

Advances in the extraction of anthocyanin from vegetables

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Abstract: Besides giving colour to plants, anthocyanins also have antioxidant and anti-hyperglycemic properties; hence, they are used as therapeutic source for many treatments of diabetes, coronary heart disease and cancer. Many anthocyanin extraction methods such as conventional acidified water (CAW), ultrasound, microwave pre-treatment, supercritical fluid extraction and pulsed electric field (PEF) have been used. Among them, ultrasound and microwave-assisted extraction are two putative methods for extraction of anthocyanins from vegetables. They have significant advantages such as cheap, easy to be manipulated, suitable for laboratory, domestic and large-scale industrial applications, less time-consuming, matrix independent, free sample particle size, less solvent used and long-term preservation. Importantly, with those properties, they help enhance the yield of anthocyanin and also suitable for application of most vegetables from nature. Furthermore, two putative methods could serve as a sound base for future large scale production of anthocyanin with high efficient and fast rate by further investigations, modifiers and optimizations.

Keywords: Anthocyanins, Extraction, Putative, Vegetables, Preservation

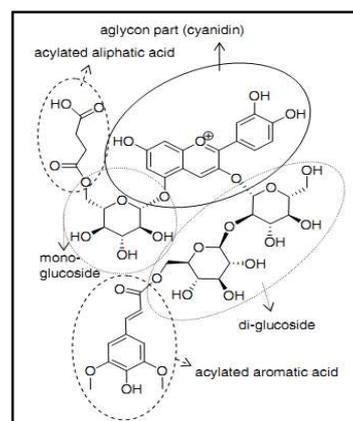
1. Introduction

Now-a-days, natural colorants have high demand for use in food industry rather than synthetic colorants which might cause adverse human health effects [1]. The neurological and behavioral effects caused by the synthetic dyes used in food industry are adverse to human health [2]. Therefore, anthocyanins, with their high potential in terms of high stability, high colorant power and low cost, have been considered as a great candidate for this requirement of new sources of pigments.

Since the mid-1970s, anthocyanins have been extracted from fruits, vegetables, cereals and flowers of a great variety of plants. Depending on pH values, they account for colour of the plant leaves, flowers and fruits with red, pink, violet, blue and green [2]. The word anthocyanins came from Greek, in which anthos means flower and kyanos means blue. Anthocyanins, belong to the flavonoid family, are a group of phenolic compound that can be soluble in water. Chemically, they are glycosides of polymethoxy and polyhydroxy derivatives of flavylium or 2-phenylbenzopyrylium salts [2],[3].

Besides giving responsibility of colouration with bright colour to plants, anthocyanins also have antioxidant and

antihyperglycemic properties [1],[2],[4]; hence, they are investigated as therapeutic source for many treatments of diabetes, coronary heart disease and cancer, preventing the process of aging [4].



Source: Xu *et al.*, 2010.

Figure 1.1. Chemical structure of acylated anthocyanins.

So far, many anthocyanin extraction methods such as conventional acidified water (Soxhlet extraction), ultrasound, high pressure extraction with CO₂ and co-solvents,

microwave pre-treatment, accelerated fluid extraction and pulsed electric field (PEF) have been proposed by researchers [2],[3],[4],[5],[6],[7],[8],[9],[10]. However, several drawbacks of the methods were reported such as time consuming, insufficient rate, degradation of anthocyanin due to high temperature use, hydrolysis of anthocyanin by using acidified organic solvents; and no single extraction method could be applied for all plants.

2. Methods for Extraction of Anthocyanins from Vegetables

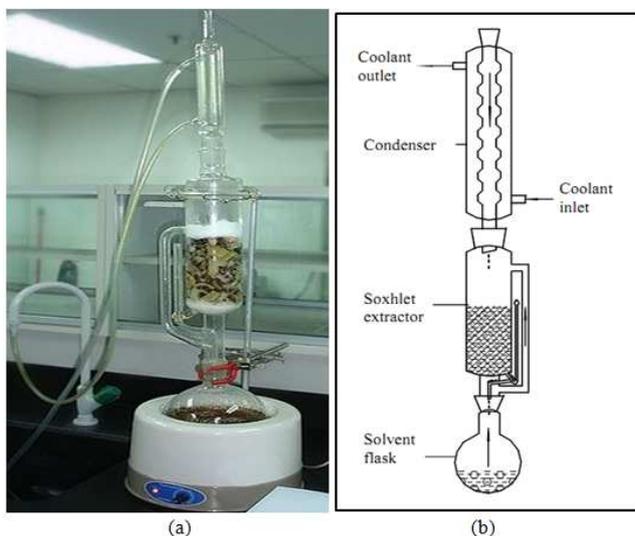
There are a number of methods for extraction of anthocyanins have been proposed and used by researchers. Each of the methods will be discussed along with principles, mechanisms, practical design and advantages and disadvantages.

