

# Extraction Methods for Anthocyanins

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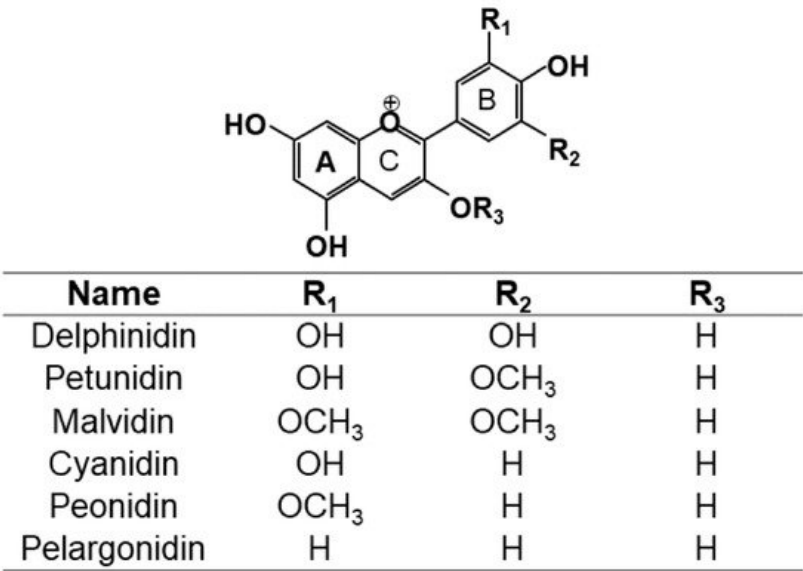
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Anthocyanins are considered high added-value compounds due to their sensory qualities, colors, and nutritional properties; they are considered bioactive ingredients. They are found in high concentrations in many by-products across the food industry. At present, expensive raw materials and production technologies make the extraction of natural anthocyanins relatively expensive.

Keywords: anthocyanins ; non-conventional extraction techniques ; anthocyanin yields ; industrial application

## 1. Introduction

In recent years, interest in food quality has increased. Consumers are increasingly becoming aware about the correlation between nutrition and health. Thus, many consumers are opting for foods that contain bioactive ingredients, which have health-promoting properties <sup>[1]</sup>. Vegetables and fruits have high amounts of antioxidant compounds that are considered bioactive ingredients. Different chemical compounds present in vegetables, fruits, and flowers have antioxidant properties. Some of these compounds belong to the group of anthocyanins, which are not only antioxidant compounds, but are also natural colorants (with aglycones of anthocyanins being the most common plant pigments). The properties of anthocyanins have been extensively studied in the literature <sup>[2][3][4][5][6][7][8][9][10]</sup>. These properties are directly related to their chemical structures. A flavylum cation acts as an acid, and it gives anthocyanins a high chemical reactivity. The structures and properties of anthocyanins depend on different factors, such as temperature, pH, and solvent. **Figure 1** shows the base structure of anthocyanins. These factors must be considered in the extraction processes to minimize changes in the quality and activity of the resulting extract <sup>[2][3]</sup>.



**Figure 1.** Structure of anthocyanins R<sub>3</sub> = sugar and anthocyanidins R<sub>3</sub> = H <sup>[2]</sup>.

The interest in anthocyanins can be attributed to the fact that they can be used as natural coloring agents rather than as synthetic coloring agents <sup>[11][12][13][14]</sup>. Anthocyanins have attractive colors, ranging from red to purple, and their use as food coloring agents has been authorized under code E163 by the European Food Safety Authority (EFSA) <sup>[11][15]</sup>. This has expanded the use of these natural coloring agents at the industrial level; they can be included in the food industry because they are innocuous and safe molecules. Moreover, anthocyanins have gained attention due to their potential health benefits, e.g., (i) anti-inflammatory, antioxidant, anti-diabetic, and anti-cancer properties; (ii) preventing cardiovascular diseases, neurological disorders, obesity, and for eye health; (iii) improving the gut microbiome; and (iv) decreasing H<sub>2</sub>O<sub>2</sub>-induced cell apoptosis of the human normal liver cell (LO2 cell) line <sup>[2][16][17][18]</sup>. Thus, anthocyanins

provide excellent added value to foods due to their dual nature as coloring agents and antioxidants, with beneficial effects on health. Therefore, they are determining factors in the quality and value of fruits and vegetables, and in processed food derived from those. In addition, they are widely used in the food and cosmetics industries.

