Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes

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Abstract Germination of legumes followed by hydrothermal treatments is an effective mean of improving nutritive value of legumes. The protein content of mungbean, chickpea and cowpea increased by 9–11, 11–16 and 8–11% after germination. A significant ($p \leq 0.05$) decrease in protein content was observed on pressure cooking and microwaving in all three legumes. The carbohydrates decreased by 1 to 3% during soaking and 2 to 6% during germination. A significant ($p \leq 0.05$) improvement in *in vitro* protein digestibility (IVPD) was observed after soaking as well as after three germination periods. Germination resulted in an increase in IVPD from 15 to 25% in mungbean, 6 to 17% in chickpea and 6 to 17% in cowpea. A significant ($p \leq 0.05$) increase in IVPD was observed when raw sprouts of three legumes were subjected to pressure cooking and microwaving. *In vitro* starch digestibility (IVSD) increased significantly ($p \leq 0.05$) after germination, the percent increase being 8 to 12% in mungbean, 9 to 11% in chickpea and 10 to 13% in cowpea. The duration of germination had significant ($p \leq 0.05$) effect on IVSD. A significant ($p \leq 0.05$) improvement in IVSD was observed when legume sprouts were subjected to pressure cooking and microwave cooking.

Keywords Germination · Hydrothermal treatments · *In vitro* protein digestibility · *In vitro* starch digestibility

Introduction

Legumes are important protein source in the diets of people especially those living in tropical and subtropical areas (Khatoon and Prakash 2005). India is the largest producer and consumer in world, it accounts for 33 and 22% of the global area and production of legumes, respectively (Sharma et al. 2007). Legumes have always been an integral part of Indian dietary system and contribute valuable nutrients. Legumes not only add variety to human diet, but also serve as an economical source of supplementary proteins for a large human population in developing countries like India where majority of the population is vegetarian (Sood et al. 2002).

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 (Colmenares De Ruiz and Bressani 1990). Germination is simple, inexpensive and improves the palatability, digestibility and availability of certain nutrients. During germination several enzymes become active; vitamins are increased, whereas there is

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reduction in phytates and tannins (Mehta and Bedi 1993). 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 increases protein and carbohydrate digestibility, enhance some of their vitamin contents, reduces the antinutritional factors and improves their overall nutritional quality (Malleshi and Klopfenstein 1996). As sprouting proceeds, the ratio of essential to non essential amino acids changes, providing more of essential amino acids. Sprouted seeds have more of maltose, therefore, improve the digestibility of carbohydrates.

Heat treatment applied to legumes improves their texture, palatability and nutritive value by gelatinization of starch, denaturation of proteins, increases nutrient availability and inactivation of heat labile toxic compounds and other enzyme inhibitors (Khatoon and Prakash 2005). 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. Cooking with various levels of added moisture and pressure has been reported to increase the efficiency of utilization of legumes to different extents as evaluated by *in vitro* enzymatic starch and legume digestion (Kelkar et al. 1996).

The legume sprouts is a popular vegetable in China and Southeast Asia and is 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. While several kinds of legumes may be eaten as sprouts, the most preferred are mungbean, chickpea and cowpea. The changes in the digestibility of legumes as affected by different germination periods and hydrothermal treatments hold a significance in view of nutritional benefits of the sprouts.

Materials and methods

Mungbean, chickpea and cowpea seeds were procured from the department of Plant Breeding and Genetics, Punjab

Agricultural University, Ludhiana, India. The seeds were germinated in a seed germinator at 25 °C and 70% relative humidity. The sprouts were then subjected to two hydrothermal treatments under optimized conditions shown in Tables 1 and 2. One hundred grams of raw mungbean, chickpea and cowpea were soaked for 10, 12 and 8 h prior to germination for three time periods for each legume (Table 1). 250 g of sprouts from each legume were subjected to two hydrothermal treatments namely pressure cooking at 15 pound 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 samples of each legume after soaking, after germination for three time periods and after two hydrothermal treatments were dried in hot air oven at 60 °C till constant weight. 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.