2.1. Conventional Extraction Method - Soxhlet Technique

2.1.1. Introduction

Soxhlet extraction was mostly used as a standard one and support as the main reference for other novel techniques available at present. Originally, Soxhlet technique was used to determine of fat in milk. It is less efficient than other novel techniques such as ultrasound-assisted extraction and/or microwave-assisted extraction, hence there is a very limited number of reports for the applications of Soxhlet technique for extraction of anthocyanins from vegetables [11].

2.1.2. Principles and Mechanisms



Source: Wang and Weller, 2006.

Figure 2.1. Schematically experimental apparatus of Soxhlet extraction technique: (a) laboratory Soxhlet extractor, (b) schematic diagram of Soxhlet extraction apparatus

Solid plant materials are placed within a thimble-holder. During the process, samples are filled with condensed solvent from a distillation flask (see figure 2.1). It is operated until the liquid reaches the overflow level. Subsequently, the

solute within the thimble-holder is aspirated by a siphon and it is also unloaded back into the distillation flask. The extracted liquid is then carried into the bulk liquid.

In the solvent flask, distillation is used to separate the achieved solute which is placed separately in flask while the fresh solvent is right back into the sample solid bed. The process is reiterated flow after flow until a target extraction solute is accomplished [11].

2.1.3. Practical Design

Soxhlet extraction method needs a suitable solvent in order to obtain the final targeted phenolic compounds. It is a very good solvent because it is soluble and easy to recover or reused. However, due to laboratory safety, human healthcares as well as environmental concerns, researchers have been selected some other less toxic solvents for the operation such as water, hydrocarbons, *d*-limonene, ethanol and isopropanol[11].

Cost of alternative solvents that have been used for Soxhlet extraction is generally higher than hexane. Therefore, co-solvent(s) sometimes is added together with solvents as a better cost deal. In addition, some of the co-solvent helps increase the polarity of the liquid phase within extraction process [6], [11]. When co-solvent(s) (i.e. isopropanol) is mixed with hexane, final extracted compounds and kinetics of extraction are increased significantly [11].

There are many factors influent the Soxhlet extraction process such as matrix characteristics and particle size of samples, and boiling temperature. Temperature not only affects to the yield and quality of final products, but also reduces visual color of the extracted compounds. It can be slightly solved by using membrane separation or vacuum [6].

2.1.4. Advantages and Disadvantages

The main outstanding advantages of Soxhlet extraction include:

- (i) It is a basic, simple and cheap technique that needs easy-taking steps to carry out;
- (ii) It is used as standard reference for a wide variety of other official methods;
- (iii) During the extraction process, the sample is taken into fresh solvent repeatedly therefore possess the displacement of the transfer equilibrium;
- (iv) The distillation flask helps maintain a relatively high temperature for extraction process hence reduce the heat applied;
- (v) After leaching, it is not necessary to carry out any filtration step[11].

The main significant disadvantages include:

- (i) It takes long time and uses large amount of solvent(s) which not only costs for extra money but also may cause unwanted environmental problems;
- (ii) The Soxhlet device cannot provide agitation to stimulate and speed up the process;
- (iii) It is mandatory to carry out the evaporation or concentration step for solvent used easy to evaporate and/or recovery;
- (iv) The sample is only extracted at the boiling point with

abundant solvent used and it occurs for a long time hence, it causes loss of extracted anthocyanins due to the thermal decomposition[6], [11].

2.1.5. Potential Applications

Soxhlet extraction technique has been widely used for industrial applications with high efficiency and re-productivity. Nevertheless, it is an old model and requires longer time and more solvents for the operation as compared with other novel fast extraction techniques such as accelerated solvent, supercritical fluid, microwave-assisted and ultrasound-assisted extractions. Soxhlet extraction technique is potentially applied for the extraction of oil and other phenolic compounds rather for extraction of anthocyanins from vegetable.

