 11.38 ± 4.34 μm, regular distribution, and without the presence of aggregates was achieved. These DBS microparticles presented some roughness with wrinkles and fissures on their surface.



(a) (b)

Figure 1. SEM micrographs of the dried DBS structures. (a) DBS microparticles obtained by the EAPG process. (b) DBS particles obtained by FD. The scale bar in both images correspond to 50 μm.

As a comparison, natural DBS was also dried by FD. This technology was selected as a control technique since this method was reported as one of the most relevant processes for the preservation of heat-sensitive bioactive compounds of application in pharmaceuticals and foods (Siracusa & Ruberto, 2019). DBS dried by FD exhibited irregular structures with a broad particle size distribution, as shown in Figure 1b. This broad size distribution could affect the dissolution rate, bioavailability, and also the organoleptic properties of the final product.

Among both techniques, EAPG provided smaller particles, with controlled size distribution and spherical morphology, which may enhance its dissolution rate when entering into contact with water and reduces or avoids the impact in the texture of a final product, for example in functional foods.

Secondly, WPC and ZN proteins were used to encapsulate natural DBS by the EAPG process in order to increase the stability of the DBS bioactive properties and functional capabilities. The morphology of the encapsulates of DBS into WPC and ZN at different encapsulant: bioactive ratios is shown in Figure 2. In all cases, spherical-like particles were obtained with uniform size distributions. Whereas particles obtained with WPC presented a smooth particle surface with the presence of some indentations, probably due to the high content of DBS in the microparticles, the particles obtained with ZN presented a rough surface, which is typical of this material, and it is produced due to the fast evaporation rate of the ethanol (Bodnár et al., 2018; Boel et al., 2020).

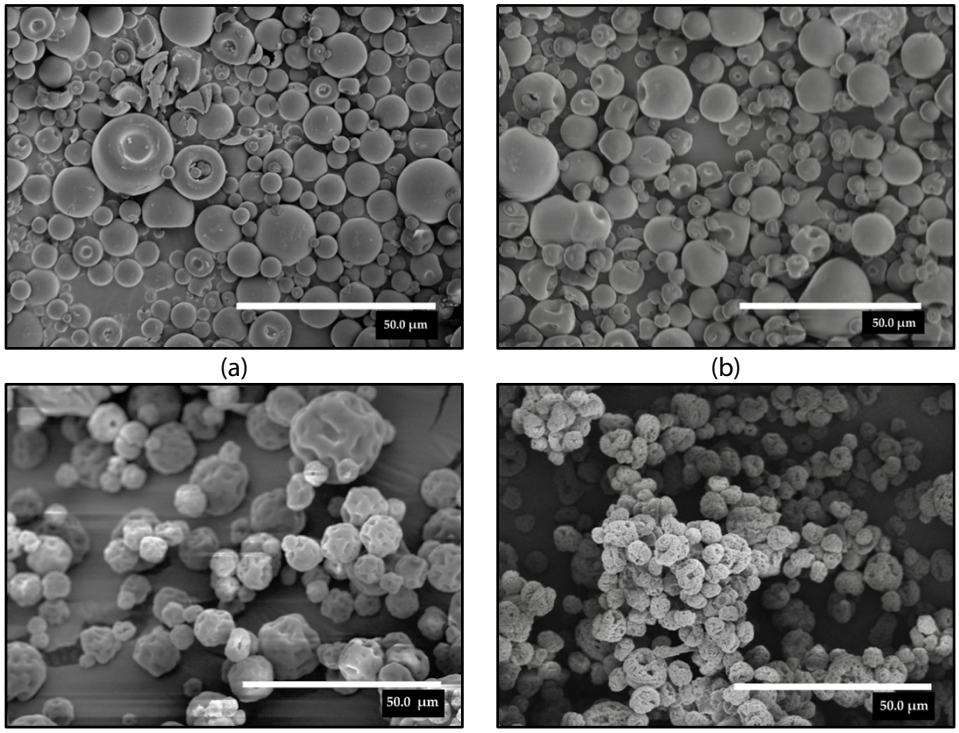


Figure 2. SEM micrographs of encapsulated DBS into WPC or ZN by the EAPG process. (a) WPC—DBS 1:1 w/w; (b) WPC—DBS 2:1 w/w; (c) ZN—DBS 1:1 w/w; (d) ZN—DBS 2:1 w/w. The scale bar in both images correspond to 50 µm.

The structure of WPC was similar for the two ratios of DBS; however, in the case of ZN, by decreasing the amount of DBS in the particle, the roughness of the surface of the particles decreased. The particle size varied significantly depending on the encapsulating material used, between 6.37 to 12.77 µm as shown in Table 1, with it being smaller for the particles obtained with ZN. However, the increase in the amount of DBS did not result in a significant variation in the particle size with any encapsulating material.

Table 1. Particle size, total soluble polyphenol content, and antioxidant activity for DBS dried by FD (DBS-FD), DBS dried by EAPG (DBS-EAPG), and encapsulated DBS into whey protein concentrate (WPC) and zein (ZN).

Sample	Particle size (μm)	TSP (mg GAE/g natural DBS)	IP-DPPH (%)
Natural DBS	-	2.05 ± 0.03 [a]	93.39 ± 0.01 [a]
DBS- FD	-	2.06 ± 0.07 [a]	96.02 ± 0.04 [b]
DBS – EAPG	11.38 ± 4.34 [a]	2.03 ± 0.06 [a]	95.27 ± 0.06 [b]
WPC - DBS 1:1	11.28 ± 4.28 [a]	2.07 ± 0.08 [a]	80.29 ± 0.02 [cd]
WPC - DBS 2:1	12.77 ± 4.54 [a]	2.03 ± 0.05 [a]	79.88 ± 0.02 [d]
ZN - DBS 1:1	7.88 ± 2.72 [b]	2.01 ± 0.12 [a]	79.64 ± 0.03 [d]
ZN - DBS 2:1	7.58 ± 2.54 [b]	2.07 ± 0.11 [a]	82.94 ± 0.01 [c]

Mean values ± standard deviation with different superscripts in the same column are significantly different (Tukey test, $p \leq 0.05$). TSP – total soluble polyphenols. GAE – gallic acid equivalent. IP-DPPH Inhibition percentage of the DPPH reactive.

3.2. Characterization of the polyphenol content and antioxidant activity

Herein, the Folin–Ciocalteu method was used to analyze the total soluble polyphenols (TSP) of natural DBS, DBS dried by EAPG and FD, and the produced DBS encapsulates. The results obtained are shown in Table 1. No significant differences were observed between the natural DBS, the DBS dried by EAPG or FD, and the encapsulates, which means that the TSP was not affected during the process.

The antioxidant activity of these materials was also characterized via the DDPH method, and the results are also shown in Table 1. These results demonstrate that natural DBS has exceptionally high antioxidant activity, which correlates with the antioxidant activity of natural DBS reported by previous authors (Oliveira et al., 2016). The antioxidant activity increased by drying the natural DBS by FD or EAPG in comparison to the natural DBS, probably due to humidity loss. However, no significant differences were observed between the

drying process, EAPG or FD, which confirms that the EAPG method is a gentle method suitable for the processing of sensitive bioactive compounds.

However, regarding the encapsulates, it was possible to observe a slight reduction in antioxidant activity between the natural DBS, the dried DBS by EAPG or FD, and the encapsulates. This fact could also be produced by the weak DBS extraction from the produced WPC and ZN capsules. Similar results were obtained with both encapsulating materials. Nevertheless, whereas no significant differences were observed between the encapsulates produced with WPC at the studied ratios; in the case of ZN, higher antioxidant activity was observed for the ratio 2:1 w/w in comparison with the ratio 1:1w/w.

3.3. Photo-oxidation stability

The photo-oxidation stability of the dried DBS samples and the encapsulates was first evaluated following the evolution of the TSP with time. The obtained results are shown in Table 2. The TSP for the dried DBS constantly decreased with time, but no significant differences were observed with the drying method since both drying methods showed similar decay rates.

According to the results shown in Table 2, both encapsulating materials provided a similar degree of protection, which could be related to the fact that the selected proteins are well-known for their capacity to block UV light and retard oxidation (Mehra et al., 2021; Minj & Anand, 2020; Silva et al., 2021). However, the ratio of encapsulant material: DBS seemed to be the most important factor. For instance, samples with a 2:1 w/w ratio presented a reduced TSP decay rate compared to examples of a 1:1 w/w ratio, which showed a similar decay rate to the dried DBS. This result could be due to a less efficient encapsulation process, as a consequence of the lack of enough encapsulating material to offer protection to the DBS present in the capsule.

Table 2. Evolution of the TSP of dried DBS by EAPG and freeze-drying (FD), and the encapsulated DBS into WPC and ZN protein at 1:1 w/w and 2:1 w/w ratios over a period time of 40 days under UV light exposure.

Sample	TSP Decay (%)		
	UV Light Exposure Time (Days)		
	0	20	40
DBS-FD	100	78.44 ± 4.97 ^{d}	59.19 ± 6.78 ^{e}
DBS-EAPG	100	85.41 ± 2.11 ^{bc}	61.69 ± 7.56 ^{e}
WPC-DBS 1:1 w/w	100	77.01 ± 1.23 ^{d}	60.87 ± 8.23 ^{e}
WPC-DBS 2:1 w/w	100	94.54 ± 2.13 ^{a}	81.69 ± 4.76 ^{ab}
ZN-DBS 1:1 w/w	100	80.12 ± 5.15 ^{bcd}	63.34 ± 2.34 ^{e}
ZN-DBS 2:1 w/w	100	94.27 ± 1.57 ^{a}	91.92 ± 6.47 ^{ab}

Mean values ± standard deviation with different superscripts in the same column are significantly different (Tukey's test, $\boldsymbol{p} \leq 0.05$).

In addition, a comparison of the photo-oxidation stability of the dried DBS samples and the encapsulation systems was performed in terms of the antioxidant activity measured via the DPPH method, and the results are shown in Table 3. According to the results, the dried DSB samples presented a significant decrease in DPPH inhibition with time, with no significant differences being observed in the decay rate between the drying methods. These results agree with the observations made of the TSP decay shown in Table 2. However, the DPPH inhibition values remained stable with time for the encapsulates, which could be related to the well-known capacity of WPC and ZN to block UV light and retard oxidation (Mehra et al., 2021; Minj & Anand, 2020; Silva et al., 2021). However, no significant differences were detected in the DPPH inhibition decay rate between these samples, neither between the proteins nor with the different ratios. In this sense, the Folin–Ciocalteu method allowed us to differentiate between the stability of the encapsulates in contrast with the DPPH method.

Table 3. Evolution of the antioxidant activity measured as DPPH inhibition percentage of dried DBS by EAPG and freeze-drying (FD) and encapsulated systems of DBS into WPC and ZN at 1:1 w/w and 2:1 w/w ratio over a period time of 40 days under UV light exposure.

Sample	DPPH Inhibition Decay (%)		
	UV Light Exposure Time (Days)		
	0	20	40
DBS-FD	100	87.92 ± 1.65 [b]	70.07 ± 0.39 [c]
DBS-EAPG	100	87.84 ± 3.34 [b]	72.56 ± 0.80 [d]
WPC-DBS 1:1 w/w	100	99.62 ± 2.78 [a]	98.79 ± 4.88 [a]
WPC-DBS 2:1 w/w	100	100.28 ± 4.85 [a]	98.35 ± 3.44 [a]
ZN-DBS 1:1 w/w	100	98.63 ± 3.66 [a]	99.76 ± 3.11 [a]
ZN-DBS 2:1 w/w	100	99.50 ± 2.10 [a]	98.92 ± 2.20 [a]

Mean values ± standard deviation with different superscripts in the same column are significantly different (Tukey's test, $p \leq 0.05$).

The analysis of the photo-oxidation stability of the DBS samples was also performed by means of ATR-FTIR. Whereas Figure 3 represents the ATR-FTIR spectral evolution of the DBS dried by EAPG, Figure 4 represents the ATR-FTIR spectral evolution of the DBS dried by FD. Since the values of the intensity are arbitrary in the ATR-FTIR mode, to compare the results, the spectra were normalized to the intensity of the peak at ca. 1519 cm^{-1}. This band was already used as an internal standard in our previous work (Escobar et al., 2018), since it is assigned to the vibration of the C=C bond, typical of aromatic systems (De Souza et al., 2015), and it did not show any variation during experimentation.

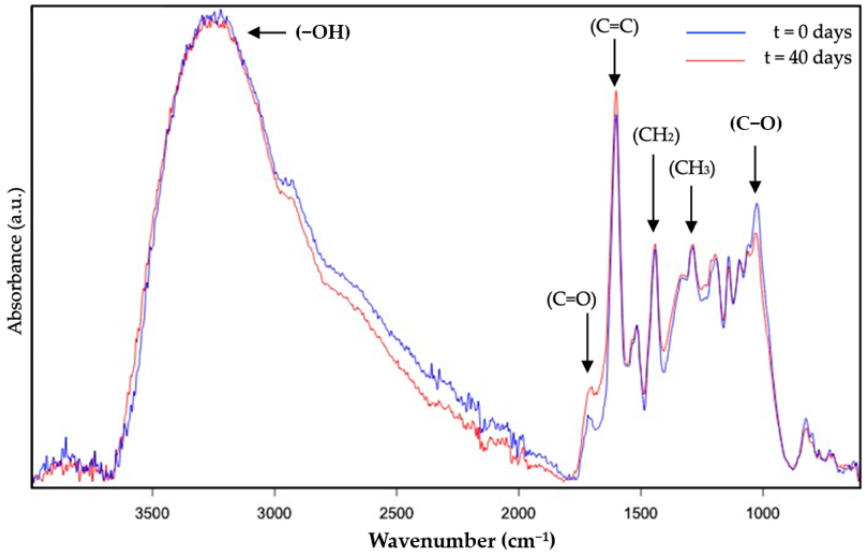


Figure 3. Attenuated total reflection—Fourier transform infrared spectroscopy (ATR-FTIR) spectral evolution over the accelerated oxidation test of DBS dried by EAPG. The spectra were normalized to the intensity of the internal standard band at ca. 1519 cm^{-1}.

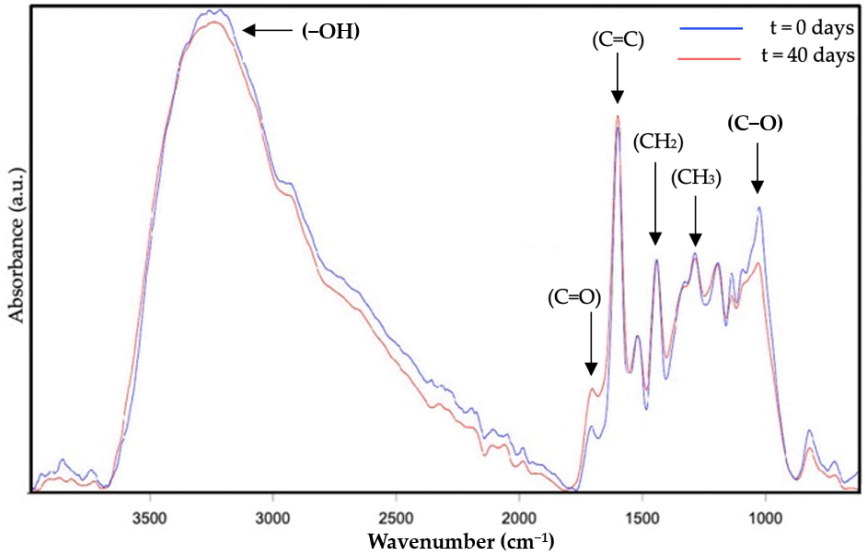


Figure 4. Attenuated total reflection—Fourier transform infrared spectroscopy (ATR-FTIR) spectral evolution over the accelerated oxidation test of DBS dried by freeze-drying (FD). The spectra were normalized to the intensity of the internal standard band at ca. 1519 cm^{-1}.

The band envelope between 3100 and 3600 cm^{-1} is associated with the stretching modes of the different -OH groups (Wang et al., 2016). This band envelope reduced its intensity probably due to a loss of water as a consequence of the heat transferred by the lamp and due to the transformation of the hydroxyl group into the ether, aldehyde, or ketone groups as a consequence of the polyphenol oxidation reactions (Syiemlieh et al., 2018). The following band at ca. 1780–1630 cm^{-1} is well defined, and it was associated with carbonyl stretching absorption (Merlic, 2012), which is characteristic of the polyphenols present in natural DBS, such as taspin or epigallocatechin, and also the carbohydrates present in the sap, and it increases its intensity as oxidation proceeds because of the transformation of the alcohol group into aldehydes and ketone groups (Syiemlieh et al., 2018). The bands at ca. 1600, 1530, and 1519 cm^{-1} are attributed to the stretching vibration of the aromatic alkenyl bond, which is also present in large amounts in polyphenols (de Souza et al., 2018; Ping et al., 2012a). Only the band at 1600 cm^{-1} shows very little modification, less than 5%, which is probably influenced by the increased intensity of the carbonyl band. This band has not been selected as an internal standard, since it could be influenced by the humidity content of the samples, since one of the characteristic bands of water overlaps with this band. A peak around 1440 cm^{-1} was attributed to -CH deformation and aromatic ring vibration (Ping et al., 2012b). The peak around 1375 cm^{-1} was assigned to methyl bending (Oxley, 2014). The peaks between 1200 and 1070 cm^{-1} were due to C-O stretching in ether groups or aromatic alcohols (Schulz & Baranska, 2007), characteristics of polyphenols, and carbohydrates. This band decreased its intensity as a consequence of the polyphenol oxidation reactions, which transformed the aromatic alcohols and ether groups into ketones, carboxylic acids, or ester groups (Dangles & Fenger, 2018a; Karonen et al., 2021). Finally, a band around 800 cm^{-1} was attributed to the out-of-plane C-H vibrations (Schulz & Baranska, 2007) and also to the humidity content of the sample, which decreased with time as explained before. Overall, these prominent characteristic peaks of the studied ATR-FTIR spectra agree with the bands previously reported for natural DBS (Escobar et al., 2018).

The -OH, C=O, and C-O characteristic bands were found to provide the most significant contribution to the oxidation of polyphenols (Dangles & Fenger, 2018a); however, only the C=O and the C-O characteristic bands were selected as the most representative to perform the comparison of the dried DBS samples in terms of photo-oxidation stability since the -OH group could be affected by the sample's humidity.

Figure 5 represents the evolution of the decay percentage of carbonyl group intensity normalized to the standard internal peak at ca. 1519 cm^{-1} of the dried DBS samples. The increase in the intensity of the carbonyl compounds could be generated from the oxidation reactions due to the formation of ester, carboxylic acids, and ketone groups (Karonen et al., 2021). The increase was a little bit more intense for the FD sample.

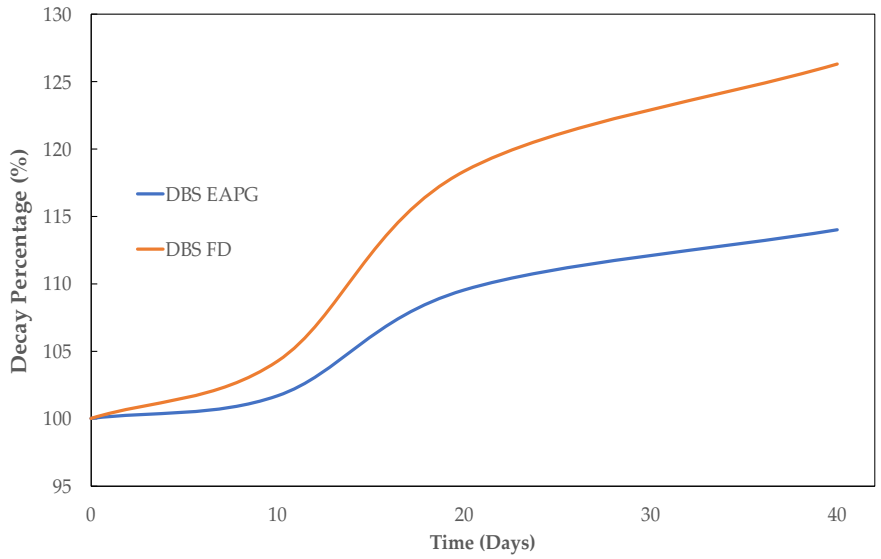


Figure 5. Evolution of the decay percentage of the DBS characteristic carbonyl functional group (C=O) (1715 cm^{-1}) normalized to the standard internal band at ca. 1519 cm^{-1} for the DBS dried by EAPG and by freeze-drying (FD) during 40 days under UV light radiation.

