UNIVERSIDADE ESTADUAL DE CAMPINAS
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MONIQUE MARTINS STRIEDER

Processing of *Genipa americana* L. using high-intensity ultrasound for obtaining natural blue colorant for the food industry: Techno-economic assessment of genipin extraction, the use of dairy and plant proteins for crosslinking and application in almond-based beverage

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RESUMO

A demanda do mercado consumidor por ingredientes e produtos alimentícios provenientes de fontes naturais, ricos em bioativos, e obtidas através de tecnologias inovadoras que promovam menores impactos sobre os recursos naturais tem impulsionado o desenvolvimento de novos produtos e processos. Assim, o objetivo dessa tese foi estudar novas rotas e perspectivas de processamento da matriz vegetal *Genipa americana* L. utilizando a tecnologia de ultrassom de alta intensidade para obtenção de novos produtos. Os frutos verdes de jenipapo contêm genipina, um iridoide precursor de compostos de cor azul, o qual também apresenta capacidade antioxidante, anti-inflamatória e de inibição de células carcinogênicas. A tecnologia de ultrassom de baixa frequência e alta potência, por sua vez, tem sido utilizada no projeto de processos eficientes e limpos. Desta forma, na primeira etapa, estudou-se a viabilidade técnica e econômica da obtenção de um extrato etanólico rico em genipina a partir de diferentes configurações de processos de extração assistido pelo ultrassom. Os impactos da aplicação de ultrassom foram avaliados por meio da utilização de processos não térmicos e térmicos sob as mesmas condições de energia específica. Processos térmicos (18 W) tiveram um melhor desempenho na extração do composto alvo e foram economicamente mais viáveis em comparação aos não-térmicos (1.5 W). Embora os processos utilizando maior potência acústica tenham aumentado a temperatura do sistema, eles favoreceram a difusão e, consequente, extração da genipina sem contribuir para degradação do precursor corante. Posteriormente, avaliou-se o efeito da energia específica (0,06, 0,10, 0,17 e 0,25 kJ/g) fornecida pelo ultrassom sobre a obtenção de um corante natural azul em uma única etapa. Para isso o sistema coloidal do leite foi utilizado como solvente, fonte de aminas primárias para reação de reticulação com a genipina e carreador dos compostos de coloração azul produzidos. A energia acústica assistiu à extração da genipina, sua reticulação com as proteínas do leite e a formação de um corante mais azul e cineticamente estável. Da mesma forma, seguindo a demanda por produtos livres de matérias-primas de origem animal, o desempenho de extratos vegetais de arroz, aveia, amendoim e amêndoa foram avaliados em substituição ao leite para a produção de corantes naturais azuis. Nesta etapa, o impacto do tratamento de termosonicação aplicado aos extratos vegetais sobre a qualidade tecnológica dos corantes foi avaliado. O extrato vegetal obtido de amêndoas foi o que apresentou as melhores características químicas e físicas para carrear o corante azul. Além disso, a termosonicação favoreceu a estabilização cinética dos corantes líquidos obtidos. Por fim, avaliou-se a aplicação do novo produto obtido a partir do extrato de amêndoa em uma bebida vegetal. Avaliou-se o tratamento dessa bebida através da termosonicação aplicada em diferentes potências acústicas (4,6, 8,5 e 14,5 W) e tempos de tratamento (5, 10 e 15 min). Todos os tratamentos avaliados preservaram a atividade antioxidante da bebida, porém os mais intensos (8,5 e 14,5 W por 10 e 15 min) promoveram a degradação de alguns ácidos graxos e compostos voláteis. Dessa forma, a partir de frutos verdes de *Genipa americana* L. foi possível estabelecer novas rotas empregando a tecnologia de ultrassom de alta intensidade para produção de corante natural azul. Além disso, nós demonstramos a viabilidade técnico-econômica da extração assistida por ultrassom para a produção de um extrato etanólico rico em genipina e a estabilidade do corante produzido a partir das proteínas de amêndoa frente a tratamentos de termosonicação que aplicaram energia acústica e térmica.

Palavras-chaves: *energia acústica, genipina, corante natural azul, produtos não-lácteos.*

ABSTRACT

New food products and processes have been developed due to the consumer demand for food ingredients and products from natural and bioactive sources obtained through innovative technologies that promote less impact on natural resources. Thus, this thesis aimed to study new routes and perspectives for processing the plant matrix *Genipa americana* L. using high-intensity ultrasound technology to obtain novel products. The unripe fruits of genipap are rich in genipin, an iridoid precursor of blue compounds that present antioxidant, anti-inflammatory, and inhibiting capacity for carcinogenic cells. Low-frequency and high-power ultrasound technology have been used to design efficient and clean processes. Thus, the technical and economic feasibility of obtaining an ethanolic extract rich in genipin from different configurations of ultrasound-assisted extraction processes was studied in the first stage. The impacts of ultrasound application were evaluated using non-thermal and thermal processes under the same specific energy conditions. Thermal processes (18 W) had a better performance in extracting the target compound and were more economically viable than non-thermal processes (1.5 W). Although higher acoustic power processes increased the system's temperature, they favored the diffusion and, consequently, the extraction of genipin without contributing to the degradation of the colorant precursor. Subsequently, the effect of specific energy (0.06, 0.10, 0.17, and 0.25 kJ/g) provided by ultrasound on obtaining a natural blue colorant in a single step was evaluated. For this, the colloidal milk system was used as a solvent, source of primary amines for crosslinking reaction with genipin, and carrier of the blue-colored compounds produced. Acoustic energy-assisted the extraction of genipin, its crosslinking with milk proteins, and the formation of a bluer and kinetically stable colorant. Likewise, following the demand for products free of animal raw materials, the performance of plant extracts of rice, oats, peanuts, and almonds was evaluated as a substitute to milk for the production of natural blue colorants. In this step, the impact of the thermosonication treatment applied to plant extracts on the technological quality of the colorants was evaluated. The plant extract obtained from almonds showed the best chemical and physical characteristics to carry the blue colorant. Furthermore, thermosonication favored the kinetic stabilization of the liquid colorants obtained. Finally, we evaluated the application of the almond-based colorant in a plant-based beverage. The beverage was processed through thermosonication applied at different acoustic powers (4.6, 8.5, and 14.5 W) and treatment times (5, 10, and 15 min). All treatments preserved the beverage's antioxidant activity, but more intense treatments (300 and 400 W for 10 and 15 min) promoted the degradation of some fatty acids and volatile compounds. Therefore, we established new routes employing high-intensity ultrasound technology to produce natural blue colorants from unripe fruits of *Genipa americana* L. Furthermore, we demonstrated the technical-economic feasibility of ultrasound-assisted extraction for producing an ethanolic extract rich in genipin and the stability of the blue almond-based colorant against thermosonication treatments that applied acoustic and thermal energy.

Keywords: *acoustic energy, genipin, natural blue colorant, non-dairy products.*

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CHAPTER 1

General introduction, objectives and thesis structure

1 General introduction

Consumers are demanding food products with high added value concerning their sensory properties and safety. In this sense, plant materials have been evaluated as a source of ingredients to produce innovative products. Genipap (*Genipa americana* L.) is a Brazilian fruit with a high content of phenolic compounds and iridoids. Geniposide is the major compound in the unripe genipap fruits, representing more than 70% of the total iridoids, but it is the genipin that stands out (Bentes & Mercadante, 2014). Genipin has an antioxidant capacity; thus, it can act against carcinogenic bladder cells and glioblastoma tumor cells (Ahani, Sangtarash, Houshmand, & Eskandani, 2019; Li et al., 2018).

Moreover, this iridoid is a precursor of blue color compounds that are produced through its crosslinking with primary amines groups (Touyama, et al., 1994). The crosslinking between genipin and primary amine groups is catalyzed by oxygen and heat. Figure 1 presents pictures of unripe genipap fruit with peel (A) and without peel (B).

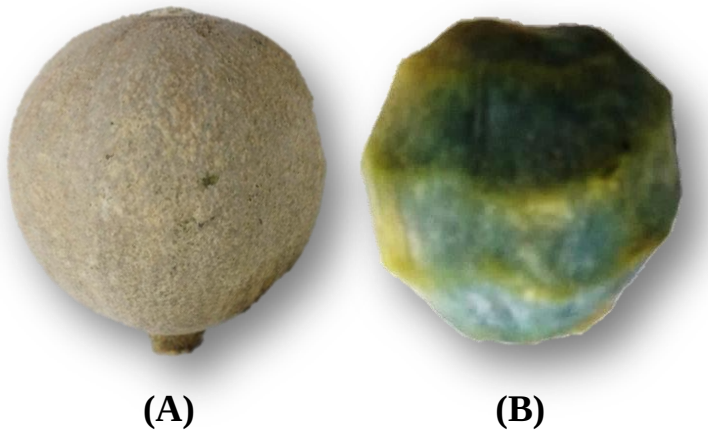

Figure 1. Unripe genipap fruit with peel (A) and without peel (B).

The blue color observed in the peeled fruit (Figure 1B) is formed immediately after peeling. The oxygen-catalyzed genipin reacts with the fruit's proteins forming the blue compounds. Some studies reported that these compounds are a mixture of high molecular weight polymers (Landim Neves, Silva, & Meireles, 2021). Due to genipin's chemical characteristics, solid-liquid extractions, using pressurized fluids, ultrafiltration, and assisted by enzymes have been studied to extract and isolate this iridoid from unripe genipap fruits (Bellé, et al., 2018; Náthia-Neves, Tarone, Tosi, Maróstica Júnior, &

Meireles, 2017; Ramos-De-La-Pena, Renard, Montañez, de la Luz Reyes-Vega, & Contreras-Esquivel, 2015; Renhe, Stringheta, Fonseca, & de Oliveira, 2010).

Among the innovative technologies, high-intensity ultrasound is more efficient for extraction processes, providing high yields in a short processing time (Chutia & Mahanta, 2021; Neves, Strieder, Vardanega, Silva, & Meireles, 2020). Besides that, ultrasound-assisted extractions allow the use of GRAS (Generally Recognized as Safe) solvents; they are flexible and require low energy (Galviz-Quezada, Ochoa-Aristizábal, Zabala, Ochoa, & Osorio-Tobón, 2019; Tiwari, 2015). Therefore, a techno-economic assessment of ultrasound technology to obtain genipin ethanolic extract becomes interesting. Among the parameters involved in these processes, the sample and solvent mass, ultrasound power, and processing time are the most evaluated. However, there is still no standardization in the operational parameters of the high-intensity ultrasound processing and many times, some of these are not informed (Strieder, Silva, & Meireles, 2019). In this regard, we proposed to evaluate the energy sourced by the ultrasound device to the system according to the specific energy (Equation 1) (Silva et al., 2018).

$$Specific\ energy\left[\frac{kJ}{g}\right] = \frac{Acoustic\ power\ (kW) \times Processing\ time\ (s)}{Sample\ and\ solvent\ mass\ (g)} \quad (1)$$

Where the acoustic power can be determined using a calorimetric technique, hydrophones, optical microscopes, or aluminum foil (Cárcel, Benedito, Bon, & Mulet, 2007; Mason, Lorimer, Bates, & Zhao, 1994).

The application of ultrasound in a liquid medium promotes acoustic cavitation. This phenomenon occurs when acoustic energy waves promote cycles of compression and rarefaction of the molecules in the solution resulting in microjetting of bubbles (Chemat et al., 2017). These microjetting facilitates the mass transfer by reaching the raw material, unblocking its channels and pores, and even causing cell ruptures that favor the diffusion of the compounds (Miano, Ibarz, & Augusto, 2016). In this way, this technology can be used in the processes of extraction, homogenization, emulsification, physical modifications, among others (Bernardo, Ascheri, & Carvalho, 2016; Chemat & Khan, 2011; Cui & Zhu, 2021; L. Zhou, Zhang, Xing, & Zhang, 2021).

In addition, to provide acoustic cavitation, the application of acoustic energy can increase the temperature of the medium. In this sense, ultrasound technology can be

evaluated through non-thermal and thermal processes by applying the same acoustic energy and comparing the extraction yield. Thus, the effect of the heat provided by the process can also be evaluated in the target compound extraction. Furthermore, as ultrasound is an emerging technology, evaluating the process's economic viability to extract a target compound can source information about the advantages of the extraction procedures and scale-up. This evaluation can be performed using experimental data and software that simulate the cost of extracting and obtaining the product. In this sense, the ultrasound treatments' energetic performance can also be compared with other technologies.

The genipin from the genipap extracts can be separated and purified to be used as a stabilizer and natural crosslinker (Bigi, Cojazzi, Panzavolta, Roveri, & Rubini, 2002; Chiono, et al., 2008; Mi, Shyu, & Peng, 2005; X. Zhou, et al., 2018). In addition to these functions, genipin can also be used to produce a natural blue colorant. This iridoid is catalyzed by heat and oxygen crosslinks with primary amines groups forming blue compounds. These blue compounds can be used as a natural food coloring. The blue color of these compounds is affected by the pH, temperature, amino source, and genipin-to-amino acids source ratio (Neri-Numa, Pessoa, Paulino, & Pastore, 2017). Therefore, some researchers extracted the genipin using water or other organic solvents and, afterward, promoted genipin reaction with primary amines such as glycine and lysine (Echeverry, Zapata, & Torres, 2011; Touyama, et al., 1994). In this regard, as an alternative to these processes that used pure amines, other innovative and less expensive protein sources can be evaluated. Dairy proteins from milk can be an alternative, considering their constituents and applicability in the development of food products. Moreover, an ultrasound-assisted process can favor genipin extraction and its crosslinking with milk's primary amines in just one step. For that, different ultrasound-specific energies must be evaluated considering the effects of acoustic cavitation and temperature on the medium. Thereby, a natural blue colorant can be produced from unripe genipap and milk for application in dairy and bakery products and desserts.

Likewise, plant proteins can replace dairy proteins for producing this natural blue colorant considering the current demand for plant-based products. Plant extracts obtained from rice, oats, peanuts, and almonds can be extracted using water or other solvents and used as primary amines sources for producing blue-colored compounds. They are easier to use when compared to the isolated plant proteins because they are obtained in a liquid state and do not require purification steps. The use of high-intensity ultrasound

technology can advantageously provide the extraction of genipin, and its crosslinking with plant proteins as well as stabilize the plant extracts through a process of thermosonication (Alcántara-Zavala, de Dios Figueroa-Cárdenas, Pérez-Robles, Arámbula-Villa, & Miranda-Castilleja, 2020; Anaya-Esparza, et al., 2017). Plant-based extracts present insoluble solids that can affect the kinetic stability of the product (Aydar, Tutuncu, & Ozcelik, 2020). Thus, thermosonication treatments can favor the stabilization employing acoustic and thermal energies to reduce the particle size and increase the homogeneity of plant extracts (Atalar, et al., 2019).

The natural blue colorants obtained from unripe genipap and dairy or plant proteins can be applied in different food products, such as beverages, dairy, and bakery products. Among these, plant beverages have been gaining market share in recent years due to the increase of consumers' intolerance or allergy to milk constituents and those who seek diversification in their diet (Vanga & Raghavan, 2018). Additionally, prebiotic carbohydrates and other functional ingredients are also a trend in beverage formulation (Guimarães, et al., 2019; Silva, Arruda, Pastore, Meireles, & Saldaña, 2020). For beverage processing, the high-intensity ultrasound technology through a thermosonication process contributes to the homogenization and stabilization of the product. Thus, avoiding the degradation of bioactive compounds due to the high temperatures used in conventional thermal treatments such as pasteurization and sterilization (Alcántara-Zavala, et al., 2020; Anaya-Esparza, et al., 2017). In this sense, the evaluation of the thermosonication processing design (acoustic power and holding time) under the phytochemical characteristics of a plant beverage can bring relevant information about the preservation of these compounds.

Therefore, the potential of unripe genipap as a natural source of genipin to obtain innovative products and high-intensity ultrasound technology as an efficient and clean alternative for producing these products can be evidenced.

2 Objectives

2.1 General objective

Study new processing alternatives for *Genipa americana* L. using high-intensity ultrasound technology to obtain innovative natural blue colorants for the food industry.

2.2 Specific objectives

- ✓ Discuss the process variables of ultrasound-assisted extractions to obtain natural food colorants;
- ✓ Observe the recent advances, trends, challenges regarding ultrasound processing applied to dairy, meat, bakery, minimally processed products, beverages, and food ingredients;
- ✓ Evaluate non-thermal and thermal ultrasound-assisted extraction processes for obtaining a genipin ethanolic extract from unripe genipap;
- ✓ Study the manufacturing costs and economic feasibility of obtaining genipin extract using high-intensity ultrasound technology;
- ✓ Evaluate the ultrasound energy on the production of a natural blue colorant from the crosslinking between milk proteins and genipin using milk as a solvent, reaction medium, and carrier for the blue color compounds;
- ✓ Verify the effects of a thermosonication pretreatment and four plant extracts, *Oryza sativa* L., *Avena sativa* L., *Arachis hypogaea* L., and *Prunus dulcis*, on the crosslinking between genipin and plant proteins for the production of natural blue colorants;
- ✓ Observe the effects of the thermosonication pretreatment on the microstructure and kinetic stability of the plant-based colorants;
- ✓ Apply the blue colorant obtained from the crosslinking between genipin and almond proteins on the formulation of a functional almond beverage;
- ✓ Evaluate the impact of thermosonication treatments on the phytochemicals, antioxidant capacity, fatty acid stability, volatile organic compounds, and color attributes of the blue plant-based beverage.

3 Thesis structure

The stages performed in this thesis are presented in 10 chapters. Figure 2 shows the topics presented in each chapter.

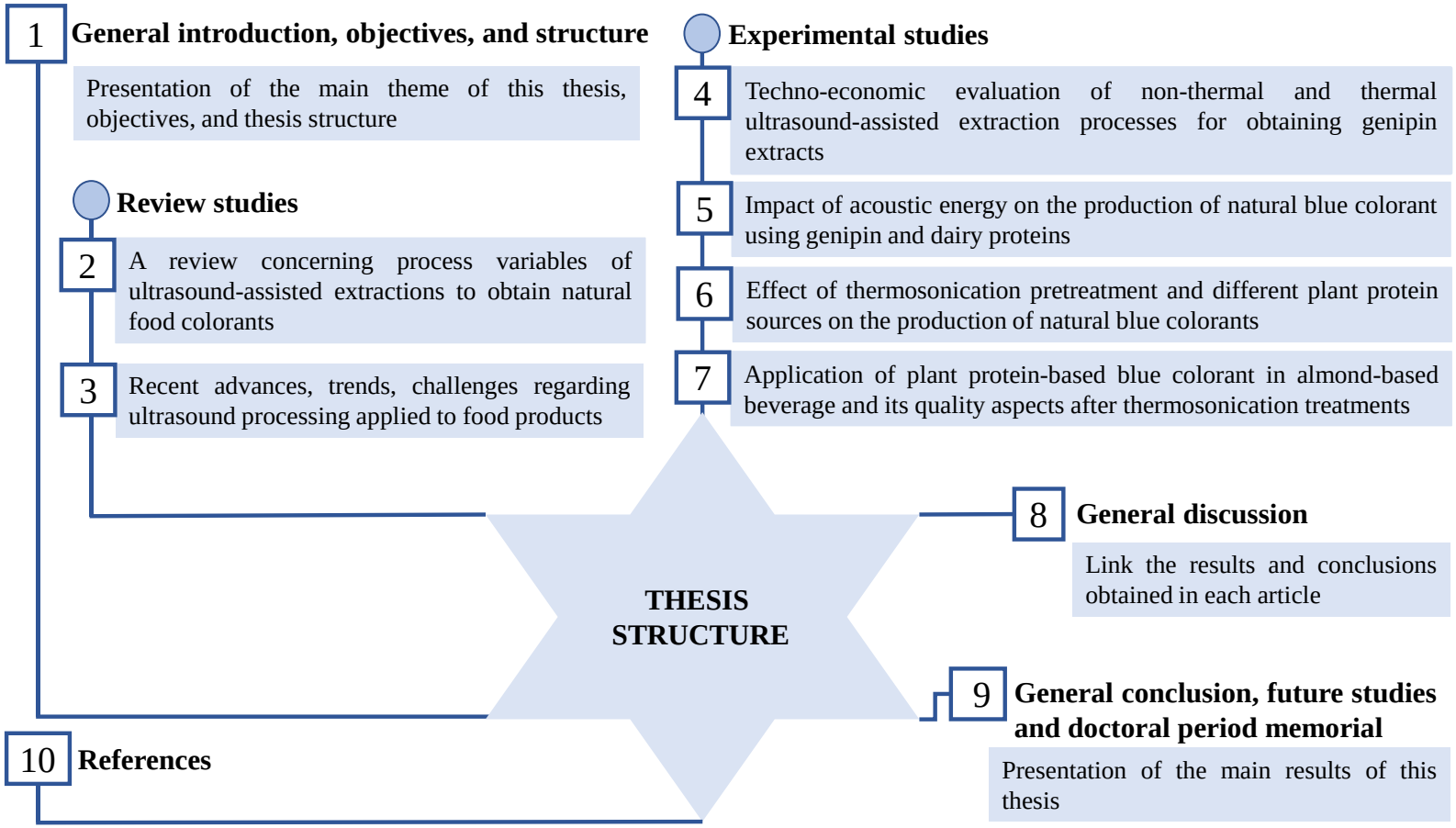

Figure 2. Scheme of the presentation of the topics covered in each thesis chapter.

The plant material chosen for this thesis elaboration was unripe genipap. Our research group had already studied this raw material as a source of phytochemicals and coloring compounds. However, high-intensity ultrasound technology had not yet been evaluated for obtaining products from genipap. Therefore, **Chapter 1** introduced genipap as a plant matrix rich in genipin, an iridoid that presents beneficial health effects for the human body. Moreover, the applications of genipin as a crosslinker and colorant were presented. Among the emerging technologies that can be used to obtain products from genipap, high-intensity ultrasound was highlighted. Thereby, we proposed the objectives also presented in **Chapter 1**. We planned to write two reviews to address all topics studied in this thesis. The first presented in **Chapter 2** we reviewed the use of high-intensity ultrasound technology for extracting natural colorants.

While in **Chapter 3** we proposed to review the recent advances, trends, challenges regarding ultrasound processing applied to dairy, meat, bakery, minimally processed products, beverages, and food ingredients. These reviews supported our experimental design in which we proposed to obtain products from unripe genipap pulp using high-intensity ultrasound technology. Thus, in **Chapter 4** we did a techno-economic evaluation of non-thermal and thermal ultrasound-assisted extraction processes for obtaining a genipin ethanolic extract from unripe genipap. In this study, we observed the efficiency of thermal ultrasound treatments for the extraction of genipin. Thus, in **Chapter 5** we proposed to obtain a blue natural colorant from the crosslinking between the genipin extracted from unripe genipap and milk proteins. The milk was used as the extracting solvent, source of primary amines, and carrier of the blue compounds. The effects of ultrasound processing were evaluated at different specific energies on the production of the natural blue colorant. One-step acoustic cavitation assisted the genipin extraction from the unripe genipap and its diffusion into the colloidal milk system favoring its crosslinking with milk proteins. Likewise, plant proteins can replace dairy proteins for producing this natural blue colorant considering the current demand for plant-based products. In this regard, in **Chapter 6** we employed plant extracts obtained from rice, oats, peanuts, and almonds as extracting solvents and sources of primary amines for producing blue compounds.

Moreover, a thermosonication treatment was applied to plant-based extracts to produce bluer colorants with better kinetic stability. The almond extract presented the best chemical and physical characteristics for producing the natural blue colorant. Thus, in the last experimental study shown in **Chapter 7** we evaluated the application of this

colorant in an almond-based beverage. Also, we evaluated the thermosonication effects on the chemical characteristics of the product. The treatments increased the total flavonoids in the beverage and decreased the total phenolic content, promoting maintenance of the antioxidant activity.

On the other hand, the thermosonication treatments promoted the oxidation of lipid and ethyl ester acids in the beverage. Furthermore, the treatments did not degrade the sensory attributes concerning the blue color. Thus, the thermal stability of the colorant previously produced was shown. Finally, **Chapter 8** presents the general discussion and **Chapter 9** the general conclusion, suggestions for future studies, and a memorial about the doctoral period. In these, we discussed the main results and conclusions observed in each review and experimental step. **Chapter 10** presents the references used in the general introduction and discussion, followed by appendices.

CHAPTER 2

A review concerning process variables of ultrasound-assisted extractions to obtain natural food colorants

Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants

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Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants

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Abstract The demand for natural colorants has boosted the search for innovative technologies to obtain them. Ultrasound-assisted extraction (UAE) is one of the new green techniques that has been studied due to its process advantages that include high yields, short extraction times and nonutilization of elevated temperatures. However, a nonstandardization of the UAE variables complicates comparisons and hinders progress in the studies of this topic. In this review, the focus is the verification and discussion of which UAE process conditions authors have used to obtain natural colorants. Thus, it is possible to confirm that some authors used ultrasonic systems that are not appropriate for performing a good extraction, that some used a great amount of solvent and a long extraction time, and that researchers did not express the main variables (nominal power, extraction time and sample mass) as a function of the specific energy applied to processing. Therefore, it is possible to conclude that some studies using UAE were not conducted to obtain the best results, and the expression of the variables as a function of specific energy can generate a standardization, which facilitates comparison among the results obtained by the scientific community.

Keywords Dyes, Sonication, Emerging technology

1. Introduction

Colors are an important characteristic of food products, and they awaken people's different expectations [1]. According to Lee, Lee, Lee and Song [2], people perceive a food via their visual perception system, and through that, predict its taste before making a decision about whether or not to purchase it. Because of this, colorants are an important food ingredient, and the global food colorant market is growing—according to Markets and Markets, a growth of approximately $0.4 bn is estimated through 2020 [3].

However, the majority of industries utilize synthetic dyes, which have been associated with health problems and cause, for instance, allergies and intolerances, especially in children [4]. Because of this, synthetic food colorants have been progressively replaced by those extracted from natural matrices [5, 6]. These are mostly carotenoids, anthocyanins, betacyanins and chlorophylls obtained from fruits and others vegetables [7].

Obtaining natural colorants, generally includes, a solid-liquid extraction, which is a separation process that involves mass transference and employs a solvent [8]. The solvent utilized for extraction depends on the vegetable matrix, the chemical properties of the pigment and the technic to be employed [9]. In many separation processes performed by industry, large quantities of volatile and flammable organic solvents are used, thus, affecting the environmental and economic performance of the overall extraction [10]. Therefore, currently there is a search for nontoxics solvents that generate less waste and reduce costs [11, 12].

In view of new tendencies, green technologies have also been studied for the extraction of different compounds [13]. For the extraction of colorants, techniques include ultrasound [14], microwave [15], pulse electric field [16], pressurized liquids [17] and supercritical fluid processing [18]. Among them, sonochemistry (the principle of ultrasound) has been mentioned as a green chemistry, evidencing various advantages, such as energy savings due to the short time of operation; major yields due to the selectivity; and a reduction in the generation of waste, by the possible use of solvents, including water [19].

The variables in ultrasound-assisted extractions (UAEs) include the system utilized, the matrix and compound to be extracted, the solvent, the proportions of solvent and feed (the amount of solid sample) and the specific energy applied to processing. Specific energy is energy per unit mass expressed as a function of the nominal power and process time. As will be seen in this review, these variables have recently been studied for the obtaining of natural food colorants. However, a lack of standardization in the

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expression of these conditions is observed, for example, the authors did not clearly indicate what specify energy was applied for the extraction.

Thus, in this review, a verification and discussion are performed with respect to the variables used in UAE to obtain natural food colorants. For this, articles published between 2016 and 2018 are reviewed.

2. Ultrasound Technology

The utilization of ultrasonic techniques has been increasing over time, and they can be utilized for the analysis (low-intensity) or modification of foods (high-intensity) [20]. High-intensity ultrasound is characterized by the use of a frequency of 20 - 24 kHz and high levels of power (10 - 1000 W/cm^2), which physically rupture the materials [20, 21].

The principle of this technique involves acoustic cavitation that is promoted by the system. The waves of acoustic energy promote cycles of compression and rarefaction of the molecules in the solution. Through pressure changes occurs the formation and collapse of microbubbles in the medium that result in microjetting. The microjetting generates effects such as surface peeling, erosion and particle breakdown [22, 23], promoting different applications such as the extraction of different compounds [24], microbial and enzymatic inactivation [25], emulsion formation [26] and physical modifications [27]. Among the applications, UAEs have recently been increasingly studied for the acquisition of natural food colorants.

3. Variables in Colorant Extraction by UAE

In the achievement of extracts by UAE, the relevant variables include the type of system utilized, the matrix and compound to be extract, the solvent employed, the relation of solvent/feed (S/F), the temperature and the specific energy applied [28, 29].

The results obtained by some recent studies on the UAE of natural colorants are shown in Table 1, and the variables and the best values as determined by the authors are presented.

3.1. Ultrasonic System

The UAE of natural colorants has been performed using a system with a probe (Figure 1-A) or a bath (Figure 1-B) [23, 30].

The probe system (Figure 1-A) contains a power generator, a transducer, an amplifier and a probe. The power generator produces high-frequency electrical energy of 20 kHz, which is converted to a mechanical energy by the transducer. The mechanical energy is amplified, and further, the acoustic energy is dissipated by the probe in the form of waves [23]. In the bath system (Figure 1-B), consisting of a power generator, a transducer and a bath, the power generator normally produces an energy of 40 kHz [30]. The transducer or transducers dissipate the acoustic energy into bath in the form of waves. In this system, the samples do not receive the waves directly, as is the case in the probe system.

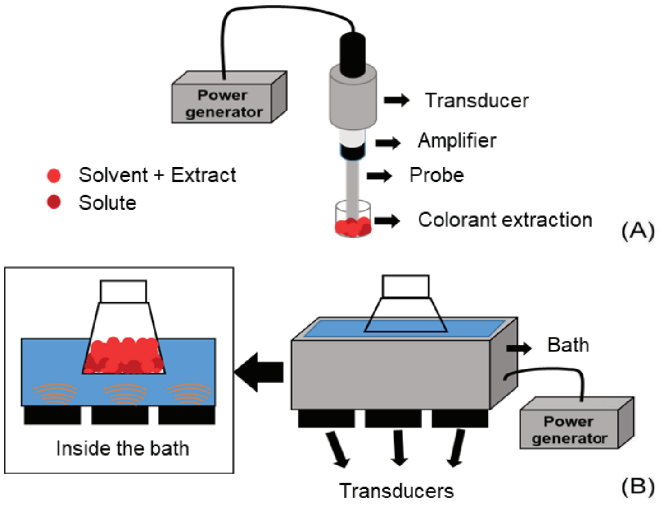

Figure 1. Ultrasonic systems (A: Ultrasound probe), (B: Ultrasound bath)

It is possible to observe in Table 1 that the current research into colorant extractions has been performed using either of the two systems, probe or bath; however, the ultrasonic bath was not the more adequate system for this. The frequencies normally employed in this system are more than 24 kHz, such as 37 kHz [31, 32], while the physical effects dominate at lower frequencies [33]. In a study to extract anthocyanins, Cai, Qu, Lan, Zhao, Ma, Wan, Jing and Li [34], using a bath

ultrasonic system, reported that the UAE is a long-time extraction method and resulted in a low anthocyanin yield. Nevertheless, they used the bath system with an operation frequency of 37 kHz, which was not the more adequate system for this purpose.

Apart from the probe and bath systems, some extraction procedures have been performed with a combination of UAE and other techniques. For example, Wizi, Wang, Hou, Tao, Ma and Yang [35] studied the ultrasound-microwave-assisted extraction of natural colorants from sorghum husk. They obtained a yield of 3.6 times that produced via the conventional shaking method. However, the authors used equipment with a frequency of 25 kHz; thus, had a system that generates a lower frequency been used, it is possible the results would have been better.

A combined treatment using a probe system and a cell grinder was realized by Jiang, Yang and Shi [28] to obtain anthocyanins from blueberry. In this work, the authors observed that the cell grinder destroyed the cell walls, which would release water-soluble anthocyanins into any concentration of ethanol.

Comparing the two systems (the probe combined with the cell grinder and the probe), the authors noted some advantages in utilizing the combined technique.

For example, they were able to use water as the only solvent, as well as acquire a higher yield (2.12 to 2.89 mg/g) and utilize a shorter extraction time (120 to 40 min). Most likely, even though the use of combined techniques to obtain the extract promotes a better yield, it is still necessary explore only the high-intensity ultrasound approach.

Table 1. Optimized Variables for the Ultrasound-assisted Extraction of Colorants

Ultrasonic System	Matrix/ Compound	Solvent	S/F (w/w) Estimated	Temperature (°C)	Process Time (min)	Power (W)	Extraction Yield	Ref.
Probe	Rhizomes of Curcuma/ Curcumin	Ethanol	25	35	60	250	9.18 mg/g	[36]
Probe	Pomegranate wastes/ Carotenoids	Soy oil	10	51.5	30	130	0.67 mg/100 g	[37]
Probe	Fig peel/ Anthocyanin	Ethanol	5	30-35	21	310	3.82 mg/g	[38]
Probe	Gomphrena globosa L. /Betacyanins	Water	73	uncontrolled temperature	22	500	46.9 mg/g	[39]
Probe	Hibiscus sabdariffa calyces/ Anthocyanin	Ethanol/ water	30	30-35	45	500	51.7 mg/g	[40]
Probe	Mulberry (*Morus nigra*) pulp /Anthocyanins	Methanol/ Water	8	48	10	200	149.9 µg/g	[41]
Probe	Undaria pinnatifida/ Carotenoids and Chlorophylls	Water	30	50	30	300	34 and 0.5 mg/mL	[42]
Probe	Red prickly pear peels and pulps/ betanin and isobetanin	Water	10	uncontrolled temperature	10	400	57.47, 89.29 and 28.25 mg/100 g	[43]
Probe/ Probe and Cell grinder	Blueberry /Anthocyanins	Ethanol and Acidified Water/ Acidified Water	20 25	25	120 40	1800 1500	2.12 and 2.89 mg/g	[28]
Bath	Purple sweet potatoes/ Anthocyanins	Ethanol/ water	10	60	60	200	214.92 mg/100 g	[34]
Bath	Wine lees/ Anthocyanins	Choline –chloride-with Malic Acid/Water	10	35	30.6	341.5	6.55 mg/g	[31]
Bath	Residues of Rubus f ruticosus, Vaccinium myrtillus and Eugenia brasiliensis/ Anthocyanins	Ethanol/ Water	20	80	90	580	2.38, 2.33 and 0.87 mg/g	[32]

3.2. Matrix and Compound

Vegetables are a typical resource in research for various applications, due to the vast diversity of molecules [44]. However, some care must be taken to avoid variations due to the matrix. For example, depending on its cultivar, the fig acquires different colors as a function of the anthocyanin concentration [45].

Furthermore, the compounds to be extracted showed different tolerances and peculiarities. For example, anthocyanins are sensitive to temperature, pH, light, oxygen and metals, which must be considered during the separation processes to avoid loss [46]. Additionally, curcumin, according Heger, van Golen, Broekgaarden and Michel [47], degrades in the presence of sunlight and visible light.

3.3. Solvent and Feed

The physical properties of the mixture (solvent and feed) strongly influence the effectiveness of the cavitation, which needs to be proper for acoustic energy transference. For example, the solvent characteristics affect the cavitation phenomenon: the steam pressure governs the intensity of the bubble collapse, and the surface tension and viscosity govern the transient threshold of cavitation [33].

With respect to the solvent, it should be well-matched with the compound [48]. For example, the anthocyanins are normally stable under acidic conditions, and because of this, employing acidified ethanol as the solvent is an option [28]. Carotenoids possess nonpolar characteristics; thus, a good option, according to Goula, Ververi, Adamopoulou and Kaderides [37], is the utilization of vegetable oils.

Machado, Pereira, Barbero and Martínez [32] demonstrated the importance in the choice of solvent to be employed. Obtaining anthocyanins from residues of *Rubus fruticosus*, *Vaccinium myrtillus* and *Eugenia brasiliensis*, the authors observed different results using different solvents (water or ethanol). They observed that using water as solvent, higher extracts yields were obtained, but in relation to the antioxidant activity, they verified the opposite. The colorants in ethanol showed a higher antioxidant activity (determined using in vitro methods) than did the ones obtained with water. This phenomenon, according the authors, occurs because more compounds are soluble in water, but not the target compounds.

In addition, the solvent utilized must be GRAS (generally regarded as safe) [49] and minimize the environmental impact. In Table 1, it is possible to observe that most of the research has been performed using green solvents such as water and ethanol. Nevertheless, some, including Shirsath and Sable (23), still studied solvents such as methanol and acetone to perform the ultrasound-assisted extractions. However, the majority of authors used GRAS solvents to do the extractions, which demonstrates a tendency in the utilization of green solvents over toxic organic solvents [50].

The amount of solvent employed is also very important to obtain an efficient extraction, but an excessive quantity must be avoided to minimize the environmental impact. Table 1 shows that a large amount of the solvent was still used. Considering that in some studies S/F relations of 5 and 8 were utilized, a relation of 73 is extremely high and must be reduced to avoid the generation of a great amount of effluent.

Backes, Pereira, Barros, Prieto, Genena, Barreiro and Ferreira [38] observed that the relation of S/F is extremely important to obtain a pure extract of anthocyanin pigments from *Ficus carica*. Out of the values of S/F studied, the of 5 obtained the best results. This result indicated the possibility of obtaining good extraction results using a low relation of S/F.

3.4. Temperature

In addition to the cavitation and mechanical effects, the thermal effects also have a significant influence on the UAE [51]. Thus, the temperature is another important variable in the extraction.

In previous studies, high-intensity ultrasound was verified as an economically feasible technology for the extraction of thermolabile compounds, but with long extractions time, it is important to pay attention to this factor. [43]. During the extraction process, there is a fast rise in the temperature of the reaction system [40]. The temperature can increase considerably, and the process can be characterized as thermal.

The increase in temperature can be favorable for the extraction of some dyes, but not for others. For example, Zhu, Wu, Di, Li, Barba, Koubaa, Roohinejad, Xiong and He [42] observed that with an increase of 10°C (40 to 50°C), the carotenoid yield increased by 8%; however, with a major increase, the yield was reduced. This result was attributed to the degradation of thermolabile carotenoids. On the other hand, the chlorophylls' recovery showed a positive increase with the temperature (40 to 60°C).

It is possible to observe in Table 1 that most of the processes recently studied were performed at controlled temperatures. The evaluated temperatures varied from 25 to 80°C; however, in some studies, the temperature was not controlled. It is necessary to emphasize that temperature control is particularly important, especially when working with thermolabile compounds. For example, in the extraction process realized by Roriz, Barros, Prieto, Barreiro, Morales and Ferreira [39], the temperature not was controlled, but they extracted betacyanins, which are thermolabile compounds [52].

3.5. Specific Energy

The energy densities applied by ultrasound in food processes have been standardized by some researchers according to Equation 1 [26, 53, 54]. It is possible to observe in Equation 1 that the energy applied in the processes depends on the nominal power, the extraction time and the sample volume. However, the volume is a function of the pressure and temperature, and because of that, another way to express the energy is as a function of mass, which does not depend on other variables. This relation is expressed in

specific energy (Equation 2), according Rajha, Boussetta, Louka, Maroun and Vorobiev [55].

$$ED = \left(\frac{J}{mL}\right) = \frac{Nominal\ power\ (W) \times Extraction\ time\ (s)}{Sample\ volume\ (mL)} \quad (1)$$

$$E = \left(\frac{J}{g}\right) = \frac{Nominal\ power\ (W) \times Extraction\ time\ (s)}{Sample\ mass\ (g)} \quad (2)$$

3.5.1. Nominal Power

The nominal power is the power provided by the ultrasound device itself; however, this is not exactly the same value that is converted into the cavitation phenomenon [56]. This occurs due to energy loss in the equipment by dissipation during the subsequent conversions of mechanical energy into cavitation. According to Mamvura, Iyuke and Paterson [57], an energy conversion from electrical to cavitation of 9% was achieved. Shirsath, Sable, Gaikwad, Sonawane, Saini and Gogate [36] also verified through a calorimetric method that the energy efficiency of the process was approximately 5.6%. Because of that, most authors have used the highest nominal power of the equipment to do the extractions [37].

A high nominal power causes great shear forces in plant materials that results from the critical pressure and temperature obtained from the oscillation and collapse of cavitation bubbles within the solvent [40]. Thus, high values of the nominal power normally result in high extraction yields.

In the majority of the studies shown in Table 1, higher nominal powers were selected as the best condition for the extractions. According to Zhu, Wu, Di, Li, Barba, Koubaa, Roohinejad, Xiong and He [42], better results for obtaining pigments are achieved with more intense ultrasonic treatments, mainly due to the cavitation effect of ultrasound.

3.5.2. Extraction Time

The extraction time is directly associated with the nominal power supplied by the ultrasound and the samples mass (solvent and feed), as given by Equation 2. Therefore, the effect of time on the extraction is one of the most important factors; if the samples are exposed to shorter or longer times than suitable, the compounds could be degraded or not be completely extracted [39].

Thus, the time to be employed depends of the other variables. For example, Espada-Bellido, Ferreiro-González, Carrera, Palma, Barroso and Barbero [41] observed that to recover anthocyanin from mulberry, the maximum time was 10 min. According to them, this time was sufficient for a quantitative extraction and to avoid anthocyanin degradation. However, the time can probably be reduced when using a higher nominal power (greater than 200 W).

It is possible to verify in Table 1 which extractions used a long time (up to 120 min). This time can be reduced substantially. From Equation 1, it is possible that a high nominal power and a small sample mass can result in a short extraction time.

3.5.3. Sample Weight or Volume

Another criticism lies with the relationship of the sample employed in the ultrasound-assisted extractions. The majority of the studies expressed values in terms of the volume and this term is not the more suitable, considering that it can change with the temperature (Boyle-Mariotte law).

Beyond that, it is possible observe that a great amount of solvent was used in relation to a little amount of feed (Table 1). This implies that little energy was applied to the sample, decreasing the friction between the particles. The friction causes cellular rupture and the release of the compounds of interest, thus, assisting the extraction.

3.5.4. The Variable Combinations

It is important to observe the combinations of the main variables through the specific energy (E) value (Equation 2), which permits an easy understanding of the UAE process employed. The E value relates the variables of time, nominal power and sample mass and clearly represents the energy involved in the process. Furthermore, typically, a bigger E value results in the best extraction yield.

It is possible to observe some combinations in Table 1. Goula, Ververi, Adamopoulou and Kaderides [37] utilized a longer time (30 min) but a smaller nominal power (130 W) for extraction; while Koubaa, Barba, Grimi, Mhemdi, Koubaa, Boussetta and Vorobiev [43], using the same S/F (10), used a shorter time (10 min) and a larger nominal power (400 W) to obtain the dye extract.

However, the authors did not express the E value utilized and doing so would have favored a comparison of the extractions realized. In the papers reviewed, it is possible to verify that only Koubaa, Barba, Grimi, Mhemdi, Koubaa, Boussetta and Vorobiev [43] approached the process by regarding the specific energy utilized in the UAE; however, the authors did not connect the specific energy to the extraction time, thus, expressing the specific energy input in kJ/kg.

4. Quality of Dyes Obtained by UAE

Extracts from vegetables contain bioactive compounds that are valuable to the nutraceutical fields, and because of this, the extraction process is a crucial step that needs to ensure that the active ingredients are not lost or destroyed during its operation [58]. Among the methods recently revised by Náthia-Neves and Meireles [59] to obtain natural colorants, the UAE is prominent because of the high purity of the final product. Thus, it is worth noting that in addition to obtaining high yields, it is also interesting to obtain pure extracts that facilitate the material's application.

The ultrasound efficiency was observed by Koubaa, Barba, Grimi, Mhemdi, Koubaa, Boussetta and Vorobiev

[43], who verified a cell denaturation after ultrasound treatment via scanning electron microscopy. According to the authors, this result can provide a better recovery of the intracellular compounds with less impurities [43]. The best results were obtained by [Backes, Pereira, Barros, Prieto, Genena, Barreiro and Ferreira [38]] who related that the UAE technique led to an extract with a greater purity of cyanidin 3-rutinoside in comparison with that of extractions assisted by heat and microwave.

Machado, Pereira, Barbero and Martínez [32] observed that among the emergent methods, UAE was the least aggressive in recovering total and individual anthocyanins, followed by the others studied, UAE + pressurized liquid (PLE) and PLE, using hydroethanolic mixtures as the solvent.

Thus, it was possible verify that ultrasound-assisted extractions are a good choice to obtain adequate results in terms of yield and quality of colorant extracts.

5. Conclusions

In this review, an evaluation of the variables that have been used in the ultrasound-assisted extractions is made, and some important aspects are observed:

- ✓ It is important to know the stability of the compound to be extracted to avoid loss due to the extraction operation.
- ✓ The solvent to be employed should be compatible with the compound of interest, be a GRAS, and be in a smaller amount than the majority of the studies have been using.
- ✓ The variation in temperature must be controlled during the process of extraction.
- ✓ The extraction time can be reduced by using a high nominal power.
- ✓ Larger nominal power values generate higher extraction yields.
- ✓ The best manner is to express the amount of solvent and feed in units of mass.
- ✓ The colorants obtained by UAE showed a high quality.

At the end of this review, is possible to suggest that the variables of nominal power, extraction time and sample mass are expressed in terms of the specific energy to standardize the form of expressing the energy applied to the sample. Thus, a comparison among the results of ultrasound-assisted extraction of colorants would be facilitated.

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CHAPTER 3

Recent advances, trends, challenges regarding ultrasound processing applied to food products

Advances and innovations associated with the use of acoustic energy in food processing: An updated review

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Advances and innovations associated with the use of acoustic energy in food processing: An updated review

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ABSTRACT

Consumers are demanding food products with high added value concerning their sensory properties and safety. In this scenario, updates in industrial food processing have been claimed by the consumer market due to the undesirable sensory properties associated with severe heat treatments. Therefore, the development of novel food processes based on innovative non-thermal technologies is one of the biggest trends for the next years. In this context, low-frequency and high-intensity ultrasound technology has been pointed out as a promising strategy to produce high-technological food products and ingredients. Thereby, the recent advances and challenges regarding the application of acoustic energy processing on the dairy, meat, bakery, and minimally processed products, beverages, and food ingredients were reviewed from the perspective of energy performance. Ultrasound has provided high-technological food products through various ultrasound systems at different amplitudes and/or powers. These have preserved or improved food products' sensory characteristics.

1. Introduction

Novel food production strategies have emerged to meet the global demand for health-promoting products with high added-value concerning their sensory properties and safety. Consumers are demanding foods with sensory attributes similar to unprocessed ones. Likewise, innovative ingredients, such as plant proteins, prebiotic carbohydrates, thickening agents of low glycemic index or even sugar-free, fat replacers, and others, are being demanded for designing of functional foods and beverages (Kumar, 2021; Neri-Numa, Arruda, Geraldi, Júnior, & Pastore, 2020; Strieder, Neves, Silva, & Meireles, 2021). Thus, developing new products of high technological quality from non-thermal processing is one of the biggest trends for the next years. Manufacturing fresh-like products and the use of non-traditional ingredients are currently critical challenges for the food industry. In this way, the interest for the processes design based on non-thermal emerging technologies has strongly grown.

Emerging technologies using supercritical fluids, high-pressure, microwaves, and ultrasound are promising to replace traditional thermal processes of extraction, homogenization, and pasteurization (Amaral et al., 2017; Gallego, Bueno, & Herrero, 2019; Huerta & Saldaña, 2018; Liu, Deng, Li, & Zou, 2018; Tremonte et al., 2014). These technologies using mild processing temperatures have promoted the desired effects on the products maintaining their endogenous bioactive compounds (Dias et al., 2016; Khemakhem et al., 2017; Zhao et al., 2019). Thus, the sensory characteristics of fresh foods are also better preserved. Among these clean technologies, ultrasound has been presenting some advantages. Ultrasound-assisted processes are easier to operate than those using supercritical and pressurized fluids, which require qualified labor to be operated (Chemat & Khan, 2011). Ultrasound can assist processes in any liquid medium, including food emulsions and suspensions. The acoustic energy supplied by ultrasound allowed better energy distribution in the sample than microwave energy application (Kumar, Joardder, Farrell, & Karim, 2018). Additionally, the phenomenon of acoustic cavitation promoted by ultrasonic waves has resulted in more efficient processes in a shorter processing time than the other emerging technologies (Chutia & Mahanta, 2021; Neves, Strieder, Vardanega, Silva, & Meireles, 2020).

Ultrasound equipment is a versatile and economic system that can be used in different operational applications depending on its configuration and the intensity of acoustic energy supplied to the food matrix (Chemat et al., 2017; Strieder, Neves, Zabot, Silva, & Meireles, 2020). The types of ultrasonic systems (probe or bath) and the operating process conditions of frequency, acoustic power, processing time, and sample mass influence the acoustic cavitation performance. These microbubbles represent the acoustic cavitation phenomenon that may cause from

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slight to severe physical and chemical modifications on sonicated products (Ashokkumar et al., 2009). The interaction between cavitation microbubbles and the food matrix directly or indirectly can result in different effects, because the acoustic cavitation produces cracks and pores in the physical structure of products and an increase in the temperature of the sonicated liquid medium (Knorr, Zenker, Heinz, & Lee, 2004). These effects have been widely observed in food matrices subjected to ultrasound-assisted processes of extraction, homogenization, stabilization, reaction, hydration, modification, synthesis, and emulsification (Balthazar et al., 2019; Bera, Mondal, Martin, & Singh, 2015; Martínez, Luengo, Saldaña, Álvarez, & Raso, 2017; Martinez-Guerra, Gude, Mondala, Holmes, & Hernandez, 2014; Miano & Augusto, 2018; Monteiro et al., 2018; Shabana et al., 2019; Silva, Rosa, & Meireles, 2015).

A search in the Scopus database using the keywords "ultrasound", "acoustic energy", and "food" found sixty-two documents (article, review, book chapter, conference paper, and short survey) and three thousand seven hundred seventeen patents in the last years from 2015 to 2020 (Fig. 1A). The number of studies on ultrasound in food processing has grown over the years. The United States, followed by Spain, China, and Australia, are the countries that most research about this theme. The largest number of patents was filed in the United States Patent & Trademark Office. Furthermore, ultrasound-processed products were classified into six food groups: dairy, meat, bakery, and minimally processed products, beverages, and food ingredients. Fig. 1B presents the results for the search performed in Scopus database with the keywords "ultrasound", "acoustic energy", and "dairy/meat/bakery/beverage/minimally processed foods/food ingredient". Food ingredients followed by beverage and minimally processed foods are the most investigated. These food groups have been processed using different ultrasound-assisted processes. Some processes more traditional, as mentioned earlier, extraction, pasteurization, homogenization, and others with more specific objectives and approaches.