2.2. Ultrasound-Assisted Extraction

2.2.1. Introduction

The application of ultrasound-assisted extraction (UAE) technique to the extraction of anthocyanins and other phenolic compounds is not only considered as a reliable, efficient and rapid alternative to the ones applied conventional extraction technique, but also one of the most economically and feasible large-scale applications in food industry^[12]. There are two commonly used laboratory UAE extractors for extraction of anthocyanins, ultrasound probe and ultrasound bath (see figure 2.2).

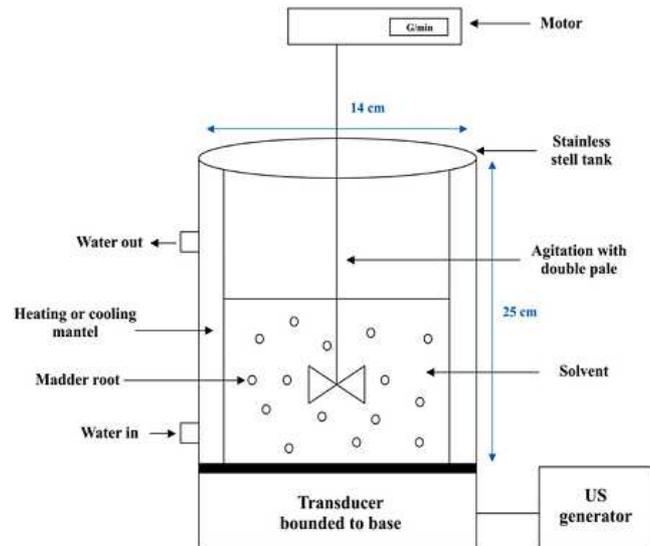


Source: Chemat *et al.*, 2011.

Figure 2.2. Two common ultrasound-assisted extraction apparatuses: (a) Ultrasound bath, (b) Ultrasound probe.

2.2.2. Principles and Mechanisms

Sound waves used to treat in the extraction process normally have frequency higher than 20 kHz (up to 10MHz)^[12]. They are mechanic waves which can mechanically vibrate in gas, liquid and solid conditions. When the ultrasounds are applied, their journey along the medium involves compression cycles and expansion, which are not involved in electromagnetic waves. In such way, their expansion may induce negative pressure and even some bubbles form in the liquid (see schematic diagram in figure 2.3). Those bubbles will collapse after reached the critical size, and release energy. The energy in association with high temperature and pressure within the ultrasonic bath will destroy the cell walls of the samples and release anthocyanins easily [11],[12].



Source: Vilku *et al.*, 2008.

Figure 2.3. Laboratory and schematic illustration diagram of 3 liter volume UAE extractor.

2.2.3. Practical Design

The apparatus of the UAE must consider some important factors including solvent used, particle size of the plant sample, moisture content, sonication time and frequency treated, temperature and pressure^{[11],[12]}.

Different sample from vegetables are affected differently by the ultrasound frequency. For laboratory design, the ultrasound-assisted extraction is usually set up with power of 200W and frequency 40 kHz^[12]. Apart from ultrasound frequency, the ultrasonic wave is also considered as an important parameter within the ultrasound-assisted operation system. From the observation, the zone located in the vicinity of the ultrasonic horn's surface is highest ultrasound power portion within an ultrasonic extractor. The more distance from the radiating surface, the less ultrasonic intensity. Frequently, shaking and/or agitation are usually supplemented to assist for commodious ultrasound waves. From that, there will be no solid free-of-ultrasonic wave region, more micro-mixing and facilitating for the highest yield and kinetics obtained^[11].

For the pressure and temperature parameters, it is stated that, the ultrasonic assistance help reduce the uses of those during the extraction process^[12]. Significantly, other thermolabile compounds which may be altered under normal or conventional operating conditions such as in Soxhlet technique, is kept original form until the final extracted products^[11].

2.2.4. Advantages and Disadvantages

The UAE technique is considered as an efficient, simple, inexpensive and good alternative to the conventional ones. Also, its operation and apparatus is easier and cheaper than other methods such as microwave and supercritical fluid extractions.

