REVIEW ARTICLE Agricultural Reviews, Volume 40 Issue 4: 249-260 (October-December 2019)

Importance of Bioactive Compounds Present in Plant Products and Their Extraction: A Review

Amardeep Kumar[1], Faizan Ahmad[2], Sadaf Zaidi[2]

10.18805/ag.R-1926

ABSTRACT

India is one of the largest producers of fruits and vegetables in the world. These are rich sources of bioactive compounds which provide health benefits and also possess antioxidant therapeutic value. A large portion of fruits and vegetables goes unutilized in the form of pulp and peels after the extraction of juice in food processing industries. This contains a large number of bioactive compounds. The compounds like phenolic compounds, flavonoids and carotenoids are ubiquitously present in fruits, vegetables and their by-products. These compounds have antioxidant, antimicrobial, anticancer, antiviral, antitumor and many more activities to a greater or lesser extent. This review focuses on the bioactive compounds present in fruits, vegetables and their by-products along with their classification and importance in day to day life. A further aim of this review is to discuss various techniques employed in the extraction of bioactive compounds from plant products. The antioxidant activity of various fruits and vegetables based on DPPH radical scavenging methods has also been reported in this work.

Key words: Antioxidant activity, Bioactive compounds, By-products of fruits and vegetables, Extraction techniques.

Fruits and vegetables possess a large number of bioactive compounds and the antioxidant properties that are present in them have a vital role in our diet. As people are becoming more and more health-conscious, the presence of natural antioxidants from the addition of fruits and vegetables, either in the whole form or in the form of juice, in their regular diets have gained increased importance. Many antioxidant compounds are naturally present in fruits and vegetables (Ahmad and Khan, 2019). Phenolic compounds, anthocyanins, carotenoids and tocopherols have been extensively studied in fruits, vegetables, cereals, legumes, spices and nuts (Naczk and Shahidi, 2006). These bioactive compounds possess an aromatic ring bearing one or more hydroxyl groups. Their structures may range from that of a simple phenolic molecule to that of a complex high-molecular-mass polymer (Balasundram *et al*., 2006).

Extensive research on these compounds has shown that these compounds have antioxidant, antimicrobial, anti-cancer, antiviral, anti-tumor and many more activities to a greater or lesser extent (Miliauskas *et al*., 2004; Cai *et al*., 2004). During oxygen metabolism some reactive oxygen species (ROS) are formed. Examples of such species are peroxyl radicals (ROO°), hydroxyl radical (°OH) and superoxide radicals (O_2°) which are responsible for causing coronary heart disease, carcinogenesis and are also associated with other health issues related to aging. These ROS radical related oxidative reactions are directly terminated by antioxidants and thus solve the problems of associated aging diseases and other health problems. Apart from ROS adverse effects on health, these oxidative reactions also develop off-flavor and rancid odour in foods and thus reduce the nutritional and organoleptic quality of processed foods. Propyl gallate (PG), Butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) are some of the synthetic antioxidants which are commonly used in food industries to prevent oxidative reactions but it has been found that they are carcinogenic in nature along with some other side effects (Borneo *et al*., 2009). These problems can be solved by replacing synthetic antioxidants with natural antioxidants, which are omnipresent in fruits, vegetables and other biological materials as they are safe and have nutritional and therapeutic value. This increased the interest in the evaluation of antioxidant properties of various biological products among which fruits have been given special attention as they are a rich source of phenolic compounds (Ajila *et al*., 2007).

The extraction of such bioactive compounds present in biological materials has attracted scientists and researchers to pay their special attention to find novel technology to increase the efficiency of extraction. Several conventional and non-conventional methods are being developed, but still, not a single method is considered as a standard method for the extraction of bioactive compounds. Conventional methods such as Soxhlet extraction and hydrodistillation along with non-conventional methods like microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and pulsed electric field extraction (PEF) are used to extract bioactive compounds (Azmir *et al*., 2013).

[1]Department of Food Engineering and Technology, School of Engineering, Tezpur University, Napaam-784 028, Assam, India.
[2]Department of Post-Harvest Engineering & Technology, Faculty of Agricultural Science, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India.

Corresponding Author: Faizan Ahmad, Department of Post-Harvest Engineering & Technology, Faculty of Agricultural Science, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India, Email: f4faizahmad1989@gmail.com

How to cite this article: Kumar, A., Ahmad, F. and Zaidi, S. (2019). Importance of bioactive compounds present in plant products and their extraction: A Review. Agricultural Reviews, 40(4): 249-260.

Source of support: Nil

Conflict of interest: None

Submitted: 10-08-2019 **Accepted:** 17-10-2019 **Published:** 14-12-2019

CLASSIFICATION OF PLANT METABOLITES

Plant metabolites can be classified into mainly two groups such as primary metabolites and secondary metabolites. Primary metabolites aim at the overall growth and development of plant products and therefore include proteins, amino acids, carbohydrates and lipids (Fig 1). On the other hand, secondary metabolites are the compounds which increase the overall ability of the plant to survive and overcome local hurdles by allowing them to interact with their surroundings. Most of the plant bioactive compounds are secondary metabolites which give plants their colour, flavour and aroma (Meltzer *et al*. 2010).

BIOACTIVE COMPOUNDS

The bioactive compounds are important nutritional components that are present universally in little amounts in fruits and vegetables and are well known for their various behavioural, immunological and physiological health benefits. A large number of bioactive compounds have been identified which are classified according to their chemical structures and functions. Some of the examples of bioactive compounds are polyphenols, flavonoids, lycopene, carotenoids, anthocyanin, tannins, terpenoids, saponins, taurine, phytoestrogens, etc.