This review summarizes the recent advances, trends, challenges regarding ultrasound processing applied to dairy, meat, bakery, minimally processed products, beverages, and food ingredients. The role of ultrasound technology on process innovation was discussed, focusing in future strategies. Also, the ultrasound effects on the sensory characteristics of these products were highlighted. The trend towards using this technology on an industrial scale was reviewed using recent scaling up studies.

2. Fundamentals of ultrasound technology

Ultrasound technology is based on mechanical waves. Thus, frequency, amplitude, and wavelength are intrinsic characteristics of ultrasound waves (Fig. 2). The frequency (Hz) represents the number of complete oscillation cycles produced in one second. The amplitude is the magnitude or intensity of ultrasound waves. It is proportional to the maximum deflection of the particles in the transmission medium. Finally, the wavelength is the characteristic time in which the same phenomenon of the complete oscillation cycle is repeated. Thus, these characteristics impact the ultrasound waves applicability to promote different effects on the sonicated medium. In this regard, according to

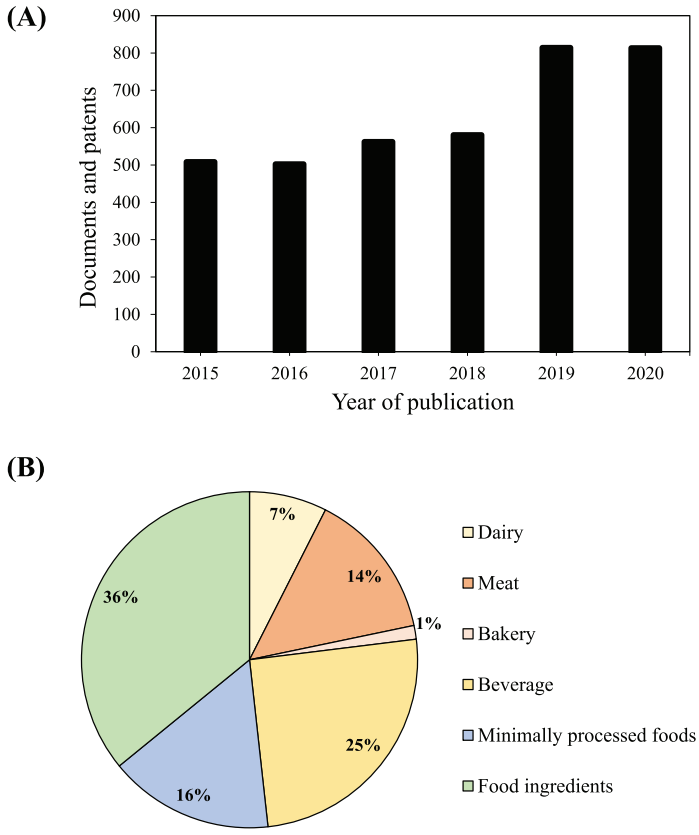

Fig. 1. Number of documents and patents in Scopus database published from 2015 to 2020 using the keywords: (A) "ultrasound", "food", and "acoustic cavitation" and (B) "ultrasound", "acoustic cavitation", and "dairy/meat/bakery/beverage/minimally processed foods/food ingredients".

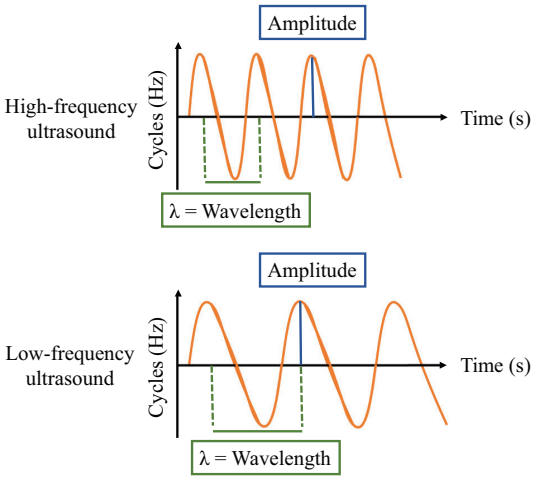

Fig. 2. Ultrasound waves at high-frequency and low-frequency.

the application, ultrasound technology can be classified by the frequency employed into high-frequency (1–100 MHz) and low-frequency (16–200 kHz) ultrasound (Dong, Udepurkar, & Kuhn, 2020). Fig. 2 exhibits the ultrasound waves at low and high frequencies.

High-frequency ultrasound has been applied in medical imaging diagnostics to produce pictures of the inside of the body. For instance, Sastry et al. (2017) described three applications for high-frequency ultrasound technology in brain tumor diagnostic: (1) intraoperative navigation, (2) assessment of the extent of resection, and (3) brain shift monitoring. The associated imaging modalities provided by three-dimensional ultrasound, contrast-enhanced ultrasound, high-frequency ultrasound, and ultrasound elastography have facilitated the analysis of neurosurgeons in the intraoperative period.

On the other hand, the application of low-frequency ultrasound promotes physical and chemical modifications on several matrices, such as food, polymers, alloys, and others (Téllez-Morales, Hernández-Santo, & Rodríguez-Miranda, 2020). These modifications also depend on other ultrasound process conditions. The ultrasonic system type, power and intensity of acoustic energy employed, and physicochemical properties of the sonicated medium affect the interaction of low-frequency ultrasound with the ultrasound-treated medium.

2.1. Ultrasound system

The ultrasound system may present different configurations to promote the desirable effects. The main ultrasonic systems used in food processing are bath or probe-type equipment. Both can present transducers with different geometries and diameters and can apply acoustic energy directly or indirectly (Fig. 3).

Fig. 3A presents the direct and indirect bath ultrasonic systems, which are formed by a power generator, a transducer, and a bath. The power generator is powered by electrical energy, which is converted into mechanical energy by the transducers and is dissipated into acoustic waves into the bath (Lukić et al., 2020; Tao, Zhang, & Sun, 2014). Sonicated samples may receive acoustic waves directly or indirectly. Directly, the sample is placed inside the bath, and indirectly, a container with the sample is placed inside the bath filled with some liquid (Miano, Ibarz, & Augusto, 2016).

Fig. 3B presents the direct and indirect probe systems, which comprise a power generator, transducer, amplifier, and probe. The power generator is powered by electrical energy, which is converted to mechanical energy by the transducer, amplified, and applied by the probe (Bhargava, Mor, Kumar, & Sharanagat, 2020). The samples may

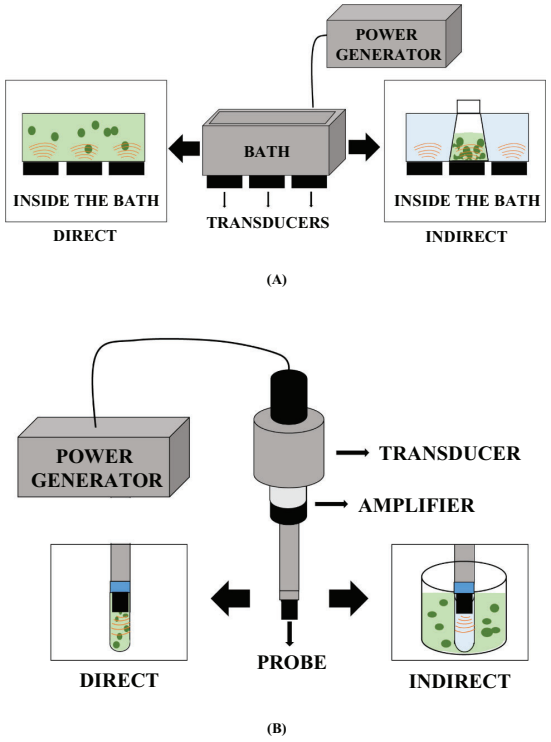

Fig. 3. Ultrasound systems: (A) Bath; and (B) Probe.

receive acoustic waves from the probe directly or indirectly. Normally, in a direct form, the sample is placed in a container coupled with the probe. In indirect form, the probe is coupled in a smaller container with water, and the sample is placed in a larger container that is kept around the first one (Miano et al., 2016).

2.2. The energy applied by ultrasound processes

The acoustic energy sourced by the ultrasound system during the sonication is not always reported. Many studies only report the nominal power set on the ultrasound equipment (Barukčić, Jakopović, Herceg, Karlović, & Božanić, 2015; Bosiljkov et al., 2009; Zabot, Silva, Azevedo, & Meireles, 2016). However, the electrical energy powered is not completely converted into acoustic energy. Throughout the sequent conversion of electrical into acoustic energy, a fraction of this energy is dissipated into other energy forms, such as thermal, chemical, sound, and others. The acoustic energy supplied by the transducers is further converted into acoustic propagation and cavitation energies (Wu, Bai, & Lin, 2020). The cavitation energy is the energy absorbed by microbubbles and responsible for acoustic cavitation. In turn, the acoustic cavitation releases this energy through mechanical, thermal, and chemical modifications of the sonicated medium. In this way, methodologies based on physical properties, which allow the direct or indirect measurement of the applied acoustic energy, are widely used. Physical methods estimate the transferred energy by measuring either chemical or physical changes in the sonicated medium (Chemat et al., 2017). The most common methodologies are the measurement of acoustic pressure using hydrophones or optical microscopes, aluminum foil, and calorimetric assays. Cárcel, Benedito, Bon, and Mulet (2007) compared the methodologies based on acoustic pressure, such as hydrophones and calorimetric assays, to determine the acoustic energy applied to the meat brining process. The calorimetric method was more efficient to measure

acoustic energy. According to the authors, the cavitation bubbles implosion could affect the hydrophone measurements when the transducer applied higher electrical power.

The calorimetric method measures the temperature increase rate due to the conversion of acoustic energy into heat $\left(\frac{dT}{dt}\right)$ (Mason, Lorimer, Bates, & Zhao, 1994). Normally, water is used as a control liquid medium to determine the acoustic power of the ultrasound system due to its physical and chemical properties that were widely known. Eq. (1) presents the acoustic power determination by the calorimetric assay.

$$Acoustic\ power\ (W) = mC_P\left(\frac{dT}{dt}\right) \quad (1)$$

Where m is the sample mass and C_P is the heat capacity of the sample.

The acoustic intensity or ultrasound intensity can be determined according to Eq. (2), considering the acoustic power and square meter of the emitting surface (Silva, Arruda, Pastore, Meireles, & Saldaña, 2020). The low-frequency ultrasound technology is classified into high-intensity ultrasound according to its acoustic intensity range. High-intensity ultrasound provides intensities greater than 1 W/cm^2 (Mason & Luche, 1996).

$$Acoustic\ intensity\left(\frac{W}{cm^2}\right) = \frac{4 \times Acoustic\ power}{\pi D^2} \quad (2)$$

Where D is the diameter of the transducer or probe.

Ultrasonic intensity also can be expressed as the energy transmitted per second considering the ultrasound processing time. This parameter is associated with the amplitude of the transducer that provides the ultrasound wave amplitude. After ultrasound application into a liquid medium, the acoustic cavitation phenomenon starts when a specific amplitude is propagated.

Additionally, terms such as power density or acoustic density (Eq. (3)) and specific energy (Eq. (4)) have been used considering the volume and mass of product, respectively (Gomes et al., 2017; Joshi & Gogate, 2019; Strieder, Silva, & Meireles, 2019; Tao et al., 2014).

$$Power\ density\left[\frac{W}{mL}\right] = \frac{Acoustic\ power}{sample\ volume} \quad (3)$$

$$Specificenergy\left[\frac{J}{g}\right] = \frac{Acousticpower \times processing\ time}{samplemass} \quad (4)$$

2.3. Acoustic cavitation

The acoustic cavitation phenomenon starts in the liquid medium when a specific value of the high-amplitude ultrasonic signal is propagated (Vanhille & Campos-Pozuelo, 2012). Ultrasonic signal, applied by the transducers or probe (Fig. 3) through acoustic waves, promotes cycles of compression and rarefaction of the molecules in the sonicated medium. Thus, after reaching a limit of pressure, the acoustic cavitation phenomenon occurs by forming and subsequent collapse of microbubbles. Vanhille et al. (2012) developed a nonlinear model that described the mechanism of acoustic cavitation in one-dimensional stationary waves. The authors observed that cavitation microbubbles strongly affect the ultrasonic field due to their dissipation, dispersion, and non-linearity. Thus, ultrasound processing can be employed in different mass transfer applications depending on the system type (bath or probe) and how the acoustic waves are applied into the sample (direct and indirect). Acoustic cavitation also presents transient and stable categories: the stable (repetitive transient) comprises bubbles regularly oscillating for various acoustic cycles. In contrast, in the transient state, the bubbles present irregular oscillations, which cause a rapid alternation of temperature and pressure (Qiu, Zhang, Chitrakar, & Bhandari, 2020). Ashokkumar et al. (2009) observed that using a probe system at 20 kHz first occurs the generation of transient cavitation bubbles. On the other hand, using a plate type transducer at 25 and 37 kHz occurs the generation of a significant amount of stable cavitation.

The bubbles structures also have been classified according to their visual appearance (Mettin, 2005). Bai et al. (2014) observed five types of bubble structures (conical bubble structure, smoker, acoustic Lichtenberg figure, tailing bubble structure, jet-induced bubble structures) using five different transducers and frequencies (20–50 kHz). In bath ultrasound systems, smoker bubble structures are usually observed at the bottom of baths or submerged transducers processes (Bai et al., 2014). Fig. 4A presents a representation of smoker bubble structures that were acquired by Bai et al. (2014) using a transducer of 50 mm of radiating surface diameter and 18.5 kHz. The smoker bubble structure consists of a cloud or plume of very small bubbles that are characterized by bounding to the surface with a small tip and a big tail. This structure moves outwards, such as clouds of smoke (Bai et al., 2014; Mettin, 2005). The conical structure is the most common in processes using sonotrode (probe systems), which presents a small emitting surface that is used for high-intensity applications (Mettin, 2005). Fig. 4B presents a schema of the structure obtained by Bai et al. (2014) with a 20-mm ultrasound probe at 20 kHz. This bubble structure seems to originate from the transducer surface (bubbly film) and reaches downwards to bundle near the symmetry axis, forming a visual cone structure (Bai et al., 2014; Mettin, 2005).

The liquid medium that the ultrasonic waves were applied also influenced the bubbles structures. Tzanakis, Lebon, Eskin, and Pericleous (2017) observed different bubble structures using water, ethanol, and glycerine as liquid media. They applied different power intensities using a probe system with a 40-mm tip diameter at 20 kHz. In water, the characteristic conical structure bubbles provided by sonotrode systems were observed. However, this behavior was not observed for ethanol and glycerine. In ethanol medium, only fairly large individual cavitation bubbles were visible. Specifically, using 50% of power, a smoker structure was observed. Glycerine exhibited a distinct behavior, the bubble in its viscous medium does not easily break up into a cloud of small bubbles. The oscillation period of the bubbles was increased due to the medium viscosity. Thus, the bubble collapse was less violently, and most of the exerted energy was dissipated.

The bubble structures formed due to the different transducer's diameters also affect the ultrasound energy efficiency. Silva and Saldaña

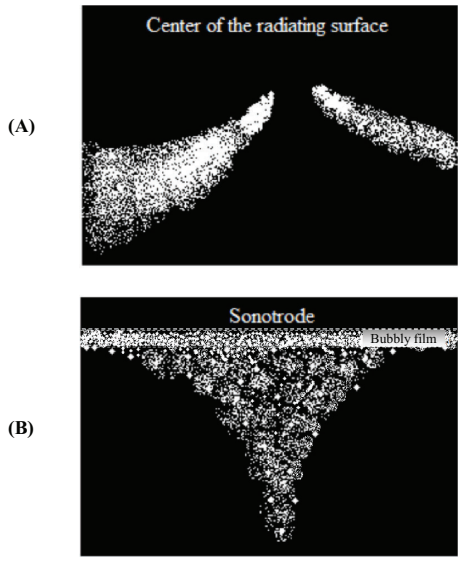

Fig. 4. Scheme of smoker (A) and conical (B) bubbles structures. (Source: Adapted of Bai et al. (2014).)

(2020) observed a higher ultrasound intensity (W/cm^2) using a 10-mm probe diameter compared to 20-mm probe at the same process conditions. According to the authors, this difference is associated with energy dissipation due to the subsequent conversion of electrical energy to acoustic cavitation. This energy loss occurs by heat dissipation. Thereby, larger probe diameters present a larger area, contributing to higher energy dissipation. Therefore, lower ultrasonic intensities are provided. Otherwise, as the probe contact area with the sample is larger, the ultrasound processing using the 20-mm probe diameter heated the sample more than the 10-mm probe. Despite that, the diameters evaluated did not influence the extraction yields of alcohol glycosides from *Rhodiola Rosea* roots. Nonetheless, Lukić et al. (2020) observed that ultrasound probe diameter was the most important process parameter for stabilizing white wines. They studied the probe diameters of 12.7, 19.1, and 25.4 mm. The best results were acquired using the 19.1 mm with higher ultrasound amplitude (50–100%). In these conditions, acoustic cavitation promoted favorable effects on the wine phenolic and volatile composition. The authors explained that is very difficult to interpret the influence of all process variables associated with low-frequency and high-power ultrasound application on the complex wine matrix. Thus, the acoustic cavitation variables must be evaluated for each type of system, since the same process conditions can cause different effects depending on the sonicated matrix.

3. Acoustic cavitation on food processing

As mentioned earlier, acoustic energy can be applied to food matrices directly or indirectly (Fig. 3). The direct application promotes rapid and subsequent compression and expansion of the molecules of the materials. This phenomenon facilitates the mass transfer by the bubbles microjets formed that reach the raw material, unblocking its channels and pores, and even causing cell ruptures that favor the diffusion of the compounds (Miano et al., 2016). Thus, this direct application can be used in processes of extraction, homogenization, emulsification, and others (Bera et al., 2015; Goltz, Ávila, Barbieri, Igarashi-Mafra, & Mafra, 2018; Silva, Gomes, Hubinger, Cunha, & Meireles, 2015; Strieder, Neves, Silva, & Meireles, 2020; Zabot et al., 2016). In contrast, the indirect application promotes microchannels in the samples. The acoustic cavitation applied in the water inside or outside the product cells promotes tissue and cell disruption causing cavities and microchannels

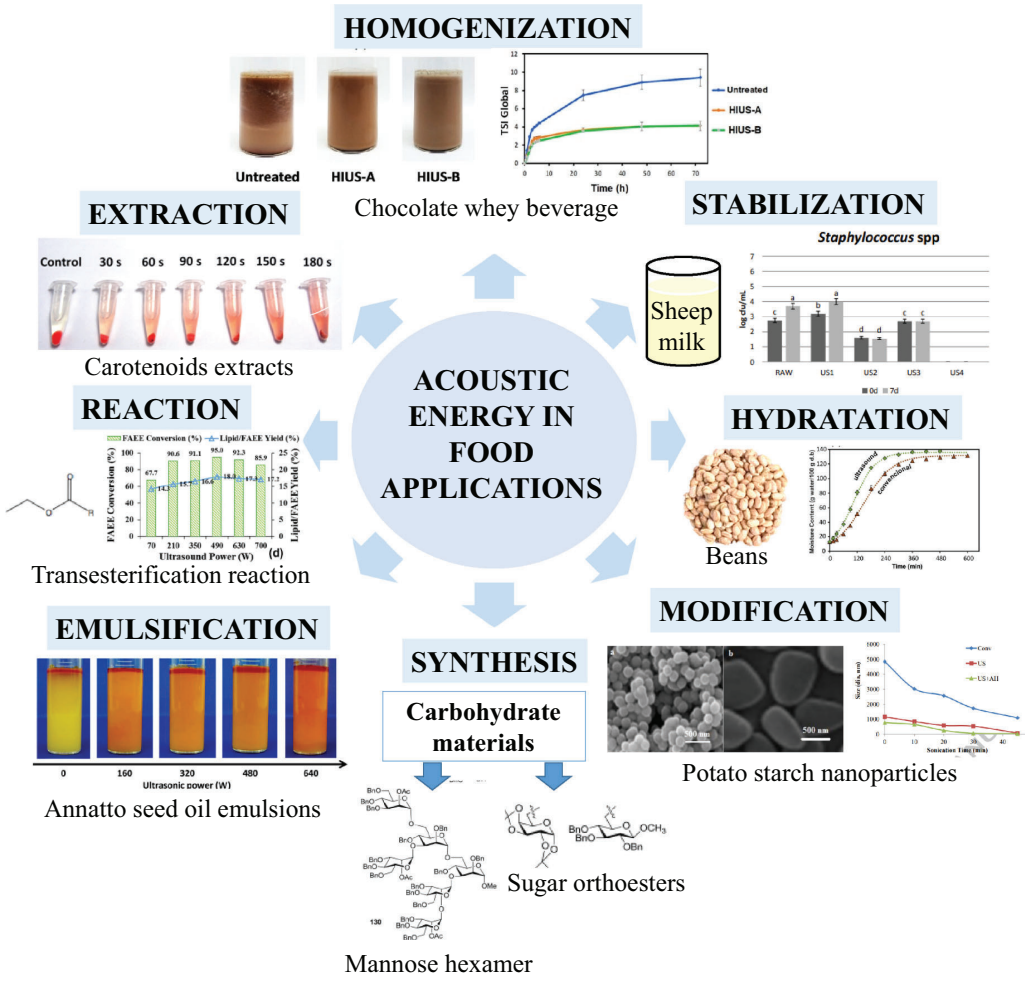

Fig. 5. Main applications of acoustic energy in food processing.
(Source: Balthazar et al. (2019), Miano and Augusto (2018), Monteiro et al. (2018), Shabana et al. (2019), Silva and Meireles (2015), Bera et al. (2015), Martinez-Guerra et al. (2014), and Martínez, Delso, Aguilar, Álvarez, and Raso (2020).)

(Miano et al., 2016). This indirect application is more used in processes of hydration in which the aim is to hydrate grains without causing gelatinization of their starch (Miano and Augusto (2018)).

In addition to the way of applying acoustic cavitation (direct and indirect), the sample's characteristics also influence the effects of acoustic cavitation (Cárcel, García-Pérez, Benedito, & Mulet, 2012). The application of the same energy intensity in media with different viscosities impacted them in different modes due to the bubble structure formation (Tzanakis et al., 2017). Furthermore, using the same liquid medium, different effects can be observed according to the interaction of the cavitation bubbles with the sample cells. Fig. 5 presents some examples of the main applications of acoustic cavitation in food processing, including homogenization, stabilization/pasteurization/sterilization, extraction, hydration, reaction, emulsification, modification, and synthesis. Also, ultrasound processing may have more than one or a specific function providing unique process conditions to obtain certain food products.

4. Acoustic energy applied to the production of innovative foods and beverages

From the review of innovative food processes using ultrasound technology, the main products obtained with this technology were divided into six food groups: dairy, meat, bakery, beverage, minimally processed products, and ingredients.

4.1. Dairy products

The most common effects of acoustic cavitation on dairy products were recently reviewed by Carrillo-Lopez et al. (2021) and Chávez-Martínez et al. (2020). They observed the main impacts of ultrasound treatments on the physicochemical, functional, and microbiological properties of milk and dairy products. This review, on the other hand, focused on observing the innovative effects of acoustic cavitation on dairy product characteristics or processing.

Table 1 presents some examples of ultrasound treatments used to promote innovative modifications in dairy products or processing. In the first example, acoustic cavitation accelerated the whey hydrolysis phase increasing the solubility and biodegradability of complex organic matter (Mainardis, Flaibani, Trigatti, & Goi, 2019). This greater solubility and availability of organic matter increase the methane yield and its production in anaerobic digestion to produce whey cheese. The lowest ultrasonic energy evaluated (40 W for 5 min) was better to acquire methane and produce it from the ultrasonicated skimmed whey. The most intense acoustic cavitation may have promoted high acceleration of hydrolysis, which did not favor the fermentation process. The organic matter of fatty whey was impacted only with the highest power treatment (80 W). In this case, the whey fat content may have difficulted the action of acoustic cavitation. Therefore, low ultrasound energy (251.4–693.7 Wh/kg) favored the production of the fermentation's compounds from skimmed whey, providing different sensory characteristics for the whey cheese. Huang et al. (2019) also evaluated ultrasound treatments to hydrolyze skim milk proteins to release more peptides for their yogurt. The best process condition (100 W/L at 28 kHz for 35 min) applied on skim milk (12.6% *w/v*) already fermented for 9 h increased in 64% the peptide content of the yogurt. In this case, acoustic cavitation increased the extracellular enzyme activities of the acid, neutral and alkaline proteases in the fermented media. The activation effect presented a short duration, but the underlying mechanism may have been responsible for the ultrasound increasing peptide content in the fermented skim milk.

In the production of Greek yogurt, Körzendörfer, Schäfer, Hinrichs, and Nöbel (2019) evaluated an ultrasound treatment during a protein-enriched milk gel fermentation to overcome a technological problem. According to them, the milk concentration before fermentation can prevent the formation of acids in the final product. However, the gels produced were firm, making it difficult for further processing. Thereby, they applied acoustic energy of 15 W/cm^2 during the fermentation to soften the gel. The ultrasonicated gels presented a lower cohesive structure and more compact microgel particles than the non-ultrasonicated ones. Acoustic cavitation may have promoted these results by breaking down whey protein aggregates and preventing their reformation. Since sonochemical cleavage can be caused by mechanical degradation due to collapsed cavitation bubbles or by a chemical reaction of the aggregates with the radicals produced by ultrasound. Thus, the acoustic energy provided a less acid milk gel for the manufacture of Greek yogurt that allow the developing of products with novel textures. Another ultrasound treatment was applied to produce butters with different texture and melting behavior (Lee & Martini, 2019). An ultrasound pre-treatment was evaluated to process the cream applying 85 W for 0, 10, 30, 60, and 90 s. The creams processed for 30, 60, and 90 s presented a lower solid fat content compared with the sample processed for 10 s. This may be due to the temperature increase (2–6 °C) promoted by acoustic cavitation during ultrasound treatments. A relatively shorter churning time of 10 s-sonicated cream compared with non-sonicated cream was observed, possibly because ultrasound weakens the fat globule membrane. This weakening may have allowed the leakage of liquid oil in the fat droplets, which resulted in the shortest time for arrives a higher degree of partial coalescence. Furthermore, the cream droplet size was not affected by 10 s of ultrasound processing. The sonication also induces the crystallization of low-melting-point triacylglycerols. These compounds, in turn, play an important role in phase inversion during butter production, resulting in a harder butter.

Likewise, an ultrasound pre-treatment was applied into an ice cream

Table 1
Acoustic energy on the innovative dairy products manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Whey cheese	Anaerobic digestion	Accelerates the hydrolysis phase	Ultrasound probe system at 20 kHz	Nominal power (40 and 80 W)	5 and 10 min	(Mainardis et al., 2019)
Yogurt	Milk fermentation	Hydrolyze the skim milk proteins	Ultrasound bath system	Frequency (28, 33, 40 and 68 kHz), pulse duration (30, 50, 100, 200 and 300 s), and ultrasonic power (60, 80, 100 and 120 W/L)	30 min	(Huang et al., 2019)
Greek yogurt	Fermentation	Reduces the cohesive structure and compacts the milk gel particles	13-mm ultrasound probe system at 20 kHz	Acoustic power (20 W) and ultrasound intensity (15 W/cm^2)	Cycle time of 1 s (0.2 s on, 0.8 s off) from pH 5.8 to 5.1	(Körzendörfer et al., 2019)
Butter	Churn	Weakens the fat globule membrane and induces the crystallization of low-melting-point triacylglycerols	1.27-cm ultrasound probe system at 20 kHz and amplitude of 108 μm	Nominal power (85 W)	0, 10, 30, 60, and 90 s	(Lee & Martini, 2019)
Soft-serve ice cream	Pretreatment for churn	Increases the incorporation of CO_2	120-mm ultrasound transducer at 205 kHz	Nominal power (30 W)	30 s	(Adhikari et al., 2020)

to promote CO_2 incorporation (Adhikari, Truong, Prakash, Bansal, & Bhandari, 2020). The application was before churning through a transducer flanged beneath the ice cream mix vessel. This ultrasound treatment allowed a reduction in 13 min of churning time. Furthermore, a consumer panel evaluation demonstrated that ultrasound treatment significantly improved the ice cream's overall sensory properties. The acoustic energy promoted the dispersion of gas bubbles in the liquid or solid phase forming a foam structure.

Low acoustic energy has been directly applied in dairy product processing (Table 1). Maximum nominal power of 120 W was required to process whey cheese, yogurt, Greek yogurt, butter, and soft-serve ice cream. Besides that, most processes used the probe-type ultrasonic system. Since the energy is applied in a smaller product volume than in bath systems, higher acoustic energy levels are applied. However, in this case, low acoustic energy was applied by the probe system. This provided products with different characteristics, in addition to reducing some costs of processing. In this regard, the acoustic energy required for dairy processing is low and, in some cases, its application reduces the processing time.

4.2. Meat and meat products

Oxidative stability and sensory attributes are important characteristics of meat and meat products. Because of this, high-intensity ultrasound technology has been used to thaw and process meat products, reducing the processing time and avoiding modifications in their characteristics (Boateng & Nasiru, 2019). Studies regarding microbial inactivation also have been carried out. Ultrasound waves through acoustic cavitation also reduce microbial loads in meat, mainly when combined with other technologies (Owusu-Ansah et al., 2020). Otherwise, the application of acoustic energy in meat processing can improve its sensory characteristics. Table 2 presents main remarks of meat products processed by acoustic energy.

Acoustic energy has been applied in meat processes of curing, tenderization, and homogenization. Its application in meat curing was studied by Sergeev et al. (2020) and Xiong et al. (2020). Sergeev et al. (2020) evaluated the interactions of ultrasound-treated salt solutions with the proteins of pork meat. The NaCl solution treated with ultrasound promoted a cured pork meat with different characteristics about the spin-spin relaxation time, the rate of chemical exchange of water protons, and the amount of unfrozen water. These meat's different characteristics were associated with interactions of the meat protein with radicals from sonochemical reactions that occurred in the saline solution. The chemical reactions occur in steam during the solution exposure to the acoustic cavitation. The main radicals are H• and OH•, and hydroperoxides (H_2O_2). The radicals quickly recombine into H_2 and H_2O and affect the protein matrix during the process. Hydroperoxides can participate in breaking the peptide bonds leading to a reduction in the molecular weight of protein. Also, they act altering the protein conformation, becoming available the hydrophilic amino acid sites previously hidden inside molecular globule for interaction with water. Additionally, the increase in NaCl solubility after ultrasound exposure also can have affected. The higher solubility may promote its faster interaction with proteins, causing its denaturation. On the other hand, Xiong et al. (2020) evaluated the application of acoustic cavitation directly to assist chicken breast meat curing with sodium bicarbonate. The chicken treated with acoustic cavitation presented the highest marinade uptake and chloride content than the samples treated with other curing treatments. Furthermore, the ultrasound treatment decreased the cooking loss and surface hydrophobicity of the chicken. The low-field nuclear magnetic resonance (LF-NMR) relaxation times (T21 and T22) decreased as they did for pork meat (Sergeev et al., 2020). This was associated with the lower fluid losses in chicken. The acoustic cavitation promotes strong shear causing damage to muscle fibers. This further contributed to a higher cleavage and dissolution of the sarcoplasmic and myofibrillar proteins resulting in an improved. Additionally, ultrasonic waves can destroy the lysosomes releasing cathepsin and calpain, enzymes that could increase the protein solubility. Therefore, the ultrasound treatment provided better meat tenderization, water holding capacity, and curing efficiency. Meanwhile, ultrasonicated cured pork and chicken breast acquired different sensory aspects.

A tenderization process applying acoustic energy into old chicken breast packaged in a vacuum packer was evaluated by Shi et al. (2020). The energy was applied to the package immersed in ice water to avoid protein denaturation. After sonication, the meat removed from the vacuum packing bag was used for further marination treatment with potassium alginate solution. The pre-treatment with acoustic energy allowed better water retention on the meat during the cooking. The ultrasound processing promoted the release of salt-soluble proteins related to the gel structure. Thereby, soluble proteins could be coated onto the droplet surfaces to form small-sized emulsion droplets, allowing more moisture to be trapped within muscle fibers. Also, the potassium alginate, which possesses a large hydration capacity, offered good accessibility to functional groups in muscle proteins, allowing undergo H-bonding interactions with water molecules, decreasing moisture loss. Thus, the ultrasound treatment favored water retention in the meat, providing a softer chicken breast.

Barretto et al. (2020) evaluated the application of acoustic cavitation on the production of low sodium restructured cooked ham. The sonication of the ham ingredients contributed to lower fluid released within thirty days of refrigerated storage. The structural changes in the product were caused by cavitation, which in turn exposed and caused microfissures in the myofibrillar proteins, facilitating their binding with the water and, thus, reducing losses by exudation. Ultrasound treatments also opened interfibrillar channels that allowed greater retention of water in the product. Thereby, acoustic cavitation favored the retention

Table 2
Acoustic energy on the innovative meat manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Pork meat	Curing (NaCl solution homogenization)	Produces radicals in the saline solution that improves its interaction with meat proteins	Ultrasound at 20 kHz	Nominal power (200 W)	10 min	(Sergeev, et al., 2020)
Marineted chicken breast meat	Curing	Damages the muscle fibers protein and releases cathepsin and calpain enzymes	6-mm ultrasound probe system at 20 kHz	Nominal power (300 W)	50 min (on-time 5 s and off-time 5 s)	(Xiong et al., 2020)
Old chicken breast meat	Tenderization	Releases salt-soluble proteins related to the gel structure	12-mm ultrasound probe system at 20 kHz	Nominal power (300 W), intensity (15.6 W/cm^2)	5 min (on-time 2 s and off-time 3 s)	(Shi et al., 2020)
Low sodium restructured cooked ham	Homogenization of the ham brine-ingredients mixture	Exposes and causes micro-fissures on the surface of myofibrillar proteins	Ultrasound probe system at 20 kHz	Nominal intensity (600 W/cm^2)	10 min	(Barretto et al., 2020)
Dry fermented sausages	Additional treatment after stuffing	Promotes proteolysis and formation of compounds derived from lipid oxidation	Ultrasound bath system at 25 kHz	Nominal power (128 W)	0, 3, 6, and 9 min	(Alves et al., 2020)

of water in the cooked ham similarly as favored in other meat products cured and tenderized by ultrasound. Likewise, the sonicated low sodium restructured cooked ham presented a better texture.

The sensory characteristics of a dry fermented sausage also were improved by ultrasound processing carried out after its stuffing (Alves et al., 2020). The samples subjected to 3 and 6 min of treatment showed higher free amino acids contents than non-sonicated samples at the end of manufacture (day 28 of ripening/day 1 of storage) and storage (day 120). Acoustic cavitation breaks myofibrils and damages the structure of cellular organelles leading to the release of cathepsin enzymes from lysosomes and calcium from the sarcoplasmic reticulum, activating the calpain system. Also, the activity of proteases can increase with the rise in the temperature throughout the ultrasound treatment. However, in the longer processing time of 9 min, the increase in temperature may have promoted protease denaturation. Thus, the processing times of 3 and 6 min were better to promote proteolysis. Acoustic cavitation also induced the production of reactive oxygen species that increased the oxidation levels of lipids. Thereby, volatile compounds were affected by the ultrasound exposure times, especially those derived from the lipid oxidation. The sample sonicated for 9 min presented a greater formation of compounds from the oxidation reactions and/or the advanced stages of lipid oxidation at 120 days of storage. Therefore, ultrasound treatments promoted proteolysis and the formation of compounds derived from lipid oxidation. Meanwhile, sausages with different sensory characteristics were obtained by ultrasound treatments.

Greater acoustic energies are required for the processing of meat and meat products (Table 2). Besides that, the energy has been applied directly by probe systems to produce many products. However, the choice of sonication time is an essential factor in meat processing because an inadequate sonication time may negatively affect the meat texture, as acoustic cavitation can favor oxidative instability of proteins leading to crosslinking (Boateng & Nasiru, 2019). Thereby, acoustic energy has modified meat products providing new ones with improved sensory characteristics.

4.3. Bakery products

Bakery industries are increasingly interested in adopting new processes for facilitating the manufacture of products with more safety, quality, and process efficiency. Since process conditions variation in bakery manufacturing can promote biochemical changes, complex reactions, and physical modifications on the products (Jerome, Singh, & Dwivedi, 2019). Additionally, the increase of celiac people boosted the development of new processes to obtain gluten-free products with good sensory characteristics (Ulasevich et al., 2020). Thus, recently, high-intensity ultrasound technology has been studied for the processing of bakery products. Table 3 presents the main remarks concerning the production of bakery products assisted by acoustic energy.

Some studies have evaluated the use of acoustic energy in the production of gluten-free products. The application of acoustic cavitation on gluten-free pan dough throughout its homogenization step decreased its firmness besides changing bread texture (Jalali, Sheikholeslami, Elhamirad, Khodaparast, & Karimi, 2020). The process employing 60 W increased the specific volume, porosity, and overall acceptability. Ultrasound waves can damage the starch granules, destroy the covalent bonds, and finally break the polymer threads of the starch molecules. Thus, the increase in starch solubility leads to better gelatinization during baking, a decrease in the bread surface, and texture firmness. Furthermore, the turbulence caused by acoustic cavitation can have promoted the increase in the contact surface near the boundary layer and, thus, favored the aeration of the dough. The increased volume and porosity are two effective factors on softness, as well as decreased compression due to more presence of the air bubbles and their homogeneous distribution. Therefore, the processes assisted by ultrasound at 30 and 70% of intensity produced the softer dough. Another gluten-free dough prepared with ultrasound presented a two-fold increase in its volume (Ulasevich et al., 2020). A processing time of 10 min was enough to manufacture the dough acceptable for automatic molding of the dumplings. Also, the increased volume of the dough allows obtaining an additional number of dumplings during the automatic molding. The mass yield of dumplings increases by 5% per kg of dough. Thus, the ultrasound processing presented an additional beneficial economic effect. As mentioned earlier, acoustic cavitation promotes better dough aeration. Besides that, it impacted the dough ingredients in different ways, favoring their homogenization. On the other hand, Ulasevich et al. (2020) investigated the impact of ultrasound processing on obtaining a sterilized dough. The sterilization process of 30 min leads to a 13-fold decrease of bacterial density compared to the non-sonicated sample. Thereby, the ultrasound technology provided a dough with better sensory characteristics by a lower cost process. Moreover, another ultrasound treatment produced a sterilized product increasing its shelf life.

Vegetarian consumers are another class in rising. Some of these consumers do not consume eggs and egg-based products. In this way, the production of egg-free food options is important. Thus, Movahhed, Mohebbi, Koocheki, Milani, and Ansarifar (2020) studied the ultrasound effects on the quality of an eggless cake batter. The intensity and holding time of sonication significantly affected the batter density (relation between batter mass and volume) of the cake. Smaller values of batter

Table 3
Acoustic energy on the innovative bakery products manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Corn flour for producing gluten-free pan bread	Bread dough homogenization	Damages the starch granules and promotes better contact between surface near the boundary layer favoring aeration	Ultrasound probe at 26 kHz	Nominal power (60 and 140 W)	3 min	(Jalali et al., 2020)
Gluten-free dough for producing dumplings	Gluten-free dough preparation and sterilization	Affects the dough starch, gum, egg yolk, and protein promoting a better dough homogenization and inactivates dough bacteria	Ultrasound bath at 35 kHz	Non-informed	Preparation (60 min), sterilization (10, 20, and 30 min)	(Ulasevich et al., 2020)
Eggless cake batter	Cake batter homogenization	Evacuates the air from the liquid phase improving the aeration	Ultrasound 22-mm probe at 24 kHz	20, 60, and 100% amplitude (4.8, 14.4, and 26 kHz)	30, 60, and 90 s	(Movahhed et al., 2020)
Cupcakes	Cake homogenization	Aerations to improve cake texture characteristics	Ultrasound bath at 37 kHz, and ultrasound probe at 20 kHz	Bath nominal power (196 and 280 W) and probe nominal power (175 and 250 W)	0, 4, 6, and 8 min	(Kenari & Nemati, 2020)
Dough	Dough freezing	Changes the way that ice crystals form and plays a role in breaking ice crystals improving dough moisture distribution and reducing the damage to protein molecular structure	Ultrasound probe at 20 kHz	Power levels (20, 40, 60, 80, and 100 W/L)	From 0 °C to −5 °C (5 s on-time and 5 s off-time)	(Zhang, et al., 2020)

density were observed for samples treated at 60% of intensity for 30 or 60 s. The batter with a lower density is more desirable for presenting higher air content. During sonication treatment, the negative pressure caused by ultrasonic waves evacuates the air from the liquid phase. Thus, ultrasound treatment could improve the aeration of baked products. This condition (60% of intensity) also increased the eggless cake volume and springiness but decreased the crumb fractal dimension, hardness, gumminess, cohesiveness, and chewiness. An increase of the sonication time to 60 s, at low and medium ultrasound intensities caused an improvement, that is, a reduction in the batter density. However, in longer times, higher batter densities were observed, which can be attributed to increased processing temperature. The temperature rise may lead to the denaturation of proteins and their inability to retain the air bubbles. Therefore, the combination of 60% intensity and 60 s of sonication time provided the best quality of the eggless cake.

Similarly, Kenari and Nemati (2020) studied the acoustic cavitation application into cupcake baking to improve its texture characteristics. The acoustic waves were applied indirectly to the cake batter by two ultrasound systems (bath and probe). The acoustic cavitation improved the porosity and the size of the cake. Therefore, the product was softer, and the staling was delayed. The sonication treatment reduced the hardness of the texture due to better aeration and uniform distribution of gas cells in the final product. The emulsification assisted by ultrasound makes the batter mixing faster and increases the quality of the batter and the texture of the cake. The probe system had more effect on the quality of the cake than the bath system. This result can be associated with the different bubble structures provided by the two ultrasound systems (Figs. 3 and 4). Thus, the interaction of the different bubble structures with the medium (cake ingredients) resulted in different cake texture characteristics. The best ultrasound treatment studied for them was employing an ultrasound probe system at 250 W for 6 min.

Ultrasound treatments are also effective for assisting dough freezing. The acoustic energy improves the distribution of moisture in the dough and reduces the damage to the molecular structure of the proteins (Zhang et al., 2020). Moreover, the ultrasound process compared to traditional freezing significantly decreased the freezing time and increased the freezing speed of the dough. The acoustic cavitation greatly impacted the freezing rate from 0 °C to −5 °C (the maximum ice crystal generation). Thereby, ultrasound changes the way that ice crystals form and plays a role in breaking ice crystals. This improved the freezing speed in the sub-cooling stage. Despite that, higher power density (>80 W/L) harms the freezing rate. Mechanical and thermal effects of acoustic cavitation can promote the formation of large ice crystals by accelerating the mass and heat transfer inside the dough tissue. Moreover, a higher power density can cause structural changes in wheat gluten, such as disulfide bond breaking, hydrophobic group exposure, and secondary structure disorder. On the other hand, the acoustic cavitation produced by lower power densities (<80 W/L) enhanced the formation of small ice crystals, improving the uniformity of crystals size and decreasing the damage to the dough proteins. Thus, lower power densities improve the overall quality of the dough. Besides that, the structural characteristics of the gluten were improved by the ultrasound treatment. The ice crystals formed in the freezing process caused the de-polymerization of the extra-stranded SS of gluten, glutenin, and gliadin, increasing the SH content. The SH is essential for protein folding and the stability of the native protein conformation. Thus, acoustic energy supplied at lower densities provided less damage to dough molecular structures than the traditional freezing processing.

Few studies regarding acoustic energy in the processing of bakery products were found (Fig. 1). The main applications are centered in the homogenization step to enhance sensory characteristics (Table 3). Acoustic energy has been applied at different intensities and mostly in a direct way from a probe system. However, the best process conditions have been observed using lower energy densities.

4.4. Beverages

Ultrasound technology has been widely studied to replace pasteurization and other conventional methods to inactivate pathogenic and spoilage microorganisms and endogenous enzymes for extending the product's shelf life (Barba et al., 2021). Ultrasound processing at high-energy densities is usually effective in disrupting microbial cells and, depending on its process conditions, may preserve vitamins, polyphenols, antioxidants, and phytochemicals (Barba, Zhu, Koubaa, Sant'Ana, & Orlien, 2016). On the other hand, low-energy densities of ultrasound can enhance biotechnological processes, such as fermentation without damage the cells. Thus, ultrasound-assisted processes can provide differentiated beverages. Table 4 presents the main remarks concerning beverages treated by acoustic energy.

In most of the examples presented, the authors evaluated the impacts of ultrasound on the quality parameters of beverages. Silva, Arruda, et al. (2020) evaluated the acoustic cavitation effects on stabilizing a prebiotic orange juice enriched with xylooligosaccharides (XOS). Prebiotic beverages containing bioactive compounds have emerged due to consumer demand for healthier products (Neri-Numa et al., 2020). Non-thermal treatments have been investigated to avoid losses of the prebiotics added to the products and, as well as the micronutrients from the raw materials (Silva et al., 2020). In this regard, ultrasound technology is a promising alternative, considering that high energy can be applied in a short holding time (Strieder et al., 2019). The working temperature in ultrasound processing depends on the acoustic energy intensity applied. The application of higher energies rises the collapse of cavitation bubbles, increasing the energy transfer and, thus, an increase in working temperature. Sonication medium characteristics, such as vapor pressure or viscosity, also affect the working temperature. Since higher viscosities promote a decrease in energy transfer efficiency (Guimarães et al., 2018). Zhang et al. (2016) observed the depolymerization of microcrystalline cellulose by an intense ultrasound treatment combined with the addition of a Fenton reagent. The treatment was carried out in a probe system at a nominal power of 800 W, a frequency range of 21–23 kHz for 2.5 h. The depolymerization occurred due to the acoustic cavitation, which caused degradation of the polymer due to the chemical reaction between the polymer and molecules, such as hydroxyl radicals produced during acoustic cavitation (Gomes et al., 2017). Ultrasound processing did not affect the XOS chemical stability. However, the process intensification by increasing nominal power from 300 to 1200 W caused significant loss in the ascorbic acid, malic acid, and citric acid content (Silva, Arruda, et al., 2020). Furthermore, the nominal power increase reduced the values of total phenolic content (TPC) and antioxidant activity by the Ferric reducing ability of plasma (FRAP). These experiments were carried out assisted by an ice bath to avoid overheating. However, the heat dissipated by the acoustic cavitation at 1200 W promoted an increase of up to 80 °C. Despite that, the ultrasound processing even at high-energy conditions did not depolymerize the XOS molecules.

Bhargava et al. (2020) observed that the combination of ultrasound with other techniques has enhanced the overall quality of the final product. In this way, the synergistic effect of acoustic energy and mild heat processing, known as thermosonication, may result in higher quality food products. Thermosonication treatment is based on the combination of mild thermal treatments from an external heat source and acoustic cavitation provided by ultrasound (Anaya-Esparza, et al., 2017). This technique has been evaluated to inactivate microbial and enzymatic load in beverages due to less exposure of the product to heat (Balthazar et al., 2019).

A thermosonication treatment to process wheat plantlet juice was studied by Ahmed et al. (2019). The working temperatures were 30, 45, and 60 °C. The treatments increased the non-enzymatic browning, viscosity, and cloud value of the juice. The lowest temperature (30 °C) for 20 and 40 min resulted in the highest value of phenolics compounds, flavonoids, antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl

Table 4
Acoustic energy on the innovative beverage manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Prebiotic orange juice	Stabilization	Increases the juice temperature leading to the loss of minor compounds and conserves the Xylooligosaccharides added	10-mm ultrasound probe at 20 kHz	Acoustic power (11.7, 19.7, 32.3, 49.6 W)	10 min	(Silva, Arruda, et al., 2020)
Wheat plantlets juice	Thermosonication	Promotes the inactivation of the lipoxygenase and breakage the cell walls releasing compounds	Ultrasound bath at 40 kHz	Nominal power (420 W)	20 and 40 min	(Ahmed et al., 2019)
Pulque (non-dairy Mexican fermented beverage)	Pasteurization	Decreases the microbial and yeasts loads and retained the pulque sensory and physicochemical properties	Ultrasound probe	Nominal powers (325, 375, 425, and 475 W)	5, 7, 10, 13, and 17 min for 325 W, 3, 6, 9, 12, and 15 min for 375 W, 2, 4, 6, 8, and 10 min for 425 W, and 1, 3, 5, 7, and 9 min for 475 W	(Alcántara-Zavala et al., 2020)
Kiwifruit juice	Stabilization	Ruptures the cell wall causing the dispersion of the intracellular components into juice, degradations in pectin and improvements of the rheological properties	Ultrasound probe at 20 kHz	Nominal power (400 W)	0, 4, 8, 12 and 16 min	(Wang et al., 2020)
Cider from Lebanese apples	Fermentation	Promotes microbial growth by separating the cell groups, increasing the membrane permeability, and impacting the cellular functions and components	Ultrasound chamber	Energy consumptions (311 J/mL) for 12 h; and (158 J/mL) for inoculation	On: 0.5 s; off: 6 s for 12 h (all fermentation) and On: 0.5 s; off: 6 s for inoculation	(Al Daccache et al., 2020)

(DPPH), carotenoid content, anthocyanin content, chlorophyll (a + b), minerals, and free amino acids. The acoustic cavitation increased the carotenoid content in the wheat plantlets juice due to the inactivation of lipoxygenase and breakage of cell walls throughout the ultrasound processing. On the other hand, the treatments at high temperatures (45 and 60 °C) for 40 min showed a significant impact on reducing microbial load. Thus, an intense thermosonication (higher temperature and holding time) favored microbial inactivation. However, phytochemicals were degraded. Thermosonication treatments performed at 45 and 60 °C promoted the polyphenols thermal degradation and decreased the antioxidant activity. Alcántara-Zavala, de Dios Figueroa-Cárdenas, Pérez-Robles, Arámbula-Villa, and Miranda-Castilleja (2020) also observed alterations in flavor and aroma of Pulque thermosonicated at 475 W and 50 °C for 3 and 5 min. Otherwise, this treatment also promoted the best yeast reduction. The higher nominal powers (425 and 475 W) also inactivated a higher microbial load of lactic acid bacteria than the other treatments. The sensitivity of the yeasts was affected by the association of the heat treatment at 50 °C with acoustic energy. Thermosonication treatments at 375 and 425 W resulted in yeasts sublethal reductions and preserved the pulque sensory and physicochemical properties. Also, the shelf life of the beverage stored at 4 °C was extended up to 24 days. Therefore, the best treatments to stabilize the pulque samples were 375 W for 6 and 9 min and 425 W for 4 and 6 min. These processing conditions inactivated lactic acid bacteria and yeasts that could develop throughout storage. Moreover, thermosonication treatments exhibited the best performance to preserve sensory and physicochemical properties of the pulque than conventional pasteurization.

Indeed, ultrasound processing at high energy densities have been applied to stabilize beverages (Table 4). Thermosonication processes seem to be a good option to achieve microbiological stabilization. However, high holding times associated with high temperatures can promote losses of phytochemical compounds in beverages. Thus, operational parameters need to be optimized to preserve the beverages' quality attributes.

