Optimization of germination time and heat treatments for enhanced availability of minerals from leguminous sprouts

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Abstract Germinated legumes are highly nutritious food especially for their enhanced iron bioavailability primarily because of reduction of phytates and increase in ascorbic acid with an advancement of germination period. Length of germination time followed by different heat treatments affect the nutritive value of leguminous sprouts. To optimize germination time and heat treatments for enhanced availability of iron from leguminous sprouts, three legumes namely, mungbean, chickpea and cowpea were germinated for three time periods followed by cooking of sprouts by two cooking methods *ie.* pressure cooking and microwaving. Optimized germination time for mungbean was 12, 16 and 20 h; 36, 48 and 60 h for chickpea and 16, 20 and 24 h for cowpea. Germination process increased ascorbic acid significantly in all the three legumes, the values being 8.24 to 8.87 mg/100 g in mungbean, 9.34 to 9.85 mg/100 g in chickpea and 9.12 to 9.68 mg/100 g in cowpea. Soaking and germination significantly reduced the phytin phosphorus in all the three legumes, the percent reduction being 5.3 to 16.1% during soaking and 25.7 to 46.4% during germination. The reduction in phytin phosphorus after pressure cooking was 9.6% in mungbean, 18.4% in chickpea and 6.1% in cowpea. The corresponding values during microwaving were 8.4, 19.7 and 4.5%. Mineral bioavailability as predicted by phytate:iron enhanced significantly with an increase in germination time. Further reduction *i.e.* 0.9 to 16.3% was observed in three legumes after the two heat treatments. The study concluded that the longer germination periods *ie.* 20 h for mungbean, 60 h for

chickpea and 24 h for cowpea followed by pressure cooking for optimized time were suitable in terms of better iron availability.

Keywords Germination · Heat treatments · Ascorbic acid · Minerals, Molar ratios

Introduction

Nutritional quality of legumes can be enhanced by three approaches *viz.* biotechnology, processing and fortification. Processing technologies should help to transform raw grains into useful products with maximum nutritional value to ensure nutrient security of population for developing countries. With the aim of improving the nutritive value of legumes, preparation techniques have been developed to significantly raise the bioavailability of nutrients. Such techniques include germination, a complex metabolic process during which the lipids, carbohydrates and storage proteins within the seed are broken down in order to provide energy and amino acids necessary for the plant development (Ziegler 1995). The metabolic changes that take place during the different stages of germination influence the bioavailability of essential nutrients. There is a decrease in caloric content of the seed after germination, hence, the nutrient-energy ratio of some vitamins is higher than in the original seeds (Colmenares De Ruiz and Bressani 1990).

Germination is simple, inexpensive and improves the palatability, digestibility and availability of certain nutrients. However, the effect of germination depends on the type of legume and on the conditions and duration of the germination process (Savelkoul *et al.* 1992). Sprouting or controlled germination of legumes reduces the antinutri-

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tional factors and improves their overall nutritional quality (Malleshi and Klopfenstein 1996). Minerals like calcium, zinc and iron are released from bound form. Phytic acid is reduced, so the availability of minerals is increased during germination (El-Adawy 2002). Cooking is important to make the food safe by killing contaminating bacteria, and also to inactivate several heat-labile anti-nutritional factors present in many foods. Microwave ovens are comparatively a recent entry for cooking or reheating foods in Indian homes. Pressure cooking is the most common method of cooking food in Indian households. An increase in the period of pressure cooking is effective in reducing antinutritional factors (Sinha et al. 2005).

The legume sprouts are a popular vegetable in China and Southeast Asia and are often used in meals. They have become increasingly popular in restaurant salad bars and US kitchens particularly with health enthusiasts as these are rich in vitamins and low in carbohydrates (Stephens 2003), however, sprouts are not well known in India where a vast potential for its commercial production, consumption and export exists. Sprout production is a simple germination process that requires neither sunlight nor soil. It has no season limitation. The process is completed in short period. The sprout production is extremely inexpensive, requiring only seeds, sprouting containers and water as inputs. It can therefore, be practical even for poor farmers in augmenting their meager resources. It has a potential of being introduced as a vegetable and as a method of product diversification. The legume sprouts serve as a good alternative vegetable and source of income. This is especially true during the hot summer and rainy seasons when there is acute shortage of fresh vegetables.

