Sprouting characteristics and associated changes in nutritional composition of cowpea (*Vigna unguiculata*)

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Abstract Cowpea (*Vigna unguiculata*), is an important arid legume with a good source of energy, protein, vitamins, minerals and dietary fibre. Sprouting of legumes enhances the bioavailability and digestibility of nutrients and therefore plays an important role in human nutrition. Improved varieties of grain cowpea viz. Pant Lobia-1 (PL-1) and Pant Lobia-2 (PL-2) and Pant Lobia-3 (PL-3) were examined for sprouting characteristics and associated changes in nutritional quality. Soaking time, sprouting time and sprouting temperature combinations for desirable sprout length of ¼ to ½ inch for cowpea seed samples were standardized. All the observations were taken in triplicate except soaking time, where six observations were taken in a completely randomized design of three treatments. Results revealed that optimum soaking time of PL-1 and PL-2 seed was 3 h whereas PL-3 required 9 h. Sprouting period of 24 h at 25 °C was found to be desirable for obtaining good sprouts. Significant improvement in nutritional quality was observed after sprouting at 25 °C for 24 h; protein increased by 9–12 %, vitamin C increased by 4–38 times, phytic acid decreased by 4–16 times, trypsin inhibitor activity decreased by 28–55 % along with an increase of 8–20 % in in-vitro protein digestibility.

Keywords Sprouting · Cowpea · Improved varieties · Nutritional quality

Introduction

Cowpeas (*Vigna unguiculata*), an important legume is a versatile crop, also commonly known as southern pea, black eye pea, crowder pea, lubia, niebe, coupe or Frijole. Cowpea grain contains, on an average, 23–25 % protein and 50–67 % carbohydrate. As in case of most legumes, amino acid profile of cowpea complements cereal grains. Presence of significant amounts of protein, calories and some water-soluble vitamins, makes cowpea a promising food ingredient.

Cooking and sprouting of legumes greatly influence nutritional quality by increasing bioavailability of nutrients as well as enhancing digestibility and utilization of nutrients (Obboh et al. 2000). During sprouting metabolic enzymes such as proteinases are activated which may lead to release of some amino acids and peptides and synthesis or utilization of these may form new proteins. As a consequence, nutritional quality of proteins may be enhanced by sprouting in legumes and other seeds (Gulewicz et al. 2008).

Sprouting characteristics like sprouting rate gives information about the age of seeds. Aging retards sprouting rate and affects viability appreciably. Sprouting characteristics are also important from sensory point of view of seeds. Storage-induced textural defects in legumes prolong cooking time and demand higher fuel inputs for food preparation (Aguilera and Stanley 1985) and presence of certain anti-nutritional factors and non-digestible components might be the reason for their underutilization. Increased utilization of legumes in general and cowpea, in specific, will depend upon development of appropriate technologies to produce food products with enhanced nutritional quality (Prinyawiwatkul et al. 1996).

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The present investigation envisages understanding the sprouting characteristics and effect of sprouting on nutritional quality of three newly released and improved varieties of cowpea for high yield and early maturity viz. Pant lobia-1 (PL-1), Pant lobia-2 (PL-2) and Pant lobia-3 (PL-3).

Materials and methods

Freshly harvested seeds of three improved varieties of cowpea viz. PL-1, PL-2 and PL-3, were procured from Breeder's Seed Production Centre, Pantnagar. The cowpea seeds were cleaned free of dust, chaff and grit and dried at 60 °C for 1 h, cooled and stored in air tight container at room temperature.

Soaking time and sprouting time and sprouting temperature combinations for desirable sprout length of $1/4$ to $1/2$ inch (www.beansprouts.com) for cowpea seed samples were standardized. The time required for complete soaking of seeds before sprouting was taken as time required in reaching maximum water uptake by seeds as shown by further negligible change in weight of soaked seeds after soaking.

Sprouting characteristics of sprouts viz. sprouting rate, sprouting capacity and sprout length of cowpea seeds were measured by Pinzino (1999) method, in which seeds were sprouted at 23 °C, with slight modifications.

