

Comparative evaluation of antioxidant potential in thermally processed, underutilized food grains of the Himalayan region

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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.

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/100g for pan cooked and 1883.11 mg GAE/100g for pressure cooked samples) and TAC by both FRAP (6423.76 mg TE/100g for pan-cooked and 4415.58 mg TE/100g 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 bean, phenolics, flavonoids, total antioxidant capacity

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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]. Underutilized 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

29 significance of minor millets lies in their richness in micronutrients like calcium, iron,
30 phosphorus, vitamins and sulphur containing amino acids. Barnyard millet is the fastest
31 growing of all millets and produces a crop within 80 days of sowing. In India, Uttarakhand is
32 the major producer of barnyard millet. The crop occupies 74, 000 hectares area with
33 production and productivity of 87, 000 tonnes, and 857 kg/ha, respectively [2]. It is an
34 important source of vital minerals like niacin, magnesium, phosphorus, manganese, iron and
35 potassium. It contains high amounts of protein, fibre, essential amino acids like methionine,
36 lecithin, and vitamin E [3].

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

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

68 Polyphenols are the primary antioxidants that donate hydrogen atoms to the free radicals
69 [15]. They delay or prevent oxidation of lipids, proteins and DNA by reactive oxygen species
70 that are produced in cells during oxidation [16]. Phenolic compounds are a large group of
71 plant substances which have aromatic ring bearing 1 or more hydroxyl groups[17].
72 Flavonoids are a large group of phenols which occur in plants in both free States and in
73 glycosides[18]. The measurement of total antioxidant capacity (TAC) is a useful tool in
74 evaluating the anti-oxidative role of the investigated compounds. The total antioxidant power
75 as an 'integrated parameter of antioxidants present in a complex sample' is often more
76 meaningful to evaluate health effects because of the cooperative action of antioxidants [19].
77 Antioxidant activity can be assessed through FRAP or DPPH analysis. DPPH has the ability
78 to donate an electron or hydrogen radical to the stable DPPH free radical whereas FRAP
79 compares antioxidants based on their ability to reduce ferric (Fe³⁺) to ferrous (Fe²⁺)ion
80 through the donation of an electron, with resulting ferrous ion(Fe²⁺). The health benefits of
81 whole grain products are in part attributed to their unique antioxidant activities[20]. It is

82 common knowledge that most grains (cereals and pulses) have to be processed/ cooked in
 83 some way before being eaten. Cooking increases digestibility and palatability of grains. The
 84 normal hydrothermal process such as boiling, pressure cooking, or canning is utilized to
 85 make these foods edible. Dry heat processes such as puffing, roasting and oil frying can
 86 also be used. Processing leads to inactivation of anti-nutritional factors as well as changing
 87 the level of bioactive compounds. In the study area pressure cooking and covered pan,
 88 cooking is commonly used processing methods for pulses and cereals. Thus these two
 89 processing techniques were adopted to assess their impact on the bioactive potential of the
 90 samples.

91
 92 Information regarding the antioxidant activity of underutilized crops will help in understanding
 93 their role in combating chronic degenerative diseases. Therefore, the present study was
 94 planned to estimate and compare the total phenol, total flavonoids and total antioxidant
 95 capacity by FRAP and DPPH in five underutilized crops of the state of Uttarakhand, India.
 96 Among grains dehusked barnyard millet and amaranth seeds were taken, while among
 97 pulses, black soybean, rice bean and horse gram were studied. All evaluations were done
 98 for the sample in their raw state, as well as after thermally processing them in the covered
 99 pan, pressure cooker

100

101 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**
 102 **(ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)**