While several kinds of legumes may be eaten as sprouts, mungbean, chickpea and cowpea are commonly used and preferred beans for sprouting. Keeping in view the nutritional benefits of sprouts, establishing optimal germination period to enhance ascorbic acid and to decrease the phytic acid that could negatively affect the utilization of minerals holds a great significance.

Materials and methods

Mungbean (PAU911), chickpea (PBG 1) and cowpea (CL367) seeds were procured from the department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. One hundred grams of raw mungbean, chickpea and cowpea were soaked for 10, 12 and 8 h prior to germination for three time periods i.e. 12, 16 and 20 h for mungbean; 36, 48 and 60 h for chickpea and 16, 20 and 24 h for cowpea. The seeds were germinated in seed germinator at 25 °C and 70% RH using 250 mm diameter petridishes. 250 g of sprouts from each legume were

subjected to two hydrothermal treatments namely pressure cooking at 15 lb pressure and microwave cooking at 800 W. The cooking time of mungbean and cowpea was 1 and 7 min for pressure cooking and microwave cooking, respectively while for chickpea, it was 5 and 15 min for the two treatments. The processed samples were dried in hot air oven at 60 °C. The dried samples were ground into fine powder to pass through 5 mm sieve and preserved in 150 gauge polythene bags and stored at ambient conditions (20 °C, 60% RH) for analysis.

Ascorbic acid was estimated in fresh samples using AOAC, 1980 method. The antinutritional factors i.e. phytin phosphorus was estimated using method given by Haug and Lantzch (1983). Calcium, iron and zinc were estimated using Inductively Coupled Plasma Optical Emission Spectrophotometer (Perkin Elmer, D 2100) after wet digestion of samples.

The millimoles of phytic acid and iron were calculated by dividing the milligrams of phytic acid by 660.0 (atomic weight of phytate ion) and the milligrams of 55.8 (atomic weight of iron). The factor used for converting phytin phosphorus to phytate was 3.55. The molar ratio was then calculated by dividing millimoles of phytic acid by millimoles of iron. The millimoles zinc was calculated by dividing the milligrams of zinc by 65.4 (atomic weight of zinc). The molar ratio was then calculated by dividing millimoles of phytic acid by millimoles of zinc. Similarly, millimoles of calcium was calculated by dividing the milligrams of calcium by 40.0 (atomic weight of calcium). The molar ratio of calcium X phytate: zinc was calculated by dividing millimoles of calcium X phytate by millimoles of zinc.

The experiments were conducted in triplicates. The average and standard deviations were computed. Analysis of variance was used to determine the variation between samples of different germination periods and hydrothermal treatments. Critical difference (C.D.) at 5% was calculated where F-ratio was significant.

Results and discussion

The chemical changes in legumes during germination process and in legume sprouts due to heat treatments is shown in Tables 1 and 2, respectively.

The negligible amount of ascorbic acid was present in mungbean, chickpea and cowpea. Germination process increased ascorbic acid enormously in all the three legumes. In all the three legumes, there was a significant ($p \leq 0.05$) reduction in ascorbic acid content when sprouts were either i.e. pressure cooked or microwaved. The percent reduction in ascorbic acid during pressure cooking was 26.9, 23.3 and 24.2% in mungbean, chickpea and cowpea, respectively.

