



Current Trends in Extraction of Plant Bioactive Molecules Valuable for Food Use

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Abstract: Fruits, vegetables, and their by-products are rich in bioactive compounds. These bioactive compounds are used in variety of industrial markets, including the medicinal, dairy, and chemical industries. Until these bioactives can be used in particular applications, they must first be isolated from natural matrices and then analysed and categorised. There is a demand of most appropriate and standard methods to extract these active components from natural sources. Subcritical water extraction (SWE), enzyme assisted extraction (EAE), microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE) are the novel techniques that offer a suitable, safe, cost-effective, low time consuming and environmentally safe alternative to extract bioactives. These methods are sustainable and green because they are energy efficient, generate less effluent, use less water, utilise fewer hazardous solvents, and are ecologically sound. This review compares the conventional methods and novel technologies along with their theoretical concepts, values and shortcomings used for the purpose of extraction.

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Introduction

In today's world there is a strong intend to consume foods useful for the metabolic functions of human body together with other chemical compounds that have beneficiary effects on our health as well. These compounds are non-nutritional ingredients and called as bioactive compounds. Plants synthesize bioactive compounds (BACs) as secondary metabolites to coordinate physiological and cellular activities and enhance their survival resistance. The growing

need for naturalness, as well as the movement toward plant-based foods, has rekindled interest in fruits, vegetables, and their by-products as sources of bioactive compounds. BACs such as hydrocolloids (polysaccharides), colorants (anthocyanins, carotenoids, betalains), antioxidants, are extracted from plants. BACs are generally found in plants at extremely low quantities and are used to replace some synthetic additives in meals nowadays. Chemical methods for synthesis of these active compounds are demanding, laborious, challenging and present low yield (Renard, 2018). For all these reasons, significant efforts are being made to extract them from plant materials.

Characteristically, bioactive compounds remain in conjugation with other compounds present in plants. These can be collected from various plant parts such as fruits, flowers, leaves and stems. Extraction of BACs from plant materials can be done by various conventional procedures like maceration, percolation, soxhlet and steam distillation. Almost all of these methods rely on the extraction efficiency of the various solvents employed, as well as the extent of heat and/or mixing. Over the last 50 years, non-traditional approaches that are more environmentally sustainable due to lower use of synthetic and organic chemicals, shorter operating times, and higher yield and quality of extract have been approached. Ultrasounds, enzymes, microwaves, and subcritical water are being investigated and exploited as non-conventional techniques to improve overall yield and criteria for selection of BACs from plant sources. Concomitantly, traditional extraction approaches, such as soxhlet, has also been used to compare the performance of newly developed methodologies. There has not been a lot of scientific study undertaken in innovative extraction methods around the world. As a result, there is a urgent need to increase understanding of extraction mechanisms, overcome technological obstacles, and enhance the design and scale-up of novel extraction systems for better industrial applications.

2. Bioactives in Fruit and Vegetables

Plants contain many active compounds like alkaloids, phenols, steroids, tannins, volatile oils, terpenoids, glycosides, resins, and flavonoids which get deposited in their particular parts such as leaves, bark, fruits, flowers, seeds, and roots. BACs derived from plants are often synthesized as secondary metabolites by various metabolic pathways, which are thought to aid the plant's potential to survive and overcome local obstacles and dangers by allowing it to interact with its surroundings. In other words, secondary metabolites are those that are frequently produced during a growth process, have little role in growth (though

they may have a survival function), are produced by specific taxonomic classes of microorganisms, have peculiar chemical structures, and are frequently created as mixtures of closely related members of a chemical family.

BACs can be categorized into following groups:

- (a) Terpenes and terpenoids (approximately 25000 types)
- (b) Alkaloids (approximately 12000 types)
- (c) Phenolic compounds (approximately 8000 types).

3. Extraction of BACs

There is great variation among bioactive compounds and their sources. It is necessary to build up a standard approach to screen out the compounds which carry human health benefits. The selection of appropriate extraction process involves separation, identification, and characterization of BACs. Nearly all the techniques have some objectives in common-

- (a) to extract target bioactive compounds from complex plant sample
- (b) to increase keenness of analytical method used
- (c) to increase sensitivity of bioassay by elevating the concentration of target compounds
- (d) to convert the bioactive compounds into a more acceptable form for detection and separation

4. Conventional Extraction Techniques

In conventional methods organic solvents or water are used at atmospheric pressure for extraction of valuable components (UNESCO). The main conventional techniques are maceration, percolation, soxhlet and steam distillation. These are simple methods, easy to use, using non-complicated utensils and equipments, do not require skilled operators and are best for certain substances which are very less soluble in solvent and requires only long term contact with solvent. So are best suited for less potent and cheap drugs (Pandey and Tripathi, 2014).

4.1. Maceration

Maceration is generally used in the handmade preparation of tonics. It quickly became a common and low-cost method of extracting essential oils and bioactive compounds from plant material. On a small scale it usually involves contact between the plant source and liquid solvent for a period of

time at room temperature. In order to increase the contact between plant cells and solvent, sample needs to be cut or crushed into minute fragments. The fragments should not be too big, since this would prevent the solvent from reaching the innermost cells. They also should not be reduced to powder, that would result in losing the volatile active ingredients in the cells. After completion of maceration, filtration is done. The plant residue needs a final press in order to recover all the remaining BACs. But separation of the active constituent from the solvent is a challenging step. When water is used as solvent there is increase in maceration time, so some amount of alcohol may be added to inhibit microbial growth (Handa *et al.* 2008). Therefore, alcohol is the most commonly used solvent for extraction. Since considerable quantity of active components left behind in the first pressing, repeated maceration can be more effective than single maceration. Where the active constituent is valuable component, repeated maceration is more effective.