Figure 6 represents the evolution of the decay percentage of the C-O band normalized to the standard internal band at ca. 1519 cm^{-1} for the dried DBS samples. This band decreased its intensity as a consequence of the polyphenol oxidation reactions, which transformed the aromatic alcohols and ether groups into ketones, carboxylic acids, or ester groups (Dangles & Fenger, 2018a; Karonen et al., 2021). Despite both samples showing a similar decay rate during the first 20 days, after this point, the decay of the C-O band remained stable for the EAPG processed sample, whereas it continued to decrease for the freeze-dried sample.

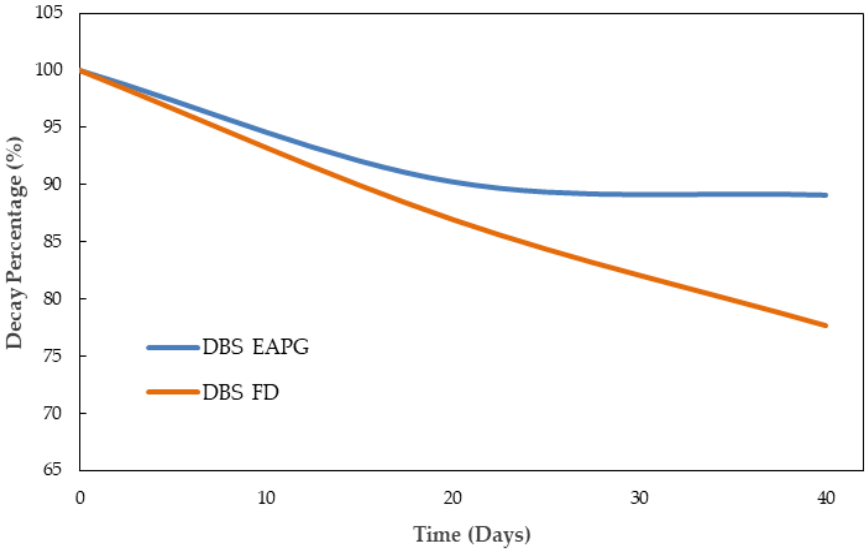


Figure 6. Evolution of the decay percentage of the DBS characteristic C-O band (1070 cm^{-1}) normalized to the standard internal band at ca. 1519 cm^{-1} for the DBS dried by EAPG and by freeze-drying (FD) during 40 days under UV light radiation.

Although no significant differences were observed between these two drying methods, the obtained results agree with the previous analysis performed in terms of TSP and DPPH inhibition and allowed us to prove that by using the EAPG process, there were no significant chemical alterations in this bioactive ingredient in comparison to FD.

Figure 7 shows the ATR- FTIR spectral evolution of protein: DBS encapsulates after 40 days of the accelerated oxidation test. Herein, the most significant spectral changes regarding DBS oxidation occurred in the wavenumber range between 2000 and 600 cm^{-1}, and for comparison purposes, the spectra were maximized to the highest intensity band in this wavenumber range.

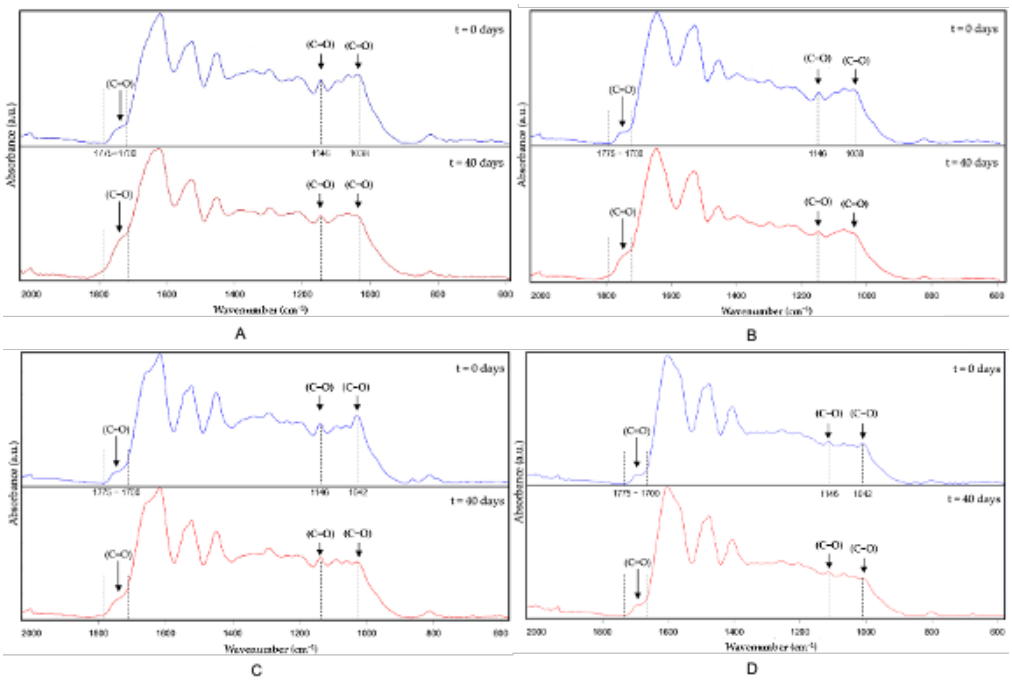


Figure 7. Attenuated total reflection—Fourier transform infrared spectroscopy (ATR-FTIR) spectral evolution over the accelerated oxidation test. (**A**) WPC—DBS 1:1 w/w, (**B**) WPC-DBS 2:1 w/w, (**C**) ZN-DBS 1:1 w/w, (**D**) ZN-DBS 2:1 w/w. The spectra were normalized to the highest intensity band in the wavelength range between 2000 and 600 cm^{-1}

The main characteristic bands of WPC (spectrum not shown) were located at ca. 1740 cm−1 ascribed to the stretching vibration of the C=O bond, at ca. 1632 cm−1 and ca. 1520 cm−1 ascribed to the bending vibration of the N-H bond of amides I and II, respectively, two bands at ca. 1448 and 1391 cm−1 ascribed to the bending vibration of the -CH bond, a band at ca. 1237 cm−1 ascribed to the axial

deformation vibrations of the C-N bond, and two bands at 1153 and 1076 cm−1 ascribed to the stretching vibration of the C-O bond (Andrade et al., 2019; He et al., 2016). Regarding the ZN (spectrum not shown), the main characteristic bands were located at ca. 1735 cm−1 ascribed to the stretching vibration of the C=O bond, the bands at ca. 1642 cm−1 and at ca. 1517 cm−1 ascribed to the bending vibration of the N-H bond of amides I and II, respectively, a band at ca. 1447 cm−1 assigned to the bending vibration of the -CH bond, a band at ca. 1235 cm−1 ascribed to the axial deformation vibrations of the C-N bond, and a band at ca. 1168 cm−1 ascribed to the stretching vibration of the C-O bond (Torres et al., 2021). Concerning the DBS, the main characteristic bands have been already described.

The characteristic bands of the DBS and proteins were observed in all the encapsulated ATR-FTIR spectra shown in Figure 7. The encapsulates of WPC-DBS 1:1 w/w and 2:1 w/w, shown in Figure 7a,b, showed a band envelope between 1775 and 1700 cm−1, which is the result of the overlapping of the contributions of the stretching vibration of the C=O bonds of the protein and the C=O bonds of the DBS, the band envelope between 1700 and 1568 cm−1 which is the result of the overlapping of the band ascribed to the bending vibration of the N-H bond of amide I of the protein and the band ascribed to the C=C bond of the DBS, the band envelope between 1568 and 1482 cm−1, which is the result of the overlapping of the contribution of the C=C bond and the bending vibration of the N-H bond of amide II, three bands at ca. 1445, 1388, and 1340 cm−1 ascribed to the bending vibration of the CH bond, and a band envelope between 1300 and 1000 cm−1, which could be ascribed to the stretching vibration of the C-O bond. The band at ca. 828 cm−1 is attributed to the out-of-plane C-H vibrations and also the humidity content of the sample.

The encapsulates of ZN-DBS 1:1 w/w and 2:1 w/w, shown in Figure 7c,d, showed a characteristic band envelope between 1775 and 1700 cm−1, which is the result of the overlapping of the contributions of the stretching vibration of the C=O bonds of the protein and the C=O bonds of the DBS, the band envelope

between 1700 and 1568 cm^{-1}, which is the result of the overlapping of the band ascribed to the bending vibration of the N-H bond of amide I and the band ascribed to the C=C bond, the band envelope between 1568 and 1480 cm^{-1}, which is the result of the overlapping of the contribution of the C=C bond and the bending vibration of the N-H bond of amide II, three bands at ca. 1444, 1368, and 1339 cm^{-1} assigned to the bending vibration of the CH bond, and a band envelope between 1300 and 1000 cm^{-1}, which could be ascribed to the stretching vibration of the C-O bond. The band at ca. 877 cm^{-1} may be due to the ethanol residual content in the particles, which disappears within 40 days with the heat provided by the lamp. The band at ca. 828 cm−1 is attributed to the out-of-plane C-H vibrations and also the humidity content of the sample.

As shown in Figure 7, the most significant changes were identified in the band envelope between 1775–1700 cm^{-1}, associated with the carbonyl stretching absorption, highly present in polyphenols, proteins, and carbohydrates and in the band envelope at ca. 1300–1000 cm^{-1} ascribed to the stretching vibration of the C-O bond of not only aromatic alcohols, ethers, and esters groups of polyphenols, but also present in carbohydrates. Whereas the carbonyl envelope increased its relative intensity with UV time exposure, some bands in the C-O bond envelope decreased their relative intensity with UV time exposure. This occurred because of the oxidation reactions of polyphenols, which transformed the aromatic alcohols and ether groups into ketones, carboxylic acids, or ester groups (Dangles & Fenger, 2018a; Karonen et al., 2021). However, the variations in the relative intensity of these band envelopes were less pronounced than in the dried DBS achieved by EAPG (Figure 3), thanks to the protection offered by the proteins.

In particular, the encapsulates obtained using WPC in both encapsulation ratios, 1:1 w/w and in 2:1 w/w (Figure 7a,b), resulted in clear alterations in the bands at ca. 1146 cm^{-1} and 1038 cm^{-1} and also in the relative intensity of the band envelope between 1775–1700 cm^{-1}. The cited spectral variations were more pronounced in the 1:1 w/w, since there is a lower amount of protein with respect to the DBS to offer UV protection. In the case of the capsules obtained using ZN

(Figure 7c,d), alterations in the relative intensity of the bands at ca. 1146 and 1042 cm^{-1} and in the band envelope between 1775 and 1700 cm^{-1} were observed with UV light exposure, similar to how it was observed for the WPC encapsulates. For this protein, also in a 1:1 w/w ratio, the most pronounced variation in the relative intensity of the cited bands was shown. The tendency observed in these bands agrees with the TSP results obtained for the encapsulates shown in Table 2. However, among the two proteins at a ratio of 2:1 w/w, ZN showed the lowest spectral variations in both band envelopes, and consequently, this formulation was proposed as the most stable.

4. Conclusions

In this work, the electrospraying assisted by pressurized gas (EAPG) method was used for the first time to dry DBS, and the characteristics of the obtained dried particles were compared with the dried DBS particles obtained by FD, a reference technique for the drying of sensitive bioactive compounds. Both techniques produced dried DBS particles with similar TSP and antioxidant properties, and similar photo-oxidation stability. According to the ATR-FTIR results, polyphenols oxidation reactions took place during the photo-oxidation test, transforming the aromatic alcohols and ether groups, into ketones, carboxylic acids, or ester groups, which could be the reason for the loss in antioxidant activity. However, whereas EAPG produced DBS spherical microparticles with controlled particle size distribution, FD produced irregular particles with a broad particle size distribution, which could affect the dissolution rate, the bioavailability, and the organoleptic impact in a final food product. Secondly, the encapsulation of DBS into protein-based systems, WPC and ZN, was successfully achieved for the first time via EAPG, with it obtaining free-flowing powder with controlled particle size. This encapsulation process contributed to protecting the DBS polyphenolic compounds without affecting their antioxidant bioactivity and TSP. Moreover, it was possible to protect the DBS from UV light radiation, increasing the stability of this bioactive ingredient, especially when using ZN as an encapsulating material

in the ratio 2:1 w/w, since the minimum chemical alterations in the ATR-FTIR bands related to polyphenol oxidation were detected. The obtained results showed that this novel process offers an innovative mild encapsulation and drying technology with the advantage, compared to, for instance, FD, in that it is a continuous process available at an industrial scale. In conclusion, considering the obtained results, these microparticles could be promoted as functional food ingredients adequate to be used in the formulation of functional foods, nutraceuticals, nutracosmetics, or pharmaceuticals, among others.

Acknowledgments

J.D.E.G. would like to thank the Spanish Ministry of Economy, Industry and Competitivity for his predoctoral grant (DI-6906). C.P. would like to thank the Valencian Ministry of Innovation, Universities, Science and Digital Society for her postdoctoral grant (CIAPOS/2021/45). The authors would like to thank Alberto Rodríguez Escrivá and Alexandra Sánchez for their technical assistance.

References

ANDRADE, J., PEREIRA, C. G., ALMEIDA JUNIOR, J. C. DE, VIANA, C. C. R., NEVES, L. N. DE O., SILVA, P. H. F. DA, BELL, M. J. V., & ANJOS, V. DE C. DOS. (2019). FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. LWT, 99, 166–172. https://doi.org/10.1016/J.LWT.2018.09.079

BASAR, A. O., PRIETO, C., & LAGARÓN, J. M. (2021). Novel Encapsulation of Bioactives: Use of Electrohydrodynamic Processing and Applications. Importance & Applications of Nanotechnology. In Importance & Applications of Nanotechnology (Vol. 6).

BELŠČAK-CVITANOVIĆ, A., DURGO, K., HUĐEK, A., BAČUN-DRUŽINA, V., & KOMES, D. (2018). Overview of polyphenols and their properties. In Polyphenols: Properties, Recovery, and Applications. Elsevier. https://doi.org/10.1016/B978-0-12-813572-3.00001-4

BODNÁR, E., GRIFOLL, J., & ROSELL-LLOMPART, J. (2018). Polymer solution electrospraying: A tool for engineering particles and films with controlled morphology. Journal of Aerosol Science, 125, 93–118. https://doi.org/10.1016/J.JAEROSCI.2018.04.012

BOEL, E., KOEKOEKX, R., DEDROOG, S., BABKIN, I., VETRANO, M. R., CLASEN, C., & VAN DEN MOOTER, G. (2020). Unraveling particle formation: From single droplet drying to spray

drying and electrospraying. In Pharmaceutics (Vol. 12, Issue 7, pp. 1–58). https://doi.org/10.3390/pharmaceutics12070625

BUSOLO, M., TORRES-GINER, S., PRIETO, C., & LAGARON, J. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. Innovative Food Science & Emerging Technologies, 51, 12–19. https://doi.org/10.1016/j.ifset.2018.04.007

DANGLES, O., & FENGER, J. A. (2018b). The chemical reactivity of anthocyanins and its consequences in food science and nutrition. Molecules, 23(8). https://doi.org/10.3390/molecules23081970

DE SOUZA, V. B., THOMAZINI, M., BALIEIRO, J. C. D. C., & FÁVARO-TRINDADE, C. S. (2015). Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (Vitis labrusca). Food and Bioproducts Processing, 93(October), 39–50. https://doi.org/10.1016/j.fbp.2013.11.001

DE SOUZA, V. B., THOMAZINI, M., ECHALAR BARRIENTOS, M. A., NALIN, C. M., FERRO-FURTADO, R., GENOVESE, M. I., & FAVARO-TRINDADE, C. S. (2018). Functional properties and encapsulation of a proanthocyanidin-rich cinnamon extract (Cinnamomum zeylanicum) by complex coacervation using gelatin and different polysaccharides. Food Hydrocolloids, 77, 297–306. https://doi.org/10.1016/j.foodhyd.2017.09.040

ESCOBAR, J. D., PRIETO, C., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2018). Dragon's blood sap: Storage stability and antioxidant activity. Molecules, 23(10). Molecules 2018, 23, 2641; https://doi.org/10.3390/molecules23102641

ESCOBAR-GARCÍA, J. D., PRIETO, C., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2021). Room temperature nanoencapsulation of bioactive eicosapentaenoic acid rich oil within whey protein microparticles. Nanomaterials, 11 (3), 1–16. https://doi.org/10.3390/nano11030575

G. GÓMEZ-MASCARAQUE, L., MARTÍNEZ-SANZ, M., FABRA, M. J., & LÓPEZ-RUBIO, A. (2019). Development of gelatin-coated ι-carrageenan hydrogel capsules by electric field-aided extrusion. Impact of phenolic compounds on their performance. Food Hydrocolloids, 90, 523–533. https://doi.org/10.1016/J.FOODHYD.2018.12.017

GÓMEZ-MASCARAQUE, L. G., & LÓPEZ-RUBIO, A. (2016). Protein-based emulsion electrosprayed micro- and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. Journal of Colloid and Interface Science, 465, 259–270. https://doi.org/10.1016/j.jcis.2015.11.061

GÓMEZ-MASCARAQUE, L. G., & LOPEZ-RUBIO, A. (2019). Production of food bioactive-loaded nanoparticles by electrospraying. Nanoencapsulation of Food Ingredients by Specialized Equipment: Volume 3. In the Nanoencapsulation in the Food Industry Series, 107–149. https://doi.org/10.1016/B978-0-12-815671-1.00003-2

GUO, Y., QIAO, D., ZHAO, S., ZHANG, B., & XIE, F. (2021). Starch-based materials encapsulating food ingredients: Recent advances in fabrication methods and applications. Carbohydrate Polymers, 270, 118358. https://doi.org/10.1016/J.CARBPOL.2021.118358

GUPTA, D., & GUPTA, R. K. (2011). Bioprotective properties of Dragon's blood resin: In vitro evaluation of antioxidant activity and antimicrobial activity. BMC Complementary and Alternative Medicine 2011 11:1, 11(1), 1–9. https://doi.org/10.1186/1472-6882-11-13

HE, Z., XU, M., ZENG, M., QIN, F., & CHEN, J. (2016). Interactions of milk α- And β-casein with malvidin-3-O-glucoside and their effects on the stability of grape skin anthocyanin extracts. Food Chemistry, 199, 314–322. https://doi.org/10.1016/J.FOODCHEM.2015.12.035

HECK, R. T., LORENZO, J. M., DOS SANTOS, B. A., CICHOSKI, A. J., DE MENEZES, C. R., & CAMPAGNOL, P. C. B. (2021). Microencapsulation of healthier oils: an efficient strategy to improve the lipid profile of meat products. Current Opinion in Food Science, 40, 6–12. https://doi.org/10.1016/J.COFS.2020.04.010

KARONEN, M., IMRAN, I. BIN, ENGSTRÖM, M. T., & SALMINEN, J. P. (2021). Characterization of natural and alkaline-oxidized proanthocyanidins in plant extracts by ultrahigh-resolution UHPLC-MS/MS. Molecules, 26(7). https://doi.org/10.3390/molecules26071873

KESAVAN, P., BANERJEE, A., BANERJEE, A., MURUGESAN, R., MAROTTA, F., & PATHAK, S. (2018). Chapter 17 - An Overview of Dietary Polyphenols and Their Therapeutic Effects. In Polyphenols: Mechanisms of Action in Human Health and Disease (2nd ed., pp. 221–235). Elsevier. https://doi.org/10.1016/B978-0-12-813006-3.00017-9

LABUSCHAGNE, P. (2018). Impact of wall material physicochemical characteristics on the stability of encapsulated phytochemicals: A review. In Food Research International (Vol. 107, pp. 227–247). Elsevier. https://doi.org/10.1016/j.foodres.2018.02.026

LOCK, O., PEREZ, E., VILLAR, M., FLORES, D., & ROJAS, R. (2016). Bioactive compounds from plants used in traditional Peruvian medicine. Natural Product Communications, 11(3), 315–337.