Moisture, crude protein, ash, crude fat and crude fibre were estimated using AOAC (1990) method. Carbohydrates were calculated by difference. The proximate principles *ie.* moisture, crude protein, ash, crude fat and crude fibre were summed up and subtracted from 100. *In vitro* protein digestibility was estimated by the method of Akesson and Stachmann (1964) modified by Singh and Jambunathan (1981). 0.5 g was digested with 5 ml of HCl solution (pH 2.0) containing 2 mg of pepsin (P2000 HD821, Sigma Chemicals Limited, USA). The flask was incubated in a water bath shaker for 16 h at 37 °C. Two ml of pancreatin (P7547 H0159, Sigma Chemicals Limited, USA) solution was added and the contents were further incubated for 24 h. (Pancreatin solution was prepared by dissolving 50 mg of pancreatin in 100 ml of 0.1 M borate buffer (pH 6.8), containing 0.025 M CaCl₂. After 24 h, the reaction was stopped by adding 7.0 ml of 10% (W/V) trichloroacetic acid (TCA) and the suspension was centrifuged (at 10,000 rpm) for 15 min. The residue was washed twice with 5 ml of 5% TCA. Finally this 25.0 ml of volume was taken to estimate the nitrogen content by the standard microkjeldhal method.

In vitro starch digestibility was estimated by the method given by Singh et al. (1982). About 50 mg of defatted

Table 1 Optimized germination periods for legumes

Legume	Weight of sample, g	Size of petri dish, mm	Temperature °C	Soaking period, h	Germination period, hours		
					1	2	3
Mungbean	100	200	25	10	12	16	20
Chickpea	100	200	25	12	36	48	60
Cowpea	100	200	25	8	16	20	24

Table 2 Optimized hydrothermal treatments for legumes

Legume	Sprouted legumes, g	Time of hydrothermal treatment, min		Sprouted legumes: Water
		Pressure cooking at 15 lb pressure	Microwave cooking at 800 W	
Mungbean	250	1	7	1: 0.24
Chickpea	250	5	15	1: 0.60
Cowpea	250	1	7	1: 0.20

sample was dispersed in 1.0 ml of 0.2 M phosphate buffer (pH 6.9). About 20 mg of pancreatic α amylase dissolved in 50 ml of the same buffer and 0.5 ml of it was added to the sample suspension and incubated at 37 °C for 2 h. About 2 ml of 3–5 dinitrosalicylic acid (10% aqueous solution) was added and the mixture was heated for 5 min in a water bath. After cooling, the solution was made up to 25 ml with distilled water and filtered prior to measurement of absorbance at 550 nm. A blank was run simultaneously

by adding first of 3–5 dinitrosalicylic acid to the same suspension before the addition of the enzyme solution and then incubated at 37 °C for 2 h. Maltose was used for the standard curve determination and the *in vitro* starch digestibility values were expressed as percent maltose released from 100% starch present in the sample.

The experiments were conducted in triplicates. The average and standard deviations were computed. Analysis of variance was used to determine the variation between

Table 3 Effect of germination period on proximate composition of legumes (g/100 g dry matter)