4.2. Percolation

In percolation solvent is steadily moved through the plant, packing itself with active ingredients, before being forced away by another pure solvent applied from above. It can be conducted at room temperature. The sample should not be too fine as it will be difficult to separate from solvent. The speed of flow of solvent is regulated so as it gets enough time to penetrate inside the plant cells to extract BACs. The filtrate obtained rich in BAC is called leachate and the residue plant material is pressed for remaining compounds. During process to improve the extraction efficiency, agitation may be provided with the use of a mechanical stirrer. Alternatively, the extract may be circulated back to the percolator on a regular basis. This process ends when the filtrate that exits the percolator is colourless i.e., devoid of active compounds (Rasul, 2018). This method takes less time when compared with maceration. It is possible to extract thermolabile constituents as it takes less time and is more thorough. Thus, is appropriate system for potent and expensive medications.

4.3. Steam distillation

As the name suggests, steam is used to separate volatile compounds from plant samples. For extraction, sample is put inside the distiller, where vapour is generated by boiling water. Therefore, plant's cell wall become more permeable until it breaks and releases the BACs, consequently volatiles get vaporized and carried away by the water vapour. The mixture of water vapour and BACs gets condensed on a cold coil circulated by cold water and brought back to

liquid state as a mixture of bioactive and water. The BAC if have high density will settle down or vice versa. Direct steam distillation reduces the extraction time significantly, which is why it's suggested for high-boiling oils and hard materials like roots, barks and woods (Handa *et al.* 2008).

4.4. Soxhlet

Soxhlet extraction is the most effective technique for continuous extraction of a solid with a hot solvent (Grigonis *et al.* 2005). This is a simple and effective method and requires approximately 8 hours for extraction. Soxhlet assembly comprise a main chamber in which a porous thimble containing a solid sample is installed. The extraction step is usually repeated several times by refluxing the solvent through the thimble using a condenser and a siphon side arm. Soxhlet extraction is a robust, well-established technique and permits unattended extraction. Over the last two decades, soxhlet extraction has become the most commonly used method for isolating organic compounds from plant samples. Traditional soxhlet extraction has some appealing advantages as no filtration is needed followed by leaching, and sample throughput can be improved by conducting multiple simultaneous extractions in parallel, which is made possible by the low cost of the basic equipment. The two significant disadvantages of soxhlet extraction are the lengthy extraction period and the vast volume of organic solvent lost, which is not only costly to dispose of but can also cause environmental hazard (Zhang *et al.* 2020).

4.5. Decoction

Decoction is a water-based preparation used to extract active compounds from medicinal plants. The liquid is prepared by heating the plant material with water. This method has been used for extraction and evaluation of antioxidant properties of different Indian vegetable and fruit peels (Chanda *et al.* 2013). Owing to boiling, plant cells swell as a result cell wall also expands, and plant constituents are hydro dispersed from the swelling membranes. Decoction is used for tough and fibrous leaves, barks, and stems, as well as plants with water-soluble components. The primitive medication in the form of yavakuta (small pieces) is inserted in earthen pots or tinned copper vessels with clay on the outside in the Ayurvedic process, historically known as kwatha. Water is applied to the pot, which is then heated over a fire. The mixture is then heated over low heat until it become one-fourth of its original amount when it comes to soft drugs and one-eighth in the case of mildly or extremely hard drugs. After cooling and straining, the filtrate is stored in clean vessels.

5. Limitations of Conventional Methods

Conventional methods have their own advantages because of which they are used from longer period of time. Unfortunately, the disadvantages are more prominent than advantages because these are very slow and time consuming process as takes up to weeks. In traditional methods various types of solvents have been used for many years. Both humans and the environment are harmed by the organic solvents utilized in extraction. Furthermore, complete removal of these solvents is challenging; hence, a solvent with high purity and ability to extract desired components is needed for efficient extraction. Also there is possibility of sample contamination with residual debris, the removal of which necessitates the use of additional filtration methods. But there are many other factors affecting the extraction efficiency. Like particle size of the raw material requires special attention throughout the process (Rasul, 2018; Mazyan *et al.* 2021). Additionally, there is a risk of thermal breakdown of some heat labile compounds found in plant material when the samples are heated to a high temperature for an extended duration of time. While in steam distillation, the plant material near the bottom comes in direct contact with fire from the furnace so, it may char the material and thus impart an objectionable odour to the BACs. Hot water during steam distillation causes some essential oil components, such as esters, to hydrolyze during the distillation process. As a result, the final product's chemical characteristics have been transformed, which is unacceptable. Taking all of these flaws into account, it is clear that there is a need to modify the method for extraction of essential components in order to make the process economical and sustainable.

6. Novel Technologies

Novel extraction techniques are proposed to overcome some of the drawbacks of traditional extraction methods. Keeping this in mind, the extraction processes are coupled with some advanced technologies, including ultrasound, enzymes, microwave and others. All of these have been widely used for extraction of bioactive compounds.

