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An Efficient Method for the Extraction of Polyphenolics from Some Traditional Varieties of Rice of North-East India

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Abstract

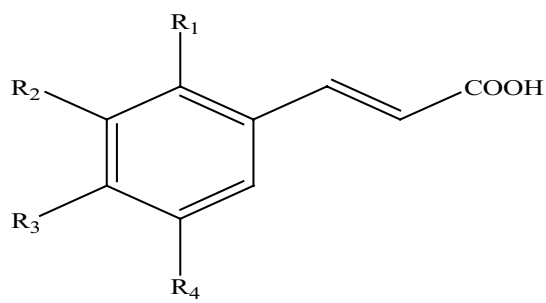
Polyphenolics are secondary metabolites having numerous health benefits. One of the potential sources of phenolics is rice. Despite being the staple food of India, phenolic extraction from rice has not gained much significance due to its concentration in the aleurone layer which is lost during processing. Two procedures were studied for the effective extraction of total phenolics from rice: ultrasound-assisted aqueous methanol extraction and aqueous methanol extraction. The results indicated that ultrasound aided extraction improved the levels of phenolic content by 1.3–2.8 times.

Introduction

Polyphenolics are widely distributed in plants as aromatic secondary metabolites. They contribute to the sensory quality (appearance, taste, and odor) as well as the antioxidant activity of fruits, vegetables, beverages and grains. Polyphenolic substances have been reported to induce health-promoting effects such as antimicrobial and anti-inflammatory action, reduce risk of cardiovascular disease, and inhibit the oxidation of human low-density lipoproteins and anticarcinogenic effects. The beneficial effects of phenols have been extensively attributed to their antioxidant properties based on the scavenging of active oxygen species and free radicals [3].Phenolic acids are an important group of polyphenolics and include hydroxybenzoic (C_6-C_1), hydroxyphenylacetic (C_6-C_2) and hydroxycinnamic (C_6-C_3) acids. Hydroxycinnamic acid is the most widely distributed in plants. The important ones include p-coumaric, caffeic, ferulic and sinapic acids.

Cereal consumption is an excellent way to increase phenolic compound intake. However, consumption of these phenolic compounds from cereals is currently neglected. The major reason for this is that these compounds are concentrated in the aleurone layer and are lost with the separation of seed coat during processing. By the same token, most phenolic compounds in rice, which is a major staple cereal all over the world, particularly in Asia, are also lost with the aleurone layer of rice [5]. This layer is very rich in bioactive molecules, mostly polyphenols.

Traditional rice varieties exhibit tremendous nutritional characteristics than the high-yielding varieties (HYV) and hence can be used as potent nutraceuticals.



R_1=R_2=R_4=H, R_3=OH	p-Coumaric acid
R_1=R_4=H, R_2=R_3=OH	Caffeic acid
R_1=R_4=H, R_2=OCH_3, R_3=OH	Ferulic acid
R_1=H, R_2=R_4=OCH_3, R_3=OH	Sinapic acid

Fig. 1.: Chemical structure of hydroxycinnamic acid

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The main step in the recovery and isolation of polyphenolics and evaluation of individual and total polyphenolics from various plant-based sources is their extraction. Solvent extraction has been established as an effective method [7]. Polyphenolics are often most soluble in lesser polar solvents than water. Effective extraction generally involves a proper solvent selection, elevated temperature and mechanical agitation to maximize polyphenol recovery.

The objective of the present study was to develop a proper extraction method for polyphenolics from some traditional rice varieties of North-East India for their maximum recovery.

Materials and Methods

Chemicals

Gallic acid was purchased from Fluka. Folin-Ciocalteau reagent was obtained from Nice Chemicals.

Rice Samples

Four traditional rice cultivars (Jengoni, Sokua, Jahingia and Betguti) of *Oryza sativa* L. were collected from the North-Eastern region of India during the period from November to February of 2010–2011.

