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Chemistry

Anthocyanin: a review of the technologies for obtaining the compound

Antocianina: uma revisão sobre as tecnologias para obtenção do composto

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ABSTRACT

Anthocyanins are phenolic compounds with high antioxidant properties obtained from plant sources, such as leaves, flowers, and fruits. As it is a thermosensitive compound, it requires a lot of control over the extraction method so that there is no degradation or reduction of antioxidant activity. In this context, this work presents a systematic review about anthocyanin extraction as well as a discussion of parameters that strongly influence the yield and amount of extracted anthocyanins, such as types of solvents and acidifiers, extraction time, solid-liquid ratio, and temperature. It was observed that solvent extraction and ultrasonic assisted extraction are the most used techniques, as well as methanol and ethanol, the most used solvents. The extraction is independent of the raw material but is optimized when performed at low pH and milder temperatures. The preference for polar solvents also stands out, due to the better solubility in relation to the bioactive ones.

Keywords: Extraction; Antioxidant activity; Phenolic compound; Anthocyanidins

RESUMO

As antocianinas são compostos fenólicos com altas propriedades antioxidantes obtidos de fontes vegetais, como folhas, flores e frutos. Por ser um composto termossensível, requer muito controle sobre o método de extração para que não haja degradação e redução da atividade antioxidante. Neste contexto, este trabalho apresenta uma revisão sistemática sobre extração de antocianinas, bem como uma discussão de parâmetros que influenciam fortemente o rendimento e a quantidade de antocianinas extraídas, tais como tipos de solventes e acidificantes, tempo de extração, relação sólido-líquido e temperatura. Observou-se que a extração por solvente e a extração assistida por ultrassom são as técnicas mais utilizadas, assim como o metanol e o etanol os solventes mais utilizados. A extração, independente da matéria-prima, é otimizada quando realizada em pH baixo e temperaturas mais amenas. Destaca-se também a preferência pelos solventes polares, devido à melhor solubilidade em relação aos bioativos.

Palavras-chave: Extração; Atividade antioxidante; Composto fenólico; Antocianidinas

1 INTRODUCTION

Antioxidant compounds are responsible for inhibiting or reducing the oxidation of molecules, preventing diseases resulting from the oxidative stress of cells. Among the components with high antioxidant power are tannins, anthocyanins, carotenoids, and others (Cömert; Gökmen, 2017).

Anthocyanins (from Greek, *anthos* = flower, *kyáneos* = purple) are originated in the secondary metabolism of plants and present in their vascular system and cellular organelles (Wang et al*.;* 2020; Da Rocha; Noreña, 2020). They are the compounds responsible for coloring (such as red, blue or purple) in leaves, fruits and flowers (Herrera-Ramirez et al*.;* 2020; Suhaimi et al*.;* 2020; Anggraeni et al*.;* 2019; Li et al*.;* 2019; Noda et al*.;* 2000), but can also be present in stems and roots (Wang et al*.;* 2020). As functions, they protect the plant against the action of ultraviolet light, regulate photosynthesis and serve as a visual attraction for animals for seed dispersal and pollination (Armbruster, 2002).

The anthocyanin molecule, belonging to the polyphenols flavonoid group, is a glycoside found in plant structure, formed by the binding of anthocyanidin (primary structure) to sugar molecules and is formed by a C_6-C_3-C_6 skeleton (Wang et al*.;* 2016). It is considered that there are about 23 types of anthocyanidins in nature, of which the molecules of greatest occurrence are cyanidin, delphinidin, petunidin, peonidin, pelargonidin and malvidin. The formation of these anthocyanin subspecies depends on the position and degree of methylation of the group -OH; and on the nature, position and number of sugars bound to structure and aliphatic or aromatic acids bound to the sugars of the molecule. They are highly soluble compounds in aqueous media however, quite unstable and easily degradable due to environmental factors such as light, oxygen, storage temperature and pH (Guo *et al.;* 2019; da Rocha; Noreña, 2020; Milea et al*.;* 2019; Zapata et al*.;* 2019).

The applications of anthocyanins range from their use in food colorants to replace synthetic dyes (Rose et al*.;* 2018; Parra-Campos; Ordóñez-Santos, 2019) to their use in medicine as anti-inflammatory, anti-carcinogenic and neuroprotective due to their antioxidant action (Popović et al*.;* 2019; Akhbari; Hamedi; Aghamiri, 2019). Studies have reported that the use of anthocyanins has a significant effect on the treatment of cancer and other chronic diseases (Condurache et al*.;* 2019; Gagneten et al*.;* 2019), due to the ability to capture free radicals by exchanging electrons with hydrogen that are linked to phenolic compounds. Some authors suggest that there is a linear correlation between the content of total polyphenols, to which anthocyanin is incorporated, and the antioxidant activity in several fruits (Casagrande et al*.;* 2019; Ravanfar et al*.;* 2018).

However, anthocyanins are not readily available, and it is necessary to perform a component extraction and subsequent purification. The approach of technologies to obtain anthocyanins plays an important role, in order to evaluate the efficiency of the processes and define which are the most suitable, according to the type of material from which the antioxidant will be extracted (Gallego; Bueno; Herrero, 2019).

The recovery of anthocyanins from natural sources, such as fruits and flowers, is not always easy and feasible for industrial scale application, since the extraction of these compounds is a delicate process due to their low stability (Albuquerque et al*.;* 2020). Several innovative technologies are being developed to extraction of anthocyanins compounds (Kumar et al*.;* 2019). Thus, the objective of the study was to conduct a systematic review on the main methods of anthocyanin extraction, including a discussion on the main factors that may interfere with the extraction yield.