CLASSIFICATION OF BIOACTIVE COMPOUNDS

Phenolic compounds, Alkaloids, Terpenes and Terpenoids, are the main classes of bioactive compounds (Croteau *et al*., 2000). Fig 2 indicates the detailed classification of bioactive compounds present in plant products.

BIOACTIVE COMPOUNDS PRESENT IN FRUITS AND VEGETABLES

Phenolic Compounds

Phenolic compounds, present naturally in plant products, are an essential part of the human diet. These compounds

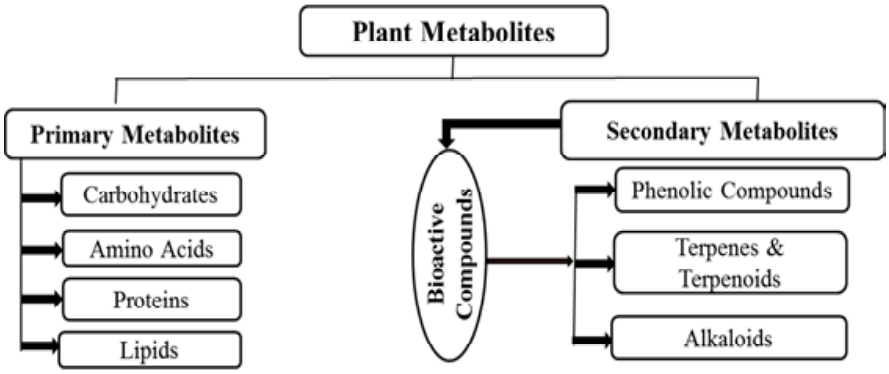


Fig 1: Classification of plant metabolites.

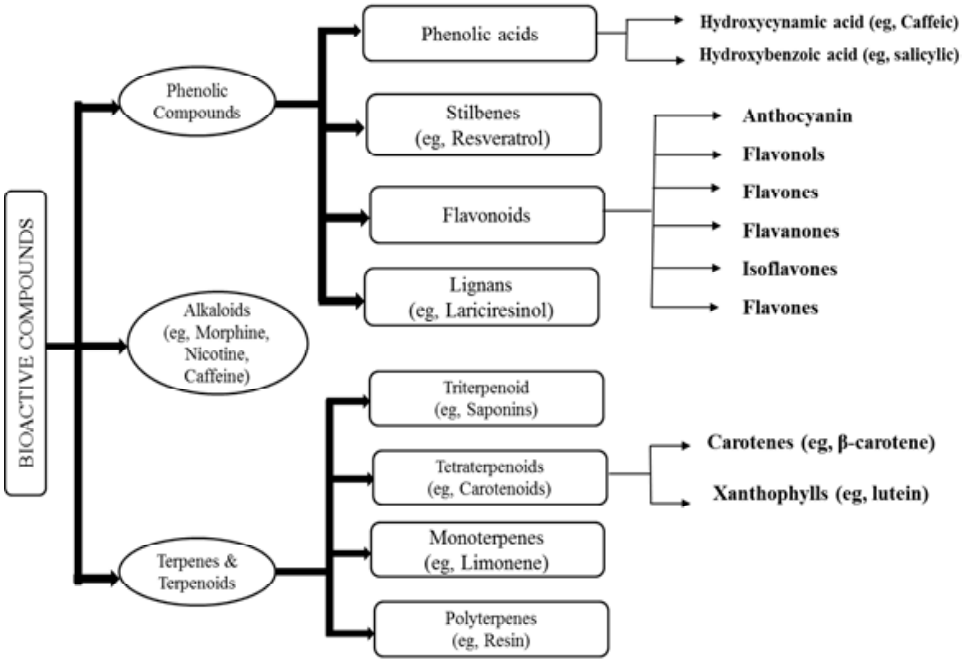


Fig 2: Classification of bioactive compounds.

have an aromatic ring that bears one or more hydroxyl groups and their structures vary from a simple phenolicmolecule to a complex high molecular weight polymer. These compounds have a large number of physiological properties such as antioxidant, anti-allergenic, anti-inflammatory, anti-atherogenic, anti-microbial and cardioprotective (Balasundram *et al*., 2006). These compounds, most widely found groups of phytochemicals, find considerable importance in the physiological and morphological activities in plants. They play a vital role in their growth and reproduction. They also provide protection against predators and pathogens apart from providing colour and sensory characteristics to fruits and vegetables (Bravo, 1998; Alasalvar *et al*., 2001).

Total phenolic content varies significantly among different fruits and vegetables and even varies among same fruits or vegetables depending upon the complexity of these groups of compounds and the methods chosen for extraction and analysis (Bravo, 1998). The recoverable quantity of these compounds also depends on their processing and storage conditions. Total phenolic content of some selected fruits, vegetables and their by-products is shown in (Table 1, 2 and 3), respectively.

Flavonoids

Flavonoids are low molecular weight compounds, made up of fifteen carbon atoms and are arranged in C_6-C_3-C_6 configuration. Structurally they have two aromatic rings joined by a 3-carbon bridge. They are further classified into flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanin. Flavonoids are an important source of antioxidants because of their high redox potential that enables them to act as reducing agents. The consumption of flavonoids in large amounts helps in preventing cancer and heart diseases (Ignat *et al*., 2011).

Table 1: Phenolic content of some selected fruits.