Preparation of Rice Samples

Rough rice was dehusked using a Rice Sheller (Indosaw, Haryana, India) and milled in a Rice Polisher (Indosaw, Haryana, India) to obtain the aleurone layer of rice. Moisture was determined by drying at 103^{0}C to constant mass according to AACC Methods of Analysis. Analysis was performed in triplicate and results were expressed on a dry basis.

Extraction of Rice Total Phenolics

Two methods were examined for the extraction of total phenolics from rice samples:

(a) Aqueous Methanol Extraction

Rice sample (10 g) was extracted with 100mL of 80 % aqueous MeOH for 2h in an orbital shaker (200rpm) at room temperature. The extracts were then filtered through a Whatman filter paper. The residue was re-extracted twice. The filtrates were then collected, evaporated to dryness under reduced pressure at 40^{0}C by a rotary-evaporator (Buchi, Switzerland), dried overnight in a desiccator and weighed.

(b) Ultrasound-assisted Aqueous Methanol Extraction

Rice sample (10 g) was mixed with 100mL of 80 % aqueous MeOH in a 250mL Erlenmeyer flask. The flask was immersed into an ultrasonic bath (Emcolite Ultrasonics) and sonicated for 15min at room temperature with occasional shaking. The mixture was filtered through a Whatman filter paper and the residue re-extracted twice. The filtrates were then evaporated to dryness in a rotary-evaporator (Buchi, Switzerland) at 40^{0}C, dried overnight in a desiccator and weighed.

The extraction procedure was done in triplicate for each of the extracts. The extraction yield was expressed as weight percentage on a dry basis. All extracts were stored in amber-colored bottles at –4^{0}C until further analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the rice extracts was determined by Folin-Ciocalteau method [6]. The rice extracts were redissolved in 10mL of methanol and then 0.5mL of appropriate diluted extract was mixed with 2.5mL of 5 % Folin-Ciocalteau reagent. The reaction was carried out for 5min in dark. Then 2mL of sodium carbonate solution (7.5 %) was added. The mixture was kept in dark at ambient temperature for 60min. The absorbance was then measured at 765 nm using a UV-Vis spectrophotometer (Perkin-Elmer). All measurements were conducted in triplicate. Gallic acid was used as the standard and the total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g of rice.

Results and Discussions

Ultrasonic extraction affected the total phenolic content of rice as indicated in Tables 1 and 2.

The total phenolic extract ranged from 12.1–19 % in ultrasound-assisted aqueous methanol extraction to 7.4–12.5 % in aqueous methanol extraction. Similarly, the total phenolic content was shown to be in the range 93.5–327.9mgGAE/100 g rice for ultrasound-assisted aqueous methanol extracts compared to 42.3–235.3mgGAE/100 g rice for aqueous methanol extracts. This may be ascribed to the cavitational phe-

Table 1: Total phenolic extract of rice (% wt, dry basis)

Samples	Ultrasound-assisted methanol extraction	Methanol extraction
Jengoni	15.36±1.02	8.47±0.45
Sokua	19.05±0.73	12.55±0.51
Jahingia	12.33±0.15	8.37±0.37
Betguti	12.16±0.15	7.48±0.50

Table 2: Total phenolic content of rice (mg GAE/100 g rice)

Samples	Ultrasound-assisted methanol extraction	Methanol extraction
Jengoni	327.93±2.1	235.33±2.51
Sokua	155.29±2.56	55.33±2.51
Jahingia	97.46±2.15	67.33±2.49
Betguti	93.51±1.5	42.33±2.4

nomenon, which is the rapid formation and collapse of countless microscopic bubbles in a solvent produced by the alternating low- and high-pressure waves generated by ultrasonic sound. This enhances the mass transfer rate and solvent penetration into cellular materials. In addition, the disruption or damage of biological cell walls by ultrasound results in facilitated release of the intracellular contents. Hence phenolic extraction is enhanced by ultrasound-aided solvent extraction.

Conclusion

Methanolic extraction with ultrasound gave a significant increase in total phenolic yield of traditional rice varieties compared to conventional reflux extraction. Thus these traditional varieties may be used as potent sources of polyphenols for the development of nutraceuticals for health benefit.

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