LOPES, M. I. L. E., SAFFI, J., ECHEVERRIGARAY, S., HENRIQUES, J. A. P., & SALVADOR, M. (2004). Mutagenic and antioxidant activities of Croton lechleri sap in biological systems. Journal of Ethnopharmacology, 95(2–3), 437–445. https://doi.org/10.1016/j.jep.2004.08.025

MCCLEMENTS, D. J. J. (2012). Requirements for food ingredient and nutraceutical delivery systems. In Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals. Elsevier Masson SAS. https://doi.org/10.1533/9780857095909.1.3

MEHRA, R., KUMAR, H., KUMAR, N., RANVIR, S., JANA, A., BUTTAR, H. S., TELESSY, I. G., AWUCHI, C. G., OKPALA, C. O. R., KORZENIOWSKA, M., & GUINÉ, R. F. P. (2021). Whey proteins processing and emergent derivatives: An insight perspective from constituents, bioactivities, functionalities to therapeutic applications. Journal of Functional Foods, 87, 104760. https://doi.org/10.1016/J.JFF.2021.104760

MERLIC, C. A. (2012). IR Absorption Table. https://webspectra.chem.ucla.edu/irtable.html

MIGUEL, G. A., JACOBSEN, C., PRIETO, C., KEMPEN, P. J., LAGARON, J. M., CHRONAKIS, I. S., & GARCÍA-MORENO, P. J. (2019). Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil. Journal of Food Engineering, 263(July), 348–358. https://doi.org/10.1016/j.jfoodeng.2019.07.019

MINJ, S., & ANAND, S. (2020). Whey Proteins and Its Derivatives: Bioactivity, Functionality, and Current Applications. Dairy, 1(3), 233–258. https://doi.org/10.3390/DAIRY1030016

NIAZ, K., & KHAN, F. (2020). Analysis of polyphenolics. Recent Advances in Natural Products Analysis, 39–197. https://doi.org/10.1016/B978-0-12-816455-6.00003-2

NIKKOLA, J. (2016). Smart multifunctional hybrid coatings with adjustable permeability for migration barriers. In Smart Composite Coatings and Membranes: Transport, Structural, Environmental and Energy Applications (pp. 351–370). Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-283-9.00013-0

OLIVEIRA, R. N., MANCINI, M. C., DE OLIVEIRA, F. C. S., PASSOS, T. M., QUILTY, B., THIRÉ, R. M. DA S. M., & MCGUINNESS, G. B. (2016). Análise por FTIR e quantificação de fenóis e flavonóides de cinco produtos naturais disponíveis comercialmente utilizados no tratamento de feridas. Revista Materia, 21(3), 767–779. https://doi.org/10.1590/S1517-707620160003.0072

OXLEY, J. (2014). Overview of Microencapsulation Process Technologies. In Microencapsulation in the Food Industry (pp. 35–46). Elsevier. https://doi.org/10.1016/B978-0-12-404568-2.00004-2

PING, L., PIZZI, A., GUO, Z. D., & BROSSE, N. (2012a). Condensed tannins from grape pomace: Characterization by FTIR and MALDI TOF and production of environment friendly wood adhesive. Industrial Crops and Products, 40(1), 13–20. https://doi.org/10.1016/J.INDCROP.2012.02.039

PIZZINO, G., IRRERA, N., CUCINOTTA, M., PALLIO, G., MANNINO, F., ARCORACI, V., SQUADRITO, F., ALTAVILLA, D., & BITTO, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. In Oxidative Medicine and Cellular Longevity (Vol. 2017). Hindawi Limited. https://doi.org/10.1155/2017/8416763

PRAKASH, D., & GUPTA, K. R. (2009). The Antioxidant Phytochemicals of Nutraceutical Importance. The Open Nutraceuticals Journal, 2(1), 20–35. https://doi.org/10.2174/1876396000902010020

PRIETO, C., EVTOSKI, Z., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2021). Bioavailability enhancement of nanostructured microparticles of carvedilol. Journal of Drug Delivery Science and Technology, 66, 102780. https://doi.org/10.1016/J.JDDST.2021.102780

PRIETO, C., & LAGARON, J. M. (2020). Nanodroplets of docosahexaenoic acid-enriched algae oil encapsulated within microparticles of hydrocolloids by emulsion electrospraying assisted by pressurized gas. Nanomaterials, 10(2), 270. https://doi.org/10.3390/nano10020270

RAHMAN, T., HOSEN, I., ISLAM, M. M. T., & SHEKHAR, H. U. (2012). Oxidative stress and human health. Advances in Bioscience and Biotechnology, 03(07), 997–1019. https://doi.org/10.4236/abb.2012.327123

RAJAN, V. K., MURALEEDHARAN, K., & HUSSAN, K. P. S. (2018). Structural evaluation and toxicological study of a bitter masking bioactive flavanone, 'eriodictyol.' In Polyphenols: Prevention and Treatment of Human Disease (2nd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813008-7.00005-9 RIBEIRO, J. S., & VELOSO, C. M. (2021). Microencapsulation of natural dyes with biopolymers for application in food: A review. Food Hydrocolloids, 112, 106374. https://doi.org/10.1016/J.FOODHYD.2020.106374

SANTOS, P. D. DE F., RUBIO, F. T. V., DA SILVA, M. P., PINHO, L. S., & FAVARO-TRINDADE, C. S. (2021). Microencapsulation of carotenoid-rich materials: A review. Food Research International, 147, 110571. https://doi.org/10.1016/J.FOODRES.2021.110571

SCHULZ, H., & BARANSKA, M. (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy, 43(1), 13–25. https://doi.org/10.1016/J.VIBSPEC.2006.06.001

SHARIF, N., GOLMAKANI, M. T., NIAKOUSARI, M., GHORANI, B., & LOPEZ-RUBIO, A. (2019). Food-grade gliadin microstructures obtained by electrohydrodynamic processing. Food Research International, 116, 1366–1373. https://doi.org/10.1016/J.FOODRES.2018.10.027

SILVA, P. M., TORRES-GINER, S., VICENTE, A. A., & CERQUEIRA, M. A. (2021). Electrohydrodynamic processing for the production of zein-based microstructures and nanostructures. Current Opinion in Colloid & Interface Science, 56, 101504. https://doi.org/10.1016/J.COCIS.2021.101504

SIRACUSA, L., & RUBERTO, G. (2019). Not Only What Is Food Is Good—Polyphenols From Edible and Nonedible Vegetable Waste. Polyphenols in Plants, 3–21. https://doi.org/10.1016/b978-0-12-813768-0.00001-3

SYIEMLIEH, I., ASTHANA, M., ASTHANA, S. K., KURBAH, S. D., KOCH, A., & LAL, R. A. (2018). Water soluble new bimetallic catalyst [CuZn(bz)3(bpy)2]PF6 in hydrogen peroxide mediated oxidation of alcohols to aldehydes/ketones and C-N functional groups. Journal of Organometallic Chemistry, 878, 48–59. https://doi.org/10.1016/J.JORGANCHEM.2018.10.001

THAKUR, M., SINGH, K., & KHEDKAR, R. (2020). Phytochemicals: Extraction process, safety assessment, toxicological evaluations, and regulatory issues. Functional and Preservative Properties of Phytochemicals, 341–361. https://doi.org/10.1016/B978-0-12-818593-3.00011-7

TORRES, S. J. V., MEDEIROS, G. B., ROSÁRIO, F., YAMASHITA, F., MATTOSO, L. H. C., & CORRADINI, E. (2021). Mechanical and water absorption properties and morphology of melt processed Zein/PVAl blends. Polímeros, 30(4), e2020042. https://doi.org/10.1590/0104-1428.10619

TORRES-GINER, S., PRIETO, C., & LAGARON, J. M. (2020). Nanomaterials to enhance food quality, safety, and health impact. Nanomaterials, 10(5), 1–6. https://doi.org/10.3390/nano10050941

WANG, H., HAO, L., NIU, B., JIANG, S., CHENG, J., & JIANG, S. (2016). Kinetics and Antioxidant Capacity of Proanthocyanidins Encapsulated in Zein Electrospun Fibers by Cyclic Voltammetry. https://doi.org/10.1021/acs.jafc.6b00540

6. CHAPTER 3

CAMU CAMU EXTRACT - CCX

Comparison of the Stability of a Camu-camu Extract Dried and Encapsulated by Means of High-Throughput Electrospraying Assisted by Pressurized Gas.

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Foods 2024, 13, 3280
https://doi.org/10.3390/foods13203280
Impact Factor (2023): 4.7
Cite Score 7.4

Abstract

This study explores the impact on the stability of drying and the encapsulation of a camu camu extract (CCX) using the non-thermal, high throughput electrospraying assisted by pressurized gas (EAPG) technique. The dried and encapsulated products by the EAPG processing techniques were compared in terms of total soluble phenolic compounds, antioxidant activity, and storage stability. Whey protein concentrate (WPC) and zein (ZN) were selected as the protective excipients for encapsulation. Dried and encapsulated products were obtained in the form of microparticles, which were smaller and more spherical in the case of the encapsulates. No significant differences were observed in the total polyphenolic content (TSP), and only relatively small differences in the antioxidant capacity were measured among samples. The generated products were subjected to various storage conditions to assess their stability and the preservation of the TSP and the antioxidant properties, i.e., 0% relative humidity (RH) and 4 °C; 0% RH and 21 °C; 23% RH and 21 °C; 56% RH and 21 °C; and UV light exposure. The results indicated that ZN encapsulation notably enhanced the retention of total soluble polyphenols and the antioxidant activity compared to WPC and dried CCX, especially in the ratio of 2:1 (encapsulating polymer: dried CCX). This study demonstrates the potential of protein-based encapsulation, particularly using ZN, for stabilizing bioactive compounds against degradation mechanisms induced by humidity, temperature, or ulraviolet radiation exposure.

Keywords: Camu camu extract; room-temperature drying; encapsulation; microparticles; whey protein concentrate; zein; EAPG.

1. Introduction

There is a growing interest among consumers in natural bioactive compounds that possess the potential to promote health benefits and contribute to the prevention and/or treatment of chronic diseases. Consequently, investigating and researching these natural bioactive compounds is a matter of great importance for numerous laboratories and industries (Alu'datt et al., 2022; Marcillo-Parra et al., 2021).

Among the bioactive compounds, phytochemicals stand out due to their ability to inhibit oxidative reactions, thereby protecting the organism from reactive oxygen species, which are implicated in numerous degenerative diseases such as cataracts, atherosclerosis, and cancer (Thakur et al., 2020).

The Amazon region boasts an extraordinary biodiversity of both native and exotic fruits. Camu camu (Myrciaria dubia (H.B.K) McVaugh) is a fruit that is distributed along Bolivia, Brazil, Colombia, Ecuador, Peru, and Venezuela, and it is emerging as a standout due to its remarkable nutritional prospects (Castro et al., 2018; Santos et al., 2022). The composition of camu camu has garnered considerable interest due to its elevated levels of bioactive compounds, such as ascorbic and citric acid and phenolic compounds, including ellagic acid, ellagitannins, and proanthocyanidins (Akter et al., 2011; Fracassetti et al., 2013; Fujita et al., 2017), being reported for its purported bioactivities encompassing antihyperglycemic, anti-inflammatory, antihypertensive, and antimicrobial, among other properties (Carmo et al., 2019; Fidelis et al., 2018; Miyashita et al., 2018). In this sense, investigations have demonstrated that camu camu exhibits potent antioxidant and anti-inflammatory properties, surpassing those observed in vitamin C tablets when tested in human subjects (Inoue et al., 2008). Moreover, studies have also reported the potential antiobesity effects of camu camu in a rat model of diet-induced obesity (Anhê et al., 2019). Additionally, several studies have suggested that camu camu possesses antidiabetic activities, indicating its

potential in treating this disease (Christensen, 2018; de Azevêdo et al., 2014; Fujita et al., 2015, 2017).

To develop functional ingredients based on the active properties of camu camu, it is necessary to extract the bioactive compounds from the fruit or the different parts of the plant (de Azevêdo et al., 2014; Miyashita et al., 2018), a process that can be followed by a drying step to facilitate its industrial handling. However, it is well-known that some bioactive compounds present low solubility in water or are unstable, making them susceptible to high temperature, light, pH, oxidative stress, and degradative enzymes, which can negatively impact their biological activities, limiting their use in functional foods or nutraceuticals. Proof of that is that Neves et al. observed a significant decrease in the total phenolic content of camu camu fruits after 5 days of cold storage under controlled conditions (Neves et al., 2017). Consequently, developing strategies to protect and preserve them, particularly under conditions of processing and storage, and to enhance their bioaccessibility and bioavailability is crucial (Dahiya et al., 2023; De Mello Andrade & Fasolo, 2013). Among these strategies, encapsulation has emerged as a promising approach to address this challenge.

Encapsulation involves entrapping the bioactive components within a protective matrix, which can slow down degradation processes, prevent the loss of their functionality, and increase bioavailability (Marcillo-Parra et al., 2021; Raddatz & de Menezes, 2021). Therefore, encapsulating bioactive compounds makes extending their shelf life possible, improving their stability throughout various processing stages and final use at consumption and enhancing their compatibility with different formulations (Alu'datt et al., 2022; Zuidam & Nedović, 2010). Furthermore, the encapsulation strategy could provide controlled release mechanisms and a sustained and targeted delivery of these compounds in the body (Shafaei et al., 2017; Sobel et al., 2014; Veršič, 2014). Thus, various encapsulation methods have been explored so far (Guldiken et al., 2021). The choice of the suitable process varies with the active ingredient's nature, the

coating material's properties, and the final product's desired characteristics based on the intended use.

Recent evidence underscores that microencapsulation is a potent process to enhance the stability and bioavailability of the naturally occurring bioactive compounds in camu camu, such as ascorbic acid and anthocyanins. Until now, Fujita et al., (2015) have reported the microencapsulation of camu camu juice into Arabic gum and maltodextrin via spray drying. de Abreu Figueiredo et al., (2020) reported the microencapsulation of the camu camu extract from pulp and peels by spray drying using maltodextrin, inulin, and oligofructose as encapsulating matrixes. Complementary findings by García-Chacón et al., (2024) reveal that spray-drying camu camu pulp with maltodextrin, whey protein, and a 50:50 mixture of both is a strategy to increase the bioactive compounds stability, modulate the fruit sensory properties, and improve their bioavailability.

However, among the techniques available, electrohydrodynamic processing, including electrospraying or electrospinning, has emerged as a promising and versatile method for encapsulating bioactive agents. Electrospraying offers several advantages over traditional encapsulation techniques: It enables the production of micro- or nano-scale particles; it is simple and versatile in terms of the materials that can be processed; it allows the production of particles at low or ambient temperatures (Gómez-Mascaraque & Lopez-Rubio, 2019). However, the main disadvantage of this technology is its low production yield, which has been solved thanks to the integration of electrospraying and gas-driven nebulization (Busolo et al., 2019). This technology has been named and patented as electrospraying assisted by pressurized gas (EAPG). It involves atomizing the solution through a pneumatic injector using compressed air that nebulizes within a high electric field. This process forms fine droplets that are dried at room temperature and collected as a free-flowing powder. Therefore, this process is suitable for sensitive bioactive compounds. Moreover, it gathers all the advantages of the electrospray process, such as high encapsulation efficiency and

control of particle size and morphology, and it is already available on an industrial scale.

Hitherto, camu camu has been encapsulated mainly into polysaccharides (Fujita et al., 2015; García-Chacón et al., 2024). However, based on our encapsulation expertise (Escobar-García et al., 2021, 2023), food-grade proteins can provide increased stability to sensitive bioactive ingredients (Jeevani et al., 2023). Therefore, this work aimed to study the potential of room-temperature EAPG technology to dry and microencapsulate a camu camu extract (CCX). Whey protein concentrate (WPC) and zein (ZN) were selected as encapsulating agents. The microcapsules were characterized by morphology, total soluble polyphenol (TSP) content, and antioxidant activity. Additionally, this study aimed to assess the storage stability of CCX EAPG-derived microcapsules under different humidity and temperature conditions and against photooxidation.

2. Material and Methods

2.2. Materials

Camu camu powder (CCP) was provided by Qomer BioActive Ingredients (Valencia, Spain). Whey protein concentrate (WPC) 80% was purchased from Beurrespa S.L. (Madrid, Spain). According to the distributor, WPC was claimed to contain 81.6% protein (on dry basis) and 7.5% fat. Maize zein (ZN), gallic acid, sodium carbonate, Folin–Ciocalteau reagent, magnesium nitrate, potassium acetate, and silica gel were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Methanol (reagent grade) was purchased from Labbox (Premia de Dalt, Spain). Ethanol 96 vol.% was purchased from Panreac Química SLU (Barcelona, Spain). Deionized water was used throughout this study.

2.2. Preparation of camu camu extract (CCX)

The CCP was extracted following a standardized adapted method as described by Fracassetti et al., (2013). A mixture of alcohol and water (85%) was used as the extraction solvent. The extraction process was carried out at a concentration of

5% (w/v), where 50 g of CCP was mixed with 1000 mL of the extraction solvent. The mixture was homogenized using a magnetic stirrer (H20 series, LBX Instruments, Premia de Dalt, Spain) at room temperature until complete dissolution of CCP. Subsequently, the mixture was sonicated in a SONOPULS HD 2200.2 (Bandelin electronic GMbH and Co. KG, Berlin, Germany) for an additional 30 min. After sonication, the solution was centrifuged at 3000 rpm for 15 min at 4 °C using an Avanti J-26 XPI Beckman Coulter centrifuge (Brea, CA, USA). The resulting supernatant was then filtered under vacuum filtration using Whatman No. 1 filter paper by Cytiva (Marlborough, MA, USA). The filtered supernatant was collected and used for further encapsulation processes.

2.3. Preparation of the polymer solution

To prepare the encapsulating solutions, a 20% (w/v) solution of whey protein concentrate (WPC) in water was prepared, and a 4% (w/v) solution of zein (ZN) in an 85% (v/v) ethanol aqueous solution. Then, the encapsulating solutions were created by slowly adding the pre-prepared camu camu extract (CCX) to the respective encapsulating matrix solutions at ratios of 1:1 w/w and 2:1 w/w (encapsulant to dry weight CCX ratio). The solutions were continuously stirred using a magnetic stirrer (H20 series, LBX Instruments, Premia de Dalt, Spain) at room temperature overnight until a homogenous mixture of polymer solutions was obtained.

2.4. EAPG process

The EAPG process was used to dry the CCX solution and encapsulate the CCX into WPC and ZN, using the CapsultekTM pilot plant from Bioinicia S.L. (Valencia, Spain). The pilot plant consists of a nebulizer that generates aerosol droplets, which are subjected to a high electric field, a drying chamber, and a cyclonic collector. The process was conducted under controlled ambient conditions of 25 °C and 30% relative humidity (RH), as detailed in the study by Busolo et al. [29]. The solution was pumped to the injection unit at a flow rate of 10 mL/min, with an air pressure of 10 L/min and a voltage of 15 kV. The resulting particles were collected from the cyclone as a free-flowing powder, stored in flasks under

vacuum at −20 °C, and protected from light to prevent oxidation until further analysis.

The theoretical loading capacity for the CCX encapsulates was 50% for the ratio of 1:1 and 33% for the ratio of 2:1. Furthermore, a 100% encapsulation efficiency was obtained, meaning no significant bioactive loss occurred during the encapsulation process.

2.5. Microscopy

Scanning electron microscopy (SEM) analysis of the particle morphology was conducted using a Hitachi S-4800 field-emission scanning electron microscope (Hitachi High Technologies Corp., Tokyo, Japan). The microscope was operated at an electron beam acceleration of 5 kV. Before analysis, approximately 5 mg of the capsules from each sample were coated with a thin layer of gold/palladium to enhance conductivity and imaging quality during SEM observations. The SEM images were captured, and the average diameters of the particles were determined using Image J Launcher v1.41 software developed by the National Institutes of Health (Bethesda, MD, USA).

2.6. Color

The color was determined using a chroma meter CR-400 (Konica Minolta, Tokyo, Japan). Color differences between samples were calculated using the CIE Lab* color space, which measures color in three dimensions: lightness (L*), red–green axis (a*), and yellow–blue axis (b*). The total color difference, ΔE*, was determined based on the Euclidean distance between the two points in the Lab space, following the formula:

$$\Delta E^* = \sqrt[2]{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]} \quad (2)$$

This metric represents the magnitude of the perceptual color difference. According to established thresholds, a ΔE* value below 1 is imperceptible to the human eye, while a value between 1 and 2 can be detected only by experienced observers. A ΔE* value between 2 and 3.5 is noticeable to most observers, values

between 3.5 and 5 indicate a clear color difference, and values above 5 indicate that the colors appear distinctly different. This method allows for an objective comparison of color changes, facilitating the assessment of product consistency during storage or processing.

2.7. Moisture

The CCX EAPG-derived samples were dried at 40 °C in a vacuum oven (Vaciotem-T, J.P. Selecta, Barcelona, Spain) until reaching a constant weight. Subsequently, the moisture content was determined by measuring the weight differential before and after the drying process.