103

104

105

2.1 Sample collection

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

108

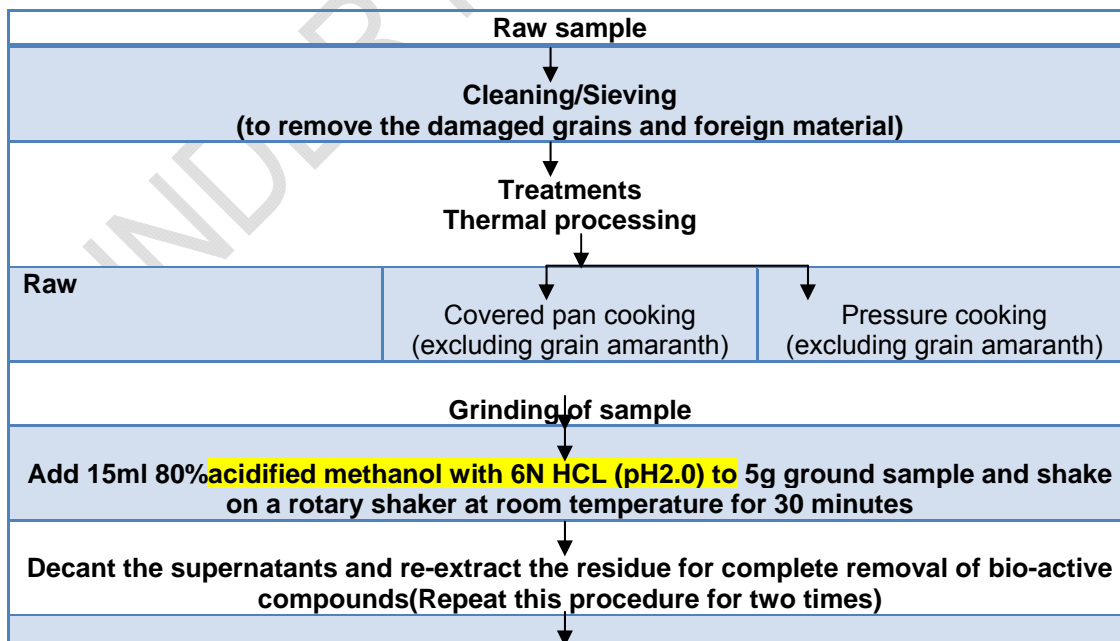
109

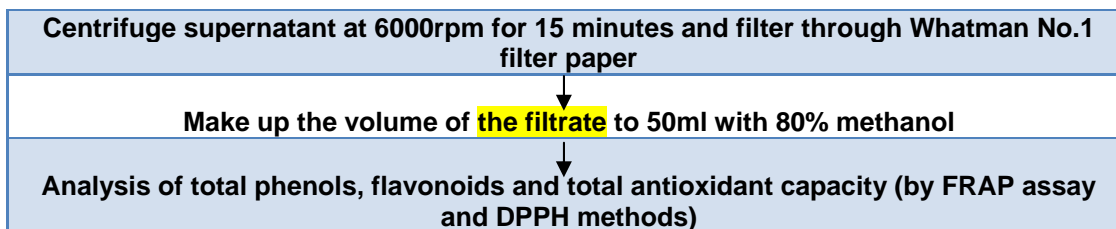
110

111

2.2 Sample preparation

The samples were prepared by using the method shown in Fig1.





112

113

114 **Fig 1: Methodology for sample preparation**

115

116 **2.3 Thermal Processing of samples**

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

122

123 **2.4 Determination of total phenolic content**

124 Total phenolic content was determined using the standard method [21]. A known aliquot of
 125 sample was taken and volume was made up to 1.5 ml with distilled water. To this 0.5ml of
 126 Folin ciocalteau reagent was added. After that 10 ml of 7.5%, Na₂CO₃ was added and
 127 incubation was done at 37° C for 60 minutes. The absorbance of the developed blue colour
 128 complex was measured at 750nm wavelength. For blank, 1.5 ml of distilled water was
 129 treated in the same way as the sample.

130 Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100g. Gallic acid
 131 (5-20 µg) was used as standard.

