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Comparative Evaluation of Antioxidant Potential in Thermally Processed, Underutilized Food Grains of the Himalayan Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the bioactive components and total antioxidant capacity (TAC) in selected underutilized crops of the Himalayan region viz. Barnyard millet, Grain amaranth, Rice bean, Black soybean and Horsegram.
Study Design: Experimental design (Lab experiment).
Place and Duration of Study: Department of Foods and Nutrition, College of Home Science, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, in the year 2016-18.

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Methodology: We applied different processing techniques (covered pan cooking and pressure cooking) in the underutilized crops and analyzed the total phenol, total flavonoids and total antioxidant capacity (Ferric reducing antioxidant power (FRAP) assay and (2, 2-Diphenyl-1-picrylhydrazyl) (DPPH) by using standard methods.
Results: It was found that black soybean had highest phenolic content after both thermal treatments (3233.76 mg GAE/100 g for pan cooked and 1883.11 mg GAE/100 g for pressure cooked samples) and TAC by both FRAP (6423.76 mg TE/100 g for pan-cooked and 4415.58 mg TE/100 g for pressure cooked) and DPPH (536.41 and 453.98 mg TE/100g for pan and pressure cooked samples, respectively) method. Among raw samples, rice bean contained the highest flavonoid content and TAC by FRAP assay. In contrast, raw grain Amaranth showed the lowest phenolic content. Further, pressure cooking was found to be better for barnyard millet, while in pulses, pan cooking yielded the best results (in terms of increased value/lower losses).
Conclusion: It can be concluded that among pulses pan cooked black soybean was found to have a good store of bioactive compounds as compared to rice bean and horse gram. The pressure cooking method was found to be suitable for millet like a barnyard.

Keywords: Underutilized; thermal processing; rice beanp; phenolics; flavonoids; total antioxidant capacity.

1. INTRODUCTION

Underutilized cereals, millets and pulses are an important group of crops which have special significance in subsistence farming and nutritional security of resource-poor masses in developing countries. The underutilized food grains have a vast scope for not only supporting the commercially grown crops by reducing pressure on their availability, but they are a cheap source of nutrients and can be raised at low management costs [1]. Under-utilized crop species which are rich in micro-nutrients can contribute effectively in making diets more balanced; hence they play an important role in combating silent hunger. Biodiversity International and International Centre for Underutilized crops listed 200 such underutilized crop species for the different eco-geographical region of the world. Minor millets like barnyard millet, pseudocereals like grain amaranth, and pulses like rice bean, black soybean, horse gram are important under-utilized crops of the Himalayan region.Minor millets are a group of grassy plants with short slender stalks and small grains possessing remarkable ability to survive under severe drought conditions. The nutritional significance of minor millets lies in their richness in micronutrients like calcium, iron, phosphorus, vitamins and sulphur containing amino acids. Barnyard millet is the fastest growing of all millets and produces a crop within 80 days of sowing. In India, Uttarakhand is the major producer of barnyard millet. The crop occupies 74, 000 hectares area with production and productivity of 87, 000 tonnes, and 857 kg/ha, respectively [2]. It is an important source of vital minerals like niacin, magnesium, phosphorus, manganese, iron and potassium. It contains high amounts of protein, fibre, essential amino acids like methionine, lecithin, and vitamin E [3].

Besides millets, among pseudocereals amaranth is a highly nutritious crop with higher protein content than other cereal species. Amaranth seeds are not true cereals. They are dicotyledonous in contrast to cereals (e.g. wheat, rice, barley) which are monocotyledonous. They are referred to as pseudocereals, as their seeds resemble in function and composition to those of the true cereals. Amaranth seeds are small (1-1.5 mm diameter), they are lenticular in shape and weight per seed ranges between 0.6-1.3 mg [4]. Many health benefits are attributed to amaranth seeds, such as decreasing plasma cholesterol levels, stimulating the immune system, antitumorigenic activity, reducing blood glucose levels and improving the condition of hypertension and anaemia patients [5]. Concerning the antioxidant activity, researchers reported that amaranth, as well as other pseudocereals, have an antioxidant capacity comparable to that of soybean and rice. Also, these authors reported that the main antioxidant compounds of pseudocereals are polyphenols [6]. Some researchers studied distinct amaranth cultivars and identified flavonoids (isoquercitrin and rutin) and phenolic acids (syringic and vanillic acids) with an antioxidant capacity [7]. Among underutilized pulses and legumes, black soya bean, rice bean and horse gram are nutritionally superior to traditionally consumed pulses. Black soybean has long been consumed in the Far East and Southeast Asia as an important source of natural antioxidants which is attributed to the anthocyanin content in its seed coat [8]. Despite the possible health benefits of coloured soybean, there is limited information related to its chemical constituents of pharmacological and nutritional importance. Another underutilized legume rice bean (*Vigna umbellate* L.), also known as climbing mountain bean, mambi bean and oriental bean, is native to Southeast Asia [9,10]. Rice bean has exhibited excellent antioxidant capacity and anti-diabetic potential of the sixteen species of beans [11]. Rice bean is also known as one of the promising pulses of mountain regions due to its high yield potential.