Germination periods	Moisture	Crude protein	Ash	Crude fat	Crude fibre	CHO ^a
Mungbean						
Raw	9.12	22.31	3.63	1.40	3.90	59.64
Soaked	7.76	23.41 (4.9)	3.88 (6.88)	1.40 (0.0)	4.77 (22.0)	58.78 (−1.4)
Germinated						
12 h	7.07	24.37 (9.2)	3.90 (7.43)	1.29 (−7.8)	4.64 (−18.7)	58.73 (−1.5)
16 h	8.41	24.49 (9.7)	3.93 (8.26)	1.25 (−10.7)	4.92 (26.1)	57.00 (−4.4)
20 h	8.32	24.93 (11.1)	3.95 (8.81)	1.26 (−10.0)	4.92 (26.0)	56.62 (−5.1)
C.D. at 5%	0.73	0.40	0.31	NS	0.55	—
Chickpea						
Raw	9.42	20.37	3.12	4.08	11.25	51.76
Soaked	8.61	21.62 (6.1)	3.42 (9.61)	4.04 (−0.9)	12.31 (9.4)	50 (−3.4)
Germinated						
36 h	7.35	23.40 (11.4)	3.79 (21.4)	3.43 (−15.9)	12.68 (12.7)	49.35 (−4.6)
48 h	7.11	23.58 (15.7)	3.84 (23.07)	3.34 (−18.1)	13.12 (16.6)	49.01 (−5.3)
60 h	7.45	23.62 (16.0)	3.85 (23.39)	3.12 (−23.5)	13.19 (17.2)	48.77 (−5.8)
C.D. at 5%	0.58	1.66	0.24	NS	0.95	—
Cowpea						
Raw	9.58	22.53	3.47	1.50	3.78	59.13
Soaked	7.87	23.06 (2.4)	3.48 (0.28)	1.49 (−0.7)	4.16 (10.1)	59.96 (−1.4)
Germinated						
16 h	9.58	24.28 (7.8)	3.55 (2.01)	1.41 (−6.0)	4.54 (20.1)	56.54 (−4.4)
20 h	9.57	24.51 (8.7)	3.61 (3.73)	1.36 (−9.0)	4.62 (22.2)	56.34 (−3.9)
24 h	9.49	24.94 (10.8)	3.72 (6.89)	1.34 (−10.7)	4.70 (24.3)	55.80 (−5.9)
C.D. at 5%	0.40	1.67	0.08	0.45	0.78	—

Values in parenthesis are percent change in comparison to raw samples.

^a CHO: Carbohydrates = $100 - (\text{Moisture} + \text{Protein} + \text{Ash} + \text{Fat} + \text{Fibre})$

NS Non Significant

Table 4 Effect of heat treatment on proximate composition of legumes sprouts (g/100 g dry matter)

Heat treatments	Moisture	Crude protein	Ash	Crude fat	Crude fibre	CHO ^a
Mungbean						
Raw						
Range	7.01–8.38	24.28–24.93	3.89–3.95	1.22–1.29	4.81–5.04	
Mean±SD	7.93±0.68	24.60±0.30	3.92±0.02	1.27±0.03	4.83±0.22	57.45
Pressure cooking						
Range	7.10–8.25	22.30–22.82	3.5–3.88	1.40–1.42	4.46–5.33	
Mean±SD	7.76±0.39	22.76±0.66 (−7.5)	3.70±0.12 (−5.6)	1.35±0.13 (−6.3)	4.94±0.37 (−2.3)	59.49 (3.5)
Microwaving						
Range	7.79–8.86	22.06–22.50	3.45–3.8	1.12–1.5	4.28–5.02	
Mean±SD	8.39±0.49	22.23±0.17 (−9.6)	3.64±0.14 (−7.1)	1.31±0.14 (−3.1)	4.73±0.30 (−2.1)	59.70 (3.9)
C.D. at 5%	NS	0.86	0.22	NS	NS	–
Chickpea						
Raw						
Range	7.02–7.87	23.18–23.68	3.64–3.94	12.38–12.86	71.95–79.43	
Mean±SD	7.30±0.31	23.42±0.24	3.82±0.10	3.30±0.39	12.67±0.16	49.49
Pressure cooking						
Range	8.04–8.16	21.43–22.75	3.45–3.81	12.08–13.54	74.94–82.05	
Mean±SD	8.12±0.08	22.35±0.49 (−4.6)	3.58±0.1 (−6.3)	3.11±0.43 (−5.8)	12.86±0.62 (1.5)	49.98 (0.9)
Microwaving						
Range	7.73–8.66	20.34–22.50	3.29–3.80	11.95–13.5	73.82–81.30	
Mean±SD	8.15±0.30	21.71±0.83 (−7.3)	3.51±0.19 (−8.1)	2.79±0.52 (−15.4)	12.82±0.62 (1.2)	51.02 (3.1)
C.D. at 5%	1.00	1.17	0.63	NS	NS	–
Cowpea						
Raw						
Range	8.31–9.55	23.62–24.95	3.52–3.74	1.24–1.56	3.98–4.75	
Mean±SD	9.21±0.53	24.58±0.55	3.63±0.08	1.37±0.14	4.60±0.38	56.61
Pressure cooking						
Range	8.08–8.20	22.06–22.93	3.48–3.67	1.26–1.36	4.33–4.98	
Mean±SD	8.13±0.07	22.50±0.39 (−8.6)	3.56±0.07 (−1.9)	1.31±0.04 (−4.4)	4.65±0.24 (1.1)	59.85 (5.7)
Microwaving						
Range	8.20–9.66	21.62–22.84	3.26–3.59	1.27–1.36	4.24–4.96	
Mean±SD	8.85±0.69	22.60±0.75 (−8.0)	3.47±0.14 (−4.4)	1.34±0.06 (−2.8)	4.55±0.33 (−1.1)	59.19 (4.5)
C.D. at 5%	0.50	1.15	0.30	NS	NS	–