Fruits	Total phenolic content	Max. absorbance wavelength	References
Apple	272.1 ± 6.2[a]	760 nm	Sun *et al*, 2002
Cranberry	570 ± 21.1[a]	760 nm	Sun *et al*., 2002
Guava (white)	247.3 ± 4.5[a]	NM	Luximon-Ramma *et al*., 2003
Guava (pink)	126.4 ± 6.0[a]	NM	Luximon-Ramma *et al*, 2003
Mango	652.59 ± 22.53[b]	700 nm	Ribeiro da Silva *et al*, 2014
Avocado	24.2 ± 1.9[a]	NM	Luximon-Ramma *et al*, 2003
Pineapple	990.76 ± 81.39[b]	700nm	Ribeiro da Silva *et al*, 2014
Tamarind	923.34 ± 53.35[b]	700 nm	Ribeiro da Silva *et al*, 2014
Strawberry	147.8 ± 1.1[a]	760 nm	Sun *et al*., 2002
Red grape	182.0 ± 2.6[a]	760 nm	Sun *et al*, 2002
Papaya	1263.70 ± 126.97[b]	700 nm	Ribeiro da Silva *et al*, 2014
Litchi	288 ± 1.7[a]	NM	Luximon-Ramma *et al*, 2003
Banana	11.8 ± 0.4[a]	NM	Luximon-Ramma *et al*, 2003

[a]mg gallic acid equivalents/100g fresh weight.
[b]mg gallic acid equivalents/100g dry basis.
NM – Not mentioned.

Table 2: Phenolic content of some selected vegetables.

Vegetables	Total phenolic content	Max. absorbance wavelength	References
Broccoli	80.76 ± 1.17[a]	760 nm	Chu *et al*., 2002
Beet root	323.0 ± 11.7[b]	650 nm	Kaur & Kapoor, 2002
Ginger	221.3 ± 9.4[b]	650 nm	Kaur & Kapoor, 2002
Spinach	79.55 ± 8.39[a]	760 nm	Chu *et al*., 2002
Cowpea	822.0 ± 85.0[a]	760 nm	Deng *et al*., 2013
Coriander	82.5 ± 1.9[b]	650 nm	Kaur & Kapoor, 2002
Cabbage	36.66 ± 6.93[a]	760 nm	Chu *et al*., 2002
Capsicum	82.30 ± 3.30[a]	NM	Sreeramulu & Raghunath, 2010
Brinjal	123.77 ± 10.62[a]	NM	Sreeramulu & Raghunath, 2010
Garlic	145.0 ± 5.9[b]	650 nm	Kaur & Kapoor, 2002
Onion	56.8 ± 1.1[b]	650 nm	Kaur & Kapoor, 2002
Soy bean (green)	1210 ± 28.0[a]	760 nm	Deng *et al*., 2013

[a]mg gallic acid equivalents/100g fresh weight.
[b]mg catechol/100g fresh weight.
NM – Not mentioned.

Table 3: Phenolic content of by-products of some selected fruits.

By-products of fruits	Total phenolic content	Max. absorbance wavelength	References
Raw mango peel (Raspuri)	109.7 ± 0.82[a]	725 nm	Ajila *et al*., 2007
Ripe mango peel (Raspuri)	100.0 ± 1.9[a]	725 nm	Ajila *et al*., 2007
Raw mango peel (Badami)	90.18 ± 0.57[a]	725 nm	Ajila *et al*., 2007
Ripe mango peel (Badami)	54.67 ± 1.5[a]	725 nm	Ajila *et al*., 2007
Pomegranate peel	249.4 ± 17.2[b]	760 nm	Li *et al*., 2006
Pomegranate pulp	24.4 ± 2.7[b]	760 nm	Li *et al*., 2006
Pineapple	27.87 ± 2.25[a]	NM	Ribeiro da Silva *et al*., 2014
Capuli cherry peel	14.94 ± 3.85[c]	750 nm	Vasco *et al*., 2008
Banana peel powder	29.2 ± 0.8[a]	740 nm	Rebello *et al*., 2014
Surinam cherry	126.96 ± 3.13	NM	Ribeiro da Silva *et al*., 2014
Papaya	7.83 ± 0.25[a]	NM	Ribeiro da Silva *et al*., 2014

[a]mg gallic acid equivalent/g dry weight.
[b]mg tannic acid equivalents/g dry weight.
[c]mg gallic acid equivalent/g fresh weight.
NM – Not mentioned.

Anthocyanins

Anthocyanins belong to the class of flavonoids and they are water-soluble vacuolar pigments that may appear as purple, red or in blue colours depending on their pH. They occur in all plant tissues, including fruits, stems, leaves, flowers and roots (Ignat *et al*., 2011). Anthocyanins donate hydrogen to highly reactive radicals and thus act as an antioxidant and prevent further radical formation. These are coloured compounds and can be used in place of synthetic dyes for colouring foods. Since they are water-soluble, that allows them to incorporate into aqueous food systems easily; thus, they are helpful in additional health benefits to such dyed food products (Kammerer *et al*., 2004).

Alkaloids

Alkaloids are secondary metabolites which are defined as active compounds, mainly composed of nitrogen. They are formed from one of the few amino acids such as lysine, tyrosine and tryptophan. Around 150 families of more than 12000 alkaloids have been identified in plants and it is estimated that around twenty percent of the 'species of flowering plant' contain alkaloids. Alkaloids are generally present in plant in the form of salts of organic acids such as acetic, malic, lactic, citric, oxalic, tartaric, tannic and other acids. Few week alkaloids such as nicotine freely exist in nature. Examples of alkaloids are also present as glycosides of sugar such as glucose, rhamnose and galactose (Zotchev 2013).