6.1. Sub Critical Water Extraction (SWE)

Water is regarded as a universal solvent, as it contains a large number of hydrogen bonds. The critical pressure and temperature of water are 218 atm and 374 °C, respectively. Subcritical water (SW) is the pressurized water whose temperature is between 100 and 374 °C. The pressure of such water is sufficiently high to retain it in liquid phase. With the increase in temperature, the strength of hydrogen bonds is reduced resulting in the reduction in water polarity and

dielectric constant. Because of the increase in the kinetic energy of the molecules at subcritical temperatures, hydrogen bonds become weaker, and the dielectric constant and polarity of water are limited to that of organic solvents (e.g., methanol and ethanol) at room temperature. Consequently, water behaves like a mixture of water-organic solvents at subcritical conditions, therefore less polar substances easily solubilise. At subcritical conditions viscosity and surface tension of water reduces, due to which there is an increase in rate of diffusivity and mass transfer. Thus, subcritical water is an effective solvent for the extraction of both polar and non-polar organic compounds.

SWE has a number of benefits over traditional techniques, including environmental compatibility, quicker extraction periods, greater yields, improved extract quality and purity, cheaper solvent supply and disposal costs, and higher selectivity (Mortazavi *et al.* 2010). In food and pharmaceuticals industries quality and purity is the topmost requirement. Water being ubiquitous non-toxic solvent; is an appropriate substitute for toxic organic solvents. Bioactive compounds such as anthocyanins and antioxidants from plants and foods, as well as organic contaminants from foodstuffs can be extracted using SW (Teo *et al.* 2010).

SWE of antioxidants and polyphenols from chestnut shells resulted higher amounts of pyrogallol and protocatechuic acid than conventional extraction. Other studies have been conducted on the SWE of phenols from avocado fruit flesh (Mazyan *et al.* 2021), polyphenols from lotus seedpods (Yan *et al.* 2020), phenolics antioxidants from kiwi fruit peels (Guthrie *et al.* 2020), polyphenolics from chestnut shells (Pinto *et al.* 2021), phenols from onion skin waste (Munir *et al.* 2018) and phenols and flavanoids from wild garlic extract (Tomsik *et al.* 2017).

However, some studies have reached the opposite conclusion. Generally, increase in temperature facilitates the release of phenolics from cell walls because ability of solvent to extract the components is also increased. This is due to the reduction in viscosity and surface tension which leads to enhanced diffusivity. On the other hand, some researchers observed thermo-degradation of the target compounds at higher temperature (Loarce *et al.* 2020) like degradation of Crocin compound found in saffron (Sarfarazi *et al.* 2019).

6.2. Enzyme Assisted Extraction (EAE)

In plants several complex compounds are present affecting the extraction of components. Like polysaccharides such as hemicelluloses, pectin and starch, are present in large amounts inside the cell wall which reduces the extraction efficiency of conventional extraction techniques. The structural diversity of

BACs and their interaction with other cellular components is insisting everyone to go for some alternate methods of extraction. Some BACs exist in soluble and insoluble conjugates, covalently attached to sugar moieties or cell wall structural components (Acosta-Estrada *et al.* 2014). As a result, a number of attempts have been conducted, with the conclusion that enzymes are effective in extracting these bound molecules due to disruption of cell wall. Simultaneously, this method is also considered eco-friendly, efficient, time saving, and mild. Furthermore, because this extraction is done at a controlled temperature, it is ideal for extracting thermo-sensitive compounds like flavours and pigments.

Many significant parameters like enzyme composition and concentration, type of extraction solvent, solid to liquid ratio, enzyme to substrate ratio, pH, extraction temperature and time affect the potential of enzymes in the degradation and disruption of cell wall structure and release of the target bioactive compounds. Among these factors, temperature is the most important one which increases the reaction rate and simultaneously mass transfer rate. But on the other hand too high temperature inactivates the enzymes. Additionally, some target bioactive components are heat sensitive and require mild temperature throughout the processing (Marathe *et al.* 2019). Also, most of the enzymes exhibit their activity within a range of pH. However, the optimal pH value varies for different substrates even for a same enzyme.

EAE is widely used in extraction of total phenolics from pomegranate peels, lycopene from tomato tissues and oil from grape seeds. Dominguez-Rodrigueza, (2021) stated that optimal conditions results in higher extraction of proanthocyanidins from sweet cherry pomace as well as higher bioactivity than alkaline and acid hydrolysis. Antioxidant compounds have also been extracted from red algae residue, rice bran, tomato processing waste, pumpkin peel waste and citrus peels. When enzymes were used to extract phenols from crude olive pomace, higher concentrations of bioactive phenolic alcohols and acids were generated (Macedo *et al.* 2021). Similarly, when spinach leaves were pre-treated with enzyme followed by extraction of chlorophyll in ethanol, it resulted in increased 39 % yield (Ozkan and Bilek, 2015). Whereas, currently available formulations of enzymes cannot fully hydrolyze plant cell walls, which limits the extraction yields of some compounds. Therefore, there is a necessity to have researches on EAE to improve its performance.