2 MATERIALS AND METHODS

The search for anthocyanin-related articles was based on a term search in Scopus' database. By combining binary operators, the term "anthocyanin" was required in the title and one of the terms deemed relevant to separation processes (“extraction”, “filtration”, “separation”, “purification”, “obtainment”) in the author’s keywords. In addition, it was stipulated that the articles should have the year of publication after 2015. Also, it was observed that the term “analysis” should not be in the title, since the objective was not to find articles of validation or optimization of analytical techniques. The search returned a total of 161 articles.

After exporting the indexing data of the articles found, new attributes were established to restrict the articles: the articles before 2018 should have at least 3 citations, and those after 2018 should have been cited at least once. With these restrictions a number of 106 articles were reached, ranked in decreasing order by the number of citations.

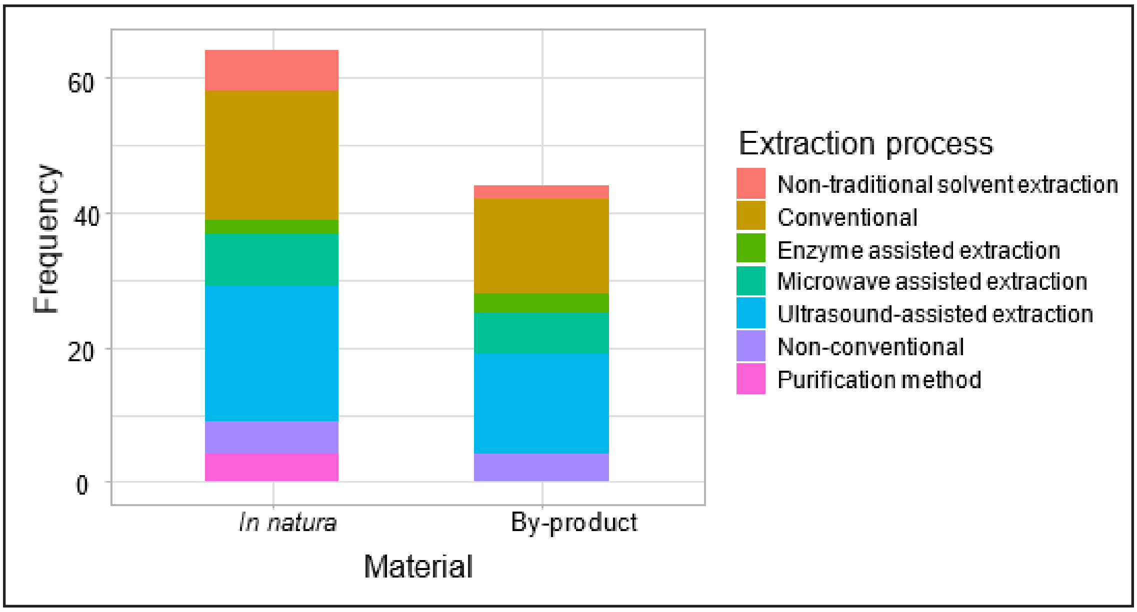
The survey of articles used the “Bibliometrix” library of RStudio® software version 7.6 (R Core Team) for bibliometric analysis and from this, the main topics and trends related to the central theme were pointed out to an evaluation of state-of-the-art throught discussions that generate new knowledge about the separation and purification of the anthocyanins.

3 RESULTS AND DISCUSSIONS

3.1 Bibliometric analysis

According to the bibliometric analysis carried out, based on research involving experimentation or the use of extraction processes, a classification was made according to the process involved (Figure 1), the type of material used as source of anthocyanins, and the parameters that configure the optimal extraction scenario, including, when applicable, temperature and time of extraction, type and concentration of solvent, type of acidifier and relationship between solid (extraction source) and liquid (solvent).

Figure 1 – Anthocyanin's extraction processes grouped by the type of material used as in natura and by-product



Source: Authors (2023)

Analyzing the Figure 1, it is noticed that conventional and ultrasound techniques have a higher frequency of citation, which may be related to the higher yield when this type of extraction process is used. Moreover, although the frequency of publications with by-products is lower than in natura materials, it is possible to observe that the by-products still have a significant amount of bioactive compounds, such as anthocyanins, being a promising source of obtain for the extraction of the component.

3.2 Anthocyanins extraction processes

Food processing by-products are often still rich in bioactive compounds which, if properly extracted and recovered, can be valorized into valuable food supplements or in nutraceutical formulations, mitigating their environmental impact and also adding economic benefits (Gallego; Bueno; Herrero, 2019).

There is no direct relationship between the different types of extraction for a given source of anthocyanin. What occurs is the existence of several investigations about different methods for increasing yield and reducing costs.

It is possible to see that conventional extraction, which uses solvents, and ultrasound-assisted extraction are the main methods used. This preference is mainly due to the yield obtained from bioactives. The use of enzymes is not very usual due to secondary reactions that these may produce, compromising the extraction rate (Meini et al.*;* 2019).

3.2.1 Conventional extraction

Solvent extraction or conventional extraction consists of keeping the sample in contact with a solvent for a certain period so that the liquid can penetrate the solid matrix, solubilizing the components and concentrating them in the extract. Purification methods must be applied later to obtain the solvent-free anthocyanins.