Values in parenthesis are percent change in comparison to raw samples.

^a CHO: Carbohydrates = 100 − (Moisture + Protein + Ash + Fat + Fibre)

NS Non Significant

samples of different germination periods and hydrothermal treatments. Critical difference (C.D.) at 5% was calculated where F-ratio was significant.

Results and discussion

Effect of germination periods and hydrothermal treatments on proximate composition of legume sprouts is shown in Tables 3 and 4, respectively.

The protein content of mungbean, chickpea and cowpea increased by 5, 6 and 2% after soaking while the corresponding increase during germination ranged between 9 to 11, 11 to 16 and 8 to 11%. A significant ($p \leq 0.05$) increase in protein content was found after germination for 12, 16 and 20 h. However, no significant variation in protein content was observed among samples germinated for different time periods. Khadar (1983) and Dogra et al. (2001) also reported that the protein content of soybean increased significantly after germination. On the other

Table 5 Effect of germination periods on *in vitro* digestibility of protein and starch of legumes (per 100 g dry matter)

Germination period	<i>In vitro</i> protein digestibility, %	<i>In vitro</i> starch digestibility, %
Mungbean		
Raw	66.4	81.1
Soaked	68.6 (3.3)	82.1 (1.3)
Germinated		
12 h	76.2 (14.7)	87.6 (8.1)
16 h	78.8 (18.7)	88.8 (9.5)
20 h	83.0 (25.0)	90.6 (11.7)
C.D. at 5%	1.6	1.8
Chickpea		
Raw	67.7	79.7
Soaked	69.8 (3.1)	81.2 (1.9)
Germinated		
36 h	72.0 (6.3)	86.6 (8.6)
48 h	75.7 (11.8)	87.6 (9.9)
60 h	79.0 (16.7)	88.1 (10.5)
C.D. at 5%	0.9	0.9
Cowpea		
Raw	73.3	80.9
Soaked	77.4 (5.6)	80.4 (−0.6)
Germinated		
16 h	77.6 (5.9)	88.8 (9.8)
20 h	80.0 (9.1)	90.9 (12.4)
24 h	85.7 (16.9)	91.1 (12.6)
C.D. at 5%	1.6	1.5

Values in parenthesis are percent change in comparison to raw samples.