Terpenes and Terpenoids

Carotenoids

Carotenoids are fat-soluble pigments present in large amounts in some fruits and vegetables. The four primary sources of carotenoids in human diets are lycopene, β-cryptoxanthin, β-carotene and lutein, but as many as 750 members of this family are identified (Degrou *et al*., 2013). They are beneficial to our health as these compounds are converted into vitamin A or retinol. β-carotene is the essential provitamin A with the highest activity, but when consumed as a separate supplement can have harmful effects. Lycopene is responsible for the red colour in vegetables and fruits including tomatoes, watermelon, red grapes and other red coloured fruits. Lycopene pigments do not convert into vitamin A but possess essential cancer-fighting properties and other health benefits (Sass-Kiss *et al*., 2005). Few examples of vegetables and fruits that contain flavonoids, anthocyanins and carotenoids are shown in (Table 4).

Antioxidant Activities

Free radicals are continuously produced by aerobic organisms which are derived from molecular oxygen called reactive oxygen species (ROS). These radicals are very unstable, containing one or more unpaired electron in the outermost orbit and thus making them quite reactive. These ROS have the potential to cause damage to the biologically important molecules such as proteins, lipids and carbohydrates. When they are in insufficient quantities in our body, they result in oxidative stress (Burnaz *et al*., 2017).

There are a number of methods to check the antioxidant capacities of these bioactive compounds based on single electron transfer (SET) reactions, hydrogen atom transfer (HAT) or methods based on other mechanisms. The reaction methods SET and HAT measure radical scavenging capacity of a sample instead of protective antioxidant capacity. The SET reaction methods measure the reducing ability of a substrate (antioxidant) while HAT reaction methods measure the capacity of a substrate to give hydrogen. HAT reaction methods include total radical trapping antioxidant parameter (TRAP), crocin bleaching assay (CBA), oxygen radical absorbance capacity (ORAC) and others. On the other hand SET reaction methods include total phenolic content by using Folin-Ciocalteu reagent (FC/TPC), (2,2-diphenyl-1-picrylhydrazyl) DPPH radical scavenging methods, Trolox equivalent antioxidant capacity (TEAC/ABTS), copper (II) ion reducing/antioxidant capacity (CUPRAC) and iron (III) ion reducing/antioxidant power (FRAP). The active antioxidant compounds have negative peaks in ABTS (414 nm) or DPPH (517 nm) tests and positive peaks in FRAP (595 nm) or CUPRAC (450 nm) tests after treatment with the reagent post-column. Examples of antioxidant capacity

Table 4: Total flavonoids, anthocyanins and carotenoids contents in some selected fruits and vegetables.

Fruits/vegetables	Total flavonoids	Total anthocyanins	Total carotenoids	References
Custard apple	418.24 ± 3.73[a]	1.55 ± 0.01[b]	2.25 ± 0.40[c]	Moo-Huchin *et al*., 2014
Red cashew	344.62 ± 4.30[a]	1.84 ± 0.03[b]	11.62 ± 0.67[c]	Moo-Huchin *et al*., 2014
Pomegranate pulp	17.2 ± 3.3[d]	5.3 ± 0.7[e]	NP	Li *et al*., 2006
Pomegranate peel	59.1 ± 4.8[d]	10.9 ± 0.5[e]	NP	Li *et al*., 2006
Litchi	9.4 ± 0.6[a]	10.0 ± 0.9[f]	NP	Luximon-Ramma *et al*., 2003
Black sapote	376.04 ± 72.29[a]	14.19 ± 0.01[b]	7.99 ± 0.38[c]	Moo-Huchin *et al*., 2014
Green sugar apple	200.92 ± 3.83[a]	0.47 ± 0.02[b]	1.44 ± 0.11[c]	Moo-Huchin *et al*., 2014
Red nance	131.98 ± 7.41[a]	0.91 ± 0.01[b]	14.22 ± 0.29[c]	Moo-Huchin *et al*., 2014
Guava pink	11.0 ± 2.1[a]	10.9 ± 1.4[f]	NP	Luximon-Ramma *et al*., 2003
Guava white	20.9 ± 1.0[a]	26.3 ± 3.1[f]	NP	Luximon-Ramma *et al*., 2003
Mango	28.1 ± 2.8[a]	16.8 ± 1.1[f]	NP	Luximon-Ramma *et al*., 2003
Papaya	37.6 ± 1.5[a]	20.8 ± 2.1[f]	NP	Luximon-Ramma *et al*., 2003

[a]mg of quercetin/100g fresh weight.
[b]mg total anthocyanins/100g fresh weight.
[c]mg of β-carotene/100g fresh weight.
[d]mg rutin equivalents/g dry weight.
[e]mg catechin equivalents/g dry weight.
[f]mg cyaniding chloride/100g fresh weight.
NP – Not performed.

Table 5: Antioxidant activity of some selected fruits and vegetables (Sreeramulu & Raghunath, 2010).

Fruits/vegetables	Antioxidant activity	
	DPPH[a]	FRAP[b]
Beet root (red)	125.10 ± 13.41	6308.09 ± 916.86
Carrot	11.06 ± 3.03	256.31 ± 65.72
Tapioca	51.07 ± 7.10	3002.4 ± 72.17
Beans	83.0 ± 11.06	1037.77 ± 195.8
Cabbage (red)	405.0 ± 68.0	10510.62 ± 1426.13
Cabbage (green)	78.0 ± 21.0	1245.99 ± 224.50
Cucumber	63.0 ± 13.0	2089.17 ± 485.85
Mango raw (green)	276.0 ± 45.0	4640.37 ± 396.95
Cauliflower	66.0 ± 0.0	1346.63 ± 308.86
Capsicum	96.0 ± 17.0	685.55 ± 117.38
Sweet potato	25.03 ± 4.07	422.56 ± 315.34
Potato	16.04 ± 8.20	704.73 ± 102.28

[a]mg trolox equivalents/100g fresh weight.
[b]mg $FeSO_4$ equivalents/100g fresh weight.

of bioactive compounds of some selected fruits and vegetables are shown in (Table 5).