6.3. Microwave Assisted Extraction (MAE)

MAE involves the heating effect of microwave, causing higher extraction temperature resulting in faster mass transfer rate. Microwaves cause direct

heating/bulk heating of the solvent body and the sample matrix due to its penetration into sample to some depth and its interaction with the polar (mostly water molecules) components. During MAE, local temperature and pressure increases, this helps the target components to move from the matrix towards the solvent body.

MAE is performed either in a closed system with increased pressure or an open system kept under atmospheric pressure. In closed MAE system extraction is performed in sealed vessel with uniform microwave heating under controlled temperature and pressure. This often results in higher working temperatures than the open system as the increased pressure in the closed vessel raises the boiling point of the extraction solvent and it reduces the requirement of solvent, but care should be taken during the process from safety point of view. Therefore, open MAE system is preferred over closed MAE system and it is more appropriate for thermo-labile components.

Dao *et al.* (2021) compared the microwave heating and conventional methods for extraction of pectin from passion fruit peels. Results showed that MAE exhibited higher yield (18.73%) as compared to the conventional one (13.58%). Likewise, polyphenol extraction from seabuckthorn leaves with the help of MAE and conventional solvent extraction (CSE) was done. It was observed that total phenolic content (TPC) extracted from CSE is less than MAE while the yield was same for both the methods (Galan *et al.* 2017). Serdar *et al.* (2016) did MAE of caffeine and catechins from green tea and were successful in extraction within 4 minutes.

Many researches have been done on the microwave extraction of tea phenols, soy isoflavones, phenolic antioxidants from peanut skins, solanesol from tobacco leaves, taxanes from *Taxus*, azadiractin from *Azadiracta*, polyphenols from cumin, coriander, turmeric and cinnamon. A rapid MAE method was established by Liu *et al.* (2021) to extract commercially important salidroside from dry plant materials of *Rhodiola crenulata*. It was seen that MAE was the most efficient and effective method in the extraction of salidroside from the dry plant materials. Various advantages of MAE are faster heating, smaller apparatus size, higher extraction yield, lower usage of solvents and better products with lower costs.

6.4. Ultrasound Assisted Extraction (UAE)

Ultrasound waves are electromagnetic waves of a frequency greater than 20 kHz. The basic principle of UAE is acoustic cavitation. These high power ultrasound waves need a medium to propagate. Due to propagation of

ultrasound waves, compression and rarefaction cycle is induced in the particles of the medium causing alterations in pressure. This results into formation of cavitation bubbles from the gas nuclei of the medium. These bubbles grow continuously until they become unstable and finally violently collapse and create hotspots and the phenomenon is called acoustic cavitation. These events cause high sheer energy waves and turbulence, resulting in disruption of cell walls of the sample. Owing to damaged cell wall, diffusion and mass transfer rate of intracellular material is facilitated. Thus, UAE can provide added benefit by increasing the extraction yield at lower temperatures and thus decreasing extraction time resulting in a better quality product.

Different bioactive compounds are extracted efficiently by UAE from a variety of fruits, vegetables and their wastes (Kumar and Rao, 2020). The UAE of anthocyanins, flavanoids and polyphenols from grape pomace was conducted and showed an increased yield of all compounds when compared to CSE at same time (Romanini *et al.* 2021). Milani *et al.* (2020) compared UAE with conventional extraction, and demonstrated that the amount of steviol glycosides from stevia through UAE is almost 3 times higher. Silva *et al.* (2020) studied the extraction of antioxidant compounds from acerola residues using UAE. The yield was better when compared with those obtained using CE. A TPC of 931.2 ± 40.1 mg GAE/100 g (dw), a TFC of 4.8 ± 0.3 mg rutin/100 g (dw), and an IC₅₀ of 5.6 ± 0.3 lg/mL was recorded. Some comparative studies between MAE and UAE were also being done, for example, Wang *et al.* (2021) extracted fucoidan from a brown macro-alga (*Sargassum siliquosum*) using MAE and UAE. It was noted that MAE performed better under the conditions of 750 W, 10 minutes, and a liquid/solid ratio of 15 mL/g, yielding a total sugar of 6.94 % per dry weight.

Besides fruits and vegetables, UAE is also applied for extraction of bioactive compounds from herbs, seeds and spices. It has been successfully applied for the extraction of polyphenols from green tea leaves (Ghasemzadeh *et al.* 2015). Moreover, under appropriate conditions it is also used for the extraction of collagen from fish. In addition, fish skin waste from the cutting process line, is also exploited as a value-added product, comprising fish skin collagen (Petcharat *et al.* 2020). Thus, it can be said that UAE can act as an effective technique in extraction as well as valorisation of numerous agro-industrial by-products for a wide range of bioactive compounds.

7. Medicinal Properties of BACs

Herbal medicine is as ancient as human culture and has evolved with it. Indian and Chinese medical systems are well developed, with written documents

dating back about 3000 years. Plants have therapeutic properties due to the existence of bioactive compounds. Most pharmacologically active molecules are alkaloids, phenolics, and terpenoids. Several plant-derived natural products, including galanthamine and triptolium, have recently been licensed by the US Food and Drug Administration (FDA) or are currently in clinical trials.

