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POLYPHENOLS: METHODS OF EXTRACTION

KARISHMA RAJBHAR*, HIMANSHU DAWDA and USHA MUKUNDAN

Plant Biotechnology Laboratory, Ramniranjan Jhunjhunwala College, Ghatkopar (West),
MUMBAI – 400086 (M.S.) INDIA

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ABSTRACT

Natural phenols and polyphenols are compounds found in plants. Polyphenols include phenolic acids and flavanoids. Polyphenols provide health benefits such as antioxidant, antiviral, anti-microbial, anti-carcinogenic, anti-inflammatory, antitumor, analgesic, antipyretic activities. In this review, a assortment of different types of extraction methods such as water bath, microwave-assisted extraction (MAE), solvents and ultrasound assisted extraction (UAE) for polyphenols is covered followed by various methods of estimation.

Key words: Polyphenols, Health benefits, Method of extraction and estimation.

INTRODUCTION

Polyphenols are a large class of chemicals found in plants. Polyphenols are abundant consumable micronutrients in our diet, and proof for their role in the prevention of degenerative diseases is fast emerging. Gallic acid and isoflavones are the well-absorbed polyphenols, followed by catechins, flavanones, and quercetin glucosides, but with different kinetics. Proanthocyanidins, galloylated tea catechins and anthocyanins are least well-absorbed polyphenols[1].

Polyphenols are polyhydroxylated phytochemicals and they represent a wide variety of compounds, which have similar structures. They can be subdivided in three main subclasses, the flavonoids, phenolic acids, and stilbenoids and again divided into several classes, i.e, hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes, and lignans[2].

Polyphenols with various structures are found in plant kingdom with a basic structure composed of a benzene ring linked to one or more hydroxyl ions, free or involved in another chemical function[3]. Polyphenols are plants secondary metabolites, produced mainly from two primary synthesis pathways-shikimate and acetate/polyketide. Both acetic acid and shikimic acid are derived from glucose metabolism[4].

Available online at www.sadgurupublications.com
*Author for correspondence; E-mail: karishmarajbhar@rjcollege.edu.in

Polyphenols have a common phenolic feature but show structural diversity, due to which their physicochemical properties differ. The frequent occurrence of polyphenols in plants and their chemical complexity makes extraction, separation, identification and analysis of polyphenols even more impressive than the recent advances in new instrumentation. Complex glycosylation and polymerization patterns resist the development of a common protocol for all polyphenols[5].

Structurally, polyphenols fall into many different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols. This structural diversity results in large variability of the physico-chemical properties influencing the extraction of polyphenols[6]. Distribution of phenolics in plants at the tissue, cellular and sub cellular levels is not always in similar. Soluble phenolics are present within the plant cell vacuoles, insoluble phenolics are found in cell walls but the outer layers of plants contain higher levels of phenolics than those located in their inner parts[7]. Polyphenolic content of the foods is greatly affected by environmental edaphic factors like soil type, sun exposure and rainfall etc.[5]

Another factor that directly affects the polyphenol content of the foods is storage. Stored foods show change in polyphenolic content due to easy oxidation of these polyphenols. Oxidation reactions result in the formation of more or less polymerized substances resulting in change in food quality, colour and organoleptic characteristics. Such changes may be beneficial, as is the case of black tea[7]. To avoid degradation of native polyphenols, samples are often dried, frozen or lyophilized before extraction because high moisture or water content aids the activity of enzymes[5].

Table 1: Health benefit of some polyphenols

Polyphenol	Benefit	References
Caffeic acid	Antitumor, antiviral, antioxidant, anti-inflammatory, selective inhibitor of 5- and 12 lipoxygenase(LO), therapeutic effect on hepatocarcinoma cells, anti-HIV (inhibitory activity against HIV-1 integrase) and antiviral supplement diet	Chung et al.[8] Bailly and Cotelle[9] Nouri et al.[10] Chiang et al.[11]
Chlorogenic acid	Antioxidant, analgesic, antipyretic, chemo preventive activity, inhibits Bcr-Abl tyrosine kinase and triggers MAP kinases p38– dependent apoptosis, inhibits tumour promoting activity of phorbol esters and activator protein-1, NF-kappaB, and MAPKs and induction of phase 2 detoxifying enzyme and inhibits DNA methylation	Lee et al.[12] Wang et al.[13]
Gallotannin	Poly (ADP-ribose) glycohydrolase (PARG), endothelial nitric oxide synthase , weak inhibitor of inducible, neuronal nitric oxide synthase and induces cyclo-oxygenase-2 (COX-2) expression, free radical scavenger, Inhibition of poly (ADP-ribose) glycohydrolase, regulates expression of proinflammatory genes, anti hydrogen peroxide-induced oxidative stress and DNA damages in IMR-90 cells	Rapizzi et al.[14] Hu[15]