Many authors compared the extraction of anthocyanins using different solvents (Hosseini et al.*;* 2016; Silva et al.*;* 2017; Coklar; Akbulut, 2017; Duy et al.*;* 2019). Although the raw material for extraction differs among these authors, some conclusions are similar, especially regarding the kinetics of degradation of these biocomposts. The extraction temperature is a crucial factor to ensure the stability of anthocyanins, because each raw material has its critical temperature that when exceeded, there is degradation of tissues and consequent degradation of anthocyanin molecules, from the separation of hydrogen atoms in benzene rings or anthocyanin glycosides (Khazaei et al.*;* 2016; Liu et al.*;* 2019), reducing the extraction potential. The density and polarity of solvents significantly affect the extraction of the compounds too. The Table 1 shows some studies that use conventional extraction and optimal extraction conditions obtained in the experiments.

Although solvent extraction is a widely used technique, there is a high solvent consumption and usually longer process times. It is necessary to minutes minimize the amount of solvent used to make the process more sustainable (Pinela *et al.;* 2019). Therefore, other methods can be associated with solvent extraction, such as the use of ultrasound, chromatography and microwaves, ensuring greater efficiency of compound extraction (Kou et al.*;* 2019).

Table 1 – Relationship between studies and optimal conditions of conventional extraction to obtain anthocyanins

(Continued)

Raw material	**Optimum extraction conditions**	**Levels of anthocyanin extracted (mg cyanidin-3-glucoside.g^{-1})**	**Reference**
Blueberry Wine Pomace	Temperature: 61°C. Extraction time: 35 minutes. Solvent: etOH 70% (v/v) acidified with HCl 0,01% (v/v).	1,72	He et al.; (2016a)
Purple sweet potatoes	Temperature: 60°C. Extraction time: 90 minutes. Solvent: etOH 80% (v/v) acidified with HCl 0,1% (v/v).	21,75 ± 0,012	Cai et al.; (2016)
Residues of blackberry	Temperature: 80°C. Extraction time: 300 minutes. Solvent: etOH.	2,82 ± 0,16	Machado et al.; (2017)
Residues of blueberry	Temperature: 80°C. Extraction time: 300 minutes. Solvent: etOH.	2,58 ± 0,13	Machado et al.; (2017)
Residues of grumixama	Temperature: 80°C. Extraction time: 300 minutes. Solvent: etOH.	0,97 ± 0,14	Machado et al.; (2017)
Peel and pulps of eggplant	Temperature: 80°C. Extraction time: 300 minutes. Solvent: water acidified with HCl 0,01 M.	23,101	Ferarsa et al.; (2018)
Saffron floral bio-residues	Temperature: 66°C. Extraction time: 30 minutes. Solvent: etOH/water mixture (59:41 v/v).	45,9 ± 0,06	Da Porto & Natolino, (2018)
Sohiong fruit	Temperature: 36,75°C. Extraction time: 120 minutes. Solvent: etOH 60,32% (v/v) acidified with citric acid 2,39%.	85,8 ± 0,06	Swer et al.; (2016)
Eggplant shells	Temperature: 65°C. Extraction time: 30 minutes. Solvent: etOH 50% (v/v) acidified with HCl 2,5%.	4,32	Akhbari, Hamedi, & Aghamiri, 2019

Table 1 – Relationship between studies and optimal conditions of conventional extraction to obtain anthocyanins

(Conclusion)

Raw material	**Optimum extraction conditions**	**Levels of anthocyanin extracted (mg cyanidin-3-glucoside.g^{-1})**	**Reference**
Peel pear	Temperature: 71°C. Extraction time: 11 minutes. Solvent: etOH 57% (v/v) acidified with trifluoroacetic acid 3%.	0,266	Belwal et al.; (2019)
Singapore Azalea	Temperature: 80°C. Extraction time: 132,43 minutes. Solvent: meOH 99,5% (v/v) acidified with trifluoroacetic acid 0,05%.	4,6 mg/L	Aziz et al.; (2020)
Whole red grapes	Temperature: 72°C. Extraction time: 100 minutes. Solvent: meOH 65% (v/v) acidified with formic acid 1%.	13,64 ± 0,07	Iglesias-Carres et al.; (2018)
Fig shells	Temperature: 25 °C. Extraction time: 180 minutes. Solvent: meOH 90% (v/v) acidified with citric acid 0,5%.	3,47	Meziant et al.; (2018)
Campbell Early grape	Temperature: 80 °C. Extraction time: 10 minutes. Solvent: water acidified with acetic acid.	0,197	Ryu et al.; (2020)
Blue Honeysuckle	Temperature: 41,56°C. Extraction time: 30 minutes. Solvent: water acidified with HCl 0,35%	24,01 ± 0,37	Li et al.; (2019b)
Grape juice pomace	Temperature: 60°C. Extraction time: 45 minutes. Solvent: acetone	640 -780	Casagrande et al.; (2019)

