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Ultrasound associated with ohmic heating, enzymes and fermentation in the extraction of curcuminoids and a review on the application of vegetable proteins as a matrix for encapsulating hydrophobic compounds

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Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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“Chuvas de Rosas caindo do céu
Sinal de que alguém olha por mim
Chuva de Rosas do céu elas veem
Teresa menina rezando por mim”
(Ricardo Sá)
Santa Terezinha do Menino Jesus, Rogai por nós.

ABSTRACT

FERNANDES, Janaina Gonçalves, D.Sc., Universidade Federal de Viçosa, July, 2024. **Ultrasound associated with ohmic heating, enzymes and fermentation in the extraction of curcuminoids and a review on the application of vegetable proteins as a matrix for encapsulating hydrophobic compounds**. Adviser: Paulo Cesar Stringheta. Co-advisers: Pedro Henrique Campelo Felix and Evandro Martins.

The selection of foods that make up people's daily diets has become increasingly rigorous. Consumers are opting for foods that offer benefits beyond basic nutrition. In this context, the market for supplements and vegan and vegetarian products is constantly growing. Turmeric, in addition to providing sensory properties to foods, has been widely consumed due to its health benefits, which are attributed to curcuminoids. However, these curcuminoids are sensitive to processing conditions, making encapsulation technology an extensively employed strategy to provide protection and controlled release of bioactive compounds like curcuminoids. Animal proteins are already widely used as encapsulating agents for hydrophobic bioactive compounds. However, to meet the demand of the vegan and vegetarian audience, studies are being conducted to utilize plant proteins as encapsulating agents, which still poses a challenge. The first study in this thesis evaluated the best techniques for curcuminoid extraction, aiming for their incorporation into food matrices and the development of supplements. The study assessed the influence of the "curing" process before turmeric drying; the effects of ultrasound-assisted extraction (UAE) process variables; and the use of auxiliary pre-treatments (enzymatic treatment, ohmic heating, and fermentation) before UAE, analyzing the yield and composition of the main curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin). Physical characterization analyses of turmeric powders subjected to different pre-treatments and colorimetric analyses of the extracts obtained were also conducted. It was observed that the traditional "curing" technique did not affect the drying time of rhizomes or alter the extraction yield of curcuminoids. The optimal UAE time was 40 minutes, whereas Soxhlet extraction took 3 hours. The ideal temperature and solid-to-liquid ratio were 35 °C and 1:30 (w/v), respectively. The enzymatic pre-treatment showed no significant difference in curcuminoid concentration compared to the control sample. Pre-treatment with ohmic heating and fermentation increased curcuminoid yield by 17% and 24%, respectively. The evaluated colorimetric parameters (L*, a*, and b*) revealed differences in values; however, the ?E parameter was 3,

indicating that the color difference is not perceptible to the human eye. Therefore, the results of this study demonstrated novel specifications of promising hybrid technologies for the more efficient recovery of curcuminoids. The second study in this thesis demonstrated that plant proteins possess interfacial properties that hinder the achievement of high encapsulation efficiency for hydrophobic compounds. However, some promising strategies have already been used to modulate the interfacial properties of certain plant proteins (such as pea and soy) through alterations in their physicochemical environment, such as pH, temperature, and ionic strength. Additionally, it was shown that auxiliary treatments, such as ultrasound, can improve encapsulation efficiency. However, studies on the application of these and other emerging technologies remain scarce. Finally, findings from the studies suggest that curcuminoids can be efficiently encapsulated using plant proteins with the assistance of modulating and auxiliary technologies.

Keywords: emerging technologies; bioactive compounds; curcumin; ultrasound-assisted extraction; plant-based

RESUMO

FERNANDES, Janaina Gonçalves, D.Sc., Universidade Federal de Viçosa, julho de 2024. **Ultrassom associado ao aquecimento ôhmico, enzimas e fermentação na extração de curcuminóides e uma revisão sobre a aplicação de proteínas vegetais como matriz de encapsulação de compostos hidrofóbicos**. Orientador: Paulo Cesar Stringheta. Coorientadores: Pedro Henrique Campelo Felix e Evandro Martins.

A seleção dos alimentos que compõem a dieta diária das pessoas está cada vez mais rigorosa. Os consumidores estão optando por alimentos que oferecem benefícios além da nutrição básica. Nesse contexto, o mercado de suplementos e produtos veganos e vegetarianos e está em constante crescimento. A cúrcuma, além de conferir propriedades sensoriais aos alimentos, tem sido amplamente consumida devido aos seus efeitos benéficos à saúde, atribuídos aos curcuminoides. No entanto, esses curcuminoides são sensíveis às condições de processamento, o que torna a tecnologia de encapsulamento uma estratégia extremamente empregada para conferir proteção e liberação controlada de compostos bioativos como os curcuminoides. Proteínas animais já são amplamente utilizadas como agentes encapsulantes de compostos bioativos hidrofóbicos. Entretanto, para atender à demanda do público vegano e vegetariano, estudos estão sendo desenvolvidos para utilizar proteínas vegetais como agentes encapsulantes, o que ainda representa um desafio. O primeiro estudo desta tese avaliou as melhores técnicas para a extração dos curcuminoides visando à sua incorporação em matrizes alimentícias e elaboração de suplementos. Foi avaliada a influência do processo de “cura” antes da secagem da cúrcuma; os efeitos das variáveis do processo de extração assistida por ultrassom (EAU) e o emprego de pré-tratamentos auxiliares (enzima, aquecimento ôhmico e fermentação) antes da EAU, analisando o rendimento e a composição dos principais curcuminoides (curcumina, demetoxicurcumina e bisdemetoxicurcumina). Também foram realizadas análises de caracterização física dos pós de cúrcuma que receberam os diferentes pré-tratamentos e uma análise colorimétrica dos extratos obtidos. Observou-se que a técnica tradicional de “cura” não afetou o tempo de secagem dos rizomas nem alterou o rendimento de extração dos curcuminoides. O tempo ideal para a EAU foi de 40 minutos, enquanto a extração por Soxhlet levou 3 horas. A temperatura e razão sólido:líquido ideais foram de 35 °C e 1:30 (m/v), respectivamente. O pré-tratamento enzimático não apresentou diferença significativa na concentração de curcuminoides em relação à amostra controle. O pré-tratamento com aquecimento ôhmico e fermentação aumentaram o

rendimento de curcuminoides em 17% e 24%, respectivamente. Os parâmetros colorimétricos avaliados (L*, a* e b*) resultaram em diferenças nos valores, entretanto, o parâmetro ?E foi = 3, indicando que a diferença de cor não é perceptível ao olho humano. Portanto, os resultados deste estudo mostraram especificações inéditas de tecnologias híbridas promissórias para a recuperação mais eficiente de curcuminoides. No segundo trabalho dessa tese foi demonstrado que as proteínas vegetais possuem propriedades interfaciais que dificultam a obtenção de uma boa eficiência de encapsulamento de compostos hidrofóbicos. No entanto, algumas estratégias promissoras já foram utilizadas para modular as propriedades interfaciais de algumas proteínas vegetais (como ervilha e soja) por meio de alterações em seu ambiente físico-químico, como pH, temperatura e força iônica, por exemplo. Além disso, foi evidenciado que tratamentos auxiliares, como o ultrassom, podem melhorar a eficiência de encapsulamento. No entanto, estudos sobre a aplicação dessa e de outras tecnologias emergentes ainda são escassos. Por fim, as descobertas desses estudos sugerem que os curcuminoides podem ser eficientemente encapsulados com proteínas vegetais, com o auxílio de tecnologias moduladoras e auxiliares.

Palavras-chave: tecnologias emergentes; compostos bioativos; curcumina; extração assistida por ultrassom; *plant-based*

SUMMARY

1 General Introduction

According to the World Health Organization (WHO), chronic diseases such as diabetes, cancer, and stroke are responsible for approximately 74% of deaths worldwide. In this context, approaches to managing these conditions generally involve various strategies, including a healthy diet, which plays a fundamental role due to the bioactive compounds found in foods. Some foods and plants, in addition to their basic nutritional values, are rich in bioactive compounds. These compounds can influence bodily functions, offering health benefits, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory activities (ZHANG et al., 2024). It is already evident that the consumption of vegetables such as fruits, and parts like peels, leaves, flowers, and roots is associated with a lower likelihood of acquiring certain diseases (KARASAWA; MOHAN, 2018).

Numerous studies have already highlighted the role of bioactive compounds in the prevention and treatment of various diseases (HUANG et al., 2024; WANG et al., 2014; ZHANG et al., 2023). Compared to medications, bioactive compounds generally exhibit lower toxicity and minimal side effects. On the other hand, their therapeutic effects are usually less significant than those of medications, hence their applications are directed towards functional foods, nutritional supplements, and dietary supplements (ZHANG et al., 2024).

Bioactive compounds can be divided into two groups: those with high water solubility (hydrophilic) and those with low water solubility (hydrophobic). Among the hydrophilic group are some phenolic acids such as caffeic acid and gallic acid; some flavonoids such as anthocyanins and catechins; vitamin C (ascorbic acid); glucosinolates, among others. Among the hydrophobic compounds are curcuminoids; carotenoids such as β-carotene, lycopene, and lutein; flavonoids such as quercetin; fatty acids (omega-3 and omega-6); vitamins A, D, E, and K, among others. The hydrophobic characteristic is predominant when it comes to bioactive compounds, however, this group still faces some challenges related to its application in the food, pharmaceutical, and nutraceutical fields. Most of these compounds are sensitive to environmental and processing factors such as temperature, light, and the presence of oxygen. Additionally, their bioavailability is compromised due to the extreme pH changes that occur throughout the gastrointestinal tract (ANAL; BOONLAO; RUKTANONCHAI, 2023).

Within this broad group, the importance of studies focused on curcuminoids stands out. A quick search for the word "curcuminoids" on the ScienceDirect platform found 5,386 papers, with 2,454

of them published in the last five years (2020-2024). The growth of these studies is evidently related to the COVID-19 outbreak, where the search for foods capable of offering health benefits was maximized (TRIPATHY et al., 2021). Studies have already demonstrated that curcuminoids have potential applications for the treatment of Alzheimer's disease (AHMED; ENAM; GILANI, 2010) and heart failure (MORIMOTO et al., 2010). It has also been found that these compounds were able to minimize renal triglyceride accumulation (SOETIKNO et al., 2013).