Otherwise, the rheological properties of kiwifruit juice were enhanced with longer processing times at high nominal powers (Wang, Wang, Vanga, & Raghavan, 2020). The acoustic cavitation disrupted the juice tissues' cell walls, releasing more water-soluble pectin than the untreated samples. Juices processed with longer ultrasound processing times presented a greater release of pectin. Moreover, the ultrasound processing enhanced the color attributes, cloudiness, and sugar content. The shear stress, apparent viscosity, storage and loss modulus also were increased using longer processing times. Therefore, in this study, higher acoustic energy promoted better effects on kiwifruit juice's rheological properties.

Ultrasound treatments at low-energy densities were evaluated by Al Daccache et al. (2020) for cider production. The effects of ultrasound-assisted fermentation on the *Hanseniaspora* sp. growth, substrate consumption, and ethanol production during cider production were investigated. Acoustic energy at low intensity was applied to either the pre-culture and in the apple juice yeast medium for a pulse duration of 0.5 s followed by a pause of 6 s. Increases in biomass concentration by 52% and 42% were observed in apple juices by ultrasound-assisted fermentation. The first concentration was observed for apple juice ultrasound-assisted fermented for 12 h. The second for the juice acquired from the ultrasound-treated pre-culture followed by apple juice fermentation. Ultrasound-assisted fermentation could promote microbial growth by separating the cell groups, increasing the membrane permeability, and impacting the cellular functions and components. Glucose consumption was higher, but ethanol production was lower in ultrasound-assisted fermentation processes. These results could be explained by the fact that the yeast cells seem to consume more glucose to produce more biomass and CO_2 rather than producing ethanol. The acoustic energy promotes a strong micro-convection in the medium, which may increase the mass transfer of substrates through the cell membrane. Since a higher content of the substrate is accessible by the cells, the consumption of glucose is greater and, thus, more biomass is produced. Despite that, according to the authors, further investigations are required to understand the mechanisms behind microbial stimulation and ethanol reduction. Thereby, this research presented new applicability of acoustic energy in obtaining fermented beverages.

4.5. Minimally processed products

Currently, minimally processed products are a strong trend. Consumers increasingly seek healthy food associated with practicality. The largest category of these products is made up of products obtained from plants. These are composed of fragile tissues, which need to be handled with care to prevent damages during their processing. A size-reduction step following by a cleaning step makes up the process used to prolong the minimally processed foods' shelf life. The cleaning step usually involves washing the product with the use of chemicals. Also, thermal treatment can be employed depending on the plant matrix. Thus, the

minimal processing include partial or minimal preservation treatments to protect foods from microbial contaminations (Bansal, Siddiqui, & Rahman, 2015). Aiming for better efficiency of this step and thus a longer product shelf life, ultrasound-assisted treatments have been studied. Table 5 presents some examples of minimally processed products obtained by ultrasound treatment.

In most of the examples presented, the authors evaluated the effects of acoustic energy on the microbiological and enzymatic stabilization of food plants. Irazoqui et al. (2019) observed that the combination of the ultrasound treatment with sodium hypochlorite improved the reduction of microbial load on the processing of fresh-cut lettuce. However, higher microbial growth throughout refrigerated storage in samples was observed. Ultrasound waves impacted the lettuce surface at a microscopic level. The epicuticular lettuce wax was removed after 30 min of treatment (130 W at 42 kHz). The wax layer is the lettuce natural defense against microorganisms. In this regard, ultrasound-treated lettuce would be more susceptible to microorganisms' growth. On the other hand, the waxy layer can make the disinfection step more difficult because particles and microorganisms can be enmeshed inside, making their removal more difficult. Thereby, acoustic cavitation enhanced the disinfection step. It improved the contact between disinfectant and product surface, removing microorganisms from the surface of the fresh product. Similarly, another ultrasound treatment associated with the addition of sodium hypochlorite sanitizer was evaluated for reducing microbial load in Chinese cabbage (Alenyorege, Ma, Ayim, Lu, & Zhou, 2019). The combined treatments presented synergistic effects on reducing the initial natural microbiota and inhibited its survival on Chinese cabbage during storage. The intense pressure gradient provided by acoustic cavitation may have facilitated the penetration of NaClO through the outer thinner peptidoglycan layer and delicate cell casing, enhancing the effectiveness of the sanitizer. Therefore, this treatment extended the storability of Chinese cabbage preserving its physical and chemical quality attributes during storage.

Ultrasound treatment was evaluated by Yildiz, Izli, and Aadil (2020) for the prevention of enzymatic browning of fresh-cut quince slices during 14 days of storage. Ultrasound treatment inhibited bacteria, mold, and yeast growth in fresh-cut quince slices at 4 °C storage. Furthermore, the samples presented higher bioactive compound contents, better color parameters, and lower enzyme activity values. Thereby, the samples showed less enzymatic browning. Since the lowest PPO and PME activities indicated less browning. Acoustic cavitation inhibits the activity of these enzymes, promoting microscopic channels in the fruits, which facilitates moisture removal. Moreover, the deformation of porous solid materials, led by ultrasonic waves, can also decrease the diffusion boundary layers and increase the convective mass transfer in the product due to microscopic channels. Thus, the lowest water content in fresh-cut quince slices can inhibit the action of these enzymes responsible for the browning of the fruit.

Ultrasound technology also can be used to provide better technological properties for minimally processed products. An ultrasound pretreatment evaluated by Zhang, Yu, Fan, and Sun (2020) reduced the potato slices' oil absorption during their frying process. The pretreatment led to an increase in the fraction of bound water in the potato slices. The formation of radicals H^+ and OH^- and the damage of samples microstructure caused by acoustic cavitation favored the combination of radicals and the generation of molecular products, such as bound water. Besides that, the explosive disruption of bubbles produced heat, local pressure, and shear forces, which increased the surface roughness of potato starch. Despite the formation of notches and grooves on the starch surface, the granules could maintain the characteristic shape and integrity. These modifications caused by the pretreatment at 360 W and 600 W, both for 60 min, resulted in potato slices that absorbed less oil than control potato slices. Thereby, the ultrasonicated fried potato slices exhibited better texture characteristics than the non-treated ones, as they absorb less oil during their frying.

Few studies evaluated the use of acoustic energy in minimally processed products. The main applications are in microbiological and enzymatic stabilizations (Table 5). Despite this, ultrasound technology also demonstrated to be promising to promote changes of technological interest in vegetables. Acoustic energy has been applied at low energy densities and by a bath system to stabilize the products. Lower energy intensities have promoted the desirable effects without causing major damage to the plant's cell walls. However, in the last example, higher acoustic energy was applied by a probe-type system to cause modification on the walls of the potato slices. Thus, the plant matrix and the process type determine the acoustic energy intensity to be applied in the minimal processing of foods.

4.6. Food ingredients

Obtaining ingredients from natural sources has been performed for the production of alternative healthier food products. In this sense, ultrasound technology has performed more efficient extractions in shorter processing times and using less and non-toxic solvents compared to other technologies (Medina-Torres, Ayora-Talavera, Espinosa-Andrews, Sánchez-Contreras, & Pacheco, 2017; Neves et al., 2020; Senrayan & Venkatachalam, 2019). Thereby, the ultrasound process variables for extracting ingredients have already been extensively studied (Chemat et al., 2017). However, the impact of acoustic waves can also promote modifications and improvements in the ingredients' technological characteristics. Table 6 presents the main remarks concerning modifications and improvements of food ingredients by acoustic energy.

Ultrasound treatments can improve protein ingredients' applicability in food products. Since these treatments have modified the functional properties of protein ingredients. These modifications were observed in the rheological behavior, aggregates size, and surface

Table 5
Acoustic energy on innovative minimally processed products manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Fresh-cut lettuce	Disinfection	Removes the epicuticular lettuce wax promoting better contact of disinfectant with the product surface, thus removing microorganisms from the fresh fresh-cut lettuce	Ultrasound bath at 42 kHz	Nominal power (130 W)	0 at 30 min	(Irazoqui et al., 2019)
Fresh-cut Chinese cabbage	Disinfection	Improves the antimicrobial efficacy of NaOCl reducing and inhibiting natural microbiota and preserves the quality of fresh-cut cabbage	Sweep frequency ultrasound at 40 kHz	28, 33, 40, and 68 kHz	10 min	(Alenyorege et al., 2019)
Fresh-cut quince fruit	Treatment for the prevention of enzymatic browning	Promotes microscopic channels in the fruits, which facilitates moisture removal and difficult enzymes action	Ultrasound bath at 28 kHz	Nominal power (50 W) and intensity (100 kW/m^3)	15 min	(Yildiz et al., 2020)
Potato slices	Pre-treatment to absorb less oil during the frying process	Promotes the formation of radicals H and OH in water and damages the microstructure of the samples increasing the fraction of bound water on the potato slices	15-mm ultrasound probe at 20 kHz	Nominal power (1200 W)	Non-informed	(Zhang, Yu, et al., 2020)

Table 6
Acoustic energy on innovative ingredients manufacturing.

Product	Ultrasound step	Acoustic cavitation effect	Ultrasound system	Ultrasound energy	Processing time	References
Mung bean protein	Modification to increase solubility	Decreases the particle size and free sulfhydryl content, and exposed the nonpolar groups by dissociation of native components	13-mm ultrasound probe at 20 kHz and 30% of amplitude	Output (36 W/cm^2)	5, 10, 20, and 30 min	(Zhong & Xiong, 2020)
Whey protein aggregates	Modification to produce a better low-fat cheese	Disintegrates the whey aggregates exposing more hydrophobic residues	11-mm ultrasound probe at 20 kHz and 50% of amplitude	Acoustic power (33 W)	1 min	(Gamlath et al., 2020)
Micellar casein powders	Modification to increase solubility	Decreases casein particles size	15-mm ultrasound probe at 20 kHz	Nominal power (60 W)	30 s on and 30 s off mode for up for 0.5, 1, 2.5 and 5 min	(Wu, Li, et al., 2020)
Potato starch gel	Modification	Reduces the amylose content of the starch gel	Ultrasound probe at 22 kHz	Nominal powers (80, 160, and 240 W)	3, 15, and 27 min	(Kalinina et al., 2020)
Potato starch nanoparticles	Modification	Breaks down the van der Waals and electrostatic forces between starch surfaces and facilitates the formation of strong interfacial interactions between oxygen-containing groups on the surface of ascorbic acid and starch	Ultrasound probe	Non-informed	15 min (3 s on 1 s off pulse mode), and 30 min (3 s on 1 s off pulse mode)	(Shabana et al., 2019)
Sweet potato and wheat flours starch	Modification	Erodes the starch surface and promotes the scission of starch chains followed by the disruption of the cluster and internal molecular structure	13-mm ultrasound probe at 20 kHz	Nominal power (750 W)	2, 4, 8, 16, and 20 h	(Cui & Zhu, 2020)
Natural blue colorant based in milk	Extraction, reaction catalysis, and homogenization	Promotes the rupture of the cell wall of the fruit, catalyzes the reaction for the formation of the colorant, and reduces the droplet size of the colorant's milk	13-mm ultrasound probe at 19 kHz	Nominal power (100, 200, 300, and 400 W)	6 min	(Strieder, Neves, Silva, & Meireles, 2020)

hydrophobicity of proteins. A thermosonication treatment carried out at 70 °C improved mung proteins solubility (>2 fold), clarity, and suspension stability (Zhong & Xiong, 2020). This treatment reduced the particle size and free sulfhydryl content of the proteins. Acoustic cavitation promoted the exposure of nonpolar groups by dissociation of native components followed by reaggregation into soluble particles. Therefore, the thermosonicated mung protein presented a better solubility that can favor its homogenization in food products. Other thermosonication treatments promoted similar effect on whey protein aggregates (Gamlath, Leong, Ashokkumar, & Martin, 2020). Thermal denaturation of whey proteins exposed hydrophobic domains that are buried within the native globular structure. Thus, the hydrophobic regions interacted to form larger aggregates, which were later disintegrated by acoustic cavitation-induced shear forces. Thereby, disintegrated aggregates present more exposed hydrophobic residues. These disintegrated aggregates of whey protein were evaluated into the kinetics of rennet gelation and protein retention in non-fat cheddar cheese. They were better retained in cheese and favored the rennet gelation improving the non-fat cheese microstructure. Thus, thermosonicated whey proteins presented better technological properties to produce low-fat cheese. Wu et al. (2020) also observed the ultrasound effects on the characteristics of powdered micellar casein. Acoustic cavitation improved the casein solubility (>95%) by decreasing its particle size from 30 μm to ~0.1 μm. Additionally, sonication did not affect the molecular weight of the individual casein molecules. Again, the acoustic energy contributed to produce a protein ingredient easier to apply in food formulations. Therefore, in the context of protein ingredients, ultrasound treatments have been combined with heat treatments to improve the solubilization of proteins. Since there is a growing demand for vegan protein products, there is still a great demand for studies that evaluate more ultrasound process conditions to improve the technological properties of plant proteins.

Starch is another ingredient widely used by food industries as raw material for improving food texture, flavor, and gelling. However, often starch passes for chemical or physical modifications to reach the necessary technological characteristics for its application. In this sense, ultrasound-assisted processes have been performed to promote different starch modifications. Kalinina et al. (2020) applied ultrasound treatment on their potato starch gel to modify its viscosity. The starch gel viscosity increased or decreased depending on the ultrasound processing time and power employed. The acoustic cavitation reduced the amylose content of the starch gel, allowing the change in gel viscosity according to the process conditions employed. Since the ratio of amylose and amylopectin determines the formation of the viscous colloidal systems. Thereby, acoustic energy can adjust the viscosity of starch solutions reducing amylopectin content. Another ultrasound treatment assisted the synthesis of crystalline potato nanoparticles (Shabana et al., 2019). The crystalline nature of the starch particles was acquired due to the modification of amylose to amylopectin, as also observed by Kalinina et al. (2020). Cavitational bubbles collapse broke down the van der Waals and electrostatic forces between starch surfaces and reduced the agglomeration of starch particles produced. Thus, their size was reduced. Besides that, the acoustic cavitation facilitated the formation of strong interfacial interactions between oxygen-containing groups on the surface of ascorbic acid and starch. Thus, the ultrasound processing increased the ascorbic acid incorporation in the starch surface. These antioxidants-loaded potato starch nanoparticles can be applied in food packaging, drug delivery, barrier coatings, and others. Acoustic energy also enhanced the sweet potato and wheat flours starches characteristics (Cui & Zhu, 2020). The acoustic cavitation induced structural modifications on starch granules morphology and reduced its crystallinity. Longer processing times (until 20 min) decreased the enthalpy of gelatinization, pasting behavior, gelling capacity and increased the digestibility of *in vitro* starch of the raw flour. Despite that, longer treatments reduced total phenolic content and antioxidant capacity of the sweet potato flours. The acoustic cavitation did not cause changes in the integrity of starch granules but eroded the surfaces progressively increased over the processing time. This was attributed to the shock waves and water impinging jets at high speeds onto the surface of starch. Also, the free radicals (OH^- and H^+) generated from ultrasound processing may have affected the integrity and rigidity of the starch. They promote the scission of starch chains followed by the disruption of the cluster and internal molecular structure. Some flour starch granules were disrupted into smaller particles. The changes in starch size were

caused by the collapse of bubbles from the acoustic cavitation in an aqueous medium. Thus, these starch modifications consequently promoted reductions in the gelatinization enthalpy, pasting behavior, and gel hardness. The flour digestibility was also increased. The reductions of total phenolic contents and antioxidant properties were mainly associated with prolonged ultrasound processing times. Thereby, the changes in starch structures caused by ultrasound provided different applicability for the starch samples.

The food colorant industry has also been changing to meet the current demand for more natural foods. In this regard, acoustic cavitation has been widely studied to assist the extraction of coloring compounds from plant raw materials to produce natural colorants (Strieder et al., 2019). The release of the colorant compounds from sonicated plant raw material is facilitated due to sonoporation effects on the plant cells and tissues. On the other hand, the acoustic cavitation phenomenon increases the average temperature of the sonicated medium, favoring the diffusion of phytochemical compounds from the plant material to the solvent. Thus, extracts with a higher content of colorant compounds have been obtained. Furthermore, acoustic cavitation favored the reaction between genipin and milk proteins to form a blue colorant (Strieder, Neves, Silva, & Meireles, 2020). In a single step, acoustic energy promoted the extraction of genipin from unripe genipap pulp and its reaction with milk proteins producing blue compounds. The acoustic cavitation favored the crosslinking of genipin with primary amine groups producing the blue colorant. Besides that, the same energy used for the extraction and reaction also reduced the size of the fat globules of the milk, resulting in the kinetic stabilization of the blue colorant.

Therefore, acoustic energy has been applied to protein and starch ingredients to enhance their technological properties, increasing their applicability in food products. The energy intensity required for each modification depends on biopolymer properties. However, the probe system was used in all the examples presented (Table 6). The acoustic energy also favored the production of a promising natural blue colorant. Although most studies that use ultrasound are related to obtaining ingredients (Fig. 1), few studies applied ultrasound to promote chemical or physical modifications. Most studies employed this technique to assist extractions. Thereby, the literature still demands more studies concerning the effects of acoustic energy on obtaining food ingredients.

5. Impact of acoustic energy on the sensory quality of foods

The consumers' purchase intention is based on food product sensory appeal, nutritional quality, safety, and health (Imtiyaz, Soni, & Yukongdi, 2021). The products obtained through ultrasound-assisted processes have shown those characteristics, as discussed in this review. However, in this topic, acoustic energy-specific effects on the sensory quality of food products were reviewed.

The sensory appeals of taste, flavor, appearance, freshness, texture, and overall liking are crucial characteristics of food products (Imtiyaz et al., 2021). Because of this, some acoustic energy-assisted processes have been developed to produce desirable sensory characteristics in foods and beverages. Gao et al. (2020), for example, processed soy sauce using a low-intensity ultrasound treatment (68 kHz and 60 W/L for 10 min) for accelerating the production of aroma in the product. Nine trained panelists evaluated the ultrasonicated soy sauce. According to them, the ultrasound treatment enhanced the sensory scores of the seven aroma characteristics as well as the overall aroma score of raw soy sauce.

Otherwise, ultrasound treatments may consequently improve or decrease food products' sensory attributes. Panelists evaluated cheeses prepared with ultrasound-modified caseins giving low grades for their overall acceptability (Lara-Castellanos, Azuara, Jimenez-Fernandez, Luna-Solano, & Jimenez, 2021). This less acceptability may be associated with the different microstructure of ultrasonicated caseins. The ultrasound-modified casein promotes great changes in the functional properties of moisture content, microstructure, and textural properties of cheese. Because of this, the panelists preferred the cheese produced with non-treated caseins. On the other hand, positive effects of sonication were observed by Kenari and Razavi (2021) on sensory properties of yogurt samples. The overall acceptability of yogurts treated by ultrasound was higher than the control yogurt treated at 90 °C for 10 min. However, the yogurt's average flavor score decreased with the increase of sonication time from 5 to 15 min, temperature from 45 to 65 °C, and amplitude from 50 to 75%. The synergistic effect of cavitation and heat generated by ultrasound energy itself denatured whey protein, producing sulfhydryl aromas. Despite that, the best texture results were observed for yogurts obtained by the most intense ultrasound treatment. The application of ultrasound at high amplitude (75%) produced a yogurt with a very firm texture that was attributed to the generation of some bitter peptides. Therefore, ultrasound parameters on dairy product processing present important effects on their sensory characteristics.

An ultrasound treatment (40 kHz, 11 W/cm^2) for 60 min was studied as a potential process to beef tenderize (Peña-Gonzalez, Alarcon-Rojo, Garcia-Galicia, Carrillo-Lopez, & Huerta-Jimenez, 2019). According to the panelists, the ultrasound process worked since they evaluated the sonicated meat as more tender and juicier than the respective control samples. Ultrasonicated beef samples also presented a higher intensity of beef and fresh meat smell than control samples. The non-sonicated samples presented the most fibrous texture. Acoustic energy increases enzyme activity by accelerating the oxidation of proteins and other components in beef, promoting changes in its color and flavor. Moreover, acoustic cavitation by the periodic oscillation of acoustic pressure softens cell membranes degrading muscle cells and some subcellular components. Thus, providing better flavor, color, and texture for the meat. These positive effects of acoustic cavitation on beef characteristics were also observed on a restructured cooked ham (Barretto et al., 2020). The sonication of restructured cooked ham (Table 2) promoted its better sensory acceptance according to panelists. Acoustic cavitation increased the diffusion of the sodium added to the meat product enhancing its sensory characteristics. Thereby, ultrasound technology has been provided better meat products' sensory properties.

Ultrasound technology applied to juice or juice vinegar processing has preserved or even improved their sensory characteristics. An ultrasound treatment to enrich bioactive components in verjuice vinegar (green grape juice) did not significantly affect its sensory characteristics compared to the untreated and the thermally treated sample (Yıkmış, Bozgeyik, & Şimşek, 2020). The ultrasound treatment (26 kHz and 68.7 amplitude for 9.4 min) that provided the greatest enrichment of bioactive compounds in vinegar was sensorially compared to untreated, and heat-treated vinegar (65 °C for 30 min). The acceptance test was performed for pungent sensation, richness in aroma, general impression, taste, aromatic intensity, and ethyl acetate odor. Ultrasound processing produced a vinegar with more phytochemical compounds preserving its sensory attributes. Likewise, in apple juice, temperature-controlled ultrasound treatments promoted better appearance, odor, and cloudiness than heat processing (Shen et al., 2021). Eight ultrasound treatments at different temperatures (30, 40, 50, 60, and 70 °C), powers (525, 975, and 1125 W), and holding times (5, 10, and 12 min) were evaluated. Pasteurization was performed at 85 °C for 10 min. Panelists evaluated with better scores the appearance, odor, cloudiness, and general acceptability of the ultrasonicated juice samples. Thereby, ultrasound treatments improved the sensory characteristics of apple juice through the microbiological stabilization process.

Otherwise, an ultrasound treatment negatively impacted the sensory quality of lettuce (Neto, Millan-Sango, Brincat, Cunha, & Valdramidis, 2019). The ultrasound treatment carried out for lettuce decontamination was performed with a probe ultrasonic system operating at 26 kHz, 90 μm, 200 W, in a continuous mode for 5 min. The acoustic energy damaged the structure of the leaves, leading to an aspect less acceptable. Indeed, this intense treatment may have caused the disadvantages observed on the sensory attributes of lettuce leaves. However, the application of less intensity acoustic energy could have promoted less

impact on the sensory characteristics of leaves. Therefore, other treatment conditions applying ultrasound need to be evaluated in the sensory properties of minimally processed foods.

Thereby, the application of acoustic energy may present controversial effects on the taste, flavor, appearance, freshness, and texture of food products. These effects would be associated with the ultrasound treatment conditions and the characteristics of the foods and beverages subjected to sonication. However, few studies were found in the literature regarding the impact of ultrasound processing on the sensory characteristics of food products. Moreover, the toxicity of food products and ingredients produced by ultrasound needs to be investigated. Since no research has been found to assess the toxicity of food produced by ultrasound technology on consumer health.

6. Scale-up and economic evaluation of ultrasound food processes

The scale-up of ultrasound-assisted processes has been evaluated since the results on the laboratory scale are promising. For this, first, the laboratory study is necessary to optimize the operational process conditions. The establishment of optimum power on a laboratory scale minimizes industrial-scale energy requirements and production costs. Besides that, the most important design aspect is to promote the uniform distribution of acoustic cavitation throughout the product volume (Patil & Gogate, 2018). Since that the technological limitation of scaling up ultrasound processes is the significant reduction in ultrasonic amplitudes in a large sample volume. This reduction implies that decreasing the intensity of cavitation-generated shear forces compromising the quality of processing. Barbell horn ultrasonic technology (BHUT) seems to overcome this limitation. BHUT allows the output surface of a probe to increase without limiting the maximum amplitude achievable by the probe (Peshkovsky, 2017). Thereby, this technology allows the construction of industrial-scale ultrasonic liquid processors that can operate at extremely high ultrasonic amplitudes. Leibtag and Peshkovsky (2020) investigated and demonstrated the feasibility of implementing BHUT for the commercial production of cannabidiol-containing nanoemulsions. The nanoemulsion sample was processed using a 1200 W bench-scale ultrasonic processor (BSP-1200, Industrial Sonomechanics, Miami, FL), equipped with a Half-Wave Barbell Horn® (HBH, Tip Diameter = 32 mm) operating in a flow-through reactor chamber at 20 kHz and the ultrasonic amplitude of 90 μm peak-to-peak. The scale-up of production of the optimized nanoemulsion allowed a processing rate of approximately 5 kg of nanoemulsion per hour. Therefore, ultrasonic processing using the BSP-1200 seems to be a promising alternative for the large-scale production of the food industry.

Zabot, Viganó, and Silva (2021) recently presented some companies specialized in the design and manufacturing of high-power ultrasonic equipment for mixing, dispersing, particle size reduction, extraction, and chemical reactions. Hielscher Ultrasonics GmbH, Reus, Sonics & Materials, Two Wheel Flavors & Botanicals, and G. Mariani & C. Spa companies have been producing ultrasound devices that apply up to 16,000 W at frequencies from 24 kHz to 30 kHz, and volumes from 0.01 mL to 2000 mL. This market demonstrates the importance of process optimization studies and economic evaluation. However, few studies have evaluated the economic viability of ultrasound-assisted processes. Ultrasound technology appears to be economically advantageous for extraction processes. Strieder et al. (2019) evaluated the cost of manufacturing (COM) for obtaining a blue ethanolic extract from unripe genipap pulp by ultrasound technology considering three scales. The laboratory scale presented the highest values of COM. The COM was lower for pilot and industrial scales. This COM decrease increasing the scale due to the optimization of some services and the higher productivity. The authors also demonstrated the low effect of the fixed capital investment on the COM of the pilot to industrial scale. Thus, indicating the low cost of the ultrasound system for large scales. Moreover, ultrasound treatments presented a faster and fixed capital investment two times cheaper than a process using pressing + low-pressure extraction. The integration of ultrasound to assist the extraction of phenolic compounds was also economically evaluated by Pereira, Zabot, Reyes, Iglesias, and Martínez (2021). They observed the COM on a laboratory scale to obtain an extract containing bioactive compounds from passion fruit rinds. However, in this case, they applied the fixed acoustic intensity of 360 W/cm^2 and evaluated different solvent mass flow rates (5, 10, or 15 g/min) on the COM. The lowest COM was obtained at 10 g/min, confirming this flow rate as the most indicated to obtain extracts enriched in phenolic compounds on the laboratory scale. Therefore, the economic viability of ultrasound-assisted processes to obtain other food products still needs to be evaluated. Comparative studies using more than one processing technique are interesting to show the feasibility of using this emerging technology.

7. Final considerations

Ultrasound technology can be largely applied in food processes based on acoustic cavitation effects. Acoustic energy at lower energy densities has been directly applied in dairy and bakery products to improve the technological properties of whey cheese, yogurt, butter, ice cream, and bread and cake dough. Otherwise, higher ultrasound intensities are required for the tenderization, curing, and homogenization of meat and meat products. Ultrasound at high-powers has been stabilizing beverages and at low-power assisted the fermentation step for cider production. Bath ultrasound systems are used to apply low-intensity acoustic energy to stabilize the minimally processed vegetables. This energy has promoted the desirable effects without causing major damage to the plant's cell walls. Proteins and starches were modified to be used in food product formulations by different intensities of acoustic energy. Thereby, ultrasound applied at different conditions by different ultrasonic systems has provided products with unique nutritional and sensory characteristics. Few studies investigated the impact of ultrasound processing on the sensory attributes of foods and beverages. However, in most evaluations, ultrasonicated products maintained or showed better sensory characteristics. Additionally, the scale-up of the ultrasound processes seems more viable as several companies have started to manufacture the equipment for larger scales. In this regard, further optimization and economic feasibility studies need to be carried out to facilitate further scale-up.

Declaration of Competing Interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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CHAPTER 4

Techno-economic evaluation of non-thermal and thermal ultrasound-assisted extraction processes for obtaining genipin extracts

A techno-economic evaluation for the genipin recovery from *Genipa americana* L. employing non-thermal and thermal high-intensity ultrasound treatments

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A techno-economic evaluation for the genipin recovery from *Genipa americana* L. employing non-thermal and thermal high-intensity ultrasound treatments

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ABSTRACT

This paper presents techno-economic results for the high-intensity ultrasound (HIUS)-assisted extraction of a valuable phytochemical. Genipin is a precussor of natural blue compounds besides presenting anticancer, antioxidative, and anti-inflammatory properties. The effects of the non-thermal and thermal HIUS treatments on obtaining of genipin-rich ethanolic extracts from the unripe *Genipa americana* L. were evaluated. The HIUS treatments were evaluated in the economic viewpoint to show their feasibility in different scenarios. The non-thermal HIUS-assisted extraction, with a maximum temperature of 30 ± 1 °C, was performed applying a nominal power of 100 W and the thermal extraction with 450 W, achieving a maximum temperature of 73 ± 1 °C. For both treatments, the HIUS specific energy levels of 1, 3, and 5 kJ/g were assessed. The unripe genipap extracts obtained were characterized during their storage time (0, 24, 48, and 72 h) at 24 ± 2 °C according to their genipin and geniposide content and visual appearance. The cost of manufacturing was evaluated in a pilot and industrial-scale for the different HIUS treatments studied. Our results demonstrated that the HIUS-assisted extractions allowed us to obtain different amounts of genipin and geniposide according to the non-thermal and thermal process conditions. The more intense acoustic cavitation provided by the nominal power of 450 W resulted in a better diffusion and, consequently, higher genipin recovery. The economic evaluation showed different scenarios to produce genipin-rich ethanolic extracts by HIUS technology. In comparison with a process using pressing + low-pressure extraction, our HIUS processes presented a faster and fixed capital investment two times cheaper.

1. Introduction

The demand for natural food products has been increasing worldwide in the last years, and to supply this new trend, food industries are searching for non-conventional processes and novel ingredients to obtain health promoters products [1,2]. Among the ingredients, the colorants for food products are essential to promote the sensory appeal. They are responsible for increasing or decreasing the consumer purchase intent since the color is one of the main sensory attributes. However, conventional colorants are associated with intolerances and allergies, especially in children [3]. Thus, studies on new natural colorants have been developed as a promising alternative to replace the synthetic ones in food manufacturing [4]. Besides, natural colorants may present beneficial effects on human health. Many of them are natural antioxidants, and thus, they can prevent cancer, cardiovascular, degenerative, and other diseases [5]. Therefore, in addition to ensuring the sensorial appeal of the food product, these colorants may bring benefits to consumers' health.

Natural colorants of different colors have been commercialized worldwide; however, natural sources of the blue colorant are still scarce concerning the other colors [6]. The blue food products such as candies, ice creams, dairy products, and other treats are mainly intended for children, demonstrating the relevance of obtaining a natural blue colorant. In this sense, studies have demonstrated that the unripe

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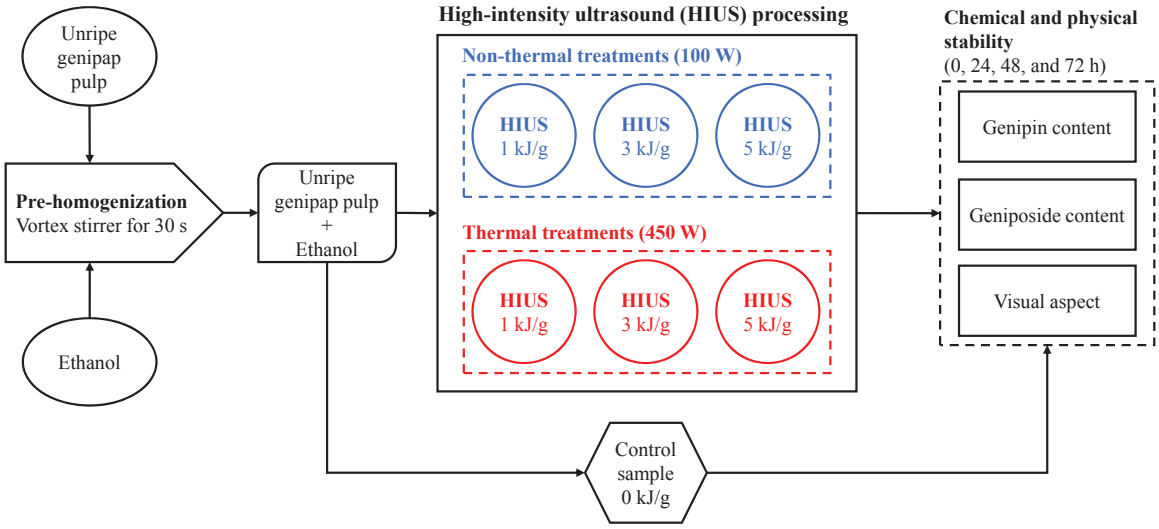

Fig. 1. Flow diagram for the high-intensity ultrasound treatments used for obtaining genipin extracts from unripe genipap.

genipap (*Genipa americana* L.) is a natural source of genipin, an iridoid that when is free (non-crosslinked with other compounds) can form blue compounds based on a reaction with primary amine groups from amino acids, peptides or proteins [7]. Also, genipin is an anticancer, antioxidative, and anti-inflammatory agent as well as a potent cross-linking drug that may find useful in novel pharmaceutical formulations [8,9]. Genipap also presents another iridoid known as geniposide, which presents a yellow color [10]. However, this compound may be hydrolyzed to genipin [11]. Both iridoids have functional properties, such as antioxidant capacity [12]. Thus, from unripe genipap fruits and a protein-rich liquid medium, a natural blue colorant could be obtained due to a polymerization reaction between genipin and primary amine groups. This reaction is induced by oxygen and heat [10].

Solid-liquid extractions with ethanol and water as solvents were evaluated by Renhe, Stringheta, Fonseca and de Oliveira [13] to obtain blue extracts from the genipap. The authors observed that the best extraction conditions were pH 4 and 75 °C. More recently, techniques such as pressurized liquid extraction and low-pressure extraction [14], enzyme-assisted extraction [15], and ultrasonic bath-assisted extraction [16] were evaluated. Among these techniques, the use of high-intensity ultrasound (HIUS) technology has gained evidence [17–19]. Extractions assisted by the HIUS technique present a shorter processing time and require reduced energy and organic solvent amount in comparison to the other extraction techniques. Thus, HIUS technology is recognized by minimizing the environmental impact and allowing a fast return of investment [20].

The HIUS-assisted extraction of genipin from the unripe genipap is a promising technique to obtain a valuable phytochemical precursor of natural blue colorants. The acoustic cavitation promotes a phenomenon observed in the liquid medium subjected to the low-frequency (16–100 kHz) and high-power ultrasound (>10 W). The cavitation phenomenon promotes the rupture of the cell walls of plant materials facilitating the diffusion of their phytochemical compounds [21,22]. Since the process temperature may influence the chemical stability of these compounds, the evaluation of the non-thermal and thermal HIUS processing is a fundamental step for the development of a novel process to obtain genipin extracts. Different types of HIUS treatments may be carried out using the same amount of specific energy based on their nominal power applied. For example, using low nominal powers and longer processing times, non-thermal HIUS treatments are performed, thus preserving thermolabile compounds like phenolic and antioxidants. In contrast, shorter processes with high nominal powers may generate an increase of temperature in the liquid medium, resulting in thermal treatment.

A techno-economic approach is a remarkable strategy for providing a panorama of different processes [23]. In food-related areas, this approach has been reported in the last years, as in the production of powdered extracts rich in bioactive compounds by green processes [24], in the extraction of clove buds oil by supercritical fluid [25], and in citric acid production by three recovery methods, including solvent extraction, ion exchange, and calcium precipitation [26], among others. Indeed, economic evaluation is a useful strategy to obtain the initial and general costs of a process to be used for further decisions.

Based on this context, this study aimed to evaluate the HIUS-assisted extraction as a non-thermal and thermal treatment for obtaining of genipin ethanolic extract from unripe *Genipa americana* L. Furthermore, a techno-economic evaluation was performed to verify the cost of manufacturing and the economic viability of these different HIUS treatments.

2. Material and methods

2.1. Raw material and sample preparation

Unripe genipap fruits of about 6 cm × 5 cm were donated by "Fazenda Lagoa" (Ponte Alta do Tocantins, TO, Brazil) and stored at −24 °C until the experimental assays. For the experiments, 50 g of thawed unripe genipap pulp were comminuted in a 400 W mechanical RI1364/07 processor (Philips-Brasil LTDA, Varginha, MG, Brazil) for 5 min.

2.2. High-intensity ultrasound-assisted extraction

Fig. 1 presents the flow diagram of the processes performed to obtain genipin extracts by using HIUS technology. We processed 25 g of ethanol (Dinâmica, Indaiatuba, SP, Brazil) and 5 g of milled unripe genipap pulp. This same ethanol mass to the genipap mass ratio was used for all experiments. Before HIUS processing, the samples were pre-homogenized in a 50 mL Falcon tube using a vortex stirrer for 30 s. The genipap ethanolic extract obtained in this step was used as a control sample and identified as "0 kJ/g". The pre-homogenized system was transferred to a 100 mL Becker with a removable inner metal basket and then HIUS-treated according to experimental design. The HIUS treatments were

Table 1
Acoustic powers, acoustic specific energies and HIUS intensities for the non-thermal and thermal treatments.

HIUS treatment	Nominal power (W)	Nominal specific energy (kJ/g)	Acoustic power (W)	Acoustic specific energy (kJ/g)	HIUS intensity (W/cm^2)
Non-thermal	100	1	1.5	0.02	1.1
		3		0.05	
		5		0.08	
Thermal	450	1	18	0.04	13.2
		3		0.12	
		5		0.19	

performed using a 13-mm ultrasound probe diameter at 19 kHz (Unique, Indaiatuba, SP, Brazil). For this, the probe contact height with the liquid medium was standardized to 15 mm. HIUS specific energy levels (1, 3, and 5 kJ/g) were applied to the system (ethanol + genipap pulp) by using nominal powers of 100 W (non-thermal treatment) and 450 W (thermal treatment). The HIUS specific energy was calculated from the Eq. (1). The experimental levels were chosen according to preliminary tests. The HIUS process conditions were selected based on ethanol boiling point (78.4 °C), aiming to avoid mass losses due to solvent evaporation. After the experimental procedures, the ethanolic extracts were separated from the genipap biomass by removing the metal basket.

$$Specific\ energy\ \left[\frac{kJ}{g}\right] = \frac{Nominal\ power\ (kW) \times Processing\ time\ (s)}{Mass\ (g)} \quad (1)$$

The effects of the HIUS treatments (non-thermal and thermal) and specific energy (1, 3, and 5 kJ/g) on the genipin and geniposide content, and visual appearance of the unripe genipap extracts were evaluated using a randomized full factorial design (2 × 4). All experiments were carried out in duplicate; thus, 16 assays were performed.

The acoustic power (Eq. (2)) or real power supplied to the model system (ethanol) was determined by calorimetric assays according to the methodology described by Mason, Lorimer, Bates and Zhao [27]. From the acoustic power, the acoustic specific energy and HIUS intensity were calculated according to Eq. (1) and Eq. (3), respectively [28].

$$Acoustic\ power\ (W) = mC_P\left(\frac{dT}{dt}\right) \quad (2)$$

$$\text{HIUS intensity}\ \left(\frac{W}{cm^2}\right) = \frac{4 \times \text{Acoustic power}}{\pi D^2} \quad (3)$$

Where m is the sample mass (g); C_P is the specific heat (J/g°C), $\frac{dT}{dt}$ is the temperature rate (°C/s), and D is the probe diameter (cm).

Table 1 presents the results of the acoustic powers, acoustic specific energies, and HIUS intensity for the non-thermal and thermal HIUS treatments studied.

2.3. Chemical and physical stability of the unripe genipap extracts

The chemical and physical stability of the unripe genipap ethanolic extracts were evaluated from the genipin and geniposide content, and changes in their visual appearance. These analyses were performed right after the HIUS processing (0 h) and afterward for an additional period until three days (24, 48, and 72 h). The genipap extracts were kept at 24 ± 2 °C during their storage time.

2.3.1. Genipin and geniposide content

The genipin and geniposide content in the genipap ethanolic extracts were determined by high-performance liquid chromatography (HPLC) on a 2695D separation module (Waters Alliance, Milford, MA, USA) equipped with a 2998 diode array detector, according to the methodology described by Náthia-Neves, Nogueira, Vardanega and Meireles [29]. The individual compounds in the genipap ethanolic extracts were separated on a Kinetex C18 column (150 × 4.6 mm id, 2.6 μm, Phenomenex, Torrance, USA) maintained at 35 °C using a flow rate of 1.5 mL/min. The mobile phase consisted of 0.1% water (v/v) in formic acid (solvent A) and 0.1% acetonitrile (v/v) in formic acid (solvent B). The iridoids were separated using the following gradient: 0 min (99% A); 9 min (75% A); 10 min (99% A); and 13 min (99% A). The compounds were detected at 240 nm and quantified using Empower 2 software (Waters Alliance, Milford, MA, USA). They were identified by comparing their retention times and UV–vis spectra to those of reference standards (Sigma Aldrich, San Louis, MO, USA).

Genipin and geniposide content were expressed regarding the weight of dried genipap and determined according to Eq. (4) and Eq. (5), respectively.

$$\text{Genipin content}\ (mg/g) = \frac{\text{Genipin mass}\ (mg)}{\text{Dried genipap mass}\ (g)} \quad (4)$$

$$\text{Geniposide content}\ (mg/g) = \frac{\text{Geniposide mass}\ (mg)}{\text{Dried genipap mass}\ (g)} \quad (5)$$

2.4. Statistical analysis

The HIUS effects on the genipin and geniposide content of the unripe genipap ethanolic extracts were evaluated by an analysis of variance (ANOVA) using the Minitab 18® software (Minitab Inc., State College, PA, USA) with a 95% confidence level (p-value ≤ 0.05).

Table 2
Input economic parameters for obtaining genipin ethanolic extracts.

Economic parameter	Value	Unit
Fixed capital investment (FCI)		
The total cost of the laboratory-scale plant (used in the experimental study; 5 g of genipap pulp)*	8000.00	US$
The total cost of the pilot-scale plant (equivalent for processing 500 g of genipap pulp per batch)*	50000.00	US$
The total cost of the industrial-scale plant (equivalent for processing 5 kg of genipap pulp per batch)*	120000.00	US$
The total cost of the industrial-scale plant with recycle (equivalent for processing 5 kg of genipap pulp per batch)*	210000.00	US$
Annual depreciation rate	10	%
Annual maintenance rate	6	%
Project lifetime	25	Years
Annual time worked (for the laboratory-scale plant)	2640	h/year
Annual time worked (for the pilot- and industrial-scale plants)	7920	h/year
Cost of raw material (CRM)		
Genipap (usual price of genipap in the region where the fruit is largely produced)**	1.42	US$/kg
Genipap (usual price of genipap in regions far from the production region)**	7.89	US$/kg
Ethanol for laboratory consumption***	2.80	US$/L
Ethanol for industrial consumption (for pilot- and industrial-scale plants)***	1.50	US$/L
Cost of operational labor (COL)		
Wage (with benefits and administration)	6.00	US$/h. worker
Number of workers per shift	1	Worker/ shift
Cost of utilities (CUT) (Main utilities)		
Water (for cooling and cleaning)***	1.00	US$/ton
Electricity***	0.20	US$/kW.h

* Estimated based on local quotations for each equipment that compose the plant.
** Based on Náthia-Neves et al. (2019).
*** Based on local quotations (Brazil).

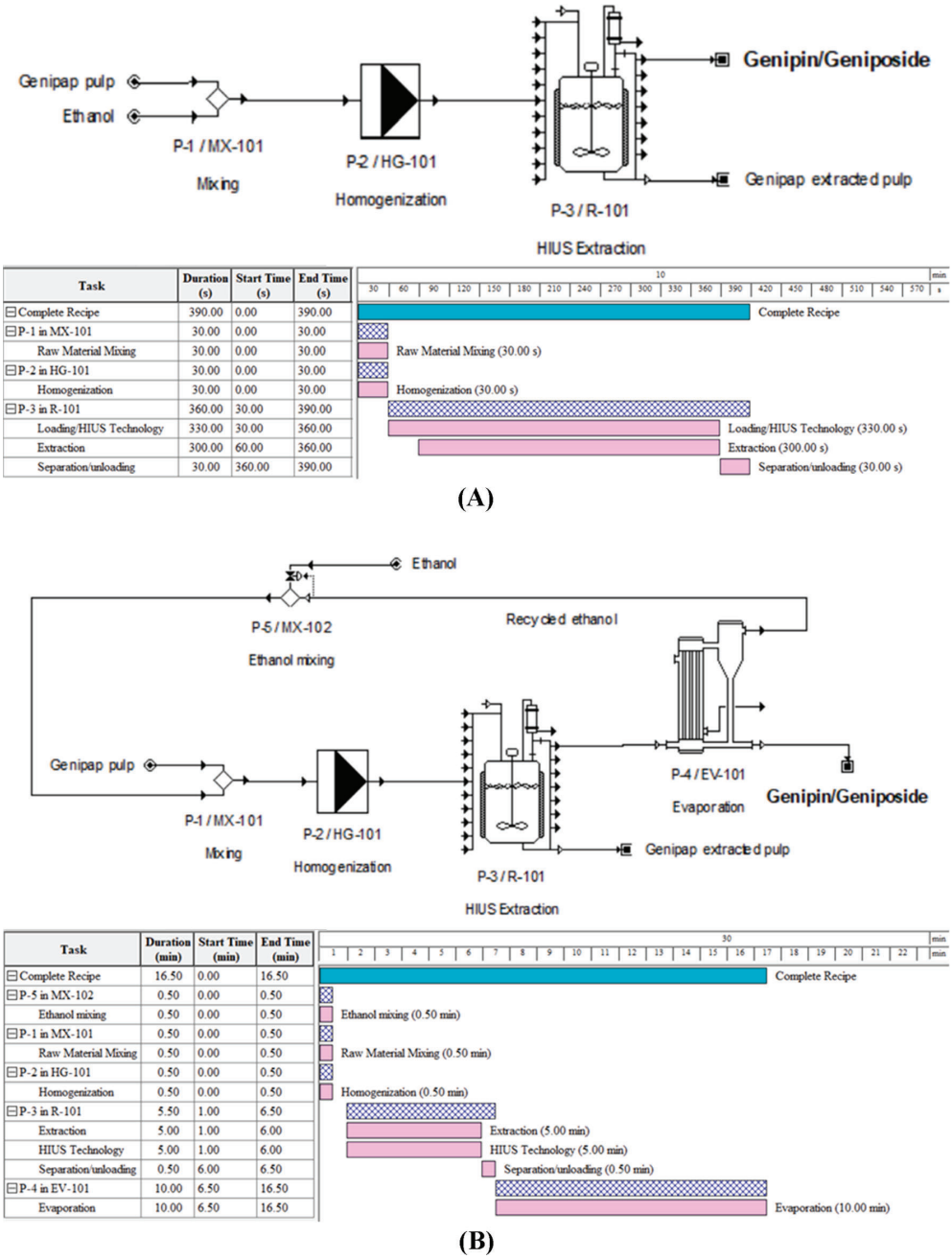

Fig. 2. Flowsheet and Gantt Chart of the HIUS process (non-thermal with 1 kJ/g) for evaluating the COM of extracts containing genipin/geniposide; (A) without ethanol recycle; (B) with ethanol recycle.

2.5. Economic evaluation

The extraction of genipin and geniposide from unripe genipap pulp was evaluated in terms of the cost of manufacturing (COM) using the SuperPro Designer 9.0® software (Intelligen Inc., Scotch Plains, NJ, USA). Firstly, the system described into Section 2.2 was designed in a flowsheet to evaluate the COM according to changes of HIUS specific energy (1, 3, and 5 kJ/g) for thermal and non-thermal treatments. The COM of the control sample (without HIUS treatment) was simulated for a comparison purpose. After that, the HIUS technology was evaluated at pilot-scale (an extraction vessel of 10 L for processing 500 g of pulp per batch) and industrial-scale (an extraction vessel of 100 L for processing 5 kg of pulp per batch). For industrial-scale, economic simulations were also done considering ethanol recovery. In these scenarios, partial evaporation of 95 wt% ethanol was done in a vacuum rotary evaporator, and the ethanol recovered was recycled in the system. The main input

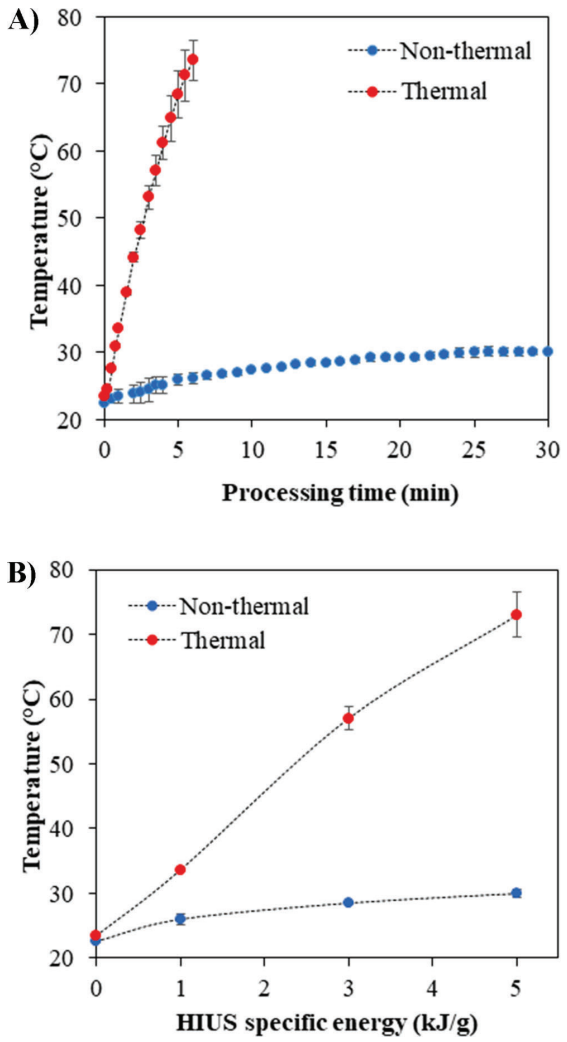

Fig. 3. Temperature profile of the system (genipap pulp + ethanol) in function of the: (A) processing time; (B) HIUS specific energy.

parameters for economic evaluation are presented in Table 2, and the flowsheets and Gantt charts are presented in Fig. 2. The main contributions on the COM, such as cost of raw material (CRM), cost of operational labor (COL), cost of utilities (CUT), and fixed capital investment (FCI) [14,30], were also evaluated. All the scenarios evaluated in this study have considered that the investors do not need bank financing to start the projects.