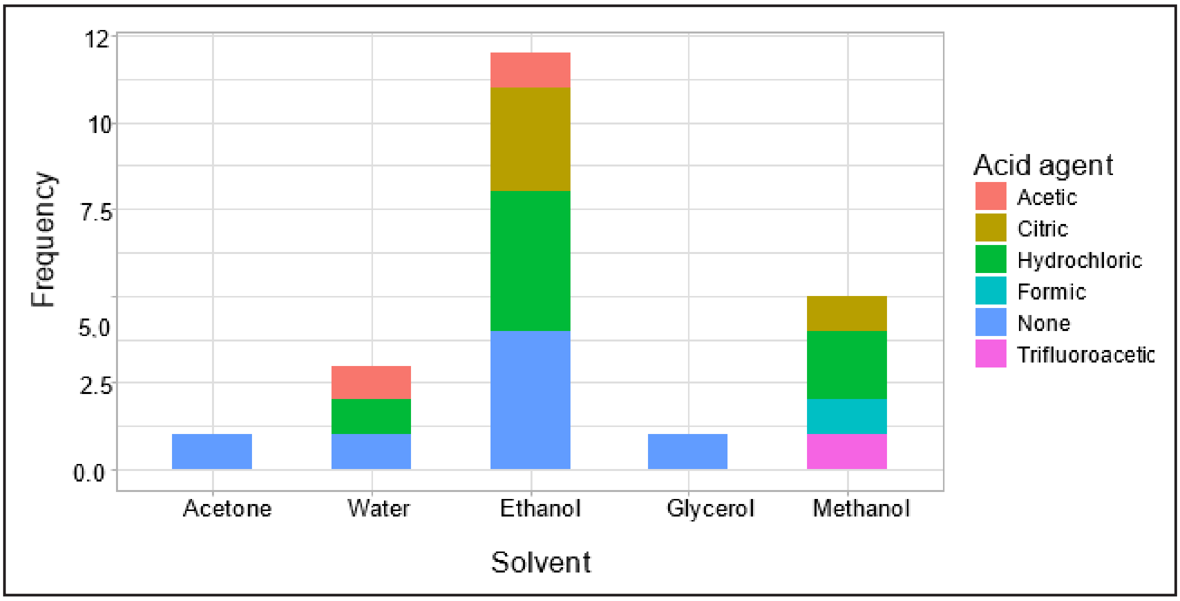
Source: Authors (2023)

3.2.1.1 Conventional solvents

Polar solvents have a higher extraction capacity, since anthocyanins are polar structures. Solid/liquid extraction is a traditional method to obtain several active compounds. In anthocyanins extraction, the commonly used solvents, due to the polar character, are aqueous mixtures of ethanol, methanol or acetone (Pérez-Orozco et al.*;* 2020). Duy et al. (2019) suggest that the addition of water in the solvent for extraction increases the anthocyanin extraction yield.

A very important factor when working with solvent extraction is the acidification of the solution to improve the extract yield. The main solvent for extraction has its pH reduced with the addition of an organic acid. The pH of the solution should preferably be low, since the acid has the ability to denature the membranes contained in the plant cells and dissolve the anthocyanin pigments. Moreover, at low pH there is the presence of anthocyanins in the form of flavylium ions, which is the easiest way to be extracted (Fernandes et al.*;* 2020). The main acidifying reagents used in the articles evaluated are presented in Figure 2.

Figure 2 – Main solvents and acidification agents used during conventional extraction



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3.2.3 Ultrasound Assisted Extraction (UAE)

Ultrasonic extraction is an auxiliary process of solvent extraction and is based on the formation of ultrasonic waves that disturb the cell walls of the solid matrix, allowing the penetration of the solvent inside the cells. It is a fast, simple and very effective technique, promoting high extraction rates. Several factors can affect the ultrasonic extraction process such as solvent composition and pH, ultrasound frequency and solid-solvent ratio (Ryu; Koh, 2019).

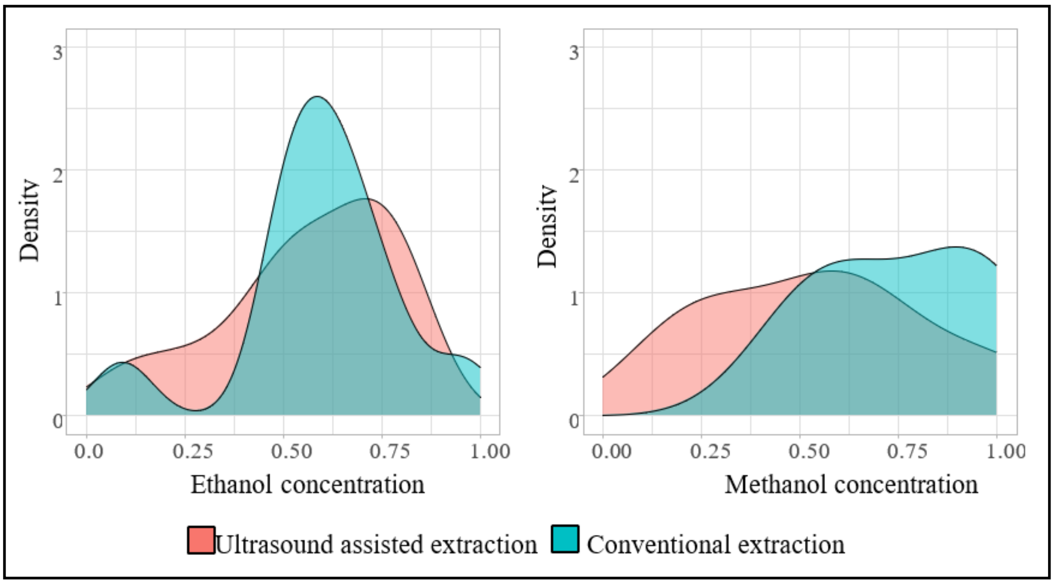
Espada-Bellido et al. (2017) tested several conditions using ultrasound for extraction of anthocyanins in mulberry pulp. The authors concluded that a temperature around 48°C, favored a better extraction of the compounds. To demonstrate that optimal conditions depend on several factors, let us compare the results presented in the previous sentence with those obtained by Sang et al. (2017), who submitted *Nitraria tangutorun* Bobr. seed bran to ultrasonic extraction. Despite being different raw materials, the most significant parameter in both studies was the temperature. For Sang et al. (2017), the optimal extraction temperature was 70°C, using a 25 kHz ultrasound probe. The difference between the values of this variable demonstrates that it is fundamental to research and optimize processes for different types of samples.

