Nutritional properties of green gram germinated in mineral fortified soak water: II. Effect of cooking on total and bioaccessible nutrients and bioactive components

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Abstract The effect of pressure and microwave cooking on total and bioaccessible nutrients and bioactive components in whole and dehulled green gram (GG) germinated in mineral fortified soak water was studied. Whole GG was soaked in water fortified with iron (100 or 200 mg/100 ml) or zinc (50 or 100 mg/100 ml), germinated, cooked by two methods and analyzed. Results showed that method of cooking did not affect the nutrient composition of whole or dehulled grains, however, phytic acid and dietary fiber were higher in microwave cooked samples. Minor differences were noted in total and percent available nutrients in differently cooked samples, significance being observed only for starch, protein and zinc in few samples. Significant differences were observed among variations, wherein the mineral fortified samples had a higher level of bioaccessibility compared to non-fortified samples. Cooking reduced the content of bioactive components in germinated GG, though bioaccessibility was higher. Comparison between raw and cooked grains showed that starch digestibility increased on cooking, though protein digestibility was not affected significantly. Bioactive components were also lower in cooked samples in comparison to the raw counterparts. In conclusion, the cooking methods did not affect the nutrient composition of green gram germinated in mineral fortified soak water, though available nutrients were higher in cooked legumes.

Keywords Pressure cooking · Microwave cooking · Mineral fortification · Digestibility · Bioaccessibility

Introduction

Pulses are a part of everyday dietaries in many parts of the world. They contain high levels of proteins among plant foods and can supply nearly 20–30% of total protein intake. They are complementary to cereals and provide essential amino acids thus raising the protein quality of vegetarian diets. Specifically, cereal based diets are poor in lysine content and pulses are rich sources of lysine, hence pulses bridge the gap of essential amino acid deficiency (Tharanathan and Mahadevamma 2003). Pulses are consumed both in whole and dehulled forms either with minimum pre-processing as soaked or germinated grains or as products processed with different modes of thermal treatments (Khatoon and Prakash 2006a; Tharanathan and Mahadevamma 2003). Dehulling reduces the dietary fiber and ash contents of pulses and increases protein, fat and starch contents as well as the availability of some nutrients. Soaking and germination also cause changes in nutritional composition and nutrient availability from grains (Devi et al. 2015; Ghavidel and Prakash 2007; Kaur et al. 2015; Lestienne et al. 2005). Along with nutrients and bioactive components, pulses also contain anti-nutritional factors which may cause gastrointestinal discomforts or in extreme cases, even adverse reactions. Many of these factors are heat-labile, hence heat processing helps to improve the nutritional quality of pulses. Heat processing also improves the digestibility and availability of nutrients from pulses apart from germination (Abd El-Hady and Habiba 2003; Egountly and Aworh 2003). Legumes can be processed using many heat processing methods such as roasting,

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boiling, steaming, frying, etc. Cooking makes the grain softer and more palatable. While conventional boiling method can be used for cooking pulses, it is comparatively time consuming and hence has been replaced by other methods. Pressure cooking (PC) and microwave cooking (MW) are quick cooking methods that are practiced in daily household cooking (Khattab et al. 2009; Khatoon and Prakash 2004, 2006a, b).

A major causative factor for micronutrient malnutrition prevalent in South East Asian and African countries is poor bioavailability of nutrients from diets. Iron availability is influenced by many dietary variables. Plant sources of iron are poorly absorbed due to the presence of many inhibitory factors like phytates, oxalates, calcium salts, polyphenols and tannins. Organic acids such as ascorbic acid, citric acid, malic acid, etc., on the other hand, promote iron absorption (Lakshmi et al. 2006). During germination, legumes also produce ascorbic acid, which helps in iron absorption (Khatoon and Prakash 2006a). Germinated grains can be a vehicle for mineral fortification as they may help in better absorption of nutrients. Hence, in the context of nutritional insecurity and multiple micronutrient malnutrition, grains germinated in mineral fortified soak water and subjected to heat processing can contribute towards improving utilization of nutrients from diets. Present study investigated the effect of pressure and microwave cooking on total and bioaccessible nutrients and bioactive components of green gram germinated in mineral fortified soak water.

Materials and methods

Materials

The material used for the study was whole green gram (mung bean, *Vigna radiata*) as detailed in Part I of the paper. All chemicals obtained and details of experiments followed were as given under Part I of the paper (Oghbaei and Prakash 2016).