Table 1 Chemical changes in legumes during germination

Germination period	Moisture, g	Ascorbic acid, mg	Iron, mg	Calcium, mg	Zinc, mg	Phytin phosphorus, mg	Phytate: Iron	Phytate: Zinc	Phytate x Calcium :Zinc
Mungbean									
Raw	9.12	0.2	5.7	123.0	3.4	141.5	7.49	14.51	27.29
Soaked	20.79	0.1	5.6	111.4	3.4	125.5	6.70	13.02	22.18
Germinated									
12 h	40.99	8.2	5.7	106.1	3.3	82.0	4.30	8.79	14.32
16 h	42.88	8.5	5.7	106.4	3.4	82.0	4.28	14.42	13.93
20 h	41.75	8.9	5.7	106.5	3.4	78.5	4.10	12.66	13.18
C.D. at 5%	NS	0.08	0.12	1.9	0.13	4.7	-	-	-
Chickpea									
Raw	9.42	0.8	6.9	153.6	2.80	252.0	11.04	31.66	74.36
Soaked	18.86	0.6	6.4	145.8	2.74	238.5	11.18	30.61	68.26
Germinated									
36 h	44.14	9.3	6.4	140.4	2.77	187.5	8.82	23.81	51.10
48 h	44.86	9.6	6.4	141.0	2.78	166.	7.80	21.07	45.42
60 h	44.63	9.9	6.5	139.9	2.82	135.0	6.26	16.84	36.03
C.D. at 5%	NS	0.15	0.16	9.0	NS	9.9	-	-	-
Cowpea									
Raw	9.58	0.3	4.36	78.8	3.0	209.5	14.42	24.81	29.90
Soaked	20.02	0.3	4.28	69.9	2.8	180.5	12.66	22.36	23.91
Germinated									
16 h	39.39	9.1	4.27	64.8	2.9	137.0	9.63	16.85	16.71
20 h	39.10	9.4	4.30	63.9	2.9	126.0	8.79	15.13	14.77
24 h	40.47	9.7	4.32	65.2	2.9	119.0	8.27	14.33	14.28
C.D. at 5%	NS	0.17	NS	1.3	0.08	10.6	-	-	-

NS=Non Significant

Values for ascorbic acid are per 100 g fresh weight.

Values for iron, calcium and zinc are per 100 g dry weight

Phytate: Iron, Phytate: Zinc and Phytate x calcium: zinc molar ratios are calculated values.

The corresponding values during microwave cooking were 25.4, 21.2 and 19.5%. The results revealed that longer germination periods significantly ($p \leq 0.05$) enhanced ascorbic acid in three legumes, while pressure cooking and microwave cooking of sprouts significantly ($p \leq 0.05$) reduced the levels. Riddoch et al. (1998) revealed that many species of legumes produced significant quantities of ascorbic acid upto five days of germination although cooking caused a marked loss of ascorbate. Barakoti and Bains (2007) observed that the ascorbic acid content of germinated mungbean was 9.06 mg/100 g which was reduced to 6.98 mg/100 g on pressure cooking. Gupta et al. (2005) revealed that germination period of 36 h optimum for obtaining maximum ascorbic acid from the seeds of *Lathyrus sativus*.

Soaking and germination of seeds significantly ($p \leq 0.05$) reduced the phytin phosphorus in all the three legumes, the percent reduction being 5.3 to 16.1% during soaking and 25.7 and 46.4% during germination. In chickpea, phytin phosphorus reduced significantly ($p \leq 0.05$) after each stage of germination

indicating that longer germination periods were useful in reducing phytin phosphorus. The germination period was longer in chickpea as compared to mungbean and cowpea. Duhan et al. (2000) reported a significant decrease in the levels of phytic acid during germination. Mbithi et al. (2001) reported that phytates decreased by 85.9% during sprouting of kidney beans for 96 h at 30 °C. The reduction in phytin phosphorus after pressure cooking was 9.6% in mungbean, 18.4% in chickpea and 6.1% in cowpea. The corresponding values during microwaving were 8.4, 19.7 and 4.5%. The greater reduction of phytic acid in chickpea was attributed to longer cooking time when compared to mungbean and chickpea. Mulimani et al. (2003) assessed phytic acid content in red gram after germination and reported a reduction in phytic acid upto the level of 10–11% after soaking, 8–20% after germination and 46–50% after cooking.