2.8. Assessment of the Stability of CCX Formulations

The stability of CCX formulations was evaluated under different storage conditions of relative humidity (RH) and temperatures and accelerated oxidation conditions under ultraviolet (UV) radiation. To simulate different humidity levels, samples of CCX formulations were placed in desiccators containing either silica gel (0% RH), potassium acetate oversaturated solution (23% RH), or magnesium nitrate oversaturated solution (56% RH). The desiccators were kept at a cooling temperature (4 °C) or ambient temperature (~21 °C) accordingly. The samples were stored under the above conditions and monitored for 40 days. Weekly samples were collected and analyzed.

Regarding UV radiation, a commercially available OSRAM Ultra-Vitalux lamp (OSRAM, Garching, Germany) irradiated the samples with UV light. This lamp consists of a quartz discharge tube and a tungsten filament, producing a blend of radiation like natural sunlight. The lamp was operated at a power of 300 W, and only wavelengths like those present in daylight passed through the special glass bulb. After 1 h of exposure, the radiation between 315–400 nm was measured to be 13.6 W, and the radiation between 280 and 315 nm was measured to be 3 W. Approximately 20 g of capsules were evenly spread on Petri dishes and positioned at 20 cm under the UV lamp. The powder in the Petri dish was stirred daily to maintain a uniform treatment. The thickness of the powder layer was maintained

below 5 mm to ensure consistent exposure to UV light. The capsules were subjected to the specified storage conditions, and their stability was evaluated over 40 days. Weekly samples were taken from the capsules for analysis.

The analysis of the samples was specifically made to focus on variations in total soluble polyphenols and antioxidant capacity, and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy was utilized.

2.9. Total Soluble Polyphenols

The total soluble polyphenol (TSP) content in the CCX formulations was determined using the Folin–Ciocalteu method described by Singleton and Rossi [33]. For the analysis, 10 mg of the CCX formulations were diluted in ethanol at a concentration of 50% (v/v). Similarly, an equivalent amount of WPC-CCX capsules was diluted in ethanol at 33% (v/v), and an equivalent amount of ZN-CCX capsules was diluted in ethanol at 75% (v/v). The ethanol concentrations utilized in this study were specifically selected to facilitate the optimal solubility of each encapsulating matrix (WPC and ZN) as well as the solubility of the CCX. After dissolution, 20 µL of the capsule's solution was mixed with 1.2 mL of water and 300 µL of sodium carbonate solution at a concentration of 7.5% (w/v) in Eppendorf tubes. The mixture was then homogenized for 1 min using a vortex shaker and left to stand for 5 min.

Next, 380 µL of water was added to the mixture, followed by 100 µL of a 10% (v/v) Folin reagent solution. The reaction proceeded for 15 min under dark conditions and at room temperature. Subsequently, the absorbance of the resulting solution was measured in triplicate at a wavelength of 765 nm using a UV/Vis spectrophotometer (UV4000 Dinko Instruments, Barcelona, Spain). The absorbance values obtained were then used to calculate the total soluble polyphenols content of the samples, expressed in gallic acid equivalents (mg GAE/g of dried CCX).

2.10. Antioxidant Activity

The antioxidant capacity of the CCX formulations was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free radical scavenging assay, a widely accepted method for assessing the ability of compounds to neutralize free radicals, which is indicative of their antioxidant activity. CCX-EAPG-derived samples were dissolved in ethanol at specific concentrations, like how TSP determination is performed. Then, they were mixed with a DPPH methanolic solution and followed the DPPH methodology from previous studies (Escobar-García et al., 2023). After the incubation period, the absorbance of the resulting mixture was measured at a wavelength of 517 nm using a UV/Vis spectrophotometer (Dinko Instruments, Barcelona, Spain).

2.11. Attenuated total reflection- Fourier transform infrared (ATR-FTIR)

The ATR-FTIR spectra of CCX formulations were measured using a Bruker Tensor 37 FT-IR Spectrometer (Bruker, Ettlingen, Germany) equipped with an ATR sampling accessory called the low-temperature Golden Gate from Specac Ltd. (Orpington, UK). This accessory ensures proper contact between the diamond crystal and the encapsulated samples, enhancing the quality of the spectra. Approximately 50 mg of the capsules were used for each measurement.

The spectra were precisely collected using ATR-FTIR spectroscopy within the 4000–600 cm^{-1} wavenumber range, capturing a comprehensive infrared frequency spectrum crucial for molecular bond characterization. The protocol averaged 10 scans per spectrum to enhance the signal-to-noise ratio, a standard analytical chemistry practice for data quality improvement. Spectral resolution was set at 4 cm^{-1}, allowing proper resolution for molecular vibration interpretation.

The OPUS 4.0 data collection software program, developed by Bruker (Ettlingen, Germany), was used to analyze the spectral data. This software enables the processing, analysis, and interpretation of the ATR-FTIR spectra, allowing for

the identification and characterization of the encapsulated samples. Measurements were performed in triplicate to ensure the reproducibility and reliability of the results. For comparison purposes, first, the baseline of the analyzed spectra was adjusted, and then the spectra were maximized to the band with the highest intensity in the wavenumber range between 1800 and 800 cm^{-1}.

2.12. Statistical Analysis

The data were expressed as mean ± standard deviation. To determine the significance of the differences observed among the different samples, an analysis of variance (ANOVA) was conducted. Following ANOVA, a Tukey test was performed to compare the means of multiple groups. In this analysis, differences were considered significant when the ***p***-value was less than 0.05. For this analysis, the software used was Statgraphics Centurion Version 17.2.04, developed by Statistical Graphics Corp. (Rockville, MD, USA).

3. Results and Discussion

This study investigates the effect of the drying of CCX and its microencapsulation using WPC and ZN via EAPG technology. The resulting microcapsules were analyzed for their morphology, color, total phenolic content, antioxidant activity, and storage stability under different ambient conditions.

3.1. Physicochemical Characterization

The obtained particles were characterized in terms of morphology, moisture, and color. Figure 1 provides a comparative analysis of particle morphologies achieved through EAPG technology. When drying neat CCX (Figure 1A), irregular, non-uniform, and agglomerated structures were predominantly observed, resulting in a particle size distribution of 10.01 ± 1.84 µm, as shown in Table 1.

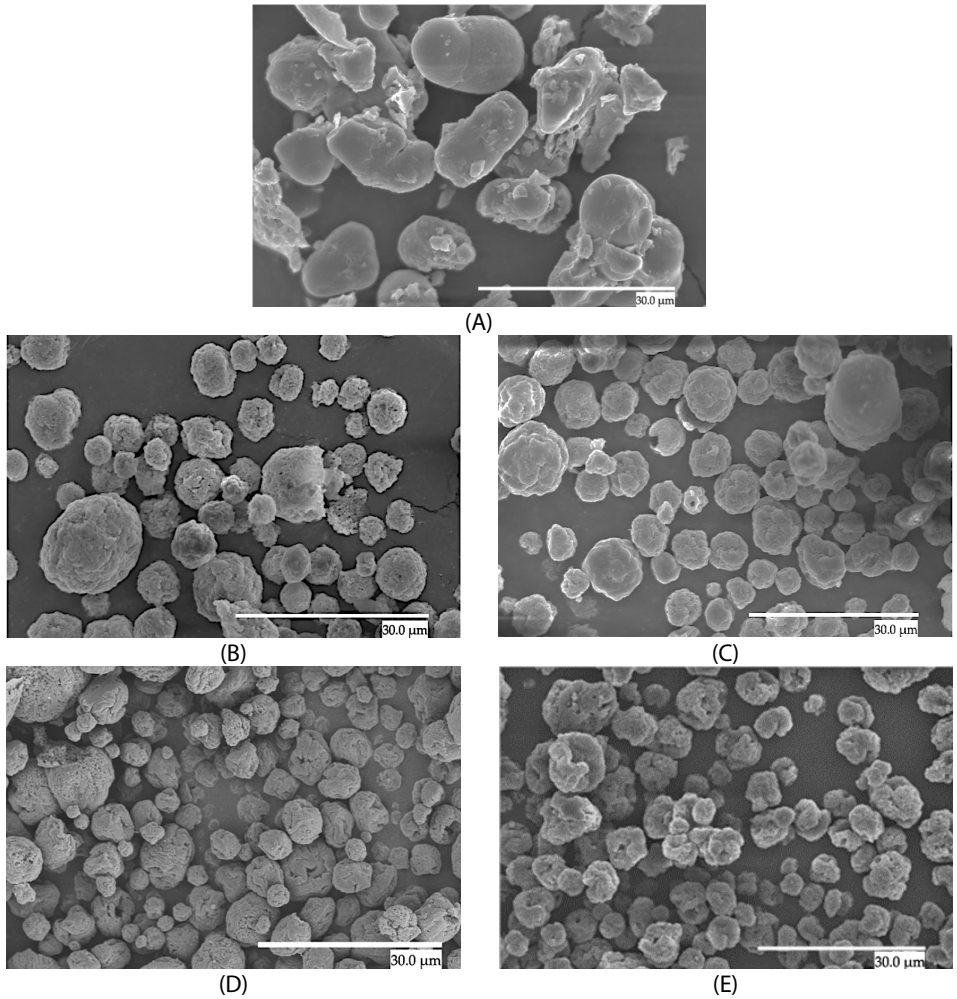

Figure 1. SEM micrographs of dried CCX structures. (A) The neat CCX dried using the EAPG process. (B) WPC—CCX 1:1 w/w; (C) WPC—CCX 2:1 w/w; (D) ZN—CCX 1:1 w/w; (E) ZN—CCX 2:1 w/w. The scale bar corresponds to 30 µm.

The CCX encapsulates exhibited a spherical morphology, and the average particle sizes are gathered in Table 1. The encapsulates showed an average particle size of around 6 µm with a narrow size distribution and with no significant differences among formulations. These results are consistent with earlier successful EAPG encapsulations of bioactive compounds, such as Eicosapentaenoic acid (EPA) (Escobar-García et al., 2021) and Dragon's blood sap

(DBS) (Escobar-García et al., 2023), which produced microparticles with average sizes around 6 µm and 11 µm, respectively.

Table 1. Physicochemical properties of CCX dried via EAPG process and encapsulated with WPC and ZN.

Sample	Particle Size (µm)	Moisture (%)	Color			
			L*	a*	b*	ΔE*
CCX-EAPG	10.01 ± 1.84 [a]	4.67 ± 1.32 [a]	37.56	5.11	7.50	
WPC-CCX 1:1	6.74 ± 2.57 [ab]	5.17 ± 1.93 [a]	39.14	4.21	8.96	2.33
WPC-CCX 2:1	7.24 ± 2.49 [ab]	4.78 ± 0.97 [a]	39.46	3.74	8.36	2.50
ZN-CCX 1:1	6.24 ± 1.72 [b]	4.15 ± 1.31 [a]	37.79	4.15	7.15	1.05
ZN-CCX 2:1	5.85 ± 1.45 [b]	4.07 ± 1.04 [a]	44.06	2.41	9.04	7.21

Mean values ± standard deviation with different superscripts in the same column are significantly different (Tukey test, $p \leq 0.05$). Color standard deviation ≤ 0.10.

Figure 2 illustrates the particle size distribution of the CCX EAPG-derived capsules. The samples were monodispersed. Broad size distribution for the neat CCX dried by EAPG and the encapsulates with WPC was observed. However, ZN encapsulates showed smaller particle sizes and a narrower particle size distribution. This difference between WPC and ZN encapsulates can be attributed to the protein composition of the WPC, i.e., 80% WP and 20% lactose, resulting in a broader particle size distribution. Similar observations were made by Prieto et al. when comparing the particle morphology and size distribution when encapsulating algae oil in WP with different protein content (Prieto et al., 2022).

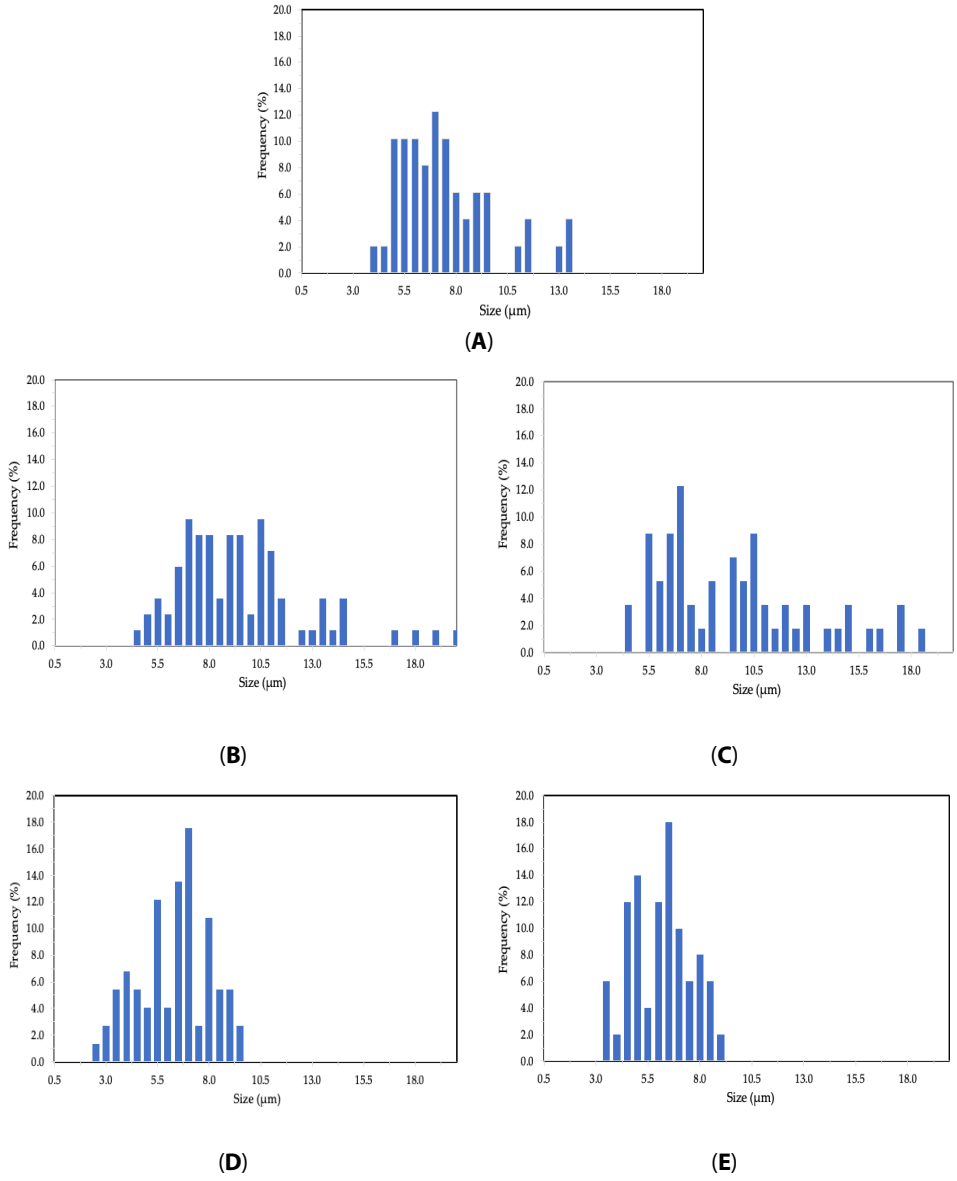

Figure 2. Histograms of dried CCX structures. (A) The neat CCX dried using the EAPG process. (B) WPC-CCX 1:1 w/w; (C) WPC-CCX 2:1 w/w; (D) ZN-CCX 1:1 w/w; (E) ZN-CCX 2:1 w/w.

Nevertheless, the particle sizes were consistently below the acceptable detection threshold of 20 µm, ensuring no detectable sensory impact (Weidner, 2009), thus preserving the potential for maintaining texture in the final product formulation. This is an advantage of the EAPG process in comparison to conventional technologies. For instance, in a recent study conducted by García-Chacón et al., the use of whey protein concentrate (WPC) and maltodextrin as coating agents were compared during the spray drying process of camu camu. Maltodextrin produced particles of sizes ranging from 1.5 to 12.5 µm, while whey protein resulted in particles ranging from 5 to 45 µm (García-Chacón et al., 2024). In another study, Figueredo et al. encapsulated a camu camu extract via spray drying using maltodextrin, inulin, and oligofructose as carrier agents, observing varying particle sizes between 5 and 54 µm (A Figueiredo et al., 2020).

Regarding the moisture content of the samples obtained, it was on average below 5%, as shown in Table 1. This moisture level is widely acknowledged as safe, as it inhibits the growth of most harmful bacteria (FAO & WHO, 2022).

Color analysis was conducted on the CCX samples produced through the EAPG technique, and the results are presented in Table 1. The CCX-EAPG sample reflects the natural color of camu camu, which is attributed to its high phenolic content, particularly anthocyanins and carotenoids, along with its notable ascorbic acid content, contributing to its visual appearance (Neves et al., 2017).

However, the color of the encapsulates resulted in a mixture between the color of the CCX and the color of the pure encapsulating matrices. WPC had L*, a*, and b* values of 57.94 ± 0.18, −0.12 ± 0.07, and 9.52 ± 0.08, respectively, while ZN showed values of 50.14 ± 0.18, 0.93 ± 0.02, and 10.93 ± 0.06, respectively. Thus, encapsulation with WPC caused a noticeable color change, with ΔE* values within the range of 2 to 3.5, meaning that the color difference would be perceptible to most observers but not strikingly different. The WPC encapsulation process slightly lightened the samples and reduced the red hues while enhancing the yellow tones, suggesting that WPC creates a subtle masking effect on the natural pigments without drastically altering the overall color.

Regarding the encapsulation with ZN, the most significant color changes were observed in ZN-CCX 2:1 ratio capsules with ΔE* well above 5. This substantial increase in lightness and reduction in redness suggests a more pronounced influence on the protein color. These results underline the critical role of the matrix composition in influencing the visual appearance of the encapsulates in addition to its protection role.

In addition, it is important to mention that the capsules were readily soluble in water or ethanol depending on the nature of the encapsulating matrix, as no thermal treatment or chemical reaction occurred during the EAPG encapsulation process.

3.2. Characterization of Antioxidant Activity and the Polyphenol Content

The antioxidant activity and polyphenol content of the CCX-EAPG-derived capsules are shown in Table 2. Previous studies have demonstrated that camu camu possesses antioxidant capabilities that significantly surpass those of various fruits by over tenfold (Fidelis, do Carmo, et al., 2020; García-Chacón et al., 2024; Grigio et al., 2021). In our investigation, DPPH inhibition percentages for the CCX were 89.06%, which increased to some small extent to 94.07% for CCX-EAPG, potentially attributed to humidity loss during drying by EAPG. Regarding the encapsulates, the 2:1 encapsulation ratio in protein-based formulations was found to be more antioxidant for the 2:1 ratio than for the 1:1 ratio, especially in the case of zein. This observation is consistent with findings from prior studies on dragon blood sap (DBS) with EAPG-delivered capsules (Escobar-García et al., 2021), where a 1:1 coating-to-core ratio led to decreased DPPH radical scavenging activity. In any case, the differences among samples are relatively small and could be within the experimental error associated with the process and the analytical method. In future studies, other antioxidant tests, such as ORAC, ABTS, or FRAP, will be considered to study the differences between the samples.

Table 2. Total soluble polyphenol content and antioxidant activity for CCX dried by EAPG (CCX-EAPG) and encapsulated CCX into whey protein concentrate (WPC) and zein (ZN).

Sample	DPPH inhibition (%)	TSPs (mg GAE/g dried CCX)
CCX	89.06 ± 0.02 [a]	1.13 ± 0.05 [a]
CCX-EAPG	94.03 ± 0.02 [b]	1.14 ± 0.07 [a]
WPC-CCX 1:1	91.60 ± 0.06 [ab]	1.15 ± 0.04 [a]
WPC-CCX 2:1	94.07 ± 0.04 [b]	1.11 ± 0.05 [a]
ZN-CCX 1:1	85.32 ± 0.02 [a]	1.21 ± 0.06 [a]
ZN-CCX 2:1	93.22 ± 0.04 [b]	1.15 ± 0.05 [a]

Mean values ± standard deviation with different superscripts in the same column are significantly different (Tukey test, $p \leq 0.05$). DPPH inhibition percentage of the DPPH reactive. TSPs—total soluble polyphenols. GAE—gallic acid equivalent.

