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Original Article

EFFECT OF PROCESSING ON NUTRACEUTICAL PROPERTIES OF GARDEN CRESS (LEPIDIUM SATIVUM L.) SEEDS

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ABSTRACT

Objective: The effects of popping and germination on nutraceutical and antioxidant activity of garden cress seeds (GCS) were investigated.

Methods: The nutraceutical properties were determined by evaluating total phenolic content (TPC), flavonoid and tannin contents. By the DPPH free radical scavenging activity and the iron reducing power assay, the antioxidant properties were studied.

Results: TPC of popped GCS increased by 18.5%, and it decreased by 5% in germinated GCS extracts compared to native GCS, whereas the flavonoid contents decreased both in popped and germinated seed extracts. The tannin contents increased while popping by 6.81% whereas, it decreased by 20.45% during germination compared to native extracts (0.44mg CEQ/100g). DPPH radical scavenging and the iron reducing power activity increased while popping but decreased during germination compared to native counterpart. Fractionation of native and processed GCS extracts by HPLC showed presence of p-coumaric acid, proto-catechuic acid and gallic acid, with p-coumaric acid showing major portion followed by proto-catechuic acid and gallic acid.

Conclusion: The results are useful for development of garden cress based health food.

Keywords: Garden cress seeds, Processing, Nutraceutical, Antioxidant, Phenolic.

INTRODUCTION

Garden Cress, botanically known as *Lepidium sativum L,* is an annual herb which belongs to Brassicaceae (Cruciferae) family. It is cultivated in India, North America and some parts of Europe. The garden cress seeds (GCS) are bitter in taste and are shown to contain medicinal properties like galactagogue, aborfacient, expectorant, aphrodisiac, antibacterial, gastrointestinal stimulant, gastroprotective, laxative, stomadic and diuretic[1, 2]. Formation of free radicals plays an important role in the origin of life and biological evolution, implying their effect on aging of organisms and cancer promotion. Several evidences have indicated that reactive oxygen species (ROS) are pathophysiological reason for aging and degenerative diseases such as Alzheimer's disease, Parkinson's disease, cardiovascular diseases and cancer [3, 4]. Living cells have a protective system of antioxidant that prevents excessive formation of ROS and causes the inactivation of ROS. Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) may be inappropriate for chronic human consumption. Thus, recently concern has risen in discovering natural antioxidants present in nature to introduce in functional foods due to their very high potential in health promotion and disease prevention, and due to higher safety and consumer acceptability. Phenolic contents in plants are responsible for the antioxidant properties.

Phenolic acids are known to act as antioxidants by donating hydrogen or electrons. Their stable radical intermediates prevent the oxidation of various food ingredients, particularly fatty acids and oils [5]. Several studies confirm that the cereal grains are rich in phenolic acids [6, 7]. Incorporation of food with components rich in phenolic acids bestow antimutagenic, antiglycemic and antioxidative properties, and health food formulations are developed by utilizing this [8]. Food and pharmaceutical industries use polyphenols as a source of conceivably health benefit phytochemicals. Much attention has been paid to study the nutraceutical and antioxidant properties of major grains. However, information on the effect of processing on phenolic compounds and antioxidant properties of garden cress seed is rather scarce. Hence, this study was carried out to investigate the effect of different processing (germination and popping) on nutraceutical (total phenolics, flavonoids, tannins and their fractionation) and antioxidant (DPPH free radical scavenging activity and reducing power assay) activities of garden cress seeds.

MATERIALS AND METHODS

Garden cress seeds used in the present study were procured from the local market. After cleaning, the seeds were subjected to different processing, such as germination and popping and were used for further research. Phenolic acid standards including 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma Chemicals Co., St. Louis, MO, USA. All chemicals used were of analytical grade. For the analysis, triple distilled water was used.

Germination

GC seeds were soaked in 100ml/100g of water for about 10 to 12 h. soaked seeds were germinated using a wet cloth for 14 h.

Popping

The seeds were mixed thoroughly with 0.15ml/g of water and tempered for about 8 h in airtight container. Tempered seeds were popped using high temperature short time process in a preheated pan at 220°C for 15 seconds.