No significant variation in iron content of mungbean and cowpea on soaking and after different germination periods was observed while chickpea had a significant ($p \leq 0.05$)

Table 2 Chemical changes in legume sprouts as influenced by heat treatments

Heat treatment	Moisture, g	Ascorbic acid, mg	Iron, mg	Calcium, mg	Zinc, mg	Phytin phosphorus, mg	Phytate : Iron	Phytate : Zinc	Phytate x Calcium: Zinc
Mungbean									
Raw	41.87±0.78	8.5±0.29	5.7±0.05	106.3±1.6	3.35±0.08	80.8±2.3	4.23	8.48	13.81
Pressure cooking	42.13±0.53	6.2±0.17	5.2±0.08	103.7±1.8	3.25±0.06	73.0±1.51	4.19	8.10	12.84
Microwaving	42.57±0.57	6.4±0.26	5.4±0.06	103.5±1.6	3.28±0.08	74.0±2.40	4.15	7.93	12.52
C.D. at 5%	NS	0.49	0.13	NS	NS	4.4	-	-	-
Chickpea									
Raw	44.54±0.30	9.6±0.24	6.4±0.07	140.4±0.5	2.8±0.06	163.3±23.8	7.62	20.55	44.11
Pressure cooking	45.10±0.72	7.4±0.09	6.2±0.03	139.7±1.8	2.6±0.12	133.2±16.6	6.47	18.09	38.64
Microwaving	45.62±0.67	7.6±0.20	6.2±0.09	140.8±1.3	2.6±0.11	131.0±13.0	6.38	17.65	38.01
C.D. at 5%	NS	0.38	0.14	NS	0.12	27.7	-	-	-
Cowpea									
Raw	39.65±0.59	9.4±0.27	4.3±0.05	64.6±0.8	2.90±0.05	127.3±8.3	8.85	15.44	15.25
Pressure cooking	40.13±0.67	7.1±0.06	4.2±0.06	63.4±1.4	2.80±0.12	119.5±4.1	8.62	15.01	14.55
Microwaving	40.73±0.40	7.6±0.20	4.2±0.09	62.5±1.4	2.82±0.09	121.5±6.2	8.68	15.15	14.48
C.D. at 5%	NS	0.39	0.13	NS	NS	NS	-	-	-

NS=Non Significant

Values for ascorbic acid are per 100 g fresh weight.

Values for iron, calcium and zinc are per 100 g dry weight

Phytate: Iron, Phytate : Zinc and Phytate x calcium : zinc molar ratios are calculated values

reduction in iron content when soaked. A significant ($p \leq 0.05$) reduction in iron content was observed after pressure cooking and microwaving the raw sprouts in all three legumes, the percent reduction in mungbean, chickpea and cowpea being 8.9, 3.8 and 3.2% in pressure cooking and 6.6, 3.7 and 2.3% in microwaving, respectively. No difference in iron content was observed when raw sprouts were subjected to either pressure cooking or microwaving. Lee and Karunanithy (1990) found that the loss of iron was less during germination. However, longer cooking periods *i.e.* 40 min decreased the iron by 25 to 47%. Trugo et al. (2000) reported no change in iron content of legumes when subjected to heat treatments.

A significant ($p \leq 0.05$) variation in calcium was observed after soaking in case of mungbean and cowpea. However, no significant ($p \leq 0.05$) difference was observed after different germination periods. A minimal reduction (0.3 to 3.2%) was seen in three legumes after two heat treatments. Lee and Karunanithy (1990) and Barakoti and Bains (2007) found that calcium was low in germinated legumes when compared to raw. Rincon et al. (1993) observed a significant loss of calcium in cowpea during pressure cooking. Contrary to this, Trugo et al. (2000) reported no change in calcium content of legume sprouts when subjected to heat treatment. A significant ($p \leq 0.05$) reduction in zinc levels was observed after soaking and germination in case of mungbean. The zinc content in cowpea decreased significantly ($p \leq 0.05$) after