Curcuminoids are hydrophobic polyphenols obtained from the rhizomes of turmeric (Curcuma longa), also known as turmeric root. The main curcuminoids present in this rhizome are curcumin (CC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). They are traditionally used as colorants, flavorings, among other applications (KIM et al., 2024). Curcuminoids have an intense bright yellow coloration, making them a potential substitute for artificial colorants such as tartrazine. The choice of a food product is still strongly influenced by color, while the search for cleaner labels and health benefits are also taken into consideration.

However, the technological application of curcuminoids remains a challenge for the food and pharmaceutical industries due to their physicochemical characteristics. In addition to low water solubility, these compounds are sensitive to processing conditions such as light, temperature, and pH (KOTHA; LUTHRIA, 2019). From a functional standpoint, curcumin and its analogs have low bioavailability due to their hydrophobic nature, which hinders their solubility in gastrointestinal fluids. Furthermore, they exhibit high chemical sensitivity at physiological pH and significantly reduced absorption in the gastrointestinal tract (SANIDAD et al., 2019).

To overcome these challenges, encapsulation techniques are being extensively studied, addressing processes capable of protecting and promoting controlled release of bioactive compounds. Encapsulation techniques involve the use of a carrier material that can form a protective barrier around the active compound of interest to maximize the application of bioactive compounds in food matrices (CULAS; POPOVICH; RASHIDINEJAD, 2024).

For the encapsulation of hydrophobic compounds such as curcuminoids, proteins are commonly used as encapsulating materials. Food proteins possess unique functional and biological properties, including amphiphilic nature, biodegradability, high nutritional value, abundant and renewable sources, and good emulsifying and film-forming capacities (MOHAMMADIAN et al., 2020). Several studies have been conducted using different proteins for curcumin encapsulation, such as bovine serum albumin (SADEGHI et al., 2013, 2014), egg albumin (ANIESRANI

DELFIYA; THANGAVEL; AMIRTHAM, 2016; FENG et al., 2016), whey protein (BOURBON; CERQUEIRA; VICENTE, 2016), zein (HU et al., 2016), and soy protein (TENG; LUO; WANG, 2012). However, within the context of this thesis, the focus is on the use of plant proteins due to the growing consumption of vegan and vegetarian products. Recent studies indicate that diets high in plant-based foods and low in meat and dairy are more sustainable and suggest that vegan diets can be considered ideal for the environment (CHAI et al., 2019).

Therefore, this thesis is divided into two chapters. The first chapter we explore strategies ranging from conventional technologies to emerging technologies to enhance the extraction of curcuminoids. Recognizing consumer demand for healthier food options and the potential benefits of curcumin and its analogs, this work aims to optimize curcuminoid extraction conditions from turmeric (*Curcuma longa*) using emerging and clean technologies. Additionally, we explore the use of hybrid extraction systems, an innovative approach that has not yet been widely addressed in the literature.

In the second chapter, we address the state of the art in the potential applications of plant proteins for encapsulating hydrophobic bioactive compounds. This research is justified by market trends and alignment with the United Nations' sustainable development goals. This chapter discusses how the interfacial properties of proteins can influence encapsulation efficiency and examines methodologies to enhance their techno-functional properties.

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ARTICLE 1

Impact of Ultrasound Operational Parameters and Hybrid Systems on the Recovery of Curcuminoids from *Curcuma longa*

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Abstract

Curcuminoids, including curcumin (CC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), are hydrophobic polyphenols derived from the rhizomes of Curcuma longa. Recent studies have demonstrated that these compounds possess antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer properties, providing significant health benefits. The growing health concerns have increased the demand for curcumin-based supplements. However, the products available on the market often have low purity and high costs due to the low scalability of currently used extraction techniques, such as Soxhlet extraction. This research aimed to study the effects of variables in the ultrasound-assisted extraction (UAE) process and to apply pre-treatments with enzymes, ohmic heating (OH), and fermentation to improve the extraction yield of curcuminoids. The results demonstrated that while the optimal extraction time for Soxhlet was 3 hours, UAE achieved 95% curcuminoid extraction in just 40 minutes. The ideal temperature for the ultrasonic bath was 35 °C, and the solid-to-liquid ratio was 1:30. Regarding the pre-treatments used, all resulted in changes in the proportion of at least one of the curcuminoids (CC, DMC, and BDMC). Enzymatic treatment did not affect the extraction yield compared to the

control sample. For OH, the extraction efficiency improved by up to 17%, while fermentation enhanced the extraction by more than 24% in terms of total curcuminoid concentration. Although colorimetric analysis showed some differences in the L*, a*, and b* parameter values, the color differences in the samples extracted by different methods were not perceptible to the human eye. Therefore, this study concludes that the application of both OH and fermentation, followed by UAE under optimized conditions, can be considered promising hybrid systems for scaling up curcuminoid extraction.

Keywords: curcumin, bioactive compounds, emerging technologies

1 Introduction

Turmeric (Curcuma longa) rhizomes have been used in food preparation as a condiment, colorant and/or flavoring agent. Dried turmeric powder is widely used to season poultry and seafood, in addition to being present in commercial product formulations such as mustard sauces and Indian curry (KHURSHEED et al., 2022; PEREIRA; STRINGHETA, 1998). Curcumin (CC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are the three main curcuminoids of this rhizome and can account for up to 10% of the total mass of the dried rhizome (HOROSANSKAIA et al., 2020). Of this total, curcumin accounts for approximately 75%, followed by DMC with about 10-20%, and BDMC with less than 5% (SHIRSATH et al., 2021).

These substances are responsible for the bright yellow coloration of the rhizomes and are of great interest for application as a natural food dye. Additionally, curcuminoids can be used as a substitute for the synthetic dye tartrazine, which has been associated with allergic reactions when ingested or applied to the skin (DEGOT et al., 2021).

Curcumin is a hydrophobic polyphenol with low molecular weight (368.38 g/mol) which have been associated to various studies have beneficial physiological activities for health, acting as antioxidants, anti-inflammatory agents, antimicrobials, and anticancer agents (FUJISAWA et al., 2004; MA et al., 2019; KHURSHEED et al., 2022; NGU et al., 2022; WANG et al., 2022). Curcumin and its analogs can inhibit viral gene infection and replication, as well as prevent the multiplicity of HIV (PRASAD; TYAGI, 2014).

Following the COVID-19 outbreak, the number of studies focused on the various health benefits of this bioactive compound has increased by more than 50 %, aiming to improve immunity and prevent the disease (TRIPATHY et al., 2021), which has generated a positive impact from an industrial perspective. The curcumin market is estimated to be USD 155 million in 2024, and it is expected to reach USD 240.68 million by 2029 (Mordor Inteligence, 2024).

However, the growth in curcumin consumption for supplementation and disease prevention purposes is still limited by the relatively high cost of the commercial product, which can range from $16.00 to $43.00 per bottle of 60 capsules containing 300 mg of curcumin (www.augmentlifeshop.com; www.lifeproductsbr.com, 2024). Part of this cost is justified by the need for investment in scalable technologies for extracting curcuminoids from the rhizomes. Additionally, much of the product available on the market is simply turmeric extract with low concentrations (from 24 to 26 % w/w) of curcuminoids (www.natuweb.com.br, www.oceandrop.com.br, 2024). The extraction process for these curcuminoids can be carried out using various techniques such as Soxhlet extraction, maceration, ultrasound, enzymes,

supercritical fluid, and microwave, among others (NURHADI et al., 2020). Industrially, curcumin is predominantly extracted using the conventional Soxhlet method, which is considered a straightforward approach (Buchi, 2024). However, this method has some disadvantages such as high operating temperatures (> 78°C), long processing times (between 6 and 14 hours), and high solvent consumption (~ 700 mL of solvent/ mg of extracted curcumin) (SHIRSATH et al., 2021). Regardless of the method used, solvent is required for the extraction process, traditionally using non-sustainable solvents such as methanol (ALTUNAY; ELIK; GÜRKAN, 2020) and acetone (DEGOT et al., 2021; MANDAL; MOHAN; HEMALATHA, 2008; SAHNE et al., 2017) . Therefore, industries are increasingly seeking "green" technologies, using ethanol as a solvent, for example.

For the broader application of curcumin and its analogs as food colorants or preventive agents against diseases to be feasible, it is necessary to improve the efficiency of extraction techniques for these compounds. In this context, ultrasonic bath extraction offers the advantage of scalable production through improved mass and heat transfer, reduced processing time (from 8 hours to 1 hour), enhanced physical mixing, lower processing temperature (from 78 to 35 °C), achieving a yield of 72 % (SHIRSATH et al., 2017).

Although ultrasound improves the efficiency of the extraction process, the yield can be further optimized by adjusting variables such as time, temperature, and the ratio of rhizome mass to solvent (JIANG; GHOSH; CHARCOSSET, 2021). Additionally, extraction techniques such as enzymatic hydrolysis of plant matrix, ohmic heating and natural fermentation of rhizomes have been related as promising strategies for the recovery of curcuminoids (KURMUDLE et al., 2013; SAHNE et al., 2017; MARATHE et al., 2019; LE-TAN et al., 2022; LIM et al., 2022). However, to date, no study has investigated the application of these techniques in combination with ultrasound-assisted bath extraction technology.

Based on the necessity to enhance hydrophobic polyphenol extraction on an industrial scale, this study aims to investigate the impact of hybrid extraction methods incorporating ultrasound (including enzymatic hydrolysis, ohmic heating, and fermentation of rhizomes with autochthonous microbiota) on the yield extracted curcuminoids from *Curcuma longa*.

The aim of this study was to investigate the raw material preparation (thermal treatment and drying time) and evaluate the effect of process variables in ultrasound-assisted extraction (time, temperature, and solid-liquid ratio) of curcuminoids using ethanol as the solvent. Additionally, we evaluated the influence of employing hybrid extraction methods, which have not yet been

explored, for the recovery of curcuminoids. Enzymatic treatment, ohmic heating, and fermentation were applied as pretreatments for ultrasound-assisted extraction to enhance extraction yield.

2 Materials and Methods

2.1 Materials

The rhizomes of *Curcuma Longa* were acquired from rural producers in the city of Viçosa, MG, Brazil, located at geographical coordinates: -20°75'58"57 latitude and -42°87'76"89 longitude. Curcumin (Fluka Chemika) with a purity grade of 96% was used as the standard. Ethanol PA. α-Amylase enzyme was acquired from Sigma-Aldrich (São Paulo, Brazil). ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were also utilized.