3. Results and discussion

3.1. HIUS temperature profile

Fig. 3 presents the temperature profiles of the system (genipap pulp + ethanol) during the HIUS-assisted extractions. The temperature increased during the processing time and, thus, we can compare the performance of each HIUS treatment to provide heat to the system. Thus, two different ways to provide the energy were performed: a non-thermal treatment, which uses a longer processing time and a lower nominal power, and a thermal treatment, which employs a shorter processing

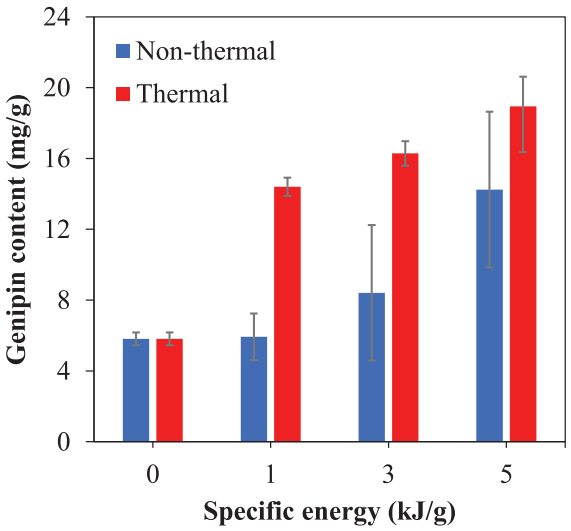

Fig. 4. Effects of HIUS treatment and specific energy on the genipin content. Specific energy was calculated from nominal power.

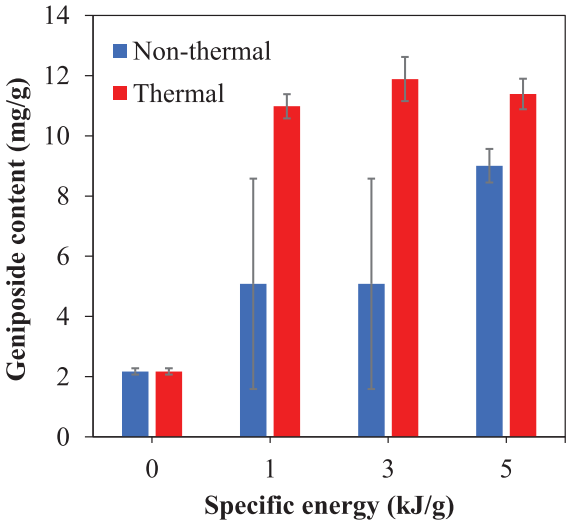

Fig. 5. Effects of HIUS treatment and specific energy on the geniposide content. Specific energy was calculated from nominal power.

time and a higher power. The HIUS specific energy for each process corresponding to one processing time calculated according to Eq. (1). Thus, for the non-thermal treatments the processing times were of 5, 15, and 25 min for the specific energies of 1, 3 and 5 kJ/g, respectively. For the thermal treatments the processing times were 1.11, 3.33, and 5.55 min for the specific energies of 1, 3 and 5 kJ/g respectively. The processes were limited to the nominal specific energy of 5 kJ/g due to the temperature increase in the thermal treatment until 73 °C (Fig. 3). Therefore, since the ethanol boiling point is 78.4 °C, the phase transition from liquid to vapor was avoided in these processes, preserving the amount of solvent mass in the system.

3.2. Genipin and geniposide content

The genipin and geniposide content in the genipap ethanolic samples were not reduced during their storage time until 72 h after their HIUS processing (complementary material). Figs. 4 and 5 present the contents of the phytochemical compounds in the ethanolic extracts obtained by non-thermal and thermal HIUS treatments. The higher genipin and geniposide recovery concerning the control sample were achieved due to acoustic cavitation provided by HIUS technology. The acoustic cavitation phenomenon has two action mechanisms: mechanical and thermal [31]. The mechanical action arises from the continuous compression and refraction of microbubbles in the ethanol. This promotes micro-jets that reach the plant material promoting its cell disruption and releasing of its phytochemical compounds into the liquid medium. At the same time, due to the abrupt microbubbles collapse, the temperature and pressure of the liquid medium are increased, promoting turbulence and accelerating, thus, the diffusion of the active compounds from the unripe genipap pulp to ethanol.

Increasing the HIUS specific energy, the genipin recovery was increased (p-value = 0.017), reaching up to 17 ± 3 mg/g for the thermal treatment applying 5 kJ/g (Fig. 4). The non-thermal treatment using 5 kJ/g recovered about 14 mg/g, the same value obtained from the thermal treatment applying 1 kJ/g. Thus, the best genipin recovery condition occurred by applying thermal treatments (p-value = 0.001). These results can be associated with the HIUS energy performance (Table 1) and, consequently, for the more intense acoustic cavitation, which promoted the temperature increase, improving the diffusion rates, leading to a greater release of the genipin from the plant material. Shirsath, Sable, Gaikwad, Sonawane, Saini and Gogate [32] also observed that the extraction yield of curcumin increased with an increase in the nominal ultrasound power. Their results were attributed to physical effects promoted by higher nominal power used in the ultrasound processing, which, according to them allowed more intense collapse of microbubbles. The HIUS process intensification by increasing nominal power enhanced the cell wall rupture improving the diffusion of the phytochemical compounds into the liquid medium. Likewise, genipin was not a thermolabile compound because increasing the liquid medium temperature (Fig. 3) favored its recovery, demonstrating the positive temperature effect on its diffusion. Zhu, Wu, Di, Li, Barba, Koubaa, Roohinejad, Xiong and He [33] observed the same behavior for chlorophyll recovery, which with the temperature increased, better yields were acquired using HIUS-assisted extraction.

For the geniposide content, as shown in Fig. 5, a different behavior was observed. In the thermal treatments, which were applied specific energies higher than 1 kJ/g, its recovery was kept constant on 11 mg/g. However, for the non-thermal treatments, increasing the specific energy the geniposide recovery was improved, reaching up to 8.8 ± 0.4 mg/g at the specific energy of 5 kJ/g. Despite this, only the treatment (non-thermal and thermal) had a significant effect on the geniposide recovery (p-value = 0.004), since the specific energy did not affect the extraction of this iridoid (p-value = 0.302). Geniposide may have been hydrolyzed in thermal treatments when specific energies greater than 1 kJ/g was applied. Temperature increase (Fig. 3) and high mechanical stress due to the acoustic cavitation intensification may be contributed to geniposide hydrolysis. However, the geniposide hydrolysis could release genipin, increasing its recovery under high specific energies [11]. For the geniposide recovery, Náthia-Neves, Tarone, Tosi, Júnior and Meireles [12] obtained 2.5 mg/g. However, this low recovery may be associated with the highest genipin content (46.5 mg/g) achieved for them. They used an extraction temperature of 50 °C. Thus the highest genipin content obtained by them could be due to geniposide hydrolysis. The geniposide recovery was not the focus of this study because it is a yellow colorant. However, this iridoid can be hydrolyzed to genipin and is a phytochemical compound with various biological activities, such as antiphlogistic, diuretic, anti-inflammatory, treating parenchyma injurie, treating ankle sprain, and anti-sepsis [12,34].

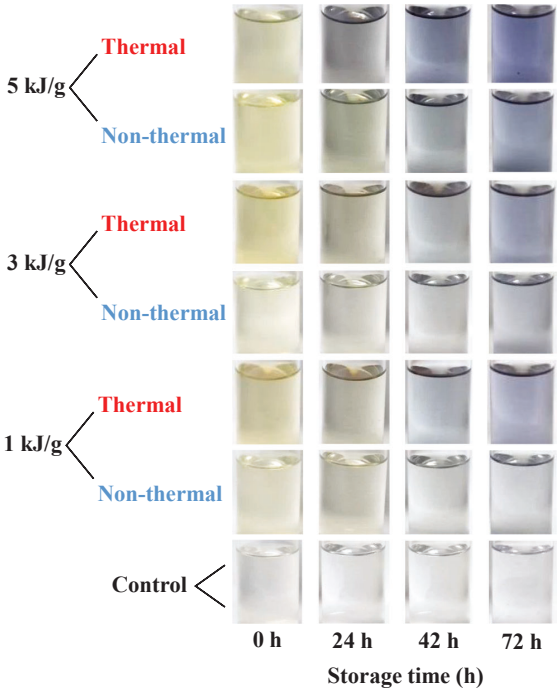

Fig. 6. Effect of the HIUS treatment and specific energy on the visual appearance of the extracts over the storage time. Specific energy was calculated from nominal power.

3.3. Physical and chemical stability of the unripe genipap ethanolic extracts

Since the genipin is the compound responsible for the blue color formation from its cross-linking with primary amine groups, a higher genipin content could not result in blue ethanolic samples. Genipin is a colorless molecule in a liquid medium. Blue color compounds are formed after its reaction with a primary amine group supply like amino acids and proteins. Neves, Strieder, Silva and Meireles [35] reported the simultaneous use of the colloidal milk system as a solvent, reaction medium, and carrier for the blue color compounds obtained from the cross-linking between genipin and milk proteins. Besides, the authors demonstrated that blue color formation is a slow process that can take up to 96 h to occur due to slow oxygen diffusion into the liquid medium over cold storage time. Therefore, the effects of the HIUS treatments (non-thermal and thermal) and specific energies on the visual appearance of the unripe genipap ethanolic extracts were evaluated during their storage time of 0, 24, 48 and 72 h (Fig. 6). Immediately after the HIUS treatments, at 0 h, yellow ethanolic samples were observed. This could be due to the geniposide content, which has this characteristic color. The yellowest extracts at 0 h also had the highest geniposide contents, which were obtained by thermal treatments (Fig. 5). During the storage time, the development of the blue color was observed in extracts due to the reaction between genipin and primary amine groups recovered from the unripe genipap pulp. Possibly a small protein content was extracted from the genipap pulp during HIUS processing. This reaction was catalyzed by oxygen from the air. After 48 h of the process, in different intensities, all ethanolic samples presented a blue color, but the bluest extracts were acquired after 72 h. The application of higher specific energies resulted in visually bluer ethanolic samples, which corroborates with the genipin content (Fig. 4). Thus, in this study, a consistent result between the genipin and geniposide content, and the

Table 3
Cost of manufacturing, extract productivity, and the main percentual contribution costs on the total cost of manufacturing.

Scenario	HIUS	Specific energy (kJ/g)	Genipin (kg/year)*	Geniposide (kg/year)*	COM (US$/g genipin + geniposide)	CRM (%)	COL (%)	FCI (%)	CUT (%)
Laboratory-scale / Genipap of US$ 7.89/kg									
1	–	0	8.64	3.23	4.59	69.80	29.55	0.43	0.22
2	Non-thermal	1	0.85	0.62	13.92	17.95	77.17	4.58	0.29
3	Non-thermal	3	0.46	0.28	24.42	7.20	87.26	5.18	0.35
4	Non-thermal	5	0.44	0.27	24.62	4.51	89.80	5.33	0.37
5	Thermal	1	7.05	5.50	2.35	42.46	53.66	3.18	0.70
6	Thermal	3	3.36	2.46	3.84	23.68	70.95	4.21	1.16
7	Thermal	5	2.31	1.49	5.37	16.42	77.64	4.61	1.34
Pilot-scale / Genipap of US$ 7.89/kg									
1	–	0	1016.39	379.61	2.21	98.28	1.54	0.05	0.13
2	Non-thermal	1	256.22	186.23	1.87	90.92	5.75	1.14	2.19
3	Non-thermal	3	138.82	84.21	1.54	77.77	13.86	2.74	5.63
4	Non-thermal	5	132.69	82.28	1.11	67.92	19.92	3.94	8.23
5	Thermal	1	2115.33	1649.67	0.71	95.47	1.78	0.44	2.31
6	Thermal	3	1009.05	736.95	0.70	88.75	3.91	0.97	6.37
7	Thermal	5	691.80	447.20	0.72	82.88	5.77	1.43	9.93
Pilot-scale / Genipap of US$ 1.42/kg									
1	–	0	1016.39	379.61	1.40	97.29	2.44	0.07	0.20
2	Non-thermal	1	256.22	186.23	1.24	86.28	8.69	1.72	3.31
3	Non-thermal	3	138.82	84.21	1.09	68.71	19.51	3.86	7.92
4	Non-thermal	5	132.69	82.28	0.80	59.01	27.56	5.45	7.98
5	Thermal	1	2115.33	1649.67	0.46	93.10	2.76	0.55	3.59
6	Thermal	3	1009.05	736.95	0.46	83.85	5.89	1.46	8.81
7	Thermal	5	691.80	447.20	0.50	75.25	8.34	2.06	14.35
Industrial-scale (without recycle) / Genipap of US$ 7.89/kg									
1	–	0	10163.86	3796.14	2.18	99.81	0.16	0.01	0.02
2	Non-thermal	1	2562.52	1862.48	1.76	96.70	0.61	0.36	2.33
3	Non-thermal	3	1388.00	842.00	1.32	90.85	1.62	0.96	6.57
4	Non-thermal	5	1327.09	822.91	0.88	85.64	2.51	1.49	10.36
5	Thermal	1	20860.62	16268.38	0.71	97.36	0.18	0.11	2.35
6	Thermal	3	10091.06	7369.94	0.67	92.68	0.41	0.24	6.67
7	Thermal	5	6919.84	4473.16	0.68	88.40	0.62	0.37	10.61
Scenario	HIUS	Specific energy (kJ/g)	Genipin (kg/year)*	Geniposide (kg/year)*	COM (US$/g genipin + geniposide)	CRM (%)	COL (%)	FCI (%)	CUT (%)
Industrial-scale / Genipap of US$ 1.42/kg									
1	–	0	10163.86	3796.14	1.37	99.70	0.25	0.02	0.03
2	Non-thermal	1	2562.52	1862.48	1.12	94.84	0.96	0.57	3.64
3	Non-thermal	3	1388.00	842.00	0.87	86.18	2.45	1.45	9.92
4	Non-thermal	5	1327.09	822.91	0.60	78.92	3.69	2.19	15.21
5	Thermal	1	20860.62	16268.38	0.46	95.66	0.28	0.17	3.69
6	Thermal	3	10091.06	7369.94	0.44	88.83	0.62	0.37	10.18
7	Thermal	5	6919.84	4473.16	0.46	82.72	0.92	0.54	15.82

COM: cost of manufacturing; CRM: cost of raw material; COL: cost of operational labor; FCI: fixed capital investment; CUT: cost of utilities.
* The purification was not considered in the economic evaluation.

color of the genipap ethanolic samples was verified. However the genipin content in our ethanolic samples was not reduced during the reaction time of 72 h (p-value > 0.05). Neves, Strieder, Silva and Meireles [35], Strieder, Neves, Silva and Meireles [36] observed a different behavior for milk samples after 24 h of the HIUS-assisted treatments, a high reduction of genipin content was verified. This difference could be associated with the primary amine group contents in our ethanolic samples, which are limited to the genipap proteins extracted. Thus, an insignificant amount of genipin in our ethanolic samples reacted to form the blue color. The blue color intensity differences can be observed from the visual appearance of the unripe genipap extracts. Thus, our genipin-rich ethanolic extracts cannot be considered blue colorants, because genipin needs to react with primary amine groups to produce the blue colorant compounds.

3.4. Economic evaluation

The economic evaluation for obtaining a blue ethanolic extract from

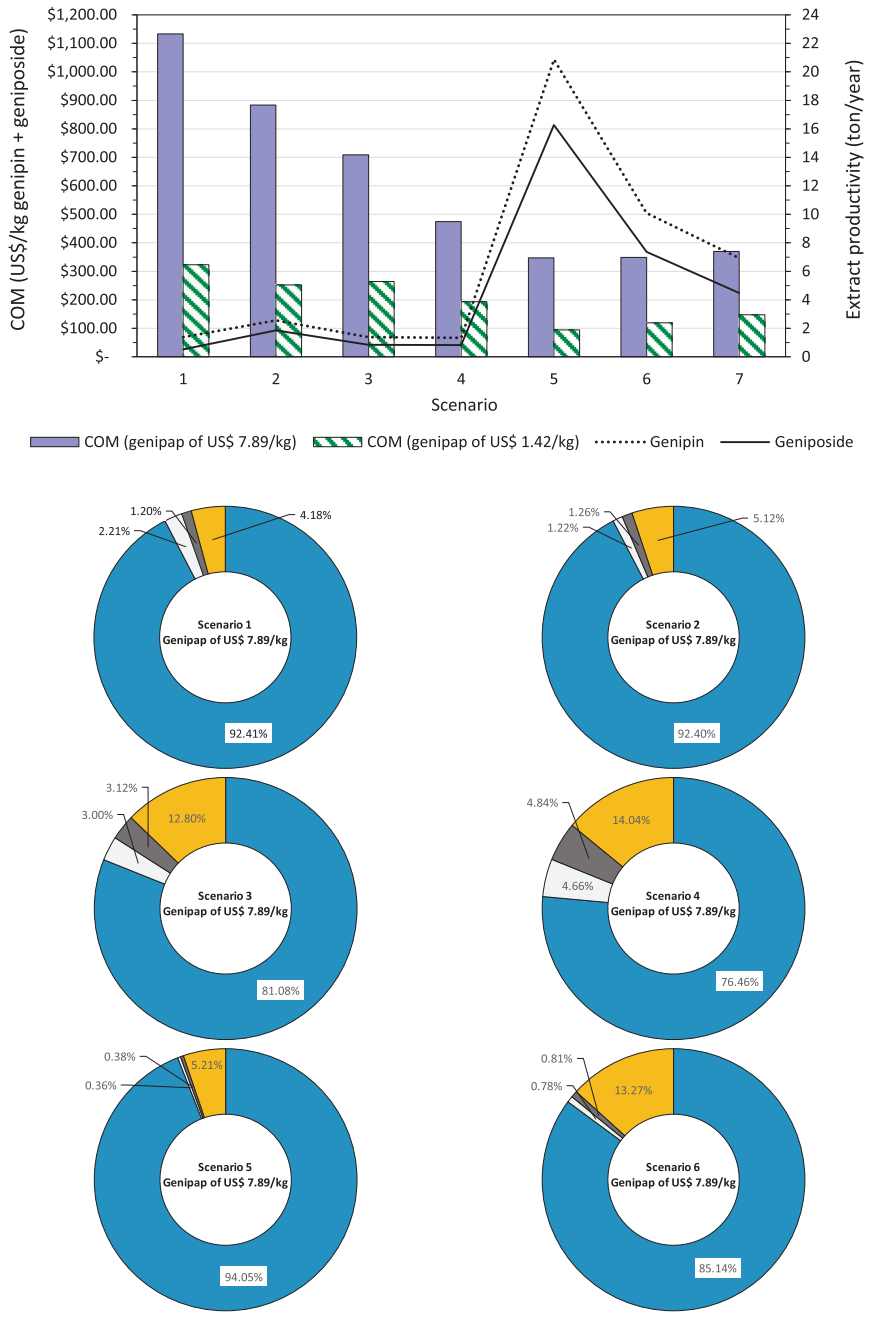

Fig. 7. Cost of manufacturing, extract productivity, and the main percentual contribution costs on the total cost of manufacturing of genipap pulp at industrial-scale with ethanol recycle.

unripe genipap pulp by HIUS technology was done for different scenarios considering three scales, two acquisition costs of genipap pulp, three specific energies (including a control), and two nominal ultrasound powers (Table 3). The scenarios for the laboratory-scale presented the highest values of COM. This behavior was expected because the COL contributes by a large extent (>53%) on the COM when low amounts of vegetal materials are processed. Otherwise, for the HIUS technology at pilot- and industrial-scales, the COM was lower than US$ 1.87/g genipin + geniposide, and the COL was lower than 28%. The COM decreases with increasing the scale because some services can be optimized, and the productivity is higher, which has been reported elsewhere [14,24]. Indeed, when comparing the pilot- and industrial-scales, the reduction is not too pronounced as that on see after comparing the laboratory- and pilot-scales. For example, taking into account the scenario 7, the COM

Fig. 7. (*continued*).

reduced approximately 8.7% (from US$ 0.50/g to US$ 0.46/g) when the pilot- and industrial-scales are compared. This behavior is attributed to a low contribution of FCI (<5.5%) and a high contribution of CRM (>59%).

Some variations on the COM were observed for the seven tested scenarios. First of all, the scenario 1 indicates the control test (without ultrasound treatment) and the largest values of COM were achieved for the pilot- and industrial-scales. Although the facility is simpler and the energy spent in the process is lower, low genipin and geniposide yields were reached (Figs. 4 and 5), thus resulting in intermediary productivities and high values of CRM as a consequence of high volumes of ethanol and genipap pulp used in the several batches. As the other scenarios have attracted the interest in the extracts' quality viewpoint, the discussion is highlighted for these scenarios. Consequently, according to the economic evaluation, the best scenarios are related to thermal processing by HIUS. In those scenarios, values of COM lower than US$ 0.72/g are presented. The responses are associated with the extract yields obtained in the experimental assays, while the productivities were the largest ones for these scenarios. According to the results (Table 3) for the largest scales, the itemized cost of utmost importance on COM is the CRM and the COM can be reduced based on some main possibilities: i) reducing the acquisition cost of genipap pulp; ii) reducing the cost of ethanol; iii) recycling ethanol. Based on these perspectives, further economic evaluations were done to reuse ethanol (possibility iii) and to

reduce the CRM. This strategy was adopted (Fig. 2B) and the results are presented in Fig. 7. It is important to point out that the vacuum evaporation was defined for 10 min. It could be fulfilled concomitantly with HIUS extraction, that is, while a sample is processed by HIUS, the previous samples can be concentrated in the evaporator, thus optimizing the operation time. Therefore, this is the reason why the productivities are the same for the industrial-scale with or without solvent recycle.

Solvent recycles demonstrated to be a positive strategy. Even though the initial investment would be higher due to the evaporator cost, the reuse of ethanol is justified. In scenario 5, the COM was US$ 94.62/kg genipin + geniposide, which indicated a 4.9-fold reduction if compared to the scenario without solvent recycle. These findings are in agreement with a study developed with genipap pulp by using integrated processes: pressing + low-pressure extraction [1414]. However, our study presents a promising technology that is approximately two times cheaper, the extraction is faster, and the process can concentrate the extracts by vacuum evaporating the ethanol. This inference is corroborated by the percentual contribution of FCI, which was the lowest one in most of the scenarios, ranging from 0.38% to 4.84% and from 1.39% to 11.85% in the case of purchasing genipap pulp of US$ 7.89/kg and US$ 1.42/kg, respectively. In the industrial-scale with recycle, the main contributions on the COM are the CRM and CUT, especially the electricity when the specific energy of 5 kJ/g is applied in the thermal processing.

4. Conclusion

Our results have demonstrated that the HIUS-assisted extraction allowed obtaining genipin-rich ethanolic, a promising phytochemical precursor of the natural blue color compounds. However, our extract cannot be considered a colorant, because genipin needs to react with primary amine groups to produce the blue colorant compounds. The temperature increase provided by the more intense acoustic cavitation in thermal treatments favored the diffusion phenomena, and thus a higher genipin recovery was obtained. Furthermore, the heat applied by these treatments may have favored the geniposide hydrolysis. The economic evaluation showed different scenarios to produce genipin-rich ethanolic extracts by using HIUS technology. HIUS treatments presented a faster and fixed capital investment two times cheaper than a process using pressing + low-pressure extraction. Besides that, our process could include the concentration of the extracts by vacuum, providing a more concentrated product. Therefore, our findings contributed to the development of a new and more economically feasible process for obtaining genipin-rich ethanolic extracts from unripe genipap pulp based on HIUS technology.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2020.117978.

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CHAPTER 5

Impact of acoustic energy on the production of natural blue colorant using genipin and dairy proteins

Low-frequency and high-power ultrasound-assisted production of natural blue colorant from the milk and unripe *Genipa americana* L.

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ABSTRACT

This study presents the production of a novel natural blue colorant obtained from the cross-linking between milk proteins and genipin assisted by low-frequency and high-power ultrasound technology. Genipin was extracted from unripe *Genipa americana* L. using milk as a solvent. Also, milk colloidal system was used as a reaction medium and carrier for the blue color compounds. The effects of ultrasound nominal power (100, 200, 300, and 400 W) on the blue color formation kinetics in milk samples were evaluated at 2, 24, and 48 h of cold storage in relation to their free-genipin content and color parameters. In addition, Fourier transform infrared (FTIR) spectrum, droplet size distribution, microstructure, and kinetic stability of the blue colorant-loaded milk samples were assessed. Our results have demonstrated that the ultrasound technology was a promising and efficient technique to obtain blue colorant-loaded milk samples. One-step acoustic cavitation assisted the genipin extraction and its diffusion into the milk colloidal system favoring its cross-linking with milk proteins. Ultrasound process intensification by increasing the nominal power promoted higher genipin recovery resulting in bluer milk samples. However, the application of high temperatures associated with intensified acoustic cavitation processing favored the occurrence of non-enzymatic browning due to the formation of complex melanin substances from the Maillard reaction. Also, the blue milk samples were chemically stable since their functional groups were not modified after ultrasound processing. Likewise, all blue colorant-loaded milk samples were kinetically stable during their cold storage. Therefore, a novel natural blue colorant with high-potential application in food products like ice creams, dairy beverages, bakery products, and candies was produced.

1. Introduction

The global demand for natural food products has increased in the last years. The new food trends are pointing to the development of functional products with health-promoting properties and high standard sensory attributes. In this sense, the worldwide colorant market has been pressed to replace the synthetic products by natural colorants [1]. Plant matrices are the main source of natural colorants and can provide colorant precursor phytochemicals [2]. Different colors and pigments may be obtained from these matrices and their phytochemical compounds. In addition, many of them present health benefits such as antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties [3]. Among all colors, blue is a high-desirable due to sensory appeal for the formulation of several products, however, natural sources of blue compounds and their precursors still are scarce. Thus, the current challenge for the food industry is to produce blue food like ice creams, dairy beverages, candies, sauces, cakes, and other products formulated from natural blue colorants [4].

Unripe genipap (*Genipa americana* L.), a native Brazilian fruit, has emerged as a promising blue color precursor due to its high genipin content [5]. Genipin reacts with primary amine groups producing blue color compounds [6]. This reaction is catalyzed by oxygen and heat [7]. Genipap extracts rich in genipin have been obtained using solvents such as methanol 80% (v/v) in water [8], ethanol and water [5]. However, the use of alternative solvents, rich in primary amine groups such as milk, could be an innovative way to simultaneously extract genipin and promote its cross-linking with milk proteins producing a novel natural blue colorant. Milk is an important source of protein and primary amine groups, therefore, a promising reaction medium for the blue color formation. Also, it is widely consumed worldwide and used for the formulation of ingredients and food products [9]. Thus, the obtaining of blue colorant-loaded milk could provide a new ingredient for the formulation of blue foods.

The recovery of genipin from genipap pulp using milk as a solvent could be performed using several extraction techniques based on high-energy technologies. An ideal extraction technique should favor the

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genipin extraction from the plant material, its diffusion into milk, and reaction with milk proteins to produce the blue color compounds. In this sense, low-frequency and high-power ultrasound technology could provide efficient tools to assist the extraction, diffusion, and reaction steps. Ultrasound processing is based on the acoustic cavitation phenomenon which promotes mechanical and thermal effects in the sonicated liquid medium [10]. Intense shear rates are associated with acoustic cavitation because it promotes the formation and subsequent collapse of microbubbles associated with extreme levels of highly localized turbulence that act as an effective homogenization technique with temperature increase [11]. Also, ultrasound technology allows the development of efficient processes with reduced operation time, energy consumption, and environmental impact [12]. Likewise, this emerging technology has been evaluated as a promising homogenization technique to produce stable dairy systems. O'Sullivan, Arellano, Pichot and Norton [13] observed that the ultrasound treatment (20 kHz, 30 W/cm^2, and 2 min) reduced the size and hydrodynamic volume of dairy proteins. According to them, the acoustic cavitation generated a decrease in the interfacial tension, which assisted the droplet break-up during emulsification. Shanmugam and Ashokkumar [14] producing a stable flax seed oil emulsion from dairy systems observed similar behavior. Applying an ultrasound treatment at 20 kHz, 176 W, and 3 min smaller sized emulsion droplets were acquired. Thus, besides to assist the genipin extraction and diffusion, and its reaction with milk proteins, ultrasound technology can promote obtaining a kinetically stable dairy emulsion able to carry the blue color compounds.

In this context, the aim of this study was to evaluate the effect of ultrasound nominal power on the production of a novel natural blue colorant obtained from the cross-linking between milk proteins and genipin extracted from unripe genipap using milk as a solvent, reaction medium, and carrier for the blue color compounds.

2. Material and methods

2.1. Raw material and sample preparation

Unripe genipap fruits were collected in "Fazenda Lagoa" (Ponte Alta do Tocantins, TO, Brazil) and were stored at −24 °C. For the experimental assays, the fruits were previously thawed, peeled, and grounded in a 400 W mechanical RI1364/07 processor (Philips Do Brasil LTDA, Varginha, MG, Brazil). The gridding procedure was carried out in batch for 5 min, using 50 g of genipap pulp. Whole milk powder was obtained from a local market in Campinas. Its fat and moisture content were 27 g/100 g and 3 g/100 g, respectively. Whole milk was reconstituted using the ratio of 10 g of milk powder to 90 g of ultrapure water.

2.2. Ultrasound processing

Fig. 1 presents the flow diagram of the processes used for obtaining blue colorant-loaded milk. Blue milk was obtained by using the ratio of 5 g of grounded genipap pulp to 25 g of reconstituted milk. Before ultrasound processing, all samples were pre-homogenized using a vortex system for 1 min. The sample obtained in this step was used as a control and named "0 W". After that, ultrasound-assisted processes were performed using a 13-mm ultrasound probe diameter at 19 kHz (Unique, Indaiatuba, SP, Brazil). The probe contact height with the liquid medium was standardized to 15 mm. The nominal powers of 100, 200, 300, and 400 W were applied to the samples for 6 min. The maximum process temperature used was 90 °C due to the milk boiling point (95 °C) [15]. After ultrasound processing, the milk was separated from the genipap pulp by filtration using a nylon filter. All experiments were performed in duplicate. Calorimetric assays were used to determine the acoustic power (Eq. (1)) supplied to the samples according to the methodology described by Mason, Lorimer, Bates and Zhao [16]. From the acoustic power, ultrasound specific energy and intensity were calculated according to Eq. (2) and (3) [17].

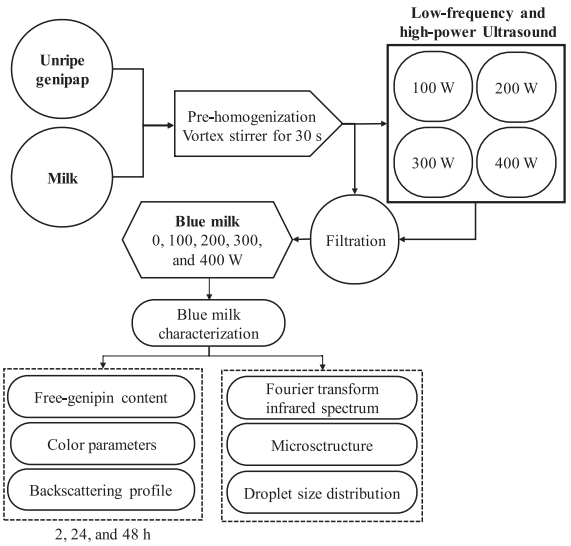

Fig. 1. Flow diagram of the processes used for obtaining blue colorant-loaded milk.

$$Acoustic\ power(W) = mC_P\left(\frac{dT}{dt}\right) \quad (1)$$

$$Specific\ energy\left(\frac{kJ}{g}\right) = \frac{Acoustic\ power \times processing\ time}{mass} \quad (2)$$

$$Ultrasound\ intensity\left(\frac{W}{cm^2}\right) = \frac{4 \times Acoustic\ power}{\pi D^2} \quad (3)$$

where, m is the sample mass (g); C_P is the specific heat (J/g °C), $\left(\frac{dT}{dt}\right)$ is the temperature rate (°C/s), and D is the probe diameter (cm). Table 1 presents the acoustic power, specific energy, and ultrasound intensity determined from each nominal power applied to the milk samples.

2.3. Blue color formation kinetics

The effects of the ultrasound nominal power on the blue color formation kinetics in the milk samples were analyzed regarding to their free-genipin content and color parameters. The measurements were made 2 h after ultrasound processing and afterward for more two days (24 and 48 h). The maximum storage time was 48 h due to the milk shelf life. The samples were kept at 7 ± 2 °C during their storage.

2.3.1. Free-genipin content

The free-genipin content was quantified in the samples using high-performance liquid chromatography (HPLC) on a 2695D separation module (Waters Alliance, Milford, Connecticut, USA) equipped with a 2998 diode array detector. An aliquot of 0.5 mL of each sample was

Table 1
Acoustic power, specific energy, and ultrasound intensity for each nominal power applied to the processing of milk samples.

Nominal power (W)	Acoustic power (W)	Specific energy (kJ/g)	Ultrasound intensity (W/cm^2)
100	4.6 ± 0.4	0.06 ± 0.01	3.5 ± 0.3
200	8.5 ± 0.1	0.10 ± 0.01	6.4 ± 0.1
300	14.5 ± 0.3	0.17 ± 0.01	10.9 ± 0.2
400	20 ± 1	0.25 ± 0.01	15 ± 1

*Mean values ± standard deviation (n = 2).

dissolved in 1 mL of ethanol manually homogenized. Then, the mixture was centrifuged at 12000 rpm for 15 min. Just after, the supernatant was filtered using nylon membrane (0.45 μm) and injected into the chromatograph. The free-genipin determination was made according to the methodology described by Náthia-Neves, Nogueira, Vardanega and Meireles [18]. The compounds were separated on a Kinetex C18 column (150 × 4.6 mm id, 2.6 μm, Phenomenex, Torrance, USA) at 35 °C using a flow rate of 1.5 mL/min. The mobile phase consisted of 0.1% water (v/v) in formic acid (solvent A) and 0.1% acetonitrile (v/v) in formic acid (solvent B). The following gradient was used: 0 min (99% A); 9 min (75% A); 10 min (99% A); and 13 min (99% A). The genipin was detected at 240 nm and quantified using Empower 2 software (Waters, Milford, MA, USA). The identification was done by comparing its retention time and UV–vis spectra to its reference standard (Sigma Aldrich, St. Louis, USA). Free-genipin content results were determined in relation to the weight of dried genipap and were expressed according to the Eq. (4).

$$\text{Free} - \text{genipin content(mg/g)} = \frac{\text{Genipin mass(mg)}}{\text{Dried genipap mass (g)}} \quad (4)$$

2.3.2. Color parameters

Color parameters of the samples were measured at room temperature in an Ultra Scan Vis 1043 (Hunter Associates Laboratory, Reston, VA, USA) with CIE (Commission Internationale de l'éclairage) coordinates L, a*, and b*. The color parameters were expressed in terms of lightness L (L = 0 for black and L = 100 for white) and chromaticity parameters a^* (green [−] to red [+]) and b^* (blue [−] to yellow [+]).

2.4. Chemical stability

The functional groups were identified using a 4100 Fourier transform infrared (FTIR) spectrometer (Jasco, Tokyo, Kantō, Japan). For this, samples acquired after 48 h of cold storage at 7 ± 2 °C were previously freeze-dried in a L101 freeze dryer (Liobras, São Carlos, SP, Brazil). The measurements were taken at room temperature and recorded in the 400–4000 cm^{-1} region.

2.5. Size distribution and kinetic stability

2.5.1. Droplet size distribution

Droplet size distribution and mean diameter of the samples were determined at 25 °C using a Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, UK). The mean diameter (D_{32}) of droplets was calculated based on the mean diameter of a sphere of a similar area (Sauter diameter), according to the Eq. (5). The polydispersity degree of droplet size was determined as the Span (Eq. (6)). The milk samples were analyzed by a wet method, with dispersion in water (refractive index of 1.52).

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (5)$$

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \quad (6)$$

where, d_i is the mean diameter of the droplets, and n_i is the number of droplets.

2.5.2. Microstructure

The samples were poured onto microscope slides, covered with glass coverslips and observed using a Carl Zeiss Model Axio Scope A1 optical microscope (Zeiss, Gottingen, Germany). A 100× objective lens and immersion oil were used to perform the measurements.

2.5.3. Phase separation kinetics

The kinetic stability of the ultrasound-treated samples was evaluated using a near infrared backscattering technique. The samples were transferred to specific glass tubes to measure the kinetic stability using a light backscatter scan analyzer Turbiscan LAB Expert (Formulaction®, Toulouse, Haute-Garonne, France). The measurements were performed after ultrasound processing (2 h) and after for more two days (24 and 48 h). After the first measurement, the samples were stored at 7 ± 2 °C until the next measurement.

2.6. Statistical analysis

The influence of the ultrasound nominal power on the formation of blue color compounds and other physical properties was verified by analysis of variance (ANOVA) using the Minitab 18® software (Minitab Inc., State College, PA, USA). Tukey's test of means was performed at a 95% confidence level (p-value ≤0.05).

3. Results and discussion

3.1. Ultrasound-assisted formation of blue color compounds

The blue color compounds were obtained from the cross-linking between milk proteins and genipin. Low-frequency and high-power ultrasound had a fundamental role in the obtaining of blue colorant-loaded milk since genipin was extracted from unripe *Genipa americana* L. using milk as a solvent and simultaneously the reaction of blue color compounds formation was started. Thus, milk colloidal system was used as a reaction medium and carrier for the blue color compounds.

Acoustic cavitation provided by ultrasound treatment promoted the genipin extraction from the plant material besides its diffusion into the milk colloidal system to react with milk proteins whereas homogenized the system. The effects of this phenomenon comprise localized heat, high pressure, and high shear rates. The acoustic cavitation effects such as temperature increase and turbulence favored the genipin recovery and diffusion and its reaction rate with milk proteins. Fig. 2 shows the nominal power effects on the temperature profiles of the unripe genipap and milk subjected to ultrasound processing. After 6 min, the system reached the maximum temperatures of 28 ± 1, 43 ± 2, 63 ± 1, and 84 ± 1 °C for the nominal powers of 100, 200, 300, and 400 W, respectively. The low frequency waves (16–100 kHz) and high powers (> 1 W/cm^2) applied by ultrasound into the liquid media promoted the phenomenon of acoustic cavitation [19]. The ultrasound process intensification by increasing nominal power intensified the acoustic cavitation effects in the liquid medium. Thus, the increase of temperature

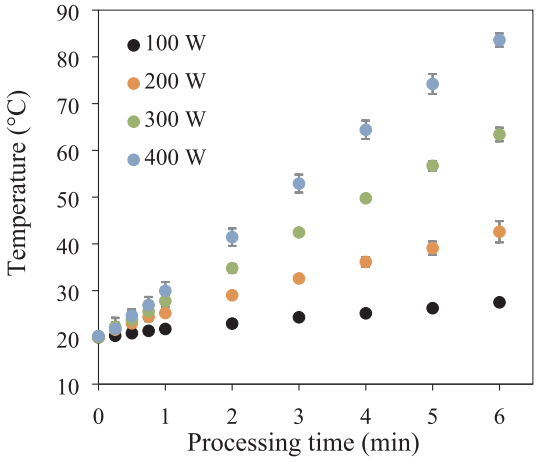

Fig. 2. Effects of ultrasound nominal power on the temperature profiles of the unripe genipap and milk system.

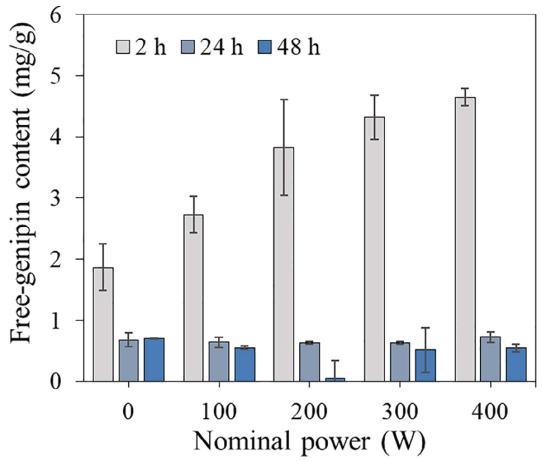

Fig. 3. Effects of ultrasound nominal power on the free-genipin content of the milk samples during their cold storage at 7 ± 2 °C.

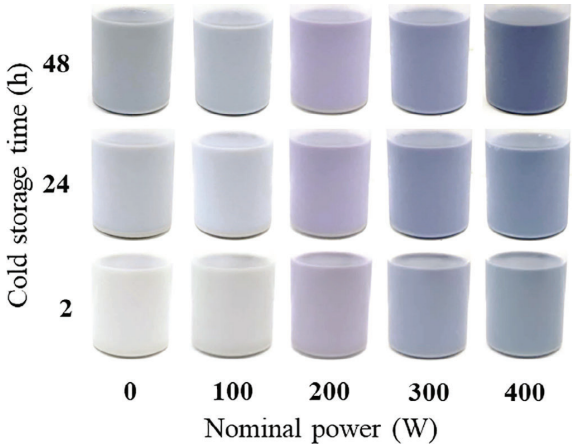

Fig. 4. Effects of ultrasound nominal power on the blue color formation kinetics of the milk samples during their cold storage at 7 ± 2 °C.

from the 20 °C is associated with the acoustic cavitation thermal effects in the liquid medium. Kang, Zou, Cheng, Xing, Zhou and Zhang [20] also observed the acoustic cavitation intensification in their system with meat protein by increasing the nominal power from 150 to 300 W. The authors reported an increase of biochemical reaction rate on their samples by intensifying acoustic cavitation.

Fig. 3 presents the effects of nominal power on the free-genipin content of the samples during their cold storage at 7 ± 2 °C. Free-genipin content expresses the amount of non-crosslinked genipin molecules with the milk proteins. Since cross-linking between them is catalyzed by oxygen and heat, right after genipin extraction from plant material, the reaction of blue color compounds formation starts. Thus, the total genipin recovered could not be quantified. After 2 h, the free-genipin content results demonstrated that the increase of nominal power applied to the ultrasound extraction step increased the genipin content recovered from unripe genipap pulp. Free-genipin content highly decreased after 24 h of cold storage in comparison to 2 h evidencing its reaction with milk proteins (p-value = 0.016). The blue milk samples presented about 20% of free-genipin in relation to the initial content after 24 h of cold storage (Fig. 3). Thus, the cross-linking between free-genipin and milk proteins kept occurring during the cold

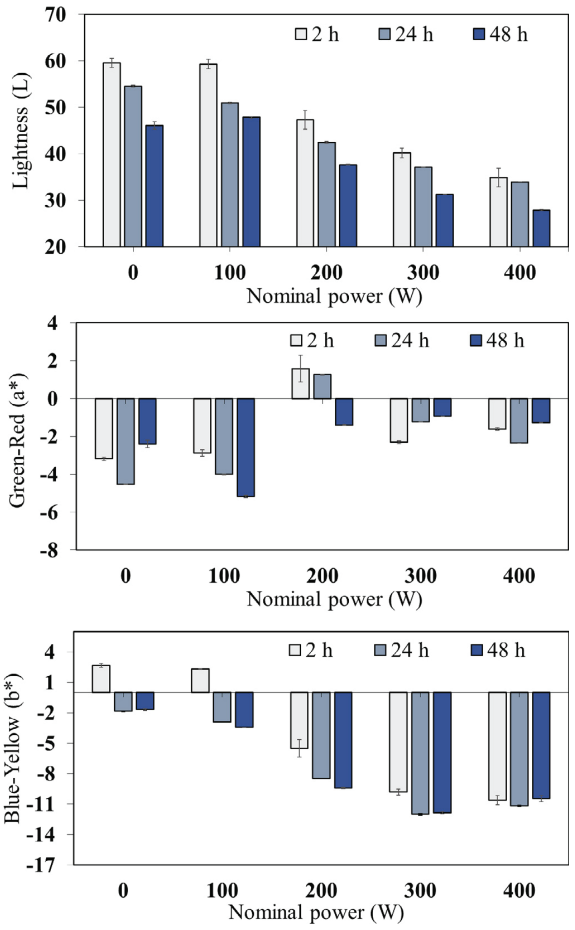

Fig. 5. Effects of ultrasound nominal power on the color parameters (L, a*, and b*) of the milk samples during their cold storage at 7 ± 2 °C.

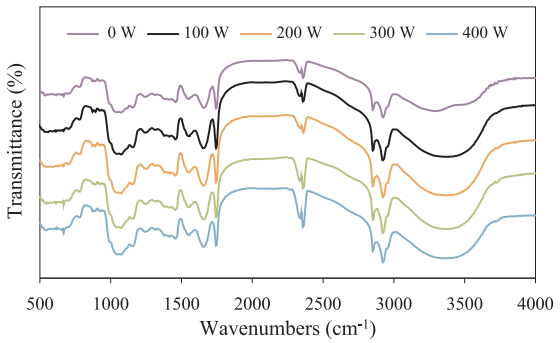

Fig. 6. Effects of ultrasound nominal power on the FTIR spectra of the blue milk samples.

storage decreasing its content in the blue milk samples. Since the reaction is catalyzed by oxygen, the formation of blue color compounds depended on oxygen diffusion into the liquid medium. This could explain the required time for blue color formation in the milk samples as shown in Fig. 4. According to Butler, Ng and Pudney [21], the oxygen induces the genipin polymerization, promoting thus the development of blue color compounds. Likewise, temperature is a critical factor for the

Table 2
Effects of ultrasound nominal power on the mean diameter (D_{32}), cumulative diameters (d_{10}, d_{50}, d_{90}), and span values of the blue milk samples.

Nominal power (W)	D_{32}(μm)	d_{10}(μm)	d_{50}(μm)	d_{90}(μm)	Span
0	1.1 ± 0.1 [a]	0.37 ± 0.01 [a]	0.49 ± 0.01 [a]	0.79 ± 0.02 [a]	0.86 ± 0.02 [a]
100	0.92 ± 0.01[b]	0.35 ± 0.01 [a,b]	0.46 ± 0.01 [a,b]	0.75 ± 0.01[a,,b,c]	0.86 ± 0.01 [a]
200	0.80 ± 0.01[c]	0.37 ± 0.01 [a]	0.49 ± 0.01 [a]	0.76 ± 0.01[b]	0.80 ± 0.01[b]
300	0.77 ± 0.02[c]	0.35 ± 0.01 [a]	0.47 ± 0.02 [a]	0.74 ± 0.04[a,b]	0.81 ± 0.02[b]
400	0.68 ± 0.03 [d]	0.33 ± 0.03[b]	0.45 ± 0.03[b]	0.7 ± 0.1[c]	0.81 ± 0.02[b]

*Mean values ± standard deviation (n = 4). Values followed by different letters in the same column show differences by Tukey's test at 95% significance (p-value ≤ 0.05).

formation of blue color compounds from the reaction between genipin and primary amine groups. Renhe, Stringheta, Silva and Oliveira [7] evaluated the effects of temperature (35, 45, 55, 65 e 75 °C) on the formation of blue color compounds from genipin-rich extracts obtained from *Genipa americana* L. using water and ethanol as solvent. The source of primary amines for the cross-linking with genipin was the genipap's own proteins extracted simultaneously with genipin. For water and ethanol, the best temperature conditions studied for them were of 75 and 55 °C, respectively. Although the reaction between genipin and milk protein also is catalyzed by heat, the milk samples were stored under refrigeration conditions due to the short milk shelf life. Thus, the oxygen diffusion into the liquid medium was delayed resulting in the formation of an intense blue color after a long storage period.

Fig. 4 presents the effects of the ultrasound nominal power on the blue color formation kinetics in the samples concerning their visual aspect. The bluer milk samples were obtained by applying 300 W and 400 W. Visual color differences could be explained by processing conditions that affect the reaction rate between free-genipin and milk proteins. The blue milk samples produced with 0 W and 100 W presented lower free-genipin contents 2 h after ultrasound processing (p-value = 0.025) (Fig. 3). Thus, these samples had less free-genipin to react with milk proteins and form the blue color compounds. Therefore, they exhibited a less intense blue color. The color parameters of the blue colorant-loaded milk samples corroborate with the effects associated with the ultrasound nominal power on the blue color formation (Fig. 5). The ultrasound process intensification by increasing the nominal power as well as cold storage time reduced the lightness values of the samples. The lightness reduction was associated with the formation of the blue color compounds because the darkest milk samples were those with the highest free-genipin content at 2 h of cold storage (Fig. 3). On the other hand, the higher temperature achieved for the nominal power of 400 W (84 ± 1 °C) could favor the occurrence of non-enzymatic browning associated with complex melanin substances due to the Maillard reaction. Different behavior was reported for the chocolate milk beverage stabilized with ultrasound treatment as described by Monteiro, Silva, Alvarenga, Moraes, Freitas, Silva, Raices, Sant'Ana, Meireles and Cruz [22]. In their system, the acoustic cavitation promoted the fat globules rupture, thus, the small globules, together with the colloidal casein particles and the calcium phosphate were responsible for higher L values. A more reddish milk sample (positive a* value) was obtained applying 200 W at 2 h of cold storage while the other treatments were greener (negative a* values). This behavior could be associated with the different temperatures observed for each ultrasound nominal power. Intermediate colors of the blue like purple could be formed due to different pH and temperature conditions. However, all blue milk samples presented similar pH values of 6.04 ± 0.02, 6.07 ± 0.04, 6.1 ± 0.1, 6.07 ± 0.04, and 6.06 ± 0.04 for 0 W, 100 W, 200 W, 300 W, and 400 W, respectively (p-value = 0.93). Regarding the blue color (negative b* values), the best result was obtained using 300 W (b* = −12.0 ± 0.1). Also, for this nominal power was not observed changes in the blue color intensity from 24 to 48 h of cold storage. However, for the less intense ultrasound treatments (100 W and 200 W), the reaction between genipin and milk proteins occurred until 48 h, which suggests that more intense acoustic cavitation increases the reaction rate even after ultrasound treatment and under refrigeration storage conditions.

3.2. Chemical stability after ultrasound processing

The effects of the nominal power on the chemical stability of the blue milk samples was evaluated by their FTIR spectra (Fig. 6). Functional groups associated with the milk structure were observed: water (3700–3000, 2400–2260 and 1700–1600 cm^{-1}), fatty acid chain (2924 cm^{-1}), proteins (at 1660 cm^{-1} and 1556 cm^{-1} for amide I and II, respectively) and lactose (at 1161 cm^{-1} and 1078 cm^{-1}) [23]. Similar spectra were observed for all blue colorant-loaded milk samples. Thus, ultrasound processing did not change the functional groups of the samples. However, an ultrasound treatment using a 13-mm probe at 20 kHz and 0.58 W/cm^2 promoted modifications on the N–H bonding of casein concentrate samples [24]. According to the authors, the acoustic cavitation favored hydrogen bonds through carbonyl groups of protein molecules, reducing the wave number corresponding to the peak for amide-I. This may promote modifications in the proteins surface hydrophobicity resulting in higher elasticity in gels. The ultrasound treatment applied to the casein concentrates samples was less intense than the applied to the blue milk samples (Table 1). Also, they used a smaller ratio between the solvent and casein concentrate mass. Thus, the acoustic cavitation effects observed by them could be associated with this greater proportion of the sample in relation to the solvent used. Xie, Wang, Wang, Wu, Liang, Ye, Cai, Ma and Geng [25] evaluated the effects of ultrasound treatment on the functional properties and assemblage structure of egg yolk. They used a higher ratio between the solvent and egg yolk and also no observed significant differences between the FTIR spectra of the samples untreated and the ultrasound-treated sample with a 6-mm diameter probe at the acoustic power of 4.57 ± 0.13 W.