Another study on ultrasound was performed by Fernandes et al. (2020) where the authors observed the kinetics of extraction of anthocyanins from jabuticaba peels. The authors tested different ultrasonic frequencies, but in both analyses, the best extraction results were obtained when the pH was 1,5. The overlap of the low pH in relation to the others discussed is due to the cations of anthocyanidins being extracted more easily at lower pH.

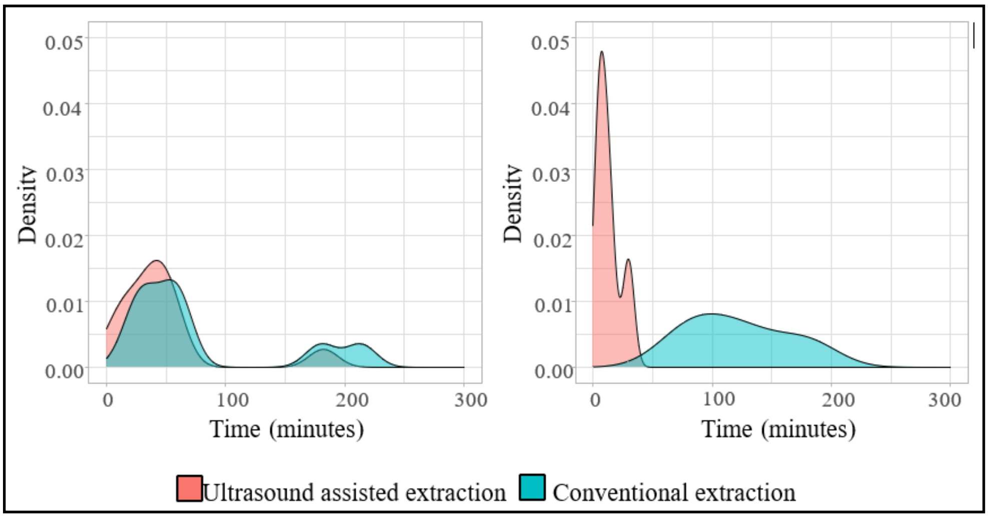
Observing that the UAE and the CE (Conventional Extraction) are the most used technologies, we conducted a discussion on the most significant parameters during the obtaining of anthocyanins, comparing the two extraction techniques. Furthermore, we evaluated the behavior of these two methods when methanol (meOH) and ethanol (etOH) are used as solvents. They are the most widely used ones due to the recognition of high extraction rate and the classification as safe and low toxicity solvents (Awika et al.; 2005; He et al.; 2016a).

When the UAE is applied, it can be seen that, in the ideal extraction scenario, the use of MeOH was observed in several concentrations, different from the EtOH in which there is greater evidence in concentrations between 50 and 70%. When evaluating the CE, it is noticed that for MeOH there is an increase in the use of concentrations over 20%, while for EtOH, the studies found optimum concentrations close to 60%. The Figure 3 shows a comparison of the frequency density of solvent concentrations, extraction times, solvent:sample ratio and temperature in the optimal scenario between conventional and ultrasonic assisted extraction.

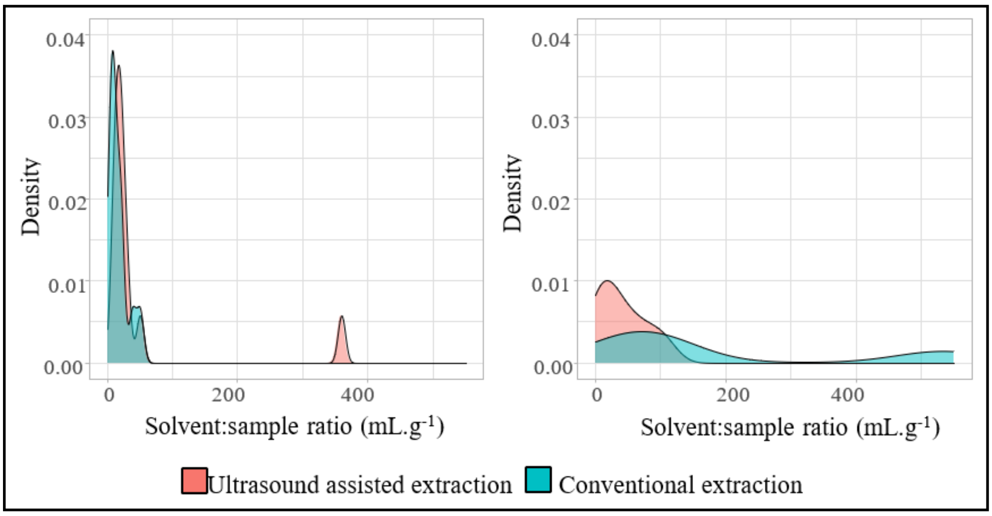
Figure 3 – Different scenarios comparing the conventional and ultrasonic assisted extraction