Processing of green gram

Whole green gram was germinated in mineral fortified soak water as given earlier. The variations were—A. Control (distilled water), B. Distilled water with 100 mg iron, C. Distilled water with 200 mg iron, D. Distilled water with 50 mg zinc, E. Distilled water with 100 mg zinc. The germinated grains were cooked using two methods, namely pressure cooking (PC) and microwave (MW) cooking. To cook grain in pressure cooker and microwave oven, appropriate glass jars were used. To 100 g germinated grain, 50 ml water was added as cooking medium which

was not decanted after cooking but used to facilitate homogenization. Pressure cooking was done for 10 min on hot plate adjusted for maximum power in a standard pressure cooker. The microwave (Sanyo- BPL- BMO-700T) was adjusted in quick set program and 100 g germinated grain was heated in oven continuously for 4 min. The cooked samples were homogenized and stored under refrigeration till further analysis.

Analysis

The cooked samples were analyzed for nutritional composition, anti-nutrients, bioactive components, and digestible/bioaccessible nutrients and bioactive components following the methods given under Part I of the paper (Oghbaei and Prakash 2016). In brief, the methods were, moisture by oven drying method, protein by Kjeldhal distillation, fat by Soxhlet extraction with petroleum ether, total starch by enzymic hydrolysis, total ash by incineration, iron by Wong's Method, calcium by titrimetric determination, zinc by atomic absorption spectrophotometer, dietary fiber by the enzymatic–gravimetric determination, phytic acid by supernatant difference method of Thompson and Erdman (1982), oxalates by the method of Baker (1952), total phenols using Folin–Ciocalteu reagent with tannic acid as standard, flavonoid content by Dowd method using quercetin as standard, and tannin using tannic acid as standard, in vitro starch digestibility (IVSD) by methods of Kon et al. (1971) and Holm et al. (1985), in vitro protein digestibility (IVPD) by method of Akeson and Stahman (1964), and bioaccessible iron, zinc, calcium, total phenols, flavonoids and tannins by simulated gastro-intestinal digestion technique (Luten et al. 1996).

Statistical analysis

Data were subjected to statistical analysis using statistical software SPSS 15.0 (SPSS Inc., Chicago, IL). Mean and standard deviation for all values were calculated. Data were analyzed using Student's 'T' test to determine significant differences between pressure cooked and microwave cooked samples for total and bioaccessible constituents. For multiple comparison, data was analyzed between raw and cooked samples using data from Part I of the paper with ANOVA and Duncan's multiple range test (Duncan 1995).

Results and discussion

The results of the study are compiled in Tables 1, 2, 3, 4, 5 and discussed below.

Table 1 Nutrients and anti-nutrients content of pressure and microwave cooked germinated green gram (per 100 g db)

Variations	Moisture (g)	Ash (g)	Starch (g)	Protein (g)	Iron (mg)	Zinc (mg)	Calcium (mg)
<i>Dehulled grains</i>							
A. Distilled water (control)							
PC	70.90 ± 0.90	2.30 ± 0.00	51.25 ± 1.12	27.10 ± 0.26	5.99 ± 0.15	3.78 ± 0.34	44.66 ± 2.73
MW	65.90 ± 1.00	2.61 ± 0.00	51.00 ± 0.53	26.80 ± 0.38	6.08 ± 0.19	4.13 ± 0.00	46.01 ± 0.00
B. With 100 mg Fe							
PC	70.60 ± 0.30	3.27 ± 0.14	46.80 ± 1.29	26.60 ± 1.09	25.66 ± 0.32	3.81 ± 0.17	32.52 ± 0.00
MW	65.40 ± 0.00	3.21 ± 0.14	51.63* ± 0.77	26.99 ± 0.58	27.09 ± 0.70	3.69 ± 0.52	34.74 ± 0.52
C. With 200 mg Fe							
PC	69.80 ± 0.30	3.64 ± 0.33	48.01 ± 1.52	26.73 ± 1.23	39.90 ± 0.00	3.64 ± 0.23	33.31 ± 0.00
MW	63.00 ± 1.80	3.84 ± 0.00	53.81 ± 1.08	26.19 ± 0.00	40.41 ± 1.71	3.27 ± 0.29	36.38 ± 2.38
D. With 50 mg Zn							
PC	71.8 ± 0.3	3.04 ± 0.06	46.81 ± 0.00	26.77 ± 1.42	6.31 ± 0.11	9.47 ± 0.53	43.46 ± 1.51
MW	69 ± 0.40	2.94 ± 0.04	49.31 ± 0.44	27.94 ± 1.10	5.68 ± 0.23	8.42 ± 0.00	45.13 ± 0.00
E. With 100 mg Zn							
PC	70.90 ± 0.70	3.11 ± 0.00	50.43 ± 0.33	27.94 ± 0.00	5.72 ± 0.36	12.06 ± 0.72	40.24 ± 1.34
MW	65.95 ± 0.45	3.30 ± 0.07	51.42 ± 0.00	26.14 ± 0.94	6.27 ± 0.39	12.10 ± 0.38	42.88 ± 0.00
<i>Whole grains</i>							
A. Distilled water (control)							
PC	68.80 ± 0.00	3.46 ± 0.00	50.58 ± 0.29	27.69 ± 0.00	4.87 ± 0.12	3.81 ± 0.35	65.19 ± 4.29
MW	65.70 ± 0.70	3.53 ± 0.29	51.20 ± 0.50	26.65 ± 0.76	4.89 ± 0.14	3.79 ± 0.00	73.21 ± 0.32
B. With 100 mg Fe							
PC	67.30 ± 0.80	3.82 ± 0.37	50.83 ± 0.24	27.80 ± 0.00	93.86 ± 0.38	3.51 ± 0.36	58.23 ± 3.18
MW	65.80 ± 0.60	3.86 ± 0.23	52.82 ± 0.28	27.02 ± 0.67	87.42 ± 2.07	3.77 ± 0.00	66.43* ± 3.16
C. With 200 mg Fe							
PC	69.60 ± 0.00	4.21 ± 0.06	49.61 ± 2.17	25.99 ± 0.00	148.35 ± 1.48	3.66 ± 0.41	49.61 ± 0.00
MW	67.20 ± 0.20	4.30 ± 0.00	51.34 ± 0.00	26.62 ± 1.07	143.99 ± 2.62	3.35 ± 0.00	53.29* ± 0.00
D. With 50 mg Zn							
PC	68.1 ± 0.20	3.00 ± 0.18	48.21 ± 0.31	25.36 ± 1.60	5.96 ± 0.00	12.10 ± 1.10	65.20 ± 1.03
MW	67.1 ± 0.00	3.31 ± 0.00	48.31 ± 0.68	25.71 ± 1.40	6.16 ± 0.11	11.85 ± 0.46	68.21 ± 1.88
E. With 100 mg Zn							
PC	68.80 ± 0.80	3.40 ± 0.00	46.57 ± 0.80	26.89 ± 1.44	5.46 ± 0.08	13.84 ± 0.89	66.28 ± 0.00
MW	65.70 ± 0.40	3.33 ± 0.04	52.68 ± 1.28	26.06 ± 0.87	5.61 ± 0.13	12.79 ± 0.49	67.29 ± 2.22