Cont…

Polyphenol	Benefit	References
Resveratrol	Antioxidant, anti–inflammatory, anti proliferative activity, inhibits diverse cellular processes associated with tumour initiation, promotion and progression, inhibition of UVB-induced skin edema and substantially reduced UVB-induced lipid peroxidation, cyclo-oxygenase and ornithine decarboxylase activities, protein expression of the latter enzyme and blocks UVB-mediated activation of NF-κB in a dose- and time-dependent manner	Svobodova et al.[16]
Phenethyl ester derivative	Anticancer, provoke leukocyte apoptosis, modulate nuclear factor-kappa B and suppresses acute inflammation, Inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF-kappa B transcription factors and induction of heme oxygenase-1 expression	King and Robinson[17] Wang et al.[13]

Mode of polyphenols extraction

Various extraction methods have been reported for polyphenol extraction; these include microwave extraction, ultrasonic extraction, Soxhlet extraction, heat reflux extraction and ultrahigh pressure extraction[18].

The hydrophilic polyphenols including aglycones, glycosides, and oligomers, are extracted using water, polar organic solvents such as methanol, ethanol, acetonitrile and acetone, or their mixture of water. The liquid extracts are sometimes partitioned with solvents such as ethyl acetate, depending on the solubility of the target polyphenols. Polyphenols are more stable in low pH as the acidic condition helps polyphenols to stay neutral, thus readily extracted into organic solvents[5,19].

Conventional extraction

Water bath is an indirect heating method used for decades. The plant material is slowly heated so as to enable maximum extraction. As the temperature rises, the plant tissue starts releasing its inner content into the medium. Longer time of extraction gives better results.

Conventional extraction and concentration of polyphenols using a water bath is typically conducted at temperatures ranging from 20 to 50°C, temperatures above 70°C cause rapid polyphenol degradation. An increase in temperature increases the efficiency of the extraction since heat renders the cell walls permeable, increasing solubility and diffusion coefficients of the compounds to be extracted and decreases the viscosity of the solvent, thus facilitating its passage through the solid substrate mass. However, the use of temperatures higher than 50°C decreases the total polyphenols and proanthocyanidins yield, which is probably due to their degradation[20].

Microwave-Assisted Extraction (MAE)

The use of MAE in natural products extraction started in the late 1980s, and through technological developments, it has now become one of the more popular and cost-effective extraction methods available with several advanced MAE instrumentations and methodologies now becoming available, e.g. pressurized microwave-assisted extraction (PMAE) and solvent-free microwave-assisted extraction (SFMAE). This

method accomplishes multiple quantitative sample extractions within minutes, with enhanced reproducibility and reduced solvent consumption. Elevated temperature and temperature control can also be achieved using this process[20].

Most plants contain water in higher amount so this is effective means of extraction. Plants with low water content take longer to heat in microwave. The temperature of the plant material heated in a microwave cannot exceed the boiling point of water (100°C). Water molecules are polar but unevenly charged. Microwave radiation consists of oscillating and magnetic fields. The microwaves used in a microwave oven need to be in the region of natural frequency of water molecules. When a microwave electric field is passed over a water molecule the positive and negative areas of the molecules interact with the positive and negative forces of the moving field. This forces the water molecules to oscillate, resulting in friction between the molecules followed by heating[20, 21].

Material/solvent ratio does not have much effect on the yield of the extraction[22]. MAE is a better extraction method for thermally sensitive and flavanols, and leads to the highest concentration of EGCG and antioxidant activity in the extract. MAE has a shorter extraction time, significant savings of energy and a reduced environmental burden by less release of CO_2 in the atmosphere[23].