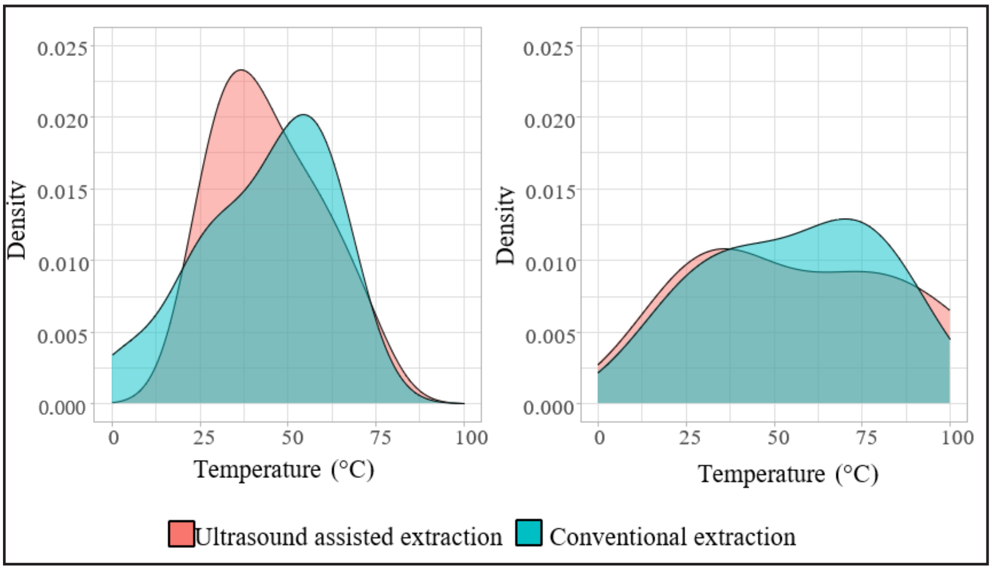
(a) Density plot of solvent concentrations in the optimal extraction



(b) Density plot of optimal extraction times



(c) Solvent:sample ratio in optimal extraction scenario



(d) Temperatures in optimal extraction scenario
Source: Authors (2023)

It is observed that the CE has a wide range of studied times, while for the UAE, the studies are concentrated in times of 30 minutesutes when methanol is used, and 50 minutesutes when ethanol is employed. Cai et al. (2016) observed that when using the UAE there is a 5.43% increase in the content of anthocyanins extracted, using half the time spent in conventional extraction. The UAE technique facilitates shorter extraction times when compared to CEs. This happens because the ultrasound generates a cavitation effect in the solvent, causing a greater movement of molecules and, therefore, a greater penetration of the solvent, leading to an acceleration of the process (Agcam; Akyildiz; Balasubramaniam, 2017).

The relationship between solvent volume and sample mass is a significant factor during the extraction process. It should be noted that a high proportion of solid:liquid, causes an extraction of low efficiency, as the capacity of dissolution is limited (Qin et al.*;* 2017). In their study, Ryu & Koh (2018) tested the conditions for maximum yield of black soy anthocyanins, in which they demonstrated that the total anthocyanin content extracted was directly proportional to the HCl concentration, in which a greater volume of solvent can dissolve the solute better, leading to a more effective yield in extraction. The optimal experimental conditions found by the authors were solvent concentration of 0.359%, sample:solvent ratio 54.2 $g.mL^{-1}$ and extraction temperature of 56.8ºC.

Pérez-Orozco et al. (2020) evaluated the effect of different temperatures, solvent polarity (water and ethanol) and sample:solvent ratio on the extraction of anthocyanins from Hibiscus rosa-sinensis, confirminutesg that all parameters affected extraction yields. The solvent/solid ratio significantly affected ($\alpha = 0.05$) the anthocyanin extraction yield at higher temperatures when using water as solvent. Temperature is a very significant factor in extraction processes, as it can promote the breaking of the phenolic matrix bond, denaturing the chemical structure of the plant cell membrane (Dranca; Oroian, 2016). Higher temperatures favor the solubility of the anthocyanins in the middle due to the increased diffusivity of the solute into the solvent, caused by the increased speed of movement of the molecules due to the energy dissipated in the environment (He et al*.;* 2016a).

Additionally, higher temperatures favor the reduction of viscosity and surface tension of solvents, which triggers greater penetration of the liquid into the membrane (Vázquez-Espinosa et al*.;* 2019). However, the increase of this parameter can also lead to the degradation of compounds in polymeric pigments (Khazael et al*.;* 2016) and also affect the antioxidant power of anthocyanins (Vergara-Salinas et al*.;* 2013).

The rise in temperature not only causes the degradation of the compound, but can also initiate solvent evaporation. It should be noted that temperatures above 70°C can cause the evaporation of methanol (Vázquez-Espinosa et al*.;* 2019), while for ethanol values above 80°C will start the evaporation of the compound.

3.2.4 Microwave assisted extraction (MAE)

Microwave assisted extraction (MAE) is a potential tool to reduce the amount of solvent to be used, time, temperature and energy consumption, resulting in better separation and recovery of compounds of interest. The principle of the method is based on the direct effect of microwaves on the ionic conduction or dipolar rotation molecules (Cassol; Rodrigues; Noreña, 2019). The electromagnetic radiation of the microwave energy results in the desolation of the cell wall matrix, rapidly increasing the penetration of solvents in plant cells and the leaching of compounds. Different chemical elements absorb the microwaves at different lengths, this behavior makes MAE an efficient method for extractions, making possible the selectivity of bioactive compounds in complex food matrices (Jafari; Khazaei; Assadpour, 2019).