2.2 Production of turmeric powder

The turmeric was washed under running water, sanitized in a 150 ppm hydrosteril solution for 10 minutes, and peeled. 5 kg of the vegetable was boiled at 100 °C for 10 minutes, while another 5 kg received no thermal treatment. The rhizomes were sliced into approximately 2 mm thick slices and dried in an oven with air circulation at 70 °C. Samples were weighed over time until reaching constant weight, and the moisture content ratio (UR) was calculated according to Equation 1. After drying, the material was ground in a mill (Tecnal, São Paulo, Brazil), sieved through a 42 mesh sieve, and stored in the refrigerator at 4°C until use.

$$\text{UR} = \frac{HTx - EH}{IH - EH} \qquad \text{Equation 1}$$

On what,

HTx = humidity at time x

EH = equilibrium humidity

IH = inicial humidity

2.3 Extraction of curcuminoids by Soxhlet (conventional method)

The Soxhlet method was used for the conventional extraction process, considered the standard method for comparison with UAE. In the literature, methods for this process vary widely

in duration, from 2.5 hours (BRAGA et al., 2003) to up to 14 hours (SHIRSATH et al., 2021). In order to find the optimal extraction time, the concentration of curcuminoids was evaluated at different extraction times. Three grams of non-thermally treated turmeric powder were placed in filter paper (Whatman 1) and placed in a Soxhlet thimble. The solvent (150 mL of ethanol) was condensed in the distillation flask and brought into contact with the turmeric powder in the thimble, where extraction took place. After extraction, the samples were quantified using spectrophotometry.

2.4 Optimization of ultrasound-assisted extraction (UAE)

A bath sonicator (Elmasonic TI-H-10, Elma, Germany) with a power of 750 W, operating at 50% amplitude and a frequency of 25 kHz, was used for the extraction process as proposed by PINGRET; FABIANO-TIXIER; CHEMAT, 2013.

The solid-to-liquid ratio and the bath temperature were set to perform the process similarly to Soxhlet extraction. A sample of 0.2 g of turmeric powder was mixed with 10 mL of ethanol and placed in the ultrasonic bath at 75°C. The effect of time was evaluated by quantifying the extracted curcuminoids after exposure to different sonication times.

To evaluate the effect of the solid-to-liquid ratio on the extraction of curcuminoids, turmeric powder was added to PA ethanol (100% v/v) at ratios of 1:10, 1:30, 1:50, or 1:70 (w/v).

Finally, for the effect of the ultrasonic bath temperature, temperatures of 35, 55, and 75 °C were used. For the temperature study, in addition to quantifying the extracted curcuminoids, the antioxidant activity (as per 2.4.1) of the samples extracted at different temperatures was also analyzed. After 40 minutes of sonication, the samples were collected, centrifuged at 4100 rpm for 10 minutes, and the supernatant was stored at 5 °C until use.

2.4.1 Determination of the antioxidant capacity of turmeric extracts

Radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The determination of the antioxidant activity of turmeric extracts using the DPPH radical was conducted according to the method described by KIM et al., 2002. Briefly, 0.5 mL of sample (5 different dilutions) was added to 3.5 mL of DPPH radical solution (0.1 mmol/L in 80% v/v ethanol). The absorbance was measured at 517 nm after 60 minutes of reaction in the dark at 25°C. The results were expressed in mmol Trolox equivalent (TE)/g of turmeric powder.

Radical scavenging activity 2,2'-azinobis-3-etil-benzotiazolina-6-sulfonado (ABTS)

The ABTS radical assay was conducted according to the method described by (RE et al., 1999). Briefly, 0.5 mL of sample (5 different dilutions) were added to 3.5 mL of ABTS radical solution (diluted in 80% v/v ethanol to an absorbance of 0.70 ± 0.05 at 734 nm). After 6 minutes of reaction in the dark at 25°C, the absorbance was measured at 734 nm. The results were expressed in mmol Trolox equivalent (TE)/g of turmeric powder.

2.5 Quantification of curcuminoids in turmeric extracts

The concentration of curcuminoids was determined using a standard curve of curcumin (Fluka Chemika 96%) with concentrations ranging from 0.3 to 10 mg of curcumin per liter of ethanol (mg/L). Absorbances were measured at 425 nm using a UV-VIS spectrophotometer (UV-M51, Bel, Monza, Italy), ensuring linearity within the Lambert-Beer law (maximum absorbance equal to 2). Twenty microliters of the samples were diluted in a 10 mL flask and subjected to readings at 425 nm (PEREIRA; STRINGHETA, 1998).

The content of curcuminoids was calculated using Equations 2 and 3, and the final results were expressed in mg of curcuminoids per gram of turmeric powder.

$$[cur]_{solução} = \frac{(ABS - 0{,}0085)}{157{,}75} \qquad \text{Equation 2}$$

$$[cur]_{turmeric} = \frac{[cur_{solution}] * V_{ethanol}}{m_{turmeric}} \qquad \text{Equation 3}$$

On what,

$[cur]_{solution}$ = concentration of curcuminoids in ethanol solution (mg/mL);

ABS = absorbance at 425 nm

$[cur]_{turmeric}$ = curcuminoids concentration in turmeric poder;

$V_{ethanol}$ = volume of ethanol used in the extraction process (mL);

$m_{turmeric}$ = mass of turmeric powder used in the extraction (g).

Identification of curcuminoids

A HPLC Shimadzu (Kyoto, Japan) series liquid chromatograph system comprising vacuum degasser, quaternary pump, thermostatted column compartment, and diode array detector (SPD-M20A) was used. The column used was an ODS C18 reversed phase Shimadzu 5 mm (250 x 4.6 mm) (Phenomenex1, UK) with a C18 guard column. Mobile phases consisted of 0.1% phosphoric acid in water (v/v) (eluent A) and 0.1% orthophosphoric acid in methanol (v/v) (eluent B). The gradient was as follows: 0–1 min, 50% B; 1–6 min, linear gradient from 50 to 100% B; Post-run time was 25 min to cleaning and recondition of column. Solvent flow rate elution was 1 mL/min. Detection was accomplished with a diode array detector and chromatograms were recorded at 254 nm. The column was maintained at 30 °C. The sample injection volume was 10 µL. Peaks were identified by comparing their retention times and UV spectra in the 300-500 nm range with authentic standards and by checking the purity of the peaks.

2.6 Study of the application of different pretreatments for the extraction of curcuminoids

Samples of fresh turmeric received different pretreatments: enzymatic, ohmic heating, and fermentation. The fresh turmeric was sanitized, peeled, and ground in a food processor. After each pretreatment, all samples were dried in a food dehydrator until reaching a water activity below 0.3, then ground and sieved through a 42 mesh sieve. The dried powders obtained were subjected to UAE process, and the curcuminoids were quantified by spectrophotometry.

2.6.1 Enzimatic

The enzymatic treatment was carried out according to the optimal parameters found in the study by SAHNE et al., (2017). For enzymatic hydrolysis, 100 g of turmeric was weighed into Erlenmeyer flasks, mixed with 60 mL of a 0.2 M sodium acetate buffer solution, and 4% (w/w) α-amylase was added. The flasks were sealed with cotton filters and incubated on a shaker at 65°C with agitation of 160 rpm for 6 hours. For this assay, the enzyme α-amylase was chosen because turmeric is composed of about 70% starch, it is inferred that α-amylase is the ideal enzyme to interact with our substrate.

After hydrolysis, the curcuminoids are extracted from turmeric by using UAE on optimal conditions (defined in this study).

2.6.2 Ohmic heating (OH)

For the ohmic heating treatment, different voltage levels were applied: 10 V, 30V, and 50V for 15 minutes each. Voltage regulation was carried out using a TDGC2-0.5 regulator, which maintains a constant current of 2 A. The voltages and time treatment applied to turmeric were defined based on pre-tests.

After OH, the curcuminoids are extracted from turmeric by using UAE on optimal conditions (defined in this study).

2.6.3 Fermentation

Thirty grams of turmeric were added to 18 mL of distilled water, maintaining the same solid-to-liquid ratio for all pretreatments. The samples were placed in Erlenmeyer flasks covered with cotton filters and stored at 25 °C for 126 hours. The pH was measured using a previously calibrated pH meter (DIGIMED model Dm20).

The count of lactic acid bacteria was performed using Rose Bengal medium with incubation at 25 °C for 7 days. For fungi, Sabouraud medium was used with incubation at 37 °C for 7 days (SILVA et al., 2017).

The quantification of total phenolics on the fermented turmeric was performed using the Folin-Ciocalteu colorimetric method (SINGLETON & ROSSI, 1965). Calibration curves were constructed using gallic acid, and the total phenolic content was expressed in milligrams of gallic acid equivalent (GAE) per gram of sample (mg GAE/g).

After fermentation, the curcuminoids are extracted from turmeric by using UAE on optimal conditions (defined in this study).

2.7 Colorimetry

The curcuminoid solutions obtained by different extraction methods were characterized using a Hinter Lab Colorquest XE colorimeter (Reston, USA). According to the CIELAB color

scale, direct readings of reflectance were taken in the L*, a*, and b* color coordinates system. From these coordinates, Chroma (C*) and Hue (h*) values were calculated using Equations 4 and 5, respectively. Additionally, the overall color difference (ΔE*) was calculated by comparing the treated samples with the control sample, as per Equation 6.

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

$$h^* = \arctan(b^*/a^*) \quad \text{Equation 5}$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{Equation 6}$$

3 Results and Discussion

3.1 The effect of heat treatment on curcuminoid extraction

During the drying at 75 °C, the turmeric treated thermally (100 °C for 10 minutes) exhibits a higher water loss rate (0.008 RU/min) compared to untreated turmeric (0.009 RU/min) during the first 150 minutes (Figure 1). However, after 200 minutes of drying, the water loss rates begin to equalize and become statistically identical after 250 minutes of the process. Thus, it was found that thermal treatment of turmeric did not lead to a reduction in drying time.