To further investigate the stability and antioxidant characteristics of the processed materials, the total soluble polyphenols (TSPs) were also determined using the Folin–Ciocalteu method. The results, shown in Table 2, unveiled consistent TSP levels across all samples, with a narrow range from 1.06 to 1.15 mg GAE/g dried CCX. The consistent retention of total soluble polyphenols (TSPs) indicates the efficiency of the EAPG method in preserving the initial TSP content during drying or encapsulation. Moreover, the comparable TSP values among samples imply that the choice of coating materials did not significantly affect the polyphenol content. These results align with prior research demonstrating the preservation of bioactive compounds, including polyphenols, throughout encapsulation processes (Escobar-García et al., 2023).

According to the literature, the total phenolic content within camu camu is influenced by factors such as fruit composition, ripeness, and postharvest processing methods. The camu camu flour used in this study was obtained through a comprehensive procedure involving handpicked fruits subjected to fluidized bed drying at temperatures below 60 °C, followed by the fruits being finely ground into a powder and packaged under controlled conditions to

safeguard their purity. Regarding the results provided by other authors, Genovese et al. (2008) reported a concentration of 19.92 mg GAE/g in commercial camu camu fruit samples. Similarly, Chirinos et al. (2010) observed varying polyphenolic content among different color stages of camu camu fruit, with concentrations ranging from 12.42 to 15.74 mg GAE/g [41]. Conversely, often overlooked seeds showcased a notably elevated phenolic content of 3.36 mg GAE/g, underscoring their potential as a concentrated source of valuable compounds. Fidelis, de Oliveira, et al., (2020) extended to seed extracts, documenting values ranging from 6.57 mg GAE/g to 54.20 mg GAE/g [42].

Furthermore, Fracassetti et al. (2013) noted substantial differences in TSP content among various components of camu camu. Specifically, processed derivatives such as flour exhibited a higher concentration of phenolics at 6.73 mg GAE/g, whereas less processed forms like pulp powder contained significantly lower levels at 0.49 mg GAE/g. Despite being rich in phytonutrients, the peel displayed a relatively modest phenolic content of 0.11 mg GAE/g, whereas fresh pulp exhibited 0.09 mg GAE/g. Therefore, this aspect must be taken into account in order to consider the potential of industrialization.

3.3. Storage stability

This study evaluated the stability of EAPG-dried CCX and CCX microencapsulates under various storage conditions. Microcapsules were exposed to diverse relative humidity (0%, 23%, and 56%) and temperatures (4 °C and 21 °C) over 40 days. An accelerated aging treatment was conducted to assess photo-oxidation stability by subjecting the microcapsules to UV light irradiation. During this storage time, particle size and morphology revealed no significant changes in the obtained samples. TSPs and DPPH were used to quantify the stability of the bioactive, whereas ATR-FTIR was used to provide a qualitative description of the chemical changes that occurred under the studied storing conditions.

3.3.1. Polyphenol Content Stability

Throughout the study, TSP evolution was monitored for each formulation to indicate its storage stability. The EAPG-dried CCX exhibited a time-dependent reduction in TSPs throughout the 40-day storage period (Figure 3A). Significant differences ($p < 0.05$) were observed among the storage conditions. Microcapsules stored under low humidity and temperature (0% RH, 4 °C) displayed the lowest TSP reduction. Conversely, exposure to UV light irradiation or higher relative humidity (23% and 56% RH) and moderate temperature (21 °C) substantially reduced TSP. This observation is consistent with prior scientific investigations emphasizing the vulnerability of polyphenols to temperature (Alean et al., 2016) and photodegradation when exposed to UV light (Lavelli et al., 2017) and humidity (Escobar et al., 2018; Moratalla-López et al., 2019).

Regarding the encapsulates, the WPC-CCX 1:1 ratio microparticles (Figure 3B) displayed significant TSP decay across all tested storage conditions throughout the 40 days, although to a lesser extent than EAPG-dried CCX. Conversely, WPC-CCX microparticles at a 2:1 ratio (Figure 3C) demonstrated substantial TSP retention under most conditions, except for the high humidity (56% RH) experiment, where significant differences in TSP decay occurred.

In contrast, ZN-CCX microparticles at both 1:1 and 2:1 ratios (Figure 3D,E, respectively) exhibited superior TSP retention under most storage conditions, highlighting the efficiency of ZN in polyphenol protection. However, ZN encapsulation at a 1:1 ratio was seen to be less effective in TSP protection under high humidity, as evidenced by a significant decrease in TSP content at 56% RH. Therefore, the encapsulation with ZN at a 2:1 ratio exhibited the best preservation even under high relative humidity and UV light.

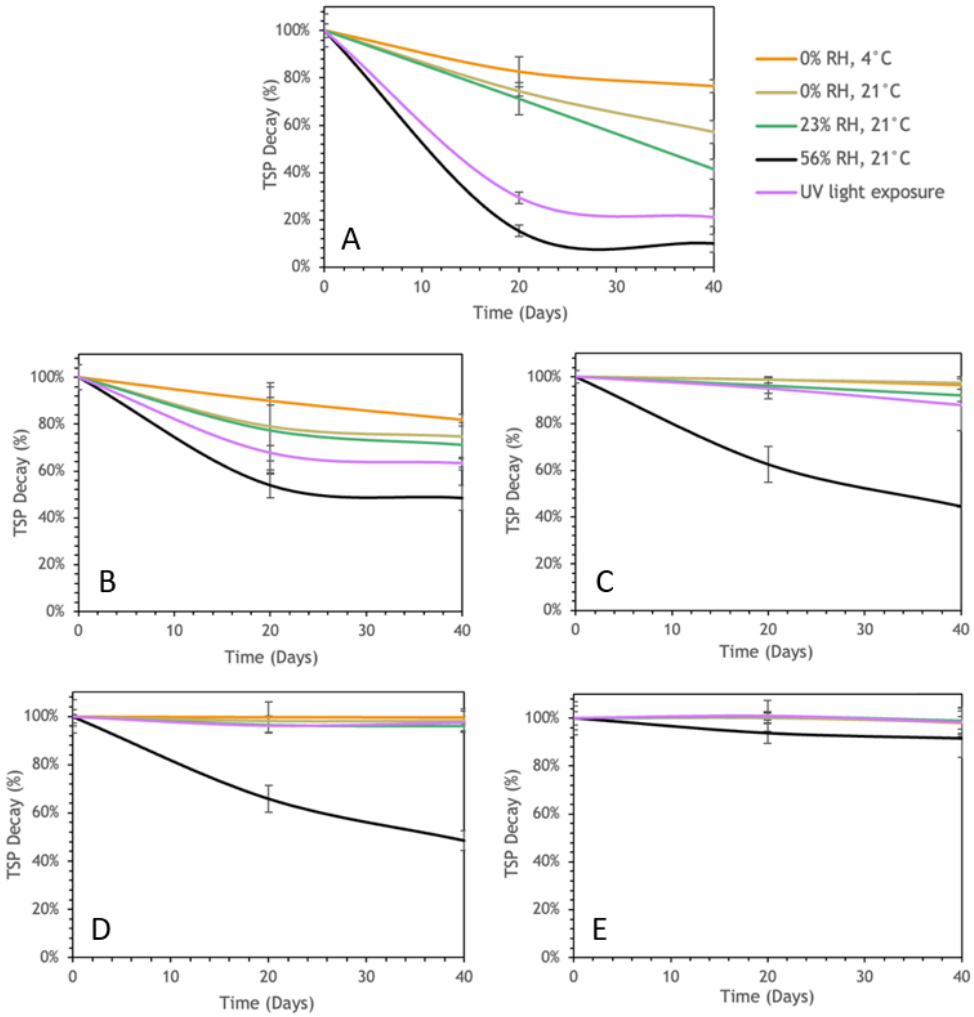

Figure 3. Evolution of the TSP Decay of CCX EAPG-derived microcapsules over 40 days under diverse storage conditions. (A) EAPG dried CCX; (B) WPC—CCX 1:1 w/w; (C) WPC—CCX 2:1 w/w; (D) ZN—CCX 1:1 w/w; (E) ZN—CCX 2:1 w/w.

3.3.2. IP-DPPH Storage Stability

This study investigated the stability of the antioxidant activity measured as DPPH inhibition in the EAPG-dried CCX and CCX microencapsulates under the studied storage conditions, including exposure to UV light as an accelerated photooxidation treatment (Figure 4).

Under low humidity conditions (0% RH), regardless of temperature, the dried CCX (Figure 4A) showed less than 20% decay in DPPH inhibition over the 40 days. Conversely, exposure to higher humidity levels (23% and 56%) or UV light resulted in a notable decrease in DPPH inhibition, up to approximately 30%. The effect of the high relative humidity and UV light on the stability of the antioxidant activity is consistent with the observations reported for dragon blood sap (Escobar et al., 2018) and curcumin (Niu et al., 2012).

Regarding the formulations of the encapsulates, it was found that the same conditions affected the stability of the antioxidant activity. However, the encapsulant materials helped to decrease the observed decay. The microcapsules with a higher encapsulating material ratio (2:1) offered better protection against degradation compared to those with a lower ratio (1:1). WPC-CCX microcapsules displayed less DPPH inhibition compared to ZN-CCX microcapsules, suggesting that ZN provides better protection against the degradation of antioxidant activity. This is consistent with the stability results for TSPs and with a previous work analyzing the stability of dragon's blood sap against different storage conditions (Escobar-García et al., 2023).

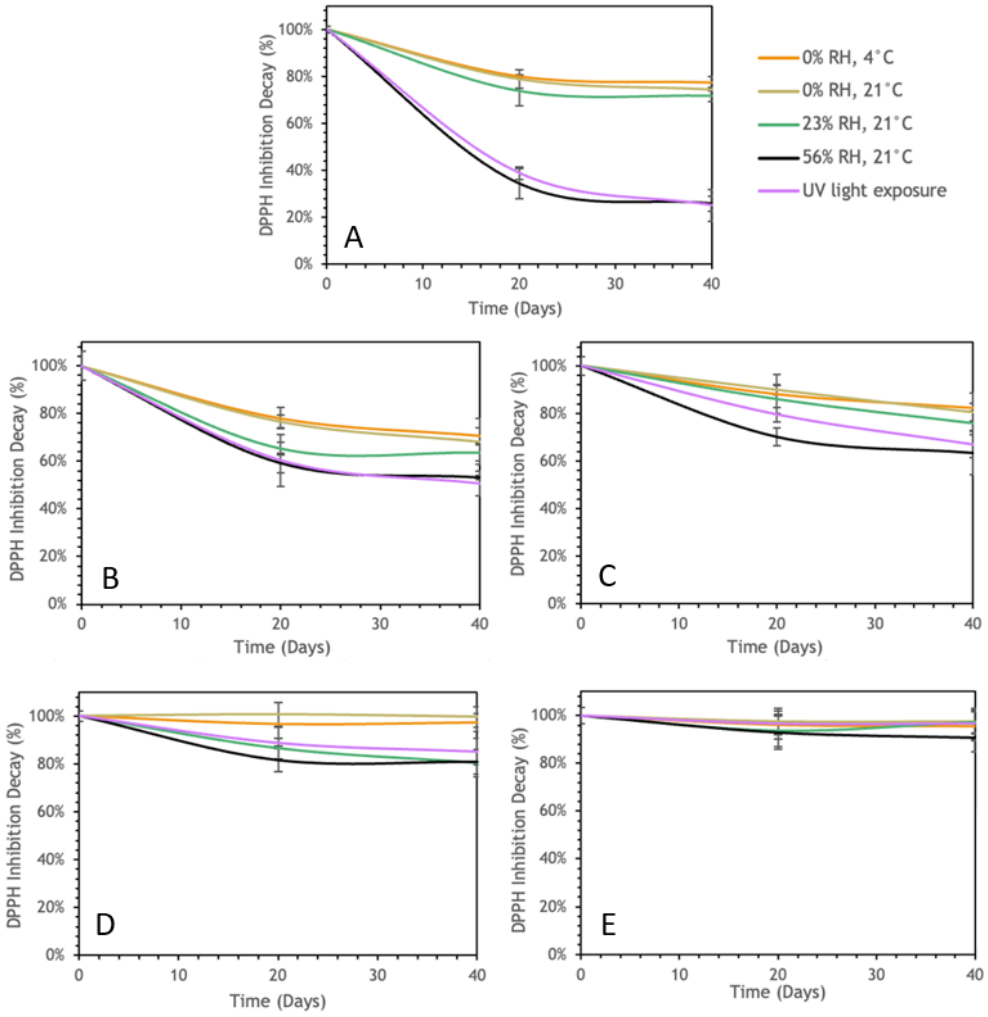

Figure 4. Evolution of the DPPH inhibition Decay of CCX EAPG-derived microcapsules over 40 days under diverse storage conditions. (A) Non-encapsulated CCX; (B) WPC—CCX 1:1 w/w; (C) WPC—CCX 2:1 w/w; (D) ZN—CCX 1:1 w/w; (E) ZN—CCX 2:1 w/w.

3.4. ATR-FTIR analysis

Attenuated total reflection–Fourier transform infrared spectroscopy (ATR-FTIR) analysis of the EAPG-dried CCX is shown in Figure 5. The band envelope at ca. 3260 cm^{-1} is primarily associated with O-H stretching vibrations, a chemical group present in the main bioactive compounds of camu camu, such as citric acid, ascorbic acid, or phenolic compounds(Escobar et al., 2018; Lavelli et al., 2017), but that may also be associated with residual moisture or the residual ethanol from the extraction process. The band at ca. 2920 cm^{-1} is attributed to alkyl -C-H stretching vibrations (Bensemmane et al., 2022). The band at around 1675 cm^{-1} is assigned to the carbonyl group (Merlic, 2012), a common chemical group in the bioactive compounds prevalent in camu camu (Akter et al., 2011; Santos et al., 2022). The band at ca. 1600 cm^{-1} is associated with alkenyl groups also characteristic of the main bioactive molecules present in camu camu (Abbas et al., 2017; Zanatta et al., 2005). The spectrum also features a band at ca. 1440 cm^{-1} linked to -CH deformation and aromatic ring vibrations (Schulz & Baranska, 2007), also characteristics of some of the bioactive molecules of camu camu such as flavonoids and proanthocyanidins (García-Chacón et al., 2024; Grigio et al., 2021). The band at around 1338 cm^{-1} is related to C-C bending vibrations, while the band envelope from 1300 to 1000 cm^{-1} is associated with C-O stretching vibrations related to ether, esters, and alcohol groups, even aromatic alcohols also present in the characteristic bioactive compounds of camu camu such as ascorbic acid, flavonoids, and proanthocyanidins (Schulz & Baranska, 2007). Lastly, out-of-plane C-H vibrations from 900 to 700 cm^{-1} provide insights into the aliphatic hydrocarbon composition of the CCX (de Abreu Figueiredo et al., 2020; Merlic, 2012).

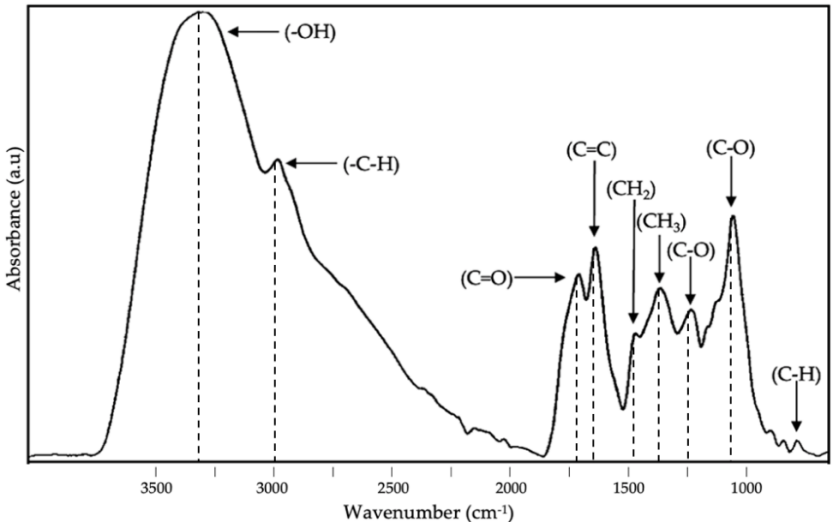

Figure 5. Attenuated total reflection—Fourier transform infrared spectroscopy (ATR-FTIR) of EAPG dried CCX.

The main characteristic bands of the camu camu dried extract were defined, and ATR-FTIR was used to perform a qualitative analysis to determine the storage stability of the different formulations. Figure 6 illustrates the ATR-FTIR spectral evolution of the EAPG-dried CCX microparticles after 40 days under the studied storage conditions. Significant changes in the spectrum were primarily observed in the 1800–800 cm^{-1} wavenumber range, which could indicate CCX degradation. The spectra were maximized to their highest intensity for clearer comparisons to compare relative changes among bands. After a 40-day storage period, the most significant changes in the dried CCX were observed when samples were subjected to high humidity conditions and exposure to UV light (as depicted in Figures 6E,F). These were mainly detected in the band envelopes at ca. 1800–1600 cm^{-1} and at ca. 1200–900 cm^{-1}. The first band envelope is ascribed to the carbonyl stretching absorption, which showed a decrease in the number of carboxylic acid groups and an increase in the number of ketone and aldehyde groups, which is aligned with the reported reactions of oxidation of the main bioactive

compounds in camu camu such as citric acid, ascorbic acid, flavonoids, and proanthocyanidins (García-Chacón et al., 2024; Grigio et al., 2021). Regarding the second band envelope at ca. 1200–900 cm^{-1}, this is associated with the stretching vibration of the C-O bond. The observed changes in band position and intensity variation were due to the formation of linear and aromatic ether groups due to the oxidation reactions of the bioactive compounds present in the dried CCX (de Abreu Figueiredo et al., 2020; Merlic, 2012).

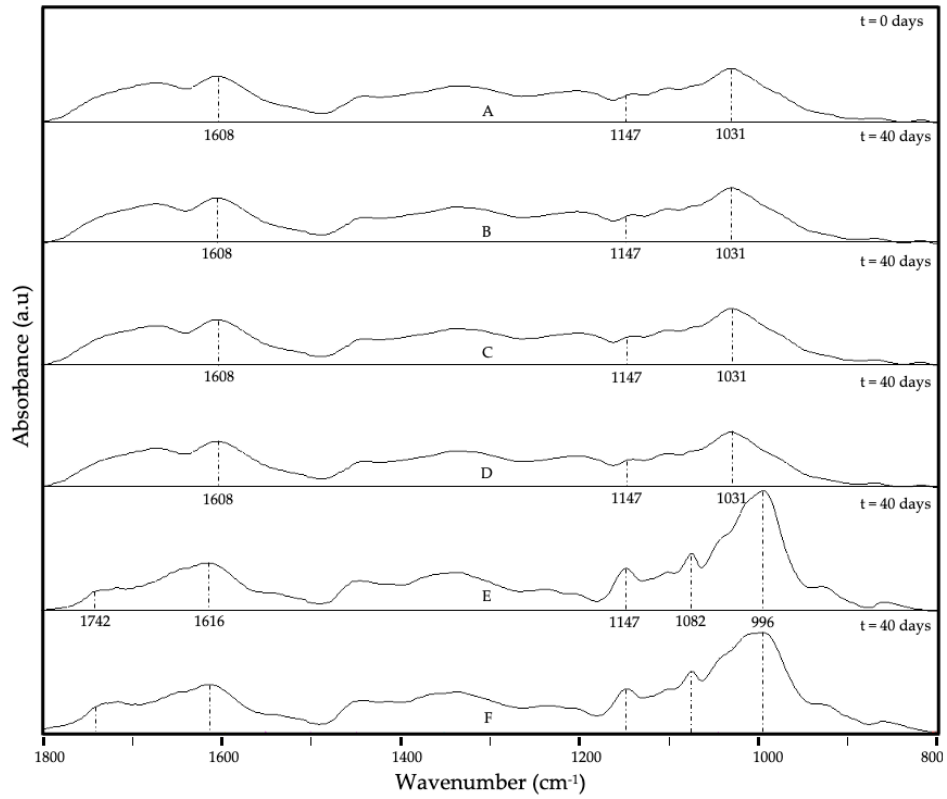

Figure 6. Spectral evolution of Attenuated total reflection—Fourier transform infrared spectroscopy (ATR- FTIR) for the EAPG dried CCX stored under various conditions (A) 0 days; (B) 40 days, 0% RH, 4°C; (C) 40 days, 0% RH, 21°C; (D) 40 days, 23% RH, 21°C; (E) 40 days, 56% RH, 21°C; (F) 40 days, UV light exposure. The spectra were maximized to the band with the highest intensity in the wavelength range between 1800 and 800 cm^{-1}.