8. Pros and Cons of Novel Technologies

Like every other technology, novel extraction technologies also have some pros and cons. These are a little bit expensive as compared to the traditional ones so everyone cannot afford. While performing SWE under higher temperatures, there is a problem of thermal degradation of thermo-labile compounds. Similarly, when UAE is done, there are chances of modification of the target molecule. But leaving all these cons aside, there are several benefits of these technologies which make them better than the conventional ones. These require fewer amounts of solvent, green solvents and that's why also called as environment friendly techniques. These also are very less time consuming. Extraction occurs in minutes whereas the conventional methods take even days for extraction. Also, the efficiency of these methods is very high due to higher extraction rate and the extracted compound is also pure in nature. However, a single extraction technique is not enough to extract different compounds. A proper extraction technique is still required in the food industry.

9. Conclusion

Ample experimental work has been done in the last 25–30 years for the development of “substitute” technologies for the extraction of BACs from plants, including fruit and vegetables. Before, during and after extraction, researchers are facing countless issues which are creating hurdles in the selection of the most sustainable extraction technique. There are many questions which are to be answered yet like; is there a purification step needed after extraction? As most of the BACs are present in very low concentrations, their extraction still leave large amounts of waste: can this waste accordingly become a co-product? Novel extraction technologies yield higher BACs in shorter extraction time, with better quality and less environmental pollution than conventional ones. Currently, various researches are being done focusing on the combination of these novel extraction technologies in order to remove all the technical barriers for efficient extraction. However, these novel extraction techniques still require a proper design and optimized conditions to develop the finest process of extraction, which will also be parallel to sustainable development.

Table 1: Different sources of BACs

<i>Phytochemical</i>	<i>Compound</i>	<i>Sources</i>	<i>References</i>
Phenols	Anthocyanins	Grape pomace	Romanini <i>et al.</i> 2021
	Caffeic acid	Apple peel	Geana <i>et al.</i> 2021
	Catechin	Blueberry peel	Wang <i>et al.</i> 2021
	Galangin, Gallic acid	Guava peel, seeds	Murakonda <i>et al.</i> 2021
	Ferulic acid	Beetroot peel	Sobhy <i>et al.</i> 2020
	Hydroxybenzoic acid	Garlic peel	Ismail <i>et al.</i> 2021
	Ferulic acid	Citrus peel	Singh <i>et al.</i> 2020
Flavonoids	Genistein	Soybean	Poasakate <i>et al.</i> 2021
	Resveratrol	Grapes	Restani <i>et al.</i> 2020
	EGCG	Green tea	Zhan <i>et al.</i> 2020
	Anthocyanins	Blackberry	Delgado-Povedano <i>et al.</i> 2021
Alkaloids	Berberine	Berberis	Malhora <i>et al.</i> 2021
	Morphine	Opium poppy	Yazici and Yilmaz, 2021.
	Nicotine	Tobacco	Tita <i>et al.</i> 2021
	Caffeine	Coffee, tea	Souza <i>et al.</i> 2020
Terpenes	Xanthophylls	Peach, papaya	Alferez <i>et al.</i> 2021
	Menthol	Mint	Lothe <i>et al.</i> 2021
	Carotenoid	Apricot, carrot	Wabg <i>et al.</i> 2021
	Geraniol	Citronella	Rihayat <i>et al.</i> 2020
	Curcumene	Turmeric	Singh <i>et al.</i> 2010

Table 2: Different solvents used for extraction (Rasul, 2018)

<i>Water</i>	<i>Ethanol</i>	<i>Methanol</i>	<i>Chloroform / acetone</i>	<i>Ether</i>
Tannins	Tannins	Tannins	Flavonoids	Alkaloids
Anthocyanins	Polyphenols	Terpenoids	Terpenoids	Terpenoids
Saponins	Flavanoids	Polyphenols		
Terpenoids	Terpenoids	Anthocyanins		
	Alkaloids	Saponins		

Table 3: Short overview of method of extraction from natural products

<i>Method</i>	<i>Solvent</i>	<i>Temperature</i>	<i>Time</i>	<i>Volume of organic solvent used</i>
Maceration	Aqueous and non aqueous	Room temperature	5-10 days	Large
Percolation	Aqueous and non aqueous	Room temperature	2-3 days	Large
Steam distillation	Water	Under heat	1-8 hrs	-nil-

Soxhlet	Organic solvent	Under heat	8 hrs	Moderate
Decoction	Water		1-5 hrs	
SWE	Water	Under heat	1-20 min	-nil-
EAE	Aqueous and non aqueous	Room temperature or heat after enzyme treatment	20-40 min	Moderate
MAE	Aqueous and non aqueous	Room temperature	10-30 min	Moderate
UAE	Aqueous and non aqueous	Room temperature or heat	15-20 min	Moderate