Solvents

The extraction of phenolic compounds in plant material is influenced by their chemical nature, the sample size, extraction time and storage conditions as well as the presence of interfering substances. Phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. Solvent extraction is frequently used to extract phenolic compounds from their plant sources due to their ease of use, efficiency, and wide applicability. Chemical extraction depends on the type of solvent, solvent polarity, extraction time and temperature, as well as on the chemical composition and physical characteristics of the samples[24].

The extraction of polyphenol is challenged by their chemical structure and their interaction with other food components. Solid-liquid extraction is mainly influenced by solvent composition, time of extraction, temperature, pH, solid-liquid ratio and particle size. A wide range of solvents like water, acetone, methanol, ethanol, N,N-dimethylformamide (DMF) or their mixtures with water, have been studied for their extraction efficiency due to their differences in polarities[25,26]. The use of an alcoholic solution provides satisfactory extraction. Methanol, acetone and water alone are inefficient solvents for extraction of total phenols from powdered plant as polyphenols are associated with other biomolecules like proteins, polysaccharides, terpenes, chlorophyll, lipids, and inorganic compounds. However, methanolic extracts have proven to be better for catechin, epicatechin and epigallocatechin extraction6. Aqueous mixtures of acetone are good solvents for polar polyphenols but undesirable residue is found in the extracts[25]. The low solubility of the polyphenols in absolute organic solvents is due to strengthening of the hydrogen bonds between polyphenols and protein. Increase in solubility with the addition of water to organic solvents is due to the weakening of the hydrogen bonds in aqueous solutions[27].

Ultrasound assisted extraction (UAE)

Ultrasound assisted extraction depends on destructive effects of ultrasonic waves. UAE are helpful to intensify mass transfer, cell disruption, more enhanced penetration and capillary effects. Very high temperature in UAE increases the solubility, diffusivity and pressure, which help the waves to penetrate the tissue and transport contents in a variety of solvents, both organic and inorganic. Ultrasonic probe and bath are two most common systems used for extraction. UAE has disadvantage of decreased experimental reproducibility[28].

Extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out the cell's content once the walls are broken. Ultrasound waves interact with the plant material to alter its physical and chemical properties. The cavitational effects of these waves facilitate the release of extractable compounds and enhance mass transport by disrupting the plant cell walls. Better swelling improves the rate of mass transfer and results in an increased extraction efficiency and/or reduced extraction time. Increase in the extraction of tanning material under ultrasonic vibration at a frequency of 8000 KHz for 15 min compared with stirring at 1400 rpm for 8 h. influence of ultrasound frequency, time and intensity on the extraction of tannins using different solvents is seen[29].

Estimation of polyphenols

For the rough quantification of polyphenols colourimetric methods are widely used mainly due to their simplicity and high sensitivity. These include the Folin- Ciocalteu and Prussian-Blue methods for total polyphenols[4]. The estimation of polyphenols can be done by various techniques viz. nuclear magnetic resonance, near-infrared reflectance spectroscopy, high performance thin layer chromatography (HPTLC), liquid chromatography coupled with mass spectroscopy (LC-MS), high performance capillary electrophoresis (HPCE) and high performance liquid chromatography (HPLC).

Besides organic solvent extraction below techniques can be applied for qualitative and quantitative analysis as well as for isolation and purification procedures for more specific result. Chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and more recently capillary electrophoresis (CE), column chromatography (CC) over Sephadex LH-20 are frequently used for final purification, due to residue-free solutions for structural analysis by degradation.[5,19]

CONCLUSION

Based on the comparison of the various extraction methods used for polyphenols, the microwave-assisted extraction method has shown best results. MAE for 6 mins is higher than that of ultrasound-assisted extraction in 60 mins, conventional heating reflux extraction in 60 mins and extraction at room temperature in 24 hrs. The advantage of microwave extraction is that high efficiency extraction can be done using minimum amount of solvent, while simultaneously reducing extraction time. In addition, easy maintenance of extraction vessels, enhancement of analytical capabilities (such as improvement of recovery and repeatability) and the possibility of simultaneous extraction of multiple samples is also better with MAE, than with conventional extraction techniques.[22,30]

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