Horse gram that belongs to the family *Fabaceae* is a grain legume having excellent nutritional and remedial properties with excellent climate resilience making it adaptable to harsh environmental conditions [12]. It is one of the most important under-exploited food legumes being grown almost all over the world. Temperate and sub-tropical regions, encompassing countries of East and Northeast Africa, Asian countries particularly, India, China, Philippines, Bhutan, Pakistan, Sri Lanka and Queensland in Australia are known to grow horse gram [13,14].

Polyphenols are the primary antioxidants that donate hydrogen atoms to the free radicals [15]. They delay or prevent oxidation of lipids, proteins and DNA by reactive oxygen species that are produced in cells during oxidation [16]. Phenolic compounds are a large group of plant substances which have aromatic ring bearing 1 or more hydroxyl groups [17]. Flavonoids are a large group of phenols which occur in plants in both free States and in glycosides [18]. The measurement of total antioxidant capacity (TAC) is a useful tool in evaluating the anti-oxidative role of the investigated compounds. The total antioxidant power as an 'integrated parameter of antioxidants present in a complex sample' is often more meaningful to evaluate health effects because of the cooperative action of antioxidants [19]. Antioxidant activity can be assessed through FRAP or DPPH analysis. DPPH has the ability to donate an electron or hydrogen radical to the stable DPPH free radical whereas FRAP compares antioxidants based on their ability to reduce ferric ($Fe3+$) to ferrous ($Fe2+$)ion through the donation of an electron, with resulting ferrous ion($Fe2+$). The health benefits of whole grain products are in part attributed to their unique antioxidant activities [20]. It is common knowledge that most grains (cereals and pulses) have to be processed/ cooked in some way before being eaten. Cooking increases digestibility and palatability of grains. The normal hydrothermal process such as boiling, pressure cooking, or canning is utilized to make these foods edible. Dry heat processes such as puffing, roasting and oil frying can also be used. Processing leads to inactivation of anti-nutritional factors as well as changing the level of bioactive compounds. In the study area pressure cooking and covered pan, cooking is commonly used processing methods for pulses and cereals. Thus these two processing techniques were adopted to assess their impact on the bioactive potential of the samples.

Information regarding the antioxidant activity of underutilized crops will help in understanding their role in combating chronic degenerative diseases. Therefore, the present study was planned to estimate and compare the total phenol, total flavonoids and total antioxidant capacity by FRAP and DPPH in five underutilized crops of the state of Uttarakhand, India. Among grains dehusked barnyard millet and amaranth seeds were taken, while among pulses, black soybean, rice bean and horse gram were studied. All evaluations were done for the sample in their raw state, as well as after thermally processing them in the covered pan, pressure cooker.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of dehusked barnyard millet, grain amaranth, black soybean, rice bean and horse gram were procured from villages of Tehri and Nainital districts of Uttarakhand, India.

2.2 Sample Preparation

The samples were prepared by using the method shown in Fig. 1.

2.3 Thermal Processing of Samples

Five gram of sample was taken for Thermal Processing of each underutilized grain. Standard cooking temperature for thermal processing (pan cooking (100°C) and pressure cooking (120°C)) was maintained. Amount of water used in covered pan cooking was 15 ml. For pressure cooking 20 ml water was used. Water after processing was not drained. Glass test was used to check the completion of cooking of the samples.

2.4 Determination of Total Phenolic Content

Total phenolic content was determined using the standard method [21]. A known aliquot of sample was taken and volume was made up to 1.5 ml with distilled water. To this 0.5 ml of Folin ciocalteau reagent was added. After that 10 ml of 7.5%, Na_2CO_3 was added and incubation was done at 37ºC for 60 minutes. The absorbance of the developed blue colour complex was measured at 750 nm wavelength. For blank, 1.5 ml of distilled water was treated in the same way as the sample.

Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100g. Gallic acid (5-20 µg) was used as standard.

2.5 Determination of Total Flavonoids

Total flavonoids content was estimated by using the standard method [22]. A known aliquot of sample was taken and volume was made up to 5 ml with distilled water. Then 0.3 ml of 5% NaNO2 was added. After 5 minutes 0.6 ml of 10% of AlCl3 was added. After another 6 minutes2.0 ml of 1N NaOH was added and contents were mixed. Then 2.1 ml of distilled water was added to make volume up to 10 ml. The absorbance of developed pink colour was measured at 510 nm wavelength against distilled water as blank and expressed as mg rutin equivalents (RE)/100 g. Rutin (50-200 µg) was used as standard.

2.6 Determination of Total Antioxidant Capacity (TAC) by Ferric Reducing Antioxidant Power (FRAP) Assay

Two methods were used to determine the total antioxidant capacity of samples. Total antioxidant capacity by FRAP assay was determined by using the standard method with some modifications [23,24]. Different aliquots of the sample were taken and the volume was made up to 0.3 ml with distilled water. Then 1.8 ml of FRAP working reagent was added and the contents were incubated at 37ºC for 10 minutes. The absorbance of the developed blue coloured complex was measured at 593 nm wavelength against 0.3 ml of distilled water as blank and expressed as mg Trolox equivalents (TE)/100 g. Trolox(5-20 µg) was used as standard.

2.7 Determination of Total Antioxidant Capacity by DPPH (2, 2-Diphenyl-1-picrylhydrazyl)

Total antioxidant capacity using DPPH radical was measured by the standard method with some modification [25,26]. Different sample aliquots were taken and the volume was made up to 1 ml with methanol. Then 3 ml of DPPH reagent was added to the sample and contents were mixed properly. Incubation was done for 20 minutes at 37ºC. The absorbance of the developed colour of the oxidized solution was measured at 517 nm wavelength against methanol as blank and expressed as mg Trolox equivalents (TE)/100g. Trolox (10-40 µg) was used as standard.

2.8 Statistical Analysis

Data from this study were reported as mean ± SD from three replicates for each test. One way ANOVA test was applied using IBM SPSS statistics version 20 programme to observe any difference among the treatments with respect to bioactive potential.

3. RESULTS AND DISCUSSION

3.1 Thermal Processing/Cooking

Barnyard millet took 15 minutes and 5 minutes to be completely cooked in covered pan and pressure cooker (Table 1). Grain Amaranth was not processed by these methods as it is not boiled or pressure cooked for usual consumption. The pulses, rice bean, black soybean and horse gram were fully cooked by 26 minutes, 23 minutes, and 25 minutes respectively, when cooked in a covered pan. Cooking time for the three pulses was 8 minutes, 6 minutes and 7 minutes respectively when cooked in a pressure cooker (Table 1). Thus among the three pulses, black soybean took less time to cook in comparison to the other 2 pulses in both covered pan and pressure cooking methods. It is thought that high-pressure rice cooker is more energy-intensive than a normal rice cooker during the cooking process [27].

3.2 Bioactive Compounds and Total Antioxidant Capacity

Each of the five samples, barring grain amaranth, were given two heat treatments and subjected to four assays to establish their bioactive potential. Results were statistically analysed to see the

Raw sample

↓

Cleaning/Sieving
(to remove the damaged grains and foreign material)

↓

Treatments
Thermal processing

↓

Raw | Covered pan cooking (excluding grain amaranth) | Pressure cooking (excluding grain amaranth)

↓

Grinding of sample

↓

Add 15 ml 80% acidified methanol with 6N HCL (pH2.0) to 5 g ground sample and shake on a rotary shaker at room temperature for 30 minutes

↓

Decant the supernatants and re-extract the residue for complete removal of bio-active compounds(Repeat this procedure for two times)

↓

Centrifuge supernatant at 6000rpm for 15 minutes and filter through Whatman No.1 filter paper

↓

Make up the volume of the filtrate to 50 ml with 80% methanol

↓

Analysis of total phenols, flavonoids and total antioxidant capacity (by FRAP assay and DPPH methods)