Table 4: Therapeutic properties of BACs

<i>Bioactive compound</i>	<i>Therapeutic effect</i>	<i>Reference</i>
Glycosides (Stevia)	Antibacterial, anti-inflammatory, hypotensive, diuretic, anti-tumors effects antibacterial, anti-inflammatory, hypotensive, diuretic, and anti-tumors effects	Milani <i>et al.</i> 2020
Flavonoids, anthocyanin, phenolic acids, and hydrolysable tannin (Pomegranate peel)	Anti-inflammatory, antioxidant, anti-microbial, anti-mutagenic properties, anti-viral, chemopreventive, anti-proliferic, and anti-fungal	Kumar and Rao, 2020
Rutin (Acerola waste)	Anti-diabetic and anti-inflammatory	Silva <i>et al.</i> 2020
Fucoidans (Brown algae)	Anti-viral, anti-inflammatory, anti-angiogenic, and anti-tumorigenic	Wang <i>et al.</i> 2021
Flavanoids (Saffron)	Anti-microbial, anti-tyrosinase, antiradical, anticancer, antifungal	Gahruie <i>et al.</i> 2020
Gallic and ellagic acids) and tannins (Chestnut shells)	Antiviral and antimicrobial	Pinto <i>et al.</i> 2021
Gallic acid derivatives(mango peel)	Anti-proliferative activity.	S´anchez-Camargo <i>et al.</i> 2021
indole alkaloids (<i>Catharanthus roseus</i>)	Anticancer, anti-diabetics, bactericides, and antihypertensive	Koel <i>et al.</i> 2020
Non extractable polyphenols (Cherry pomace)	Antioxidant and anti-hypertensive	Dominguez-Rodriguez <i>et al.</i> 2021
Phlorotannins (algae)	Cholesterol-lowering effect, improvement of bone health, anti-hypertensive, prevention of gut-related diseases	Del Pilar Sanchez-Camargo <i>et al.</i> 2021

Table 5: Application of different BACs in food industry

Extracts	Applications	Functionality	References
Picrocrocin	Alcoholic and non- alcoholic drinks	Anti depressant	De Monte and Cesa, 2021
Safranal	Bakery	Anti anxiety	Nanda and Madan, 2021
Crocin	Colouring agent in cookery	Pigmentation	De Monte and Cesa, 2021
Chlorophyll	Green food colorant	Pigmentation	Ozkan and Bilek, 2015
Pholoro-tanins	Functional foods	Anti inflammatory Anti diabetic Anti microbial	Cassani <i>et al.</i> 2020
Antho-cyanins	Food additive E163, Purple -coloured jam, confectionaries, and beverages	Pigmentation	Wahyuningsih <i>et al.</i> 2017
Rebauside-A	Soy sauce, pickle, boiled fish paste	Sweetener	Baines and Seal, 2012

References

- Acosta-Estrada BA, Gutierrez-Uribe JA, Serna-Saldivar SO. 2014. Bound phenolics in foods, a review. *Food Chemistry*, 152, 46-55.
- Alferez F, de Carvalho DU, Boakye D. 2021. Interplay between abscisic acid and gibberellins, as related to ethylene and sugars, in regulating maturation of non-climacteric fruit. *International Journal of Molecular Sciences*, 22(2), 669.
- Baines D and Seal R. (Eds.). 2012. *Natural food additives, ingredients and flavourings*. Elsevier.
- Cassani L, Gomez-Zavaglia A, Jimenez-Lopez C, Lourenço-Lopes C, Prieto MA, Simal-Gandara J. 2020. Seaweed-based natural ingredients: stability of phlorotannins during extraction, storage, passage through the gastrointestinal tract and potential incorporation into functional foods. *Food Research International*, 109676.
- Chanda S, Amrutiya N, Rakholiya K. 2013. Evaluation of antioxidant properties of some Indian vegetable and fruit peels by decoction extraction method. *Am J Food Technol*, 8(3), 173-182.
- Dao TAT, Webb HK, Malherbe F. 2021. Optimization of pectin extraction from fruit peels by response surface method: conventional versus microwave-assisted heating. *Food Hydrocolloids*, 113(4), 106475.
- De Monte C and Cesa S. 2021. Use of saffron as a functional food and saffron nutraceuticals. In *Saffron*, pp. 241-273. Academic Press.
- Delgado-Povedano MDM, de Villiers A, Hann S, Causon T. 2021. Identity confirmation of anthocyanins in berries by LC-DAD-IM-QTOFMS. *Electrophoresis*, 42(4), 473-481.