Table 2.1. Advantages of UAE technique compared with other techniques

	UAE	SPE	MAE	ASE
Name	Ultrasound-assisted extraction	Supercritical fluid extraction	Microwave-assisted extraction	Accelerated solvent extraction
Brief description	Sample is immersed in solvent and submit into ultrasound using a US probe US bath	Sample is placed in a high pressure vessel and crossed by the supercritical fluid	Sample is immersed in solvent and submitted to microwave energy	Sample is heated by a conventional oven and crossed by the extraction solvent under pressure
Extraction time	10-60 mins	10- 60 mins	3-30 mins	10-20 mins
Sample size	1-30g	1-5g	1-10g	1-30g
Solvent use	50-200 ml	2-5ml (solid trap) 30-60 ml (liquid trap)	10-40 ml	15-60 ml
Investment	low	High	Moderate	High
Advantages	Easy to use	Rapid low solvent consumption concentration of the extract No filtration necessary Possible high selectivity	Rapid, simple and cost effective Moderate solvent use high efficiency, less labor work	Rapid, no filtration necessary Low solvent consumption, high yield short treatment time
Disadvantages	Costly for scaling up, matrix dependent	Many parameters to optimize Filtration step required	Costly for scaling up, degradation of anthocyanins Extraction solvent must absorb microwave energy	Uncertain rate, require meticulous control of time-temperature in certain phases Possible degradation of thermolabile analytes

Source: Chemat *et al.*, 2011.

The significant disadvantages of UAE include:

- (i) The kinetics and yields are depended on the nature of plant matrix;
- (ii) The ultrasound wave may be attenuated due to the presence of dispersed phase during the operation;
- (iii) The active part of ultrasound within the apparatus is restricted to a zone sited nearby the ultrasonic emitter. This factor needs to be highly considered when build up the UAE process[11],[12].

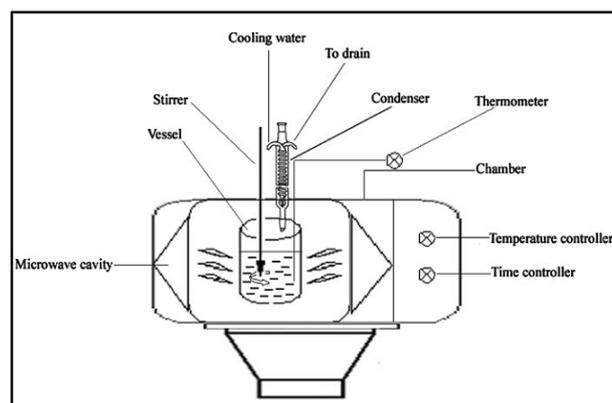
2.2.5. Potential Applications

There are some reports for the extraction of anthocyanins using UAE technique. It is suitable for scaling up for industrial applications with big bath extractors of 50, 500 and 1000 liters. However, it needs optimization of the parameters or modifiers for the large-scale operator, which is highly required for high yield and purified extraction of the final products[11],[12].

2.3. Microwave-Assisted Extraction

2.3.1. Introduction

Microwave-assisted extraction (MAE) is another novel technique has been used for oil and nutraceuticals such as anthocyanins and other phenolic compounds recently [13],[14]. It can be applied for extraction of a wide variety of materials with the assistance of a microwave applicator as energy source (see figure 2.4). It not only allows for extracting selective compound from plant materials, but also provides a high yield of final products in a very short period of time (Belanger and Pare, 2006; Tong *et al.*, 2010; Routray and Orsat, 2012).



Source: Routray and Orsat, 2012.

Figure 2.4. Schematic diagram of MAE system.

2.3.2. Principles and Mechanism

There are two types of MAE apparatuses, focused microwave ovens and closed extraction vessels. They both requires with supplement controlled temperature and atmospheric pressure. The first one can be adjusted with maximum temperature and the second is normally conducted and run in drastic conditions with high temperature. Boiling points of the solvents along with the total volume within the vessel determine for the pressure of extracting condition. Therefore, based on those parameters, the MAE system can be adjusted with sufficient temperature and pressure to obtain high yield of compound(s) (anthocyanins)[14].

2.3.3. Practical Design

Solvents used for microwave-assisted extraction include some polar solvent such as water, methanol and ethanol. In contrast, hexane and toluene, which are non-polar ones, are not recommended for microwave-assisted extraction.

However, mixtures of hexane and acetone are highly recommended and widely used in microwave-assisted extraction process[13].