132

133 **2.5 Determination of total flavonoids**

134 Total flavonoids content was estimated by using the standard method [22]. A known aliquot
 135 of sample was taken and volume was made up to 5 ml with distilled water. Then 0.3 ml of 5%
 136 NaNO₂ was added. After 5 minutes 0.6 ml of 10% of AlCl₃ was added. After another 6
 137 minutes 2.0 ml of 1N NaOH was added and contents were mixed. Then 2.1 ml of distilled
 138 water was added to make volume up to 10 ml. The absorbance of developed pink colour
 139 was measured at 510 nm wavelength against distilled water as blank and expressed as mg
 140 rutin equivalents (RE)/100g. Rutin (50-200 µg) was used as standard.

141

142 **2.6 Determination of total antioxidant capacity (TAC) by ferric reducing** 143 **antioxidant power (FRAP) assay**

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

152

153 **2.7 Determination of total antioxidant capacity by DPPH (2, 2-Diphenyl-1-** 154 **picrylhydrazyl)**

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

162

163 2.8 Statistical analysis

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

167

168 3. RESULTS AND DISCUSSION

169

170 3.1 Thermal processing/cooking

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

180

181 **Table 1: Processing of selected samples**

182

Sr. No.	Samples	Processing time (in minutes)		Amount of water used for processing (ml)	
		Covered pan cooking	Pressure cooking	Covered cooking	pan Pressure cooking
Millet					
1	Barnyard millet (dehusked) (<i>Echinochloa frumentacea</i>)	15	5	15	20
Pulses					
1	Rice bean (<i>Vigna umbellata</i>)	26	8	15	20
2	Black soybean	23	6	15	20

(*Glycine max*)

3	Horsegram (<i>Macrotyloma uniflorum</i>)	25	7	15	20
---	---	----	---	----	----

183

184

3.2 Bioactive compounds and total antioxidant capacity

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

228

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 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/ 100g (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/100g). Total Flavonoid content was found to be minimum (31.42 mg RE/100g) in covered pan cooked barnyard millet and maximum (157.14mgRE/100g) in raw barnyard millet, while in pressure cooked barnyard millet, total flavonoid content was found to be 60.75mgRE/100g (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 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 ($p \leq 0.05$) 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/100g 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) than both processed samples of the pulse. In contrast, covered pan cooked soybean exhibited the highest significant ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) maximum value in raw rice bean (4818.18mgTE/100gm), pan cooked soybean (6423.76) and pan cooked horse gram (2542.60), similarly TAC analysed by DPPH method exhibited significant ($p \leq 0.05$) highest value in raw rice bean(204.626), pan

229 cooked black soybean (536.41) and pan cooked horse gram(145.5 mg TE/100gm) (Table 2).
 230 A study showed similar values for total phenolic content and total flavonoid content in raw
 231 black soya bean as 613.00 mg GAE/100g and 219 mg RE/100g respectively [30].
 232
 233
 234

Table2: Bioactive components and total antioxidant capacity of samples

Sr.No.	Sample	Raw/processed	Total phenolic content(mg GAE/100g)	Total Flavonoids (mg RE/100g)	TAC by FRAP assay (mg TE/100g)	TAC by (DPPH) (Mg TE/100g)
Millet and Pseudocereal						
1.	Barnyard millet (dehusked) (<i>Echinochloa frumentacea</i>)	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 (<i>Amaranthes cadatuslinn</i>)	Raw	4.41±0.004	60±0.004	59.13±0.008	67.83±0.003
Pulses and legumes						
1	Rice bean (<i>Vigna umbellata</i>)	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 (<i>Glyci</i>	Raw	200±0.010	422.07±0.	2204.30±0.	206.45±0.0

<i>ne max)</i>			003	001	01	
	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(<i>Macrotyloma uniflorum</i>)	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

235 S- Significant, NS- Non-significant

236

237 3.3 Proximate composition

238 Proximate composition of the five underutilized grains as reported in the literature (Table 3).
 239 reveals moisture content in the range of 7.10 to 11.39g%, ash content ranged from 3.05% to
 240 4.27%, crude protein ranged from 6.93 to 35.35g %, crude fat ranged from 0.62 to 18.1g%,
 241 carbohydrate ranged from 24.45 to 71.87g% and energy was ranged from 302.63 to 356.22
 242 Kcal in the selected samples (Table 3).