EXTRACTION OF BIOACTIVE COMPOUNDS

Extraction is a separation process in which bioactive compounds are separated from the sample matrix using a variety of solvents. Conventional, as well as non-conventional methods, have been used to quantify the different bioactive compounds present in plant products. The limitations of conventional methods of extraction are replaced by using new non-conventional methods of extraction. These methods are being discussed below.

CONVENTIONAL METHODS OF EXTRACTION

In these methods, the extracting power of various solvents is used along with the application of mixing and/or heating. Some of the conventional methods that are widely used in the extraction of bioactive compounds such as hydrodistillation and soxhlet extraction method are discussed below.

Hydrodistillation Method

It is a conventional method that is generally used for the extraction of bioactive compounds and essential oils from biological materials. In this method, organic solvents are not utilized and this technique is performed prior to dehydration of materials. Hydrodistillation is of three types: (a) with water distillation, (b) with water and steam distillation and (c) with direct steam distillation (Silva *et al*., 2005).

Extraction of bioactive compounds by hydrodistillation involves several steps, First, the plant material is packed into a still compartment and sufficient amount of water is

added to it; second, the whole mass is brought to boil. Alternatively, steam is directly injected into the plant material. Steam and hot water are the main factors that are used to free the bioactive compounds present in the plant materials (Vankar, 2004). The main drawback of this process is that some volatile components get lost because of the high temperature.

Soxhlet Extraction Method

Soxhlet extraction method is widely used for the extraction of bioactive compounds. Mostly, it is used as a reference model for comparing the results obtained from other new extraction techniques. In this method, the sample is first grounded and placed in a thimble holder. The condensated fresh solvent is gradually filled from a distillation flask and when the liquid reaches overflow level, the solution is aspirated by a siphon from the thimble holder. The solution comes back into the distillation flask through the siphon and thus the extracted solute comes into the bulk liquid. Then the fresh solvent repeats this cycle till the extraction is completed. The main disadvantage of this process is that it is time consuming process and a large amount of solvent is wasted with the degradation of some volatile compounds due to high temperature (Luquede Castro & Garcia-Ayuso, 1998). Some of the examples of bioactive compounds extracted from the various solvent using soxhlet extraction methods are shown in (Table 6).

NON-CONVENTIONAL METHODS OF EXTRACTION

There are many limitations associated with the conventional extraction methods such as more time-consumption, costly operations, requirement of a high purity solvent, degradation of some bioactive compounds because of higher operating temperature and longer duration of extraction, solvent selectivity and evaporation of a large amount of solvent. In order to avoid such limitations, new extraction techniques have been introduced which are known as the non-conventional methods of extraction. These are explained in the following subsection.

Ultrasound-Assisted Extraction (UAE)

Ultrasound is a sound wave that has a frequency higher than the upper limit of human hearing. In its physical properties, it is not different from the normally audible sound except that humans cannot hear it. Sound waves propagate into media creating alternating compression and rarefaction depending on their frequencies. During rarefaction, small vacuum bubbles or voids are created in the liquid and subsequent growth of bubbles takes place. These bubbles tend to collapse violently at the point when they no longer absorb energy during compression (Fig 3). During this process, a large amount of energy is produced. The pressure and temperature at that moment have been estimated to be up to 2000 atmosphere and 5000K (Chemat *et al*., 2011).

UAE is an emerging technology which can accelerate heat and mass transfer. It is successfully used in the extraction of bioactive compounds since it alters its physical and chemical properties and cavitational effects that facilitate the release of extractable compounds. It also accelerates mass transfer by disrupting plant cell walls. Sonocapillarity and Sonoporation, are able to improve the penetration of liquid through the channels produced by the bubble implosion and the alteration of the permeability of the cell membranes, respectively (Fig 4). The main advantage of the UAE is that it reduces extraction time, energy consumed and solvent used. The energy of ultrasound also increases mixing efficiency, energy transfer and reduces thermal gradients, the temperature of extractions and the size of equipment (Chemat *et al*., 2008).

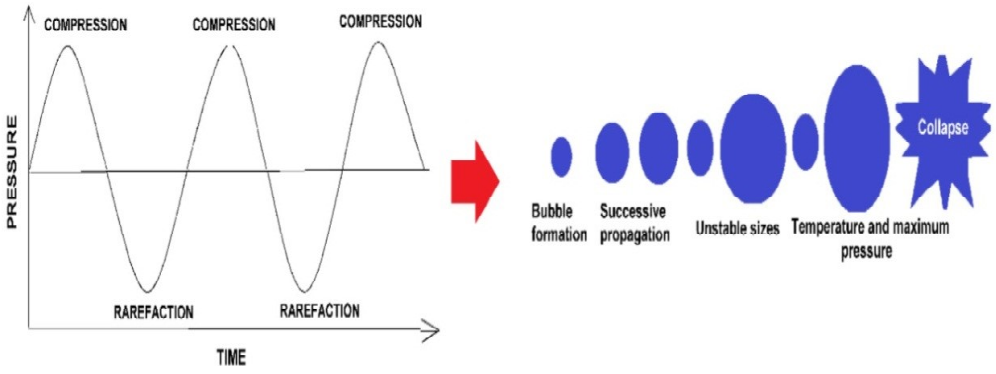


Fig 3: The principle of acoustic cavitation (Soria and Villamiel, 2010).