Variations	Phytic acid (mg)	Dietary fiber (g)		
		Insoluble	Soluble	Total
Anti-nutrients in Control sample				
Dehulled				
PC	84.23 ± 0.00	11.99 ± 1.34	2.06 ± 0.14	14.05 ± 1.79
MW	101.26* ± 2.14	14.07 ± 0.00	1.83 ± 0.07	15.90 ± 0.19
Whole				
PC	125.50 ± 2.10	15.66 ± 0.58	2.04 ± 0.05	17.70 ± 0.40
MW	157.39* ± 1.79	18.06 ± 0.99	1.84 ± 0.09	19.90 ± 1.27

Significant differences between PC and MW grains on application of 'T' test

PC pressure cooked, MW Microwave cooked

* $P \leq 0.05$, values without notation are not significantly different

Nutritional composition

The effect of cooking method on nutritional composition of green gram germinated (GGG) in mineral fortified soak water is compiled in Table 1. There was a difference in moisture content of PC and MW cooked samples, the latter having a lower range of moisture, which was obviously due to evaporation of moisture during microwave cooking. As pressure cooking is done in closed container, there is no loss of moisture. All other constituents are presented on dry weight basis. Ash content was in higher range in all fortified samples of dehulled grain and iron fortified samples of whole grain in comparison to control. The starch and protein contents of grains cooked by two different methods did not vary significantly except for starch content of dehulled variation B which was higher in MW cooked sample. Similarly, no differences were seen for iron or zinc content in differently cooked samples, though fortified samples had higher mineral contents. Calcium was lower in samples with iron fortification as seen in raw samples (Part I of paper). In whole grains, calcium content of MW cooked samples was higher than PC samples, the difference being marginally significant. Overall cooking method did not affect the nutritional quality of germinated grains and minerals were retained in fortified grains. Khatoon and Prakash (2006a) studied effect of PC and MW cooking on nutritional quality of GGG and reported that, moisture contents of raw, PC and MW cooked samples were 65.8, 79.1, 75.8% respectively. Protein and starch contents of germinated pulses decreased after PC and MW cooking by 3.6 and 6.4 g/100 g respectively. Ash content (2.9%) remained same in PC but decreased to 2.5% after MW cooking.