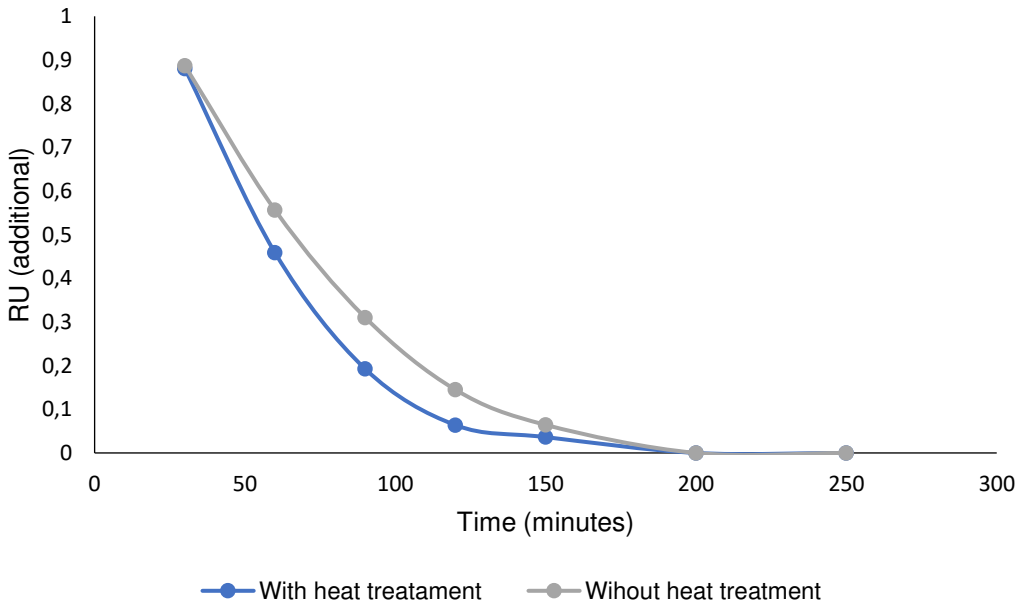

Figure 1: Turmeric drying behavior with and without heat treatment

By the contrary, GOVINDARAJAN; STAHL, 1980 reported that thermal pretreatment can reduce sun drying time from weeks to 10 to 15 days probably because the heating helps to break down the plant's cellular structures, aiding in mass transfer during drying. However, differently of our study the authors applied more intense cooking, without temperature control (heating is done over country-type furnaces for 1 to 6 hours) which helps to explain the difference with our results. Although better results were found by (GOVINDARAJAN; STAHL, 1980), the use of higher heating treatments can degrade the cucurminoids impacting the extraction yield (PRATHAPAN et al., 2009).

After drying, no significant difference ($p > 0.05$) was observed in the concentration of curcuminoids between the thermally treated turmeric (44.35 ± 0.04 mg/g) and untreated turmeric (46.54 ± 0.015 mg/g). These results are consistent with, PRATHAPAN et al., (2009) concluded that thermal treatments (60 to 100°C for 10 to 60 minutes) did not significantly alter the concentration of curcuminoids compared to untreated turmeric (which ranged between 53 to 55 mg/g). However, the same study demonstrated that the concentration of curcuminoids was lower (48 mg/g) when using the sun drying process due to the extended period (15 days) of exposure to light and temperature. On the other hand, (SURESH; MANJUNATHA; SRINIVASAN, 2007) observed a significant decrease (from 25.7 to 18.8 mg/g) in curcumin concentration when turmeric was subjected to boiling. The bioactive concentration reported by these authors was nearly half of what was found in our study, which may be related to the extraction process, as their extraction was performed from fresh plant material without prior drying. It is worth noting that drying assists in solvent penetration into the interior of plant tissue cells, thereby increasing the efficiency of curcuminoid extraction, as documented by González et al., (2017).

In terms of the proportion of extracted curcuminoids, thermally treated turmeric contained approximately 71.1% curcumin (CC), 22% demethoxycurcumin (DMC), and 6.9% bisdemethoxycurcumin (BDMC) (Figure 2A). In contrast, the sample that did not undergo thermal treatment showed about 72.8% CC, 21.1% DMC, and 6.1% BDMC (Figure 2B).

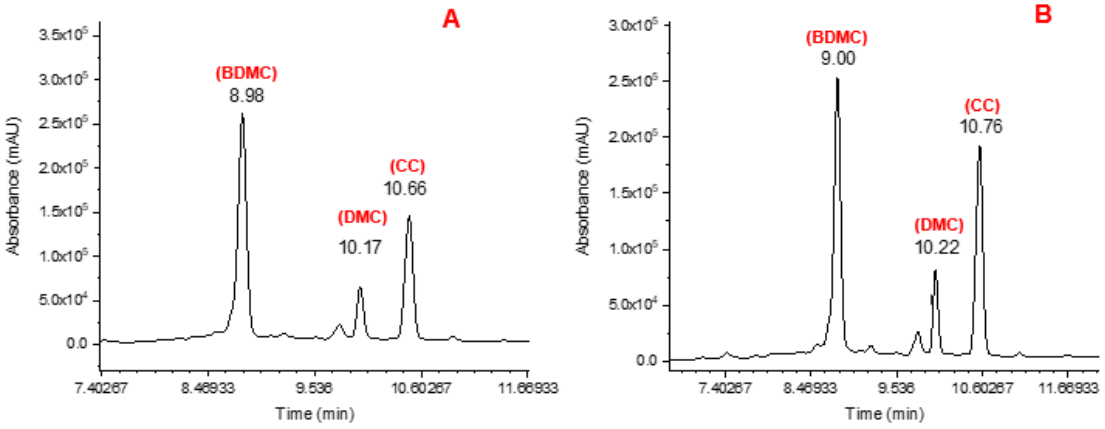

Figura 2: The chromatographic profile of the extracted curcuminoids from thermally treated (A) or untreated (B) turmeric: Bisdesmethoxycurcumin (BDMC), Demethoxycurcumin (DMC), and Curcumin (CC).

Considering that thermal treatment did not significantly affect drying time, curcumin concentration, or the proportion of curcuminoids, the remaining stages of the study were conducted using untreated turmeric.

3.2 The Effect of Operational Parameters in the Extraction Process

3.2.1 The Effect of Extraction Time on Curcuminoid Extraction using Soxhlet and UAE Methods

Curcuminoids reached maximum concentrations after 480 and 240 minutes of extraction using the Soxhlet method (53.17 mg/g or 5.3%) and UAE method (50.07 mg/g or 5.0%), respectively. These values are within the range defined in the literature, curcuminoids can constitute between 1 to 10% of turmeric powder depending on factors such as soil conditions, and climatic factors, among others (HOROSANSKAIA et al., 2020; MUNEKATA et al., 2021; THANGAVEL; DHIVYA, 2019). A study conducted with samples from 8 different cities in the state of Minas Gerais, Brazil, it was found curcuminoid levels ranging from 1.4 to 6.14% (SOUZA; GLÓRIA, 1998). Similar ranges were also observed in turmeric samples from Sri Lanka and India (3.76 and 5.05%), while slightly higher values were found for rhizomes from Iran (5.19 to 8.28%) subjected to different extraction methods (MADHUSANKHA et al., 2018).

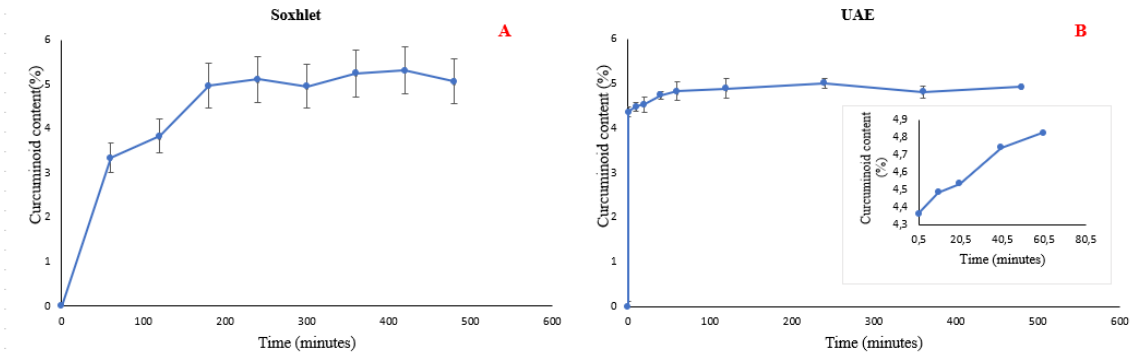

Figure 3: Extraction behavior of curcuminoids over time using different extraction methods. A) Soxhlet extraction; B) Ultrasound-assisted extraction, where the inset shows the behavior in the first 60 minutes

For the reference method, Soxhlet extraction, the concentration of curcuminoids reached approximately 50 mg/g after 3 hours of extraction and remained unchanged until the end of the 8-hour extraction process (Figure 3).

For UAE (Figure 3B), the maximum concentration of curcuminoids (~49 mg/g) was achieved after 40 minutes of extraction (Figure 3). Similar results from UAE have been observed in other studies which the optimal extraction time for curcuminoids from turmeric ranged between 40 and 60 minutes (JIA et al., 2021; MANDAL; MOHAN; HEMALATHA, 2008).

In UAE, it was observed that even in very short processing times (30 seconds), the majority of curcuminoids (43.6 mg/g) were extracted (Figure 3, inset). This occurs because curcuminoids are predominantly located on the surface of the plant matrix, facilitating their extraction. On the other hand, when this solution was subjected to an ultrasonic bath for just 40 minutes, there was an approximately 8% increase in yield. This is due to ultrasound promoting the disruption of the plant matrix, enhancing mass transfer, and facilitating the extraction of curcuminoids present inside the cells.

Based on these results, it was determined that curcuminoid extraction should be performed for 3 hours using the Soxhlet method or 40 minutes using UAE. Exceeding these established times does not affect the extraction yield.

3.2.2 The Effect of Bath Temperature in UAE

To enhance the ultrasound-assisted extraction of curcuminoids, the procedure was conducted while varying the temperature (35, 55, or 75 °C) for 40 minutes of sonication. It was found that temperature did not significantly influence the extraction yield, which ranged between 46.03 and 47.30 mg/g across the treatments.

In line with our results, RASO et al., (1999) demonstrated that temperatures between 20 to 70 °C have little effect on ultrasound power output. However, the same authors found that temperatures above 70 °C significantly decrease power output, thereby reducing the efficiency of ultrasound in extraction processes (RASO et al., 1999). On the other hand, some studies indicate that increasing the temperature up to 60 °C can enhance the efficiency of curcumin extraction using ultrasound (SHIRSATH et al., 2017, 2021). This improvement has been attributed to temperature aiding in the opening of cellular matrices, thereby increasing curcumin availability, reducing viscosity, and enhancing solvent diffusivity (SHIRSATH et al., 2017). It's important to note that this divergence with reported results could be partly explained by differences in extraction methodologies, types of equipment used, raw materials, and solvents employed.

Despite higher temperatures seemingly favoring the UAE process, curcumin is sensitive to temperature increases and undergoes degradation, resulting in a reduction in curcuminoid extraction yield from 3.7% to 3.0% when exposed to temperatures of 40 °C (SAHNE et al., 2017).