Table 6: Examples of some extracted bioactive compounds by various solvents (Azmir *et al*., 2013).

Methanol	Ethanol	Water	Ether	Chloroform	Acetone	Dichloro--methane
Polyphenols Anthocyanin Flavones Tannins Terpenoids Saponins	Polyphenols Flavonol Tannins Terpenoids Alkaloids	Anthocyanins Terpenoids Tannins Saponins	Terpenoids Alkaloids	Flavonoids Terpenoids	Flavonoids	Terpenoids

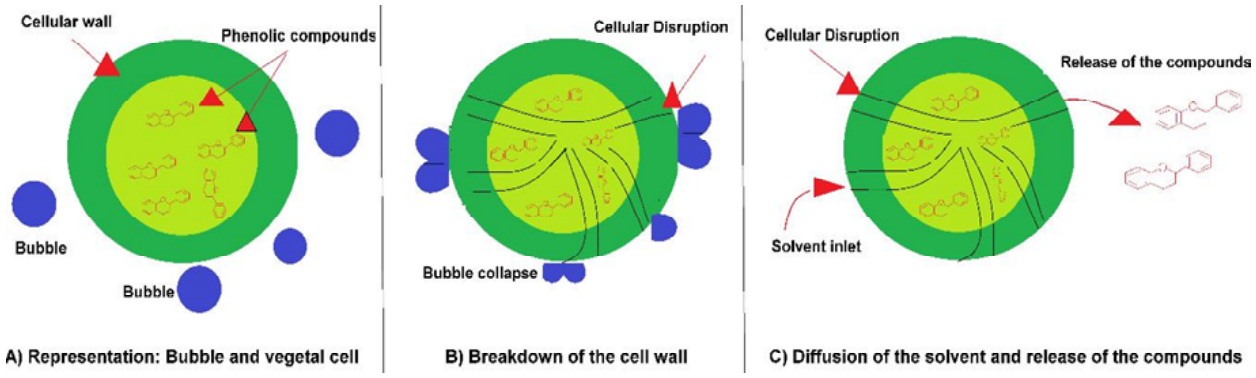


Fig 4: Graphical representation of cavitation-bubble collapsing and releasing plant material in three steps. (a) Bubble and vegetal cell representation, (b) Breakdown of the cell wall and bubble collapse, finally (c) Diffusion of the solvent through the cellular disruption and release of the compounds.

Guo *et al*. (2017) showed that ultrasound-assisted extraction of polysaccharides was 9.428% higher than hot water extraction methods for 12 hours for the same solid-liquid ratio and extraction temperature. Also in in-vitro antioxidant activity tests, UAE showed higher radical scavenging activity for DPPH, superoxide and hydroxyl than polysaccharides extraction by hot water. Similarly, Vázquez *et al*. (2014) reported that the UAE provided an extraction yield of 168 mg/g dry weight (DW) in the recovery of anthraquinones from stems of Rubiaceae, exceeding the extraction yield obtained by the Soxhlet method (34 mg/g DW). In addition, there was a reduction on the extraction time of 16 to 2 h; a reduction in the amount of solvent used (from 36 mL/g to 20 mL/g) was also observed.

Microwave-Assisted Extraction (MAE)

Microwave is an electromagnetic wave that consists of a magnetic field and electric field which oscillates perpendicular to each other. The frequency range of microwave ranges from 300MHz to 300 GHz. Penetration of microwave into certain materials and interaction with the polar components generate heat which directly acts on the molecules by ionic conduction and dipole rotation. Thus, only specific materials based on their dielectric constant can be heated (Chan *et al*., 2011). During ionic conduction, heat is generated due to the resistance of the flow of ions in the medium. On the other hand, during dipole rotation, ions continue changing their direction along with field charge, which results in collisions between molecules and thus heat is generated.

There are three steps involved in the microwave-assisted extraction process. First, the solute is separated from the active site of sample matrix under increased pressure and temperature; second, the solvent is diffused across sample matrix; and third, solutes get released from sample matrix to solvent (Alupului, 2012).

MAE is a faster operational technique through which the efficiency of extraction of high-value bioactive compounds is enhanced. The various advantages include faster heating of the material, better extraction yield, better quality of extracts, reduced time required for extraction and reduced solvent quantity as compared to conventional extraction methods and also restricts the degradation of thermolabile compounds. Green tea leaves were used for the extraction of polyphenols and caffeine. It was reported that MAE has a higher extraction yield at 4 min than by other methods of extraction for 20 h at room temperature (Pan *et al*., 2003).

Supercritical Fluid Extraction (SFE)

SFE is a sustainable green technology and finds a wider range of applications in the extraction of bioactive compounds. It is based on the solvating properties of the supercritical fluid. It can be obtained by applying temperature and pressure above the critical point of an element, compound or mixture. Extraction depends on the intrinsic properties of supercritical fluid like pressure, temperature and some extrinsic features such as properties of the sample matrix, interaction with targeted compounds and other environmental factors (Sharif *et al*., 2014).

The ideal solvent for SFE is considered to be carbon dioxide, which has its critical temperature of 31.2°C (close to room temperature) and critical pressure of 72.9 atm.This low pressure offers the possibility to operate at moderate pressure (Temelli and Guclu-Ustundag, 2005). The only limitation of CO_2 is that it has a low polarity that makes it unsuitable for most of the drug and pharmaceuticals samples, but it is ideal for fat, lipid and non-polar substances such as hydrocarbons. Use of chemical modifier successfully overcomes the limitation of the low polarity of CO_2 and can enhance the extraction efficiency and can also reduce the extraction time (Lang and Wai, 2001). For example, the addition of 0.5 ml of dichloromethane (CH_2Cl_2) to 500 mg sample in 2.5 ml SFE cell reduced the extraction time from 90 min to 30 min, while maintaining consistency of extraction efficiency for 4 hours of hydrodistillation (Hawthorne *et al*., 1994).