Temperature affects to the recovery capability and yield of extracted products. Sufficient temperature can result with a high extracted yield. Remarkably, for thermolabile compounds which are very heat-sensitive and unstable, it is necessary to moderate the temperature correctly to avoid any degradation of those extracts[14]. The dielectric constant between solvent and matrix is again, one of the most important issues in microwave-assisted extraction[14]. Last but not least, particle size of the samples also impacts to the efficiency of microwave-assisted extraction. Normally, particle size in range of 0.01 to 2 mm is suitable for extraction process[13].

2.3.4. Advantages and Disadvantages

The most outstanding advantages of MAE include: (i) complete the extraction process within a very short time; (ii) less solvents used for the operation; (iii) high yield of extracted products; (iv) the process of is generally simple and cost effective as compared to others such as supercritical fluid or accelerated solvent extraction. In terms of practical and economical aspects, MAE deserves one of the most efficient extraction techniques anthocyanins from vegetables[13],[14].

The main drawback of microwave-assisted extraction is the requirement of additional steps after extraction process such as centrifugation and/or filtration process; and choosing a correct solvent for the extraction process[13],[14].

2.3.5. Recent Applications

It is popular used not only for extraction of tea polyphenol, ginsenosides of ginseng and cocaine of some leaves, but also for extraction of anthocyanins from vegetables[14].

3. Supercritical Fluid Extraction

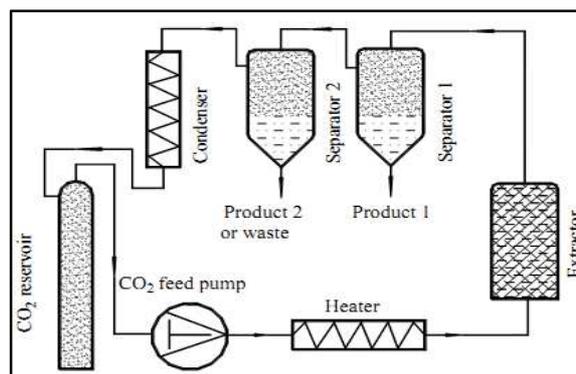
3.1. Introduction

Supercritical fluid extraction (SFE) is considered as an environmental friendly and less time-consuming alternative to the conventional extraction technique. It is rapid, selective and less solvent used as compared to the conventional technique. SFE also helps reduce the degradation processes which may occur in the traditional methods due to the absences of air and light during their extraction process. It can also be known as pressurized fluid extraction (PFE) [15].

3.2. Principles and Mechanism

The SFE possesses properties of both liquids and gases. As the pressure as well as the temperature of a substance is increased over its critical value, the operation of SFE reaches supercritical state. SFE have a lot of better characteristics such as: (i) the dissolving possibility depend on solvent density within the operator, which can be modulated by the adjustment of temperature or pressure; (ii) compared with liquid solvents, SFE is less surface tension and viscid and

more diffusion coefficient properties.



Source: Wang *et al.*, 2011.

Figure 2.5. Schematic diagram of supercritical fluid extractor.

During the process, plant sample is placed within the extractor, which can be modulated in terms of temperature and pressure for an ideal extraction conditions (see figure 2.5). In fact, this extractor is controlled in side, outlet and inlet. A CO₂ feed pump will pressurize the extractor. Next, the fluid which is contented sample and solvents used will be transferred into the separator 1 and then the separator 2. At this state, the temperature and/or the pressure are adjusted in order to decrease the salvation power of the fluid. The product at a valve located at the bottom of the separators and the process is recycled then [11].

3.3. Practical Design

There are several factors influenced the success of supercritical fluid extraction process. They include extraction conditions, modifiers, plant material preparation and firstly the selection of supercritical fluids[15].

Carbon dioxide is the major solvent used in the supercritical fluid extraction. It is cheap, non-flammable and less toxic than other solvents. Moreover, it facilitates for the extraction process to reach the critical point only with 7.3 MPa and 30°C for pressure and temperature, respectively. Hexane and nitrous oxide are also be used as solvents for the process, however, they are toxic and affect to the surrounded environment. Hence, they are less mentioned than carbon dioxide^[11]. Although water is sometimes used for the extraction process, it is not suitable for thermolabile compounds^[15].

Because anthocyanins are chemically glycosides, which are less soluble in carbon dioxide, therefore, SFE technique is less usually used for anthocyanins extraction than for other phenolic compounds and oils [11],[15]

3.4. Advantages and Disadvantages

The temperature and/or pressure of the fluid in the supercritical fluid extraction can be modulated. Therefore, the manipulation of the solubility of a chemical within the process results with a very selective extracted compounds. SFE is also good at fractionation of phenolic compounds in

addition to the extraction possibility. Moreover, at high pressure, the supercritical fluids can solubilize solid sample due to the density of a liquid within the extractor. Compared with the conventional extraction technique, it is rapid, less solvent consumption and does not require any filtration step after the extraction [11,12],[15].