Regarding the encapsulates, Figures 7 and 8 provide the evolution of the ATR-FTIR spectra at the different storage conditions for the system WPC-CCX at 1:1 and 2:1 ratios, respectively. In contrast, Figures 9 and 10 represent the spectra of the system ZN-CCX at 1:1 and 2:1 ratios, respectively. Both encapsulant matrices have a protein nature, and consequently, similar characteristic bands were detected; notably, the band located at ca. 1740 cm^{-1} ascribed to the trenching vibration of the C=O bond, the band at ca. 1632 cm^{-1} and at ca. 1520 cm^{-1} ascribed to the bending vibration of the N-H bond of amides I and II, respectively (Andrade et al., 2019; He et al., 2016).

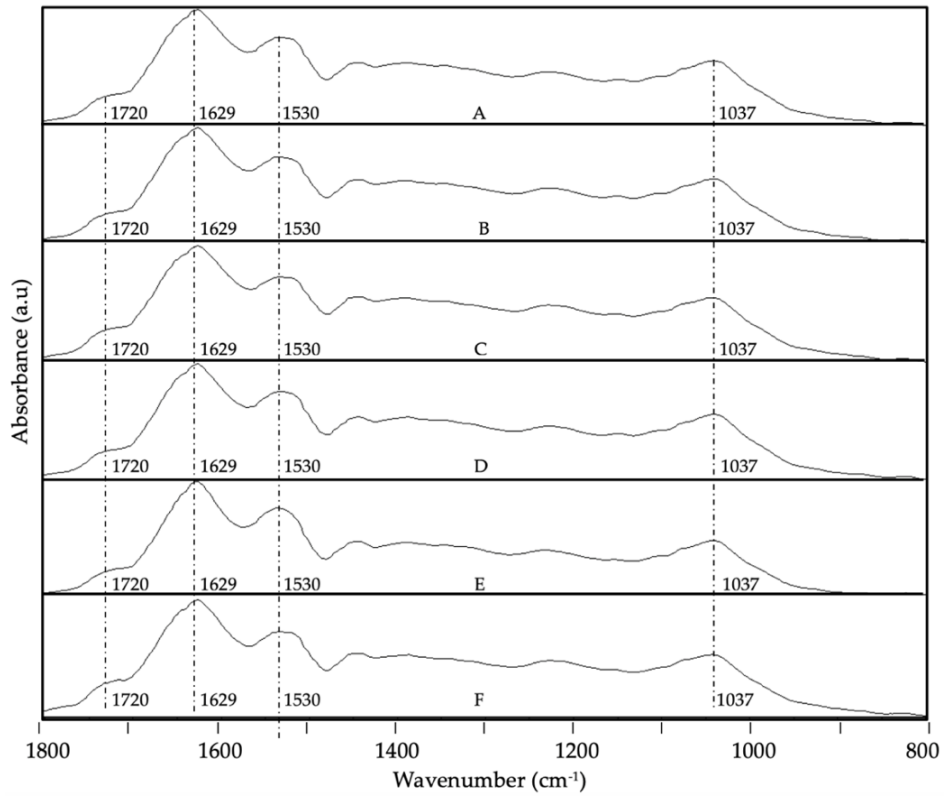

Figure 7. Spectral Evolution of Attenuated total reflection—Fourier transform infrared spectroscopy (ATR- FTIR) for WPC-CCX 1:1 w/w EAPG-derived microcapsules stored under various conditions (A) 0 days; (B) 40 days, 0% RH, 4°C; (C) 40 days, 0% RH, 21°C; (D) 40 days, 23% RH, 21°C; (E) 40 days, 56% RH, 21°C; (F) 40 days, UV light exposure. The spectra were maximized to the band with the highest intensity in the wavelength range between 1800 and 800 cm^{-1}.

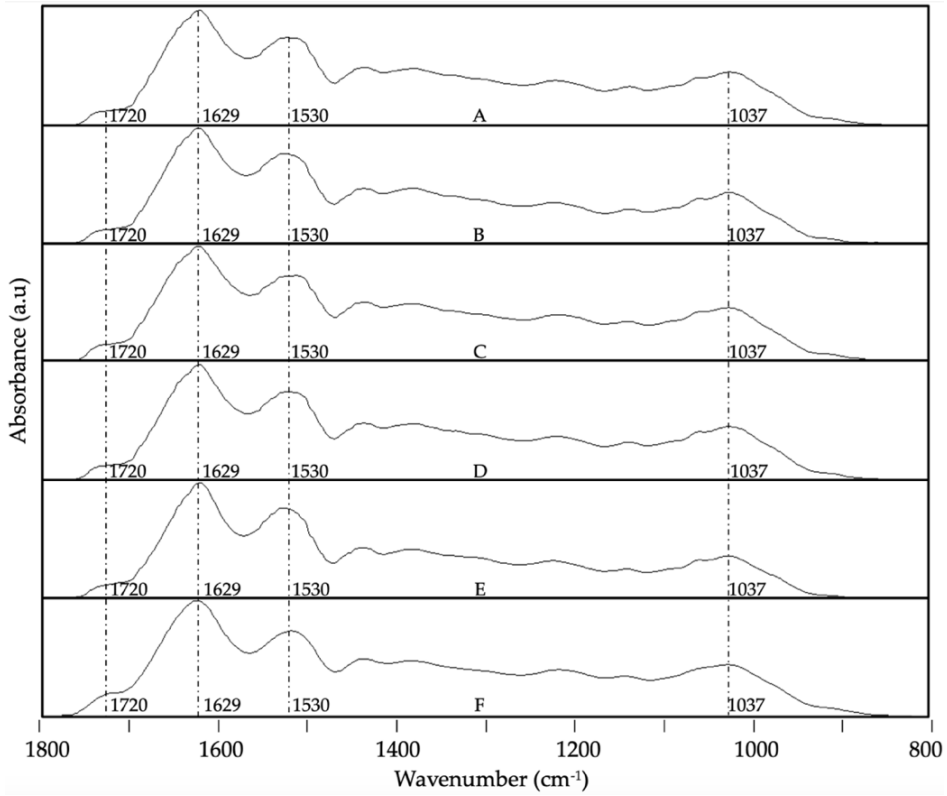

Figure 8. Spectral Evolution of Attenuated total reflection—Fourier transform infrared spectroscopy (ATR- FTIR) for WPC-CCX 2:1 w/w EAPG-derived microcapsules stored under various conditions (A) 0 days; (B) 40 days, 0% RH, 4°C; (C) 40 days, 0% RH, 21°C; (D) 40 days, 23% RH, 21°C; (E) 40 days, 56% RH, 21°C; (F) 40 days, UV light exposure. The spectra were maximized to the band with the highest intensity in the wavelength range between 1800 and 800 cm^{-1}.

The ATR-FTIR spectroscopy analysis of WPC-CCX encapsulates revealed small shifts in the spectral evolution. The most significant changes were observed when samples were subjected to high humidity (Figures 7E and 8E) and UV light (Figures 7F and 8F), as an intensity variation in the band envelope between 1600 and 1800 cm^{-1} as a consequence of the formation of aldehydes, ketones, and esters as oxidation products of the bioactive compounds of camu camu (Dangles & Fenger, 2018; Karonen et al., 2021; Kuyper, 1933; Speisky et al., 2022; Tu et al., 2017) and a small decrease in the band associated with the secondary amine at ca 1520 cm^{-1}, probably due to a slight degradation of the protein. The band envelope between

1200 and 900 cm^{-1} did not show significant differences, probably because the changes in the spectra might be hidden by the bands of the protein. No significant differences were detected for the ratios studied.

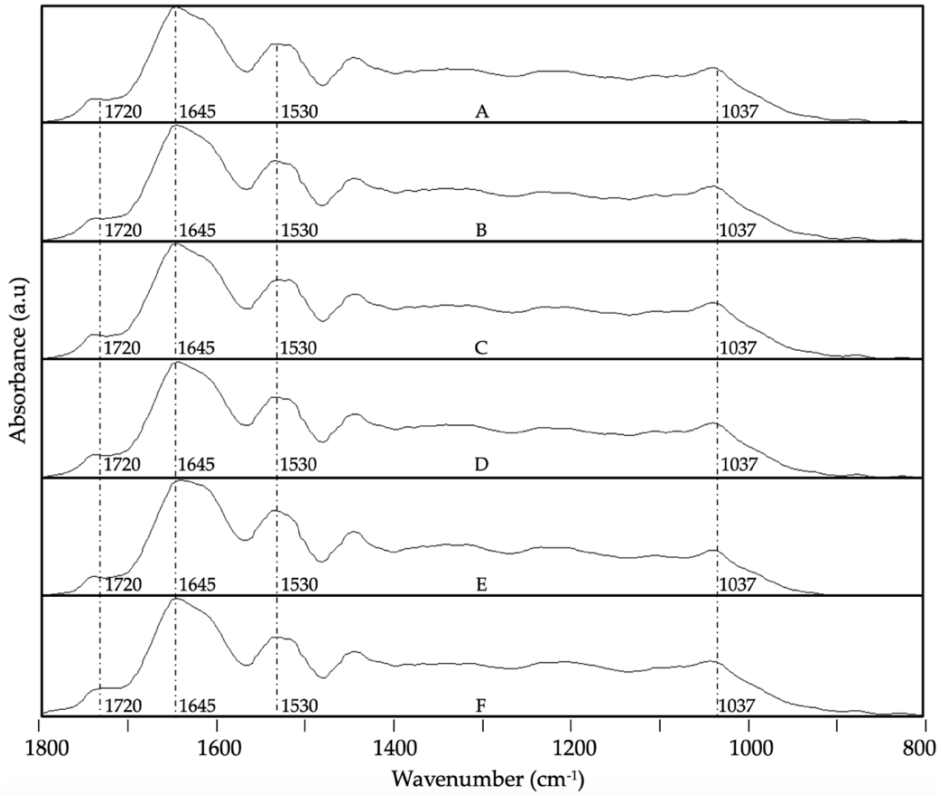

Figure 9. Spectral Evolution of Attenuated total reflection—Fourier transform infrared spectroscopy (ATR- FTIR) for ZN-CCX 1:1 w/w EAPG-derived microcapsules stored under various conditions (A) 0 days; (B) 40 days, 0% RH, 4°C; (C) 40 days, 0% RH, 21°C; (D) 40 days, 23% RH, 21°C; (E) 40 days, 56% RH, 21°C; (F) 40 days, UV light exposure. The spectra were maximized to the band with the highest intensity in the wavelength range between 1800 and 800 cm^{-1}.

The spectral evolution of ATR-FTIR for ZN-CCX encapsulates at 1:1 and 2:1 encapsulation ratios is shown in Figures 9 and 10, respectively. After 40 days of experimentation, slight changes become discernible at high relative humidity and under UV light radiation. Main variations were detected in the 1800–1600 cm^{-1} band envelope as a consequence of the increase in aldehyde and ketone as ester groups due to the progression of the oxidation reactions (Dangles & Fenger,

2018; Karonen et al., 2021; Kuyper, 1933; Speisky et al., 2022; Tu et al., 2017) and a slight decrease in intensity in the band related to the amide II of the protein. The band envelope between 1200 and 900 cm^{-1} did not show significant differences, probably because the changes in the spectra might be hidden by the bands of the protein. The ZN-CCX 2:1 encapsulation ratio (Figure 10) exhibits fewer changes than the 1:1 encapsulates, results that agree with the observations made in previous sections.

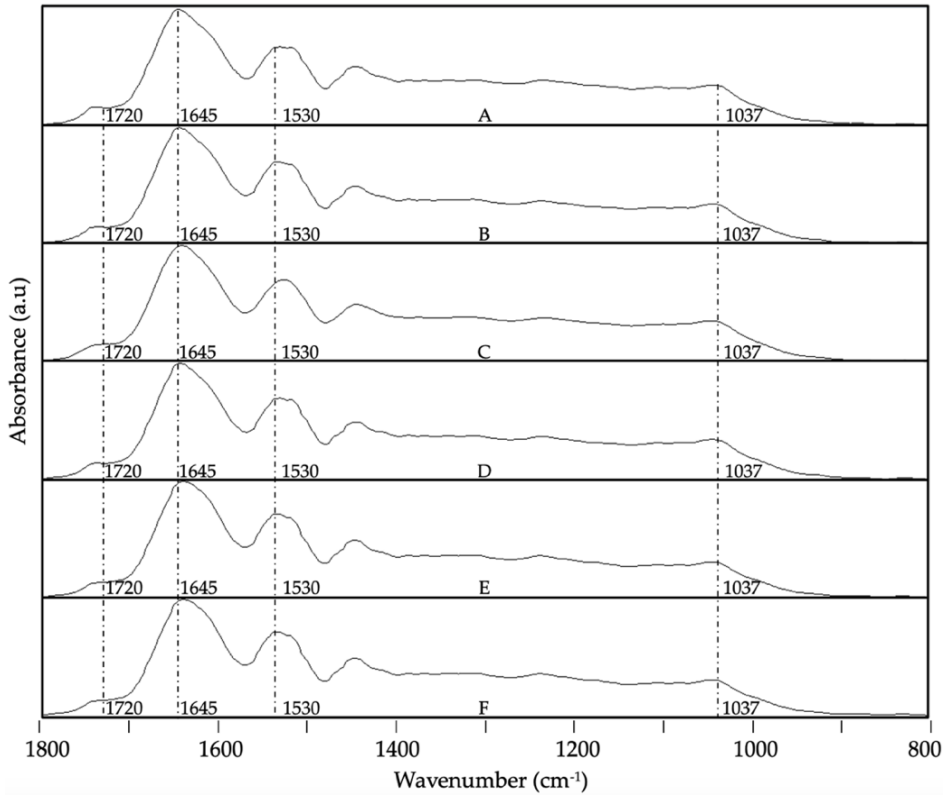

Figure 10. Spectral Evolution of Attenuated total reflection—Fourier transform infrared spectroscopy (ATR- FTIR) for ZN-CCX 2:1 w/w EAPG-derived microcapsules stored under various conditions (A) 0 days; (B) 40 days, 0% RH, 4°C; (C) 40 days, 0% RH, 21°C; (D) 40 days, 23% RH, 21°C; (E) 40 days, 56% RH, 21°C; (F) 40 days, UV light exposure. The spectra were maximized to the band with the highest intensity in the wavelength range between 1800 and 800 cm^{-1}.

4. Conclusions

This study demonstrates for the first time the impact on the stability of the electrohydrodynamic EAPG technique to dry or encapsulate camu camu extract (CCX) at room temperature. The resulting microparticles exhibit favorable attributes for application in food products, effectively preserving the bioactive properties of CCX and maintaining consistent levels of total soluble polyphenols (TSPs) and antioxidant activity (DPPH inhibition). The comparative analysis conducted in this study demonstrates the superior effectiveness of the encapsulates, especially of ZN as an encapsulation material compared to WPC, in protecting the bioactive properties of camu camu when subjected to challenging storage conditions. The encapsulation system with a 2:1 ratio also demonstrated a markedly reduced degree of degradation compared to its 1:1 counterpart, particularly in environments with high humidity and UV light exposure. Considering the outcome of this work, it is possible to state that the EAPG technique stands out as a versatile and promising approach for creating small particle size and size distribution powders and encapsulated powders of camu camu. The obtained results show the potential of this technology to positively impact the existing drying and encapsulation procedures, establishing a new standard to guarantee the stability and effectiveness of bioactive compounds across various applications, such as functional foods, cosmetics, agricultural inputs, or nutraceutical products.

Acknowledgments

J.D.E.G. would like to thank the Spanish Ministry of Economy, Industry, and Competitivity for his predoctoral grant (DI-6906). C.P. would like to thank the Valencian Ministry of Innovation, Uni-versities, Science, and Digital Society for her postdoctoral grant (CIAPOS/2021/45). The Accredi-tation as Center of Excellence Severo Ochoa CEX2021-001189-S funded by MCIU/AEI/10.13039/501100011033

is also fully acknowledged. The authors thank Alberto Rodríguez Escrivá and Alexandra Sánchez for their technical assistance.

References

A FIGUEIREDO, J., MT LAGO, A., M MAR, J., S SILVA, L., A SANCHES, E., P SOUZA, T., A BEZERRA, J., H CAMPELO, P., A BOTREL, D., & V BORGES, S. (2020). Stability of camu camu encapsulated with different prebiotic biopolymers. Journal of the Science of Food and Agriculture, 100(8), 3471–3480. https://doi.org/10.1002/jsfa.10384

ABBAS, O., COMPÈRE, G., LARONDELLE, Y., POMPEU, D., ROGEZ, H., & BAETEN, V. (2017). Phenolic compound explorer: A mid-infrared spectroscopy database. Vibrational Spectroscopy, 92, 111–118. https://doi.org/10.1016/J.VIBSPEC.2017.05.008

AKTER, M. S., OH, S., EUN, J. B., & AHMED, M. (2011). Nutritional compositions and health promoting phytochemicals of camu-camu (Myrciaria dubia) fruit: A review. Food Research International, 44(7), 1728–1732. https://doi.org/10.1016/J.FOODRES.2011.03.045

ALEAN, J., CHEJNE, F., & ROJANO, B. (2016). Degradation of polyphenols during the cocoa drying process. Journal of Food Engineering, 189, 99–105. https://doi.org/10.1016/J.JFOODENG.2016.05.026

ALU'DATT, M. H., ALROSAN, M., GAMMOH, S., TRANCHANT, C. C., ALHAMAD, M. N., RABABAH, T., ZGHOUL, R., ALZOUBI, H., GHATASHEH, S., GHOZLAN, K., & TAN, T. C. (2022). Encapsulation-based technologies for bioactive compounds and their application in the food industry: A roadmap for food-derived functional and health-promoting ingredients. In Food Bioscience (Vol. 50). https://doi.org/10.1016/j.fbio.2022.101971

ANDRADE, J., PEREIRA, C. G., ALMEIDA JUNIOR, J. C. DE, VIANA, C. C. R., NEVES, L. N. DE O., SILVA, P. H. F. DA, BELL, M. J. V., & ANJOS, V. DE C. DOS. (2019). FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. LWT, 99, 166–172. https://doi.org/10.1016/J.LWT.2018.09.079

ANHÊ, F. F., NACHBAR, R. T., VARIN, T. V., TROTTIER, J., DUDONNÉ, S., LE BARZ, M., FEUTRY, P., PILON, G., BARBIER, O., DESJARDINS, Y., ROY, D., & MARETTE, A. (2019).Treatment with camu camu (Myrciaria dubia) prevents obesity by altering the gut microbiota and increasing energy expenditure in diet-induced obese mice. Gut, 68(3), 453–464. https://doi.org/10.1136/gutjnl-2017-315565

BENSEMMANE, N., BOUZIDI, N., DAGHBOUCHE, Y., GARRIGUES, S., DE LA GUARDIA, M., & HATTAB, M. EL. (2022). Prediction of total phenolic acids contained in plant extracts by PLS-ATR-FTIR. South African Journal of Botany, 151, 295–305. https://doi.org/10.1016/J.SAJB.2022.10.009

BUSOLO, M. A., TORRES-GINER, S., PRIETO, C., & LAGARON, J. M. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the

microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. Innovative Food Science and Emerging Technologies, 51, 12–19.

https://doi.org/10.1016/j.ifset.2018.04.007

CARMO, M. A. V. DO, FIDELIS, M., PRESSETE, C. G., MARQUES, M. J., CASTRO-GAMERO, A. M., MYODA, T., GRANATO, D., & AZEVEDO, L. (2019). Hydroalcoholic Myrciaria dubia (camu-camu) seed extracts prevent chromosome damage and act as antioxidant and cytotoxic agents. Food Research International, 125, 108551. https://doi.org/10.1016/J.FOODRES.2019.108551

CASTRO, J. C., MADDOX, J. D., COBOS, M., & IMÁN, S. A. (2018). Myrciaria dubia "Camu Camu" Fruit: Health-Promoting Phytochemicals and Functional Genomic Characteristics. In Breeding and Health Benefits of Fruit and Nut Crops. InTech. https://doi.org/10.5772/intechopen.73213

CHIRINOS, R., GALARZA, J., BETALLELUZ-PALLARDEL, I., PEDRESCHI, R., & CAMPOS, D. (2010). Antioxidant compounds and antioxidant capacity of Peruvian camu camu (Myrciaria dubia (H.B.K.) McVaugh) fruit at different maturity stages. Food Chemistry, 120(4), 1019–1024. https://doi.org/10.1016/J.FOODCHEM.2009.11.041