However, their supply currently depends, to a large extent, on the complex extractions of these compounds from the matrix. At present, expensive raw materials and production technologies make the extraction of natural anthocyanins relatively expensive [1][19]. For this reason, new sources of anthocyanins are being studied, in order to extract these compounds at lower costs. Recent studies have explored the extraction performances of anthocyanins in different matrices, such as microalgae [20], potatoes [21][22], and rice [19][23]. In order to comply with current regulations regarding environmental sustainability, to improve economic performance and reduce waste in the food industry, there is growing interest in investigating the possibility of extracting anthocyanins from by-products and waste generated at different industrial food productions [17][24][25][26][27][28][29]. Anthocyanins recovered from food waste could have high potential in being used in different food and biotechnological applications, e.g., as food supplements, nutraceuticals, and/or food additives.

The food industry is currently looking for new sources of bioactive compounds, such as anthocyanins, to meet consumer demands. By-products and waste from some food industries represent low-cost sources of anthocyanins that are of interest to the industry. The extraction methods applied to anthocyanin extraction in different natural matrices have been extensively studied [6][17][30][31][32][33][34][35]. Conventional techniques, such as maceration and heat-assisted extraction (HAE), do not require sophisticated instrumentation and are easy to apply at the industrial level. However, they have a number of limitations, such as the following: the toxicity of the solvents used, possible solvent residues in the extracts, safety risks associated with the use of large volumes of solvents, deterioration of the extracts due to heating, and low yields in the extraction of anthocyanins. The latter limitation may be due to the fact that anthocyanins are found in the vacuoles of plant cells. In order to extract them with cost-effective yields, it is necessary to apply extraction methods that reduce the mass transfer resistance of the plant cell wall. To avoid the resistance of the cell wall, emerging extraction technologies have been proposed, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), high-pressure liquid extraction (HPLC), pulsed electric fields (PEFE), high voltage electrical discharge (HVED), and enzyme assisted extraction (EAE). These techniques require more sophisticated instrumentation than conventional techniques, but have some advantages: in general, they do not use heat for extraction, reducing energy costs, or improving the stability of the extracts. In addition, the volume of solvent used is lower (or zero) compared to conventional techniques, reducing greenhouse gas emissions and complying with the legal requirements of green chemistry. Thus, selecting the most appropriated extraction techniques is the primary goal for many industries, so that they can increase profitability by decreasing energy costs, and be responsible with the “green chemistry”.

## **2. Extraction Methods Used to Extract Anthocyanins**

The first step is extraction. In this way, the desired natural products can be separated from the raw materials. Solvent extraction, distillation method, pressing, and sublimation are some extraction methods, according to the extraction principles. The selection of the extraction process, to extract anthocyanins, is based on the preservation of the stability and shelf life of these compounds, which is directly related to the beneficial properties that these compounds provide. Sometimes, prior to applying the selected extraction technique, it is necessary to eliminate other compounds present in the sample matrix that may hinder the extraction of the anthocyanins, such as lipids, proteins, or contaminants. In this section, the conventional extraction techniques applied to extract anthocyanins are described and classified, with their advantages and disadvantages highlighted.