To measure the degree of curcumin degradation during ultrasound-assisted extraction (UAE) at temperatures of 35, 55, and 75 °C, DPPH and ABTS assays were performed. For the DPPH assay, values ranged from 135.68 to 143.27 μmol Trolox equivalents/g of turmeric. For the ABTS assay, values varied from 178.30 to 182.26 μmol Trolox equivalents/g of turmeric. Both assays demonstrated that temperature did not significantly influence the antioxidant activity of the curcuminoids. However, studies indicate that temperatures exceeding 75 °C may lead to the degradation of bioactive compounds (MILOŠEVIĆ et al., 2024).

Some studies have reported that the antioxidant activity of curcuminoids, as assessed by ABTS and DPPH assays, can vary depending on the temperature used in the turmeric drying process (KUMAR et al., 2023; VICHAKSHANA; FOO; CHOO, 2023). However, drying processes tend to be prolonged, often exceeding five hours. In contrast, the curcuminoid extraction process was carried out in a significantly shorter period of only 40 minutes. Therefore, it is assumed that the short exposure time of the extracts to heat did not compromise the antioxidant activity of the curcuminoids.

In brief, the temperature increase (from 35 to 75 °C) did not affect the extraction yield and the degradation of curcumin. Considering that there was no significant improvement in extraction efficiency with increasing temperature, and considering the higher energy expenditure required to maintain higher temperatures, we conclude that 35 °C is ideal for the UAE process of curcuminoids.

3.2.3 The Effect of Mass-to-Solvent Ratio in UAE

The concentrations of curcuminoids obtained in the extraction process using mass-to-solvent ratios (w/v) of 1:10, 1:30, 1:50, and 1:70 were respectively: 42.06 ± 0.84, 47.03 ± 1.63, 47.61 ± 0.15, and 45.37 ± 0.68 (mg/g). The lowest extraction yield was obtained when the 1:10 ratio was used. Conversely, adopting the 1:30 ratio resulted in an increase of over 10% in extraction yield, while larger solvent ratios (1:50 and 1:70) yielded extraction efficiencies statistically equivalent ($p < 0.05$) to the 1:30 ratio.

The increase in curcuminoid concentration was facilitated by a larger difference in concentration between the mass of turmeric and the volume of ethanol, which enhanced mass transfer. This phenomenon occurs because less acoustic pressure is required for bubble formation in the fluid when the cycle is in the rarefaction zone. Conversely, an uneven cavitation phenomenon is provoked when the solid-to-solvent ratio increases (MESSINO'; SETTE; WANDERLINGH, 1967).

SHIRSATH et al., (2021) studied the effect of solid-to-liquid ratio on curcumin extraction from *Curcuma aromatica* using probe ultrasound and observed that varying the ratio from 1:10 to 1:50 increased the extracted curcumin amount (from 2.34 mg/g to 3.86 mg/g). PATIL; PATHAK; RATHOD, (2021) optimized the extraction process of curcuminoids using probe ultrasound with deep eutectic solvent. They found that reducing the solid loading (from 20 % to 2.5 %) significantly increased the extraction yield (from 7.83 mg/g to 77.12 mg/g).

Another study evaluated the application of a new imidazolium-based ionic solvent for curcumin extraction, examining the effects of time, temperature, and solvent quantity. The yield obtained ranged from 0.76 % to 2.94 % curcumin. Among the investigated parameters, the solvent volume (ranging from 10 to 30 mL per 0.05 g) was found to be the most influential factor on the extraction yield, followed by time and temperature, respectively (GÖKDEMIR; BAYLAN; ÇEHRELI, 2020).

Therefore, the 1:30 ratio was chosen as optimal. Adjusting the appropriate solvent ratio helps minimize costs associated with both solvent consumption and the energy required for its subsequent removal.

3.3 Influence of Pretreatment Application on Curcuminoid Extraction

To investigate the efficiency of hybrid methods of extraction, i.e., UAE combined with enzymatic hydrolysis, ohmic heating (OH) or fermentation, the concentrations of curcuminoids were obtained by spectrophotometry, and the proportions of the three main curcuminoids were identified by HPLC, yielding the concentrations of curcumin (CC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), as shown in Table 1.

Table 1: Yield of UAE of curcuminoids applying different pre-treatments.

Pr-treatament	Curcuminoids (mg/g)	CC (mg/g)	DMC (mg/g)	BDMC (mg/g)
Control	41.21 ± 0.39^{de}	19.63 ± 0.39^{e}	13.96 ± 0.18^{ab}	8.62 ± 0.08^{b}
Enzimatic	43.33 ± 1.10^{cd}	22.84 ± 0.58^{c}	13.58 ± 0.34^{bc}	6.91 ± 0.18^{c}
OH 10 V	48.44 ± 0.98^{b}	24.74 ± 0.28^{b}	13.95 ± 0.16^{ab}	9.74 ± 0.11^{a}
OH 30 V	44.18 ± 0.98^{c}	22.58 ± 0.50^{c}	13.08 ± 0.29^{c}	8.51 ± 0.19^{b}
OH 50 V	41.23 ± 0.26^{e}	20.96 ± 0.13^{d}	11.68 ± 0.08^{d}	8.58 ± 0.05^{b}
Fermentation	51.29 ± 0.39^{a}	27.21 ± 0.26^{a}	14.52 ± 0.14^{a}	9.56 ± 0.09^{a}

Average values ± standard deviation (SD), n = 3. Significance ($p < 0.05$): means with at least one same letter in the column do not differ significantly according to Tukey's test.

The enzymatic pre-treatment with α-amylase did not result in a significant difference in the total concentration of curcuminoids compared to turmeric that did not receive any treatment. However, a 16% increase in the concentration of curcumin (CC) and a 20% decrease in bisdemethoxycurcumin (BDMC) were observed. (SAHNE et al., 2017) reported a 60% increase in CC extraction by applying enzymatic pre-treatment (α-amylase and amyloglucosidase) followed by extraction using ionic liquid carbamate. The authors attributed this improvement to the

enzyme's role in breaking glycosidic bonds in the turmeric cell wall, allowing better diffusion of the solvent (N, N-dipropyl ammonium N', N'-dipropylcarbamate) (SAHNE et al., 2017). An increase of 26.04% and 31.83% in CC extraction yield was also observed when applying pre-treatments with α-amylase and glucoamylase, respectively, followed by Soxhlet extraction. In this case, the improvement was attributed to the breakdown of the starch matrix (KURMUDLE et al., 2013).

In this context, the application of α-amylase can be a promising alternative if the compound of interest is solely CC. Although treatment with α-amylase is considered an efficient method for CC extraction, there was no evidence found indicating that this enzyme maximizes the total curcuminoid extraction yield when used as a pre-treatment for subsequent UAE. By optimizing the operational conditions of UAE, the ultrasound phenomena provided good solvent diffusion and promoted the breakdown of the plant's starch matrix. A similar enzymatic treatment, but with the application of magnetic nanoparticles co-immobilized with enzymes (MNPs) and UAE, was evaluated for curcumin extraction. The extraction yield from the sequential application of enzymatic treatment and UAE was the same as when only one of the treatments (enzyme or ultrasound) was employed (PATIL; RATHOD, 2022).

On the other hand, the positive effect of combining enzymatic treatment with UAE has been reported for the extraction of total phenolic compounds from mango peel (ESTRADA-GIL et al., 2022) *Trapa quadrispinosa* Roxb. (LI et al., 2017) and lycopene extraction from tomato residues (AMIRI-RIGI; ABBASI; SCANLON, 2016), for example. However, to date, this relationship has not been evidenced for curcuminoid extraction. A more detailed evaluation of the optimal conditions for enzymatic treatment prior to UAE is still needed, such as studying different enzyme concentrations, as well as the combination of two or more enzymes, incubation time, temperature, among others.

For the ohmic heating (OH) pre-treatment, three different voltages were evaluated: 10, 30, and 50 V. The initial temperature of all samples was 23°C, and all samples underwent OH for 15 minutes. After this period, the final temperatures were 24.9, 36.7, and 67.6°C for the treatments at 10, 30, and 50 V, respectively. As expected, the higher the applied voltage, the greater the heating generated. After the UAE process, the curcuminoid extraction yield from turmeric treated with OH at 50 V (41.23 ± 0.26) was statistically similar to the sample that did not receive any pre-treatment (42.21 ± 0.39). However, OH at 30 V improved the curcuminoid extraction yield by 7%. For these two treatments, the proportions of each curcuminoid varied similarly, with the extraction of CC being slightly favored while a smaller amount of DMC was obtained. For OH at 10 V, the

extraction efficiency of curcuminoids improved by 17%. There was a significant increase in the content of CC (28.7%) and BDMC (13%), while the content of DMC was preserved. Therefore, we can affirm that OH is an efficient alternative for improving curcuminoid extraction, but the operational conditions must be carefully evaluated. The data obtained in this evaluation suggest that DMC is the most sensitive compound to OH, as it was the only curcuminoid whose extraction yield was negatively affected when voltages higher than 10 V were employed.

The ohmic heating (OH) technology applied as a pre-treatment for extracting bioactive compounds is based on the synergy of thermal and non-thermal effects such as electroporation, electropermeabilization, and electrical degradation (CAPALDI et al., 2024). Regarding the involved phenomena, it is suggested that the heating generated by applying higher voltage may have caused degradation of the curcuminoids, specifically demethoxycurcumin (DMC). Although the present study demonstrated that for UAE, temperatures above 70°C did not affect the extraction yield, the combined effect of temperature and electrical current needs further investigation. While UAE evaluates the combined effect of temperature and cavitation, OH deals with temperature and electrical current. It is noteworthy that in OH, turmeric was dispersed in water, whereas ethanol was used as the solvent in UAE. Given that curcuminoids are lipophilic, upon release from the cellular matrix, they have a higher affinity for ethanol than water. Thus, it can be suggested that ethanol may provide greater thermal stability to curcuminoids compared to water.

Additionally, key parameters of OH technology include the applied time and voltage. In this case, it was observed that lower voltage was more efficient for improving extraction yield. However, the variation of time was not studied, suggesting that evaluating the application of higher voltages (> 10 V) for shorter durations (< 15 minutes) would be highly relevant. LE-TAN et al., (2022) valuated the application of a much higher electrical voltage (1000 V/cm) for a rapid period (119 seconds), followed by aqueous extraction conducted in a water bath with agitation. The temperature increased from 20 to 95°C and led to a 1.24-fold increase in curcumin recovery. It was also reported that increasing voltage (from 16 to 80 V/cm) and reducing time (from 20 to 1 minute) improved anthocyanin extraction from grape skin. The authors also observed the non-thermal effect of cellular permeabilization in the same study (PEREIRA et al., 2020).