Cooking method influenced the phytic acid content which was significantly higher in MW processed samples. This could be due to temperature of cooking. In pressure cooking temperature reaches up to 121 °C, whereas the temperatures in MW cooking is equivalent to boiling as in conventional cooking. The anti-nutrients like phytate and tannin that are the main binders of minerals change due to different thermal processing so in many cases availability of minerals from cooked foods can be more than raw counterparts (Parada and Aguilera 2007). Abd El-Hady and Habiba (2003) reported an increase in protein digestibility of legume seeds on soaking and extrusion which was said to be due to a significant reduction in anti-nutritional factors. MW cooked samples also showed higher insoluble and lower soluble dietary fiber in comparison to PC samples. Thermal treatments are known to cause alterations in dietary fiber contents of foods. Aguilera et al. (2009) reported an increase in insoluble and soluble dietary fiber contents in chick pea, white bean and pink mottled cream

bean on soaking and cooking. Heat treatment in wet conditions usually determines the hydrolysis of the fiber (the more severe the treatment the higher the hydrolysis). Khatoon and Prakash (2006b) reported an increase in dietary fiber content in dehulled legume subjected to either pressure or microwave cooking. Processing can modify the solubility of the fiber by reducing its molecular weight due to enzymatic or mechanical actions (Quaglia and Carletti 1995).

In vitro availability of selected nutrients

Effect of cooking on in vitro available nutrients is given in Table 2. The range of digestible starch was 38.71–45.09 g/100 g for dehulled and 31.16–43.09 g/100 g for whole grains. Protein content ranged from 15.32 to 20.14 g/100 g for dehulled and 12.71–18.01 g/100 g for whole grains. Cooking method did not affect the protein or starch digestibility significantly with few exceptions which were marginally significant. Cooking conditions can affect digestibility of nutrients due to breaking of cell walls and bonds among nutrients and heating of non-germinated legumes has been reported to increase in vitro enzyme hydrolysis of proteins (Torres et al. 2016). However, in case of germinated grains the effects were lesser due to modification of grain structure through enzyme activity. Germination is known to activate enzymes in legumes. In our study germination exhibited a similar effect. Guzel and Sayar (2012) reported that cooking under high pressure may result in formation of resistant starch and reduce starch digestibility in legumes.

Similar results were seen for mineral bioaccessibility, wherein major differences were not observed in any of the constituents. Iron and zinc bioaccessibility were exceptionally higher in samples fortified with respective minerals. Bioaccessible calcium was lower in iron fortified samples as seen for raw grains (Part I of the paper). The data was further computed as percent digestibility/bioaccessibility and is presented in Table 3. As expected, the method of cooking did not influence the percent availability of nutrients except in some instances. Dehulled grains had more percent digestible starch and protein than whole grains. The percent bioaccessibility of iron and zinc was higher in fortified samples. However, in whole gains iron fortification interfered with zinc absorption on account of competitive inhibition, however, iron was not affected by presence of zinc.

When all variations were compared for significant differences in percent availability of nutrients, results were very interesting. Starch digestibility of all variations of both PC and MW cooked whole grains and protein digestibility of dehulled PC grains were similar, rest of the samples showed significant differences. For some

Table 2 In vitro availability of nutrients in pressure and microwave cooked germinated green gram (per 100 g db)

Variations	Digestible (g)		Bioaccessible (mg)			Digestible (g)		Bioaccessible (mg)		
	Starch Dehulled	Protein	Fe	Zn	Ca	Starch Whole	Protein	Fe	Zn	Ca
A. Distilled water										
PC	43.57 ± 1.89	20.14 ± 0.45	1.18 ± 0.04	1.16 ± 0.00	24.54 ± 0.65	35.83 ± 0.48	18.01 ± 0.35	0.96 ± 0.06	1.02 ± 0.01	30.18 ± 0.59
MW	40.09 ± 0.18	18.74 ± 0.44	1.15 ± 0.01	1.25 ± 0.03	23.24 ± 0.45	34.96 ± 0.23	16.78* ± 0.31	0.97 ± 0.02	1.03 ± 0.03	33.57* ± 0.89
B. With 100 mg Fe										
PC	43.13 ± 0.61	16.82 ± 0.29	7.82 ± 0.23	1.04 ± 0.02	15.97 ± 0.15	43.09 ± 0.31	15.64 ± 0.29	31.77 ± 0.33	0.69 ± 0.04	14.10 ± 0.00
MW	42.79 ± 0.16	17.49 ± 0.00	7.34 ± 0.19	0.95 ± 0.02	16.76 ± 0.26	39.80* ± 0.56	15.19 ± 0.19	29.62* ± 1.07	0.775 ± 0.00	16.27 ± 0.51
C. With 200 mg Fe										
PC	42.20 ± 0.65	16.41 ± 0.51	15.84 ± 0.19	0.92 ± 0.00	15.18 ± 0.31	36.74 ± 1.51	14.56 ± 0.21	60.00 ± 0.00	0.57 ± 0.02	10.26 ± 0.00
MW	43.86 ± 0.41	15.97 ± 0.35	15.54 ± 0.25	0.73* ± 0.00	16.41* ± 0.27	36.25 ± 0.00	14.02 ± 0.21	58.11 ± 1.52	0.50 ± 0.04	10.84 ± 0.81
D. With 50 mg Zn										
PC	39.10 ± 1.33	15.32 ± 0.50	1.27 ± 0.00	3.62 ± 0.10	28.78 ± 0.59	33.89 ± 0.53	13.17 ± 0.00	1.16 ± 0.00	4.79 ± 0.12	30.86 ± 2.12
MW	38.71 ± 0.61	15.35 ± 0.00	1.16 ± 0.05	3.15* ± 0.05	27.61 ± 0.90	31.16* ± 0.27	12.71* ± 0.00	1.29 ± 0.03	4.54 ± 0.08	31.41 ± 0.96
E. With 100 mg Zn										
PC	45.09 ± 0.27	16.74 ± 0.00	1.03 ± 0.00	5.51 ± 0.06	23.81 ± 0.65	37.96 ± 0.27	14.09 ± 0.30	1.00 ± 0.05	6.41 ± 0.12	28.24 ± 2.92
MW	42.11* ± 0.68	16.39 ± 0.00	1.10 ± 0.05	5.36 ± 0.25	23.86 ± 0.66	40.44* ± 0.00	13.59 ± 0.44	0.94 ± 0.02	5.72 ± 0.13	27.35 ± 0.38