### **2.1. Conventional Extraction Techniques**

Depending on the procedure used to mix the powdered solid sample with the solvent, the conventional techniques are commonly classified as: (i) maceration, when the powdered crude sample is mixed with solvent; (ii) infusion, when a maceration is carried out with water; (iii) digestion, when a maceration is carried out with mild heating, also known as heat-assisted extraction (HAE); (iv) decoction, when an infusion is made with boiling water; (v) percolation and filtration, when the powdered sample is mixed with a continuously renewed solvent in a percolator, with a filtration process applied afterwards. More recently, the Soxhlet extraction technique emerged, which consists of mixing the powdered solid with the solvent inside a “Soxhlet apparatus”, allowing continuous cyclical repetitions of the extractions during a controlled period of time.

These techniques are based on the use of different types of solvents and/or heat. Considering the law that “like dissolves like”, the solvents commonly used to extract anthocyanins are: methanol, ethanol, water, acetone, or mixtures thereof.

Acid solutions are often added to these solvents to help stabilize the flavylum cation, which is stable in highly acidic conditions (pH ~ 3). To achieve this, the use of weak acids (e.g., formic acid, citric acid, or acetic acid) is recommended, since the use of strong concentrated acids may lead to destabilizing the anthocyanin molecule. In view of the polar structure of the anthocyanins, the addition of water to the solvent mixture can improve the extractive yield.

In addition to the solvents, the powder size of the solid, the solvent-to-solid ratio, and the time and temperature of the extraction are other analytical parameters that should be optimized to ensure the maximum yields. Different authors have optimized some of these analytical parameters to extract the maximum yield of total anthocyanin content (TAC) in different natural matrices. Paludo et al. [36] concluded that the optimal conditions for extracting anthocyanins and phenolic compounds from the skin and seed of Jabuticaba (*Plinia cauliflora*) fruits, respectively, was a solvent mixture of methanol/water/acetic acid (80:20:0.5 v/v/v), with a solid–liquid ratio of 0.01 g/mL, with two hours of constant agitation. Other authors consider the use of temperature necessary to optimize the extraction yield, as is the case of Albuquerque et al. [11], who claim that the optimal conditions to extract anthocyanins in the skins of Jabuticaba fruits involved a solvent mixture of ethanol (9.1% v/v) acidified with citric acid at pH 3, mixed and centrifuged with the powdered sample (~20 mesh), in a solid–liquid ratio of 50 g/L during 21.8 min at 47.1 °C. They conclude that the total amount of anthocyanins extracted increase with mild temperatures and decrease with high ethanol concentrations and with long times of extraction. The latter could be explained by the fact that long times could lead to the breakdown of the structures of sensitive compounds, such as cyanidin-3-O-glucoside [11]. Other studies carried out with other natural matrices found similar extraction conditions in the optimization of anthocyanin extraction. Thus, Demirdöven et al. [37] determined that a solvent mixture of ethanol (42.39% v/v) acidified with formic acid in a solid–liquid ratio of (1:3 w/v) heated at 40 °C for 75 min were optimal conditions to extract anthocyanins in red cabbage. Backes et. [38] affirmed that the optimal analytical parameters to improve the yield of TAC in fig skin was a solvent mixture of ethanol (100% v/v) acidified with citric acid (pH = 3), mixed and centrifuged with the powdered sample (~20 mesh) in a solid–liquid ratio of 50 g/L for 13.74 min at 35.64 °C. These authors revealed that the high content of ethanol and the temperature increase the yield of the extraction. In the particular case of cyaniding-3-rutinoside, the maximum extraction was obtained when 100% of ethanol was used, in contrast to what was stated by Albuquerque et al. [11]. It is well-known that high values of ethanol in the solvents increase the extraction of bioactive compounds from plant materials. However, these studies pointed out the importance of the amount of ethanol in the solvent mixture in the selective extractions of individual anthocyanins [11][12][37]

These studies show that, at the laboratory level, the analytical parameter, solid–liquid S/L ratio, has little effect on TCA extraction [12]. However, Backes et al. [38] conducted a study to optimize the solid-to-liquid (S/L) ratio in order to apply HAE at the industrial scale. They determined that a S/L ratio higher than 200 g/L does not allow a homogenous mixture. Furthermore, they established that, depending on the TAC in the natural material, the optimal S/L ratio changes, since highly concentrated samples will saturate the solvent earlier and need lower S/L than less concentrated samples. Their results applied to fig skin concluded that a S/L ratio higher than 100 g/L provoked saturation of the solvent, leading to a decrease in cyanidin-3-rutinoside levels. Fernandes et al. [12] affirmed that, in basil leaves, when the S/L ratio increased from 15 to 30 g/L, any significant proportional decrease was not observed. Thus, they concluded that ratios lower than 30 g/L do not provoke saturation in the extraction of anthocyanins from basil leaves.