Another study evaluated the application of different electric fields (20, 30, and 40 V/cm) as pretreatment, followed by ultrasound for the extraction of phenolic compounds from cornelian cherry. The extraction yield of total phenolic compounds (TPC) was enhanced with increasing applied voltage from 20 to 30 V/cm. In this study, the authors employed a scheme to maintain a constant temperature (50 °C) to assess the effect of voltage and time. It was observed that varying

the time from 1 to 10 minutes did not result in significant changes in TPC concentration for all evaluated voltages. However, at 40 V/cm, extending the time to 20 minutes increased TPC by 26% (KUTLU et al., 2021).

Finally, it was found that applying OH as a pretreatment for curcuminoid extraction, using a voltage of 10 V for 15 minutes, was efficient in enhancing the extraction process. However, it is still necessary to better understand the individual and combined effects of the parameters involved in the process, such as time, temperature, and voltage. Additionally, it is important to evaluate sample preparation characteristics, such as solvent type, solid:liquid ratio and particle size, for example

Lastly, the fermentation process occurred spontaneously over 126 hours through microbial succession. During the first 20 hours, there was a significant drop in pH (from 6.95 to 5.51), predominantly due to the growth of lactic acid bacteria. The initial and final concentrations of these bacteria were 1.1 x 10^7 and 1.5 x 10^7, respectively. Fungal concentration increased from 0 CFU/g to 6.0 x 10^4 CFU/g. The fermentation pretreatment led to an increase of over 24% in curcuminoid concentration compared to turmeric that did not receive any pretreatment. The increase in extraction yield was corroborated by the quantification of total phenolic compounds (TPC). The TPC in turmeric extract increased from 30.19 ± 0.32 to 34.85 ± 0.73 mg GAE/g at the end of the fermentation process. Fermentation was the pretreatment that provided the best performance for curcuminoid extraction (51.29 mg/g) among the evaluated treatments, statistically differing from all others. This process improved the extraction yield of the three evaluated curcuminoids, especially curcumin (CC), which showed a higher concentration than all analyzed treatments. It was possible to extract 38% more CC compared to the sample subjected only to UAE.

It is known that curcuminoids are phenolic compounds; however, it has been evidenced that the fermentation process favors the extraction of various other phenolics (LIM et al., 2022; MOHAMED et al., 2016). Additionally, despite CC, DMC, and BDMC being the main curcuminoids present in turmeric, studies have identified other curcuminoids that are presumably present in turmeric metabolites, such as curcumin sulfate, hexahydrocurcumin, tetrahydrocurcumin, dihydrocurcumin, curcumin glucuronide, and hexahydrocurcuminol (LI, 2011). In a study on turmeric fermentation using the fungi *Monascus purpureus* and *Eurotium cristatum*, the authors identified 115 curcuminoids. This study observed that after fermentation, the content of various minor curcuminoids significantly increased, while the content of the main

curcuminoids decreased (XIANG et al., 2020). Therefore, it is suggested that the fermentation process assists in the biotransformation of curcuminoids, facilitating their extraction.

Although most studies focus on the beneficial effects of CC, justified by its predominant concentration, the bioavailability of curcuminoids is related to water solubility. Research indicates that curcuminoids with greater water affinity may be more bioavailable (PRASAD; TYAGI; AGGARWAL, 2014). In this context, studies suggest that the fermentation process can help obtain more bioavailable extracts, as this process can favor the extraction of some curcuminoids that have higher polarity than the three main curcuminoids (CC, DMC, BDMC) (XIANG et al., 2020).

3.4 Colorimetric Characterization of Curcuminoid Extracts

The colorimetric parameters of the curcuminoid solutions extracted by different methods are presented in Table 2. There was a significant difference in brightness (L*) among the treatments, with samples treated with OH exhibiting the highest brightness, followed by fermented samples, the control sample, and lastly, the enzyme-treated sample. The variation in these values is related to the concentration of curcuminoids in each sample, where extracts with higher concentrations of curcuminoids tend to have higher L* values.

Table 2: Colorimetric Parameters of Curcuminoid Solutions from UAE Applying Different Pretreatments.

Pre-treatament	L*	a*	b*	C*	h*
Control	71.81 ± 0.05^{d}	-0.78 ± 0.04^{d}	74.98 ± 0.12^{c}	74.98 ± 0.12^{c}	-1.56 ± 0.00^{b}
Enzimatic	70.43 ± 0.02^{e}	2.98 ± 0.06^{a}	74.12 ± 0.05^{d}	74.18 ± 0.05^{d}	1.53 ± 0.00^{a}
OH 10 V	72.42 ± 0.02^{a}	-0.83 ± 0.02^{d}	76.64 ± 0.04^{a}	76.64 ± 0.04^{a}	-1.56 ± 0.00^{b}
OH 30 V	72.42 ± 0.02^{a}	-0.80 ± 0.03^{d}	76.63 ± 0.04^{a}	76.63 ± 0.04^{a}	-1.56 ± 0.00^{b}
OH 50 V	72.26 ± 0.01^{b}	-0.40 ± 0.04^{c}	76.52 ± 0.04^{a}	76.52 ± 0.04^{a}	-1.57 ± 0.00^{c}
Fermentation	72.03 ± 0.01^{c}	-0.07 ± 0.02^{b}	76.05 ± 0.02^{b}	76.05 ± 0.02^{b}	-1.57 ± 0.00^{c}

Average values ± standard deviation (SD), n = 3. Significance ($p < 0.05$): means with at least one same letter in the column do not differ significantly according to Tukey's test.

In Figure 4, the colorimetric plot of the different extracts is presented. For the parameter a* (red-green axis), only the enzyme-treated sample showed a positive value, indicating a tendency towards the red direction, while all others resulted in negative values, suggesting a tendency towards the green color direction. The parameter b* (yellow-blue axis), responsible for the yellow color, as well as C* (saturation), resulted in higher values for samples treated with OH, followed by fermentation, control, and enzyme-treated samples, respectively.

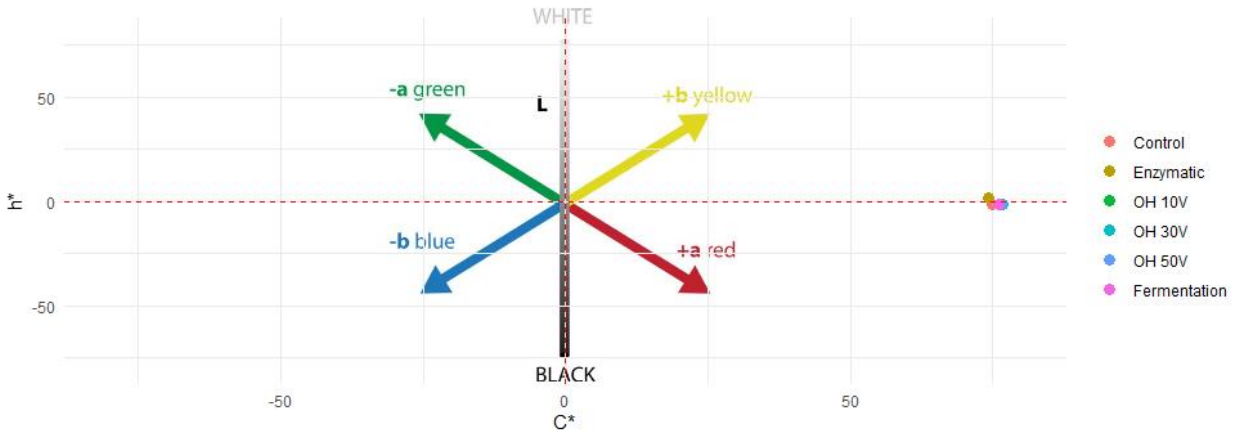

Figura 4: Colorimetric Plan of Curcuminoid Extracts.

The variation in these parameter values is associated with the different proportions of curcuminoids present in the samples. It has been demonstrated that CC and DMC are associated with the red direction (+ a*), while DBMC tends towards the green direction (- a*). Additionally, regarding C* (saturation), CC exhibits higher vividness, whereas BDMC appears more opaque (PÉRET-ALMEIDA et al., 2005).

Although the control sample showed significant differences in the evaluated parameters compared to the samples subjected to pretreatment, calculating the magnitude of the overall color difference (ΔE*) resulted in values below 5. This indicates that the color difference is not perceptible to the human eye (OBÓN et al., 2009).

4 Conclusions

This study investigated comprehensive strategies, ranging from turmeric powder production to the identification of the primary curcuminoids extracted. During the production of turmeric powder, it was found that 250 minutes were required for drying in an oven with air circulation at 75 °C, with the thermal pre-treatment before drying being deemed unnecessary.

Ultrasound-assisted extraction technology proved to be efficient in recovering curcuminoids, allowing the extraction of 95% of the curcuminoids in just 40 minutes, compared to the 3 hours required by the Soxhlet method. The optimal bath temperature was 35 °C, with a mass-to-solvent ratio of 1:30.

The application of hybrid extraction processes enhanced the extraction of curcuminoids. All pre-treatments evaluated (enzymatic, ohmic heating, and fermentation) induced changes in the proportions of at least one of the extracted curcuminoids, although the total concentration of curcuminoids remained unchanged for the enzymatic treatment. For ohmic heating (OH), it was observed that lower voltages (10V and 30V) were more effective in releasing curcuminoids, resulting in up to a 17% increase in yield. The fermentation pre-treatment maximized curcuminoid extraction by 24%, also suggesting the possible formation of other curcuminoids besides the known curcumin (CC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC).

Additionally, it was observed that although the colorimetric parameters (L*, a*, and b*) showed variations, the overall color difference of the extracts was not perceptible to the human eye. In conclusion, the techniques evaluated in this study demonstrate significant potential for scaling up curcuminoid extraction for commercial applications, whether for incorporation into food matrices or for supplementation.

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Declaration of Competing Interest

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

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ARTICLE 2

Encapsulation of hydrophobic active ingredients in plant proteins: modulation of interfacial properties and encapsulation efficiency

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Highlights

- Protein interaction with oil-in-water interfaces boosts encapsulation efficiency.
- Protein solubility aids in the encapsulation of hydrophobic actives.
- pH, ionic strength, and temperature influence encapsulation efficiency.
- Auxiliary technologies help enhance encapsulation efficiency.