Significant differences between PC and MW on application of 'T' test

* ≤0.05, the values without notation differences are not significant

Table 3 Percent in vitro availability of selected nutrients in pressure and microwave cooked germinated green gram

Variations	Digestibility			Bioaccessibility			Digestibility			Bioaccessibility		
	Dehulled						Whole					
	Starch	Protein	Fe	Zn	Ca		Starch	Protein	Fe	Zn	Ca	
A. Distilled water												
PC	85.08 ± 3.66	74.47 ± 1.67	19.89 ± 0.78	30.80 ± 0.00	55.01 ± 1.45		70.87 ± 0.93	65.09 ± 1.23	19.87 ± 1.43	26.83 ± 0.38	46.30 ± 0.90	
MW	78.65 ± 0.35	69.93* ± 1.63	19.09 ± 0.31	30.42 ± 0.78	50.55 ± 1.01		68.29 ± 0.45	63.05 ± 1.15	19.98 ± 0.57	27.25 ± 0.85	45.89 ± 1.21	
B. With 100 mg Fe												
PC	92.14 ± 1.31	62.85 ± 1.10	30.50 ± 0.90	27.44 ± 0.76	49.18 ± 0.48		84.77 ± 0.60	56.30 ± 1.05	33.86 ± 0.35	19.66 ± 1.22	24.21 ± 0.00	
MW	82.91* ± 0.30	64.77 ± 0.00	32.42 ± 0.00	25.83* ± 0.52	48.10 ± 0.56		75.36* ± 1.06	56.22 ± 0.70	33.89 ± 1.23	20.56 ± 0.04	24.53 ± 0.77	
C. With 200 mg Fe												
PC	87.92 ± 1.38	61.40 ± 1.93	39.72 ± 0.49	25.44 ± 0.00	45.61 ± 0.94		74.07 ± 3.05	56.01 ± 0.82	40.45 ± 0.00	16.77 ± 0.67	20.74 ± 0.00	
MW	81.55* ± 0.75	60.99 ± 1.36	38.47 ± 0.63	22.40** ± 0.00	45.16 ± 0.74		70.66 ± 0.00	52.69 ± 0.80	40.37 ± 1.06	16.66 ± 0.15	20.37 ± 1.53	
D. With 50 mg Zn												
PC	83.565 ± 2.835	57.21 ± 1.85	20.12 ± 0.00	38.2 ± 1.1	66.66 ± 1.32		70.31 ± 1.085	51.9 ± 0.00	19.44 ± 0.00	39.55 ± 1.00	47.3 ± 3.27	
MW	78.575 ± 1.255	54.96 ± 0.00	20.42 ± 0.78	37.46 ± 0.64	61.21 ± 2.03		64.52* ± 0.595	49.64* ± 0.04	21.11 ± 0.47	38.32 ± 0.67	46.05 ± 1.4	
E. With 100 mg Zn												
PC	89.47 ± 0.53	59.90 ± 0.00	18.20 ± 0.00	45.71 ± 0.50	59.19 ± 1.63		81.52 ± 0.58	57.00 ± 1.23	18.51 ± 1.09	46.31 ± 0.91	42.60 ± 4.40	
MW	81.90* ± 1.30	62.69** ± 0.00	16.93 ± 0.09	44.32 ± 2.12	55.65 ± 1.55		76.81* ± 0.00	52.12 ± 1.68	16.84 ± 0.39	44.74 ± 1.08	40.66 ± 0.56	
<i>Multiple comparisons between different variations for percent in vitro availability of nutrients</i>												
Pressure cooked samples												
A. Control	b	a	c	b	a		a	a	c	bc	bc	
B. Fe (100 mg)	a	a	b	bc	b		a	b	b	cd	cd	
C. Fe (200 mg)	a	a	a	c	b		a	b	a	d	d	
D. Zn (50 mg)	a	a	c	a	a		a	b	c	b	a	
E. Zn (100 mg)	a	a	c	a	ab		a	b	c	a	b	
Microwave cooked samples												
A. Control	b	a	c	c	a		a	a	c	c	bc	
B. Fe (100 mg)	a	b	b	d	b		a	b	b	cd	cd	
C. Fe (200 mg)	b	bc	a	e	b		a	b	a	d	d	
D. Zn (50 mg)	c	c	c	b	a		a	b	c	b	a	
E. Zn (100 mg)	a	bc	c	a	a		a	b	c	a	b	