Cowpea samples (raw and sprouted) were milled in Wiley mill to pass through 60 mesh size sieve to obtain homogenous ground sample for chemical analysis and stored in air tight containers till further use. Proximate composition, minerals like calcium, iron and vitamin C of raw and sprouted seeds were estimated by AOAC (1995) methods. Phytic acid was estimated by method of Wheeler and Ferrel (1971). Trypsin inhibitor was estimated by Roy and Rao (1971) method and in-vitro protein digestibility by Akeson and Stahman (1964) method with slight modifications. All the quantitative data was computed in terms of mean and standard deviation (Snedecor and Cochran 1967). The significance difference between cowpea genotypes under study for nutritive quality was determined by one way analysis of variance in a completely randomized design at 5 % level of significance.

Results and discussion

Time required for complete soaking of cowpea seeds The time required for complete soaking of seeds in both PL-1 and PL-2 at room temperature was observed to be 3 h with the PL-3 requiring exceptionally longer time of 9 h (Table 1). Higher soaking time of PL-3 may be attributed to its firmer attachment of seed coat to cotyledons (Olapade et al. 2002).

Best sprouting time and temperature combination for cowpea seeds Considering the desirable characteristics of sprouts enlisted in Table 2, 24 h germination at 25 °C was taken as standard. It was used for sprouting in the experiment to observe effect of sprouting on nutritional composition.

Sprouting characteristics The sprouting characteristics of the genotypes under study are shown in Table 3. Variety PL-2 had highest sprouting rate whereas PL-1 had lowest (Table 3). There was significant difference in sprouting rate of all genotypes. Reduction in the rate of sprouting may be expression of aging of embryo and changes in the remainder of the seed (Pinzino 1999). Significant difference was observed in sprouting capacity for different time periods in all genotypes. Similar observations were made by Murugkar and Jha (2009) in soybean seeds. It was observed that sprout length of cowpea increased with increase in sprouting time (Table 3). In 24 h of sprouting, PL-2 had significantly higher sprout length than PL-1 and PL-3. No significant difference was observed between PL-1 and PL-3. It can be noted that after sprouting for 48 h, sprout length of PL-3 came at par with that of PL-2, however significant difference re-emerged after 72 h of sprouting. Of all three cowpea genotypes under study, PL-2 showed maximum potential in terms of sprouting rate, sprouting capacity and sprout length.

Effect of sprouting on nutritional composition of cowpea seeds Sprouting effect was observed by comparing nutritional composition viz. proximate principles, two minerals (calcium and iron), vitamin C and anti-nutrients (phytic acid and trypsin inhibitor activity) and in-vitro protein digestibility of raw and sprouted cowpea at 25 °C for 24 h and the results are shown in Table 4.

Proximate composition Effect of sprouting on proximate composition of cowpea sprouts is shown in Table 4. The moisture content of cowpea significantly increased in all the genotypes after sprouting. After entry of water in seed coat, seed swelling starts and initiates sprouting. During sprouting, the water intake of seed varies. The percent increase was highest in PL-1 i.e., 22.9 %. Murugkar and Jha (2009) also observed an increase in moisture 5.4 to 56.1 % after 48 h of sprouting in soybean. Uwaegbute et al. (2000) observed that the moisture increased from 15.6 to 17.6 % after sprouting cowpea. Usually during sprouting, seeds absorb water by a process called imbibitions (Nonogaki et al. 2010, Sampath et al. 2008).

Significant increase in the ash content was observed after sprouting in each genotype. Similar results were reported by Ranhotra et al. (1977). Increase in ash content may be apparent due to loss of starch (Lorenz 1980).

There was significant increase in crude protein after sprouting in all genotypes. Mehta et al. (2007) have found that

Table 1 Time required for complete soaking of cowpea seed samples under study ($n=6$)

Genotypes	Weight of sample (g) in different soaking time (h)											
	0	1	2	3	4	5	6	7	8	9	10	11
PL-1	100	145.6±2.44	188.6±1.14	202.6±1.03	204.7±1.71	207.3±3.78	207.2±4.81	–	–	–	–	–
PL-2	100	142.5±3.71	197.5±1.73	203.0±2.96	204.0±3.30	208.0±2.60	209.0±2.63	–	–	–	–	–
PL-3	100	105.8±2.48	111.4±1.62	116.3±2.99	126.3±4.51	133.3±7.61	148.4±4.42	175.2±3.86	199.1±1.74	205.7±1.58	205.8±1.77	205.7±2.19

19.15 % increase in crude protein content of cowpea after 28 h of sprouting. Uppal and Bains (2012) observed crude protein increase from 8 to 11 % after sprouting. Apparent increase in protein content may be attributed to loss in dry matter, particularly carbohydrates through respiration during sprouting (Uppal and Bains 2012). Higher sprouting temperature and longer sprouting time would mean greater loss in dry weight and more increase in crude protein content. There is reawakening of protein synthesis upon imbibitions (Nonogaki et al. 2010), which leads to increase in protein content in sprouted seeds.