Abstract

The increasing desire for vegan products has prompted the exploration of plant proteins such as those derived from peas, soy, and sunflowers. Despite offering sustainable alternatives, plant proteins pose certain challenges, particularly in achieving high encapsulation efficiencies for hydrophobic actives. Modulating the techno-functional characteristics of plant proteins through changes in their physicochemical environment, such as pH and temperature, is crucial. Despite being limited in interfacial properties compared to animal proteins, plant proteins can be optimized for successful encapsulation systems, especially with small alterations or pre-treatments like heating or enzymatic hydrolysis. The potential of auxiliary technologies, including ultrasound and

high pressure, in enhancing plant protein-based encapsulation systems remains an underexplored area, necessitating further research for industrial applications.

1 Introduction

The global vegan food market was valued at $16,532.3 million in 2022, with a projected compound annual growth rate (CAGR) of 8.8% from 2023 to 2030. The continuous expansion of the vegan food market is attributed to the rising environmental concerns, increasing awareness of animal welfare, and the health benefits associated with a vegan diet, particularly in weight management and the prevention of chronic diseases (www.coherentmarketinsights.com). Also driven by some of these factors, the global vegan cosmetics market is projected to have a CAGR of 8.0% between 2023 and 2024, while the vegan supplements sector (vitamins, minerals, amino acids, botanical supplements, and others) is forecasted to have a CAGR of 10.35% from 2018 to 2030 (www.polarismarketresearch.com; www.grandviewresearch.com).

Over the years, animal-derived proteins such as gelatin, whey proteins, casein, and egg albumin have been utilized as encapsulation materials for various active ingredients intended for the formulation of food, cosmetics, and pharmaceuticals [1, 2, 3, 4]. The widespread use of these protein sources is justified by their availability for purchase, relatively low prices, and techno-functional characteristics (high water solubility (>85% at pH 7), high emulsifying, gelling, and foaming capacity) that facilitate the entrapment and protection of the active principle [5●●, 6●●]. However, with the increasing demand for vegan products, encapsulation techniques have also needed to be adapted to the new context. This has driven studies in the field employing pea proteins (vicilin and convicilin), soy proteins (conglycinin and glycinin), sunflower proteins (albumins), legumes (glutenins and albumins), and proteins from oats, rice, and wheat [7●].

As advantages, plant proteins are abundant in nature and offer a more sustainable large-scale production compared to animal proteins, aligning with market trends. On the other hand, their techno-functional properties differ from those of animal origin, necessitating adjustments to established encapsulation protocols. Due to being a relatively unexplored field, the encapsulation of actives with plant proteins can be challenging and often result in lower encapsulation efficiencies than those with animal-derived proteins.

Thus, an understanding of the influence of the physicochemical environment on the interfacial properties of plant proteins, specifically how they interact with oil-water interfaces in industrial processes, can serve as a starting point for modulating their techno-functional characteristics. This modulation aims to broaden their range of applications in the encapsulation of hydrophobic actives, such as vitamins (vitamin D, vitamin E), polyphenols (curcumin,

resveratrol, quercetin), carotenoids (β-carotene, lycopene, lutein, α-carotene), and polyunsaturated fatty acids (omega-3) [8●●].

2 Influence of interfacial properties on encapsulation efficiency

Proteins are amphiphilic molecules that have hydrophilic and hydrophobic domains that tend to interact with water and oil, respectively. To achieve both events simultaneously, proteins deposit at the oil-water interface, forming a film around the oil droplets. In other words, proteins cover the surface of the oil droplet, producing microcapsules with an oil core and a protein membrane. The encapsulation of oil using oil-in-water emulsification techniques is particularly employed in cosmetics, food, and pharmaceuticals to reduce the oily appearance of the product, promote controlled release of actives, minimize lipid oxidation, and prevent phase separation during storage [9]. In general, high solubility aids in the adsorption of plant proteins to oil-water interfaces, potentially enhancing the oil encapsulation efficiency through emulsification techniques [10,11,12]. However, excessively high solubilities can compromise the performance of proteins in stabilizing oil-in-water emulsions by reducing of viscoelasticity of the protein films. In this sense, it was demonstrated that homogenization at 30 kpsi improved faba bean protein solubility from 35% to 99% at neutral pH. Nevertheless, the emulsifying activity was reduced from 27.0 to 19.7 m^2/g while the emulsion stability decreased from 39.9 to 16.6 minutes [13]. It is presumed that the higher proportion of supramolecular aggregation in the high-pressure homogenized faba bean protein may decrease the viscoelasticity of the protein film, thereby reducing encapsulation/emulsification efficiency [13].

Despite there being a certain correlation between solubility and emulsifying capacity, the molecular structure of the protein, aggregation, colloidal state and environmental factors can influence its interfacial properties [14, 15]. As an example, compared to globulins, albumins tend to form more stable films at the oil-water interface due to their smaller size and higher surface hydrophobicity, which favors protein-protein interfacial interactions [16]. It makes clear that the molecular structure of the protein has a primordial role in its emulsion stabilization capacity.

In a recent study, Yang et al. (2024) demonstrated that despite cruciferin being less soluble (82%) than napin (96%), cruciferins were able to form rigid viscoelastic layers at oil-in-water interfaces with high deformation resistance. According to the authors, this type of interface was likely formed due to the high hydrophobicity of the protein surface and the β-sheet content in the

secondary protein structure, allowing for greater interaction in-plane between adsorbed proteins, leading to stable emulsions [17]. In terms of environmental factors that can affect the emulsifying capacity, Ntone et al. (2021) suggest that under acidic conditions, cruciferins dissociate from hexamers into trimers, resulting in the exposure of hydrophobic residues, thereby increasing their binding to sinapic acid naturally present in rapeseed protein extract. Thus, the cruciferin-phenol interaction reduces the adsorption of proteins at oil-water interfaces, potentially compromising emulsion stability [18].

In summary, the wide variety of factors that influence the interfacial protein behavior justifies the need for in-depth studies before practical application, especially for plant proteins that are still relatively unexplored.

In addition to aiding in the stabilization of oil-water interfaces, proteins in their soluble state present more exposed hydrophobic domains, which promotes interaction with other hydrophobic active ingredients. In the case of flavors used in the food industry, these can interact with proteins reversibly through van der Waals forces, ionic bonding, hydrogen bonding, and hydrophobic interactions [19]. However, some flavor molecules can interact irreversibly with protein side chains through covalent binding, including aldehyde-lysine and amines-carbonyl group interactions [19].

Weak or covalent chemical interactions between the active ingredient and plant proteins assist in retaining the active substance in the wall material, providing better encapsulation yields. This type of interaction is particularly important in coacervation and spray drying encapsulation techniques, aiding in the retention of the active substance inside the capsule, enhancing its protection, and enabling controlled release for specific applications.

Plant proteins are rich in hydrophobic amino acids such as alanine, leucine, isoleucine, valine, proline, and glycine, making them inherently hydrophobic proteins. Soybean, bean, pea, and faba proteins contain more than 30% hydrophobic amino acids in their composition, reaching up to 38% in the case of pea protein [20]. From a physiological perspective, plant proteins exhibit storage characteristics, and high hydrophobicity helps maintain a more compact structure to stay within seeds [5●●]. However, from a techno-functional standpoint, high hydrophobicity significantly reduces the solubility of the protein in aqueous media, leading to the tendency to form aggregates. In hydrophobic active encapsulation, this structural characteristic is disadvantageous as it reduces the electrochemical interaction of the active with the encapsulating agent.

However, plant proteins can vary structurally depending on the extraction method, pH, ionic strength, temperature, and the variety of the plant [5●●]. Small changes in the physicochemical environment or the application of treatments (heating, ultrasound, high hydrostatic pressure, enzymatic hydrolysis) can result in alterations in their interfacial properties, making them more efficient encapsulating agents.

3 Physicochemical environment and auxiliary treatments for improving encapsulation efficiency

As mentioned, the interfacial properties of plant proteins are directly related to their solubility. Thus, physicochemical changes promoting an increase in protein solubility assist in improving the encapsulation efficiency of oil and hydrophobic substances. The protein solubility and its surface electric charge are interconnected, such that a higher surface charge leads to a greater tendency for intermolecular repulsion and, consequently, increased protein solubility in aqueous media. Therefore, it is expected that electrically charged proteins serve as better emulsion stabilizers and exhibit chemical interactions with hydrophobic actives.

The solubility curve of plant proteins as a function of pH typically follows a "U" shape [21]. This indicates that at acidic and alkaline pH values, proteins are more soluble, reaching maximum solubility at pH above 8. This occurs because plant proteins possess acidic characteristics, as the sum of Aspartic acid and Glutamic acid residues (acidic amino acids) is greater than the sum of Lysine, Arginine, and Histidine residues (basic amino acids). In soybean, bean, and faba proteins, the sum of acidic amino acids varies from approximately 24 to 34%, while the sum of basic amino acids ranges from approximately 13 to 19% [20].

In an alkaline pH, acidic amino acids become ionized, increasing the protein's surface charge and, consequently, its solubility [21]. It is also in alkaline pH that plant proteins exhibit enhanced emulsifying properties, being more effective in stabilizing emulsions. Despite being electrically charged, it's worth noting that under these conditions, hydrophobic sites have fewer interactions among themselves, making them more susceptible to interactions with hydrophobic actives. In summary, charged proteins can enhance the efficiency of emulsification encapsulation processes while efficiently adsorbing hydrophobic actives to their structure.

On the other hand, in the pH range between 4 and 5, proteins tend to reach their isoelectric point, resulting in reduced solubility and high tendency for aggregation [21,22,23●,24].

Some studies have shown that it is possible to modulate the interfacial properties of plant proteins by varying the pH [25,26,27]. Shen et al. (2022) highlighted the improvement in the emulsifying capacity of pea protein isolates through pH 10-shifting treatment. According to the authors, the relative quantities of legumins and albumins increased at the oil-water interface, while the abundances of vicilins decreased due to the effect of competitive adsorption, resulting in a stronger emulsifying activity at pH 10-shifting [25]. Using a similar approach, Dai et al. (2022) demonstrated that the particle size of rice protein isolate decreased during the alkali shift treatment (pH 10–11) due to the depolymerization of protein aggregates. This pH-shifting resulted in the best emulsion stabilization property among the treatments [26].

In encapsulation of limonene droplets in corn zein, it was observed that the dynamics of coacervate droplet deposition and the interactions among coacervate droplets, which prevent coacervate droplet coalescence, are significantly influenced by pH. This leads to a well-defined pH optimum (around pH 8) for the formation of capsules [28].