hand, heating of soaked and germinated grains significantly decreased the protein content. The results of the study by Sood et al. (2002) reported an increase of 3 to 7% in crude protein content of chickpea on soaking and 23 to 26% on germination. Akinyele and Akinlosotu (1991) reported that soaking of cowpeas for 4 h increased the protein content by 5.7%. Germination increased the protein content by 26.2 and 33.4% in two varieties of cowpea. These however, decreased after indirect cooking. The increase in protein content was attributed to loss in dry weight, particularly carbohydrates through respiration during germination. A significant ($p \leq 0.05$) decrease in protein content was observed on pressure cooking and microwaving the germinated legumes. However, no significant difference was formed between the samples subjected to pressure cooking or microwaving. Opuku et al. (1981) reported an increase in protein content on sprouting ranging from 14 to 40%. Pressure cooking, solar cooking and parching significantly decreased the levels of protein in chickpea sprouts. Decrease in protein after cooking was also reported by Khalil et al. (1986) in fieldpea, mothbean and pigeonpea. King and Puswastein (1987) reported an increase in the protein on sprouting of legumes. They attributed this to synthesis of new proteins.

A significant ($p \leq 0.05$) increase in ash content was observed after germination periods in all the three legumes when compared to raw samples. However, no significant difference was observed when three germinated periods were compared with each other. A significant ($p \leq 0.05$) decrease in ash content was observed on pressure cooking and microwaving the germinated legumes. However, no significant difference was found between the samples subjected to pressure cooking or microwaving. A significant decrease in ash content in chickpea was observed by Sood et al. (2002) after germination and cooking. Barakoti (2004) also reported a significant ($p \leq 0.05$) reduction in ash content of mungbean after germination and cooking.

Fat in mungbean, chickpea and cowpea did not alter significantly after soaking and germination, though there was a gradual decline in fat contents of all the three legumes, the percent decrease being 8 to 11 in mungbean, 16 to 24 in chickpea and 6 to 11% in cowpea during different germination periods. A non significant change was observed in case of germinated legumes subjected to pressure cooking and microwaving. Dhaliwal and Aggarwal (1999) reported that the fat content of soybean decreased with increased germination time. The results of the study are par with the observation made in the reported study.

Table 6 Effect of heat treatments on *in vitro* digestibility of protein and starch of legume sprouts (per 100 g dry matter)

Heat treatment	<i>In vitro</i> protein digestibility, %	<i>In vitro</i> starch digestibility, %
Mungbean		
Raw		
Range	75.8–83.7	87.6–91.6
Mean±SD	79.5±3.0	89.0±1.5
Pressure cooking		
Range	78.1–86.3	94.6–95.8
Mean±SD	82.2±3.0 (3.4)	94.9±2.5 (6.6)
Microwaving		
Range	77.6–85.3	93.0–93.6
Mean±SD	81.5±2.9 (2.5)	93.3±2.3 (4.8)
C.D. at 5%	NS	3.3
Chickpea		
Raw		
Range	71.9–78.5	86.2–88.2
Mean±SD	75.6±3.1	87.4±0.8
Pressure cooking		
Range	74.9–82.0	91.9–94.0
Mean±SD	78.0±3.0 (3.7)	93.1±0.9 (6.5)
Microwaving		
Range	73.8–81.3	91.9–93.8
Mean±SD	77.2±2.7 (2.1)	93.0±0.9 (6.4)
C.D. at 5%	NS	1.7
Cowpea		
Raw		
Range	77.1–86.6	88.3–91.2
Mean±SD	81.1±3.8	90.3±1.2
Pressure cooking		
Range	79.5–87.9	94.1–95.6
Mean±SD	82.5±3.0 (1.7)	94.8±0.7 (5.0)
Microwaving		
Range	80.2–88.6	93.8–95.5
Mean±SD	82.9±3.93 (2.2)	94.6±0.7 (4.8)
C.D. at 5%	NS	1.8

NS Non significant

Out of three legumes, chickpea had the highest fibre content (11 g/100 g dry matter) followed by mungbean and cowpea (4 g). There was a significant ($p \leq 0.05$) change in crude fibre after soaking. Germination for different periods increased the fibre content, the range being 19 to 26% in mungbean, 13 to 17% in chickpea and 20 to 24% in cowpea. No significant change was observed when germinated legumes were subjected to either pressure cooking or microwave cooking. A significant increase in fibre during germination process of chickpea was also reported by Sood et al. (2002).