The high pressure during the operation process may lead to the degradation of anthocyanins if in cases they are extracted. SFE is only suitable for some specialized fields such as extraction of oil and few other phenolic compounds. Additionally, high cost and sophisticated modifiers for the operation conditions for large-scale industrial applications also cause the limited use of SFE of anthocyanins from vegetables as compared to others [11],[15].

3.5. Potential Applications

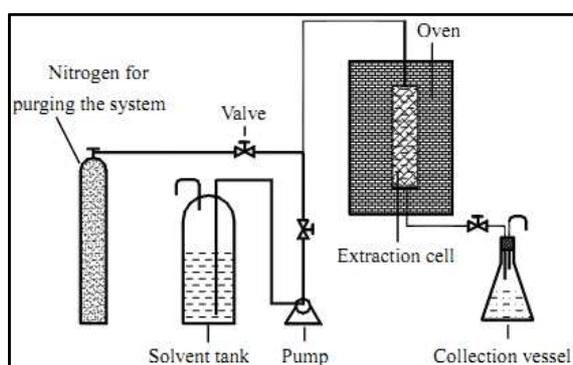
It is not efficient to use SFE for the extraction of anthocyanins from vegetables. In term of recovery of solvent and degradation of extracted anthocyanins, SFE is less efficient than the other novel methods. Together with some of its significant drawback, SFE has not been widely used by researchers for extraction of anthocyanins from vegetables in the recent time[15].

4. Accelerated Solvent Extraction

4.1. Introduction

Accelerated solvent extraction (ASE) is a widely used technique using high pressure and high temperature in its protocol for extraction of phenolic compounds. In this technique, extraction time and solvent consumption are significantly less than in the conventional ones. Similar to supercritical fluid extraction, it is also known as pressurized fluid extraction technique.

4.2. Principles and Mechanism



Source: Wang *et al.*, 2011.

Figure 2.6. Schematic diagram of Accelerated solvent extraction.

ASE is basically a solid-liquid extraction process with temperature at 50-200°C and pressure at around 10-15 MPa for the operation system. The solvent used is maintained in its liquid state during extraction periods. Different with SFE, during the ASE process, the solvent used do not reach the

critical point. The extraction kinetics and high yield are obtained by elevated temperatures and increased pressure.

Solvent used in the ASE is commonly organic solvents. Apart from it, others such as subcritical water and pressurized hot water can also be used in the extraction process. In such cases, the processes are known as subcritical water extraction and pressurized hot water extraction, respectively.

4.3. Advantages and Disadvantages

The outstanding advantages of the ASE include: (i) less solvent used and less extraction time than the conventional technique; (ii) economically and environmentally beneficial extracts due to the solvents such as water and carbon dioxide; (iii) it is an alternative of SFE for nutraceuticals; (iv) no filtration step is required after the extraction process [11].

The main significant disadvantage of this technique is mainly on the possible degradation of thermolabile compounds due to the high temperature in the extraction procedure. Moreover, more adjustments or modifiers required for the scale-up industrial applications are also significant limitations of this kind of technique for extraction of anthocyanins from vegetables [11].

4.4. Potential Applications

Red cabbage is one of the most abundant sources of anthocyanins among vegetables from the nature. There are several studies have been done for the extraction of anthocyanins from red cabbage using ASE. However, this technique is not highly recommended for use of extraction of anthocyanins from vegetables recently [15].

5. Pulse Electric Field Extraction

5.1. Introduction

Pulsed electric field extraction (PEF) is non-thermal technology used for extraction of anthocyanins from vegetables. Initially, since the mid-1900s, the pulsed electric field was used for drying processes of fruits and vegetables, enhanced juice extraction and microbial inactivation [16].