The fat content of sprouted cowpea decreased significantly when compared to its unsprouted counterparts in all three genotypes. Decrease in fat content may be due to depletion of the fat stored that contributed to the catabolic activities of the seeds during sprouting (Onimawo and Asugo 2004). Kornberg and Beevers (1957) reported that degradation of reserve nutrients (lipids and carbohydrates) during sprouting is a process whose essential purpose is to provide the energy required for protein synthesis in plant growth. Kwon (1994) suggested that fatty acids are oxidized to CO_2 and H_2O to generate energy for sprouting and there is synthesis of certain structural constituents in young seedlings. Hahm et al. (2009) reported that there was 44 % decrease in fat content in sesame seeds after 4 days of sprouting. Results of the study are at par with the observation made in the study of Uppal and Bains (2012).

Significant increase in crude fibre was observed after sprouting. Increase in crude fibre was also significant when compared between genotypes. Result obtained was in accordance with that of Uppal and Bains (2012) where 20 to 24 % increase has been reported after sprouting cowpea. A significant increase in fibre during sprouting process of chickpea was also reported by Sood et al. (2002). Ranhotra et al. (1977) also noted increase in crude fibre content due to sprouting. Increase in crude fibre content has been considered only as apparent and may be attributed to the disappearance of starch.

Carbohydrate content of sprouted cowpea was significantly decreased from their raw counterparts. Comparison between genotypes also showed significant decrease in carbohydrate content. Uppal and Bains (2012) reported 5.6 % decrease and Jirapa et al. (2001) reported 2.34 % decrease in carbohydrate content after 24 h of sprouting in cowpea. Rusydi et al. (2011) showed decrease in carbohydrate content of rice after 24 h of sprouting. Vidal-Valverde et al. (2002) explained that during sprouting, carbohydrate was used as source of energy for embryonic growth which could explain the changes of carbohydrate content after sprouting. Additionally, β -amylase activity that hydrolyzes the starch into simple carbohydrate was increased (Suda et al. 1986). Starch in cotyledon was broken down into smaller molecules such as glucose and fructose to provide energy for cell division while the seeds mature and

Table 2 Observation for standardization for best sprouting temperature and time for desirable sprout length

Temperature (°C)	24 h	48 h	72 h
22	Few seeds were sprouted.	Seeds had developed a mild foul smell.	Seeds developed off colour and odour.
25	Desirable sprout length and good percentage of sprouted seeds. No signs of off colour, flavour and odour	Sprout length within desirable limit but seeds were slimy and had mild foul smell	Very long sprouts and developed off colour, flavour and odour.
28	Sprout length desirable but less sprouted seeds. Developed sliminess and mild off flavour.	Long sprouts but seeds were dried. Developed off colour	Very long sprouts but seeds were very dried and shrunken. Developed off colour.

grow (Nonogaki et al. 2010, Vidal-Valverde et al. 2002). Apart from starch oligosaccharide content of cowpea also decreased with sprouting. Sampath et al. (2008) observed total loss of oligosaccharide during sprouting after 48 h of sprouting. This may be due to breakdown by enzyme into simple sugars.

Minerals Sprouting significantly increased calcium content of cowpea by 9.97, 5.94 and 7.24 % for PL-1, PL-3 and PL-2, shown in Table 4. Dave et al. (2008) observed 24 to 62 % increase in calcium content of cowpea after sprouting. Increase in calcium content may be attributed to presence of calcium salts in water used during sprouting process (Ranhotra et al. 1977).

There was slight increase in iron content after sprouting of cowpea genotypes but statistical analysis did not show any significant increase. Bains et al. (2011) also reported that soaking and different sprouting periods do not give any significant variation in iron content of mungbean and cowpea.