By using a spray drying technique for the encapsulation of fish oil in brewer's spent grain proteins, an oil encapsulation efficiency of 87% was achieved at pH 5, while a higher efficiency (99%) was obtained at pH 11 [29]. Also using spray drying but at acidic pH values (2.5 to 5.0), Chen et al. (2023) conducted a study on the encapsulation of spice essential oil in quinoa protein isolate and gum Arabic. They found that a pH of 3.6 was optimal for forming quinoa protein-gum Arabic coacervates, leading to an encapsulation efficiency of up to 88% after spray-drying [30].

Now considering the effect of ionic strength on protein solubility, at neutral pH, the solubility curve of the protein versus ionic strength exhibits a "Ω" shape, indicating reduced solubility at low and high salt concentrations, reaching a maximum point at intermediate concentrations. In an aqueous medium, the hydrophobic residues of proteins interact with each other; however, as salt is added, metal ions begin to interact with these residues. Consequently, the hydrophobic residues move away from each other, increasing the solubility of the protein (salting in). On the other hand, when the salt concentration becomes excessive, metal ions compete for the solvation water of proteins and the hydrophobic residues interact again, reducing the solubility (salting out) [31].

Despite plant proteins exhibiting similar behaviors in response to ionic strength, variations in the solubility-ionic strength curve profiles may occur depending on the protein type, extraction method, pH and plant variety.

At pH values significantly higher than the isoelectric point, certain proteins display a hill-valley-hill-valley solubility pattern in response to increasing ionic strength [32]. This behavior is attributed to diverse protein-protein, protein-water, and protein-ion interactions: i) at extremely low salt concentrations, proteins experience repulsion due to the pronounced net negative charge; ii) in the intermediate range of salt concentrations, solubility initially declines due to the ionic interaction between the cationic counter ions (Na^+) with the anionic groups on protein surfaces ($-COO^-$), reducing the electrostatic repulsion between protein molecules; iii) at elevated salt concentrations, anionic counter ions (Cl^-) bind to cationic patches on protein surfaces (NH_3^+), increasing the superficial negative charge and enhancing the protein solubility [31].

For active encapsulation processes, higher encapsulation efficiencies are expected at saline concentrations where protein solubility is maximum. Under these conditions, binding sites for the active ingredient on the protein become more exposed, favoring the entrapment of the active substance within the protein structure. It is also at intermediate salt concentrations that proteins are more prone to migrate to oil-water interfaces, potentially enhancing the encapsulation efficiency of emulsion-type systems. Although ionic strength seems to play a crucial role in the encapsulation of hydrophobic actives in plant proteins, no recent study has been found in the literature that elucidates the effect of salt concentration on encapsulation efficiency.

In addition to pH and ionic strength, temperature also plays an important role in the structure of plant proteins and consequently their degree of interaction with oil-water interfaces or hydrophobic actives. The temperature increase enhances the kinetic energy of molecules (actives and proteins), increasing the likelihood of interactions. Further elevations in temperature may promote a moderate protein denaturation, consequently intensifying the active-protein interaction. At pH 3.5 or 7, it has been demonstrated that heating at temperatures between 30 and 80 °C for 1 hour improves the solubility of diluted dispersions (1% w/w) of potato, rice, and pea proteins and, consequently, their emulsification capacity [33].

In another study focused on the encapsulation of lutein in high internal phase emulsions, pea protein was utilized as the encapsulating material. Before emulsion production, pea protein in pH 2 was heated at 85 °C for 20 hours to induce its hydrolysis and re-assembly, forming amyloid

fibrils [34]. Pea protein amyloid fibrils were capable of stabilizing emulsions with an internal phase volume fraction as high as 90%, resulting in colloidal systems with high viscosities [34].

However, it is worth noting that rigorous heat treatments can induce extensive protein denaturation, promoting interactions between peptides through van der Waals forces, hydrogen bonds, and even disulfide bonds between sulfur-containing amino acids.

Depending on the protein concentration and thermal treatment intensity, the extent of these interactions can lead to aggregation/gelation phenomena, compromising the interfacial properties of the protein and its encapsulating ability. Baune et al. (2021) demonstrated the formation of emulsion gels upon heating to 72 °C at protein concentrations around 10% (w/w) for soy, potato, and pea proteins. It was claimed that the high degree of protein denaturation favored electrostatic, hydrophobic, and hydrophilic interactions among the polypeptide chains, forming a solid gel [35].

In addition to controlling chemical (pH and ionic strength) and physical parameters (temperature), the application of auxiliary treatments such as ultrasonication and enzymatic hydrolysis has also proven to be effective in enhancing the interfacial properties of plant proteins, thereby optimizing the encapsulation efficiency.

Ultrasonication is a technique employed in emulsification/encapsulation processes, involving the application of high-frequency sound waves to protein dispersions to enhance their solubility and ability to interact with oil-water interfaces. The cavitation phenomenon induced by sound waves creates mechanical shocks in the protein structure, causing partial unfolding that leads to an increase in solubility.

Using ultrasound as pre-treatment, Sha & Xiong (2022) demonstrated that pea protein isolate exhibited a pronounced response to ultrasound perturbation, resulting in a 38.6% increase in solubility and a reduction in particle size (from 1.4 to 1.3). However, due to their high solubility (>99%), the legumin (11S) and vicilin (7S) globulin fractions were less sensitive to ultrasound. The ultrasound treatment leaded to increased emulsifying capacity and stronger interfacial adsorption for all protein samples [36]. The encapsulation of resveratrol in zein-gum Arabic by complex coacervation was enhanced by ultrasound treatment, increasing the encapsulation efficiency from 55.85% to 74.2%, and raising the loading capacities from 2.79% to 6.01% after sonication [37].

Soybean protein ultrasound-treated showed an encapsulation efficiency of luteolin and solubility to approximately 90% and >90%, respectively [38]. Also using soybean lipophilic

protein ultrasonically treated as a carrier of vitamin E, it was demonstrated that an ultrasonic power of 240 W for 20 min resulted in the highest emulsion stability and encapsulation efficiency [39]. In a similar approach, soybean lipophilic protein under alkaline conditions and ultrasound treatment (20 kHz and 240 W for 20 min) reaches an encapsulation of vitamin E efficiency above 80% [40]. Already combined ultrasound and heat treatment, the encapsulation of β-carotene within the pickering emulsion (pea protein isolate and mung bean starch complexes) resulted in improved stability, exhibiting a retention rate of 73.58% under optimal conditions [41].

Despite the encapsulation of hydrophobic actives with the aid of ultrasound being the subject of research in various studies, including recent investigations, the industrial-scale application is not yet a practical reality. On the other hand, enzymatic hydrolysis has significant potential, considering that this technique has already been used by the food, cosmetics, and pharmaceutical industries. Through enzymatic cleavage, plant proteins are converted into smaller and more soluble peptides, enabling interaction with the active ingredient, especially in processes involving highly hydrophobic proteins [42●●,24].

To demonstrate the potential of plant protein hydrolysis on hydrophobic active encapsulation processes, potato protein hydrolyzed with protease Protamex® and maltodextrin were used to encapsulate flaxseed oil through spray drying. A 2% degree of hydrolysis increased the emulsifying capacity from 359 to 416 g oil/g protein, and high encapsulation efficiency (92–96%) was observed after spray drying [43]. Soy protein hydrolyzed with Flavorzyme, Neutrase, and Alcalase was also used to fabricate β-carotene-loaded nanoparticles, resulting in an increased encapsulation efficiency from 30.46% to values up to 81.18%, depending on the enzyme used [44].

In summary, commercial proteinases exhibit different specificities with the substrate, generating distinct peptide profiles [42●●]. Thus, it becomes possible to partially control the peptide profiles formed by choosing the hydrolytic enzyme. However, it is crucial to control the process to prevent the formation of very small peptides, which may impart a bitter taste and fail to provide an effective barrier, especially in the encapsulation of hydrophobic actives.

4 Conclusion

Plant proteins exhibit limited interfacial properties, especially when compared to proteins of animal origin, and for this reason, they may not be suitable for encapsulation systems involving immiscibility of phases. The enhancement of interfacial properties of plant proteins, focusing on

solubility and interaction with oil-water interfaces, is crucial for these proteins to become better carriers of hydrophobic actives.

Despite scientific evidence that the modulation of interfacial properties through changes in the physicochemical environment (pH, ionic strength, and temperature) increases encapsulation efficiency, few studies focus on optimizing these variables by adopting pre-established values.

Regarding auxiliary technologies, such as ultrasound, high pressure, and enzymatic hydrolysis, defining their individual effects on encapsulation efficiency is challenging since studies combine different technologies and electrochemical environments. In recent years, many works have focused on the use of ultrasound in the encapsulation of hydrophobic actives, showing high encapsulation efficiencies. Despite its promising potential of hydrostatic high pressure, research evaluating plant proteins treated with high pressure as carriers for hydrophobic actives is quite scarce. High pressures are typically used in the preparation of oil-in-plant protein emulsions, making it challenging to assess the structural changes that occur in proteins.

As a perspective for future research, alternative sources of plant proteins (such as lesser-known or commercially underexplored plants) may be the key to obtaining proteins with techno-functionalities better suited for use in encapsulation processes of hydrophobic actives. As highlighted here, the interfacial properties of proteins are shaped by physicochemical factors, and these should be explored within broader ranges in future studies to ensure an understanding of protein behavior under different processing conditions. Despite ultrasound, another emerging technologies poor explored such as cold plasma, ohmic heating, and pulsed electric fields, for example, have potential to modulate the interfacial properties of plant proteins, opening the door to the development of innovative ingredients. However, it is worth noting that a feasibility study of the economic application of these and other technologies should also be an object of research, considering that the cost of some processes may render the use of plant proteins as encapsulating agents impractical.

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General conclusion and perspectives

This study evaluated strategies to improve curcuminoid extraction by applying hybrid extraction systems. In addition to demonstrating that evaluating the effects of operational parameters in ultrasonic baths is crucial for achieving better extraction efficiency, it became clear that combining techniques such as ohmic heating (OH) or fermentation, followed by ultrasound-assisted extraction (UAE), can improve extraction efficiency by up to 24%. The second paper highlighted that despite the abundance of plant protein sources, they exhibit interfacial properties distinct from animal proteins. The study pointed out that physico-chemical modifications, such as pH, ionic strength, and temperature, have been used to improve these properties. Emerging technologies, such as ultrasound, high pressure, and enzymatic hydrolysis, have also shown potential to optimize encapsulation. However, there is a need to explore a range of physico-chemical modifications and optimize the variables for each technology to better understand their effects. Finally, it is concluded that both papers addressed the potential of emerging technologies, both in maximizing the yield of curcuminoid extraction and improving the encapsulation process efficiency.