3.3. Size distribution and kinetic stability

Table 2 presents the effects of ultrasound nominal power on the mean diameter (D_{32}), cumulative diameters (d_{10}, d_{50}, d_{90}), and span values of the blue milk samples. The ultrasound process intensification by increasing the nominal power linearly reduced the D_{32} values (p-value = 0.003). The d_{10}, d_{50}, and d_{90} values also were reduced with ultrasound treatment (p-value = 0.002). Salve, Pegu and Arya [26] observed similar results for the ultrasound-treated peanut milk samples. The authors evaluated the effects of nominal power levels of 200, 300, and 400 W on the size distribution reduction of the peanut milk by comparing its d_{50} values after ultrasound treatment. They reported a size reduction from 0.22 ± 0.03 μm to 0.02 ± 0.00 μm by intensifying ultrasound process from 200 to 400 W, respectively.

Fig. 7 shows the effects of ultrasound nominal power on the droplet size distribution and microstructure of the blue milk samples. The size distribution corroborated with optical micrographs for the milk fat globules. The untreated sample (0 W) presented visually bigger fat globules than the ultrasound processed blue milk samples. The milk sample treated with 100 W presented a droplet size distribution and

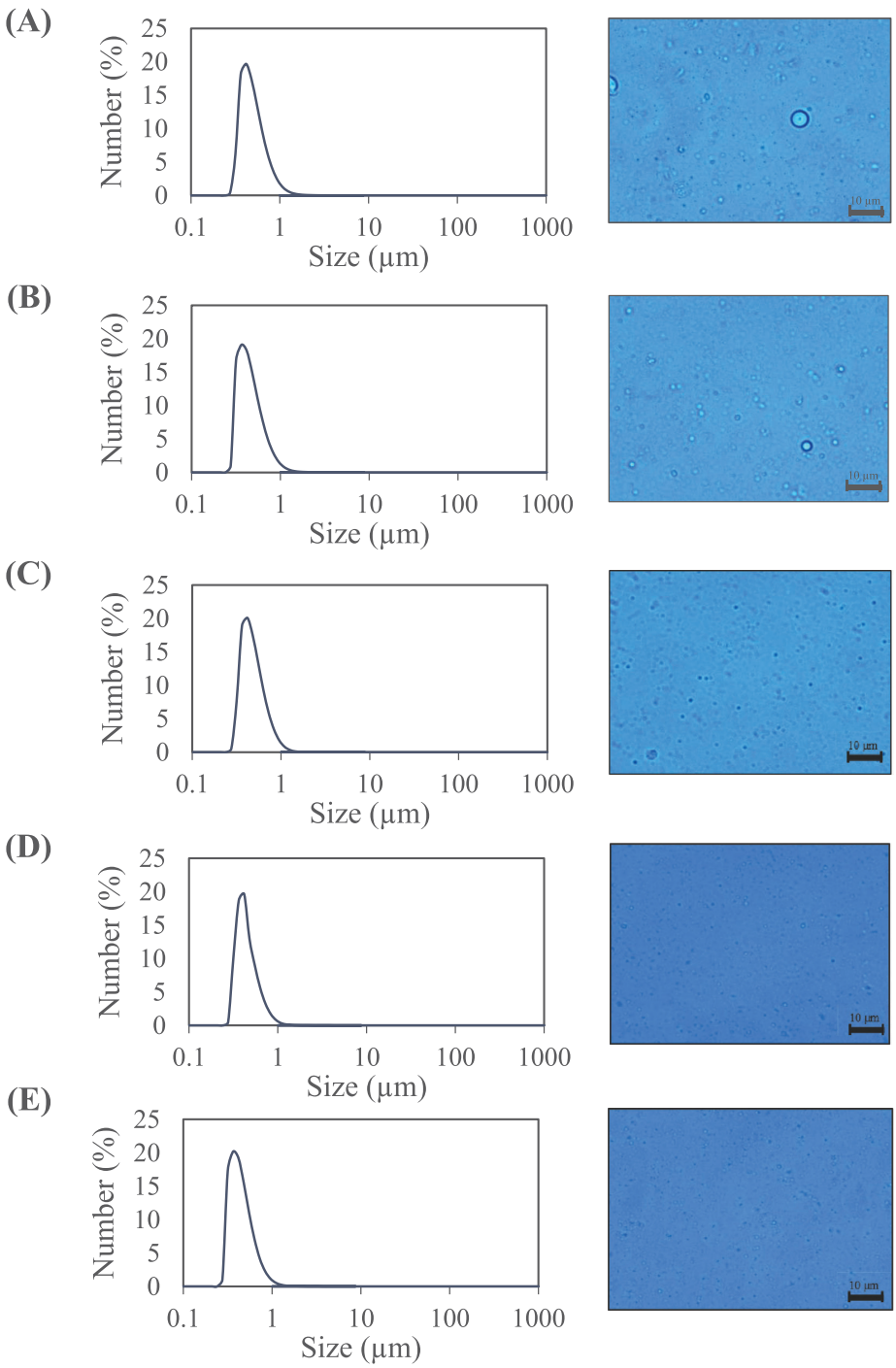

Fig. 7. Effects of ultrasound nominal power on the droplet size distribution and microstructure of the blue milk samples: A) 0 W; B) 100 W; C) 200 W; D) 300 W; and E) 400 W.

microstructure similar to the untreated sample. The ultrasound treatments applying 200 W, 300 W, and 400 W provided smaller droplet diameters, indicating that these nominal powers promoted a better homogenization for the blue colorant-loaded milk samples. Span values for the blue milk samples processed with these nominal powers confirm their lower polydispersity degree (Table 2). Small span values indicate a narrow droplet size distribution. Thus, higher nominal powers promoted a more intense homogenization resulting in a finer dairy

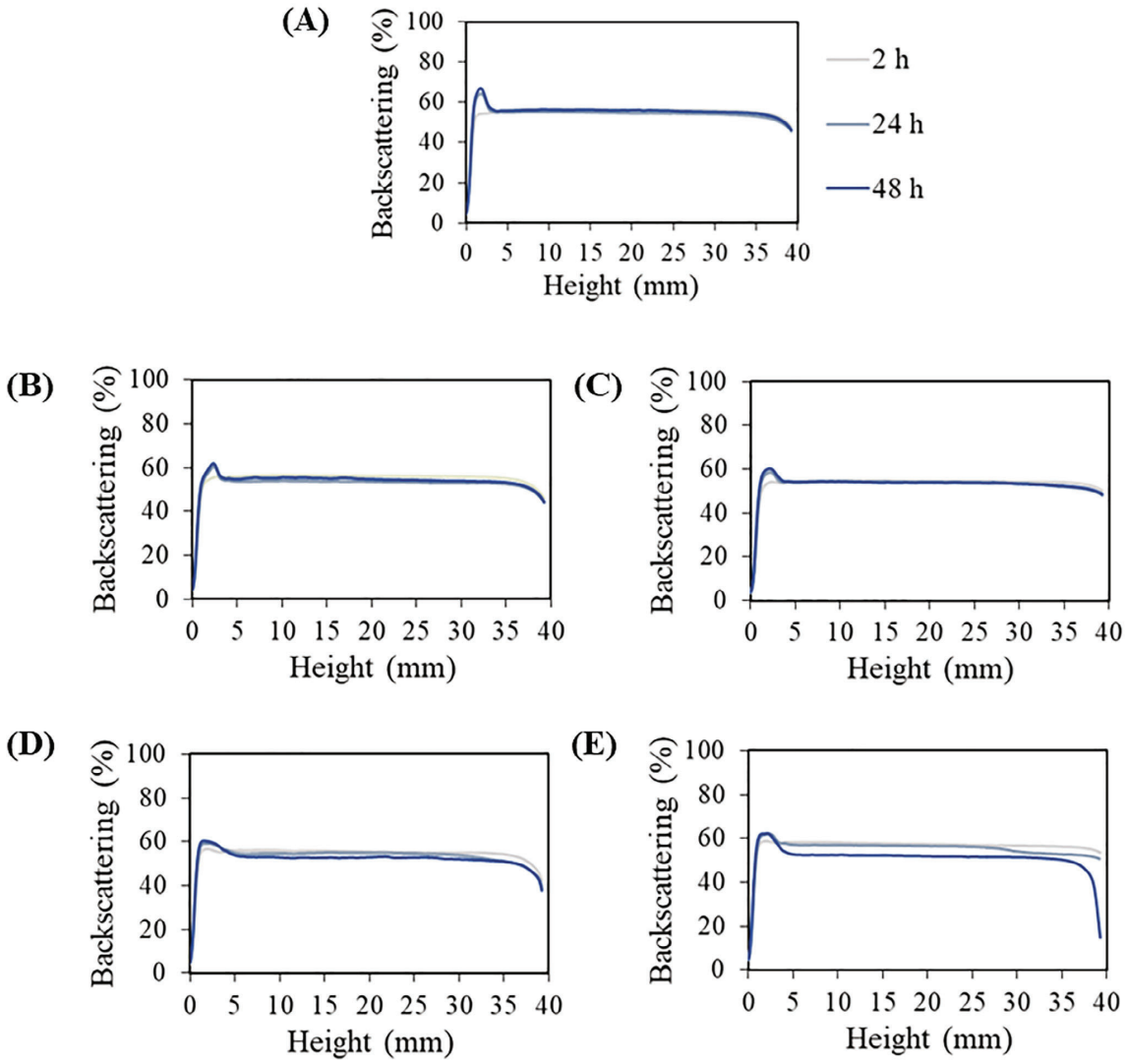

Fig. 8. Effects of ultrasound nominal power on the backscattering profiles of the milk samples: (A) 0 W; (B) 100 W; (C) 200 W; (D) 300 W; and (E) 400 W.

emulsion. Acoustic cavitation due to its action mechanism has been highlighted as an efficient emerging treatment to reduce the mean diameter of suspensions and emulsions. The formation and collapse of ultrasonic microbubbles result in micro shearing effects on the surface of fat globules reducing their size distribution. A higher nominal power promotes a more intense acoustic cavitation resulting in the narrower size distribution for the blue colorant-loaded milk [27–29].

The evaluation of the kinetic stability of the blue milk samples is an important step for their use as a new food ingredient. For this, blue milk samples were analyzed according to their backscattering profile (Fig. 8). The results were obtained by monitoring the backscattering profile (BS) in relation to the height (mm) of the glass tubes containing the milk samples, considering the initial time after the ultrasound processing (2 h), and for more a cold storage time of 24 and 48 h. Similar BS profiles were observed for the blue colorant-loaded milk samples, indicating that the different nominal powers applied did not affect their kinetic stability. In the bottom of the measurement cell (0–4 mm), a higher percentage of BS was observed after 24 and 48 h of cold storage. This high percentage could be attributed to a small sedimentation due to the reaction between genipin and milk proteins. This phenomenon was observed for all treatments, but it was more intense for the untreated sample. Smaller droplet diameters favored the kinetic stability of the blue milk samples. According to Stokes law, the speed with which a droplet moves is proportional to the square of its radius; therefore, the kinetic stability of a colloidal system may be increased by reducing the size of the droplets. Thus, the good kinetic stability observed even for the untreated sample could be associated with its smaller droplet diameter.

4. Conclusion

Our results have demonstrated that the low-frequency and high-power ultrasound technology was a promising and efficient technique to obtain blue colorant-loaded milk samples from the reaction between

genipin and milk proteins. One-step acoustic cavitation assisted the genipin extraction from the unripe *Genipa americana* L. and its diffusion into the milk colloidal system favoring its cross-linking with milk proteins. Ultrasound process intensification by increasing the nominal power promoted higher genipin recovery resulting in bluer milk samples. However, the application of high temperatures associated with intensified acoustic cavitation processing favored the occurrence of non-enzymatic browning due to the formation of complex melanin substances from the Maillard reaction. Also, the blue milk samples were chemically stable since their functional groups were not modified after ultrasound processing. Likewise, all blue colorant-loaded milk samples were kinetically stable during their cold storage. Therefore, a novel natural blue colorant with high-potential application in food products like ice creams, dairy beverages, bakery products and candies was produced.

Declaration of Competing Interest

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

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CHAPTER 6

Effect of thermosonication pretreatment and different plant protein sources on the production of natural blue colorants

Impact of thermosonication pretreatment on the production of plant protein-based natural blue colorants

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Impact of thermosonication pretreatment on the production of plant protein-based natural blue colorants

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ABSTRACT

The increase in the consumption of natural and non-dairy products has boosted the worldwide food colorant industry. Thus, the use of non-animal protein sources to produce natural blue compounds from the cross-linking between primary amines and genipin extracted from unripe *Genipa americana* L. pulp was evaluated in this study. Plant extracts obtained from white rice, oat, peanut, and almond were examined as amine suppliers, extraction media, and carrier systems for the blue compounds. The four plant extracts were thermosonicated at 70 °C and 400 W for 3 min and then subjected to ultrasound-assisted extraction of genipin from unripe genipap pulp at 300 W for 6 min. Thus, this study aimed to evaluate the effects of thermosonication pretreatment and plant material on natural blue colorant production. Four new blue plant-based colorants were produced. Protein availability was the most significant limiting factor in the blue color formation reaction. The acoustic cavitation associated with moderate heat treatment during the thermosonication promoted the rupture of fat globules and starch gelatinization. Furthermore, thermosonication pretreatment showed promising to improve the kinetic stability of the natural blue colorants.

1. Introduction

The consumption of non-dairy products is growing as the number of allergies towards bovine milk, lactose intolerance, and seeking alternative food consumption has increased (Crittenden et al., 2007; Munekata et al., 2020). Therefore, a series of plant-based products have been developed by the food industry to supply this demand. These products include beverages, yogurt, ice cream, cheese, and curd produced with non-dairy and all-natural ingredients, aiming to reach consumers looking for an alternative and healthier diet. Thus, cereals, seeds, fruits, and tubers are the main ingredients used to obtain plant-based products. Natural colorants have also been obtained from plant matrices for food and beverage formulations to increase their nutritional and sensory appeal (Albuquerque et al., 2020; Backes et al., 2020).

In this sense, a strong trend is the use of blue colorants (Spence, 2018). Blue-colored products are gaining space in the market due to their ability to capture our attention, such as bread, dairy beverages, ice creams, yogurts, and desserts. Most of these products have bovine milk in their formulation. Recently, our research group developed a natural blue colorant for application in dairy products (Neves et al., 2020). The colorant was obtained from a cross-linking between genipin extracted from unripe genipap (*Genipa americana* L.) and primary amine groups of the milk proteins (Strieder et al., 2020). Genipin is an iridoid precursor of blue compounds. Studies have also demonstrated that genipin present effects against bladder cancer cells and glioblastoma tumor cells (Zheng et al., 2018; Ahani et al., 2019). Likewise, following the trend of non-dairy foods, plant proteins can be used as an alternative to animal origin proteins to produce this natural blue colorant.

Plant proteins can be extracted from plant matrices through mechanical techniques employing water as a solvent. Additionally to containing proteins, these extracts also are a good source of phenolic compounds, unsaturated fatty acids, and bioactive compounds sourced from plant materials such as cereals, seeds, fruits, and oilseeds (Aydar et al., 2020). Rice (*Oryza sativa* L.), oat (*Avena sativa* L.), peanut (*Arachis hypogaea* L.), and almond (*Prunus dulcis*) are interesting sources of proteins to obtain the blue colorants since their extracts present a white color. Their white aqueous extract presents a colloidal suspension with a lighter background. However, they exhibit different chemical and physical characteristics as solvents, reaction media, and carriers for blue compounds.

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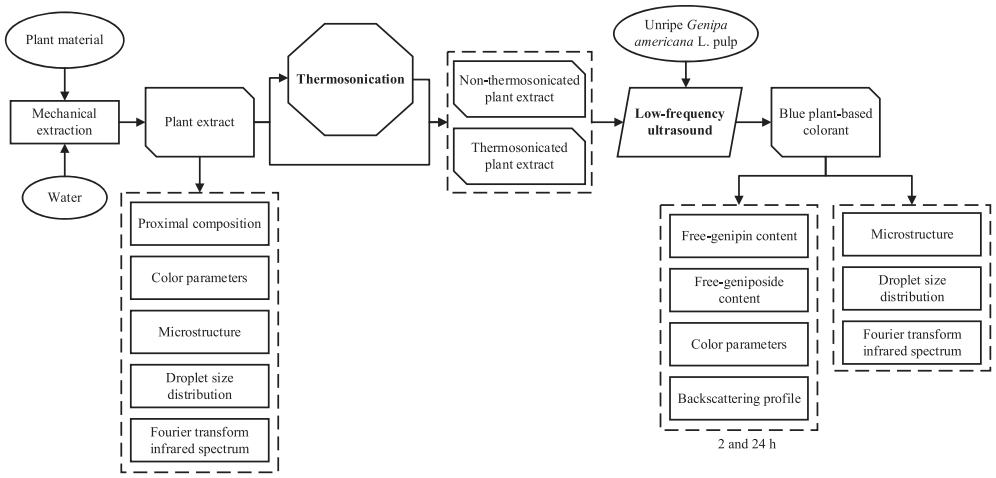

Fig. 1. Flow diagram of the processes used for obtaining blue plant-based colorants. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

After obtaining plant extracts, stabilization treatments such as pasteurization and homogenization are required. Plant extract contains insoluble particles such as protein, starch, fiber, and other cellular material that can sediment (Aydar et al., 2020). They also present a microbial load and endogenous enzymes of the own plant matrix (Mäkinen et al., 2016). Pasteurization and UHT treatments employing high temperatures (>100 °C) are unit operations used in commercial production to improve their kinetic stability and shelf life. However, conventional thermal treatments may promote undesirable effects on suspension kinetic stability, flavor, aroma, and color (Mäkinen et al., 2016). Thus, thermosonication treatments are promising alternatives for pasteurizing and homogenizing colloidal suspensions in just one step, avoiding undesirable thermal effects (Balthazar et al., 2019; Bermúdez-Aguirre et al., 2008). This emerging process is based on the combination of mild thermal treatments and acoustic energy provided by low-frequency (16–100 kHz) and high-power (>10 W) ultrasound. Thermosonication preserves the sensorial and nutritional attributes, avoiding product exposure to high temperatures. Furthermore, this treatment enables a short processing time and low energy consumption (Akdeniz and Akalın, 2019; Anaya-Esparza et al., 2017). The thermosonication pretreatment of plant extract can provide favorable chemical and physical modifications. In bovine milk samples, a thermosonication treatment promoted fat globule rupture, reducing the diameter of the samples processed at 63 °C for 30 min using a 22 mm probe operating at a nominal power of 400 W and 24 kHz (Bermúdez-Aguirre et al., 2008). In mung proteins, a thermosonication process allowed the formation of soluble aggregates, reducing their particle size and free sulfhydryl content by a process at 70 °C for 30 min, using a 10 mm probe at 1200 W and 20 kHz (Zhong and Xiong, 2020). Therefore, producing blue plant-based colorants from unripe genipap employing a thermosonication pretreatment can kinetically stabilize the plant extract and modify its chemical and physical characteristics. These modifications may favor genipin extraction and its reaction with primary amine groups.

In this context, this study aimed to evaluate the effects of thermosonication pretreatment and four plant extracts, *Oryza sativa* L., *Avena sativa* L., *Arachis hypogaea* L., and *Prunus dulcis*, on the cross-linking between genipin and plant proteins for the production of natural blue colorants. Additionally, the impact of the thermosonication pretreatments on the microstructure and kinetic stability of the plant protein-based blue colorants was evaluated.

2. Material and methods

2.1. Plant material

The plant materials white rice (*Oryza sativa* L.), oat (*Avena sativa* L.), shelled peanut (*Arachis hypogaea* L.), and almond (*Prunus dulcis*) were purchased from a local market in Campinas, SP, Brazil. Unripe genipap fruits were donated by "Fazenda Lagoa" (Ponte Alta do Tocantins, TO, Brazil). They were stored at - 24 °C until the experimental assays.

2.2. Production of blue plant-based colorants

The blue plant-based colorants were produced from the cross-linking between genipap pulp-extracted genipin and plant proteins found in rice, oat, peanut, and almond extracts. A thermosonication pretreatment was applied to plant extracts before their use as a solvent to extract genipin employing the low-frequency ultrasound extraction process. Thus, non-thermosonicated and thermosonicated plant extract was used as a solvent. Fig. 1 presents the experimental design and flow diagram of this study.

2.3. Preparation and characterization of plant extract

One hundred grams of white rice, oat, peeled peanut, and almond were previously soaked in water for 12 h at 7 °C. Then, the almonds were peeled. All plant materials were washed under running water, ground, and homogenized for 5 min with 200 g of ultrapure water in a 400 W mechanical RI1364/07 processor (Philips Do Brasil LTDA, Varginha, MG, Brazil). The samples were separated from the material cakes by filtration using a nylon filter.

The raw plant extracts of rice, oat, peanut, and almond were characterized according to their moisture and protein proximal contents. The moisture was measured according to the methodology described by Bradley (2010). The protein content was determined by the Dumas combustion method using a conversion factor of 6.25 (Ribadeau-Dumas and Grappin, 1989). The standard nitrogen curve was determined using a standard of rice flour (carbon: 40.9 ± 0.2%; nitrogen: 1.38 ± 0.05%; water: 9.50 ± 0.05%).

2.4. Thermosonication pretreatment

The raw plant extracts were thermosonicated immediately after their

preparation using a water bath and a 13-mm ultrasound probe diameter at 19 kHz (Unique, Disruptor, 500 W, Indaiatuba, Brazil). The process was carried out with 25 g of each raw extract for 3 min at 70 °C, using a nominal power of 400 W (acoustic power of 20 ± 1 W according to a previous study (Strieder et al., 2020)). For this, the probe contact height with the extract was standardized to 60 mm.

2.5. Low-frequency ultrasound-assisted extraction

The low-frequency ultrasound-assisted extraction was carried out according to Strieder et al. (2020). Unripe genipaps were previously thawed, peeled, and grounded in a 400 W mechanical RI1364/07 processor. The extraction process was carried out using 5 g of ground unripe genipap pulp and 25 g of plant extract. A 13-mm ultrasound probe diameter assisted the extraction at 19 kHz. The nominal power of 300 W (acoustic power of 14.5 ± 0.3 W) was applied to the samples using the same processing time of 6 min, which was selected according to a previous study (Strieder et al., 2020). The extraction process was performed using the four non-thermosonicated and thermosonicated plant extracts of rice, oat, peanut, and almond, resulting in eight blue plant-based colorants. All experiments were performed in duplicate, totalizing 16 experiments.

2.6. Blue color compound formation kinetics

The blue plant-based colorants were analyzed concerning free-genipin content, free-geniposide content, visual aspects, and color parameters to evaluate the samples' blue color formation kinetics. The measurements were made after the extraction assisted by low-frequency ultrasound (2 h) and after 24 h of cold storage at 7 ± 2 °C. The color parameters were also determined for the raw plant-based milk samples as a color control.

2.7. Free-genipin and free-geniposide contents

Free-genipin and free-geniposide were quantified in the samples using high-performance liquid chromatography (HPLC) on a 2695D separation module (Waters, Milford, MA, USA) equipped with a 2998 diode array detector. The sample preparation was carried out according to the methodology described by Neves et al. (2020). The genipin and geniposide determination were performed as described by Náthia-Neves et al. (2018). The compounds were detected at 240 nm and quantified using Empower 2 software (Waters, Milford, MA, USA). The identifications were made by comparing its retention time and UV–vis spectra to its reference standards (Sigma Aldrich, St. Louis, USA). Free-genipin and free-geniposide content results were determined concerning the weight of dried genipap. They were expressed according to Equations (1) and (2).

$$Free-genipin\ content\ (mg/g) = \frac{Genipin\ (mg)}{Dried\ genipap\ mass\ (g)} \quad (1)$$

$$Free-geniposide\ content\ (mg/g) = \frac{Geniposide\ mass\ (mg)}{Dried\ genipap\ mass\ (g)} \quad (2)$$

2.8. Color parameters

Color parameters were measured in an Ultra Scan Vis 1043 (Hunter Associates Laboratory, Reston, VA, USA) with CIE (Commission Internationale de l'éclairage) coordinates L (L = 0 for black and L = 100 for white), a* (green [−] to red [+]), and b* (blue [−] to yellow [+]). The color measures were made at room temperature for the plant extracts, as a control, and for the blue plant-based colorants.

2.9. Chemical stability

The identification of their functional groups assessed the chemical stability of the samples. For this, the plant extracts were previously freeze-dried in an L101 freeze dryer (Liobras, São Carlos, SP, Brazil) to determine the Fourier transform infrared (FTIR) spectrum. The measurements were taken in an FTIR spectrometer (4100 Jasco, Tokyo, Japan) at room temperature and recorded in the 400–4000 cm^{-1} region.

2.10. Microstructure characterization and kinetic stability

Their particle size distribution and microstructure evaluated the acoustic cavitation effects on the samples' physical stability before and after low-frequency ultrasound processing. The blue plant-based colorants' kinetic stability was evaluated by the backscattering profiles determined at 2 and 24 h of the ultrasound treatments.

2.11. Particle size distribution

A Mastersizer 2000 laser diffraction analyzer (Malvern Instruments Ltd., Worcestershire, UK) was used to determine the particle size and the mean diameter. The samples were analyzed by a wet method at 25 °C, with dispersion in water (refractive index of 1.52). The Sauter mean diameter ($D_{3,2}$) of particles and the polydispersity degree (*Span*) were calculated according to Equation (3) and Equation (4).

$$D_{3,2} = \frac{\sum_{i=1}^{k} n_i.d_i^3}{\sum_{i=1}^{k} n_i.d_i^2} \quad (3)$$

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \quad (4)$$

where d_i is the mean diameter of the particles, and n_i is the number of particles.

2.12. Microstructure

The sample microstructures were observed using a Carl Zeiss Model Axio Scope A1 optical microscope (Zeiss, Gottingen, Germany). The images were acquired using immersion oil and a 100 × objective lens.

2.13. Kinetic stability

The kinetic stability of the blue plant-based colorants was assessed using the near-infrared backscattering technique. The samples in a specific glass tube were measured for their kinetic stability using a light backscatter scan analyzer Turbiscan LAB Expert (Formulaction®, Toulouse, France). The measurements were performed immediately after the extraction assisted by ultrasound (2 h) and 24 h of cold storage (7 ± 2 °C).

2.14. Statistical analysis

The effects of thermosonication pretreatment and plant matrix on the production of blue plant-based colorants were verified by analysis of variance (ANOVA) using Minitab 18® software (Minitab Inc., State College, PA, USA). Tukey's test of means was performed with a 95% confidence level (p-value ≤ 0.05).

3. Results and discussion

3.1. Blue color formation kinetics

The formation kinetics of plant protein-crosslinked genipin complexes using plant extract as genipin extractor solvent and source of primary amine groups was evaluated because the cross-linking is a

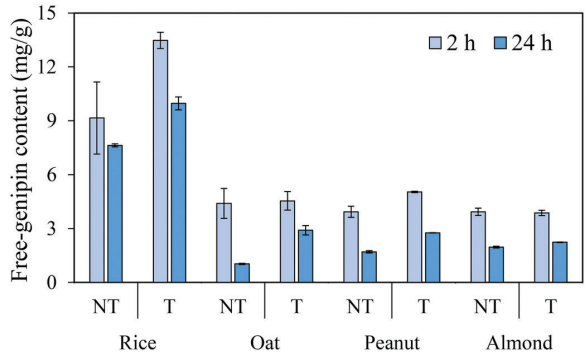

Fig. 2. Free-genipin content of the blue plant-based colorants obtained from non-thermosonicated (NT) and thermosonicated (T) plant extract over the cold storage (7 ± 2 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Chemical characterization of the plant extracts regarding their moisture and protein content, and pH value.

Plant extract	Moisture (g/100 g)	Protein (g/100 g)	pH value
Rice	96.5 ± 0.1[a]	0.46 ± 0.02[d]	6.64 ± 0.03[c]
Oat	88 ± 1[b]	1.92 ± 0.01[c]	6.86 ± 0.01[a]
Peanut	83.8 ± 0.2[c]	6.43 ± 0.03[a]	6.75 ± 0.01[b]
Almond	81.7 ± 0.1[d]	5.98 ± 0.03[b]	6.33 ± 0.02[d]

*Mean values ± standard deviation (n = 2). Values followed by different letters in the same column show differences by Tukey's test at 95% significance (p-value ≤ 0.05).

noninstant reaction. Additionally, the cross-linking between genipin and proteins is catalyzed by heat and oxygen. On the other hand, geniposide also extracted from the unripe genipap may favor the formation of blue compounds because its hydrolysis releases genipin (Lee et al., 2003; Náthia-Neves and Meireles, 2018). Thus, the reaction kinetics of blue color formation was monitored in the samples by the amounts of free-genipin and free-geniposide immediately after the extraction process assisted by low-frequency ultrasound (2 h) and after 24 h of cold storage (7 ± 2 °C).

Fig. 2 presents the results obtained for the free-genipin content in the blue plant-based colorants. Rice extract was the best genipin extractor (p-value = 0.005), recovering up to 13.5 ± 0.5 mg/g. The plant extracts of oat, peanut, and almond recovered the same genipin amount (p-value = 0.1). This result can be associated with the rice extract moisture content (Table 1). Genipin is a polar compound. Its diffusion can be favored by the water concentration gradient in the extractor medium (Náthia-Neves and Meireles, 2018). After 24 h of cold storage, all blue plant-based colorants showed a reduction in their free-genipin contents concerning the initial time of 2 h (p-value = 0.05), evidencing that the reaction for blue color compound formation continued to occur after the extraction assisted by low-frequency ultrasound. The acoustic cavitation effects on the formation of the blue compounds can be observed immediately after the ultrasound-assisted extractions (Fig. 3). Ultrasonic waves applied to the extraction medium promote the formation and subsequent collapse of microbubbles that generate shear forces on the surface of unripe genipap pulp (Strieder et al., 2020). Cavitational shear force promotes the rupture of the genipap cell wall, favoring the diffusion of intracellular compounds such as genipin and geniposide into the liquid medium used as an extractor. During the sonication process, energy in heat form is dissipated, increasing the temperature of the sonicated liquid medium, which also favors the diffusion of the blue color's precursor compounds into the plant extract. The non instantaneous reaction continues to occur after the extraction step, mainly due to oxygen that continues to catalyze the formation of blue complexes (Butler et al., 2003). The thermosonication pretreatment positively affected genipin recovery by peanut extract (p-value = 0.038). Regarding the other plant materials, thermosonication pretreatment did not significantly affect (p-value = 0.1) the genipin recovery. In peanut extract, thermosonication may have promoted physical changes, such as the breakdown of fat globules, increasing the system polarity, and favored a greater

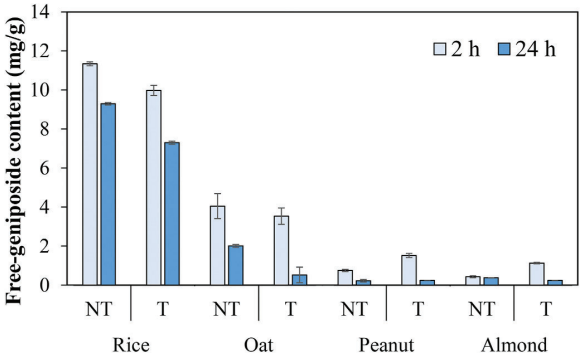

Fig. 4. Free-geniposide content of the blue plant-based colorants obtained from non-thermosonicated (NT) and thermosonicated (T) plant extract over the cold storage (7 ± 2 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

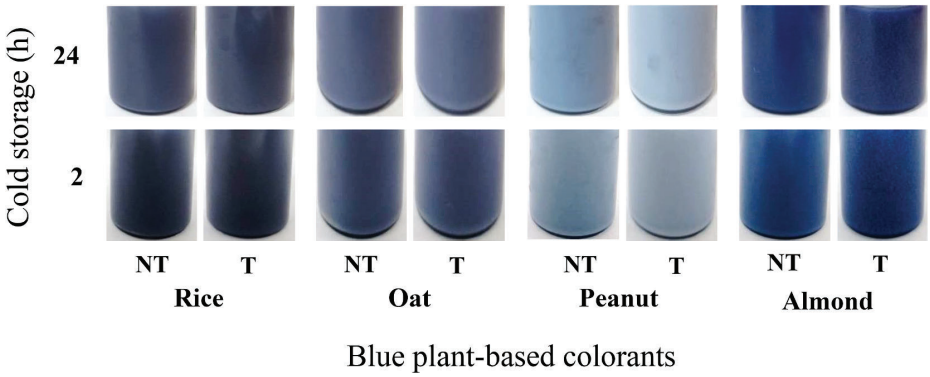

Fig. 3. Visual appearance of the blue plant-based colorants obtained from non-thermosonicated (NT) and thermosonicated (T) plant extract. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

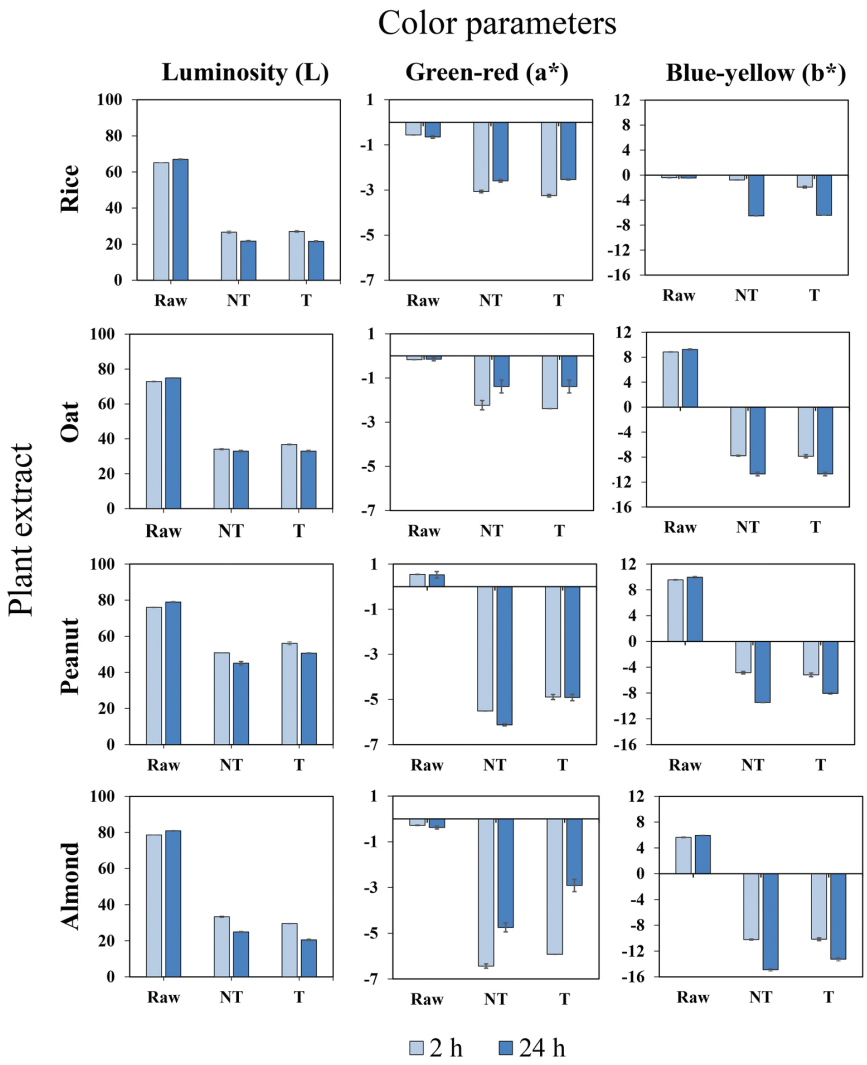

Fig. 5. La*b* parameters of the raw plant extracts, and blue plant-based colorants obtained from non-thermosonicated (NT) and thermosonicated (T) plant extract over the cold storage (7 ± 2 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

extraction of genipin (Bermúdez-Aguirre et al., 2008; Náthia-Neves and Meireles, 2018).

Fig. 4 shows the results for free-geniposide content in the blue plant-based colorants. The rice extract provided the greatest extraction of geniposide (p-value = 0.002), followed by the oat extract (p-value = 0.001). Peanut and almond extract presented the same content of free-geniposide (p-value = 0.8). Again, the higher moisture content in the raw plant extracts of rice and oat (Table 1) may have favored the diffusion of compounds, generating blue plant-based colorants richer in geniposide. The geniposide recovery from unripe genipap was also observed by Náthia-Neves et al. (2019) through a pressurized extraction using ethanol and water as solvents. Using bovine milk with different compositions, we did not observe this compound (Neves et al., 2020). This difference occurs because geniposide presents higher polarity than genipin and thus has more affinity for more polar solvents. Therefore, the higher moisture content (Table 1) can have favored its extraction. The blue plant-based colorants also showed lower free-geniposide contents after 24 h of cold storage. However, this reduction was smaller than that observed for free-genipin contents. In our previous study, in ethanolic extracts of genipap stored for 72 h at room temperature, we did not observe losses of free-geniposide. Thus, we can infer that geniposide was hydrolyzed, releasing free-genipin into the plant extract liquid medium. Thermosonication pretreatment had a positive effect on geniposide recovery by peanut extract (p-value = 0.01) and almond extract (p-value = 0.01) but had no effect on rice extract (p-value = 0.08) or oat extract (p-value = 0.4). Therefore, the thermosonication pretreatment favored a higher geniposide extraction by the plant extract samples, extracting a smaller content of this iridoid concerning genipin content. This difference may also be associated with the smaller fat globule sizes obtained after thermosonication pretreatment (Bermúdez-Aguirre et al., 2008).

All plant-based colorants obtained from non-thermosonicated and thermosonicated plant extracts presented a blue visual aspect but different shades of color (Fig. 3). This difference may be associated with each raw plant extract's characteristics, including its moisture and protein contents, availability in primary amine groups, and color. In this

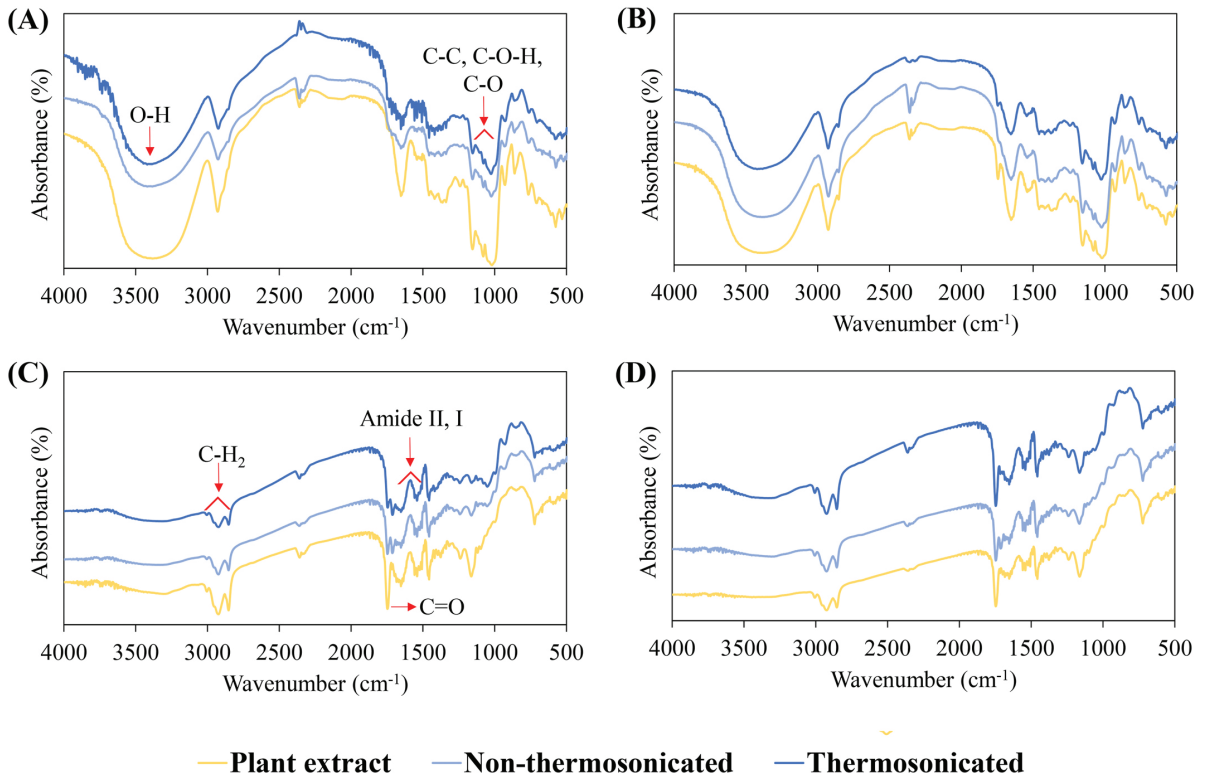

Fig. 6. FTIR spectra of the plant extracts and blue plant-based colorants obtained from non-thermosonicated and thermosonicated plant extract of (A) rice, (B) oat, (C) peanut, and (D) almond. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sense, almond and peanut extracts presented similar chemical compositions (Table 1) and extraction yields of genipin and geniposide (Figs. 2 and 4). On the other hand, they presented different shades of blue (Fig. 3). Thus, the shade difference may be related to the availability of primary amine groups to react with genipin and with the own plant extract color. The samples stored for 24 h at 7 ± 2 °C did not change the blue visual color. In whole bovine milk, we observed a different behavior. There was an intensification in the blue color during storage (Strieder et al., 2020). Different blue colorant tones were acquired for them, depending on the nominal power employed (100, 200, 300, and 400 W) and the cold storage time (2, 24, and 48 h). The differences between plant extract samples' chemical composition and their availability in primary amine groups to react may have influenced the different results regarding milk.

Fig. 5 presents the color L a* b* parameters for the raw plant extracts and the blue plant-based colorants obtained after the extraction of unripe genipap compounds. The addition of genipap compounds promoted the browning of the extracts. The blue plant-based colorants presented a lower luminosity (L) value than the raw plant extracts (p-value = 0.001). The thermosonication pretreatment provided lighter blue colorants of oat and peanut (p-value = 0.01), a darker almond (p-value = 0.01) and did not affect the rice L parameter (p-value = 0.5). This difference in the L results can be associated with each plant extract (Table 1). In hazelnut-based milk, Atalar et al. (2019) observed a small reduction in the L parameter after the thermosonication process (300 W, 25 min, which reached 65 °C). According to them, the small L reduction was not visible. It may be associated with acoustic cavitation and an increase in the medium's temperature, which may have caused the browning by the Maillard reaction. Monteiro et al. (2018) observed an increase in the L parameter value of dairy beverages treated with low-frequency ultrasound. The smaller fat globules obtained after ultrasound processing are associated with the casein colloidal particles and calcium phosphate, generating higher L values. The a* parameter values were also reduced after the extraction step (p-value = 0.006). Thus, the blue plant-based colorants showed a greener coloration than their control samples. After 24 h of cold storage, the a* value increased in the blue plant-based colorants of rice, oat, and almond (p-value = 0.01). In the blue plant-based colorant of peanut, this value was reduced in the non-thermosonicated sample (p-value = 0.03) and kept in the thermosonicated sample (p-value = 0.9). The plant-based colorants produced from thermosonicated plant extract presented a lower intensity green color (lower values of a*) than the non-thermosonicated samples. The same samples also presented lower geniposide contents (Fig. 4). The geniposide has a yellow color combined with the blue compounds formed, promoting the blue plant-based colorants' greenish hue appearance. However, the plant-based colorants presented small differences in a* values and visually unnoticeable differences (Figs. 3 and 5). The blue color formation in the plant extracts can be verified by the b* parameter value reduction after extraction (p-value = 0.001) and after 24 h of cold storage (p-value = 0.005). The thermosonication did not affect the blue color formation in the plant-based colorants of rice and oat (p-value = 0.7) but promoted higher values of b* (p-value = 0.006) in the samples of peanut and almond. The bluest plant-based colorant (b* = −14.9 ± 0.2) was obtained from almond extract after 24 h of cold storage. We observed a similar result (b* = −12.0 ± 0.1) for our blue bovine milk obtained from unripe genipap by the same ultrasound-assisted extraction conditions (Strieder et al., 2020).

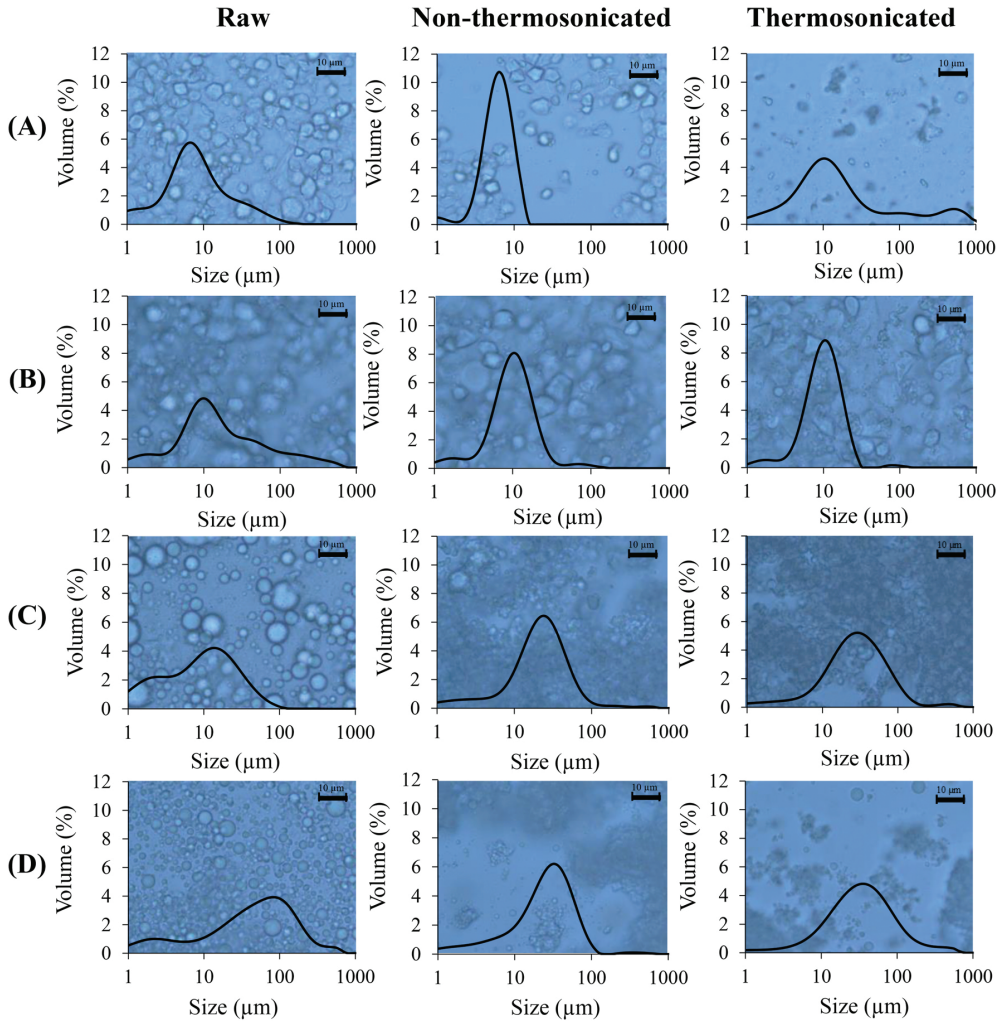

Fig. 7. Droplet size distribution and micrographs of the (raw) plant extracts and blue plant-based colorants obtained from non-thermosonicated and thermosonicated plant extracts of (A) rice, (B) oat, (C) peanut, and (D) almond. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Chemical stability

The effects of thermosonication pretreatment on the functional groups of the blue plant-based colorants were evaluated by FTIR spectroscopy. The spectra of the rice (A) and oat (B) samples were similar, as were those obtained for peanut (C) and almond (D) samples (Fig. 6). These similarities are related to the chemical compositions of the plant extracts (Table 1). The peanut and almond samples that contain higher protein contents also presented more pronounced peaks related to amides I and II (1660 cm^{-1} and 1556 cm^{-1}). Peanut and almond samples that showed more fat globules in their micrographs (Fig. 7) also presented more intense peaks associated with lipids. These are attributed to the asymmetric stretch bands of CH_2, symmetrical CH_2, and double bonds of C═O (2920, 2850, and 1745 cm^{-1}) (Ye et al., 2017). Rice and oat samples composed of higher carbohydrate content (due to their vegetable matrices) also presented more pronounced peaks related to starch: vibrational stretches of groups O–H (3700-3000 cm^{-1}), C–C, C–*O*–H, and C–O (1150-1000 cm^{-1}) (Amagliani et al., 2016; García--Tejeda et al., 2018; Punia et al., 2020; Shahrokh et al., 2019). The blue plant-based colorant spectra were similar to those obtained for their respective raw plant extracts (Fig. 6). However, in the blue plant-based colorant spectra of rice (A) and peanut (C), a reduction in the intensity of the 1660 cm^{-1} and 1745 cm^{-1} peaks was observed, referring to the stretching of the C═O bond of amide and ester groups. This difference may be associated with thermal and acoustic cavitation effects provided by low-frequency ultrasound. These may have disrupted the functional group double bonds. Despite this, the samples' functional groups were not modified. Stefanović et al. (2018) also observed changes in the intensity of functional group vibrations of egg protein samples after a low-frequency ultrasound treatment (20 kHz, 13 mm probe, 15 min, 25 °C). These were associated with hydrophobic interactions and conformational changes promoted by acoustic cavitation. We cannot observe new bonds in the blue colorants' spectra related to the complexes formed between genipin and plant proteins. However, Butler et al. (2003) observed reductions and increases in chitosan peakes added of genipin and a new peak at 1630 cm^{-1} after the reaction.

Table 2
Effects of the thermosonication pretreatment on the $D_{3,2}$, size distribution, and *Span* of the blue plant-based colorants.