Vitamin C Increase in vitamin C content of all three cowpea genotypes after sprouting was remarkably significant (Table 4). Vitamin C in PL-1 increased 37.87 times of raw vitamin C content, 9.42 times increase in PL-2 and four-fold increase in vitamin C content in PL-3 after sprouting. Uppal and Bains (2012) reported 9.4 times increase in vitamin C content after 24 h of sprouting. They reported that longer the sprouting periods significantly enhanced ascorbic acid in

chickpea, cowpea and mungbean. Sangronis and Machado (2005) also observed 10 times increase in vitamin C content of pigeon pea after 2 h sprouting. It was observed that during germination vitamin C are synthesized (Bibi et al. 2008) as a consequence of the reactivation of **vitamin C** biosynthesis undergone in the seeds (Mao et al. 2005). This may be the reason for the increase in vitamin C level in all three sprouted cowpea genotypes.

Anti-nutrients Significant reduction in anti-nutrient content was observed after sprouting, which is shown in Table 4. Decrease in phytic acid was 16.2, 8.4 and 4.8 times over raw values for PL-1, PL-2 and PL-3, respectively. Modgil et al. (2009) observed 36.02 % decrease of phytic acid in fenugreek seeds after 24 h sprouting. Chopra and Sankhalla (2004) observed 24 % decrease in phytic acid in horse gram and 32 % in moth beans after sprouting. Uppal and Bains (2012) observed 43.19 % decrease in phytic acid in cowpea after sprouting for 24 h. During soaking, phytate ions get leached in soaking water due to concentration gradient (Modgil et al. 2009). Enzymatic hydrolysis of phytate phosphorous due to increased phytase activity during sprouting decreases phytic acid content and also releases soluble proteins and minerals (Shah et al. 2011).

Statistical analysis showed significant decrease in trypsin inhibitor activity (TIA) of cowpea after sprouting. PL-3 (54.77 %) showed highest percent decrease in TIA followed by PL-2 (44.94 %) and PL-1 (28.53 %). Ramakrishna et al.

Table 3 Sprouting characteristics[#] of cowpea at 23 °C (n=3)

Sprouting characteristics		PL-1	PL-2	PL-3	CD at 5 %
Sprouting rate		38.96±0.97 ^a	146.08±1.21 ^b	59.42±1.68 ^c	2.36
Sprouting capacity (%)	At 24 h	7.33±0.57 ^c	44.67±0.57 ^a	15.00±1.00 ^b	1.48
	At 48 h	17.67±0.57 ^c	50.67±0.57 ^a	23.33±0.57 ^b	1.15
	At 72 h	21.67±0.57 ^c	53.00±1.00 ^a	27.00±1.00 ^b	1.75
Sprout length (cm)	At 24 h	0.67±0.05	0.91±0.08 ^a	0.67±0.01	0.12
	At 48 h	1.27±0.25	2.11±0.09	2.03±0.03	0.32
	At 72 h	2.25±0.09 ^a	3.32±0.12 ^b	2.49±0.05 ^c	0.19

Values with different superscripts shows significant difference from one another within the row

[#] Mean ± SD

Table 4 Effect of sprouting on chemical composition and in-vitro protein digestibility (IVPD)[#] after sprouting (per 100 g dry matter) (*n*=3)