CHRISTENSEN, L. P. (2018). The role of direct and indirect polyphenolic antioxidants in protection against oxidative stress. In Polyphenols: Mechanisms of Action in Human Health and Disease (2nd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813006-3.00013-1

DAHIYA, D., TERPOU, A., DASENAKI, M., & NIGAM, P. S. (2023). Current status and future prospects of bioactive molecules delivered through sustainable encapsulation techniques for food fortification. In Sustainable Food Technology (Vol. 1, Issue 4, pp. 500–510). Royal Society of Chemistry. https://doi.org/10.1039/d3fb00015j

DANGLES, O., & FENGER, J. A. (2018). The chemical reactivity of anthocyanins and its consequences in food science and nutrition. In Molecules (Vol. 23, Issue 8). MDPI AG. https://doi.org/10.3390/molecules23081970

DE ABREU FIGUEIREDO, J., ANDRADE TEIXEIRA, M., HENRIQUE CAMPELO, P., MARIA TEIXEIRA LAGO, A., PEREIRA DE SOUZA, T., IRENE YOSHIDA, M., RODRIGUES DE OLIVEIRA, C., PAULA APARECIDA PEREIRA, A., MARIA PASTORE, G., APARECIDO SANCHES, E., ALVARENGA BOTREL, D., & VILELA BORGES, S. (2020). Encapsulation of camu-camu extracts using prebiotic biopolymers: Controlled release of bioactive compounds and effect on their physicochemical and thermal properties. Food Research International, 137. https://doi.org/10.1016/j.foodres.2020.109563

DE AZEVÊDO, J. C. S., FUJITA, A., DE OLIVEIRA, E. L., GENOVESE, M. I., & CORREIA, R. T. P. (2014). Dried camu-camu (Myrciaria dubia H.B.K. McVaugh) industrial residue: A bioactive-rich Amazonian powder with functional attributes. Food Research International, 62, 934–940. https://doi.org/10.1016/J.FOODRES.2014.05.018

DE MELLO ANDRADE, J. M., & FASOLO, D. (2013). Polyphenol Antioxidants from Natural Sources and Contribution to Health Promotion. In Polyphenols in Human Health and Disease (Vol. 1). Elsevier Inc. https://doi.org/10.1016/B978-0-12-398456-2.00020-7

ESCOBAR, J. D., PRIETO, C., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2018). Dragon's blood sap: Storage stability and antioxidant activity. Molecules, 23(10). https://doi.org/10.3390/molecules23102641

ESCOBAR-GARCÍA, J. D., PRIETO, C., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2021). Room temperature nanoencapsulation of bioactive eicosapentaenoic acid rich oil within whey protein microparticles. Nanomaterials, 11(3), 1–16. https://doi.org/10.3390/nano11030575

ESCOBAR-GARCÍA, J. D., PRIETO, C., PARDO-FIGUEREZ, M., & LAGARON, J. M. (2023). Dragon's Blood Sap Microencapsulation within Whey Protein Concentrate and Zein Using Electrospraying Assisted by Pressurized Gas Technology. Molecules, 28(10). https://doi.org/10.3390/molecules28104137

FAO, & WHO. (2022). Ranking of low-moisture foods in support of microbiological risk management: Meeting report and systematic review. Ranking of Low-Moisture Foods in Support of Microbiological Risk Management: Meeting Report and Systematic Review. https://doi.org/10.4060/CC0763EN 0

FIDELIS, M., DE OLIVEIRA, S. M., SOUSA SANTOS, J., BRAGUETO ESCHER, G., SILVA ROCHA, R., GOMES CRUZ, A., ARAÚJO VIEIRA DO CARMO, M., AZEVEDO, L., KANESHIMA, T., OH, W. Y., SHAHIDI, F., & GRANATO, D. (2020). From byproduct to a functional ingredient: Camu-camu (Myrciaria dubia) seed extract as an antioxidant agent in a yogurt model. Journal of Dairy Science, 103(2), 1131–1140.https://doi.org/10.3168/jds.2019-17173

FIDELIS, M., DO CARMO, M. A. V., DA CRUZ, T. M., AZEVEDO, L., MYODA, T., MIRANDA FURTADO, M., BOSCACCI MARQUES, M., SANT'ANA, A. S., INÊS GENOVESE, M., YOUNG OH, W., WEN, M., SHAHIDI, F., ZHANG, L., FRANCHIN, M., DE ALENCAR, S. M., LUIZ ROSALEN, P., & GRANATO, D. (2020). Camu-camu seed (Myrciaria dubia) – From sidestream to an antioxidant, antihyperglycemic, antiproliferative, antimicrobial, antihemolytic, anti-inflammatory, and antihypertensive ingredient. Food Chemistry, 310. https://doi.org/10.1016/j.foodchem.2019.125909

FIDELIS, M., SANTOS, J. S., ESCHER, G. B., VIEIRA DO CARMO, M., AZEVEDO, L., CRISTINA DA SILVA, M., PUTNIK, P., & GRANATO, D. (2018). In vitro antioxidant and antihypertensive compounds from camu-camu (Myrciaria dubia McVaugh, Myrtaceae) seed coat: A multivariate structure-activity study. Food and Chemical Toxicology, 120, 479–490. https://doi.org/10.1016/J.FCT.2018.07.043

FRACASSETTI, D., COSTA, C., MOULAY, L., & TOMÁS-BARBERÁN, F. A. (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (Myrciaria dubia). Food Chemistry, 139(1–4), 578–588. https://doi.org/10.1016/j.foodchem.2013.01.121

FUJITA, A., SARKAR, D., GENOVESE, M. I., & SHETTY, K. (2017). Improving antihyperglycemic and anti-hypertensive properties of camu-camu (Myriciaria dubia Mc. Vaugh) using lactic acid bacterial fermentation. Process Biochemistry, 59, 133– 140. https://doi.org/10.1016/J.PROCBIO.2017.05.017

FUJITA, A., SARKAR, D., WU, S., KENNELLY, E., SHETTY, K., & GENOVESE, M. I. (2015). Evaluation of phenolic-linked bioactives of camu-camu (Myrciaria dubia Mc. Vaugh)

for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation. Food Research International, 77, 194–203.

https://doi.org/10.1016/j.foodres.2015.07.009

GARCÍA-CHACÓN, J. M., RODRÍGUEZ-PULIDO, F. J., HEREDIA, F. J., GONZÁLEZ-MIRET, M. L., & OSORIO, C. (2024). Characterization and bioaccessibility assessment of bioactive compounds from camu-camu (Myrciaria dubia) powders and their food applications. Food Research International, 176. https://doi.org/10.1016/j.foodres.2023.113820

GENOVESE, M. I., DA SILVA PINTO, M., DE SOUZA SCHMIDT GONÇALVES, A. E., & LAJOLO, F. M. (2008). Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. Food Science and Technology International, 14(3), 207–214. https://doi.org/10.1177/1082013208092151

GÓMEZ-MASCARAQUE, L. G., & LOPEZ-RUBIO, A. (2019). Production of food bioactive loaded nanoparticles by electrospraying. Nanoencapsulation of Food Ingredients by Specialized Equipment: Volume 3 in the Nanoencapsulation in the Food Industry Series, 107–149. https://doi.org/10.1016/B978-0-12-815671-1.00003-2

GRIGIO, M. L., DE MOURA, E. A., CHAGAS, E. A., DURIGAN, M. F. B., CHAGAS, P. C., DE CARVALHO, G. F., & ZANCHETTA, J. J. (2021). Bioactive compounds in and antioxidant activity of camu-camu fruits harvested at different maturation stages during postharvest storage. Acta Scientiarum - Agronomy, 43. https://doi.org/10.4025/ACTASCIAGRON.V43I1.50997

GULDIKEN, B., GULSUNOGLU, Z., BAKIR, S., CATALKAYA, G., CAPANOGLU, E., & NICKERSON, M. (2021). Innovations in functional foods development. Food Technology Disruptions, 73–130. https://doi.org/10.1016/B978-0-12-821470-1.00008-2

HE, Z., XU, M., ZENG, M., QIN, F., & CHEN, J. (2016). Interactions of milk α- And β-casein with malvidin-3-O-glucoside and their effects on the stability of grape skin anthocyanin extracts. Food Chemistry, 199, 314–322

https://doi.org/10.1016/J.FOODCHEM.2015.12.035

INOUE, T., KOMODA, H., UCHIDA, T., & NODE, K. (2008). Tropical fruit camu-camu (Myrciaria dubia) has anti-oxidative and anti-inflammatory properties. Journal of Cardiology, 52(2), 127–132. https://doi.org/10.1016/J.JJCC.2008.06.004

JEEVANI, M. M., WIJEKOON, O., MAHMOOD, K., ARIFFIN, F., NAFCHI, A. M., & ZULKURNAIN, M. (2023). Recent advances in encapsulation of fat-soluble vitamins using polysaccharides, proteins, and lipids: A review on delivery systems, formulation, and industrial applications. International Journal of Biological Macromolecules, 241, 124539. https://doi.org/10.1016/j.ijbiomac.2023.124539

KARONEN, M., IMRAN, I. BIN, ENGSTRÖM, M. T., & SALMINEN, J. P. (2021). Characterization of natural and alkaline-oxidized proanthocyanidins in plant extracts by ultrahigh-resolution UHPLC-MS/MS. Molecules, 26(7). https://doi.org/10.3390/molecules26071873

KUYPER, A. C. (1933). The Oxidation of Citric Acid. Journal of the American Chemical Society, 55(4), 1722–1727. https://doi.org/10.1021/ja01331a071

LAVELLI, V., SRI HARSHA, P. S. C., LAUREATI, M., & PAGLIARINI, E. (2017). Degradation kinetics of encapsulated grape skin phenolics and micronized grape skins in various water activity environments and criteria to develop wide-ranging and tailor-made food applications. Innovative Food Science & Emerging Technologies, 39, 156–164. https://doi.org/10.1016/J.IFSET.2016.12.006

MARCILLO-PARRA, V., TUPUNA-YEROVI, D. S., GONZÁLEZ, Z., & RUALES, J. (2021). Encapsulation of bioactive compounds from fruit and vegetable by-products for food application – A review. In Trends in Food Science and Technology (Vol. 116). https://doi.org/10.1016/j.tifs.2021.07.009

MERLIC, C. A. (2012). IR Absorption Table. https://webspectra.chem.ucla.edu/irtable.html

MIYASHITA, T., KOIZUMI, R., MYODA, T., SAGANE, Y., NIWA, K., WATANABE, T., & MINAMI, K. (2018). Data on a single oral dose of camu camu (Myrciaria dubia) pericarp extract on flow-mediated vasodilation and blood pressure in young adult humans. Data in Brief, 16, 993–999. https://doi.org/10.1016/j.dib.2017.12.009

MORATALLA-LÓPEZ, N., LORENZO, C., CHAOUQI, S., SÁNCHEZ, A. M., & ALONSO, G. L. (2019). Kinetics of polyphenol content of dry flowers and floral bio-residues of saffron at different temperatures and relative humidity conditions. Food Chemistry, 290, 87–94. https://doi.org/10.1016/J.FOODCHEM.2019.03.129

NEVES, L. C., DE CAMPOS, A. J., CISNEROS-ZEVALLOS, L., COLOMBO, R. C., & ROBERTO, S.R. (2017). Postharvest behavior of camu-camu fruits based on harvesting time and nutraceutical properties. Scientia Horticulturae, 217, 276–284. https://doi.org/10.1016/j.scienta.2017.01.030

NIU, Y., KE, D., YANG, Q., WANG, X., CHEN, Z., AN, X., & SHEN, W. (2012). Temperature-dependent stability and DPPH scavenging activity of liposomal curcumin at pH 7.0. Food Chemistry, 135(3), 1377–1382. https://doi.org/10.1016/J.FOODCHEM.2012.06.018

PRIETO, C., TALÓN, E., NOREÑA, C. Z., & LAGARON, J. M. (2022). Effect of Whey Protein Purity on the Characteristics of Algae Oil-Loaded Encapsulates Obtained by Electrospraying Assisted by Pressurized Gas. Nanomaterials, 12(18). https://doi.org/10.3390/nano12183096

RADDATZ, G. C., & DE MENEZES, C. R. (2021). Microencapsulation and co-encapsulation of bioactive compounds for application in food: challenges and perspectives. Ciência Rural, 51(3), 1–8. https://doi.org/10.1590/0103-8478CR20200616

SANTOS, I. L., MIRANDA, L. C. F., DA CRUZ RODRIGUES, A. M., DA SILVA, L. H. M., & AMANTE, E. R. (2022). Camu-camu [Myrciaria dubia (HBK) McVaugh]: A review of properties and proposals of products for integral valorization of raw material. Food Chemistry, 372, 131290. https://doi.org/10.1016/J.FOODCHEM.2021.131290

SCHULZ, H., & BARANSKA, M. (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy, 43(1), 13–25. https://doi.org/10.1016/J.VIBSPEC.2006.06.001

SHAFAEI, Z., GHALANDARI, B., DIVSALAR, A., & SABOURY, A. A. (2017). Controlled release nutrition delivery based intelligent and targeted nanoparticle. In Nutrient Delivery. Elsevier Inc. https://doi.org/10.1016/b978-0-12-804304-2.00009-3

SINGLETON, V. L., & ROSSI, J. A. (1965). Colorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic., 16, 144–158.

SOBEL, R., VERSIC, R., & GAONKAR, A. G. (2014). Introduction to Microencapsulation and Controlled Delivery in Foods. In Microencapsulation in the Food Industry. https://doi.org/10.1016/b978-0-12-404568-2.00001-7

SPEISKY, H., SHAHIDI, F., COSTA DE CAMARGO, A., & FUENTES, J. (2022). Revisiting the Oxidation of Flavonoids: Loss, Conservation or Enhancement of Their Antioxidant Properties. Antioxidants, 11(1), 133. https://doi.org/10.3390/antiox11010133

THAKUR, M., SINGH, K., & KHEDKAR, R. (2020). Phytochemicals: Extraction process safety assessment, toxicological evaluations, and regulatory issues. Functional and Preservative Properties of Phytochemicals, 341–361. https://doi.org/10.1016/B978-0-12-818593-3.00011-7

TU, Y.-J., NJUS, D., & SCHLEGEL, H. B. (2017). A theoretical study of ascorbic acid oxidation and HOO ˙/ O 2 ˙ − radical scavenging. Organic & Biomolecular Chemistry, 15(20), 4417–4431. https://doi.org/10.1039/C7OB00791D

VERŠIČ, R. J. (2014). Inventing and Using Controlled-Release Technologies Microencapsulation in the Food Industry, 551–555. https://doi.org/10.1016/b978-0-12-404568-2.00042-x

WEIDNER, E. (2009). High pressure micronization for food applications. The Journal of Supercritical Fluids, 47(3), 556–565. https://doi.org/10.1016/J.SUPFLU.2008.11.009

ZANATTA, C. F., CUEVAS, E., BOBBIO, F. O., WINTERHALTER, P., & MERCADANTE, A. Z. (2005). Determination of anthocyanins from Camu-camu (Myrciaria dubia) by HPLC-PDA, HPLC-MS, and NMR. Journal of Agricultural and Food Chemistry, 53(24), 9531–9535. https://doi.org/10.1021/JF051357V

ZUIDAM, N. J., & NEDOVIĆ, V. A. (2010). Encapsulation technologies for active food ingredients and food processing. Encapsulation Technologies for Active Food Ingredients and Food Processing, 1–400. https://doi.org/10.1007/978-1-4419-1008-0

7. GENERAL DISCUSSION

In today's world, chronic diseases, such as cardiovascular disease, diabetes, obesity, and age-related illnesses, have increased in incidence due to factors including an aging population, sedentary lifestyles, poor dietary habits, and environmental pollution. For this reason, society is demanding solutions to support overall well-being, prevent disease, and address specific health needs. In this context, novel products such as functional foods, dietary supplements, and nutraceuticals containing bioactive ingredients have surged. Bioactive ingredients, also known as bioactive compounds, are a family of compounds, including proteins, minerals, polyunsaturated fatty acids, vitamins, polyphenols, probiotics, and prebiotics, widely recognized for their significant direct and indirect impacts on human health and their extensive therapeutic benefits (Al-Dujaili & Al-Dujaili, 2015; Montserrat-De la Paz, 2021).

While the health benefits of these bioactive components are well-established, incorporating them directly into food products poses challenges. Many of these compounds are sensitive to processing conditions, including temperature and pH, which can degrade their bioavailability and efficacy (Crowe & Francis, 2013). Moreover, maintaining their stability during food production and storage is complex, often requiring advanced technologies like encapsulation or modified atmosphere packaging to preserve their functional integrity (Lisboa et al., 2024).

A wide range of encapsulating techniques is available (Saha et al., 2023) Selecting the most effective method remains challenging due to the diversity of encapsulation processes, each with advantages and disadvantages, highlighting the need for tailored approaches to achieve the desired product qualities (Castro Coelho et al., 2021).

Among the various techniques available, electrohydrodynamic processes—particularly electrospinning and electrospraying—have emerged as straightforward, adaptable, scalable, and cost-effective methods for producing high-performance nano- and microfibers, along with nano- and microcapsules, at room temperature (Jacobsen et al., 2018; Niu et al., 2020). These advantages are significant for encapsulating thermolabile bioactive compounds, as they

minimize the risk of losing bioactivity. Furthermore, the use of high-voltage technology ensures efficient encapsulation and enables precise control over the distribution of particle sizes.

Nevertheless, a significant limitation of traditional electrospraying lies in its low production yield, which has constrained its application on a large scale and its adoption in industrial contexts. In response to this challenge, Bioinicia S.L., in collaboration with the research group led by Dr. Lagaron, has developed the patented Electrospraying Assisted by Pressurized Gas (EAPG) technology. This innovative technique seamlessly integrates electrospraying with gas-driven nebulization, employing a pneumatic injector to atomize polymer solutions under controlled environmental conditions. The EAPG technology achieves enhanced production efficiency while ensuring the preservation of particle quality, thus enabling industrial-scale production (Busolo et al., 2019).

The main objective of this doctoral thesis was to evaluate the potential of the high-throughput Electrospraying Assisted by Pressurized Gas (EAPG) technology to develop microencapsulation formulations for three underexploited bioactive ingredients, concretely EPA, which is recognized for its essential omega-3 fatty acid profile (Publication I), dragon's blood sap which is esteemed for its potent antioxidant and therapeutic properties (Publication II and III) and camu camu extract, which is noted for its exceptionally high concentration of antioxidant compounds (Publication IV)., perform their characterization, and evaluate their stability under different storage conditions for their potential use in end-consumer health-enhancing products.

In the first chapter, the encapsulation of EPA utilizing WPC as coating material via EAPG technology has emerged as a potential strategy to mitigate oxidative degradation, a prevalent challenge associated with omega-3 oils. WPC was selected as wall material due to its amphiphilic, gelation, and protective qualities (Gunasekaran et al., 2007). The obtained results suggested that this technology is suitable to encapsulate 80% PUFAs- enriched oils at ambient temperatures without compromising their functional properties, which was corroborated by

the reduced differences observed before and after the encapsulation in the peroxide values (PV), a key indicator of lipid oxidation.

The combination of the use of emulsions together with the EAPG process allowed to obtain nanostructured microparticles with an average size of around 5.6 µm, with the oil homogeneously distributed within the particle in submicron droplets with an average size of 0.7 µm. This structure favored oil protection, as demonstrated by the reduced amount of extractable oil, i.e. below 30%. This fact could also favor gastric absorption. Additionally, this reduced particle size, below 20 µm, does not affect the organoleptic properties (Weidner, 2009) and, more concretely, the final product's texture.

The preservation of functional properties during storage was corroborated under accelerated oxidation conditions via exposure to ultraviolet (UV) light following the assessments of peroxide value (PV) and Fourier-transform infrared (FTIR) spectroscopy. According to the results, the PV remained significantly lower in EAPG-encapsulated EPA than in non-encapsulated samples. This result highlights the protective effect of WPC combined with the structure of the microparticles obtained by EAPG, thereby enhancing the overall stability and functionality of the encapsulated compound.