243

244 **Table 3: Proximate composition of selected samples**

245

S	Samples	Moisture %	Ash%	Crude protein %	Crude fat %	Crude fibre %	Carbohydrate(By difference)	Energy (Kcal)
1	Millet and Pseudo cereal Barnyard millet (dehusked) (<i>Echinochloafrumentacea</i>)*	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 (<i>Amaranthescad atuslinn</i>)**	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 (<i>Vigna umbellata</i>)**	11.12	3.54	19.97	.74	-	51.26	302.63
2	Black soybean (<i>Glycine max</i>)***	7.10	4.25	35.35	18.15	10.70	24.45	-
3	Horsegram (<i>Macrotyloma uniflorum</i>)**	9.28	3.24	21.73	0.62	-	57.24	329.90

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

247

248

249

4. CONCLUSION

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

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.

265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303

COMPETING INTERESTS

“Authors have declared that no competing interests exist.”

REFERENCES

1. Sankhala A, Chopra S, Sankhala A K. Effect of processing on tannin, phytate and invitro iron in underutilized millets-Bajra, The Indian J Nutr and Dietetics.2004;41: 55-61.
2. Gupta A, Sood S, Agrawal PK, Bhatt JC. Under-Utilized Food Crops Of Himalayan Region: Utilization And Prospective. Newer Approaches To Biotechnology.2013.
3. Chandrasekhar U. Appropriate technology for utilization of nutrient dense local plant foods. ProclXth Asian Congress Nutrition.2003; 48: 12-16.
4. Bressani R. Amaranth. In: B. Caballero, editors. Encyclopaedia of Food Sciences and Nutrition (pp. 166e173). Oxford: Academic Press; 2003.
5. Caselato-Souza VM, Amaya-Fafran J. State of knowledge on amaranth grain: a comprehensive review. Journal of Food Science. 2012; 77(4): r93-r104.
6. Gorinstein S. The total polyphenols and the antioxidant potentials of some selected cereals and pseudo-cereals. European Food Research and Technology.2007; 225(3-4): 321-328.
7. Rosa B D L, Fomsgaard I S, Laursen B, Mortensen A G, Olvera-Martínez L, Silva-Sanchez C, Mendoza-Herrera A, González-Castaneda J, Leon-Rodriguez A. Amaranth (*Amaranthus hypocondriacus* in italics) as an alternative crop for sustainable food production: phenolic acids and flavonoids with potential impact on its nutraceutical quality. J Cereal Sci. 2009; 49(1):117-121.
8. Astadi IR, Astuti M, Santoso U, Nugraheni PS. In vitro antioxidant Activity Of Anthocyanins Of Black Soybean Seed Coat In Human LDL. Food Chem. 2009; 112: 659–663.
9. Bolivar A, Luis CZ. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chem. 2010; 119: 1485–1490.
10. Saikia P, Sarkar CR, Borua I. Chemical Composition, Antinutritional Factors And Effect Of Cooking On Nutritional Quality Of Rice Bean [*Vigna Umbellata* (Thunb; Ohwi And Ohashi)]. Food Chem. 1999; 67:347–352.