Sample Preparation

The germinated GCS samples were dried in a mechanical drier 50°± 2°C for 4h. Native (unprocessed) and processed garden cress seeds milled in the hammer mill and passed through 500µm aperture sieve. The flour was defatted using hexane in Soxhlet apparatus. The defatted powdered samples were ground in a coffee grinder to pass through 250µm aperture sieve.

Extraction of polyphenols

The defatted powder (1g) was extracted by refluxing in 1% HCl–methanol at 60 °C for 1h (100ml×4), and the extracts were pooled and concentrated under vacuum in a rotary flash evaporator [9].

Determination of Total Phenolic Contents (TPC)

Using Folin and Ciocalteu reagent by a spectrophotometric method TPC of the GCS extracts were determined [10]. This involves mixing

0.1 ml of acidified methanolic extract with 5 ml of distilled water in a 50ml volumetric flask and treating it with 2.5 ml of Folin–Ciocalteu's reagent (1:2 dilution with water) and 7.5 ml of 15% sodium carbonate solution, mixing thoroughly and makeup to 50ml. The blue colour developed was read at 760nm after 30 min. A calibration curve was prepared using a standard solution of gallic acid (R^2=0.999). The result was expressed as mg of gallic acid equivalents (GAE)/100 g of sample (dry basis).

Determination of total flavonoids

The total flavonoid contents of the GCS extracts were determined according to the method of Zhishen *et al* [11] with some modifications. Acidified methanolic extract (0.1 ml) was mixed with 4.9 ml of distilled water. At zero min time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5min, 0.3 ml of (10% w/v) $AlCl_3$ and at 6 min, 2 ml of 1 M NaOH was added, and immediately the volume was made up to 10 ml with distilled water. The absorbance was read at 510 nm after vigorous shaking. A calibration curve was prepared using a standard solution of catechin (R^2 = 0.999). The result was expressed as mg catechin equivalents (CEQ)/100 g of sample (dry basis).

Determination of tannins

The tannin contents of the garden cress seed extracts were determined by the modified vanillin–HCl method [12]. Acidified methanolic extract (0.1 ml) was made up to 1 ml with distilled water and 5 ml of Vanillin–HCl reagent was immediately added. The tubes were allowed to stand at ambient temperature (~30 ^{0}C) for 20 min and the color developed was measured at 500 nm. A calibration curve was prepared using a standard solution of catechin (R^2= 0.998). The result was expressed as mg catechin equivalents (CEQ)/100 g of sample (dry basis)

Radical scavenging activity

The antioxidant activity of native and processed garden cress seed extracts was also measured by the DPPH radical scavenging method [13]. An aliquot (10µl) of the acidified methanolic extract was mixed with distilled water (90µl) and 3.9ml of methanolic 0.1mM DPPH solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured at 515nm. The result was expressed as the percentage of inhibition of the DPPH radical.

Reducing Power Assay

The reducing power assay was determined according to the method of Oyaizu [14]. 100µl of acidified methanolic extract was added in a test tube and the volume adjusted to 1 ml with methanol. 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide were added to the tube and vortexed. The mixture was left for 20 min at 50ºC in the water bath. After incubation, 2.5ml of 10% trichloroacetic acid was added, and the mixture centrifuged at 6000 rpm for 10min. 2.5ml of the supernatant was mixed with 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride in a test tube, and the absorbance measured at 700nm.

A higher value of absorbance indicates a higher reducing power.

Characterization of Phenolic Acids

The phenolic components in native and processed GCS extracts were separated and quantified by reverse phase HPLC (model LC-8A, Shimadzu Corporation, Japan), on a C_{18} column (4.6 × 25cm, 5µm, Supelco, Sigma), using a diode array detector operating at 280nm the peaks were identified by comparing with the retention times of standard phenolic acids.

An isocratic solvent system, consisting of water: acetic acid: methanol (83:2:15, v/v), was used as the mobile phase at a flow rate of 1 ml/min [15].