- Dominguez-Rodriguez G, Marina ML, Plaza M. 2021. Enzyme-assisted extraction of bioactive non-extractable polyphenols from sweet cherry (*Prunus avium* L.) pomace. *Food Chemistry*, 339, 128086.
- Gahrue HH, Parastouei K, Mokhtarian M, Rostami H, Niakousari M, Mohsenpour Z. 2020. Application of innovative processing methods for the extraction of bioactive compounds from saffron (*Crocus sativus*) petals. *Journal of Applied Research on Medicinal and Aromatic Plants*, 19, 100264.
- Galan AM, Calinescu I, Trifan A, Winkworth-Smith C, Calvo-Carrascal M, Dodds C, Binner E. 2017. New insights into the role of selective and volumetric heating during microwave extraction: Investigation of the extraction of polyphenolic compounds from sea buckthorn leaves using microwave-assisted extraction and conventional solvent extraction. *Chemical Engineering and Processing: Process Intensification*, 116, 29-39.
- Geana EI, Ciucure CT, Ionete RE, Ciocarlan A, Aricu A, Fikai A, Andronescu E. 2021. Profiling of phenolic compounds and triterpene acids of twelve apple (*Malus domestica* Borkh.) cultivars. *Foods*, 10(2), 267.
- Ghasemzadeh-Mohammadi V, Zamani B, Afsharpour M, Mohammadi A. 2017. Extraction of caffeine and catechins using microwave-assisted and ultrasonic extraction from green tea leaves: an optimization study by the IV-optimal design. *Food Science and Biotechnology*, 26(5), 1281-1290.
- Grigonis D, Sivik P, Sandahl M, Eskilsson C. 2005. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloe odorata*). *Journal of Supercritical Fluids*, 33(15), 223–233.
- Guthrie F, Wang Y, Neeve N, Quek SY, Mohammadi K, Baroutian S. 2020. Recovery of phenolic antioxidants from green kiwifruit peels using subcritical water extraction. *Food and Bioproducts Processing*, 122, 136-144.
- Handa S, Khanuja SP, Longo G, Rakesh DD. 2008. Extraction Technologies for Medicinal and Aromatic Plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology, 3(8), 40-46.
- Ismail T, Hashmi MS, Akhtar S. 2021. Biochemical characterization of vegetables wastes and development of functional bread: vegetables' wastes based functional bread. *Journal of Microbiology, Biotechnology and Food Sciences*, 10(4), 691-696.
- Koel M, Kuhtinskaja M, Vaher M. 2020. Extraction of bioactive compounds from *Catharanthus roseus* and *Vinca minor*. *Separation and Purification Technology*, 252: 117438.
- Kumar A and Rao PS. 2020. Optimization of pulsed-mode ultrasound assisted extraction of bioactive compounds from pomegranate peel using response surface methodology. *Journal of Food Measurement and Characterization*, 14(6), 3493-3507.
- Loarce L, Oliver-Simancas R, Marchante L, Diaz-Maroto MC, Alanon ME. 2020. Implementation of subcritical water extraction with natural deep eutectic solvents for

- sustainable extraction of phenolic compounds from winemaking by-products. *Food Research International*, 137, 109728.
- Lothe NB, Mazed A, Pandey J, Patariya V, Verma K, Semwal M, Verma RK. 2021. Maximizing yields and economics by supplementing additional nutrients for commercially grown menthol mint (*Mentha arvensis* L.) cultivars. *Industrial Crops and Products*, 160, 113110.
- Macedo GA, Santana ÁL, Crawford LM, Wang SC, Dias FF, de Moura Bell JM. 2021. Integrated microwave-and enzyme-assisted extraction of phenolic compounds from olive pomace. *LWT*, 138, 110621.
- Malhotra B, Kulkarni GT, Dhiman N, Sharma AK, Kharkwal H. 2021. Recent advances on *Berberis aristata* emphasizing berberine alkaloid including phytochemistry, pharmacology and drug delivery system. *Journal of Herbal Medicine*, 100433.
- Marathe SJ, Jadhav SB, Bankar SB, Dubey KK, Singhal RS. 2019. Improvements in the extraction of bioactive compounds by enzymes. *Current Opinion in Food Science*, 25, 62-72.
- Mazyan WI, O'Connor E, Martin E, Vog A, Charter E, Ahmadi A. 2021. Effects of temperature and extraction time on avocado flesh (*Persea americana*) total phenolic yields using subcritical water extraction. *Processes*, 9(1), 159.
- Milani G, Vian M, Cavalluzzi MM, Franchini C, Corbo F, Lentini G, Chemat F. 2020. Ultrasound and deep eutectic solvents: an efficient combination to tune the mechanism of steviol glycosides extraction. *Ultrasonics Sonochemistry*, 69, 105255.
- Mortazavi SV, Eikani MH, Mirzaei H, Jafari M, Golmohammad F. 2010. Extraction of essential oils from *Bunium persicum* Boiss. using superheated water. *Food and Bioproducts Processing*, 88(2-3), 222-226.
- Munir MT, Kheirkhah H, Baroutian S, Quek SY, Young BR. 2018. Subcritical water extraction of bioactive compounds from waste onion skin. *Journal of Cleaner Production*, 183, 487-494.
- Murakonda S and Dwivedi M. 2021. Powders from Fruit Waste. *Food Powders Properties and Characterization*, 155-168.
- Nanda S and Madan K. 2021. The role of Safranin and saffron stigma extracts in oxidative stress, diseases and photoaging: A systematic review. *Heliyon*, 7(2), 06117.
- Ozkan G and Bilek SE. 2015. Enzyme-assisted extraction of stabilized chlorophyll from spinach. *Food Chemistry*, 176, 152-157.
- Pandey A, Tripathi S. 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, 2(5), 115-119.
- Pinto D, Vieira EF, Peixoto AF, Freire C, Freitas V, Costa P, Rodrigues F. 2021. Optimizing the extraction of phenolic antioxidants from chestnut shells by subcritical water extraction using response surface methodology. *Food Chemistry*, 334, 127521.