Significant differences between PC and MW on application of "T" test, * $P \leq 0.05$, the values without notation are not significantly different. For multiple comparisons different alphabets in a column are statistically different

constituents, (protein, iron, zinc, and calcium of whole grains, and iron and calcium of dehulled grains) the pattern was similar for PC and MW samples, while for others slight differences were observed. In general, thermal heat processing is reported to reduce the anti-nutritional factors and improve the starch and protein digestibility as well as bioavailability of other nutrients in legumes by many authors (Khatab and Arntfield 2009; Khatoon and Prakash 2004, 2006a, b). A significant improvement in iron and zinc availability on sprouting in green and white faba bean was reported by Luo and Xie (2014).

Total and bioaccessible bioactive components

Total and bioaccessible bioactive components (content and percentage) are presented in Tables 4 and 5. The total phenol contents of mineral fortified samples were lower than controls for both dehulled and whole grains. In all the MW cooked samples, contents were lower than PC samples, and the differences were also significant for some variations. Bioaccessible phenols followed similar pattern. In dehulled grains both total and bioaccessible tannins were significantly lesser in MW cooked sample. Whole grains followed similar pattern with differences being non-significant for most. Both total and bioaccessible flavonoids were higher in MW cooked samples in all variations of whole grains and few variations of dehulled grains. Percent bioaccessibility of phenolic constituents and tannin in cooked grains was very high for many of the control and fortified samples. Differences arising out of cooking methods were observed for some samples. Percent bioaccessibility of flavonoids was more in dehulled than whole grains. Multiple comparisons between variations revealed significant differences between total phenols, tannins and flavonoids of whole grains cooked by both methods. Significant difference was also observed in total phenols in dehulled grains while rest of the variations were similar.

Comparison between raw and cooked germinated green gram

All data obtained for cooked GGG was also compared with values obtained for raw GGG (presented in part I of the paper) and the level of significant differences was determined. (Data is compiled in Table 6 under supplementary files). Important observations can be summarized as follows—few variations showed reduction in ash content which can be due to lixiviation of inherent or added mineral of germinated grain to cooking water. Major differences were observed in starch content (few variations) and digestible starch (all variations). All variations (whole and dehulled) of GGG exhibited significantly higher starch digestibility after cooking with both methods. Cooking

starchy foods (legumes, cereals and different vegetables) results in starch gelatinization and increases digestibility of starch (Khatoon and Prakash 2006b; Chung et al. 2008). A slight decrease in starch content, ranging from 7.5 to 25.1%, in MW and pressure cooking has been reported. This may be because cooking in water media results in more solid loss. Pressure cooking of germinated legumes lead to loss of 3.2–9.6% starch and MW cooking showed 9.8–16.5% loss (Khatoon and Prakash 2006a). Cooking did not change protein content of majority of variations but few whole germinated grains lost protein significantly. Reduction of protein contents of legumes during thermal treatments has been reported earlier (Fagbemi 2007). Pal et al. (2016a) studied the effect of dehulling, germination and cooking on nutrients and anti-nutrients in lentil (*Lens culinaris*) and found that while dehulling did not show any significant effect on protein content, germination and cooking decreased the protein content in many varieties of lentil. For iron, zinc and calcium, differences were observed in few samples. As seen for raw variations, grains germinated in iron fortified water exhibited significant differences in iron content of whole and dehulled grain (zinc penetrated to endosperm and differences between whole and dehulled grain were not significant). This would imply that a large quantity of iron was attached to outer layer. During cooking external sources of mineral moved in cooking water. While the cooked water was not discarded and used for homogenization during mixing, still some part of mineral may adhere to vessels and result in a reduction of their content. Four of seven significant changes occurred in variations with iron and two in variation with zinc, so in addition to effect of cooking on reduction of phytic acid, the interaction of minerals and their high concentrations could explain these changes. Zinc bioaccessibility of GGG increased following thermal treatments and significant changes were observed mostly among variations with iron or zinc. Both raw and cooked (PC and MW), GGG were found to have least calcium bioaccessibility in variations C (with 200 Fe) and highest value was in control for cooked and raw grains. For bioactive components, major differences were observed in both content and percent bioaccessibility of all variations (Table 6). For many of the variations, the total content decreased significantly on cooking, while the bioaccessible content did not show major differences with few exceptions. The reduction in dehulled grains was more than in whole grains. In terms of percent bioaccessibility, cooking showed a significant increase among most of the variations. Our observations are supported by many studies in literature, some of which are mentioned below.