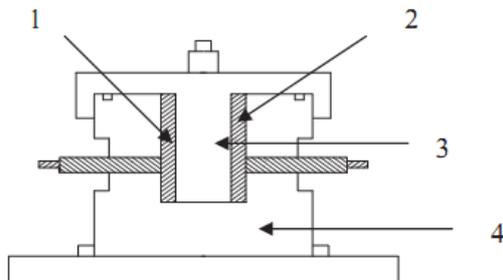
5.2. Principles and Mechanism

There are many factors influent the degree of electroporation such as number of pulse applied, treatment time, type of pulse wave, intensity of the electric field, and product constituents. It helps breakdown the cell membrane or wall by applying PEF. Meaning, the permeability of the cell walls will be increased, hence facilitate for the release of the intracellular contents[16],[17].

5.3. Practical Design

After the preparation step, PEF treatment will be applied. Figure 2.7 shows the pulsed electric filed treatment for extraction of anthocyanins from red cabbage. The voltage adjusted within the chamber is in the range of 0.5-5.0 kV/cm.

The frequency of pulse is 1 Hz. A total of 50 pulses are usually applied in the treatment chamber. The samples are placed in the capacitor with voltage electrode and ground electrode (see figure 2.7). Depending on the sample size, number of pulses will be modulated in order for an ease of cellular content release[17].



Source: Gachovska *et al.*, 2010.

Figure 2.7. Sketch of the pulsed electric field treatment chamber: (1) high voltage electrode, (2) ground electrode, (3) sample, (4) spacer or insulator.

5.4. Advantages and Disadvantages

The PEF helps increase the breakage of cellular membranes resulting in higher yield or mass transfer. It also leads to more free movement of the intercellular liquid, which either contains anthocyanins. Using this technique for the extraction of anthocyanins, more non-acylated anthocyanins are obtained than the acylated ones. It not only results with a better extraction efficiency, but also reduces the light and thermal degradation[12],[17].

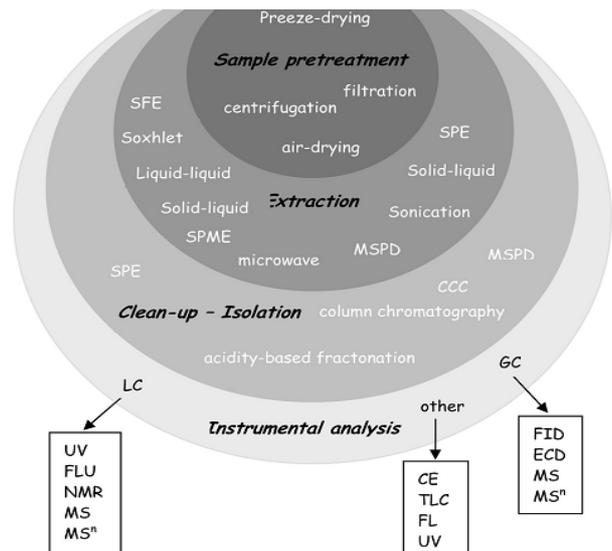
Most of the anthocyanins obtained from PEF process are non-acylated which are turned to purple colour and easy to be degraded after long time of storage. There is approximately 80% of the total extracted anthocyanins using PEF is degraded after 135 days of storage[17].

5.5. Recent Applications

PEF was used for enhancing juice yields extracted from various kinds of fruits and vegetables such as beets, apples, alfalfa, carrots. It is a promising technique for the extraction of anthocyanins however, more modifiers as well as experiments need to be conducted in order for industrial applications in future[17].

6. Comparisons among Methods for Extraction of Anthocyanins from Vegetables

Depends on the source of anthocyanins from vegetables to be extracted, the appropriate extraction methods will be selected (see figure 3.1). Soxhlet extraction is still considered as a standard one in most cases.



Source: Stalikas, 2007.

Figure 3.1. Summary of the determination of anthocyanins including the extraction techniques have been used recently. (SFE) supercritical fluid extraction, (SPME) solid-phase microextraction, (MSPD) matrix solid-phase dispersion, (ECD) electron capture detection, (FL) fluorescence, (CCC) counter-current chromatography.

General speaking, MAE and UAE are two promising techniques for the extraction of anthocyanins from vegetables due to their outstanding benefits (see table 3.1). They are widely used with numerous advantages in terms of extraction time, volume used of solvent(s), high yield and quality of the final products. The pre-treatments can help disrupt cell wall and ease of release cellular contents, hence, facilitate high extraction for the final products[11],[14].

An advantage of MAE compared to SFE and other techniques is that no drying step of sample is required and the contact surface between sample and solvent used for better rapid heating which facilitates for rupture of cell walls. It is also less matrix dependent than other techniques such as accelerated and supercritical fluid extraction. In MAE, the solvent is usually methanol and water, which are cheap, non-toxic and environmental friendly (see figure 3.1)[13],[14].