Plant material	Sample	$D_{3,2}$ (μm)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	*Span*
Rice	Plant extract	4.2 ± 0.1^{c}	1.9 ± 0.1^{c}	6.9 ± 0.4^{c}	30 ± 4^{b}	4.0 ± 0.3^{b}
	NT colorant	4.50 ± 0.01^{b}	3.18 ± 0.01^{a}	5.82 ± 0.01^{b}	9.64 ± 0.01^{c}	1.10 ± 0.01^{c}
	T colorant	6.2 ± 0.2^{a}	2.8 ± 0.1^{b}	11 ± 1^{a}	158 ± 40^{a}	14 ± 4^{a}
Oat	Plant extract	6.7 ± 0.2^{a}	2.9 ± 0.1^{c}	12.2 ± 0.3^{a}	110 ± 11^{a}	9 ± 1^{a}
	NT colorant	6.4 ± 0.4^{b}	3.8 ± 0.3^{b}	9.4 ± 0.2^{b}	19.4 ± 0.3^{b}	1.64 ± 0.03^{b}
	T colorant	7.05 ± 0.03^{a}	4.41 ± 0.01^{a}	9.37 ± 0.03^{b}	17.3 ± 0.2^{c}	1.37 ± 0.02^{c}
Peanut	Plant extract	3.9 ± 0.1^{c}	1.46 ± 0.03^{c}	9 ± 1^{c}	30 ± 4^{c}	3.2 ± 0.2^{a}
	NT colorant	9.2 ± 0.4^{b}	5 ± 1^{b}	21 ± 1^{b}	50 ± 3^{b}	2.2 ± 0.2^{c}
	T colorant	11.8 ± 0.3^{a}	6.9 ± 0.1^{a}	26 ± 1^{a}	78 ± 7^{a}	2.7 ± 0.2^{b}
Almond	Plant extract	9 ± 1^{b}	2.9 ± 0.3^{c}	46 ± 6^{a}	165 ± 16^{a}	3.5 ± 0.1^{a}
	NT colorant	9.7 ± 0.1^{b}	4.6 ± 0.1^{b}	24.7 ± 0.2^{c}	57 ± 1^{c}	2.13 ± 0.04^{c}
	T colorant	14.5 ± 0.3^{a}	8.2 ± 0.2^{a}	31 ± 1^{b}	108 ± 9^{b}	3.2 ± 0.2^{b}

* Colorant obtained from NT: non-thermosonicated plant extract and T: thermosonicated plant extract.
**Mean values ± standard deviation (n = 4). Values followed by different letters in the same column for the same plant extract show differences by Tukey's test at 95% significance (p-value ≤ 0.05).

3.3. Physical and kinetic stability

The effects of thermosonication pretreatments on the size distribution of the blue plant-based colorants can be observed in the results of Sauter mean diameter ($D_{3,2}$), diameters based on cumulative volumes (d_{10}, d_{50}, and d_{90}), and *Span* (Table 2). Similar behavior was observed in the plant-based colorants obtained from thermosonicated plant extract. The mean diameter increased, and the *Span* values decreased. This behavior can be associated with the thermosonication treatment, where heat and acoustic energy are applied in the samples that present starch and other insoluble materials in their compositions. The heat sourced by the process can favor the gelatinization phenomenon. Thus, the water-retaining starch molecules have larger diameters.

Additionally, the re-coalescence may have promoted the increase in the $D_{3,2}$ values. Silva et al. (2016) observed an increase in the $D_{3,2}$ values of their oil-in-water emulsions stabilized with modified starch after a process intensification using acoustic energy. According to them, a re-coalescence phenomenon occurred due to the "overprocessing," resulting in higher Sauter mean diameters. Thus, the higher values obtained for $D_{3,2}$ may be related to the starch granules' larger diameters due to gelatinization and re-coalescence of the droplets. Despite this, thermosonication pretreatment allowed greater homogeneity (*Span* reduction) in the blue plant-based colorants of oat, peanut, and almond. The rice-based blue colorant showed the highest *Span* values associated with this plant material's high starch content (Amagliani et al., 2016). Our recent study observed that the genipap compounds' ultrasound-assisted extraction caused a reduction in the $D_{3,2}$ of the bovine milk samples (Strieder et al., 2020). This reduction may have occurred because of the breakdown of milk fat globules due to micro shearing caused by acoustic cavitation (Strieder et al., 2020). This behavior was only observed for the oat-based blue colorant produced from a non-thermosonicated sample, which presented a similar composition to bovine milk in terms of its protein and moisture content (Table 1). The other plant-based colorants obtained from non-thermosonicated samples showed higher $D_{3,2}$ values than their respective raw plant extracts. Therefore, the ultrasound treatments promoted different effects on the samples depending on their composition (Table 1).

In the optical micrographs and sample size distributions (Fig. 7), some thermosonication pretreatment effects can be observed. Blue plant-based colorants presented smaller fat globules than their respective raw plant extracts. The formation and subsequent eclosion of microbubbles in the liquid medium promote micro shearing, causing fat globules' breakdown. The same behavior was observed in a recent study by Guimarães et al. (2018) for bovine milk samples processed using low-frequency ultrasound technology (Strieder et al., 2020). The gelatinized starch microstructures were observed mainly in the images obtained for the plant-based colorants produced from thermosonicated samples. Thus, the increase in the starch particle diameter, which may have promoted an increase in D_{32} values (Table 2), also caused displacement in the size distribution curve for the right, indicating larger particles. The heat provided by thermosonication pretreatment (70 °C for 3 min) may have been responsible for this phenomenon. However, the second processing using acoustic cavitation for genipin recovery may also have favored the increase in particle size.

The impact of thermosonication pretreatment on the blue plant-based colorants' kinetic stability was evaluated through their backscattering (BS) profiles (Fig. 8). The BS profile was monitored concerning the height (mm) of the sample glass tube, considering the initial time after the treatments assisted by low-frequency ultrasound (2 h) and after 24 h of cold storage (7 ± 2 °C). Thermosonication pretreatment improved the kinetic stability of the samples. After 24 h of cold storage, the samples obtained from non-thermosonicated plant extracts showed more instability phenomena than the thermosonicated samples. Rice-based colorant produced from non-thermosonicated samples exhibited two phenomena: flocculation/coalescence of its particles, which promoted a lower percentage of BS in the tube's center due to sedimentation, evidenced by the higher percentage of backscattering at the tube bottom. This rice-based colorant sample did not show phase separation after 24 h of cold storage, indicating that the thermosonication pretreatment promoted its kinetic stabilization. Oat and peanut-based colorants produced with non-thermosonicated plant extracts showed clarification in the top of the tube after 24 h of cold storage. This behavior was not observed for the colorants obtained from the same thermosonicated plant extracts. Almond-based colorant produced with non-thermosonicated plant extract showed a phase separation at the bottom of the tube minimized by the thermosonication process. However, in this case, the thermosonication pretreatment also promoted creaming in the middle part of the tube. The fat globule reduction and starch gelatinization provided by acoustic cavitation associated with milder thermal treatment allowed us to obtain samples with lesser phase separation effects. Thus, in general, the thermosonication pretreatment provided blue plant-based colorants that were more homogeneous and kinetically stable.

4. Conclusion

Our results demonstrated that the chemical and physical characteristics of the raw plant extracts affected the technological properties of the natural blue colorants. Protein availability was the most significant limiting factor in the blue compound formation reaction since the almond and peanut samples showed the same protein content but different color results. The color differences between the samples were also associated with the raw plant extract moisture and protein contents in addition to their natural color. The almond plant extract presented the best conditions for the cross-linking between primary amine groups and

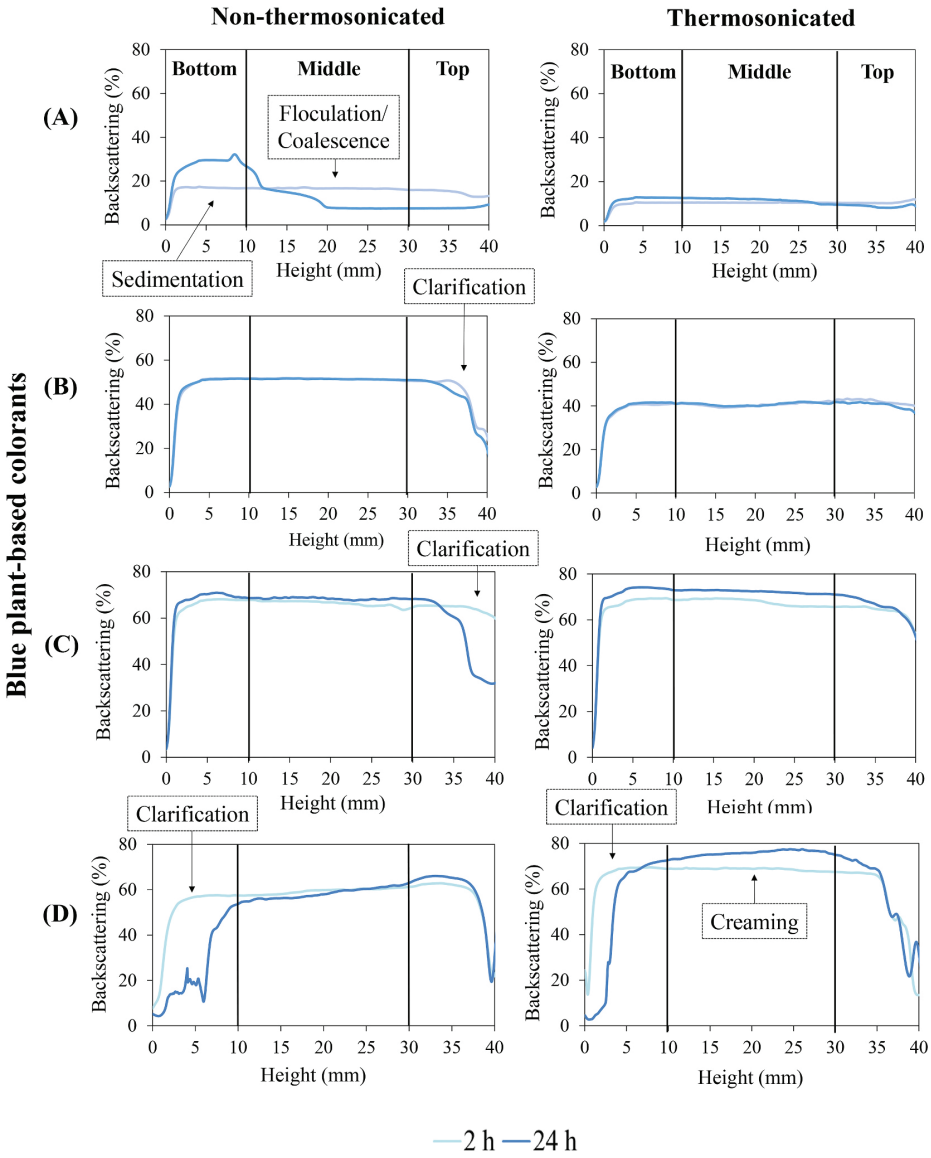

Fig. 8. Backscattering profile for the blue plant-based colorants obtained from non-thermosonicated and thermosonicated plant extracts of (A) rice, (B) oat, (C) peanut, and (D) almond. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

genipin extracted from the unripe genipap. This plant-based colorant showed the most intense blue color ($b^* = -14.9 \pm 0.2$). Acoustic cavitation associated with moderate heat treatment promoted the rupture of fat globules. However, starch gelatinization and re-coalescence of droplets increased the Sauter mean diameter. The thermosonication pretreatment provided blue plant-based colorants kinetically more stable. The blue colorants produced from thermosonicated plant extracts exhibited better kinetic stability than the non-thermosonicated samples. Therefore, our results indicated that plant extract is an excellent option to replace bovine milk in obtaining natural blue colorants from the cross-linking of its proteins with genipin. Additionally, thermosonication pretreatment showed promise for improving blue plant-based colorant kinetic stability.

CRediT author statement

Monique Martins Strieder: Investigation, Formal analysis, Writing – original draft. Maria Isabel Landim Neves: Investigation, Formal analysis. Eric Keven Silva: Investigation, Supervision, Writing – review & editing. Maria Angela A. Meireles: Resources, Supervision, Project administration.

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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CHAPTER 7

Application of plant protein-based blue colorant in almond-based beverage and its quality aspects after thermosonication treatments

Impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage

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Impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage

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ABSTRACT

Consumers are demanding not animal-derived food products produced by clean technologies. An almond-based functional beverage enriched with coconut oil, xylooligosaccharides, and natural blue colorant was processed by low-frequency ultrasound combined with mild heat treatment. This study aimed to evaluate the effects of thermosonication treatments on the phytochemicals, antioxidant capacity, fatty acids stability, volatile organic compounds, and color attributes of the plant-based beverage. Thermosonication treatments were carried out at 50 °C using three levels of acoustic power (4.6, 8.5, and 14.5 W) and holding time (5, 10, and 15 min). The acoustic energy increased the working temperature up to 25 °C beyond that provided by the external heat source. However, thermosonication treatments did not affect the antioxidant capacity of the beverage. These promoted the release of some bioactive compounds that were bound. Likewise, the degradation of others that were free. Higher acoustic powers and longer holding times degraded stearic and palmitic fatty acids. The thermosonication treatment at 14.5 W for 15 min degraded ethyl esters of the samples modifying the natural flavor of the beverage. Additionally, all thermosonication treatments intensified the blue color of the almond-based beverage maintaining its sensory appeal concerning color attributes.

1. Introduction

Consumer demand for novel food products from natural sources has increased worldwide over the years. Additionally, the number of people with lactose intolerance and allergy to milk proteins are also rising (Flom & Sicherer, 2019). Thus, the industry of natural vegan foods has been developing a series of innovative products from plant raw materials. Among these products, plant-based beverages have gained evidence due to their phenolic compound content, unsaturated fatty acids, and bioactive compounds, such as phytosterols and isoflavones. They make them an excellent alternative for those who also seek a healthier lifestyle (Aydar, Tutuncu, & Ozcelik, 2020).

Among plant beverages, those produced from almond have been the most consumed replacing dairy beverages in the United States (Wolf, Malone, & McFadden, 2020). Almonds are a source of essential nutrients such as alpha-tocopherol (vitamin E), unsaturated fatty acids, manganese, calcium, magnesium, selenium, potassium, and others (Sethi, Tyagi, & Anurag, 2016). Besides these essential compounds, almond beverages may also be added with other bioactive and prebiotic ingredients. Plant-based beverages have been produced with the addition of coconut oil due to its high content of lauric acid (dodecanoic acid). Antibiotic, antiviral, antibacterial, and antifungal properties were reported for this fatty acid (Yani, Aladin, Wiyani, & Modding, 2018). Prebiotic carbohydrates, such as xylooligosaccharides, fructooligosaccharides, inulins, and galactooligosaccharides, have also been added to plant-based beverages to increase their functionality (Silva, Arruda, Pastore, Meireles, & Saldaña, 2020). Flavoring additives, xylitol, and vanilla extract have been used (Luo, Mu, Sun, & Chen, 2020; Villegas, Carbonell, & Costell, 2009). Likewise, natural colorants also have been incorporated into plant-based beverage formulations to improve their sensorial and nutritional appeal (Khan et al., 2015).

Plant-based beverages are prepared from aqueous plant suspensions obtained through solid-liquid extractions assisted by mechanical stress. The disintegration of plant matrices facilitates the aqueous extraction of their phytochemical and hydrocolloid compounds. For this reason, plant extracts often present large insoluble particles and are not kinetically

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stables (Aydar et al., 2020). Stabilizers and emulsifiers can be added to the beverages to improve their kinetic stability. Also, thermal treatments, such as pasteurization and ultra-high temperature (UHT), are used for microbiological and enzymatic stabilization of these products improving their shelf life (Munekata et al., 2020). However, in these thermal treatments, the endogenous bioactive compounds of the plant-based beverages may be degraded due to the high temperatures employed (Valencia-Flores, Hernández-Herrero, Guamis, & Ferragut, 2013). Therefore, alternative stabilization processes, such as high pressure and ultra-high pressure homogenization, have been studied to homogenize and stabilize plant beverages avoiding losses in their bioactive compounds (Chourio Chavez, 2019; Valencia-Flores et al., 2013). These techniques combine different energy sources (heat and mechanical), allowing mild heat treatments.

Thermosonication technique has emerged as an efficient homogenization and stabilization treatment of foods and beverages. This innovative process combines acoustic energy with moderate heat treatments (50–70 °C) (Anaya-Esparza et al., 2017). The acoustic energy is provided by low-frequency at 16–24 kHz and high-power ultrasound. Ultrasound processing requires a lower investment cost, present a shorter processing time, and are easier to operate than high pressure-based techniques (Farid Chemat et al., 2017). Thermosonication processes have been recently studied to inactivate pathogenic and spoilage microorganisms and endogenous enzymes, avoiding losses in phytochemicals, flavor, and aroma of wheat plantlets juice, camu–camu nectars, and pulque (a traditional Mexican beverage) (Ahmed et al., 2019; Alcántara-Zavala, de Dios Figueroa-Cárdenas, Pérez-Robles, Arámbula-Villa, & Miranda-Castilleja, 2020; Souza et al., 2019). Higher nominal power (425 and 475 W) promoted higher microbial reduction. However, the treatment using 475 W at 50 °C for 3 and 5 min promoted pulque flavor and aroma alterations. In plantlet juice, the thermosonication treatment using the lowest temperature (30 °C) for 20 and 40 min provided the highest total phenolics content, flavonoids, total antioxidant capacity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay, carotenoids, anthocyanin contents, chlorophyll (a + b), minerals and free amino acids. Additionally to these thermosonication impacts, the breakdown of fat globules is another effect widely observed in products processed by acoustic energy (Strieder, Neves, Silva, & Meireles, 2020; Zhou, Zhang, Lorenzo, & Zhang, 2021).

In this context, this study aimed to evaluate the impact of thermosonication treatments on the phytochemicals, antioxidant capacity, fatty acids stability, volatile organic compounds, and color attributes of an innovative plant-based beverage. Almond-based beverage was enriched with coconut oil, xylooligosaccharides, and natural blue colorant produced from crosslinking between almond proteins and genipin.

2. Material and methods

The almond-based functional beverage was formulated, thermosonicated, and characterized according to the flow diagram exhibited in Fig. 1.

2.1. Beverage ingredients

The almond, xylitol, organic coconut oil (Copra Indústria Alimentícia, Maceió, AL, Brazil), and vanilla (Dr. Oetker, São Paulo, SP, Brazil) were purchased from a local market in Campinas, SP, Brazil. The PreticX™, a prebiotic ingredient from non-genetically modified corn (Xylooligosaccharides, DP = 2 to 6), instant gum BB acacia gum, and low-acyl gellan gum (Kelcogel® F) were sourced by AIDP Inc. (City of Industry, CA, United States), Nexira Comercial Ltda (São Paulo, SP, Brazil), and Kelco Biopolymers (San Diego, CA, United States), respectively. "Fazenda Lagoa" (Ponte Alta do Tocantins, TO, Brazil) donated the unripe genipap fruits used to produce the natural blue colorant.

The almond extract used in the beverage formulation was produced using 50 g of peeled almond previously soaked in water for 12 h at 7 °C. The almonds were ground and homogenized with 450 g of ultrapure water in a 400 W mechanical RI1364/07 processor (Philips Do Brasil LTDA, Varginha, MG, Brazil) for 3 min. The almond extract was separated from the solid material by filtration. The blue almond-based colorant was produced from unripe *Genipa americana* L. pulp and concentrated almond extract according to our previous study (Strieder, Neves, Silva, & Meireles, 2021).

2.2. Formulation and proximal characterization of almond-based functional beverage

The almond-based functional beverage was prepared from almond extract (82.95 g/100 g), blue almond-based colorant (7 g/100 g), xylitol (5 g/100 g), acacia gum (2.5 g/100 g), coconut oil (1 g/100 g), xylooligosaccharides (1 g/100 g), vanilla (0.5 g/100 g), and gellan gum (0.05 g/100 g). The acacia gum, previously soaked in almond-based extract for 12 h at 7 °C, was added to coconut oil, xylooligosaccharides, xylitol, blue almond-based colorant, vanilla, and gellan gum. These ingredients were mixed in a RI1364/07 mechanical processor for 30 min.

The non-processed sample, named "0 W", was characterized according to its proximal moisture, fat, and protein content. The moisture content was measured by drying the sample in an air ventilated oven at

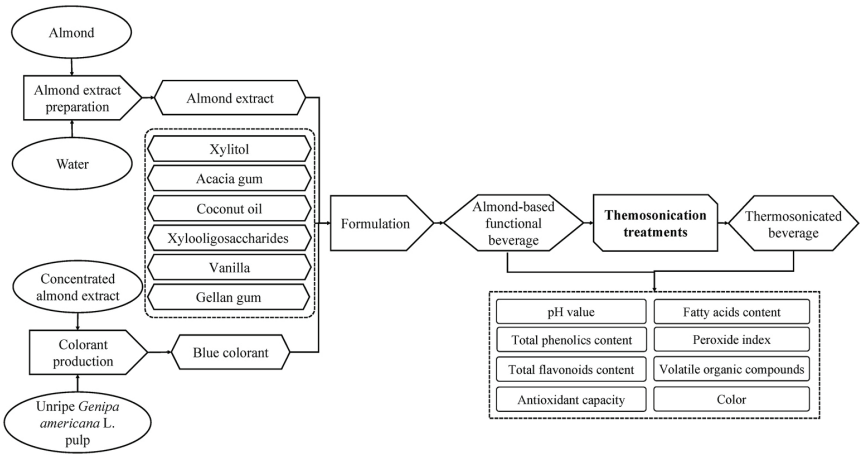

Fig. 1. Obtaining and characterization of thermosonicated almond-based functional beverage.

105 °C for 24 h. The fat content was determined using 2 g of dried samples (105 °C for 24 h) with 150 mL of hexane under condensing temperature. The samples were placed in a paper cartridge to be refluxed for 6 h in a Soxhlet apparatus. The protein content was measured by the Dumas combustion method using a conversion factor of 6.25. The almond-based functional beverage presented 87.3 ± 0.2 g/100 g, 0.8 ± 0.1 g/100 g, and 1.39 ± 0.02 g/100 g of moisture, fat, and protein content, respectively.

2.3. Thermosonication treatments

The almond-based functional beverage was thermosonicated at 50 °C using three levels of acoustic power (4.6, 8.5, and 14.5 W) and holding time (5, 10, and 15 min). The working temperature of 50 °C was chosen because thermosonication processes are classified as sub-lethal (<45 °C) and lethal (>45 °C) (Anaya-Esparza et al., 2017). Thereby, using 50 °C possible thermal degradations promoted by higher temperatures were avoided. For the thermosonication treatments, 30 g of sample were heated to 50 °C in a 50 mL Falcon tube. After the sample reached the temperature, the 13-mm ultrasound probe at 19 kHz was dipped in the Falcon tube center. The acoustic energy was provided for the samples according to the acoustic power and holding time of the assay. The contact height of the beverage with the probe was standardized to 20 mm. The external heat source was kept constant during the thermosonication processes by a jacketed Becker connected to a heating water bath. The graphical abstract presents an illustrative scheme of the system used for thermosonication treatments. All experiments were performed in duplicate, totalizing 18 experiments. Thermosonicated samples were frozen immediately after treatments at −24 °C for further analysis.

The nominal powers of 100, 200, and 300 W provided acoustic powers of 4.6, 8.5, and 14.5 W, respectively. The calorimetric technique described by Mason, Lorimer, Bates, and Zhao (1994) was used to determine the acoustic powers (Strieder et al., 2020).

2.4. Bioactive compounds and antioxidant capacity

The impact of thermosonication treatments on bioactive compounds was evaluated by the total phenolic content and total flavonoid content. The antioxidant capacity was evaluated from the ability of the sample to sequester free radicals using the DPPH and ABTS as chromophore radicals.

2.4.1. Total phenolic content

The total phenolics content was determined using Folin-Ciocalteu colorimetric method according to the methodology described by Arruda et al. (2019) with some modifications proposed by Silva and Saldaña (2020). The samples were mixed with ethanol in the proportion of 1:11 (v:v) using vortex for 3 min and after were centrifuged at 1200 g for 10 min. 300 μL of the supernatant diluted in ethanol, 300 μL of Folin-Ciocalteu reagent, and 2400 μL of sodium carbonate (5 g/100 mL) were mixed. Then, the reaction solution was kept in the dark for 20 min. The absorbance was measured at 760 nm against a blank using a UV-VIS spectrophotometer (Femto 800 XI, São Paulo, SP, Brazil). A calibration curve ($R^2 = 0.99$) using the gallic acid standard from 10 to 90 μg/mL was used to quantify the total phenolic content. The results were expressed as μg gallic acid equivalents per mL of almond-based functional beverage (μg GAE/mL).

2.4.2. Total flavonoids content

The total flavonoid content was determined according to the methodology proposed by Zhishen, Mengcheng, and Jianming (1999). The absorbance of the samples was measured at 510 nm in the spectrophotometer. A calibration curve ($R^2 = 0.99$) using the catechin standard from 20 to 300 μg/mL was used to measure the total flavonoid content. The results were expressed as μg catechin equivalent per mL blue almond beverage (CE μg/mL).

2.4.3. Capacity to sequester free radicals (DPPH)

The capacity of sequester free radicals by samples using DPPH was determined according to Brand-Williams, Cuvelier, and Berset (1995). The absorbance of DPPH that did not react with the sample was read at 517 nm against the blank. A calibration curve ($R^2 = 0.99$) using the Trolox standard from 5 to 45 μg/mL was used to measure the antioxidant capacity. The results were expressed as μmol Trolox equivalent per mL blue almond beverage (μmol Trolox eq. per mL).

2.4.4. Capacity to sequester free radicals (ABTS)

The capacity of sequester free radicals by samples using ABTS was carried out according to Leite et al. (2011). The absorbance of ABTS that did not react with the sample was read at 734 nm against the blank. A calibration curve ($R^2 = 0.99$) using the Trolox standard from 5 to 45 μg/mL was used to measure the antioxidant capacity. The results were also expressed as μmol Trolox equivalent per mL blue almond beverage.

2.5. Fatty acids content

The fatty acids content was determined using a Shimadzu – CG17A gas chromatography (Shimadzu, CG17A, Kyoto, Japan) equipped with a flame ionization detector and split/splitless injector liner both at 250 °C. The samples were automatically injected (1 μL) using a 1:20 split ratio. Helium was employed as carrier gas using 1.05 mL/min. A DB-5 (J&W Scientific, Folsom, USA) capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was operated under programmed temperature as following conditions: 70–180 °C at 3 °C min^{-1} and then until 280 °C at 4 °C min^{-1} totalizing 63.67 min. The quantification of five fatty acids (Lauric, Myristic, Palmitic, Oleic, and Stearic) was performed by preparing a calibration curve using a fatty acid mixture solution from 25 to 120 μg/mL. The identification of each fatty acid was performed by individual injection of them, and the characteristic retention time was used.

2.6. Peroxide index

The peroxide value refers to the content of oxygen as peroxide, especially hydroperoxides in a sample. This parameter indicates the occurrence of oxidation. The peroxide index was determined for the almond-based functional beverage samples according to Ani, Amove, and Igbabul (2018). For this, 2 mL of sample was placed into a 250 mL conical flask, 10 mL of chloroform was added and swirled gently for 30 s. After that, 15 mL of glacial acetic acid and 1 mL of fresh saturated aqueous KI solution were added. The flask was shaken for 1 min and placed for exactly 1 min in the dark. Finally, 5 mL of distilled water was added, mixed, and the mixture was titrated with 0.01 M sodium thiosulfate solution using the soluble starch solution (1 g/100 mL) as an indicator. A blank reagent determination was carried out.

2.7. Volatile organic compounds identification

The identification of volatile organic compounds was carried out in a gas chromatograph (Agilent 7820A, Santa Clara, USA) coupled to a mass spectrometer. (Agilent 5977E, Santa Clara, USA). The samples were homogenized with ethyl ether (1:10 v/v) using a vortex, and they were centrifuged at 1200 g for 10 min. The supernatants resulting from the centrifugation were injected into the chromatograph. Thus, the ethyl ester acids were identified from the sample profiles of volatile compounds using patterns.

2.8. Color stability

The beverage's color stability was evaluated through its visual appearance, color parameters (L, a*, and b*), and browning index. Color

parameters were measured in a Hunter Lab colorimeter (CR-400, Konica Minolta, Ramsey, NJ, USA) at room temperature. The color measurement was carried out by transferring 1 mL of the sample to a white container (20 mm in diameter). The results were expressed in the values of L (luminosity), a * (red-green), and b * (blue-yellow). The browning index (BI) was also determined for each sample according to Maskan (2001) by Equations (1) and (2).

$$BI = \frac{[100(x - 0.31)]}{0.172} \quad (1)$$

$$x = \frac{(a^* + 1.75L)}{(5.645L + a^* - 3.012b^*} \quad (2)$$

2.9. Statistical analysis

The effects of thermosonication treatments on the almond-based functional beverage were verified by analysis of variance (ANOVA) at a 95% of confidence level (p-value ≤ 0.05) using Minitab 18® software (Minitab Inc., State College, PA, USA).

3. Results and discussion

The thermosonication treatment inactivates microorganisms through the combined effect between acoustic cavitation provided by high-intensity ultrasound and heat provided by an external source. Acoustic cavitation promotes the sonoporation of the cell membranes and organelles by shear stress. This effect weakens or ruptures the cells releasing their internal content (Bermudez-Aguirre, 2017, pp. 15–37). Thereby, a lethal or sub-lethal effect on microorganism cells and spores is observed. However, acoustic energy is not enough to inactivate all microorganisms and their spores found in food products (Huang et al., 2017). In this regard, thermal energy at low temperatures has been associated with acoustic energy to favor microbial inactivation. Therefore, many studies have evaluated the effects of thermosonication treatments on the inactivation of microorganisms found in beverages. Manzoor et al. (2021) observed that almond beverages thermosonicated at 45 °C for 40 min and 60 °C for 10–40 min exhibited no detectable microbial growth in total plate count and yeast and mold count. Furthermore, all thermosonication treatments evaluated by them (30–60 °C and 10–40 min) reduced more the microbial load compared to the pasteurized beverage at 90 °C for 1 min. However, their beverage samples were thermosonicated in an ultrasonic bath at 40 kHz applying a nominal power of 600 W. In contrast, our study evaluated the impact of a 13 mm-probe ultrasound at 19 kHz to perform thermosonication treatments. Thus, the acoustic energy applied by us was higher than that used by them since we applied the ultrasound waves at a lower frequency and in a smaller volume of sample. Some studies have been demonstrated that ultrasound lower frequencies produce larger bubbles (greater amplitude), which results in higher temperatures and pressures in the cavitation zone. In this way, lower frequencies have promoted higher shear intensity on the cell membranes and organelles of plants favoring the recovery of phytochemical compounds (Chukwumah, Walker, Verghese, & Ogutu, 2009; Machado et al., 2019). The effects of the ultrasound frequency have not been evaluated on microbial inactivation. However, higher shear stress promoted by lower frequencies may weaken more the cellular membranes of microorganisms. Thus, the thermosonication treatments performed by us applying 100, 200, and 300 W at 19 kHz and 50 °C for 5, 10, and 15 min may have promoted the same or greater effect on the inactivation of microorganisms. Moreover, thermosonication processes carried out at temperatures higher than 45 °C are classified as lethal (Anaya-Esparza et al., 2017).

Thermosonication treatments have been also evaluated to improve the sensory properties of products that need to undergo thermal treatments. Indeed, some studies verified that thermosonicated products have better sensory characteristics than thermally treated products (Alcántara-Zavala et al., 2020; Manzoor et al., 2021). Therefore, our research aimed to evaluate other effects of thermosonication processing on the almond beverage characteristics since this is an emerging technology for beverage treatment.

3.1. Thermosonication thermal history

Acoustic energy and moderate heat treatment are coupled for the application of thermosonication treatment. In this way, the thermal energy in thermosonication processes is supplied for samples by two means. The acoustic energy also promotes an increase in the temperature of the liquid medium. Thus, the heat supplied to samples is an association of the thermal energy constantly sourced by the heating water bath and acoustic cavitation. Fig. 2 presents the temperature profiles for each thermosonication treatment. The initial temperature of the beverage was standardized to 18 °C. In the first 6.5 min of the holding time, the sample was heated to the working temperature of 50 °C. After this initial heating, depending on the acoustic power and the holding time employed in the thermosonication process, different temperature profiles were observed.

The acoustic power of 4.6 W was the only one that maintained the working temperature of 50 °C, regardless of the holding time. Thermosonication treatments performed at 8.5 W and 14.5 W had their working temperatures increased in the holding time function. The acoustic cavitation promoted the temperature increased in these processes. The formation and subsequent collapse of cavitation microbubbles in the liquid medium promote shocks of molecules, leading to an increase in temperature (Farid Chemat et al., 2017). The greater ΔT (maximum temperature – working temperature) was observed for thermosonication treatments carried out at 14.5 W. For this acoustic power, ΔT values of 18.3, 23.5, and 24.9 °C were observed for the holding times of 5, 10, and 15 min, respectively. In this way, the process carried out at 14.5 W for 15 min also reached the highest working temperature (75 ± 1 °C). Thus, an increase in temperature of up to 25 °C was observed in the thermosonication treatments, demonstrating the relevance of monitoring of thermal histories in food processing due to critical changes in nutritional and sensory attributes of thermally treated foods and beverages.

3.2. pH value

The pH value was 6.41 ± 0.01 for control sample (0 W) and was of 6.35 ± 0.01, 6.36 ± 0.01, and 6.39 ± 0.02 for the samples treated at 4.6 W, 6.35 ± 0.01, 6.35 ± 0.01, and 6.38 ± 0.01 for 8.5 W, and 6.34 ± 0.01, 6.32 ± 0.01, and 6.33 ± 0.01 for 14.5 W for the holding times of 5, 10, and 15 min, respectively. The interaction between acoustic power and holding time had no significant effect on pH (p-value = 0.063). However, the linear effects of acoustic power (p-value = 0.001) and the holding time (p-value = 0.014) promoted significant change on the pH value. The almond-based functional beverage had its pH value decreased after thermosonication treatments. The smaller pH values were observed for the beverage treated at 14.5 W. The same pH behavior was observed for the fruit smoothie after its thermosonication treatment, as reported by Amador-Espejo et al. (2019). The thermosonication parameters of amplitude and holding time significantly reduced the pH of the fruit smoothie samples. The authors treated the samples using a probe ultrasound system at 20 kHz and 1500 W. They studied the amplitudes (70–85), holding time (15–25 min), and temperature (40–55 °C). The effect observed for them in the pH values was due to the acoustic cavitation because the thermosonication temperature had no significant impact. Therefore, the reduction of pH values in the almond-based beverage can be associated with the acoustic cavitation provided by ultrasound. O'Sullivan, Arellano, Pichot, and Norton (2014) observed that sonication treatments using an ultrasonic probe system at 20 kHz and intensity of 34 W/cm^2 for 0, 15, 30, 60, and 120 s reduced the pH values of the sodium caseinate, whey protein, and milk protein

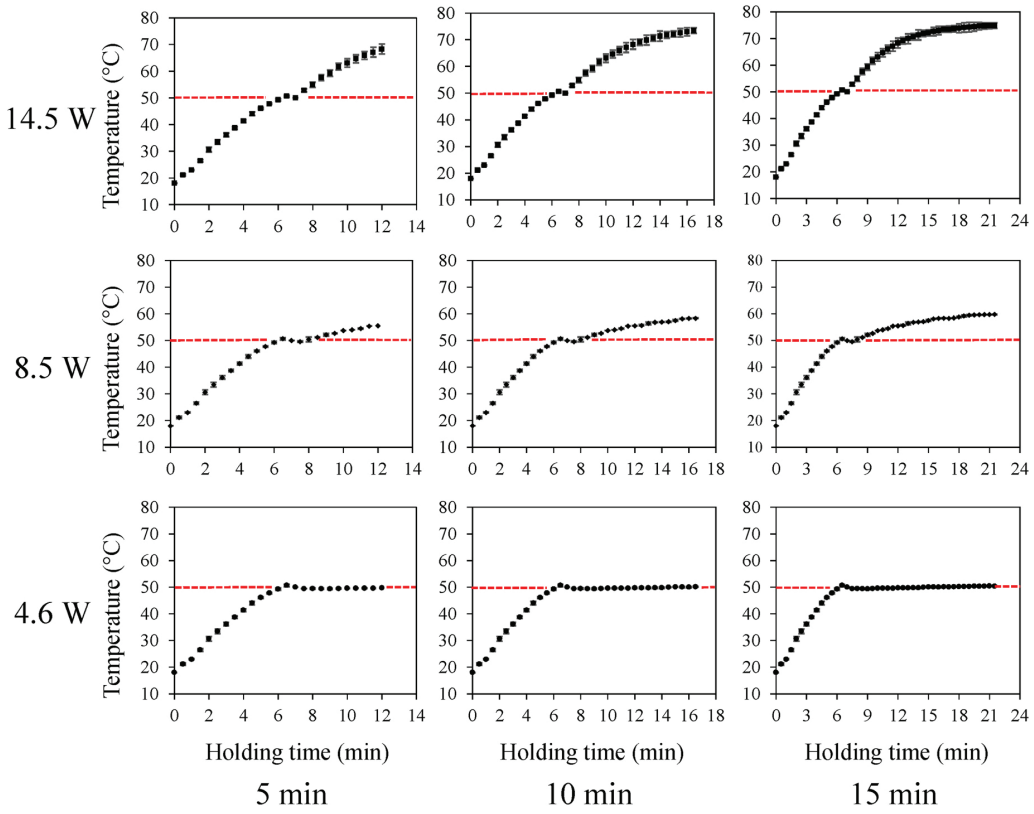

Fig. 2. Thermal histories of the thermosonication treatments applied to the almond-based functional beverage. The red dotted line on the graph represents the moderate heat treatment supplied by the external source. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

samples. The decrease in the pH value of the protein samples was observed as the time of ultrasound treatment increased. These reductions could be associated with the exposure of acidic amino acid residues that were contained within the aggregated structure of the protein micelles before the sonication treatment. Thus, the decrease in pH values of the almond-based functional beverage may be associated with the acoustic cavitation effects on the protein molecules. Almond proteins have an isoelectric pH range from 4.5 to 5.5 (Li & He, 2004). This pH reduction may not have promoted changes in the technological characteristics of the beverage.

3.3. Bioactive compounds and antioxidant capacity

The bioactive compounds found in plant beverages are responsible for their nutritional value bringing more healthiness to their consumers. Thus, the preservation of these compounds after microbiological and enzymatic stabilization treatments is very important to ensure the bioactivity of these beverages. Table 1 exhibits the thermosonication impacts on bioactive compounds and antioxidant capacity of the almond-based functional beverage.

The interaction between acoustic power and holding time (p-value = 0.7), as well the linear effect of power (p-value = 0.8) and holding time (p-value = 0.6) did not promote significant effects on the total phenolic content. Despite that, the thermosonicated samples had their total phenolic content reduced to 10% compared to the untreated beverage (control sample). This slight loss in phenolic compounds may be associated with the moderate heat treatment and the acoustic energy provided to the samples. Rawson et al. (2011) also observed a similar effect

Table 1
Thermosonication effects on bioactive compound content and antioxidant activity of the almond-based functional beverage.

Thermosonication process conditions		Total phenolic content (GAE μg/mL)	Total flavonoids content (CE μg/mL)	DPPH (μmol Trolox eq. per mL)	TEAC (μmol Trolox eq. per mL)
Acoustic power (W)	Holding time (min)				
0	0	657 ± 8	2599 ± 356	65.4 ± 0.1	168 ± 3
4.6	5	602 ± 8	6288 ± 237	56 ± 14	148 ± 20
	10	552 ± 16	6246 ± 415	70 ± 14	168 ± 43
	15	602 ± 39	6037 ± 119	62 ± 14	176 ± 11
8.5	5	552 ± 94	6749 ± 59	33 ± 4	167 ± 17
	10	580 ± 23	6288 ± 119	62.2 ± 0.1	196 ± 2
	15	574 ± 16	5743 ± 178	63 ± 9	180 ± 4
14.5	5	591 ± 8	5030 ± 949	64 ± 3	197 ± 16
	10	558 ± 39	5869 ± 237	55 ± 11	180 ± 8
	15	585 ± 47	5869 ± 356	53 ± 13	173 ± 6

of the thermosonication treatment in their watermelon juices. They used a 1500 W ultrasonic processor with a 19 mm diameter probe at 20 kHz. Temperatures (25–45 °C), amplitudes (24.4–61.0 μm), and holding times (2–10 min) were evaluated. The temperature had a significant effect on phenolic compounds degradation. A phenolic content decrease was verified as the temperature was increased from 25 to 45 °C. This effect was more pronounced at longer holding times. In our study,

although all the treatments were carried out at temperatures equal to or greater than 50 °C, the acoustic power or holding time did not generate higher phenolic compound degradation (Fig. 2). This difference may be associated with the highest acoustic energy employed by them to treat melon juice.

Additionally, the almond-based beverage is a colloidal system that may have better protected phenolic compounds by encapsulation (Lu, Kelly, & Miao, 2016). Atalar et al. (2019) observed an increase in total phenolic compounds of their thermosonicated samples of hazelnut milk. The phenolic compounds are incorporated into the vacuole in soluble form or bond to cell walls of pectin, cellulose, hemicellulose, and lignin. Thus, they attributed the increase in the phenolics content in thermosonicated samples to the release of bound phenolics. The acoustic cavitation promotes the rupture of the cell walls facilitating the release of bound compounds. Thus, despite the reduction in the phenolic compounds content in the samples, this was small and may be due to the degeneration of free compounds and the release of an amount bound to the cell wall.

The total flavonoid content increased after the thermosonication treatments (Table 1). However, the interaction between acoustic power and holding time (p-value = 0.08) and the linear effect of the holding time (p-value = 0.6) did not significantly affect the total flavonoids. In contrast, the acoustic power, impacted the flavonoids content (p-value = 0.03). The samples processed at 14.5 W presented lower values than the samples processed with the other acoustic powers. This difference may be related to the effects of acoustic cavitation and the temperatures that each treatment reaches (Fig. 2). Souza et al. (2019) also observed an increase in the content of flavonoids in the camu - camu nectar after most of the thermosonication treatments were studied by them. However, the treatment at 60 °C/60 min of nectar did not increase its flavonoid content. Despite that, this sample maintained the same flavonoid content as the untreated sample. Therefore, thermosonication treatments increased the release of flavonoids, increasing their content in the beverage. The acoustic cavitation promotes the rupture of the cell walls facilitating the release of bound compounds. Acoustic cavitation due to microshearing can release these compounds that are bonded with cell walls. However, higher thermosonication temperatures can reduce the content of these compounds due to thermal degradation.

The DPPH and TEAC values for thermosonicated samples were similar to those obtained for the untreated beverage. Moreover, the interaction between process variables (acoustic power and holding time) and their linear effects have not affected the results of DPPH (p-value $\geq$ 0.2) and TEAC (p-value $\geq$ 0.2). Thereby, the antioxidant capacity of thermosonicated beverages may have been maintained due to increased flavonoid content in contrast to decreased total phenolics (Table 1). Souza et al. (2019) observed that most thermosonication treatments evaluated by them promoted an increase in the nectar antioxidant capacity. Only the treatment at 40 °C/60 min promoted a reduction in the DPPH value of the nectar compared to the control sample. According to them, the acoustic energy provided by thermosonication can provide these increases and decreases in the antioxidant capacity of the nectar. Acoustic cavitation can favor the bioavailability of bioactive compounds in the sonicated medium. On the other hand, acoustic cavitation can promote the production of hydroxyl radicals that can oxide phenolic compounds. Yıkmış, Aksu, Çöl, and Alpaslan (2019) observed a linear increase in quince juice antioxidant values of DPPH and CUPRAC as temperature, duration, and amplitude of thermosonication treatment increased. They thermosonicated the juice at 26 kHz at different holding times (2–10 min), temperatures (30–50 °C), and amplitudes (40–60%). The best results were obtained by the treatment parameters of 50%, 40 °C for 6 min, and 50%, 40 °C for 6 min. The release of antioxidant compounds from the quince during thermosonication promoted the higher juices antioxidant activity. The disintegration of the cell wall caused by the pressure created by cavitation during thermosonication favored this release.

3.4. Lipid stability

Almond beverages contain polyunsaturated fatty acids that are susceptible to several oxidation mechanisms by the presence of oxygen, light, high temperature, and others. Besides that, sonication processing can also promote lipid oxidation due to cavitational microshearing. Acoustic cavitation promotes the reduction of fat globules, promoting better homogenization and kinetic stability in samples containing lipids (Balthazar et al., 2019; Strieder et al., 2020). On the other hand, the cavitational bubbles microjets may also promote some undesirable effects as lipid oxidation.

Fig. 3 shows the fatty acids content quantified in samples mostly provided from almond extracts and coconut oil. The main fatty acids found in almond-based functional beverage samples were oleic acid, followed by stearic, lauric, palmitic, and myristic acid. According to Maguire, O'sullivan, Galvin, O'connor, and O'brien (2004), oleic acid is the main fatty acid present in almonds, followed by palmitic, stearic, and myristic. Coconut oil contains more lauric acid as well as palmitic, oleic, stearic, and myristic acid. Thermosonication treatments at 4.6 and 8.5 W increased the content of oleic, lauric, and myristic acid in the samples (p-value < 0.001) (Fig. 3). The content of stearic and palmitic acid was reduced in some of these treatments. However, all fatty acids had their amount reduced in the samples at 14.5 W for 10 and 15 min. Chemat, Grondin, Sing, and Smadja (2004) also observed that the fatty acids content in sunflower oil was modulated due to the ultrasound processing at 20 and 60 °C for 1 h. According to them, this variation may indicate oil degradation by acoustic cavitation. Lipid oxidation occurs by the degradation of triglycerides in free fatty acids, hydroperoxides, and peroxyl free radicals and, later, amides, alcohols, aldehydes, hydrocarbons, and ketones. Oxygen, heat, time, light, water, and catalysts such as trace metal ions, metalloproteins, and inorganic salts favors oxidation. Thus, lipid oxidation in the samples can be caused by thermosonication treatments by thermal and sonochemistry degradation. The high temperatures achieved during thermosonication processing and the free radicals generated can favor lipid oxidation in samples. Therefore, thermosonication treatments performed at 4.6 and 8.5 W were better to preserve the fatty acid content in samples. The thermosonicated samples in any holding time remained lauric and oleic acid content. Among saturated fatty acids, lauric acid was associated with lower fat accumulation. Furthermore, antimicrobial activity against bacteria, fungi, and viruses was reported for the lauric acid (Dayrit, 2015). Oleic acid is an essential fatty acid that has been used to reduce cardiovascular risk by reducing blood lipids, mainly cholesterol.

The thermosonication treatments did not promote the formation of hydroperoxides in the samples, although there were degradations in fatty acids. The samples did not present hydroperoxides before and after the thermosonication treatments. Valencia-Flores et al. (2013) studied high-pressure homogenization treatment for evaluating its effect on the microbiological, physical, and chemical quality of almond beverages. They observed a higher peroxide value for their samples treated by high-pressure homogenization than their untreated or thermal-treated samples. They applied 200 and 300 MPa at 55, 65, and 75 °C. Our study used practically the same working temperatures, but the holding times we used were shorter. Thus, shorter holding times and less exposure to heat may have prevented the production of these oxidation compounds in the almond-based beverage samples.

3.5. Volatile organic compounds

Ethyl ester acids are natural flavorings that contribute to the formation and enhancement of food flavorings. Ethyl octanoate, for example, presents a sour apple aroma, and ethyl decanoate a floral odor (Saerens et al., 2008). Thus, the loss of these can de-characterize the product's flavor. In this sense, we evaluated the maintenance of these compounds in the samples after thermosonication treatments. Table 2 present the ethyl ester acids identified in samples. Octanoic, decanoic,

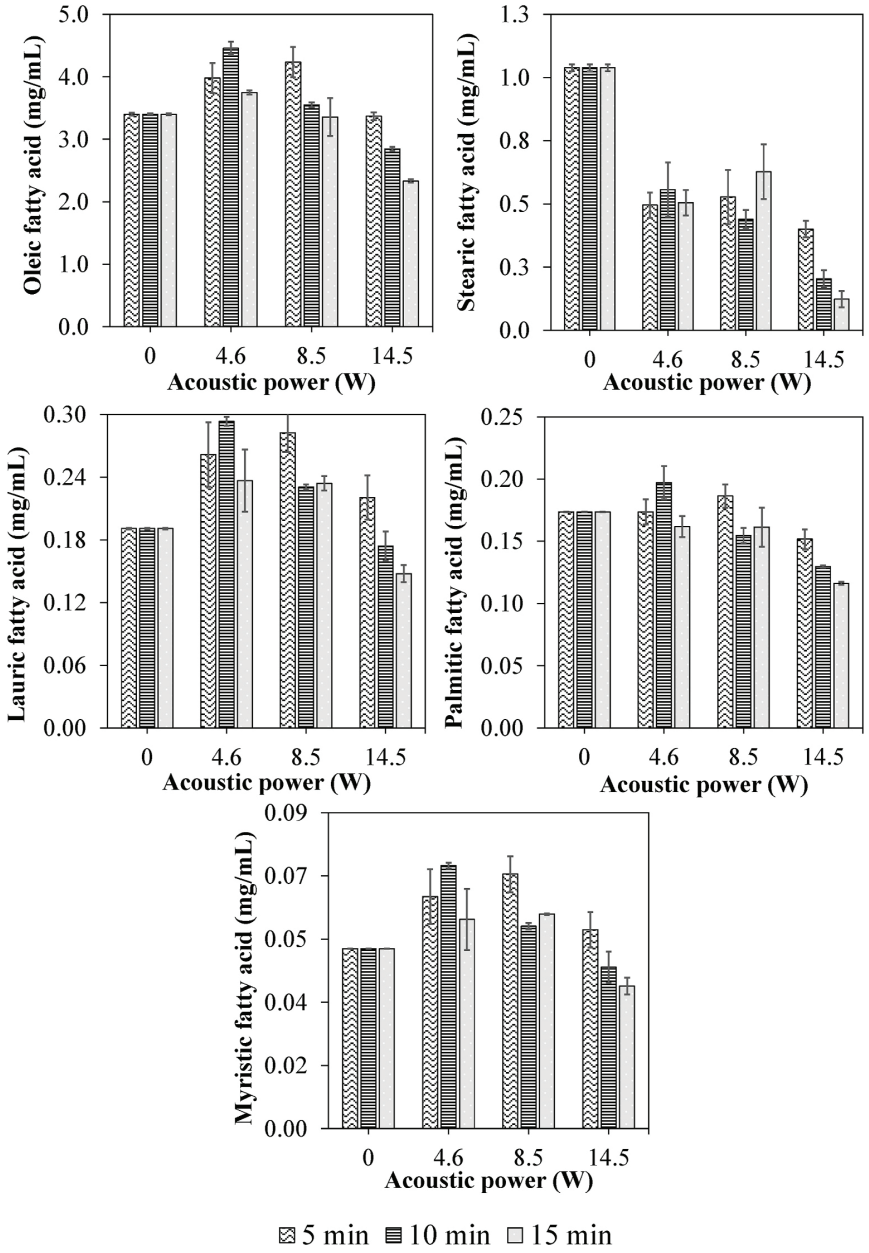

Fig. 3. Thermosonication effects on the fatty acid content (oleic, stearic, lauric, palmitic, and myristic acid).

dodecanoic, heptadecanoic, e-11-hexadecenoic, linoleic, and e-9-octadecanoic ethyl ester acids were identified in the untreated beverage. The treatments carried out at 4.6 W kept all the esters according to the control sample. The thermosonication treatments performed at 8.5 W and 14.6 for 5 min only did not maintain the heptadecanoic ester. However, the samples processed at 14.5 W for 10 and 15 min presented more significant ethyl ester acid losses. The sample thermosonicated for 15 min only presented the octanoic and e-9-octadecanoic acids. The loss of these compounds may be associated with the temperatures reached in thermosonication processes (Fig. 2). The heat treatment in the most intense thermosonication treatments may have promoted the thermal degradation of these compounds. Dayrit et al. (2011) evaluated the headspace of coconut oils by quantifying the contents of volatile organic compounds. The octanoic and dodecanoic ethyl esters were the compounds quantified. Additionally, they observed that different treatments to obtain coconut oil (centrifuge, expeller, fermentation without heat, and with heat) caused different effects on volatile organic compounds. The fermentation without heat, for example, produced an oil with a higher octanoic acid content than the fermentation with heat. Thus, in our study, the heat supplied by the thermosonication treatments may have been the cause of the loss of ethyl ester acids.