These types of extractions are currently the most widely used in the industry, particularly in natural dye industries, likely because these extraction methods have low instrumentation costs. Despite their wide use, these methods also have some disadvantage: (i) high energy consumption; (ii) the use of environmentally unfriendly organic solvents; (iii) the need for expensive and high purity solvents; (iv) the use of a large volume of solvents; (v) the application of a long extraction time to extract compounds with lower yields; (vi) the need for moderate–high temperatures in some cases, which could cause deterioration of antioxidants; (vii) the need for evaporation of a huge amount of solvents; and (viii) the low selectivity of extraction [24][29][39]. Thus, conventional extraction techniques are currently applied in the industry. However, they do not provide high yields in the extraction of anthocyanins, although the yield can improve by increasing the temperature of the extraction. It can also provoke change in the color or in the properties of the extract. The most important factors to optimize are the S/L ratios, which depend on the TAC in the matrix and the solvent used for the extraction. The optimization of the solvent amount is essential to minimize the use of contaminants and work, in agreement with the legal requirements, and in order to reduce the evaporation costs.

## 2.2. Non-Conventional Extraction Techniques

To overcome the above-mentioned disadvantages of conventional extraction methods, new and promising extraction techniques have been introduced over the year. These techniques are more environmentally-friendly and have important industrial focuses, as they aim to improve the extraction efficiency and yield. However, they have not been employed on a massive scale yet. Among these extraction methods, the most applied techniques to extract anthocyanins are: ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), high-pressure liquid

extraction (HPLE), pulsed electric fields (PEFE), high voltage electrical discharge (HVED), and enzyme assisted extraction (EAE).

### 3. Advantages and Disadvantages of the Promising Green Extraction Techniques

According to the results presented above, the most promising green techniques to improve the anthocyanin yield and the extraction efficiency from natural matrices have been presented. These techniques are considered as 'green non-conventional techniques'. All of them have the following in common: they reduce processing time, temperature, energy consumption, and the use of organic solvents, in comparison with conventional techniques. In addition, they have previously been shown to be effective in the extraction of anthocyanins; thus, which of these techniques is the most suitable for extracting anthocyanins at an industrial scale? It is not possible to give a clear answer to this question, as each technique has a number of advantages and disadvantages. In general, the selection of extraction methods depends mainly on many factors, such as the physicochemical properties of the compound and solvent, the economic value of the compound, environmental concerns, the cost of the process, the required instrumentation, among others. **table 1** shows a comparison between these techniques based on their strengths, weaknesses, and suitability to extract anthocyanins.

**table 1.** Strengths, weaknesses, and suitability to extract anthocyanins of non-conventional extraction techniques.

Technique	Strengths	Weaknesses	Suitability
UAE	Versatile, flexible, low cost, and very easy to use; fast energy transfers; low solvent usage; extraction time (5–60 min); can be combined with heating to improve the yield or with enzymatic treatment to improve the anthocyanin yield and the bioactivity of the extract; available on a large scale.	Lack of homogeneity in the process improved by probe system (PUE); the large-scale application could be limited by the higher cost and nonlinearity of process; after the extraction, a filtration and clean-up step is required; the process can lead to operator fatigue.	😊😊😊
MAE	Quick and homogeneous heating; low solvent usage; extraction time (1–40 min); currently, vacuum microwave extraction has been developed to provide a MAE method with a lower reactor temperature; possible application on a large scale.	The solvent must absorb microwaves; the heating could damage the structure and the activity of some compounds; after the extraction, a filtration and clean-up step is required.	😊😊
SFE	CO <sub>2</sub> as a solvent; easy to remove after extraction; reduced the thermal degradation. Extraction time (up to 1 h); it does not require an alternative energy source; it is available on a large scale.	Needs a co-solvent to extract polar compounds. The amount and type of co-solvent need to be optimize together with other parameters. SWE present the limitation of need high temperature to reach the subcritical condition, ethanol could be used instead of water.	😊😊
PLE	Low solvent consumption; protection for oxygen and light sensitive compounds; it needs temperature; possible application on a large scale.	Expensive equipment required; after the extraction, a clean-up step is required; extraction time (1–2 h).	😊😊