Liu et al*.* (2019) varied the microwave power in two stages to obtain anthocyanins from blueberry powder. The highest rates were extracted at 800 W in the first stage and 280 W in the second, at a transition temperature of 36°C. The authors also observed a 17.75% increase in compound extraction when compared to microwave extraction at constant power. A lower degradation of anthocyanin was also reported.

In the article by (Cassol; Rodrigues; Noreña, 2019), the authors compared the extraction of phenolic compounds from hibiscus flowers using MAE; extraction in acidified aqueous solution followed by MAE; and MAE followed by extraction with citric acid solution. The results found indicate that the maximum extraction occurred in the MAE methodology with subsequent acid extraction, in a scenario of 700 W for 8 minutes of MAE, and 6 hours of extraction in acidified aqueous solution.

Fernandez-Aulis *et al.* (2019) used MAE to extract anthocyanins from purple maize by-products at a power of 600 W and using as solvent a mixture of ethanol:water:lactic acid (80:19:1). The optimal extraction time was 60 seconds with an extraction capacity of 24.4 $mg.g^{-1}$. When comparing MAE with other methods, it is possible to notice that its efficiency is much higher due mainly to the high amount of bioactive that can be extracted in such a short time. For conventional extraction in a time of 30 minutes, the authors obtained a content of 21.89 $mg.g^{-1}$, while for extraction with ultrasound a content of anthocyanins of 25.80 $mg.g^{-1}$ in 20 minutes was achieved.

3.2.5 Enzyme assisted extraction

The investigation of enzymes for the extraction of bioactive compounds is a promising field, since it does not require technological investments and is easily tested on a laboratory scale. The purpose of enzymes is to degrade the plant cell wall, releasing the intracellular content and improving the extraction of compounds of interest (Swer; Chauhan, 2019).

Nogales-Bueno et al. (2020) used grape samples with low anthocyanin extraction potential which were macerated in the presence of different enzymes that compose enzymatic preparations applied in the wine industry. For the extraction, cellulase, glucosidase and pectinase enzymes were applied individually and in mixtures, using a model wine hydroalcoholic solution for a 72 hours maceration period. Cellulase had a positive effect on the extraction of phenolic and anthocyanin compounds compared to the control assay. To a lesser extent, glucosidase showed a similar effect to cellulase, while the enzymatic mixture produced inconsistent results. Treatments with pectinase showed lower phenolic contents than those obtained in the controls.

Swer et al. (2019) studied enzyme assisted extraction to obtain anthocyanins from Sohiong, a dark colored fruit, using the cellulase enzyme. The extracts obtained were lyophilized and stored appropriately for later addition in food. Its results presented itself as an economical and ecologically viable alternative for commercial extraction of anthocyanins from Sohiong fruit that can be used as a natural dye with applicability in the food industry.

Jiang, Wang, & Yang, (2019) extracted anthocyanins from red radish and hydrolyzed them using α- and β-glucosaccharase, respectively, to trace which enzyme performs best. Their results showed that β-glucosaccharase showed better hydrolysis performance than α-glucosaccharase, showing a higher rate of hydrolysis, reaching 49.87±1.05%. Also, it was possible to observe that hydrolysis significantly increased the antioxidant and anti-proliferative capacities of radish anthocyanins. According to Aggarwal; Jain, (2019), enzyme-assisted processes for extraction of anthocyanins showed positive results with strawberry and chokeberry. However, it is necessary to find more enzymes able to extract the bioactive, without compromising the antioxidant capacity of the extract obtained.

3.2.6 Alternative solvents

The use of alternative solvents has been increasingly employed in research involving the extraction of anthocyanins. They are considered substitutes to conventional solvents due to low environmental impact, higher anthocyanin extraction efficiency and better stabilization of these biocomposts (Panic et al.; 2019; Xie et al.; 2019; Cassol; Rodrigues; Noreña, 2019; Guo et al.; 2019; Oktaviyanti; Kartini; Mun'im, 2019; Wang et al.; 2020).

3.2.6.1 Natural deep eutectic solvents (NADES)

Natural deep eutectic solvents (NADES) are liquid solvents composed of hydroxyl, carboxyl and amines groups, joined by hydrogen bonds. These solvents are characterized by low volatility, solubilization resistance, biodegradability and low cost.

Dai et al. (2016) evaluated the extraction capacity of anthocyanins from petals of the species *Catharanthus roseusem* using NADES and compared the results with acidified methanol extraction. More than one NADES was evaluated: 1,2-propanediol-choline chloride (PCH); lactic acid-glucose (LGH); proline-malic acid (PMH); malic acid-choline chloride (MCH); glucose-choline chloride (GCH) and glucose-fructose-sucrose (FGSH). The authors verified that PCH, LGH and FGSH solvents achieved a similar yield to conventional solvents in the extraction of anthocyanins and that the less viscous solvents achieved the highest yields. The stability of cyanidin (anthocyanidin in greater proportion in the study) proved to be greater when extracted with natural solvents.

Blueberry anthocyanins were extracted with NADES composed of choline chloride, glycerol, citric acid in the proportion of 0.5:2:0.5 respectively, by Silva et al. (2020). The extraction efficiency was compared with extraction using organic solvents (methanol:water:formic acid 3% and water:citric acid 1%). It was observed that the use of NADES showed a yield of 76% when compared to the solvent with methanol and a higher proportion of arabinoside anthocyanin.