Table 2
Thermosonication effects on the ethyl ester acids of the almond-based functional beverage.

Thermosonication process conditions		Octanoic RT: 13.95 RI: 1196	Decanoic RT: 22.197 RI: 1396	Dodecanoic RT: 30.01 RI: 1595	Heptadecanoic RT: 37.256 RI: 2094	E-11-Hexadecenoic RT: 43.059 RI: 1974	Hexadecanoic RT: 43.756 RI: 1993	Linoleic RT: 48.715 RI: 2162	E-9-Octadecnoic RT: 48.91 RI: 2174
Acoustic power (W)	Holding time (min)								
0	0	X	X	X X		X	X	X	X
4.6	5	X	X	X X		X	X	X	X
	10	X	X	X X		X	X	X	X
	15	X	X	X X		X	X	X	X
8.5	5	X		X		X		X	X
	10	X		X		X	X	X	X
	15	X		X		X	X	X	X
14.5	5	X		X		X	X	X	X
	10	X		X				X	X
	15	X							X

3.6. Color stability

Color is an important characteristic of food products. It can awaken people's different expectations. Blue food products have been increasingly explored since they attract consumers' attention, mainly children. Thus, the thermosonication effects on the color of the beverage were evaluated because we added in its formulation a natural blue colorant previously produced by our research group (Strieder et al., 2021). The blue colorant presented the values of 23 ± 2, −0.23 ± 0.04, and −11.4 ± 0.8 for the L, a*, and b* color parameter, respectively. The visual appearance, color parameters, and browning index of the almond-based functional beverage before and after thermosonication treatments are exhibited in Figs. 4 and 5. Visually, all samples presented a blue color with different shades (Fig. 4). Additionally, the samples thermosonicated for 5 min presented a lighter color. Luminosity parameter (L) also was higher for samples obtained employing 5 and 15 min (p-value = 0.047) (Fig. 5). The untreated sample presented the smaller values of the a* parameter. This value was raised in the samples in function of the holding time (p-value = 0.002) and acoustic power (p-value = 0.02), but the interaction between the two process variables did not affect the a* parameter (p-value = 0.5). Similar behavior was observed for the b* parameter values. The untreated sample presented a higher b* value. It was decreasing along holding time (p-value = 0.001) and increasing with the acoustic power raise (p-value < 0.001). The interaction between the two variables also did not affect the b* parameter (p-value = 0.1). Thus, the sample thermosonicated at 14.5 W and 15 min showed the bluest color (lowest values of b*) and the most reddish color (highest value of a*). The heat provided in this treatment may have favored this result. The previously obtained natural blue colorant is produced through a crosslinking between genipin provided by unripe genipap pulp and primary amines provided by almond extracts (Strieder et al., 2021). The formation of blue complexes is favored by heat and oxygen. Thus, the more prolonged thermosonication treatment, which also provided the highest processing temperature (Fig. 2), may have favored the crosslinking of genipin with amines, intensifying the blue color of the almond beverage. The acoustic power increase also promoted lower BI values (p-value = 0.01). Thus, higher acoustic powers produced fewer brown compounds in the beverage (Maskan, 2001). The higher temperature achieved for the thermosonication treatment at 14.5 W could favor non-enzymatic browning associated with complex melanin substances due to the Maillard reaction. Despite that, thermosonication treatments at high acoustic power and longer holding time promoted better results of color in the almond-based beverage.

4. Conclusion

Our results demonstrated that thermosonication treatments preserved the antioxidant activity of the almond-based functional beverage, even with an increase of up to 25 °C in working temperature due to acoustic cavitation. The thermosonication treatments promoted the

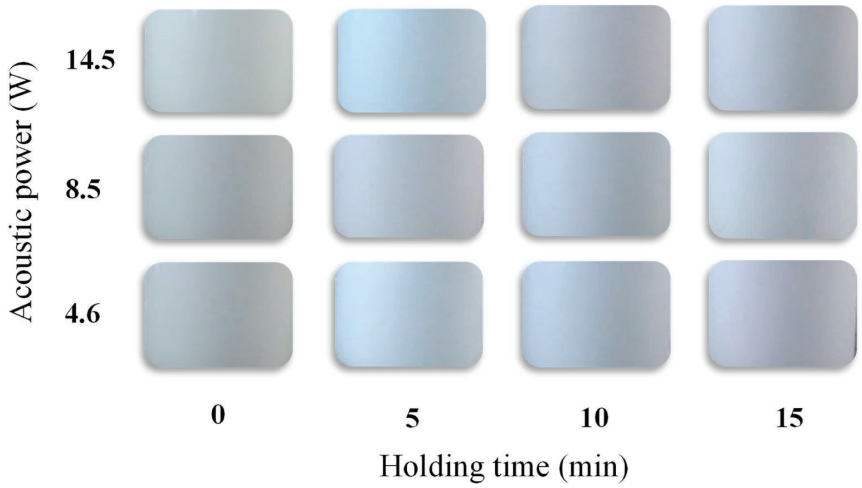

Fig. 4. Thermosonication effects on the visual appearance of the almond-based functional beverage.

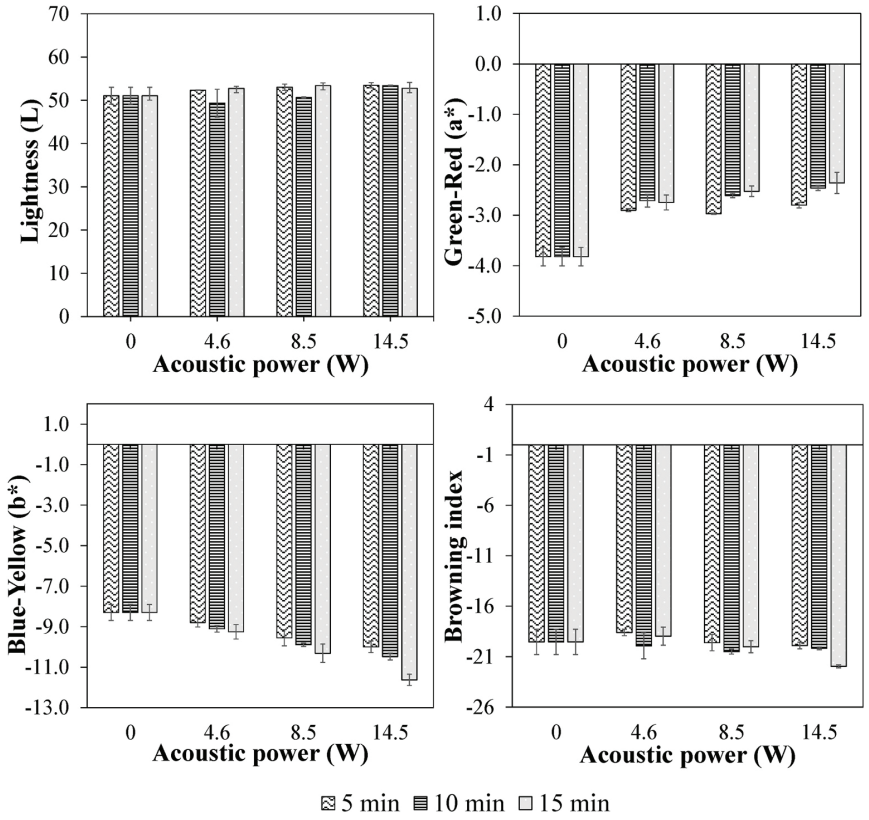

Fig. 5. Thermosonication effects on the color parameters L, a*, and b* and on the browning index of the almond-based functional beverage.

release of some bioactive compounds that were bound. Likewise, the degradation of others that were free. The more intense thermosonication treatments (higher acoustic power for longer holding time) degraded stearic and palmitic fatty acids. However, the peroxide index of the beverage has not changed. These more intense thermosonication treatments also degraded volatile organic compounds modifying the natural flavor of the beverage. Furthermore, all treatments intensified the blue color of the almond-based beverage maintaining its sensory appeal concerning color attributes.

CRediT authorship contribution statement

Monique Martins Strieder: Investigation, Formal analysis, Writing – original draft. **Maria Isabel Landim Neves:** Investigation, Formal analysis. **Joao Raul Belinato:** Methodology, Validation. **Eric Keven Silva:** Conceptualization, Supervision, Project administration, Visualization, Writing – review & editing. **Maria Angela A. Meireles:** Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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CHAPTER 8

General discussion

General discussion

The plant material chosen for this thesis elaboration was unripe genipap pulp. This Brazilian fruit presents a rich composition in iridoids that can produce innovative food products. Our research group had already studied this raw material as a source of phytochemicals and coloring compounds. However, high-intensity ultrasound technology had not yet been evaluated for obtaining products from genipap. Thus, our initial idea had been to perform a genipap biorefinery using ultrasound technology to obtain colorant and starch from the unripe fruits. However, in the first stage of this study, we realized that obtaining the colorant from the fruit still had many gaps to be studied.

In this regard, **Chapter 1** introduced genipap as a plant matrix rich in genipin, an iridoid that presents beneficial health effects for the human body. Moreover, the applications of genipin as a crosslinker and colorant were presented. Among the emerging technologies that can be used to obtain products from genipap, high-intensity ultrasound was highlighted. This green technology, through acoustic cavitation, favors mass transfer in liquid media, enabling a series of applications in food processes. Besides that, the application of acoustic energy increases the medium temperature, which may favor the crosslinking of genipin with the primary amines to form the blue-colored compounds. Thus, we concluded that high-intensity ultrasound technology could provide the energy to produce blue natural colorants and other products from unripe genipap fruits. Thereby, we proposed the objectives also presented in **Chapter 1**. We planned to write two reviews to address all topics studied in this thesis. In the first one, we searched and discussed the process variables of ultrasound-assisted extractions to obtain natural food colorants. In contrast, our second review paper examined the recent advances, trends, challenges regarding ultrasound processing applied to dairy, meat, bakery, minimally processed products, beverages, and food ingredients. These reviews supported our experimental planning in which we proposed to evaluate the design of high-intensity ultrasound processes to obtain a genipin ethanolic extract, blue colorants using milk and plant extracts, and an almond-based beverage.

Chapter 2 presents the review article "**Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants"** published in the ***Food and Public Health*** journal. This article was developed using a PDSA improvement cycle in the discipline of "Tópicos em Engenharia de Alimentos" during the second semester of 2018. We searched and discussed the variables of ultrasound-assisted extractions to obtain natural food colorants. In this sense, we observed a non-standardization in

reporting the variables used in the ultrasound-assisted extractions. Some researches did not present the main variables as nominal power, extraction time, and sample mass used in the procedures. Since the acoustic cavitation can be affected by the frequency, power and sample employed in the process, the results cannot be reproducible if using different parameters as devices and power supply. Thus, we proposed expressing the energy supplied by ultrasound processes through specific energy or energy density as some authors have proposed (Arruda, et al., 2019; Rajha, Boussetta, Louka, Maroun, & Vorobiev, 2014; Silva, et al., 2018). This way, the power, processing time, and sample mass applied in the ultrasound processes will be informed. Furthermore, we reviewed the operational conditions studied for extracting colorants from plant sources. In this sense, we also observed a lack of information regarding the temperature used in the extraction processes. Considering that acoustic cavitation increases the temperature of the medium, this information is also relevant. Therefore, from this review, we could observe and understand the process variables and control them to carry out the ultrasound-assisted extractions to obtain standardized processes.

The second review article presented in **Chapter 3** "**Advances and innovations associated with the use of acoustic energy in food processing: An updated review,**" was published in the ***Innovative Food Science and Emerging Technologies*** journal. This review, written between 2020 and 2021, presents the more recent applications of acoustic cavitation on the processing of dairy, meat, bakery, beverage, minimally processed products, and food ingredients. We observed that ultrasound applied at different conditions by different ultrasonic systems had provided products with unique nutritional and sensory characteristics. Besides that, the association of acoustic and thermal energies through thermosonication treatments has provided milder processes, keeping the bioactive compounds present in food products. Few studies have investigated the impact of ultrasound processing on the sensory attributes of foods and beverages. However, ultrasonicated products maintained or showed better sensory characteristics in most evaluations. Additionally, the scale-up of the ultrasound processes seems more viable as several companies have started to manufacture the equipment for larger scales. In this regard, further optimization and economic feasibility studies need to be carried out to facilitate further scale-up. Thus, from this review, in addition to reviewing process conditions that favored obtaining high-quality products by protecting bioactive compounds, we were able to observe gaps in the literature for future studies.

The first experimental study presented in **Chapter 4** "**A techno-economic evaluation for the genipin recovery from *Genipa americana* L. employing non-thermal and thermal high-intensity ultrasound treatments,**" was published in ***Separation and Purification Technology***. In this, we did a techno-economic evaluation of non-thermal (100 W) and thermal (450 W) ultrasound-assisted extraction processes for obtaining a genipin ethanolic extract from unripe genipap. We employed the same specific energies (1, 3, and 5 kJ/g) at different nominal powers (100 and 450 W) and processing times to observe the effects of the acoustic cavitation applied at the same energy amount (same specific energy) but at different power. For the extraction, ethanol was used since this is a GRAS (generally recognized as safe) solvent and had already been used for genipin extraction by other solid-liquid extraction techniques. Thereby, first, we performed preliminary tests to select the better ratio between ethanol and genipap mass (S/F). These results are presented in **Appendix I**. We selected the S/F of 5 because this ratio provided a good yield of genipin by the non-thermal (100 W) and thermal (450 W) extraction processes. Regardless of the specific energy, the thermal treatments were better to extract genipin from unripe genipap fruits. Thus, the heat provided by 450 W processes favored the diffusion of genipin from the fruit to ethanol. Besides that, genipin showed to be a thermostable (until about 75 °C) and stable compound in ethanol for 72 h at 24 ± 2 °C. We also determined the cost of obtaining extracts at different scales, demonstrating the economic feasibility of the extraction process using the proposed systems. Thermal processes promoted by ultrasound showed to be economically more advantageous than the non-thermal ones. Additionally, ultrasound treatments presented a faster and fixed capital investment two times cheaper than a process using pressing + low-pressure extraction. The experimental article 1 raw data and the statistical results of the data were presented in **Appendix II** to **VI**.

Despite our extract presenting a slightly negative b* value (**Appendix II**) indicating the blue color, we cannot consider it a blue colorant. Some studies also report that they obtained a blue colorant from the extraction of genipin using ethanol or water. However, the blue compounds obtained from unripe genipap fruits result from the crosslinking between genipin and primary amine groups (Touyama, et al., 1994). Thus, our ethanolic extract presented a blue color because the genipap fruits contain a small number of proteins. In this way, free genipin crosslinked with the amino groups of these proteins, forming blue-colored compounds. However, the genipap fruits present a low protein content, limiting crosslinking and the formation of blue compounds in the ethanol

extract. Therefore, the later addition of primary amines groups to the extract is necessary to obtain a blue colorant from a genipin aqueous or ethanolic extract. In this sense, in the following experimental steps of the thesis, we planned to use solvents that naturally contain proteins.

Thereby, the second experimental article presented in **Chapter 5** was "**Low-frequency and high-power ultrasound-assisted production of natural blue colorant from the milk and unripe** *Genipa americana* L." published in the ***Ultrasonics Sonochemistry*** journal. In this study, we evaluated the ultrasound-specific energy (0,06, 0,10, 0,17 e 0,25 kJ/g) on producing a blue colorant from the crosslinking between genipin and milk proteins. For this, we used the colloidal system of milk as a solvent, source of primary amines for crosslinking reaction with genipin, and carrier of the blue-colored compounds produced. One-step acoustic cavitation assisted the genipin extraction from the unripe genipap and its diffusion into the colloidal milk system favoring its crosslinking with milk proteins. Ultrasound process intensification by increasing the nominal power (100 to 400 W) promoted higher genipin recovery resulting in bluer colorants. However, the higher temperature achieved for the nominal power of 400 W (84 ± 1 °C) could favor the occurrence of non-enzymatic browning associated with complex melanin substances due to the Maillard reaction. Thus, the best nominal power evaluated to obtain the blue colorant was 300 W. Nevertheless, all colorants acquired a blue color after 96 h of cold storage, even the obtained at 200 W (**Appendix VII**). That happens because the crosslinking reaction between genipin and primary amines is catalyzed by heat and oxygen. Thereby, crosslinks continue to occur in extracts with high protein content after ultrasound processing. **Appendix VIII** also demonstrated that the pH value of the colorants was almost unchanged after 48 h of cold storage. Additionally, some experimental raw data of the second experimental article were presented in **Appendix IX** to **XII**.

Likewise, plant proteins can replace dairy proteins for producing this natural blue colorant considering the current demand for plant-based products. Therefore, the third experimental paper presented in **Chapter 6** was "**Impact of thermosonication pretreatment on the production of plant protein-based natural blue colorants,**" published in the ***Journal of Food Engineering***. In this study, we evaluated the thermosonication pretreatment and four plant extracts, *Oryza sativa* L., *Avena sativa* L., *Arachis hypogaea* L., and *Prunus dulcis* effects on the crosslinking between genipin and plant proteins for the production of natural blue colorants. First, we performed some tests

using commercial plant-based beverages. **Appendix XIII** shows the appearance of beverages and the colorants obtained from them. We noticed that the whiter plant-based beverages favored the obtainment of bluer colorants in this preliminary test. Therefore, we used laboratory-prepared white plant-based extracts to standardize the preparation and obtain a purer system without other ingredients. Thus, rice, oat, peanut, and almond extracts were evaluated to obtain blue plant-based colorants.

Moreover, a thermosonication treatment was applied to plant-based extracts to produce bluer colorants with better kinetic stability. We obtained blue colorants using all plant extracts. However, almond extract presented the best characteristics for producing the natural blue colorant. Protein availability was the most significant limiting factor in the blue compound formation reaction since the almond and peanut samples showed the same protein content but different color results. The color differences between the samples were also associated with the raw plant extract moisture and protein contents in addition to their natural color. Thermosonication had almost no effect on the coloration of the colorants but improved their kinetic stability since thermosonicated colorants presented fewer phase separation effects than non-thermosonicated ones. **Appendix XIV** presents a scheme of article 3, and **Appendix XV to XVIII** shows its raw data.

In **Chapter 7**, we propose to evaluate the application of the blue almond-based colorant in a beverage since the almond extract allowed the best physicochemical characteristics to obtain the colorant. Thus, we presented the last experimental article, "**Impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage,**" published in the **LWT - Food Science and Technology** journal. In this study, we also evaluated the effects of thermosonication applied at different acoustic powers (4.6, 8.5 and 14.5 W) and holding times (5, 10 and 15 min) on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage. For this, we first studied the formulation of the beverage. **Appendix XIX** presents all the tests performed to define the almond-based beverage formulation (**Table XIX.1 to XIX.6**). We visually evaluated the percentage of gellan gum needed to emulsify the fat (coconut cream) with water (**Table XIX.1**). An amount between 2.5 and 3% of gum was necessary to emulsify the water and fat fraction. Thus, the second test was carried out with more formulation ingredients, varying the proportion of gum (2.5 and 3%). Additionally, we evaluated the addition of 0.1 and 1% of pectin in the formulation (**Table XIX.2**). In this test, we observed the formation of insoluble solids that we associate with pectin. Furthermore, we observed that

the proportion of colorant needed to increase to achieve a bluer beverage. Thus, we tested a smaller pectin fraction and a higher proportion of colorant in the following formulation (**Table XIX.3**). These beverages still presented some solids in suspension, so we decided not to use pectin and replace the coconut cream with coconut oil. Therefore, the coconut oil tests were carried out with the formulations presented in **Table XIX.4**. In this way, we observed a product with a better homogenization but with a faint blue color. Thereby, we decided to use coconut oil and 7% of colorant. In the last formulation test, we studied the addition of a thickener (**Table XIX.5)**. Thus, we reached the final formulation (**Table XIX.6**) since the gellan gum provided a better visual viscosity to the beverage. After establishing the beverage formulation, we evaluated the effects of different thermosonication treatments on the beverage's chemical characteristics. The acoustic power and holding time did not significantly affect the total phenolic, but the thermosonicated samples had their total phenolic content reduced to 10% compared to the untreated beverage (control sample). Other studies determined that the thermosonication temperature significantly affected phenolic compounds degradation. In our study, although all the treatments were carried out at temperatures equal to or greater than 50 °C, the acoustic power or holding time did not generate higher phenolic compound degradation. In this sense, the almond-based beverage is a colloidal system that better protects phenolic compounds by encapsulation. On the other hand, the total flavonoid content increased after the thermosonication treatments. However, the acoustic power increase decreased the beverage flavonoids content. This behavior may be related to the effects of acoustic cavitation and the temperatures that each treatment reaches at the cell walls. However, higher thermosonication temperatures can reduce the content of these compounds due to thermal degradation. In this way, the treatments increased the total flavonoids in the beverage and decreased the total phenolic content, promoting maintenance of the antioxidant activity. Moreover, the treatments promoted beverage lipid and ethyl ester acids oxidation. The most intense thermosonication treatments (14.5 W for 10 and 15 min) applying higher acoustic and thermal energy promoted greater fatty acids and ethyl ester acids oxidation. Despite that, the treatments did not degrade the blue compounds of the beverage. Thus, demonstrating the thermal stability of the natural blue colorant previously produced. **Appendices** from **XX** to **XXI** show the raw data of the fourth experimental article, and **Appendix XXII shows** the article's statistical analysis results.

Figure 3 illustrates the experimental products acquired and the main ultrasound effects observed in this thesis.

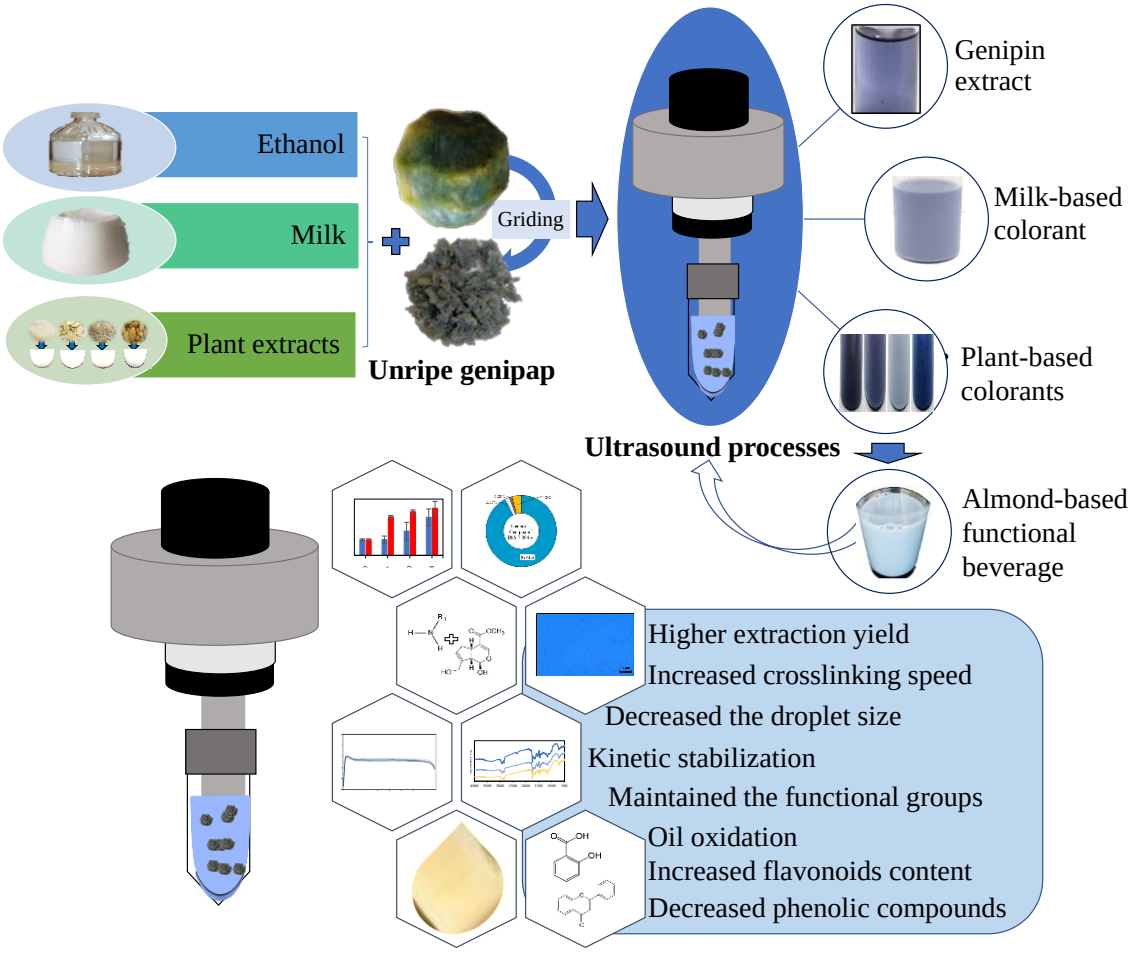

Figure 3. Illustration of the main results acquired in this thesis by studying ultrasound technology to obtain products from unripe genipap fruits.

This chapter (**Chapter 8**) presented a brief and general discussion about the main results observed in each stage carried out in this thesis. **Chapter 9** presents the general conclusion, and **Chapter 10** shows the references used in the general introduction and discussion **(Chapter 1 and 8)**. Then, we proposed future studies and presented a memorial of the doctoral period. **Appendix XXIII** presents the authorizations of the publishers to show the published articles in this thesis, and **Appendix XXIV** the publications carried out in partnership during the doctoral period.

CHAPTER 9

General conclusions, future studies and doctoral period memorial

1 General conclusions

In this thesis, we demonstrate the potential of high-intensity ultrasound technology as a new approach to obtain innovative products from unripe genipap fruits. The reviews presented in **Chapters 2 and 3** were the basis for understanding ultrasound technology and its process variables to assist food processes. In the first review, we observed high-intensity ultrasound as a promising technology to assist the extraction of natural colorants from plant raw materials. Furthermore, we verified the ultrasound variables for extraction and the lack of information about some processes using this technology. In the second review article, we realized each process requires a specific amount and way of acoustic energy supply. The recent advances, trends, challenges regarding ultrasound processing applied to dairy, meat, bakery, minimally processed products, beverages, and food ingredients were reviewed. Furthermore, we observed the association of acoustic energy with an external thermal source (thermosonication) as another promising process for food manufacturing. In this way, we could understand and select the process variables to be evaluated in this thesis experimental portion.

Thereby, in the first experimental step presented in **Chapter 4** we observed the effects of acoustic specific energy (1, 3, and 5 kJ/g) supplied at different powers (100 and 450 W) for obtaining a genipin-rich ethanolic extract. The higher ultrasound power (450 W) provided thermal processes by increasing the extraction medium temperature through acoustic cavitation. Thus, the genipin extraction was favored by acoustic cavitation and heat since the extracts acquired at 450 W presented higher genipin content. Besides that, genipin showed to be a thermostable (until about 75 °C) and stable compound in ethanol for 72 h at 24 ± 2 °C. Moreover, ultrasound-assisted extractions were faster and had a fixed capital investment twice as cheap as a low-pressure pressing + extraction process. In this experimental design, we noticed that the genipin extracts showed a slight blue color due to the reaction of genipin with the proteins of the genipap fruits themselves. However, the crosslinking reaction for the formation of blue compounds was limited to the protein content of the fruits. Thus, we proposed to study the use of solvents containing proteins to obtain the colorant in fewer operational steps.

Therefore, in **Chapter 5** we evaluated ultrasound nominal power (100, 200, 300, and 400 W) to obtain a natural blue colorant from genipin and milk proteins. Colloidal milk system was used as a solvent, a source of primary amines for the crosslinking reaction with genipin, and as a carrier for the blue-colored compounds produced. The ultrasound processes extracted genipin from unripe genipap and favored its diffusion in

the milk system, promoting its crosslinking with milk proteins in a single step. Moreover, the process intensification by increasing the nominal power (from 100 to 400 W) provided a higher genipin recovery resulting in bluer colorants. However, non-enzymatic browning by the Maillard reaction was also observed due to the temperature increase by the power rise. In this way, the nominal power of 300 W provided a blue colorant less dark than 400 W. The colorants remained kinetically stable during the storage (48 h of cold storage), indicating that ultrasound processes also assisted in the kinetic stabilization of the product.

Likewise, plant proteins can replace dairy proteins for producing this natural blue colorant considering the current demand for plant-based products. In this sense, in **Chapter 6** we evaluated a thermosonication pretreatment and four plant extracts, *Oryza sativa* L., *Avena sativa* L., *Arachis hypogaea* L., and *Prunus dulcis* effects on the crosslinking between genipin and plant proteins for the production of natural blue colorants. All evaluated plant proteins were able to crosslink with genipin forming blue colorants. However, the almond extract presented the best chemical and physical characteristics for producing the colorant. The availability of proteins present in the extracts was one of the factors that most impacted the formation of blue compounds. Besides the availability of proteins, the macronutrient composition and the color of the extracts also affected the obtainment of plant-based blue colorants. Furthermore, the thermosonication treatment efficiently produced blue colorants more kinetically stable since the samples thermosonicated presented fewer phase separation phenomena than untreated ones. In this way, we demonstrated the obtainment of plant alternatives to the colorant previously obtained from animal proteins.

Finally, in **Chapter 7**, we evaluated and demonstrated the applicability of the blue almond-based colorant in an almond beverage. Besides that, the effects of different thermosonication conditions were evaluated on the phytochemical characteristics of the plant beverage. Acoustic cavitation and heat provided by thermosonication treatments promoted the degradation of phenolic compounds and the extraction of flavonoids. In this way, the beverage antioxidant activity was maintained. However, the most intense treatments (higher acoustic power and longer holding time) affected the non-polar composition of the beverage, promoting oxidation. Despite that, the treatments did not degrade the blue compounds of the beverage. Thus, the thermal stability of the natural blue colorant previously produced was evidenced.

Therefore, this thesis presents the potential of ultrasound technology as a new route to produce blue natural colorants from unripe fruits of *Genipa americana* L. Furthermore, we demonstrated the technical-economic feasibility of ultrasound-assisted extraction to obtain an ethanolic extract rich in genipin and the stability of the almond-based colorant against processes that applied acoustic and thermal energies. In this way, we demonstrated how ultrasound technology and its process variables could assist the obtainment of a genipin extract, blue natural colorants, and a blue plant-based beverage from a Brazilian plant matrix.

2 Future studies

From the results of the present Thesis, other gaps emerged that can be studied in the future:

- ✓ An economic evaluation of the processes for obtaining the different colorants: based on milk and plant proteins;
- ✓ Evaluate the effects of the conditions of ultrasound treatments on the sensory characteristics of the products obtained (colorants and beverage);
- ✓ Study the toxicity of the genipin-based colorants obtained;
- ✓ Apply the natural blue colorants in different food products and verify the acceptability through sensory tests.

3 Doctoral period memorial

Ph.D. candidate Monique Martins Strieder joined Unicamp in 2018 through the selection process of the Food Engineering Postgraduate Program and with the financial assistance of CNPq (141110/2018-0). During the first year (2018), she attended 4 mandatory subjects of the program: Fenômenos de Transporte I (TP322), Termodinâmica (TP320), Fenômenos de Transporte II (TP323 -A), and Seminários (TP199) and 1 elective subject offered by the department: Tópicos em Engenharia de Alimentos (TP121), totaling 15 credits. In the second semester of the first year, the Ph.D. candidate performed the first experiments in LASEFI related to research parallel to her doctorate. Together with her research group, she developed the experimental part of the study published in RSC Advances: "Biorefinery of turmeric (*Curcuma longa* L.) using non-thermal and clean emerging technologies: an update on the curcumin recovery step." Besides that, she assisted an undergraduate student in developing her experimental project, “Processing of inulin-enriched apple juice using high-intensity ultrasound technology.” The experimental development of these researches was very useful for the Ph.D. candidate, as she learned to use the LASEFI infrastructure. Additionally, both studies employed high-intensity ultrasound technology, her thesis topic.

The Ph.D. candidate started the experimental activities of her project in 2019. The research related to her doctoral project was conducted in cooperation with Maria Isabel Landim Neves' doctoral project. Also, she participated in the organizing committee of the V Iberoamerican Conference on Supercritical Fluids of that year. Despite supercritical fluids not being her specific area of research, the participation in the organization of the event and the presentation of the paper “Production and characterization of emulsion-filled gels based on geranylgeraniol-rich extract obtained by supercritical CO_2 extraction” were very enriching activities for the Ph.D. candidate. The paper was developed with her colleagues from the Research Group she worked with because this is the Group’s strongest field. From September 7 to 13, she also had the opportunity to participate in the exchange program “Sakura Science” promoted by the Japan Science and Technology Agency at Kumamoto University in Kumamoto/Japan. In this event, the Ph.D. candidate presented some of her preliminary experimental results among the various academic activities carried out. During this period, she also assisted undergraduate students in the development of their experimental projects: “Effect of high-intensity ultrasound specific energy on the bixin and geranylgeraniol recovery from defatted annatto seeds” and

“Efeito da temperatura de armazenamento na cinética de formação da cor azul em extratos de jenipapo (*Genipa americana* L.)”.

In the first months of 2020, the student developed part of her thesis experiments and started a voluntary teaching training internship (PED C - CD003). The internship was carried out on the subject of Mechanics of the materials. The experience was enriching since the discipline was administered remotely due to the social isolation resulting from COVID-2019. In 2020, the Ph.D. candidate focused on writing the experimental articles presented in Chapters 4, 5, 6, and 7. Moreover, our research team developed a novel process and wrote the report for the patent application “Processo de produção de corante natural azul”. It was filed in the Instituto Nacional da Propriedade Industrial (BR 10 2020 026302 1) on December 21, 2020.

In the first semester of 2021, the student completed her experimental activities and finished writing the last experimental and review articles. She also contributed to the experimental activities and writing of articles of a master's student of the LASEFI's Research Group about recovering bioactive compounds from fennel. In October 2021, the Ph.D. candidate was awarded a 6-month sandwich doctoral scholarship from CNPq. She did the sandwich period at the University of Alberta in Edmonton/Canada under Professor Marleny Saldaña supervision. The Ph.D. candidate studied emerging technologies to produce a barley-based beverage during this exchange period. Figure 4 presents the main activities developed during her internships at the University of Campinas and the University of Alberta.

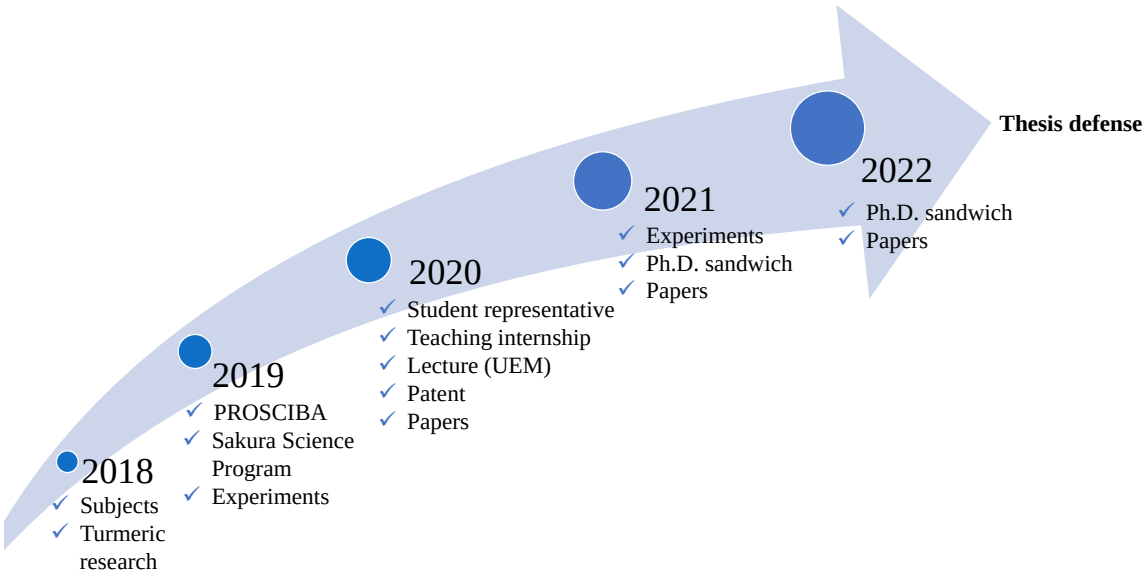

Figure 4. Main activities performed during the Ph.D. period.

Then, her doctoral project's development resulted in 2 review articles and 4 experimental articles. Furthermore, together with her Research Group, she produced 1 patent, 6 experimental articles, 1 review article, and 5 abstracts published in conference proceedings.

Publications during the doctoral period:

Patent

Meireles, M. A. A.; Silva, E. K.; Neves, M. I. L.; **Strieder, M. M.** Blue natural colorant process. (2020). INPI - Instituto Nacional da Propriedade Industrial. Country: Brazil. Patent of Invention. Register number: BR10202002630. Deposit date: 12/21/2020. Depositor/Holder: University of Campinas.

Articles published in peer-reviewed journals

Strieder, M. M., Silva, E. K., Meireles, M. A. A. (2019). Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants. *Food and Public Health* v. 9, 45-52.

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CHAPTER 10

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Appendices

Appendix I - Article experimental 1

Preliminary test: determination of the ratio (S/F) between ethanol and genipap mass

Table I. S/F effects on the global yield and genipin content of extracts

Ultrasound-assisted extraction process	S/F	Global yield (g dried extract/100 g dried pulp genipap)	Global yield (g genipin/ 100 g dried extract)	Genipin content (g genipin/100 g dried pulp genipap)
Non-thermal (100 W)	5	45.2	2.02	0.91
	7.5	41.9	1.15	0.48
	10	40.8	1.33	0.54
Thermal (450 W)	5	47.5	1.77	0.80
	7.5	53.6	1.47	0.79
	10	44.4	2.12	0.94

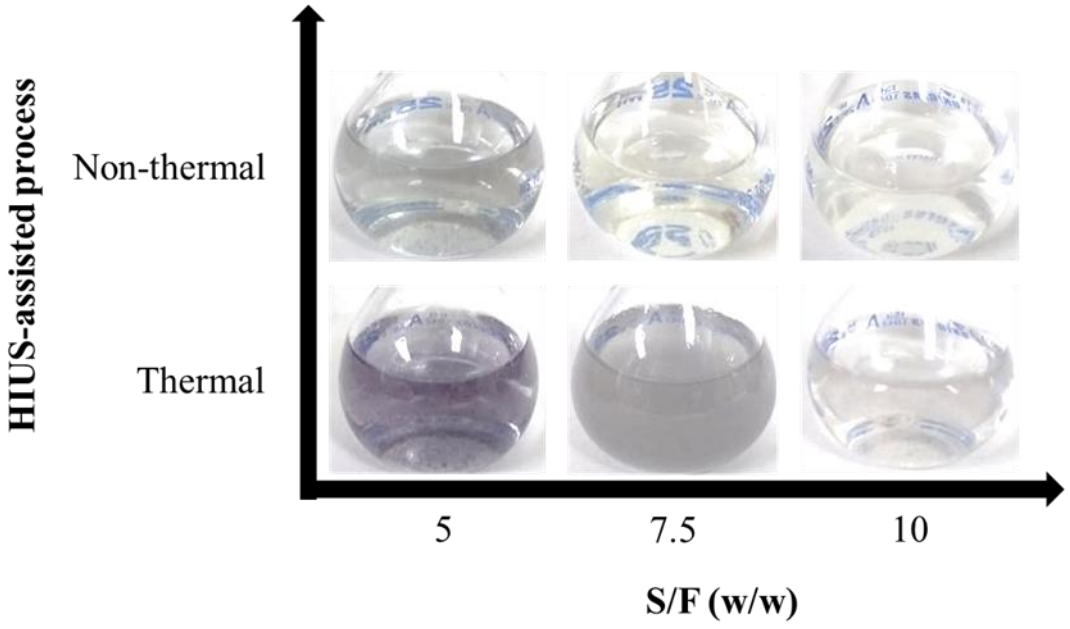


Figure I. S/F effects on the visual appearance of genipap extracts. Images acquired after 48 h of the ultrasound-assisted extractions.

Appendix II - Article experimental 1

Additional result: pH and color parameters of ethanolic genipap extracts produced in article experimental 1

Table II. pH and color CIE coordinates of ethanolic genipap extracts obtained after 72 h of storage (at room temperature)

Process	**Specific energy (kJ/g)**	**pH**	**L**	**a***	**b***	**C***	**H***
Control	0	6.25 ± 0.06	9 ± 4	-0.8 ± 0.6	-2 ± 1	2.3 ± 0.8	242 ± 26
Non-thermal	1	6.01 ± 0.05	12 ± 3	-1.1 ± 0.7	-0.6 ± 0.1	1.2 ± 0.7	212 ± 14
	3	5.95 ± 0.05	7 ± 1	-1.2 ± 0.8	-0.77 ± 1	1.8 ± 0.1	211 ± 56
	5	5.90 ± 0.06	7 ± 1	-0.7 ± 0.6	-1.2 ± 0.1	1.4 ± 0.3	239 ± 23
Thermal	1	5.91 ± 0.03	11 ± 1	-1.0 ± 0.3	-0.7 ± 0.6	2 ± 1	225 ± 45
	3	5.98 ± 0.04	7 ± 4	-0.9 ± 0.9	-1.3 ± 0.7	1.8 ± 0.1	235 ± 37
	5	5.91 ± 0.08	7 ± 4	-0.8 ± 0.7	-1.3 ± 0.1	1.4 ± 0.8	233 ± 10

*Mean values ± standard deviation (n = 4).

Appendix III - Article experimental 1

Raw data: temperature profiles of ultrasound-assisted extractions

Chart III. Temperature profile (genipap + ethanol) data

Processing time (s)	**Non-thermal (R1)**	**Non-thermal (R2)**	**Thermal (R1)**	**Thermal (R2)**
0	22.6	22.4	23.4	23.4
15	22.6	22.6	24.3	24.7
30	22.5	23.6	29.2	27.5
45	22.5	24.2	31.3	30.6
60	22.5	24.4	33.7	33.5
90	22.5	24.4	38.5	39.4
120	22.5	25.1	43.4	45.0
150	22.5	25.6	47.0	49.4
180	22.7	26.1	51.3	54.9
210	23.8	26.3	54.8	59.3
240	23.9	26.4	58.7	63.7
270	24.0	26.6	61.5	68.3
300	25.1	26.8	65.0	72.0
330	25.2	26.8	67.5	75.0
360	25.3	26.9	70.5	76.5
390	25.3	26.9	72.8	76.5
420	26.0	27.0		
450	26.6	26.9		
480	26.4	27.1		
510	26.6	27.3		
540	26.6	27.3		
570	26.8	27.3		
600	27.4	27.4		
630	27.7	27.6		
660	27.6	27.6		
690	27.7	27.9		

Chart III. Continuation of temperature profile data (genipap + ethanol)

720	27.6	27.9
750	27.9	28.0
780	28.3	28.0
810	28.7	28.0
840	28.9	28.0
870	28.8	28.1
900	28.8	28.2
930	28.7	28.2
960	28.7	28.4
990	28.9	28.5
1020	29.3	28.5
1050	29.6	28.6
1080	29.7	28.7
1110	29.7	28.8
1140	29.7	28.8
1170	29.9	28.9
1200	29.7	29.0
1230	29.8	28.9
1260	29.7	28.9
1290	29.7	29.1
1320	30.0	29.1
1350	30.0	29.1
1380	30.1	29.2
1410	30.5	29.2
1440	30.6	29.2
1470	30.6	29.2
1500	30.7	29.3
1530	30.7	29.3
1560	30.8	29.4
1590	30.7	29.4
1620	30.7	29.5

Chart III. Continuation of temperature profile data (genipap + ethanol)

1650	30.6	29.5
1680	30.6	29.5
1710	30.6	29.6
1740	30.5	29.7
1770	30.5	29.7
1800	30.5	29.7

Appendix IV - Article experimental 1

Raw data: global, genipin, and geniposide yields

Chart IV. Experimental design with global yield data

Run Order	Ultrasound process	Specific energy (kJ/g)	Global yield (g dried extract/ 100 g dried pulp genipap)		Global yield (g genipin/ 100 g dried extract)		Global yield (g geniposide/ 100 g dried extract)	
			0 h (R1)	0 h (R2)	0 h (R1)	0 h (R2)	0 h (R1)	0 h (R2)
1	**Thermal**	**3**	41.87	44.93	3.22	2.76	0.229	0.072
2	**Thermal**	**1**	39.67	38.64	2.85	2.91	0.144	0.087
3	**Thermal**	**5**	44.55	43.94	3.98	2.58	0.145	0.081
4	**Non-thermal**	**5**	54.87	48.07	2.44	1.58	0.113	0.053
5	**Non-thermal**	**1**	38.42	34.06	1.63	1.18	0.108	0.035
6	**Non-thermal**	**3**	45.91	33.81	2.45	1.21	0.101	0.045
Control			18.58	17.68	2.98	2.47	0.033	0.083

Appendix V - Article experimental 1

Raw data: genipin and geniposide contents

Chart V. Experimental design with genipin and geniposide content data

Run Order	Ultrasound process	ê (kJ/g)	Genipin content (mg genipin/g dried pulp genipap)				Geniposide content (mg geniposide/g dried pulp genipap)			
			0 h (R1)	0 h (R2)	72 h (R1)	72 h (R2)	0 h (R1)	0 h (R2)	72 h (R1)	72 h (R2)
1	**Thermal**	**3**	16.77	15.79	16.86	15.52	12.40	11.37	11.18	6.25
2	**Thermal**	**1**	14.03	14.76	14.07	14.12	10.70	11.26	7.07	11.50
3	**Thermal**	**5**	20.12	17.75	20.56	16.86	11.74	11.03	11.61	6.76
4	**Non-thermal**	**5**	17.35	11.13	15.64	10.25	9.40	8.61	9.03	4.47
5	**Non-thermal**	**1**	4.99	6.86	5.32	6.99	7.55	2.61	8.63	3.04
6	**Non-thermal**	**3**	5.70	12.24	5.87	11.93	3.67	7.30	5.34	8.08
Control			5.55	6.07	5.59	6.76	2.04	2.20	2.3	3.05

Appendix VI - Article experimental 1

Statistical analysis results:

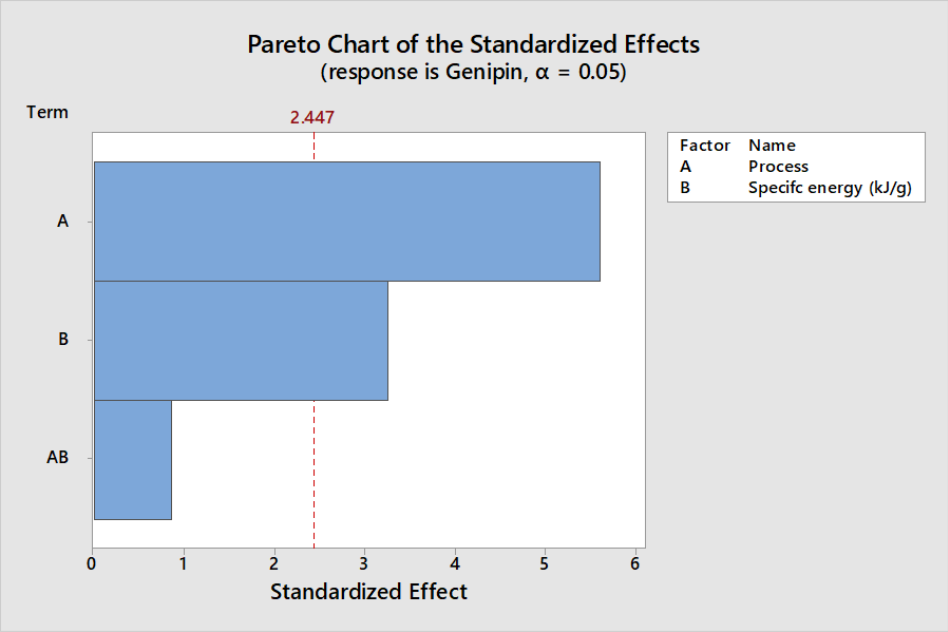


Figure VI.1. Effect of the extraction process and specific energy on genipin content (mg/g) of extracts. Where: A: Processes non-thermal and thermal; B: Specific energies of 1, 3, and 5 kJ/g.

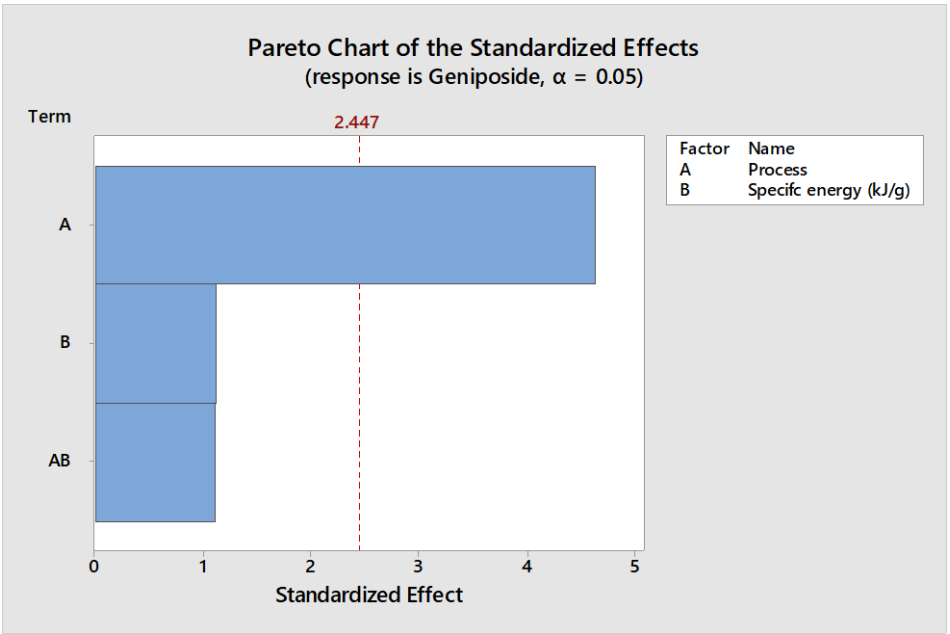


Figure VI.2. Effect of the extraction process and specific energy on geniposide content (mg/g) of extracts. Where: A: Processes non-thermal and thermal; B: Specific energies of 1, 3, and 5 kJ/g.