Technique	Strengths	Weaknesses	Suitability
<b>HHPE</b>	Short extraction time (~ 5 min); performed at room temperature; higher repeatability; smaller amount of solvents; possible application at large scale.	High investment cost and cost maintenance and service; high pressure could affect the structure or activity of some compounds. The parameter should be optimized to avoid it.	😊😊😊
<b>PEFE</b>	Short extraction time (less than 1 s); performed at room temperature; low energy and monetary costs; possible application on a large scale.	Some compounds could be affected by high electric fields; it is desirable to reduce the electrical conductivity of the matrix before the extraction. For industrial application there are some problems related to: non-uniform distribution of the electric pulses, the suitable solvents are very limited and cooling system is necessary to control the temperature when extracting thermolabile compounds if high electrical pulses are applied.	😊😊😊
<b>HVED</b>	Low temperature; short extraction time and energy input; possible application on a large scale.	High cost maintenance and service; high voltage electrical discharges may generate chemical products and free reactive radicals, which can react with antioxidant compounds decreasing their bioactive activity.	😊😊
<b>EAE</b>	Moderate extraction conditions; eco-friendly; selectivity due to the specificity of enzymes; can be combined with ultrasonic extraction to improve the yield and the bioactivity of the extract.	Expensive cost of enzymes; activity of enzymes varying with the pH, temperature and nutrients of the matrix; after the extraction, a filtration and clean-up step is required. Difficulties to be applied on a large scale; extraction time (1–12 h); low availability of commercial enzyme types; sometimes they have low selectivity and variability.	😊😊

These techniques have been used not only in isolation to extract anthocyanins, but their combined use has also been explored to improve the extraction of anthocyanins. In general, this alternative produces a more effective extraction than the use of a single one, as is the case of UAE combined with microwave UMAE <sup>[40]</sup>, enzyme-assisted supercritical fluid extraction (EASCFE) <sup>[41][42]</sup>, microwave-assisted sub-critical water extraction (MA-SWE) <sup>[19]</sup>, ultrasound-assisted with pressurized liquid extraction (US-PLE) <sup>[43]</sup>, ultrasound and pulsed electric field US-PEF <sup>[44]</sup>, or ultrasound-assisted enzymatic extraction (UAEE) <sup>[45][46]</sup>. In some cases, the combination of these techniques could provoke deterioration of the extract. In order to avoid the deterioration of the extract during the process, a specific optimization of the extraction parameters should be done according to the matrix and the extracted compounds. In addition, innovative approaches are needed for overcome these shortcomings. Furthermore, the combination of various techniques implies even greater complexity for large-scale implementation. The scaling of the extraction methods to industrial scale presents some disadvantages, such as insufficient recovery, degradation due to excessive heating and extraction time, which ultimately results in high-energy consumption <sup>[29]</sup>. To choose a suitable extraction process for anthocyanins it is necessary to consider the extraction efficiency, economic feasibility, and environment aspects. Extraction efficiency of non-conventional extraction techniques is clearly advantageous compared with the conventional extraction methods in regard to time, energy, and extraction yield. Nevertheless, the extraction efficiencies among non-conventional extraction techniques are different (**table 1**). For these reasons, nowadays, conventional techniques are still used at the industrial scale <sup>[39]</sup>.

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