- 304 11. Yao Y, Cheng XZ, Wang LX, Wang SH, Ren GX. Biological potential of
305 sixteen legumes in china. *Int J Mol Sci.* 2011; 12: 7048–7058.
- 306 12. Kumar D. Horsegram Research: An Introduction. In: Kumar D. (Ed.),
307 Horsegram In India. 2006. Pp1-10.
- 308 13. Durga KK. Variability and Divergence in horsegram (*Dolichos Uniflorus*). *J*
309 *Arid Land.* 2012; 4(1): 71–76.
- 310 14. Krishna KR. Legume agro ecosystems of south India: nutrient dynamics,
311 ecology and productivity. Brown Walker Press, Florida, USA. 2010; Pp 372-
312 382
- 313 15. Singh B, Singh JP, Kaur A, Singh N. Bioactive compounds in banana and
314 their associated health benefits—a review. *Food Chem.* 2016; 206:1–11.
- 315 16. Amarowicz R, Estrella I, Hernández T, Robredo S, Troszyńska A, Kosińska
316 A, Pegg RB. Free radical-scavenging capacity, antioxidant activity, and
317 phenolic composition of green lentil (*Lens Culinaris*). *Food Chem.* 2010;
318 121(3):705-11.
- 319 17. Harborne JB, Herbert H. Phtochemical dictionary: a handbook of bioactive
320 compounds from plants. London: Taylor and Francis; 1993.
- 321 18. Bhat SV, Nagasampagi BA, Sivakumar M. Chemistry of natural products.
322 New Delhi: Narosa publishing house; 2005.
- 323 19. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a
324 tool to assess redox status: critical view and experimental data. *Free Radical*
325 *Bio Med.* 2000; 29(11):1106-14.
- 326 20. Tian L, Liu J, Ma L, Zhang L, Wang S, Yan E, Zhu H. Isolation and
327 Purification of Antioxidant and ACE-Inhibitory Peptides from Yak
328 (*Bosgrunniens*) Skin. *J Food Processing Preservation,* 2017; 41(5), e13123
329
- 330 21. Singleton VI, Orthofer R, Lamuela-Raventos RM. Analysis of total phenol and
331 other oxidation substrates and antioxidants by means of Folin-Ciocalteu
332 reagent. *Methods of Enzymology.* 1999; 299:152-158.
333
- 334 22. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid
335 contents in mulberry and their scavenging effects on superoxide radicals.
336 *Food Chem.* 1999; 64(4):555-559.
337
- 338 23. Benzie I, Strain JJ. Ferric reducing/ antioxidant power assay: direct measure
339 of total antioxidant activity of biological fluids and modified version for
340 simultaneous measurement of total antioxidant power and ascorbic acid
341 concentration. *Methods in Enzymology.* 1999; 299:15-27.

- 342 24. Tadhani MB, Patel VH, Subhash R. In vitro antioxidant activities of stevia
343 rebaudiana leaves and callus. J Food Compos Ana. 2009;20(3):323-329.
- 344 25. Brand-Williams W, Cuvelier ME, Berset CLWT. Use Of A Free Radical
345 Method To Evaluate Antioxidant Activity. LWT-Food Sci Tech.1995;
346 28(1):25-30.
- 347 26. Lee JH, Kim HJ, Lee BW, Lee YY, Lee BK, Ko JY, Woo KS.
348 Physicochemical and antioxidant properties of rice cooked with different
349 proportions of black soybeans and cooking methods. J Food bioch. 2018;
350 42(6):E12671.
- 351 27. Goudar G, Sathisha GJ. Effect of extrusion and flaking on the retention of
352 nutrients and phenolic compounds in millet grains. Int J Food SciNutr.2016;
353 1(4):08-11.
- 354 28. Pasko PA, Barton H , Zagrodzki P, Gorinstein S , Fołta M , Zachwieja Z.
355 Anthocyanins, total polyphenols and antioxidant activity in amaranth and
356 quinoa seeds and sprouts during their growth. Food Chem.2009; 115 :994–
357 998.
- 358 29. Katoch R.Nutritional potential of rice bean (*VignaUmbellata*): an
359 underutilized legume. J Food Sci Tech. 2013; 78(1):C8-16.
- 360 30. Malencic D, Cvejic J, Miladinovic J. Polyphenol content and antioxidant
361 properties of colored soybean seeds from central Europe J Medicinal
362 Food.2012; 15(1):89-95.
- 363
364