Data Analysis

Data were analyzed using the GraphPad Instat software. Each experiment was performed in triplicate, and the results were expressed as the mean values ± standard deviation. Statistical significance was determined by one way analysis of variance (ANOVA) followed by multiple comparison test (Tukey's test) at 5% level of significance. Values with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSION

Nutraceutical Properties

The total phenolic content in GCS varied in popped and germinated seeds as compared to native GCS (Table 1). The TPC contents were 1.78g GAE/100g, 2.11g GAE/100g and 1.69g GAE/100g in native, popped and germinated GCS respectively. The increase in TPC while popping may be due to an increase in the extractability of bound phenolics by the thermal degradation of cellular constituents. The increase in TPC during processing has also been reported by other investigators [16].

The flavonoid contents exhibited a significant reduction during the processing of GCS. Decrease in the flavonoid contents was observed to be highest in case of the germinated GCS extract (28.64%), compared to native GCS (1.92 g/100 g). This may be attributed to the biochemical changes of GCS during germination, and popping which leads to a reduction in these plant secondary metabolites. Similar result was also reported by Pradeep SR and Guha M during processing of little millet [17].

The tannin contents of the garden cress seed extracts increased (6.81%) while popping whereas it decreased (20.45%) during germination as compared to the native GCS extract. Increase in the tannin contents may be due to the degradation of high molecular weight insoluble polymer into a low molecular weight soluble polymer while popping. The increase in the tannin contents during processing was also reported by other investigators [18, 19].

Table 1: Nutraceutical Properties of native and processed garden cress seed extracts

Sample	Total Polyphenols (g/100g)[A]	Flavonoids (g/100g)[B]	Tannins (g/100g)[C]
Native	1.78± 0.049[a]	1.92 ± 0.080[a]	0.44 ± 0.07[a]
Popped	2.11 ± 0.175[b]	1.49 ± 0.055[b]	0.47 ± 0.03[a]
Germinated	1.69 ± 0.020[a]	1.37 ± 0.055[b]	0.35 ± 0.025[a]

Results are mean of three determinations ±SD. Values with same letters (a, b, c, within columns) are not significantly different at $p < 0.05$.

[A] g gallic acid equivalent (GAE)/100g of sample, dry basis, [B] g catechin equivalents (CEQ)/100g of sample, dry basis, [C] g catechin equivalents (CEQ)/100g of sample, dry basis

Table 2: Phenolic Acid Composition of native and processed garden cress seed extracts*

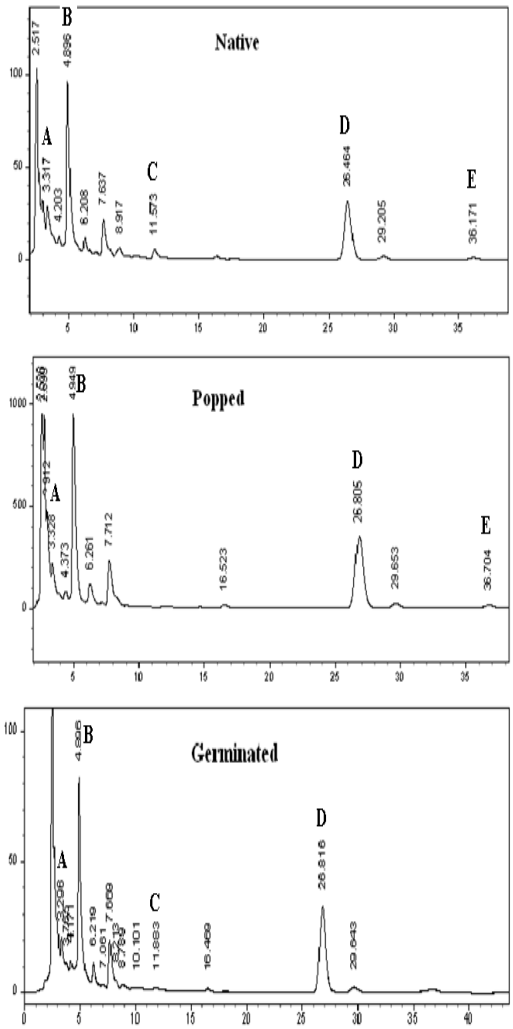
Phenolic acids	Native	Popped	Germinated
Gallic acid	1.5[3.3]	1.94[3.3]	0.36[3.2]
p-coumaric acid	8.86[4.9]	27.13[4.9]	1.93[4.8]
Vanallic acid	0.14[11.5]	ND	0.010[11.8]
Caffeic acid	ND	ND	ND
Syringic acid	ND	ND	ND
Proto catechuic acid	2.13[26.5]	6.94[26.8]	0.62[26.8]
Ferrulic acid	0.0026[36.1]	0.0128[36.7]	ND
Cinnamic acid	ND	ND	ND