It offers several operational benefits over conventional methods. Since it uses supercritical solvents having different physicochemical properties like viscosity, density, diffusivity and dielectric constant, these supercritical fluids have lower viscosity with relatively higher diffusivity, having enhanced transport properties than liquids and diffuse easily through solid materials, giving faster extraction rates (da Silva *et al*., 2016). The critical properties of some solvents used in SFE are shown in (Table 7).

Table 7: Critical properties of some solvents used in SFE (da Silva *et al*., 2016).

Solvent	Critical Property		
	Temperature (°C)	Pressure (atm)	Density ρ_{SFC}(g/mL)
Carbon Dioxide	31.2	72.9	0.470
Methanol	-34.4	79.9	0.272
Ethane	32.4	48.2	0.200
Nitrous Oxide	36.7	71.7	0.460
Ethene	10.1	50.5	0.200
n-Butene	-139.9	36.0	0.221
Water	101.1	217.6	0.322
n-Pentane	-76.5	33.3	0.237
Sulfur hexafluoride	45.8	37.7	0.730

Pressurized Liquid Extraction (PLE):

Pressurized liquid extraction (PLE) is the application of high pressure and temperature to extract bioactive compounds beyond their normal boiling point. This method is known by different names: accelerated solvent extraction (ASE), high-pressure solvent extraction (HPSE), pressurized fluid extraction (PFE) and enhanced solvent extraction (ESE) (Nieto *et al*., 2010). It is an alternate method of extraction as it allows faster extraction and also reduces the consumption of the solvent. It is a green technology and one can also adjust its process parameters for a particular group of compounds (Machado *et al*., 2015). The solvents used in PLE are water and/or ethanol are even more promising as they are generally recognized as safe (GRAS) solvents (Monrad *et al*., 2010). This process has been used successfully for the extraction of thermally sensitive bioactive compounds present in biological material.

The main advantage of this process is that the medium is closed and inert and therefore, the extraction is performed under high pressure and temperature. Since at higher pressures, the solvents remain in a liquid state even at the temperature above their boiling points, thus it enhances the solubility of the target compounds and desorption kinetics from the matrix (Mustafa and Turner, 2011).

Pulsed Electric Field Extraction (PEF)

The treatment of biological materials using pulsed electric field (PEF) is one of the novels and most promising physical methods for improving the efficiency of various processes employed in the food processing industry such as extraction, drying, dehydration and juice expression (Barba *et al*., 2015). In this method, the material is placed between two electrodes and electric field pulses of short duration (ranging from microseconds to milliseconds) and moderate-intensity (0.5 – 10 kV/cm) are applied. This causes electroporation of cell membranes (Bouzrara and Vorobiev, 2003) where some metabolites are contained, which increases the permeability of the cell membranes for the transportation of ions and macromolecules. Therefore, the resistance for the diffusion from cell membranes is reduced and thus, the extraction of bioactive compounds becomes easier and results in increased extraction yield (Redondo *et al*., 2018).

The extraction yield of phenolic compounds, anthocyanins and flavonoids from agricultural by-products obtained from food processing of grapes, papaya peels, seeds and mango peels are enhanced when PEF assisted extraction with solvents is carried out (Quagliariello *et al*., 2016). They showed that not only the yield of antioxidant compounds present in brown rice like polyphenols, γ-oryzanol and phenolic acids and of saturated and unsaturated fatty acids were enhanced but it also increased the cytotoxicity effects on cancer when PEF assisted extraction was performed.

In the past several years, various studies have been conducted in order to extract the bioactive compounds from the by-products of fruits and vegetables using different and novel non-conventional extraction methods. Major findings of some of the studies based on the extraction of compounds from the waste of fruits and vegetables using different extraction method are given in (Table 8).

IMPORTANCE AND UTILIZATION OF COMPOUNDS EXTRACTED FROM THE BY-PRODUCTS OF FRUITS AND VEGETABLES

Mango seed kernel is a good source of polyphenols, tocopherols and phytosterols. These compounds have many activities such as antimicrobial, antidiabetic and anticancer activity. They have been widely used in the viper bite treatment as well as in herbal medicines (Abdel-Aty *et al*., 2018). Due to the presence of a significant amount of fat, protein and some natural antioxidants, it can be used as a valuable ingredient in functional foods and is also used in cosmetics (Kittiphoom 2012). Other than seed, mango peel is also a good source of ploy phenols such as flavonols, galotannins and xanthones and it can also be used as a livestock feed and as an adsorbent (Kanjilal *et al*., 2014). Several studies have shown that the compounds present in banana peel have shown great potential to cure ailments like stomach ulcer, depression and anaemia (Pereira and Maraschin 2015). The peel of banana includes carbohydrates, protein, lipids and some essential minerals like potassium due to which it can also be used as livestock feed and as an organic fertilizer.

Other than the by-products of mango and banana, the by-products of pomegranate and pineapple are also good sources of dietary fiber and polyphenols (Faizan and Kumar 2018). Due to the presence of dietary fiber the pomegranate peel can be used in chicken and meat products to increase their shelf life by 2-3 weeks during cold storage (Kanatt *et al*., 2010). Similarly, the waste of pineapple has also been used as a meat tenderizer and bread batter improver (Kodagoda and Marapana, 2017).