Appendix VII - Article experimental 2

Additional result: blue color development of the colorants throughout 96 h of storage

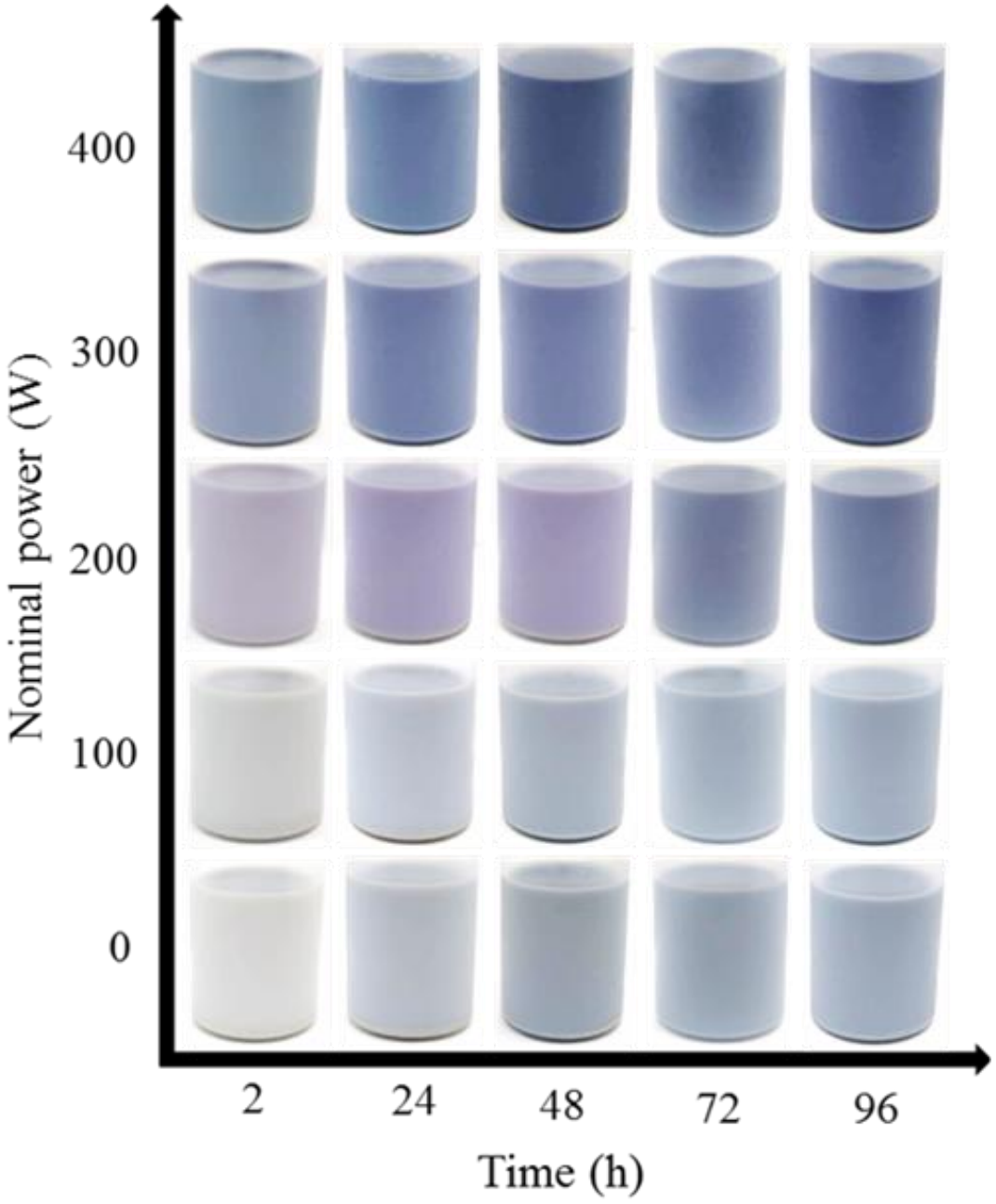


Figure VII - Effect of the nominal power on the kinetic of blue color formation over the storage time (96 h)

Appendix VIII - Article experimental 2

Additional result: pH value of the colorants

Table VIII - Colorant pH values

Storage time (h) / **Nominal power (W)**	**2 h**		**48 h**	
	Temperature (°C)	**pH**	**Temperature (°C)**	**pH**
Control 1	17.3	6.02	8.80	6.04
Control 2	17.0	6.07	12.5	6.04
100 (R1)	17.3	6.01	8.90	6.05
100 (R2)	18.0	6.10	11.1	6.10
200 (R1)	17.2	5.97	9.60	6.04
200 (R2)	17.7	6.13	12.5	6.11
300 (R1)	17.0	6.01	9.80	6.06
300 (R2)	18.0	6.10	13.3	6.09
400 (R1)	16.8	6.04	11.3	6.02
400 (R2)	17.1	6.10	12.6	6.09

Appendix IX - Article experimental 2

Raw data: Temperature profiles of ultrasound processes

Chart IV. Temperature profile (genipap + milk) data

Processing time (min) / **Nominal power (W)**	**0**	**0.25**	**0.5**	**0.75**	**1**	**2**	**3**	**4**	**5**	**6**
100 (R1)	20.0	20.1	20.8	20.9	21.6	22.4	24.1	24.9	25.9	27.0
100 (R2)	20.0	20.6	21.0	21.9	22.0	23.5	24.5	25.4	26.6	28.0
200 (R1)	20.2	20.9	22.4	23.9	25.0	28.6	32.8	36.9	40.1	44.2
200 (R2)	20.0	22.4	23.6	24.9	25.4	29.4	32.4	35.4	38.1	41.0
300 (R1)	20.1	20.9	22.5	24.1	26.9	34.2	42.5	50.0	57.4	64.4
300 (R2)	20.0	23.6	25.1	27.0	28.7	35.4	42.4	49.5	56.0	62.4
400 (R1)	20.6	23.8	26.6	29.4	32.6	44.1	55.6	67.2	77.2	85.6
400 (R2)	20.0	19.8	22.7	24.4	27.3	38.8	50.2	61.6	71.2	81.6

Appendix X - Article experimental 2

Raw data: free-genipin content

Chart X. Free-genipin content (mg genipin/g dried colorant) data of the samples

Storage time (h) / **Nominal power (W)**	**Genipin content (mg genipin/g dried colorant)**		
	2 h	**24 h**	**48 h**
Control 1	2.12	0.75	0.60
Control 2	1.61	0.61	0.72
100 (R1)	2.98	0.66	0.46
100 (R2)	2.48	0.63	0.64
200 (R1)	3.18	0.64	0.05
200 (R2)	4.47	0.63	0.05
300 (R1)	4.29	0.60	0.45
300 (R2)	4.35	0.67	0.57
400 (R1)	4.69	0.73	0.55
400 (R2)	4.61	0.60	0.45

Appendix XI - Article experimental 2

Raw data: color parameters

Chart XI. Color parameters (L, a*, and b*) data of the samples

Color parameters / **Nominal power (W)**	**L**	**a***	**b***
2 h			
Control 1	59.08	-3.23	2.70
Control 2	60.51	-3.09	2.57
100 (R1)	58.96	-3.07	2.26
100 (R2)	59.98	-2.77	2.32
200 (R1)	49.25	0.76	-4.51
200 (R2)	46.33	1.99	-6.00
300 (R1)	39.58	-2.25	-9.61
300 (R2)	40.73	-2.36	-10.04
400 (R1)	33.14	-1.56	-10.30
400 (R2)	36.66	-1.66	-10.92
24 h			
Control 1	54.39	-4.53	-1.82
Control 2	54.64	-4.52	-1.89
100 (R1)	51.07	-3.99	-2.93
100 (R2)	50.86	-4.03	-2.92
200 (R1)	42.60	1.29	-8.46
200 (R2)	42.32	1.25	-8.5
300 (R1)	37.07	-1.23	-11.96
300 (R2)	37.15	-1.21	-12.06
400 (R1)	33.96	-2.35	-11.13
400 (R2)	33.98	-2.36	-11.23
48 h			

Chart XI. Continuation of color parameters (L, a*, and b*) data of the samples

Control 1	45.55	-2.26	-1.75
Control 2	46.61	-2.53	-1.61
100 (R1)	47.68	-5.14	-3.43
100 (R2)	47.94	-5.22	-3.44
200 (R1)	37.68	-1.42	-9.47
200 (R2)	37.54	-1.38	-9.37
300 (R1)	31.22	-0.93	-11.78
300 (R2)	31.19	-0.93	-11.94
400 (R1)	27.94	-1.28	-10.68
400 (R2)	27.75	-1.25	-10.24

Appendix XII - Article experimental 2

Raw data: drop size distribution

Chart XII. Mean diameter (D_{32}), cumulative diameters (d_{10}, d_{50}, d_{90}), and *span* values data of the samples

Nominal power (W)	D_{32}	d_{10}	d_{50}	d_{90}	***Span***
Control 1	1.07	0.37	0.50	0.80	0.85
Control 2	1.11	0.35	0.48	0.78	0.89
100 (R1)	0.92	0.35	0.48	0.76	0.85
100 (R2)	0.92	0.34	0.46	0.74	0.87
200 (R1)	0.81	0.37	0.49	0.76	0.79
200 (R2)	0.79	0.37	0.49	0.76	0.80
300 (R1)	0.75	0.35	0.46	0.72	0.80
300 (R2)	0.80	0.36	0.48	0.76	0.82
400 (R1)	0.73	0.35	0.47	0.75	0.83
400 (R2)	0.67	0.33	0.44	0.68	0.81

Appendix XIII - Article experimental 3

Preliminary test: blue color formation in commercial plant-based beverages used as extractor solvent of genipin

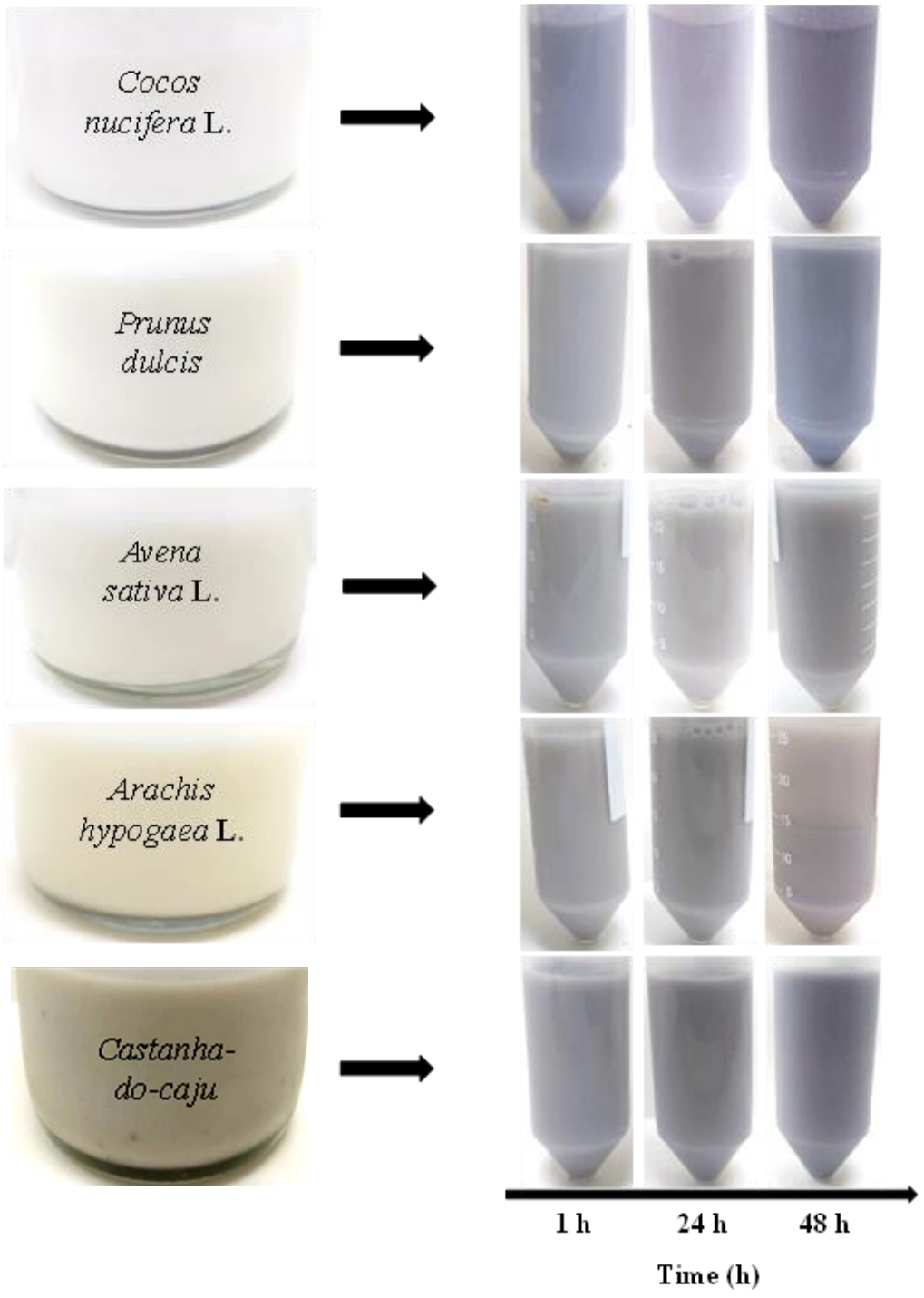


Figure XIII - The visual appearance of plant-based colorants obtained from commercial plant-based beverages and unripe genipap using an ultrasound treatment (300 W, 6 min)

Appendix XIV - Article experimental 3

Additional result: scheme of article experimental 3

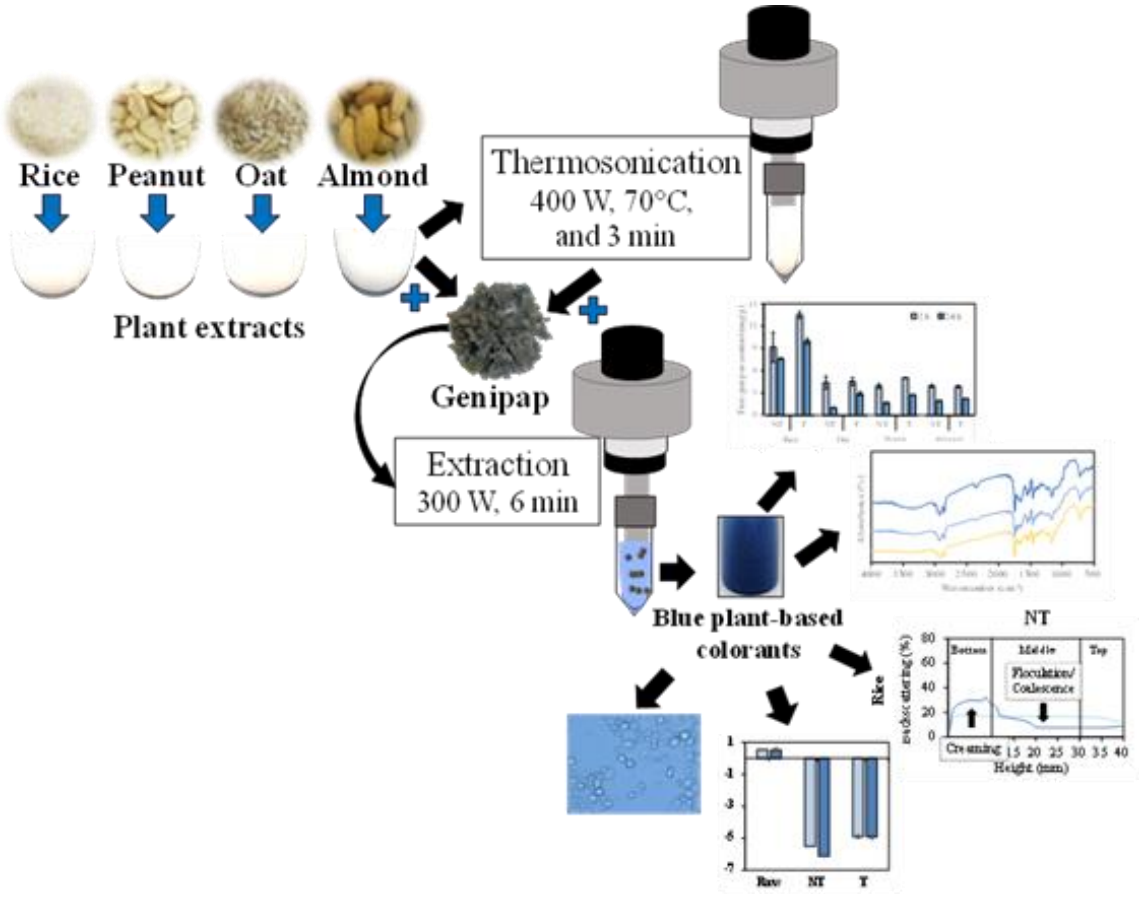


Figure XIV - Scheme of article experimental 3

Appendix XV - Article experimental 3

Raw data: free-genipin and free-geniposide content

Chart XV. Free-genipin and free-geniposide contents (mg/g dried colorant) data of the samples

Storage time (h) / **Plant-based colorant**	**Genipin content (mg genipin/g dried colorant)**		**Geniposide content (mg geniposide/g dried colorant)**	
	2 h	**24 h**	**2 h**	**24 h**
NT Rice (R1)	17.0	12.4	5.93	5.83
NT Rice (R2)	9.2	7.6	11.34	9.29
T Rice (R1)	20.4	14.9	6.29	7.30
T Rice (R2)	13.5	10.0	5.44	4.72
NT Oat (R1)	6.3	3.5	1.68	1.06
NT Oat (R2)	4.4	1.0	4.05	2.01
T Oat (R1)	5.6	2.9	1.94	0.52
T Oat (R2)	4.5	1.2	3.53	1.79
NT Peanut (R1)	1.8	0.2	1.99	1.32
NT Peanut (R2)	3.9	1.7	0.75	0.22
T Peanut (R1)	5.3	1.2	0.92	0.25
T Peanut (R2)	5.0	2.8	1.52	0.24
NT Almond (R1)	3.7	0.3	0.75	0.24
NT Almond (R2)	3.9	2.0	0.43	0.37
T Almond (R1)	4.5	0.6	0.90	0.23
T Almond (R2)	3.9	2.2	1.13	0.24

Appendix XVI - Article experimental 3

Raw data: moisture and protein content and pH values

Chart XV. Moisture and protein contents and pH values data of the samples

Plant-based extract	**Moisture content (g/100 g)**	**Protein content (g/100 g)**	**pH value**
NT Rice (R1)	94.5	0.445	6.67
NT Rice (R2)	95.9	0.470	6.62
T Rice (R1)	93.8		
T Rice (R2)	95.9		
NT Oat (R1)	83.6	1.917	6.86
NT Oat (R2)	84.0	1.919	6.86
T Oat (R1)	84.1		
T Oat (R2)	84.1		
NT Peanut (R1)	82.9	6.457	6.76
NT Peanut (R2)	83.5	6.411	6.75
T Peanut (R1)	83.8		
T Peanut (R2)	87.4		
NT Almond (R1)	80.1	5.964	6.35
NT Almond (R2)	83.9	6.004	6.32
T Almond (R1)	83.2		
T Almond (R2)	84.3		

Appendix XVII - Article experimental 3

Raw data: color parameters

Chart XVII. Color parameters (L, a*, and b*) data of the samples

Color parameters / **Sample**	**L**	**a***	**b***
2 h			
Rice extract (R2)	70.11	-0.6	-0.11
Rice extract (R2)	68.89	-0.57	-0.12
NT Rice (R1)	51.75	-0.03	2.59
NT Rice (R2)	51.5	0.01	2.52
T Rice (R1)	37.34	-1.59	-1.86
T Rice (R2)	36.73	-1.46	-2.1
Oat extract (R1)	76.68	-0.36	7.04
Oat extract (R1)	77.21	-0.33	7.11
NT Oat (R1)	59.13	-1.79	2.24
NT Oat (R2)	59.08	-1.76	2.06
T Oat (R1)	56.4	-1.81	2.16
T Oat (R2)	56.34	-1.73	1.98
Peanut extract (R1)	76.51	0.36	8.41
Peanut extract (R2)	76.83	0.4	8.54
NT Peanut (R1)	56.39	-4.91	-2.34
NT Peanut (R2)	56.27	-4.82	-2.78
T Peanut (R1)	58.69	-3.12	-4.65
T Peanut (R2)	58.67	-3.16	-4.83
Almond extract (R1)	79.48	-0.26	6.74
Almond extract (R2)	78.24	-0.35	6.37
NT Almond (R1)	57.52	-5.06	-0.83
NT Almond (R2)	56.17	-5.24	-1.74

Chart XVII. Continuation of color parameters (L, a*, and b*) data of the samples

T Almond (R1)	55.08	-4.28	-5.21
T Almond (R2)	54.07	-4.4	-5.74
24 h			
NT Rice (R1)	46.47	0.45	6.33
NT Rice (R2)	46.33	0.49	6.24
T Rice (R1)	33.2	-0.33	-0.43
T Rice (R2)	32.54	-0.32	-0.61
NT Oat (R1)	48.7	0.39	-1.45
NT Oat (R2)	48.75	0.42	-1.5
T Oat (R1)	46.09	0.99	-1.81
T Oat (R2)	46.26	1.08	-1.79
NT Peanut (R1)	46.5	-4.76	-7.65
NT Peanut (R2)	46.42	-4.61	-7.8
T Peanut (R1)	55.82	-0.24	-5.08
T Peanut (R2)	55.4	-0.02	-5.3
NT Almond (R1)	44.11	-6.24	-8.68
NT Almond (R2)	43.81	-6.09	-9.08
T Almond (R1)	44.27	-3.27	-9.8
T Almond (R2)	43.99	-3.17	-9.92

Appendix XVIII - Article experimental 3

Raw data: particle size distribution

Chart XVIII. Mean diameter (D_{32}), cumulative diameters (d_{10}, d_{50}, d_{90}), and *span* values data of the samples

Sample	D_{32}	d_{10}	d_{50}	d_{90}	***Span***
Rice extract (R1)	4.110	1.849	6.594	26.546	3.745
Rice extract (R2)	4.384	1.928	7.325	33.729	4.341
NT Rice (R1)	4.496	3.186	5.824	9.646	1.109
NT Rice (R2)	4.505	3.192	5.823	9.641	1.107
T Rice (R1)	6.049	2.681	10.554	112.850	10.439
T Rice (R2)	6.359	2.818	11.155	181.021	16.021
Oat extract (R1)	6.715	2.975	12.411	116.857	9.173
Oat extract (R1)	6.521	2.856	11.899	96.842	7.899
NT Oat (R1)	6.111	3.648	9.305	19.185	1.670
NT Oat (R2)	6.754	4.107	9.607	19.636	1.617
T Oat (R1)	7.077	4.415	9.384	17.461	1.391
T Oat (R2)	7.023	4.400	9.352	17.082	1.357
Peanut extract (R1)	4.112	1.498	9.454	35.000	3.544
Peanut extract (R2)	3.873	1.444	8.511	27.462	3.058
NT Peanut (R1)	8.912	4.690	19.847	51.038	2.334
NT Peanut (R2)	9.433	5.331	20.928	50.398	2.158
T Peanut (R1)	12.046	7.113	26.837	84.370	2.879
T Peanut (R2)	11.719	6.921	25.880	75.101	2.632
Almond extract (R1)	9.527	3.092	50.357	174.968	3.412
Almond extract (R2)	8.404	2.633	40.076	149.393	3.663
NT Almond (R1)	9.763	4.707	24.804	57.999	2.148
NT Almond (R2)	9.603	4.569	24.564	56.303	2.107
T Almond (R1)	14.165	8.064	30.456	101.198	3.057
T Almond (R2)	14.772	8.368	32.344	114.649	3.286

Appendix XIX - Article experimental 4

Preliminary test: beverage formulation

Table XIX.1. Emulsification test in a model system with different percentages of gum

Essay	Water (%)	Gellan gum (%)	Coconut cream (%)
1	98.5	0.5	1
2	98	1	1
3	97	2	1
4	96.50	2.5	1
5	96	3	1

Table XIX.2. Emulsification test with different percentages of gellan gum and pectin

Essay	Almond extract (%)	Gellan gum (%)	Coconut cream (%)	Pectin (%)	XOS (%)	Xylitol (%)	Colorant (%)
1	88.8	2.5	1	1	1	5	0.67
2	89.4	3	1	0.1	1	5	0.5

Table XIX.3. Emulsification test with different percentages of pectin and colorant

Essay	Almond extract (%)	Gellan gum (%)	Coconut cream (%)	Pectin (%)	XOS (%)	Xylitol (%)	Colorant (%)
1	89.4	2.5	1	0.05	1	5	1
2	88.4	2.5	1	0.1	1	5	2

Table XIX.4 - Emulsification test with coconut oil or cream

Essay	Almond extract (%)	Acacia gum (%)	Coconut oil/cream (%)	XOS (%)	Xylitol (%)	Colorant (%)	Vanilla (%)
1	87.4	2.5	1 (oil)	1	5	3	0.1
2	87.4	2.5	1 (cream)	1	5	3	0.1
3	85	2.5	1 (oil)	1	5	6.67	0.5

Table XIX.5. Emulsification test with different thickener

Essay	Almond extract (%)	Acacia gum (%)	Coconut oil (%)	XOS (%)	Xylitol (%)	Colorant (%)	Vanilla (%)	Thickener (%)
1	82.95	2.5	1	1	5	7	0.5	0.05 (CMSO)
2	82.95	2.5	1	1	5	7	0.5	0.05 (gellan gum)
3	82.95	2.5	1	1	5	7	0.5	0.05 (pectin)

*CMSO: carbamoyl methyl sulfoxide

Table XIX.6. Final formulation

Almond extract (%)	Acacia gum (%)	Coconut oil (%)	XOS (%)	Xylitol (%)	Colorant (%)	Vanilla (%)	Gellan gum (%)
82.95 (24.88 g)	2.5 (0.75 g)	1 (0.3 g)	1 (0.3 g)	5 (1.5 g)	7 (2.1 g)	0.5 (0.15 g)	0.05 (0.015 g)

Appendix XX - Article experimental 4

Raw data: temperature profiles of ultrasound processes

Chart XX.1. Heating temperature profile data of the samples in the water bath at 63 °C

Heating time (min)	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5
Heating time (s)	0	30	60	90	120	150	180	210	240	270	300	330	360	390
R1	18	21.1	23.1	26.4	30.6	33.4	36.1	38.7	41.3	43.7	45.9	47.7	49.2	50.9
R2	18	21.2	22.9	27.1	31.6	34.5	36.9	39.3	41.9	44.9	46.5	47.8	49.2	50.5
R3	18	20.7	22.7	26.2	30.7	33.5	36.3	38.7	41.4	44.0	46.1	48.1	49.8	51.0
R4	18	21.6	23.1	26.1	29.5	32.4	35.3	38.2	40.7	43.4	45.8	47.5	48.9	50.1

Chart XX.2. Thermosonication temperature profile data of the samples

Time (min)	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14
Time (s)	0	450	480	510	540	570	600	630	660	690	720	750	780	810	840
100 W	50	49.4	49.3	49.3	49.2	49.4	49.6	49.7	49.7	49.7	49.8	49.9	49.8	49.9	49.9
	50	49.4	49.6	49.5	49.5	49.5	49.5	49.5	49.5	49.5	49.6	49.5	49.6	49.6	49.6
200 W	50	49.4	49.8	51.1	51.8	52.6	53.7	54.0	54.6	55.4	55.5	55.9	56.8	56.9	57.0
	50	49.7	51	51.3	52.5	52.7	53.7	54	54.4	55.4	55.5	55.5	56.1	56.7	56.8

Chart XX.2. Continuation of thermosonication temperature profile data of the samples

300 W	50	52.4	54.2	56.8	58.4	60.8	62.0	63.6	64.9	65.8	67.0	68.3	68.6	69.8	70.0
	50	53.2	55.6	58.4	60.1	62.6	64.2	65.7	67	68.4	69.6	70.0	71.2	71.5	72.7

Chart XIX.3. Continuation of thermosonication temperature profile data of the samples

Time (min)	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22
Time (s)	870	900	930	960	990	1020	1050	1080	1110	1140	1170	1200	1230	1260	1290	1320
100 W	50.0	50.1	50.2	50.1	50.2	50.1	50.2	50.2	50.3	50.3	50.4	50.3	50.3	50.4	50.3	50.4
	49.9	50.0	49.9	49.9	50.0	50.0	50.0	50.1	50.2	50.2	50.3	50.3	50.4	50.4	50.4	50.4
200 W	57.2	57.7	58.2	58.2	58.3	58.3	58.4	58.6	58.9	59.3	59.5	59.6	59.7	59.7	59.7	59.7
	56.9	57.3	58	58.3	58.4	58.3	58.5	59.0	59.5	59.6	59.7	59.7	59.7	59.7	59.8	59.8
300 W	71.0	71.4	71.5	71.9	72.7	72.7	72.8	72.7	72.9	73.0	73.3	73.6	73.9	74.0	74.1	74.1
	72.7	72.9	73.7	74.1	74.2	74.3	74.5	75.0	75.4	75.5	75.6	75.6	75.6	75.6	75.6	75.7

Appendix XXI - Article experimental 4

Raw data: flavonoids and TPC content, antioxidant capacity by DPPH and TEAC, pH values, and color parameters

Chart XXI. Experimental article data

TS condition (W and min)		Essay	Flavonoids content	TPC	AC DPPH	TEAC	pH value	L	a*	b*	BI
100	5	6	6120	596	46	162.5	6.35	52.3	-2.88	-8.95	-18.8294
		18	6456	607	65	134.1	6.34	52.36	-2.92	-8.63	-18.3739
	10	3	6540	563	80	198.4	6.36	47.05	-2.62	-9.22	-20.8251
		15	5953	541	61	137.2	6.36	51.62	-2.8	-8.99	-19.0136
	15	5	6120	574	52	183.9	6.4	53.11	-2.64	-9	-18.3279
		11	5953	629	72	168.8	6.37	52.38	-2.85	-9.5	-19.5955
200	5	9	6707	486	36	178.2	6.35	52.53	-2.96	-9.83	-20.1814
		14	6791	618	31	154.9	6.35	53.52	-2.98	-9.27	-19.0369
	10	2	6372	563	62	194.7	6.36	50.73	-2.58	-9.83	-20.325
		4	6204	596	62	197.8	6.34	50.54	-2.64	-9.95	-20.6606
	15	7	5617	563	57	177.6	6.37	53.01	-2.45	-9.99	-19.5927
		13	5869	585	69	183.3	6.39	53.82	-2.6	-10.63	-20.4424
300	5	1	4360	596	61	207.9	6.33	53.59	-2.82	-10.19	-20.1626
		10	5701	585	66	185.2	6.34	53.36	-2.78	-9.8	-19.6177

Chart XXI. Continuation of experimental article data

300	10	8	6037	530	47	185.2	6.32	53.82	-2.6	-10.63	-20.4424
		16	5701	585	63	174.5	6.32	52.99	-2.32	-10.32	-19.9196
	15	12	5617	552	62	177.0	6.33	51.79	-2.21	-11.43	-21.844
		17	6120	618	43	168.8	6.32	53.71	-2.51	-11.82	-22.0705

TS: Thermosonication; TPC: Total phenolic content; AC DPPH: antioxidant capacity by 2,2-difenil-1-picril-hidrazil; TEAC: trolox equivalent antioxidant capacity; L: luminosity; a: red/green coordinate; b*: yellow/blue coordinate; BI: Browning index.

Appendix XXII - Article experimental 4

Statistical analysis results:

General Factorial Regression: Flavonoids content versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	3656064	457008	2.99	0.061
Linear	4	1822174	455544	2.98	0.080
Power	2	1631600	815800	5.34	0.030
Time	2	190575	95287	0.62	0.557
2-Way Interactions	4	1833890	458473	3.00	0.079
Power*Time	4	1833890	458473	3.00	0.079
Error	9	1374246	152694		
Total	17	5030310			

General Factorial Regression: Total phenolic content versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	6229.2	778.7	0.46	0.855
Linear	4	2708.4	677.1	0.40	0.803
Power	2	826.0	413.0	0.24	0.788
Time	2	1882.3	941.2	0.56	0.591
2-Way Interactions	4	3520.9	880.2	0.52	0.722
Power*Time	4	3520.9	880.2	0.52	0.722

Error	9	15173.6	1686.0	
Total	17	21402.8		

General Factorial Regression: DPPH versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	1776.8	222.1	2.02	0.158
Linear	4	716.9	179.2	1.63	0.249
Power	2	284.0	142.0	1.29	0.321
Time	2	432.8	216.4	1.97	0.195
2-Way Interactions	4	1059.9	265.0	2.41	0.126
Power*Time	4	1059.9	265.0	2.41	0.126
Error	9	989.5	109.9		
Total	17	2766.3			

General Factorial Regression: TEAC versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	3599.3	449.9	1.33	0.336
Linear	4	1651.8	412.9	1.23	0.366
Power	2	1297.9	649.0	1.93	0.201
Time	2	353.8	176.9	0.52	0.609
2-Way Interactions	4	1947.6	486.9	1.44	0.296

Power*Time	4	1947.6	486.9	1.44	0.296
Error	9	3033.5	337.1		
Total	17	6632.8			

General Factorial Regression: pH versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	0.008000	0.001000	9.00	0.002
Linear	4	0.006533	0.001633	14.70	0.001
Power	2	0.004933	0.002467	22.20	0.000
Time	2	0.001600	0.000800	7.20	0.014
2-Way Interactions	4	0.001467	0.000367	3.30	0.063
Power*Time	4	0.001467	0.000367	3.30	0.063
Error	9	0.001000	0.000111		
Total	17	0.009000			

General Factorial Regression: L versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	32.632	4.079	2.67	0.083
Linear	4	22.506	5.627	3.68	0.048
Power	2	9.084	4.542	2.97	0.102
Time	2	13.422	6.711	4.39	0.047
2-Way Interactions	4	10.126	2.532	1.66	0.243

Power*Time	4	10.126	2.532	1.66	0.243
Error	9	13.761	1.529		
Total	17	46.393			

General Factorial Regression: a* versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	0.66314	0.08289	5.43	0.010
Linear	4	0.60759	0.15190	9.96	0.002
Power	2	0.18621	0.09311	6.10	0.021
Time	2	0.42138	0.21069	13.81	0.002
2-Way Interactions	4	0.05556	0.01389	0.91	0.498
Power*Time	4	0.05556	0.01389	0.91	0.498
Error	9	0.13730	0.01526		
Total	17	0.80044			

General Factorial Regression: b* versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	11.7825	1.47281	17.18	0.000
Linear	4	10.9199	2.72997	31.84	0.000
Power	2	8.1750	4.08751	47.68	0.000
Time	2	2.7449	1.37244	16.01	0.001
2-Way Interactions	4	0.8626	0.21566	2.52	0.115

Power*Time	4	0.8626	0.21566	2.52	0.115
Error	9	0.7716	0.08573		
Total	17	12.5541			

General Factorial Regression: BI versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	14.593	1.8241	4.18	0.024
Linear	4	10.140	2.5350	5.80	0.014
Power	2	6.947	3.4733	7.95	0.010
Time	2	3.193	1.5967	3.66	0.069
2-Way Interactions	4	4.453	1.1132	2.55	0.112
Power*Time	4	4.453	1.1132	2.55	0.112
Error	9	3.931	0.4368		
Total	17	18.524			

General Factorial Regression: Lauric (mg/mL) versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	0.32028	0.040035	13.44	0.000
Linear	4	0.27631	0.069077	23.19	0.000
Power	2	0.21207	0.106035	35.60	0.000
Time	2	0.06424	0.032118	10.78	0.004

2-Way Interactions	4	0.04397	0.010993	3.69	0.048
Power*Time	4	0.04397	0.010993	3.69	0.048
Error	9	0.02681	0.002979		
Total	17	0.34709			

General Factorial Regression: Miristic (mg/mL) versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	0.018054	0.002257	10.53	0.001
Linear	4	0.014831	0.003708	17.30	0.000
Power	2	0.011392	0.005696	26.58	0.000
Time	2	0.003439	0.001719	8.02	0.010
2-Way Interactions	4	0.003223	0.000806	3.76	0.046
Power*Time	4	0.003223	0.000806	3.76	0.046
Error	9	0.001929	0.000214		
Total	17	0.019982			

General Factorial Regression: Palmitic (mg/mL) versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	0.093420	0.011677	14.79	0.000
Linear	4	0.076057	0.019014	24.09	0.000
Power	2	0.060128	0.030064	38.08	0.000
Time	2	0.015929	0.007965	10.09	0.005

2-Way Interactions	4	0.017363	0.004341	5.50	0.016
Power*Time	4	0.017363	0.004341	5.50	0.016
Error	9	0.007105	0.000789		
Total	17	0.100524			

General Factorial Regression: Oleic (mg/mL) versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	63.981	7.9976	21.73	0.000
Linear	4	56.250	14.0625	38.21	0.000
Power	2	42.006	21.0028	57.08	0.000
Time	2	14.244	7.1222	19.35	0.001
2-Way Interactions	4	7.731	1.9327	5.25	0.018
Power*Time	4	7.731	1.9327	5.25	0.018
Error	9	3.312	0.3680		
Total	17	67.293			

General Factorial Regression: Stearic (mg/mL) versus Power and Holding time

Factor Information

Factor	Levels	Values
Power	3	100, 200, 300
Time	3	5, 10, 15

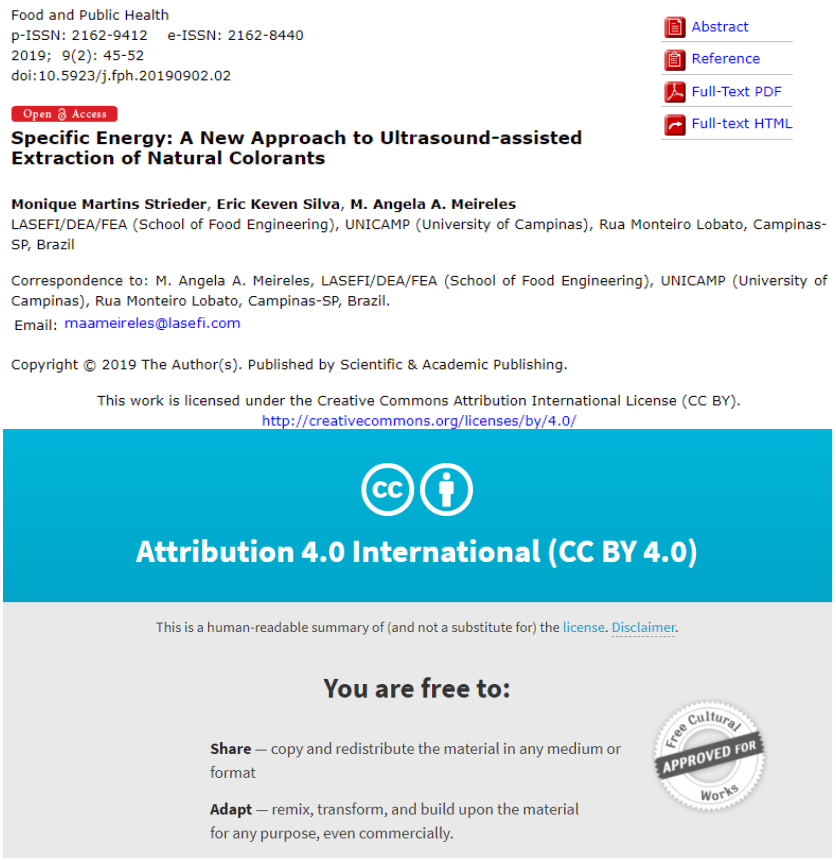
Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	3.9557	0.49447	4.40	0.020
Linear	4	3.0277	0.75692	6.74	0.009
Power	2	2.8644	1.43218	12.75	0.002
Time	2	0.1633	0.08166	0.73	0.510

2-Way Interactions	4	0.9281	0.23202	2.07	0.168
Power*Time	4	0.9281	0.23202	2.07	0.168
Error	9	1.0111	0.11235		
Total	17	4.9669			

Appendix XXIII - Publishers' authorization

✓ Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants:



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Abstract
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Specific Energy: A New Approach to Ultrasound-assisted Extraction of Natural Colorants

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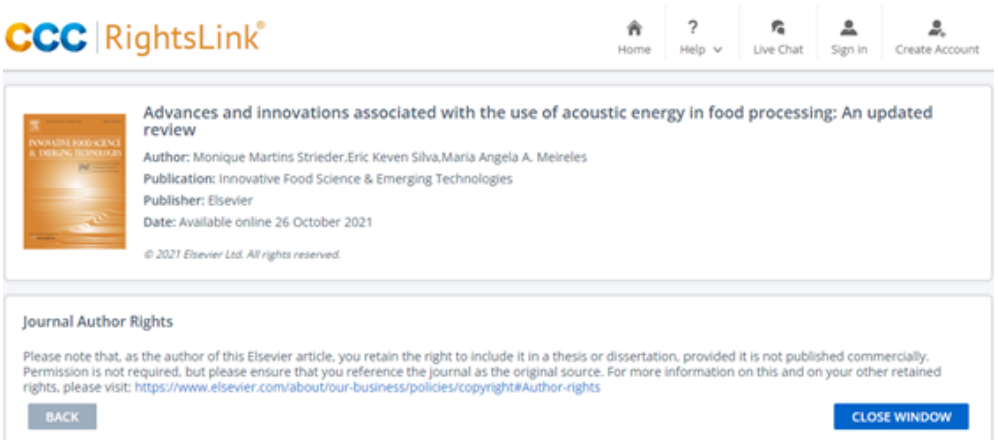
✓ Advances and innovations associated with the use of acoustic energy in food processing: An updated review:



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Advances and innovations associated with the use of acoustic energy in food processing: An updated review

Author: Monique Martins Strieder,Eric Keven Silva,Maria Angela A. Meireles
Publication: Innovative Food Science & Emerging Technologies
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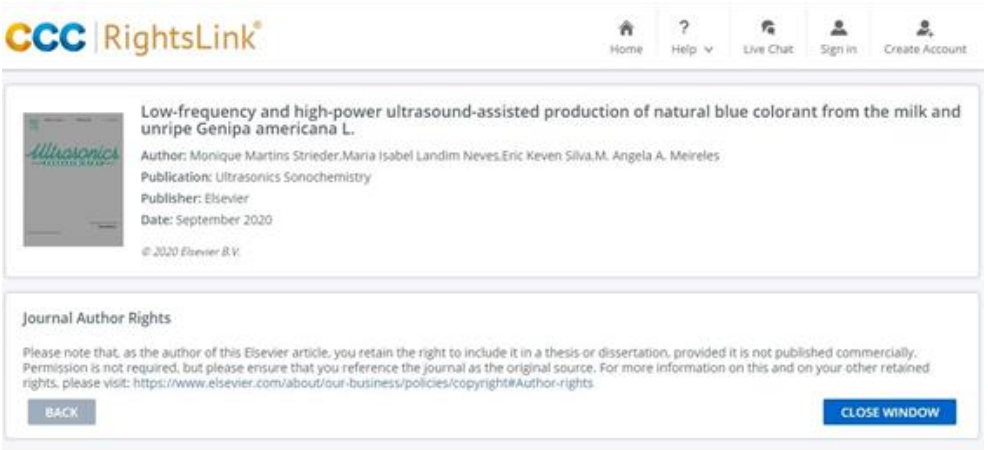
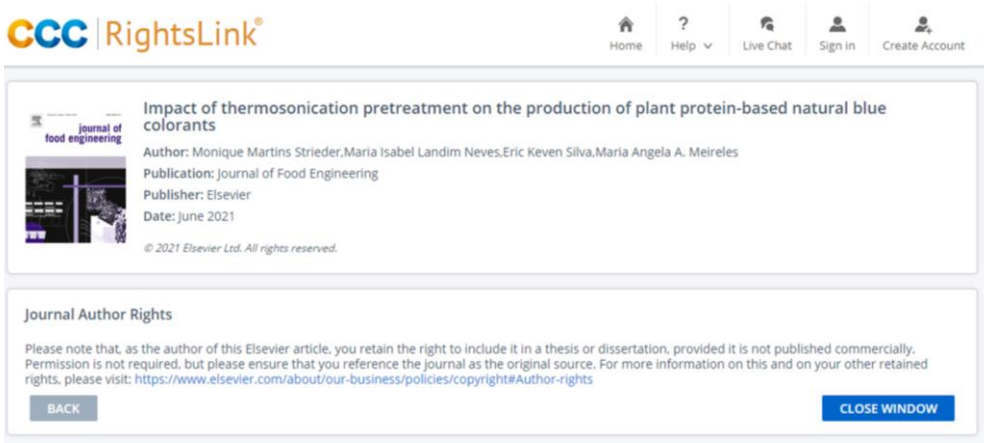
BACK CLOSE WINDOW

✓ A techno-economic evaluation for the genipin recovery from *Genipa americana* L. employing non-thermal and thermal high-intensity ultrasound treatments:

✓ Low-frequency and high-power ultrasound-assisted production of natural blue colorant from the milk and unripe *Genipa americana* L.:



✓ Impact of thermosonication pretreatment on the production of plant protein-based natural blue colorants:



✓ Impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage:

Appendix XXIV – Publications performed in partnership during the Ph.D. period

- ✓ Patent: "Processo de produção de corante natural azul". It was filed in the Instituto Nacional da Propriedade Industrial (BR 10 2020 026302 1) on December 21, 2020.

INPI INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

21/12/2020 870200159779 18:51

29409161923626387

Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2020 026302 1

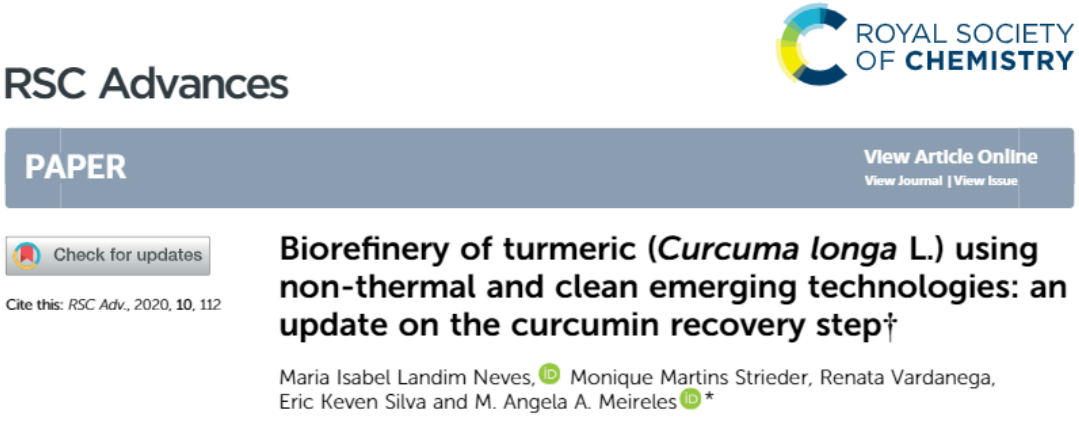
Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social: UNIVERSIDADE ESTADUAL DE CAMPINAS - UNICAMP
Tipo de Pessoa: Pessoa Jurídica
CPF/CNPJ: 46068425000133
Nacionalidade: Brasileira
Qualificação Jurídica: Instituição de Ensino e Pesquisa
Endereço: Cidade Universitária Zeferino Vaz
Cidade: Campinas
Estado: SP
CEP: 13084-971
País: Brasil

Inventors: Maria Angela de Almeida Meireles, Eric Keven Silva, Maria Isabel Landim Neves e Monique Martins Strieder

- ✓ Biorefinery of turmeric (*Curcuma longa* L.) using non-thermal and clean emerging technologies: an update on the curcumin recovery step.



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Cite this: *RSC Adv.*, 2020, **10**, 112

Biorefinery of turmeric (*Curcuma longa* L.) using non-thermal and clean emerging technologies: an update on the curcumin recovery step†

Maria Isabel Landim Neves, Monique Martins Strieder, Renata Vardanega, Eric Keven Silva and M. Angela A. Meireles *

✓ Milk colloidal system as a reaction medium and carrier for the natural blue colorant obtained from the cross-linking between genipin and milk proteins.

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Milk colloidal system as a reaction medium and carrier for the natural blue colorant obtained from the cross-linking between genipin and milk proteins

Maria Isabel Landim Neves, Monique Martins Strieder, Eric Keven Silva*, M. Angela A. Meireles

LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), Rua Monteiro Lobato, 80, Campinas, SP 13083-862, Brazil

✓ Fructans with different degrees of polymerization and their performance as carrier matrices of spray dried blue colorant.

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Carbohydrate Polymers

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Fructans with different degrees of polymerization and their performance as carrier matrices of spray dried blue colorant

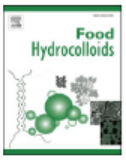
Maria Isabel Landim Neves, Monique Martins Strieder, Ana Silvia Prata, Eric Keven Silva *, Maria Angela A. Meireles

✓ Xylooligosaccharides as an innovative carrier matrix of spray-dried natural blue colorant.

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



Xylooligosaccharides as an innovative carrier matrix of spray-dried natural blue colorant

Maria Isabel Landim Neves, Monique Martins Strieder, Ana Silvia Prata, Eric Keven Silva *, Maria Angela A. Meireles

✓ Manufacturing natural blue colorant from genipin-crosslinked milk proteins: Does the heat treatment applied to raw milk influence the production of blue compounds?.

Contents lists available at ScienceDirect

Future Foods

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Manufacturing natural blue colorant from genipin-crosslinked milk proteins: Does the heat treatment applied to raw milk influence the production of blue compounds?

Maria Isabel Landim Neves, Monique Martins Strieder, Eric Keven Silva*, Maria Angela A. Meireles

✓ Thermosonication process design for recovering bioactive compounds from fennel: A Comparative study with conventional extraction techniques.

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Article

Thermosonication Process Design for Recovering Bioactive Compounds from Fennel: A Comparative Study with Conventional Extraction Techniques

Adela Cristina Martinez Urango, Monique Martins Strieder, Eric Keven Silva * and Maria Angela A. Meireles