Sharma and Gujral (2010) studied effect of MW cooking and sand roasting on total polyphenol and flavonoid content of 8 different varieties of barley and it was observed

Table 4 Total and bioaccessible bioactive components in pressure and microwave cooked germinated green gram (mg/100 g db)

Variations	Sample	Total Phenols		Tannins		Flavonoids	
		Total	Bioaccessible	Total	Bioaccessible	Total	Bioaccessible
Dehulled grains							
A. Control	PC	164.78 ± 3.51	163.37 ± 0.81	217.82 ± 1.84	225.37 ± 7.80	6.87 ± 0.55	3.59 ± 0.15
	MW	148.14* ± 2.98	147.09* ± 4.08	204.75* ± 0.53	200.36* ± 0.89	7.39 ± 0.56	3.42 ± 0.13
B. Fe (100 mg)	PC	128.84 ± 1.53	119.22 ± 2.89	144.51 ± 2.06	139.50 ± 5.01	8.30 ± 0.44	4.11 ± 0.07
	MW	120.55* ± 2.40	105.51 ± 3.47	122.95* ± 3.50	112.82* ± 5.07	6.79 ± 0.46	3.73* ± 0.06
C. Fe (200 mg)	PC	135.93 ± 3.01	130.26 ± 2.98	143.34 ± 2.19	138.73 ± 4.29	5.00 ± 0.00	2.42 ± 0.03
	MW	91.08** ± 1.81	92.17** ± 0.00	126.23* ± 3.20	123.27* ± 0.00	5.03 ± 0.38	2.50* ± 0.04
D. Zn (50 mg)	PC	138.55 ± 2.73	131.08 ± 1.72	165.78 ± 1.28	167.98 ± 0.00	6.28 ± 0.78	3.12 ± 0.04
	MW	115.92 ± 10.11	109.35* ± 0.00	156.23* ± 1.16	151.56** ± 1.89	6.87 ± 0.00	3.68 ± 0.03
E. Zn (100 mg)	PC	128.87 ± 7.49	130.22 ± 1.41	205.07 ± 1.84	202.30 ± 5.33	3.33 ± 0.52	1.74 ± 0.05
	MW	115.95 ± 6.40	114.90 ± 5.20	193.41 ± 4.74	181.32* ± 1.47	4.58 ± 0.50	2.35* ± 0.09
Whole grains							
A. Control	PC	162.69 ± 9.01	163.62 ± 3.43	264.98 ± 6.30	276.35 ± 6.93*	10.80 ± 0.00	4.90 ± 0.00
	MW	155.44 ± 2.23	153.31 ± 1.38	266.55 ± 5.20	252.94 ± 5.83	12.33* ± 0.00	5.54 ± 0.15
B. Fe (100 mg)	PC	121.12 ± 8.73	84.62 ± 0.09	160.60 ± 1.54	150.14 ± 8.06	7.98 ± 0.98	3.26 ± 0.11
	MW	81.89* ± 3.52	73.98 ± 3.95	152.09 ± 3.52	157.63 ± 1.61	13.30 ± 0.00	5.80* ± 0.07
C. Fe (200 mg)	PC	118.95 ± 4.41	82.20 ± 1.15	162.73 ± 1.74	118.09 ± 3.16	8.91 ± 0.00	3.50 ± 0.12
	MW	100.03* ± 2.04	82.59 ± 2.04	158.05 ± 5.61	131.20* ± 3.25	12.41 ± 0.91	5.26** ± 0.08
D. Zn (50 mg)	PC	129.80 ± 9.80	119.92 ± 1.43	175.94 ± 2.21	174.08 ± 3.92	9.40 ± 0.00	4.34 ± 0.08
	MW	116.35 ± 1.19	119.88 ± 4.89	160.58* ± 0.00	152.24 ± 1.58	14.65* ± 0.73	6.87* ± 0.12
E. Zn (100 mg)	PC	124.65 ± 0.00	130.24 ± 1.78	212.80 ± 2.58	204.55 ± 5.10	10.74 ± 0.00	4.58 ± 0.00
	MW	112.78* ± 1.73	116.49* ± 2.00	216.69 ± 11.36	189.80* ± 4.08	13.44 ± 0.93	6.06** ± 0.00

Significant differences between pressure and microwave cooked samples on application of 'T' test