Table 8: Extraction of compound from the by-products of fruits and vegetables using different extraction methods.

Extraction Method	Fruit and Vegetable By-product	Compounds	References
MAE	Grape seeds (*Vitisvinifera*)	Phenolic antioxidants	(Krishnaswamy *et al*. 2013)
MAE	Grape Skins	Anthocyanins	(Liazid *et al*. 2010)
MAE	Apple Pomace	Polyphenols (chlorogenic acid, phlorizin and quercetin)	(Bai *et al*. 2010)
MAE	Apple Pomace	Pectin	(Wang *et al*. 2007)
MAE	Orange Peels	Pectin	(Prakash Maran *et al*. 2013)
UAE	Apple Pomace	Flavan-3-ols, Procyanidins, Flavonols	(Virot *et al*. 2010)
UAE	Grape by-products	Anthocyanin	(Corrales *et al*. 2008)
UAE	Orange peel(*Citrus sinensis L*.)	Flavanone glycosides	(Khan *et al*. 2010)
SFE	Grape seed (*Vitisvinifera L*.)	Linoleic, stearic and oleic acids	(Prado *et al*. 2012)
SFE	Grape bagasse(*Vitisvinifera*)	syringic, vanillic, gallic, ρ-hydroxybenzoic and quercetin	(M. Farías-Campomanes *et al*. 2013)
SFE	Orange pomace	Flavonoids	(Benelli *et al*. 2010)
SFE	Apple peels	Glycosides-derivatives, Quercetin glycosides	(Díaz-Reinoso *et al*. 2006)
SFE	Apple and peach pomace	Phenolic compounds	(Hasbay *et al*. 2007)
PLE	Pressed palm ûbre	Carotenoids	(Cardenas-Toro 2015)
PLE	Sea buckthorn (*Hippophaër hamnoides* L.)seed	Antimicrobial andantioxidant components	(Michel *et al*. 2012)

MAE: Microwave Assisted Extraction, UAE: Ultrasound Assisted Extraction, SFE: Supercritical Fluid Extraction, PLE: Pressurised Liquid Extraction.

APPLICATION OF BIOACTIVE COMPOUNDS IN ACTIVE PACKAGING AND EDIBLE COATING

Bioactive compounds are extra nutritional constituents that typically occur in small quantities in foods. Nowadays, the potential uses of bioactive compounds in active packaging systems and edible coatings are getting more attention from packaging industries due to the increasing consumer demands for minimally processed and preservative-free food products (Valdés *et al*., 2015). The addition of antioxidants in edible films and coatings can add value to the packaged food products by increasing their shelf life and by making them eco-sustainable and cost-competitive products (Leites *et al*., 2017). The bioactive substances such as phenolic compounds, phytoestrogens, carotenoids, organo sulphur compounds, plant sterols, monoterpenes, soluble dietary fibers, plant extracts, essential oils, prebiotics, bacteriocins and enzymes are mainly suitable for incorporation into the package wall. (Kris-Etherton *et al*., 2002; Lopez-Rubio *et al*., 2006; Juneja *et al*., 2012).

The bioactive substances can be incorporated into packaging materials by any of the following methods (Cagri-Mehmetoglu, 2015).

- Direct application of bioactive edible coatings to the food
- Incorporation of bioactive substances directly into the package wall.
- Incorporation of bioactive substances into a sachet included in the package.
- Coating of the packaging materials with a matrix that serves as a carrier of the bioactive substances.

Since synthetic antioxidants are associated with possible toxic effects mentioned earlier, hence, their application has been avoided in the foods. A wide range of natural antioxidants such as ascorbic acid, tocopherol, plant extracts and essential oils have been incorporated into edible coatings in order to increase their bioactivity. The coating, with antioxidants, increases the shelf life of the food. Application of antioxidants in edible coatings also reduces the browning in various foods (Eca *et al*., 2014). The added antioxidants also help to preserve food, inhibit browning of food and reduce the undesirable effects of nutrients oxidation (Pastor *et al*., 2011; Bonilla *et al*., 2013).

Antimicrobial packaging films are also used for the inhibition of pathogenic microorganisms. The major potential food application of antimicrobials include some sensitive foods like fresh products such as fruits and vegetables, bakery products, meat and fish products, dairy products (Radusin *et al*., 2013).

Application of various natural additives, particularly those having antimicrobial properties in the development of active packaging systems and edible films have been studied in various commodities such as fresh-cut broccoli, grapes, fish, against listeria and fish spoilage bacteria. The shelf life of perishable fresh foods could be improved by 20 percent by the use of new active compounds. Active packaging also minimizes losses from spoilage and could increase the useful life of the product by one or two additional days (AIMPLAS, 2012). According to Silva-Weiss (2013), incorporation of natural extracts together with vitamin C and E, represent a promising approach for the development of edible films with improved bioactive, physicochemical properties and applications. Since the antioxidant activity of the coating with added bioactive compounds can decrease with time, controlled delivery studies of bioactive compounds in coatings should be performed.

CONCLUSION

Fruits, vegetables and their by-products are an important source of bioactive compounds mainly as natural antioxidants that can replace the use of synthetic antioxidants. The study revealed that the by-products of fruits and vegetables like peel which is discarded in the processing industry can be efficiently used for the extraction of valuable bioactive compounds and for extracting these compounds, a number of techniques can be used. However the current trend is to extract these compounds using non-conventional methods as these techniques maintain the quality of the extract and also reduce the extraction time.

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ISSN: 0976-0539 (Online), 0253-1496 (Print), Naas Rating: 4.37