- Poasakate A, Maneesai P, Rattanakanokchai S, Bunbupha S, Tong-Un T, Pakdeechote P. 2021. Genistein prevents nitric oxide deficiency-induced cardiac dysfunction and remodeling in rats. *Antioxidants*, 10, 237.
- Rasul G. 2018. Conventional extraction methods use in medicinal plants, their advantages and disadvantages. *International Journal of Basic Sciences and Applied Computing (IJBSAC)*, 2(6), 394-397.
- Renard C. 2018. Extraction of bioactives from fruit and vegetables: State of the art and perspectives. *LWT - Food Science and Technology*, 93, 390–395.
- Restani P, Fradera U, Ruf JC, Stockley C, Teissedre PL, Biella S, Lorenzo CD. (2020). Grapes and their derivatives in modulation of cognitive decline: a critical review of epidemiological and randomized-controlled trials in humans. *Critical Reviews in Food Science and Nutrition*, 1-11.
- Rihayat T, Hasanah U, Siregar JP, Jaafar J, Cionita T. 2020. Geraniol quality improvement on citronella oil as raw material for making anti-bacterial perfumes. In *IOP Conference Series: Materials Science and Engineering*, 788(1), p. 012028). IOP Publishing.
- Romanini EB, Rodrigues LM, Finger A, Chierrito TPC, da Silva Scapim MR, Madrona GS. 2021. Ultrasound assisted extraction of bioactive compounds from BRS violet grape pomace followed by alginate-Ca²⁺ encapsulation. *Food Chemistry*, 338, 128101.
- Sarfarazi M, Jafari SM, Rajabzadeh G, Feizi J. 2019. Development of an environmentally-friendly solvent-free extraction of saffron bioactives using subcritical water. *LWT*, 114, 108428.
- Serdar G, Demir E, Bayrak S, Sokmen M. 2016. New approaches for effective microwave assisted extraction of caffeine and catechins from green tea. *International Journal of Secondary Metabolite*, 3(1), 3-13.
- Silva PB, Mendes LG, Rehder AP, Duarte CR, Barrozo MA. 2020. Optimization of ultrasound-assisted extraction of bioactive compounds from acerola waste. *Journal of Food Science and Technology*, 57, 4627-4636.
- Singh B, Singh JP, Kaur A, Singh N. 2020. Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Research International*, 132, 109114.
- Singh G, Kapoor IPS, Singh P, De Heluani CS, De Lampasona MP, Catalan CA. 2010. Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (*Curcuma longa* Linn.). *Food and Chemical Toxicology*, 48(4), 1026-1031.
- Sobhy ES, Abdo E, Shaltou O, Abdalla A, Zeitoun A. 2020. Nutritional evaluation of beetroots (*Beta vulgaris*) and its potential application in a functional beverage. *Plants*, 9(12), 1752.
- Souza MC, Santos, MP, Sumere, BR, Silva LC, Cunha DT, Martinez J, Rostagno MA. 2020. Isolation of gallic acid, caffeine and flavonols from black tea by on-line coupling of pressurized liquid extraction with an adsorbent for the production of functional bakery products. *LWT*, 117, 108661.

- Teo CC, Tan N, Yong JWH, Hew CS, Ong ES. 2010. Pressurized hot water extraction (PHWE). *Journal of Chromatography A*, 1217(16), 2484-2494.
- Tita GJ, Navarrete A, Martín Á, Cocero MJ. 2021. Model assisted supercritical fluid extraction and fractionation of added-value products from tobacco scrap. *The Journal of Supercritical Fluids*, 167, 105046.
- Tomsik A, Pavlic B, Vlastic J, Cindric M, Jovanov P, Sakac M, Vidovic S. 2017. Subcritical water extraction of wild garlic (*Allium ursinum* L.) and process optimization by response surface methodology. *The Journal of Supercritical Fluids*, 128, 79-88.
- UNESCO. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – Paris, France, 1996, 129.
- Wahyuningsih S, Wulandari L, Wartono MW, Munawaroh H, Ramelan AH. 2017. The effect of pH and color stability of anthocyanin on food colorant. In IOP conference series: Materials Science and Engineering, 193(1), 012047.
- Wang H, Fang XM, Sutar PP, Meng JS, Wang J, Yu XL, Xiao HW. 2021. Effects of vacuum-steam pulsed blanching on drying kinetics, colour, phytochemical contents, antioxidant capacity of carrot and the mechanism of carrot quality changes revealed by texture, microstructure and ultrastructure. *Food Chemistry*, 338, 127799.
- Wang SH, Huang CY, Chen CY, Chang CC, Huang CY, Dong CD, Chang JS. 2021. Isolation and purification of brown algae fucoïdan from *Sargassum siliquosum* and the analysis of anti-lipogenesis activity. *Biochemical Engineering Journal*, 165, 107798.
- Wang Z, Barrow CJ, Dunshea FR, Suleria HA. 2021. A comparative investigation on phenolic composition, characterization and antioxidant potentials of five different australian grown pear varieties. *Antioxidants*, 10(2), 151.
- Yan Z, Zhan, H, Dzah CS, Zhang J, Diao C, Ma H, Duan Y. 2020. Subcritical water extraction, identification, antioxidant and antiproliferative activity of polyphenols from lotus seedpod. *Separation and Purification Technology*, 236, 116217.
- Yazici L and Yilmaz G. 2021. Investigation of alkaloids in opium poppy (*Papaver somniferum* L.) varieties and hybrids. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi)*, 27(1), 62-68.
- Zhan C, Chen Y, Tang Y, Wei G. 2020. Green tea extracts egcg and egc display distinct mechanisms in disrupting A β 42 protofibril. *ACS Chemical Neuroscience*, 11(12), 1841-1851.
- Zhang J, Wen C, Zhang H, Duan Y, Ma H. 2020. Recent advances in the extraction of bioactive compounds with subcritical water: a review. *Trends in Food Science & Technology*, 95, 183-195.