Finally, sensory evaluations indicated that when EPA was encapsulated and incorporated into milk, the encapsulated product offered a reduced impact, even after 10 days of UV light exposure, even if the effect was not significant. However, when EPA and DHA encapsulated were combined, a significant difference was observed for the encapsulated product for the freshly prepared samples and after 10 days of UV exposure. This finding is particularly significant, as the characteristic fishy flavor of EPA has historically limited its utilization in food applications. Consequently, the EAPG technique not only preserves the stability and bioactivity of EPA but also improves its sensory acceptability, facilitating its incorporation into dairy products and aligning with consumer preferences for health-oriented foods with desirable sensory qualities.

The second chapter deals with the characterization, drying, and encapsulation of Dragon's Blood Sap (DBS). This is a natural resin extracted from Croton lechleri rich in bioactive compounds, primarily proanthocyanidins and catechin, epicatechin, and taspin (Lock et al., 2016; Rossi et al., 2011). These polyphenolic compounds exhibit potent antioxidant, anti-inflammatory, antimicrobial, and cicatrizing properties (Jones, 2004), making DBS a valuable natural remedy with broad applications in health and cosmetic products. However, the bioactivity of DBS is highly susceptible to environmental factors (Volf et al., 2014), which can degrade its functional components and significantly reduce its therapeutic potential. For this reason, the storage stability and the photo-oxidation of DBS were studied by FTIR and UV-Vis spectrophotometry for 39 days at different temperatures (4-21ºC) and relative humidities (0-56%), as well as under UV light exposure. The degradation of phenolic compounds was reduced at 0% RH, which did not significantly affect temperature in the range studied. Exposure to UV light resulted in a 20% reduction in DBS integrity. Additionally, DBS showed a high and stable antioxidant content (≈93% inhibition percentage), which makes it an interesting functional ingredient for consumer product formulations. However, despite DBS stability, it is interesting to explore effective stabilization strategies to preserve its bioactive properties and extend its applicability.

As a first approximation in the stabilization of this extract, the strengths and weaknesses of EAPG and freeze-drying were compared to dry DBS. No significant differences were detected between both samples regarding total soluble polyphenol content and antioxidant activity characterized by the inhibition of DPPH. However, the most significant difference was observed in morphology. Whereas EAPG obtained spherical microparticles with an average size of 11 μm, freeze-dried DBS exhibited irregular structures with broad particle size, which could affect the dissolution rate, the bioavailability, and the organoleptic impact in a final food product. The freeze-dried sample was also more affected after the accelerated oxidation study under UV light, showing a reduced DPPH inhibition and a smaller total soluble polyphenol content.

Secondly, DBS was encapsulated using EAPG technology to produce microparticles that protect it from environmental degradation. The process leverages food-grade encapsulation matrices, such as whey protein concentrate (WPC) and zein (ZN), because these proteins have effective microencapsulating and antioxidant and photo-protective properties. Additionally, two encapsulant materials, bioactive compound ratios, were explored. Encapsulation resulted in particles with smooth surfaces using WPC and rough textures with ZN, with particle sizes ranging between 6 µm and 12 µm, depending on the encapsulant matrix and the encapsulant-to-bioactive ratio.

The mild conditions of the EAPG process were corroborated once again since the TSP content remained stable during encapsulation, with only a minor decrease in antioxidant activity detected post-process not affected during the encapsulation process, and a little reduction in antioxidant activity was observed after encapsulation.

The accelerated photo-oxidation test under ultraviolet light confirmed that the encapsulated DBS showed an increase in oxidative stability compared to the neat bioactive, with the stability enhanced for the ratio 2:1, especially when ZN was used as an encapsulating matrix.

The third chapter dealt with the drying, encapsulating, and characterizing camu camu extract (CCX). This extract is derived from ***Myrciaria dubia***, and it is recognized for its exceptional antioxidant capacity, largely attributed to its high content of bioactive compounds such as vitamin C, ellagic acid, ellagitannins, and proanthocyanidins (Akter et al., 2011; Fracassetti et al., 2013; Fujita et al., 2017). These phytochemicals provide significant health benefits, including anti-inflammatory, antihyperglycemic, and antimicrobial effects (Carmo et al., 2019; Fidelis et al., 2018; Miyashita et al., 2018). However, CCX's inherent instability under environmental conditions—particularly fluctuations in temperature, humidity, and ultraviolet (UV) exposure—severely limits its practical application in long-term formulations.

The drying of CCX via EAPG produced irregular, non-uniform, and agglomerated structures with an average particle size of 10 µm and a reduced moisture content below 5%, which is widely acknowledged as safe, as it inhibits the growth of the most harmful bacteria. No loss of antioxidant activity or total soluble polyphenols content was detected compared to the neat CCX.

The encapsulation of CCX within WPC and ZN via EAPG technology exhibited distinct morphologies depending on the matrix. WPC encapsulates were smooth, while ZN encapsulates had rougher surfaces. Particle sizes ranged from 7.2 µm to 12.5 µm, with ZN consistently producing smaller, more uniform particles.

Encapsulation effectively maintained the bioactive properties of CCX and improved its stability across a range of storage conditions, including temperatures from 4–21ºC, relative humidity levels of 0–56%, and UV light exposure. While WPC provided adequate stability, ZN's hydrophobic nature and photo-protective properties were particularly effective against environmental degradation. Especially at a 2:1 encapsulant-to-bioactive ratio, it was notably more effective than WPC in retaining total soluble polyphenols and antioxidant activity during exposure to stressors such as humidity and UV radiation.

From a scientific perspective, this research advances the understanding of protein-based encapsulation and its role in stabilizing natural bioactives. These findings serve as a foundation for future work aimed at optimizing encapsulation materials and developing tailored solutions to meet the specific needs of bioactive compounds.

References

AKTER, M. S., OH, S., EUN, J. B., & AHMED, M. (2011). Nutritional compositions and health promoting phytochemicals of camu-camu (myrciaria dubia) fruit: A review. Food Research International, 44(7), 1728–1732. https://doi.org/10.1016/J.FOODRES.2011.03.045

AL-DUJAILI, E., & AL-DUJAILI, E. A. (2015). Cronicon NUTRITION Natural Polyphenols: Potential for Disease Prevention. In Review Article, EC Nutrition (Vol. 2). https://www.researchgate.net/publication/28818236

BUSOLO, M. A., TORRES-GINER, S., PRIETO, C., & LAGARON, J. M. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. Innovative Food Science and Emerging Technologies, 51, 12–19. https://doi.org/10.1016/j.ifset.2018.04.00

CARMO, M. A. V. DO, FIDELIS, M., PRESSETE, C. G., MARQUES, M. J., CASTRO-GAMERO, A. M., MYODA, T., GRANATO, D., & AZEVEDO, L. (2019). Hydroalcoholic Myrciaria dubia (camu-camu) seed extracts prevent chromosome damage and act as antioxidant and cytotoxic agents. Food Research International, 125, 108551. https://doi.org/10.1016/J.FOODRES.2019.10855

CASTRO COELHO, S., NOGUEIRO ESTEVINHO, B., & ROCHA, F. (2021). Encapsulation in food industry with emerging electrohydrodynamic techniques: Electrospinning and electrospraying – A review. Food Chemistry, 339(December 2019), 127850. https://doi.org/10.1016/j.foodchem.2020.127850

CROWE, K. M., & FRANCIS, C. (2013). Position of the Academy of Nutrition and Dietetics: Functional Foods. Journal of the Academy of Nutrition and Dietetics, 113(8), 1096–1103. https://doi.org/10.1016/j.jand.2013.06.002

FIDELIS, M., SANTOS, J. S., ESCHER, G. B., VIEIRA DO CARMO, M., AZEVEDO, L., CRISTINA DA SILVA, M., PUTNIK, P., & GRANATO, D. (2018). In vitro antioxidant and antihypertensive compounds from camu-camu (Myrciaria dubia McVaugh, Myrtaceae) seed coat: A multivariate structure-activity study. Food and Chemical Toxicology, 120, 479–490. https://doi.org/10.1016/J.FCT.2018.07.043

FRACASSETTI, D., COSTA, C., MOULAY, L., & TOMÁS-BARBERÁN, F. A. (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (Myrciaria dubia). Food Chemistry, 139(1–4), 578–588. https://doi.org/10.1016/j.foodchem.2013.01.121

FUJITA, A., SARKAR, D., GENOVESE, M. I., & SHETTY, K. (2017). Improving anti-hyperglycemic and anti-hypertensive properties of camu-camu (Myriciaria dubia Mc. Vaugh) using lactic acid bacterial fermentation. Process Biochemistry, 59, 133–140. https://doi.org/10.1016/J.PROCBIO.2017.05.017

GUNASEKARAN, S., KO, S., & XIAO, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. Journal of Food Engineering, 83(1), 31–40. https://doi.org/10.1016/j.jfoodeng.2006.11.001

JACOBSEN, C., GARCÍA-MORENO, P. J., MENDES, A. C., MATEIU, R. V, & CHRONAKIS, I. S. (2018). Use of Electrohydrodynamic Processing for Encapsulation of Sensitive Bioactive Compounds and Applications in Food Supplemental Material. https://doi.org/10.1146/annurev-food-030117

JONES, K. (2004). Review of Sangre de Drago (Croton lechleri) - A South American Tree Sap in the Treatment of Diarrhea, Inflammation, Insect Bites, Viral Infections, and Wounds: Traditional Uses to Clinical Research. The Journal of Alternative & Complementary Medicine 9(6), 877–896. https://doi.org/10.1089/107555303771952235

LISBOA, H. M., PASQUALI, M. B., DOS ANJOS, A. I., SARINHO, A. M., DE MELO, E. D., ANDRADE, R., BATISTA, L., LIMA, J., DINIZ, Y., & BARROS, A. (2024). Innovative and Sustainable Food Preservation Techniques: Enhancing Food Quality, Safety, and Environmental Sustainability. Sustainability, 16(18), 8223. https://doi.org/10.3390/su16188223

LOCK, O., PEREZ, E., VILLAR, M., FLORES, D., & ROJAS, R. (2016). Bioactive compounds from plants used in peruvian traditional medicine. Natural Product Communications, 11(3), 315–337.

MIYASHITA, T., KOIZUMI, R., MYODA, T., SAGANE, Y., NIWA, K., WATANABE, T., & MINAMI, K. (2018). Data on a single oral dose of camu camu (Myrciaria dubia) pericarp extract on flow-mediated vasodilation and blood pressure in young adult humans. Data in Brief, 16, 993–999. https://doi.org/10.1016/j.dib.2017.12.009

MONTSERRAT-DE LA PAZ, S. (2021). Active ingredients from foods: Biochemical and processing aspects. Foods, 10(6). https://doi.org/10.3390/FOODS10061240

NIU, B., SHAO, P., LUO, Y., & SUN, P. (2020). Recent advances of electrosprayed particles as encapsulation systems of bioactives for food application. In Food Hydrocolloids (Vol. 99). Elsevier B.V. https://doi.org/10.1016/j.foodhyd.2019.105376

ROSSI, D., GUERRINI, A., MAIETTI, S., BRUNI, R., PAGANETTO, G., POLI, F., SCALVENZI, L., RADICE, M., SARO, K., & SACCHETTI, G. (2011). Chemical fingerprinting and bioactivity of Amazonian Ecuador Croton lechleri Müll. Arg. (Euphorbiaceae) stem bark essential oil: A new functional food ingredient? Food Chemistry, 126(3), 837–848. https://doi.org/10.1016/j.foodchem.2010.11.042

SAHA, D., KHATANIAR, A., SINGH, A. K., & JHA, A. N. (2023). Review of methods for encapsulation of nutraceutical compounds. In Nutraceuticals: Sources, Processing Methods, Properties, and Applications (pp. 127–156). Elsevier. https://doi.org/10.1016/B978-0-443-19193-0.00010-1

VOLF, I., IGNAT, I., NEAMTU, M., & POPA, V. I. (2014). Thermal stability, antioxidant activity,

and photo-oxidation of natural polyphenols. Chemical Papers, 68(1), 121–129. https://doi.org/10.2478/s11696-013-0417-6

WEIDNER, E. (2009). High pressure micronization for food applications. The Journal of Supercritical Fluids, 47(3), 556–565. https://doi.org/10.1016/J.SUPFLU.2008.11.009

8. CONCLUSIONS

This doctoral thesis has effectively demonstrated the transformative potential of electrospraying assisted by pressurized gas (EAPG) as an advanced drying and encapsulation technique, offering robust stabilization for sensitive bioactive compounds, such as eicosapentaenoic acid (EPA), Dragon's blood sap (DBS), and camu camu extract (CCX).

A notable advantage of EAPG is its ability to dry and encapsulate bioactives at ambient temperatures without compromising their bioactivity. This feature positioned EAPG as superior to traditional drying and encapsulation methods that may expose sensitive compounds to damaging heat or solvents. Consequently, the implications of these findings are substantial for the food, nutraceutical, pharmaceutical, and cosmeceutical industries, which are increasingly focused on developing stable, bioactive-rich products to satisfy consumer demand for health-promoting ingredients. The enhanced stability and efficacy of encapsulated bioactives introduced by this thesis open new avenues for creating functional foods, dietary supplements, and therapeutic products with improved shelf life and bioavailability.

Additionally, integrating EAPG with natural protein matrices presented a viable approach to encapsulate and consequently to extend the shelf life of bioactive compounds while maintaining their functional properties and enhancing their sensory qualities. The encapsulation of EPA significantly reduced oxidation rates, preserving its essential omega-3 fatty acid profile over extended storage periods. For DBS and CCX, encapsulation preserved their high antioxidant capacities, effectively shielding them from environmental degradation and enabling their potential applications in various products.

This thesis highlights the critical importance of matrix selection in ensuring bioactive stability. Both WPC and ZN offer unique protective advantages, with ZN demonstrating exceptional efficacy in protecting phenolic compounds from photo-oxidation, especially at a encapsulant matrix : bioactive compound ratio of 2:1.

Additionally, the encapsulation techniques and formulations explored in this thesis notably enhanced stability and sensory attributes in model food products.

In conclusion, this thesis makes a significant contribution to the field of encapsulation science, paving the way for advancements in the development of robust, effective, and sustainable bioactive ingredients designed to meet the needs of the modern consumer.

Future perspectives

These findings serve as a cornerstone for future studies aimed at scaling up production, validating real-world applications, and ensuring market viability.

Several critical research directions must be pursued to ensure a successful market introduction of the encapsulated bioactives developed in this thesis. The proposed areas of further work are as follows:

8.1. Stability studies in food products

Evaluating the encapsulates' performance in real food matrices under commercial conditions is interesting in demonstrating their compatibility and stability in diverse applications such as functional beverages, snacks, or supplements. These studies will assess how temperature, pH, and interactions with other food components affect the encapsulates' integrity, bioavailability, and sensory properties. Success in this area will provide robust evidence to attract food manufacturers and validate the scalability of these encapsulation technologies in industrial food production.

8.2. Testing alternative matrices

Exploring new encapsulation materials, such as polysaccharides or hybrid combinations with proteins, can optimize the functionality and cost-effectiveness of the encapsulation systems. These studies will also focus on improving the sensory profiles of the encapsulates, environmental stability, and biodegradability. Developing versatile encapsulation matrices tailored to specific bioactives or applications will enhance market adaptability and broaden the potential product portfolio.

8.3. In Vitro digestive studies

Understanding the bioavailability and release kinetics of encapsulated bioactives in simulated gastrointestinal environments is vital for product development. These studies will ensure that the encapsulates are designed for optimal release at the target absorption sites in the digestive system, enhancing their efficacy as nutraceutical or pharmaceutical agents. Detailed in vitro data will also serve as a critical prelude to regulatory submissions, streamlining the pathway to market approval.

8.4. In Vivo studies

Animal trials are essential to establish the physiological effects and therapeutic potential of the encapsulated bioactives. These studies will provide data on pharmacokinetics, tissue distribution, and potential toxicity, ensuring the safety and effectiveness of the formulations. Demonstrating consistent and reproducible benefits in animal models is key to gaining stakeholder confidence and attracting investment for scaling production.

8.5. Clinical trials

Human clinical trials are the gold standard for validating the safety, efficacy, and health benefits of bioactive compounds. These trials will test the encapsulates in specific target populations, such as individuals with inflammatory disorders or those seeking enhanced antioxidant support. Clinical data will also serve as a cornerstone for regulatory approvals, marketing claims, and product differentiation in a competitive marketplace.

The proposed studies address critical bottlenecks in transitioning encapsulation technologies to market-ready solutions. Ensuring the viability of encapsulates under commercial conditions and confirming their health benefits will have an impact on the industry by differentiating these products in the highly competitive functional foods and nutraceutical sectors.

9. APPENDICES

Appendix I. Abbreviations and Acronyms

ATR	Attenuated Total Reflectance
BIOENCAP	Electrohydrodynamic Atomization Technology Development (INNCAD00-18-31)
BIOPAPERTECH	Sustainable Packaging Innovation Project (PID2021-128749OB-C31)
CAGR	Compound Annual Growth Rate
CAPSULTEK	Scaling Electrospray Technology for Bioactive I ngredients (H2020-EIC-SMEinst-873827)
CCX	Camu Camu Extract
CSIC	Consejo Superior de Investigaciones Científicas
DBS	Dragon's Blood Sap
DHA	Docosahexaenoic Acid
EAPG	Electrospraying Assisted by Pressurized Gas
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic Acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FODIAC	Dietary Solutions for Diabetes and Cognitive Support (H2020-MSCA-RISE-2017-778388)
PUFAs	Polyunsaturated Fatty Acids
UN	United Nations
UPV	Universitat Politècnica de València
WPC	Whey Protein Concentrate
ZN	Zein

Appendix II. Other scientific contributions

Articles

Peña, N., Minguez, S., & Escobar, J.-D. (2023***). Current production scenario and functional potential of the whole amaranth plant: A review.*** IntechOpen. https://doi.org/10.5772/INTECHOPEN.111881

Escobar García, J. D., Alcaniz, M., Masot, R., Fuentes, A., Bataller, R., Soto, J., & Barat, J. M. (2013). ***Quantification of organic acids using voltammetric tongues.*** Food Chemistry, 138, 814–820. https://doi.org/[DOI if available]

Mejía Giraldo, L. M., Cifuentes Wchima, X., & Escobar García, J. D. (2011). ***Determinación de factores multivariantes que influyen en la sostenibilidad y la seguridad agroalimentaria del sector rural en el departamento del Quindío, Colombia.*** Revista de Investigaciones Universidad del Quindío, 1(1), 32–36.

Mejía, L. F., & Escobar, J. D. (2006). ***Fortificación de un producto cárnico con micronutrientes en Industrias Alimenticias Rex S.A.*** Vector, 1(1), 57–62.

Oral presentations:

Escobar García, J. D. (2020, September). ***The innovation trajectory of Q'OMER: Achievements and challenges.*** Oral presentation at the International Online Forum on Entrepreneurship and Innovation in Agro, Brazil.

Prieto López, C., Escobar García, J. D., Pardo Figuérez, M. M., Torres Guiner, S., & Lagarón Cabello, J. M. (2020). ***Microencapsulation of omega-3 polyunsaturated fatty acids by an innovative high-throughput technique based on electrospraying.*** Oral presentation at the Controlled Release Society Annual Meeting & Exposition, Valencia, Spain.

Escobar García, J. D. (2019, May). ***Scientific and sustainable entrepreneurship.*** Oral presentation at the XX Ibero-American Annual Meeting and VI MOTIVA International Congress, Valencia, Spain.

Prieto López, C., Escobar García, J. D., Pardo Figuérez, M. M., & Lagarón Cabello, J. M. (2019, May). ***Microencapsulation of bioactives by an innovative high-throughput technique based on electrospraying***. Oral presentation at EUPOC 2019 – Electrospinning and Related Techniques: From Design to Production of Advanced Polymer Materials and Devices, Como, Italy.

Escobar García, J. D., Prieto, C., & Lagarón Cabello, J. M. (2018, October). ***Dragon's Blood Sap: A natural antioxidant material for advanced polymer materials.*** Oral presentation at PolyMar - 2nd Conference for Early Stage Researchers in Polymer Science through the Aegean, Athens, Greece.

Torres Giner, S., Escobar García, J. D., Cherpinsky, A., Melendez, B., Busolo, M., & Lagarón Cabello, J. M. (2017, December). ***High throughput electrospinning/electrospraying applications of interest in food, pharma, biomedicine and packaging.*** Oral presentation at the International Conference of the African Materials Research Society (AMRS2017), Gaborone, Botswana.

Escobar García, J. D. (2017, October). ***Bioeconomy: A model for social innovation.*** Oral presentation at the International Symposium on Innovation Ecosystems, Palmira, Colombia