* $P \leq 0.05$; ** $P \leq 0.01$, values without notation are not significantly different

that both methods reduced polyphenol and flavonoid contents significantly. Zhang and Hamauzu (2004) observed a loss of phenolic compounds for boiled and MW broccoli. MW cooking and roasting of buck wheat flour reduced total polyphenol and flavonoid compounds greatly (Zhang et al. 2010). Effect of thermal processing on different group of food polyphenols is not similar, hence, the content and the functionality of some phenolic compounds might also increase during heating. The increase in polyphenol content can be due to degradation of tannin or other component to smaller molecule that carries polyphenol properties (Schütz et al. 2004). Bugianesi et al. (2004) reported that in vivo availability of carotenoids and polyphenols in cooked cherry tomato was significantly superior than the raw one. Compounds which were trapped in the matrix of membrane of ripe fruit were strongly bound with insoluble polyesters that are constituents of tomato fiber. As stated by the authors, the mechanical and heat treatments may provide the energy necessary to break the interactions and improving naringenin bioaccessibility in vivo. In general, the process of germination and dehulling was reported to

improve the nutritive value, antioxidant properties and reduce the anti-nutrients in horse gram by Pal et al. (2016b). Parada and Aguilera (2007) reviewed effect of processing on nutrients and bioactive components bioaccessibility and stated that thermal processing increases the bioaccessibility through softening cell wall and release of compounds from food matrix. Results clearly showed that cooking assisted in better absorption of nutrients, specifically minerals from fortified samples.

Conclusion

The results of the study can be summarized as follows—cooking methods did not affect the nutritional composition of germinated grains and minerals were retained in fortified grains on cooking. Phytate and dietary fiber was higher in microwave cooked products than in PC samples. In vitro protein and starch digestibility was higher in dehulled than in whole grains. Bioactive components were not affected by cooking though bioaccessibility was higher in cooked

Table 5 Percent bioaccessible bioactive components in pressure and microwave cooked germinated green gram

Variations	Sample		Total Phenols				Tannins				Flavonoids			
Dehulled grains														
A. Control	PC		99.14 ± 0.21				103.47 ± 3.58				52.31 ± 1.99			
	MW		99.29 ± 2.75				97.86 ± 0.44				46.31 ± 1.89			
B. Fe (100 mg)	PC		92.53 ± 2.24				96.54 ± 4.49				49.59 ± 0.87			
	MW		93.69 ± 1.07				91.76 ± 4.13				55.16** ± 0.75			
C. Fe (200 mg)	PC		95.83 ± 2.19				96.77 ± 2.99				48.69 ± 0.81			
	MW		101.20* ± 1.05				97.66 ± 0.00				50.07* ± 0.74			
D. Zn (50 mg)	PC		94.61 ± 1.24				101.32 ± 0.00				50.07 ± 0.73			
	MW		94.35 ± 0.93				97.01* ± 1.21				53.70 ± 0.49			
E. Zn (100 mg)	PC		101.05 ± 1.09				98.70 ± 2.64				52.56 ± 1.75			
	MW		99.10 ± 4.48				93.75 ± 0.76				51.58 ± 2.13			
Whole grains														
A. Control	PC		100.57 ± 2.11				104.29 ± 4.12				45.34 ± 0.00			
	MW		98.62 ± 90.89				94.89 ± 2.20				44.99 ± 1.19			
B. Fe (100 mg)	PC		69.86 ± 0.09				93.49 ± 5.02				41.02 ± 1.28			
	MW		90.35* ± 4.82				103.65 ± 1.06				43.71 ± 0.49			
C. Fe (200 mg)	PC		69.11 ± 0.97				72.56 ± 1.94				39.47 ± 1.19			
	MW		82.56* ± 2.04				83.01** ± 2.05				42.51 ± 0.71			
D. Zn (50 mg)	PC		92.40 ± 1.10				98.94 ± 2.22				46.31 ± 0.90			
	MW		103.04* ± 4.205				94.81 ± 2.65				46.99 ± 0.75			
E. Zn (100 mg)	PC		104.48 ± 1.43				94.10 ± 4.42				42.70 ± 0.00			
	MW		103.35 ± 1.83				87.59 ± 1.88				45.19** ± 0.00			
Variations	PC		MW		PC		MW		PC		MW			
	D	W	D	W	D	W	D	W	D	W	D	W		
Multiple comparisons between different variations for percent bioaccessibility of bioactive components														
A. Control	a	a	a	a	a	a	a	bc	a	ab	a	ab		
B. Fe (100 mg)	ab	ab	ab	ab	a	b	a	ab	a	ab	a	ab		
C. Fe (200 mg)	bc	b	bc	b	a	b	a	d	a	b	a	b		
D. Zn (50 mg)	c	a	c	a	a	a	a	a	a	a	a	a		
E. Zn (100 mg)	a	a	a	a	a	b	a	cd	a	a	a	ab		

Different alphabets in a column are statistically different for multiple comparisons

Significant differences between MW and PC on application of 'T' test, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$, values without notation are not significantly different

products. It can be concluded that cooking of green gram germinated in mineral fortified soak water did not affect the composition significantly but nutrient availability was better. Hence, mineral fortified grains can also be used in cooked form.

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