There was a reduction in carbohydrates on germination, the range being 2 to 5% in mungbean, 5 to 6% in chickpea and 4 to 6% in cowpea. Increased popularity of sprouts in United States particularly with health enthusiasts is because

sprouts are rich in vitamins and low in carbohydrates (Stephens 2003).

There was gradual increase in protein, ash and fibre with an increase in germination time in case of all legumes. The fats and carbohydrates decreased with an increase in germination time indicating that germinated legumes had lower energy content because of low fat and carbohydrates. Colmenares De Ruiz and Bressani (1990) observed a decrease in carbohydrate and fat content of the legumes after germination, hence, the nutrient-energy ratio of some vitamins is higher than in the original seeds. For all the proximate principles, no significant ($p \leq 0.05$) difference was found when legumes were germinated for different time periods. Two hydrothermal treatments namely pressure cooking and microwaving reduced the protein content by

8 to 10% in mungbean, 5 to 7% in chickpea and 8 to 9% in cowpea. The percent reduction in ash was by 6 to 7 in mungbean, 6 to 8 in chickpea and 2 to 4% in cowpea. No significant effect of two hydrothermal treatments was observed on crude fat and fibre contents for all the three legumes.

Effect of germination periods and hydrothermal treatments on IVPD and IVSD of legume sprouts is shown in Tables 5 and 6, respectively. A significant ($p \leq 0.05$) improvement in IVPD was observed after soaking as well as after three germination periods. The percent increase in IVPD during soaking was 3 to 6% in three legumes, whereas during germination it was increased from 15 to 25% in mungbean, 6 to 17% in chickpea and 6 to 17% in cowpea. There was a significant ($p \leq 0.05$) improvement in IVPD after every stage of germination in three legumes, thus indicating that longer germination periods helped to improve IVPD. A significant ($p \leq 0.05$) increase in IVPD was also observed when germinated legumes were subjected to pressure cooking and microwaving. Jimenez et al. (1985) revealed that the protein digestibility increased as the germination period advanced and obtained better values when autoclaved. Preet and Punia (2000) and Trugo et al. (2000) observed a progressive increase in protein digestibility as the germination advanced. Protein digestibility of germinated seeds further improved with hydrothermal treatments.

Very little change in IVSD was found after soaking, however IVSD increased significantly ($p \leq 0.05$) after germination, the percent increase being 8 to 12% in mungbean, 9 to 11% in chickpea and 10 to 13% in cowpea. The duration of germination had significant ($p \leq 0.05$) effect on IVSD i.e. 12 h germination of mungbean had significantly ($p \leq 0.05$) higher IVSD when compared to 20 h germination. Similar trend was observed in chickpea and cowpea. The results indicated that mungbean, chickpea and cowpea germinated for 20, 60 and 24 h had significantly ($p \leq 0.05$) higher IVSD than shorter germination periods.

A significant ($p \leq 0.05$) improvement in IVSD was observed when raw sprouts were subjected to pressure cooking and microwaving. However, no difference was observed when legume sprouts were either pressure cooked or microwaved. The results revealed that long germination periods followed by either of two hydrothermal treatments were useful in improving IVSD. Kelkar et al. (1996) reported that the germination of legumes increased the digestibility by 23 to 35% and was attributed to the degradation of starch into smaller fragments and formation of reducing sugars. The study concluded that germination of legumes followed by hydrothermal treatments is an effective mean of improving protein and starch digestibility of legumes. It is recommended that longer germination periods i.e. 20 h for mungbean, 60 h for chickpea and 24 h for cowpea followed by pressure cooking for optimized

time are suitable to enhance protein and starch digestibility of three legumes.

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