Values given in parenthesis indicate retention time (min). ND, not detected., * mg/100 g of extract.

Fractionation of Phenolic acids

The isolation and identification of the phenolic fractions of the crude extracts from native and processed garden cress are presented in

Fig. 1. The HPLC profile revealed the complex nature of garden cress seed polyphenols, and the phenolic constituents were identified using nine known standards. The phenolic acid composition of native and processed GCS extracts is shown in Table 2. p-coumaric acid was the major phenolic acid detected in the extract of popped garden cress seed extracts (27.13mg/100g) followed by native (8.86mg/100g) and germinated (1.93mg/100g) extracts. Proto catechuic acid comes next to the p-coumaric acid detected in native and processed garden cress seed extracts, its concentration increased while popping (6.94mg/100mg) and decreased during germination (0.62mg/100mg) as compared to the native garden cress seed extract (2.13mg/100mg). Moreover, Cinnamic acid, Caffeic acid and Syringic acids were not detected both in the native and processed garden cress seed extracts.



A- Gallic acid; B- p-coumaric acid; C- Vanallic acid; D- Proto catechuic acid; E- Ferrulic acid

Fig. 1: Fractionation of Phenolics of the native and processed Garden Cress Seed extracts

Antioxidant Properties

From Table 3, it was observed that the radical scavenging activity of popped garden cress seed extracts increased while decreased in germinated garden cress seed extracts compared to the native sample. This change may be due to the presence of highest TPC in popped garden cress seed extracts since the %DPPH inhibition is directly correlated with TPC [20]. The higher antioxidant properties of popped garden cress seed extracts may be due to the formation of higher Maillard products during the high temperature short time processing. Similar findings have been reported by Nicoli *et al* [21] while roasting of coffee brews and also by Rocha-Guzman *et al* [22] during pressure cooking of common bean.

The results for iron reducing power of native and processed garden cress seed extracts followed a similar trend to the DPPH free radical scavenging activity. The popped garden cress seed extract showed 5.87% increase in the absorbance spectra as compared to the native sample. Germinated garden cress extracts exhibited a decrease in the absorbance value by 14.95% compared to native garden cress seed extract.

Table 3: Antioxidant Properties of native and processed garden cress seed extracts

Sample	% DPPH Inhibition	Reducing Power Assay (absorbance at 700 nm)
Native	94.18 ± 0.27[a]	0.749 ± 0.274[a]
Popped	94.87 ± 0.15[b]	0.793 ± 0.370[a]
Germinated	93.57 ± 0.04[c]	0.637 ± 0.344[a]

Results are mean of three determinations ±SD. Values with same letters (a, b, c, within columns) are not significantly different at $p < 0.05$.

CONCLUSION

Polyphenols are well known to be important nutraceuticals having antioxidant properties. The antioxidant activity of native and processed GCS polyphenols was determined in terms of the DPPH reduction capacity as well as the reducing power of ferrous to ferric iron state. The present study showed that popping improved the nutraceutical properties of GCS by increasing its content in phenolic compounds and also its antioxidant activities. Therefore, popped GCS holds a good potential as a source of nutraceuticals in health food formulations.

CONFLICT OF INTEREST

Hema Panwar declares that she has no conflict of interest.

Manisha Guha declares that she has no conflict of interest.

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