Fig. 1. Methodology for sample preparation

Table 1. Processing of selected samples

Sr. No.	Samples	Processing time (in minutes)		Amount of water used for processing (ml)	
		Covered pan cooking	Pressure cooking	Covered pan cooking	Pressure cooking
	Millet				
1	Barnyard millet (dehusked) (*Echinochloa frumentacea)*	15	5	15	20
	Pulses				
1	Rice bean (*Vigna umbellata)*	26	8	15	20
2	Black soybean (*Glycine max*)	23	6	15	20
3	Horsegram (*Macrotyloma uniflorum*)	25	7	15	20

impact of different cooking methods on the bioactive compounds, as well as establish significant differences between samples. The comparison was done among the treatments and among the selected samples. Following were the findings of the study:

Total phenolic content of barnyard millet ranged from 21.32 (pressure cooked) to 33.08 (raw) mg GAE/ 100 g (Table 2). Similar results for total phenolic content (22.905 to 36.90 mg GAE/100g) of raw dehusked barnyard were reported [28]. However, pan cooking led to lesser losses of total phenolic content (29.41 mg GAE/100 g). Total Flavonoid content was found to be minimum (31.42 mg RE/100g) in covered pan cooked barnyard millet and maximum (157.14 mgRE/100 g) in raw barnyard millet, while in pressure cooked barnyard millet, total flavonoid content was found to be 60.75 mgRE/100 g (Table 2). The results indicate that loss of flavonoids was less in pressure cooked barnyard millet samples as compared to pan cooked sample. This has also been statistically

Table 2. Bioactive components and total antioxidant capacity of samples

Sr.No.	Sample	Raw/processed	Total phenolic content (mg GAE/100 g)	Total Flavonoids (mg RE/100 g)	TAC by FRAP assay (mg TE/100 g)	TAC by(DPPH) Mg TE/100 g
Millet and Pseudocereal						
1.	Barnyard millet (dehusked) (*Echinochloa frumentacea)*	Raw	33.08±0.001	157.14±0.004	60.75±0.004	59.891±0.002
		Pan cooked	29.41±0.003	31.42±0.001	80.51±0.002	46.506±0.003
		Pressure cooked	21.32±0.002	54.29±0.002	60.75±0.003	53.517±0.002
		F value	42.609	544.517	170.855	1182.932
		S/NS	S	S	S	S
2.	Amaranth seeds *(Amaranthes cadatuslinn)*	Raw	4.41±0.004	60±0.004	59.13±0.008	67.83±0.003
Pulses and legumes						
1	Rice bean (*Vigna umbellata)*	Raw	1029.41±0.005	857.14±0.000	4818.18±0.003	204.626±0.004
		Pan cooked	544.117±0.003	642.86±0.003	3428.57±0.003	186.678±0.002
		Pressure cooked	352.94±0.001	514.28±0.001	3798.70±0.002	178.092±0.004
		F value	168.557	15.967	102.415	45.038
		S/NS	S	S	S	S
2.	Black soybean (*Glycine max*)	Raw	200±0.010	422.07±0.003	2204.30±0.001	206.45±0.001
		Pan cooked	3233.76±0.003	512.98±0.004	6423.76±0.001	536.41±0.013
		Pressure cooked	1883.11±0.001	428.57±0.003	4415.58±0.002	453.98±0.005
		F value	6179.570	5.441	390.901	34813.938
		S/NS	S	NS	S	S
3.	Horse gram (*Macrotyloma uniflorum*)	Raw	156.06±0.030	238.96±0.028	340.36±0.002	33.74±0.002
		Pan cooked	146.96±0.012	356.09±0.002	2542.60±0.004	145.55±0.004
		Pressure cooked	107.35±0.007	100.83±0.005	2097.40±0.005	114.81±0.005
		F value	14.616	7.339	5731.097	1232.177
		S/NS	S	NS	S	S

S- Significant, NS- Non-significant

Table 3. Proximate composition of selected samples

Sr. No.	Samples	Moisture%	Ash%	Crude protein %	Crude fat %	Crude fibre %	Carbohydrate (By difference)	Energy (Kcal)
	Millet and Pseudo cereal							
1	Barnyard millet (dehusked) (*Echinochloa frumentacea)**	11.39±0.05	4.27±0.02	6.93±0.13	2.02±0.06	2.98±0.12	71.87±0.10	333±0.35
2.	Amaranth seeds *(Amaranthus caudatus linn)***	9.20±0.40	3.05±0.30	13.27±0.34	5.56±0.33	-	61.46±0.6	356.22±0.10
	Pulses							
1	Rice bean (*Vigna umbellata)***	11.12	3.54	19.97	.74	-	51.26	302.63
2	Black soybean (*Glycine max*)***	7.10	4.25	35.35	18.15	10.70	24.45	-
3	Horsegram (*Macrotyloma uniflorum*)**	9.28	3.24	21.73	0.62	-	57.24	329.90