Nutrients	PL-1	PL-2	PL-3	CD at 5 %
Moisture (g)				
R	6.91±0.71 ^b	6.98±0.06 ^b	9.78±0.28 ^a	0.88
S	8.49±0.39 ^b (22.9)	8.47±0.51 ^b (21.3)	10.57±0.03 ^a (8.1)	0.74
Ash (g)				
R	3.78±0.12 ^b	4.26±0.22 ^a	3.93±0.11 ^b	0.31
S	3.94±0.16 ^b (4.2)	4.50±0.19 ^a (5.6)	4.11±0.03 ^b (4.3)	0.29
Crude protein (g)				
R	27.15±0.26 ^b	30.87±0.75 ^a	24.99±0.08 ^c	0.92
S	29.72±0.04 ^c (9.5)	33.62±0.58 ^a (8.8)	28.14±0.12 ^b (12.7)	0.68
Crude fat (g)				
R	2.63±0.27	2.91±0.38	2.75±0.06	0.54
S	1.99±0.13 ^c (−24.3)	2.19±0.04 ^b (−24.7)	2.50±0.07 ^a (−9.1)	0.18
Crude fibre(g)				
R	4.43±0.54	4.99±0.19	4.26±0.11	0.67
S	6.47±0.13 ^a (46.0)	6.51±0.11 ^a (30.5)	5.10±0.18 ^b (20.0)	0.29
CHO (g)				
R	66.50±0.06 ^b	61.92±1.19 ^c	68.16±0.21 ^a	1.34
S	63.92±0.19 ^a (−3.9)	59.69±0.53 ^c (−3.6)	65.25±0.18 ^b (−4.3)	0.68
Calcium (mg)				
R	105.99±1.24 ^b	109.65±2.84 ^b	138.18±2.56 ^a	4.63
S	116.56±2.75 ^b (10.0)	116.17±2.75 ^b (5.9)	148.18±2.33 ^a (7.2)	5.22
Iron (mg)				
R	5.91±0.14 ^b	6.75±0.18 ^a	4.85±0.05 ^c	0.27
S	5.99±0.01 ^b (1.4)	6.78±0.06 ^a (0.4)	4.86±0.04 ^c (0.4)	0.13
Vitamin C (mg)				
R	0.08±0.14 ^b	0.19±0.33 ^b	0.73±0.11 ^a	0.42
S	3.03±0.31 ^a (37.9)	1.79±0.63 ^b (9.4)	3.20±0.57 ^a (4.4)	1.04
Phytic acid (mg)				
R	308.83±5.21 ^c	380.34±3.01 ^a	352.72±3.11 ^b	7.81
S	19.07±3.06 ^c (−16.2)	45.56±3.06 ^b (−8.4)	73.87±3.13 ^a (−4.8)	6.15
TIA (TIU/mg protein)				
R	5.65±0.02 ^c	6.03±0.01 ^a	5.77±0.02 ^b	0.03
S	4.05±0.17 ^a (−28.5)	3.32±0.05 ^b (−44.9)	2.61±0.01 ^c (−54.8)	0.21
IVPD %				
R	59.76±1.93 ^b	63.83±2.48 ^a	52.65±1.69 ^c	4.11
S	64.99±1.97 ^b (8.7)	71.66±3.35 ^a (12.3)	63.61±1.69 ^c (20.8)	6.06

Values with different superscripts show significant difference from one another within the row

Values in parenthesis are percent change in comparison to raw samples except for vitamin C and phytic acid where these represent manifold change over raw values

R raw, S sprouted, CHO- carbohydrate = 100-(Moisture + Protein + Ash + Fat + Fibre), TIA trypsin inhibitor activity, TIU trypsin inhibition unit

[#] Mean ± SD

(2006) observed 17 % decrease in TIA of mung bean. Murugkar and Jha (2009) observed high decrease in TIA with increasing sprouting period in soybean. The decreased in TIA after sprouting may be due to proteolytic activity of enzymes, which are activated during sprouting (Chauhan and Chauhan 2007).

In-vitro protein digestibility (IVPD) After sprouting, IVPD of cowpea increased by 8.73, 12.27 and 20.82 % for PL-1, PL-2 and PL-3, respectively (Table 4). Sinha et al. (2005) observed 11 % increase in IVPD of cowpea after 24 h of sprouting. There were significant improvement in IVPD by 17.00 and 20.80 % when period of sprouting increased from

48 to 60 and 60 to 72 h, respectively. Increase in IVPD may be ascribed as sprouting increases protein digestibility of pulses as seed proteins are metabolized and anti-nutrients including protease inhibitors, phytate, polyphenols etc., are catabolised during sprouting (Sinha et al. 2005).

Conclusion

Sprouting significantly improved nutritional attributes except crude fat and carbohydrates in cowpea genotypes (PL-1, PL-2 and PL-3). Manifold increase in vitamin C was observed after sprouting in all the genotypes. Anti-nutritional factors like phytic acid and trypsin inhibitor also decreased after sprouting. Considerable increase in IVPD was noted after sprouting with highest increase in PL-3 followed by PL-2 and PL-1. Present study shows that sprouting is an effective method for removing anti-nutritional factors in cowpea without application of heat processing methods, which may reduce content of heat sensitive nutrients.

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