soaking. No significant ($p \leq 0.05$) difference was observed after germination and between the stages of germination. There was a significant ($p \leq 0.05$) reduction observed in zinc after the two heat treatments in case of chickpea which could be due to leaching of zinc during cooking). However, no difference was found when sprouts were cooked either by pressure cooking or microwaving. Urbano et al. (2006) found that soaking process prior to seed germination was responsible for the losses of zinc in small quantities. Rincon et al. (1993) also found that zinc levels in cowpea reduced significantly during cooking.

The PA:Fe molar ratios was the highest *i.e.* 14.42 in cowpea followed by chickpea (11.04) and mungbean (7.49). The reduction ranged between 1.3 and 12.2% after soaking and 20.10 to 45.25% after germination. Further, reduction *i.e.* 0.9 to 16.3% was observed in three legumes after the two heat treatments. A greater reduction was observed in chickpea due to the longer germinating time. Bains et al. (2006) reported that PA:Fe ratio for mungbean was 8.33. Germination and cooking of mungbean showed a decrease in the PA:Fe ratio when compared to raw due to massive breakdown of phytates. A greater reduction in PA:Fe ratio was reported in cooked germinated samples (3.49) as compared to raw germinated samples (4.16) of mungbean in the reported study. Chickpea had the highest PA:Zn molar ratio of 31.66 followed by cowpea (24.81) and mungbean (14.51). Though soaking resulted in a reduction in PA:Zn ratio between 3.3 and

10.3%, a greater reduction *i.e.* 32 to 46.8% was observed after germination. PA:Zn ratios decreased with the advancement of stages of germination, the most pronounced in chickpea which might be due to longer germination periods. The heat treatments given to chickpea resulted in a greater reduction in PA:Zn when compared to PA:Zn ratio of mungbean and cowpea, the reason being longer cooking times in chickpea. Phytates found in legumes are thought to be major contributor to reduced availability of zinc (Forbes et al. 1984). The PA:Zn molar ratios has been suggested to be an important determinant of zinc bioavailability from human diets (Oberleas and Harland 1981). Morris and Ellis (1980) reported that PA:Zn molar ratio 10:1 or less provides adequate dietary zinc. Bains et al. (2006) reported a PA:Zn ratio of 8.57 in mungbean which was reduced to 6.25 on germination. A further reduction (6.14) was observed when germinated mungbean was cooked. The PA X Ca:Zn was highest in chickpea followed by cowpea and mungbean. Though soaking helped to reduced PA X Ca:Zn (8.2 to 20.0%), the germination process resulted in greater reduction (31.3 to 52.2%). The more pronounced reduction was observed in chickpea as compared to other two legumes. Heat treatments namely pressure cooking and microwaving resulted in a further reduction in the ratio, the range being 4.6 to 45.6%. Fordyce et al. (1987) suggested that PA X Ca:Zn is a better prediction of zinc utilization and PA X Ca:Zn molar ratios above 50 mM/100 g dry diet may be of concern for poor zinc status. Bains et al. (2006) reported that PA X Ca:Zn molar ratio was 29.2 in raw mungbean which was reduced to 17.7 after germination and further reduced to 16.7 after cooking the germinated mungbean. The results of present study were close to the reported study.

The study concluded that there was a gradual increase in ascorbic acid as the germination advanced. Advancement in germination period helped to reduce phytates and tannins. Both pressure cooking and microwave cooking were beneficial in reducing antinutrients in raw sprouts, however, no difference was observed between two heat treatments. The PA:Fe, PA:Zn and PA X Ca:Zn molar ratios as the predictors of mineral availability indicated that longer germination periods *i.e.* 20 h for mungbean, 60 h for chickpea and 24 h for cowpea followed by pressure cooking or microwaving for optimized time enhanced the mineral availability to a significant level.

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