-Verma et al. (2015), **- Longvah et al. (2017), *-Ciabotti et al. (2016)*

established. Regarding results of total antioxidant capacity by FRAP; raw and pressure cooked barnyard millet showed a similar value of 60.75, while covered pan cooking increased the total antioxidant capacity (TAC) to 80.51 mg TE/100 g. Total Antioxidant capacity (TAC) by DPPH method of raw barnyard millet exhibited the highest significant ($p \le 0.05$) value (59.81 mg TE/100 g)as compared to the thermally processed samples. In covered pan cooking and pressure cooking treatment, TAC was reduced to 46.506 and 53.517 mg TE/100 g, respectively. However, pressure cooking treatment was found better than pan cooking as the latter method caused a higher loss of TAC (by DPPH).

In raw amaranth seeds, total phenolic and total flavonoid contents were found to be 4.41 mg GAE/100 g and 60 mg RE/100g, respectively. While total antioxidant capacity by FRAP and DPPH was found to be 59.13 and 67.83mgTE/100gm respectively (Table 2). Some researchers reported lower values than that of the present study of total phenol in *Amaranthus cruentus* v. *Aztek* i.e. 2.95 mg GAE/g, further, they also reported the antioxidant activity by FRAP in amaranth seeds as 3.37 mmol Fe^{2+} per kg dry weight [28].

Regarding raw and thermally processed pulses, a significantly high ($p \le 0.05$) phenolic content of 1029 mg GAE/100g was found in raw rice bean (Table 2). These values were higher than that of 16 cultivars of raw rice bean (1.63-1.82 per cent) [29]. In raw Horse gram, the phenolic content was 156.06 mg GAE/100g which was significantly higher ($p \le 0.05$) than both processed samples of the pulse. In contrast, covered pan cooked soybean exhibited the highest significant ($p \le 0.05$) phenolic content (3233.76 mg GAE/100g.) as compared to the raw and pressure cooked samples.

Total Flavonoid content of raw rice bean showed the statistically significant and highest value of 857.14mgRE/100g as compared to the two processed samples (Table 2). Similarly, pan cooked black soybean and horse gram showed significant ($p \le 0.05$) highest value of 512.98 and 356.09 mg RE/100g respectively when compared to their raw and pressure cooked forms.

Total Anti-oxidant capacity (TAC) measured by FRAP method had significant ($p \le 0.05$) maximum value in raw rice bean (4818.18 mgTE/100gm), pan cooked soybean (6423.76) and pan cooked horse gram (2542.60), similarly TAC analysed by DPPH method exhibited significant ($p \le 0.05$) highest value in raw rice bean (204.626), pan cooked black soybean (536.41) and pan cooked horse gram (145.5 mg TE/100gm) (Table 2). A study showed similar values for total phenolic content and total flavonoid content in raw black soya bean as 613.00 mg GAE/100 g and 219 mg RE/100 g respectively [30].

3.3 Proximate Composition

Proximate composition of the five underutilized grains as reported in the literature (Table 3). reveals moisture content in the range of 7.10 to 11.39g%, ash content ranged from 3.05% to 4.27%, crude protein ranged from 6.93 to 35.35g%, crude fat ranged from 0.62 to 18.1 g%, carbohydrate ranged from 24.45 to 71.87g% and energy was ranged from 302.63 to 356.22 Kcal in the selected samples (Table 3).

4. CONCLUSION

It was concluded from the study that bioactive components were reduced due to processing in barnyard millet. However, pressure cooking was found better than covered pan cooking as it led to greater losses. This may be due to the prolonged cooking time in the latter method. Thermal processing (both covered pans cooked and pressure cooked) of the samples showed black soybean had the highest phenolic content and total antioxidant capacity by both FRAP and DPPH methods. Thermally processed (both covered pan and pressure cooked) rice bean had the highest flavonoid. In contrast, raw grain Amaranth showed the lowest phenolic content and lowest total antioxidant capacity by FRAP assay. Thus it may be concluded that black soybean is the richest source of bioactive compounds among all selected samples. This reinforces the health benefits of black soybean followed by rice bean whereas barnyard millet and amaranth have lower bioactive potential as compared to pulses. Further, it may also be concluded that in the case of pulses covered pan cooking yielded better results as compared to pressure cooking in terms of lesser losses of bioactive compounds and total antioxidant capacity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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