Additionally, ultrasound-assisted extraction is another promising technique. The ultrasonic transducers, generators with high power/amplitude are now available commercially (Vilkhu *et al.*, 2008; Vatai *et al.*, 2008). Together with good points such as cost effective, matrix independent, free sample particle size, the significant advantages of the ultrasound-assisted extraction also involve low maintenance, capability of large-scale applications and short payback period for the maintenance of operation process (Wand and Weller, 2006; Ignat *et al.*, 2011).

Table 3.1. Summary of comparisons of the characteristics of the methods has been used for extraction of anthocyanins from vegetables.

Methods	Description	Sources	Sample size	Solvent used	Extraction time	Advantages	Disadvantages	Ref.
Soxhlet technique	Sample is mix up with solvent used within the thimble-holder	Red cabbage, sweet potato, Roselle, radish	1-30 g	Hexan with more than 200 ml	4 hours and further	Low cost, ease of use, ranging applicability, no filtration steps required	Time-consuming, insufficient rate, degradation and hydrolysis of acylated anthocyanins	Lapomik et al., 2005. Naczk and Shahidi, 2004. Wand and Weller, 2006
Ultrasound-assisted extraction	Sample is immersed in solvent and submit into ultrasound using a US probe US bath	Red cabbage, back, carrots, radish	1-30g	50-200ml	10- 60 mins	Easy to use less labor intensive, short treatment time	Costly for scaling up, matrix dependent	Wand and Weller, 2006. Vilkhu et al., 2008. Chemat et al., 2011.
Microwave-assisted extraction	Sample is immersed in solvent and submitted to microwave energy	Red cabbage, back, carrots, radish, red onions, potato stems and leaves	1-10g	10- 40ml	3-30 mins	Rapid, simple and cost effective Moderate solvent use high efficiency, less labor work	Costly for scaling up, degradation of anthocyanins Extraction solvent must absorb microwave energy	Routray and Orsat, 2012 Tong et al., 2010
Accelerated solvent extraction	Sample is heated by a conventional oven and crossed by the extraction solvent under pressure	Red cabbage, red-flashed potato	1-30g	15-60ml	10-20 mins	Rapid, no filtration necessary Low solvent consumption, high yield short treatment time	Uncertain rate, require meticulous control of time-temperature in certain phases Possible degradation of thermolabile analytes	Arapitsas and Turner, 2008. Wand and Weller, 2006. Corrales et al., 2009
Supercritical fluid extraction	Sample is placed in a high pressure vessel and crossed by the supercritical fluid	Red cabbage, potato, Roselle	1-5g	30-60ml	10-60 mins	Rapid low solvent consumption concentration of the extract No filtration necessary Possible high selectivity	Many parameters to optimize Filtration step required	Bleve et al., 2008; Mario et al., 2010; Wand and Weller, 2006
Pulse electric field	Sample s are placed in the capacitor with voltage electrode and electrode	Red cabbage beets, alfalfa, carrots, broccoli	1-30g	10-60ml	6-11 mins	Rapid low solvent consumption reduces the light and thermal degradation	Possible degradation of thermolabile analytes	Gachovsks et al., 2006. Gachovsks et al., 2010; Chelemchat et al., 2004

7. Conclusion

Over the past two decades, many techniques have been developed including ultrasound-assisted, microwave-assisted extraction, supercritical fluid extraction, accelerate solvent extraction. For future study, attention should be devoted to a putative method with clean-up of biological extracts characteristics.

Normally, the extraction processes are influenced by several factors including sample characteristics, pressure, anthocyanins structure (acylated or non-acylated), temperature. In terms of temperature parameter, at high temperature, the extraction efficiency will be increased. However, it will significantly affect to the stability of extracted anthocyanins. Hence, some modern techniques with high efficiency but they are not recommended as an

alternative method that can be used for most vegetables. MAE and USE are suitable for novel alternatives. They are independent to particle size of the sample to be extracted hence they are promising for extraction of anthocyanins from most vegetables. In conclusion, the food industry is now-a-days requires more and more natural products such as colorants from natural pigments, anthocyanins. Methods for extraction with cost effective, accuracy, extensive validation, ease of controls and monitoring are highly needed for better yield and quality of the products.

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