3.2.6.2 β-cyclodextrin

β-cyclodextrin is a cyclic derivative prepared from partially hydrolyzed starch (maltodextrin) using an enzymatic process (Estevinho; Rocha, 2018) and has wide application in the food and pharmaceutical industries, as it is generally recognized as safe for human consumption (Xie *et al.;* 2019). The use of β-cyclodextrin for anthocyanin extraction is an uncommon method, but it was studied by Yu et al. (2018). The authors concluded that the increased concentration of β-cyclodextrin slowed the degradation process of the anthocyanins and helped to preserve the color of the compound.

In the work of Xie et al. (2019), the authors evaluated β-cyclodextrin as a solvent for the extraction of anthocyanins from four blackberries varieties, observing the thermal stability of the extracted compounds. The treatments with β-cyclodextrin surpassed the extractions using aqueous and ethanolic solutions for all cultivars, determinate using an optimal extraction scenario of 44.95 minutes, with a temperature of 20°C and a concentration of β-cyclodextrin of 45 $g.L^{-1}$. In addition, the use of the alternative solvent provided an increase in the stability of the extracted anthocyanins.

3.2.7 Non-conventional extraction

Some researchers are investing in atypical methods for obtaining products. This section highlights some methods that have been little studied when it comes to obtaining anthocyanins, but which are promising.

3.2.7.1 Pulsed electric field

The use of the pulsed electric field allows the electric rupture of the cell membranes, for minutes pores that alter the permeability of the matrix walls. Some factors such as plant tissue structure and cell size and shape may require specific parameters for the release of content through the membranes. The major disadvantage of this method is the selection of parameters for the extraction from different raw materials, which are often not available in the literature. In contrast, the method is highly efficient, low cost and reduces process times (Barba et al*.;* 2015).

Pataro et al*.* (2017) used pulsed electric field as pre-treatment for blueberry juice extraction and later obtaining of anthocyanins. The application was performed with a field force of 3 $kV.cm^{-1}$ with a change in the number of pulses applied at a frequency of 10 Hz. This first treatment aims to increase the permeability of cell membranes, which facilitates the release of juice and intracellular compounds. The authors observed that there was a greater amount of individual anthocyanins extracted when compared to control samples (without electric pulse).

3.2.7.2 Pressurized liquid extraction

This technique is based on the use of liquid solvents at high temperatures and pressures, which are capable of increasing the solubility of the compounds to be extracted, reducing the viscosity and interfacial tension of the solvent to facilitate its penetration into the matrix and improve mass transfer (Mustafa; Turner, 2011). The method is characterized for being quick, uses little amounts of solvent, and is indicated for the extraction of thermosensitive compounds (Feuereisen et al.; 2017).

Factors such as particle size and matrix structure can affect extraction yield. With this in minutes, it is recommended to pre-treat the sample, such as drying or freeze-drying, to create a porous structure and allow the solvent to enter the matrix more easily; besides ensuring, the stability of sensitive compounds (Eliasson; Labrosse; Ahrné, 2017).

Machado et al. (2017) combined ultrasound assisted extraction (UAE) and pressurized liquid (PLE) to recover anthocyanins from blackberry, blueberry and grumixam residues. The efficiencies of the methods were also evaluated individually and it was observed that the UAE was the most promising, followed by UAE + PLE and PLE. The low yield of PLE can be justified by the high pressures and temperatures employed in the process, which may have caused the degradation of anthocyanins.

Kitrytè et al. (2020) have developed an effective biorefining system to recover functional components of blueberry pomace, among other methods, using pressurized liquid extraction. This proved to be more efficient when compared to conventional enzyme assisted extractions, reaching a rate of 84% of polar constituents recovered from blueberry pomace, combining supercritical CO_2 and PLE, optimized with green food grade solvents. Approximately 89-94% of antioxidants, anthocyanins and proanthocyanidins were recovered using PLE-EtOH, with cyanidin-3-galactoside being the main anthocyanin.

3.2.7.3 Carbon dioxide supercritical extraction

Supercritical extraction occurs with the use of carbon dioxide at a temperature of 31.1°C and 7.38 MPa of pressure. Lighter conditions than these do not lead the gas to a supercritical condition (Elst et al*.;* 2018). The use of pressurized carbon dioxide (CO_2) to obtain anthocyanins from the filter pie after extraction of bilberry juice was performed by Eliasson et al. (2017). Ethanol was used as a co-solvent and a carbon dioxide flow rate of 3 $g.min^{-1}$. The study evaluated different particle sizes and types of drying (lyophilization, hot air and microwave assisted hot air). Furthermore, the authors compared supercritical extraction with conventional methanol extraction, and observed that methanol was able to obtain higher content of extracted anthocyanins for larger particle sizes. When extracting from small particles, allied with any pre-treatment of drying, the use of CO_2 proved to be an efficient technique, since there was an increase in surface area that directly influences a smaller diffusion path, allowing a more efficient extraction.

4 CONCLUSIONS

Several factors can affect the anthocyanin content during the extraction process such as temperature, solid: liquid ratio, time, type of solvent and acidification of the medium. High levels of these variables can degrade the compound or affect its antioxidant properties, and for low levels it can cause lower performance of the operation.

The use of residues to obtain anthocyanins has shown to be a promising alternative due to the concentration of the bioactive still present in the extract. However, the reuse of this residue can generate another by-product with greater toxicity due to the use of solvents for extraction. Therefore, alternative solvents appear to help in this aspect and minutes minimize the environmental impact.

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