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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 781-786

www.thepharmajournal.com
Received: 07-08-2022
Accepted: 14-09-2022

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Study on the effect of different treatments on the nutritional quality of cowpea (*Vigna unguiculata*) flour

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Abstract
Cowpea (*Vigna unguiculata*) is an important pulse consumed worldwide. It is the richest source of nutrients including protein, dietary fibre, carbohydrates, amino acids, vitamins and minerals. Apart from nutrients, it is also a drought-resistant crop which can be cultivated in arid and semi-arid areas. The present study was conducted in the Department of Food Science and Environmental Science, Dr Yashwant Parmar University of Horticulture and Forestry Nauni Solan (HP) to determine the effect of different treatments such as soaking, roasting, germination and both soaking and germination on the nutritional quality improvement of cowpea flour. Soaking of seeds in warm water (1:3) for 12 h followed by germination for 24, 36 and 84 hours significantly increased moisture (7.13-11.21%), crude protein from (24.98 - 29.24%), crude fibre (9.72-11.54%), ascorbic acid (5.19-11.98 mg/100 g) and ash content (2.78-3.67%) while total carbohydrates and total energy decrease significantly i.e. (61.17-51.53%) and (408.58-362.23%), respectively. Similarly, the minerals increase significantly in soaked and germinated treatments as compared to the raw sample. There is an increase from (109.24 to 118.46 mg/100 g) for sodium, (1275.34 to 1458.55 mg/100 g) potassium, (301.54 to 406.55 mg/100 g)phosphorous, (174.21 to 197.45 mg/100 g) calcium, (4.25 to 7.16 mg/100 g) iron, (8.95 to 12.67 mg/100 g) copper, (5.14 to 8.12 mg/100 g) zinc, (1.75 to 7.32 mg/100 g) manganese and (4.79-10.23 mg/100 g) magnesium. The flour is recommended for use in value-added products to boost the nutritional quality and prevent nutrition deficiency in a vulnerable group.

Keywords: Cowpea seed flour, germination, minerals, roasting, soaking

Introduction
Cowpea (*Vigna unguiculata* L. Walp) is a popular legume crop grown in 45 countries around the world with an estimation of 14.5 million ha /year. India cultivates cowpea as a minor pulse in arid and semi-arid areas of Punjab, Delhi, West UP, Rajasthan, Karnataka, Kerala, Tamilnadu and Gujarat. The top ten largest producers in African countries include Nigeria, Niger, Burkina Faso, Tanzania, Cameroon, Mali, Kenya, Myanmar and Mozambique (Boukar *et al.*, 2018) [9]. In Tanzania, cowpea is grown and consumed in the regions of Mwanza, Shinyanga, Dodoma, Singida, Iringa and Kilimanjaro (Mamiro *et al.*, 2011) [20]. It is high in glutamic and aspartic acid, as well as critical amino acids like leucine, arginine, methionine and phenylalanine. Tryptophan is the limiting amino acid (Iqbal *et al.*, 2006) [15]. For pre-school children and adults, cowpea proteins provide appropriate levels of the most essential amino acids (Rangel *et al.*, 2004) [27]. The seeds have a relatively modest lipid content (1.30–1.90%) (Antova *et al.*, 2014) [1]; nonetheless, levels as high as 4.8 per cent have been documented (Iqbal *et al.*, 2006) [15]. It contains a lot of physiologically active substances (tocopherol, sterols, and phospholipids). Palmitic and linoleic acid is the most abundant fatty acids in the seeds oil, followed by linoleic and oleic acid (Antova *et al.*, 2014) [1]. Carbohydrates account for 56.0-66.0 per cent of total calories (Gómez, 2004) [14]. Antova *et al.* (2014) [1] found that the seed contains 28.30–36.20 per cent starch, as well as indigestible oligosaccharides, raffinose, stachyose and verbascose (Phillips *et al.*, 2003) [24]. Cowpeas have fibre and ash values of 1.70-3.80 and 3.20-4.20 per cent (Antova *et al.*, 2014) [1]. Cowpeas are high in phosphorus, calcium and iron, according to Gómez (2004) [14].
Aside from the minerals, low digestibility, a lack of sulfur-containing amino acids and the presence of ant nutritional elements are the key limiting factors of cowpea consumption in today's diet. Because they tend to bind proteins and chelate divalent metal ions, phenolic substances such as proanthocyanidins (Ojwang *et al.*, 2013) [23], phytic acid (Sinha and Kawatra, 2003) [29], tannins (Lattanzio *et al.*, 2005) [18], haemagglutinins, cyanogenic glucosides, oxalic acid (Afiukwa *et al.*, 2011) [2], dihydroxyphenylalanine and saponins may

Be nutritionally unfavourable to humans. Apart from that, enzyme inhibitors in cowpea, such as protease inhibitors, are anti-nutritional compounds. The phytic acid concentration of unprocessed cowpeas was 836.0 mg/100 g, according to Sinha and Kawatra (2003) [29]. The enzyme inhibitors in cowpea, particularly protease inhibitors, disrupt the body's normal protease control, which can lead to a variety of health problems. However, proper processing methods can be utilized to eliminate anti-nutritional components and increase bioavailability, especially when used as a diet for infants and children (Chuwa, 2022) [10]. Sprouting is the process of germinating seeds to activate numerous metabolic enzymes such as proteinases. This could result in the release of amino acids and peptides, which could be used to make new proteins (Devi *et al.*, 2015) [12]. Cowpea crude protein levels increased from 8 to 11% after sprouting, Uppal and Bains (2012) [31]. Furthermore, according to Devi *et al.* (2015) [12], sprouting may increase total mineral content and Owuamanam *et al.* (2014) have found that the mineral composition of sprouted cowpea flour is much higher than that of non-sprouted cowpea flour. Most importantly, Devi *et al.* (2015) [12] discovered that sprouted cowpea contained significantly less fat and carbohydrate than non-sprouted cowpea. The decline could be attributed to the seed's stored fat and carbohydrates being depleted, which contributed to their catabolic activities during sprouting (Onimawo and Asugo, 2004) [22].

Further, Uppal and Bains (2012) [31] discovered a 20 to 24 per cent rise in crude fibre content after cowpea sprouting. Many researchers have also noticed a considerable drop in anti-nutrient content after sprouting cowpea seeds (Devi *et al.*, 2015) [12]. The contribution of cowpea to both food and nutrition security put more attention on food Scientists to pay attention to proper processing before utilization. Therefore, the current study used different processing methods including soaking, germination and roasting to improve the nutritional quality of cowpea flour.

Materials and Method

The present investigation was conducted in the Department of Food Science and Technology, Dr Yashwant Parmar University of Horticulture and Forestry for nutritional characterization of different treatments for cowpea seed flour and mineral analysis was determined in the Environmental Science Department, Dr Yashwant Parmar University of Horticulture and Forestry. Cowpea seeds and packaging materials (PET jars) were purchased from local market Solan while chemicals were procured from LobaChemie, International Scientific and Surgicals, Solan (HP). Each treatment was replicated thrice and results were recorded on a dry weight basis.

Preparation of raw and soaked cowpea flour: The cowpea seeds (2kg) were washed and soaked in warm water (1:3) overnight (12 hours). The washed and soaked seeds were drained and dried in a mechanical dehydrator at 60 ± 2°Cfor 10 hours in separate lots. Each lot was sieved via a 36 mm mesh sieve after being ground in a mixer cum grinder (Havells, Model MX-1155). The flour of each lot was packed in PET jars, sealed properly and kept under ambient temperature for further analysis. The procedures for the preparation of raw and soaked cowpea seeds flour are depicted in Fig. 1

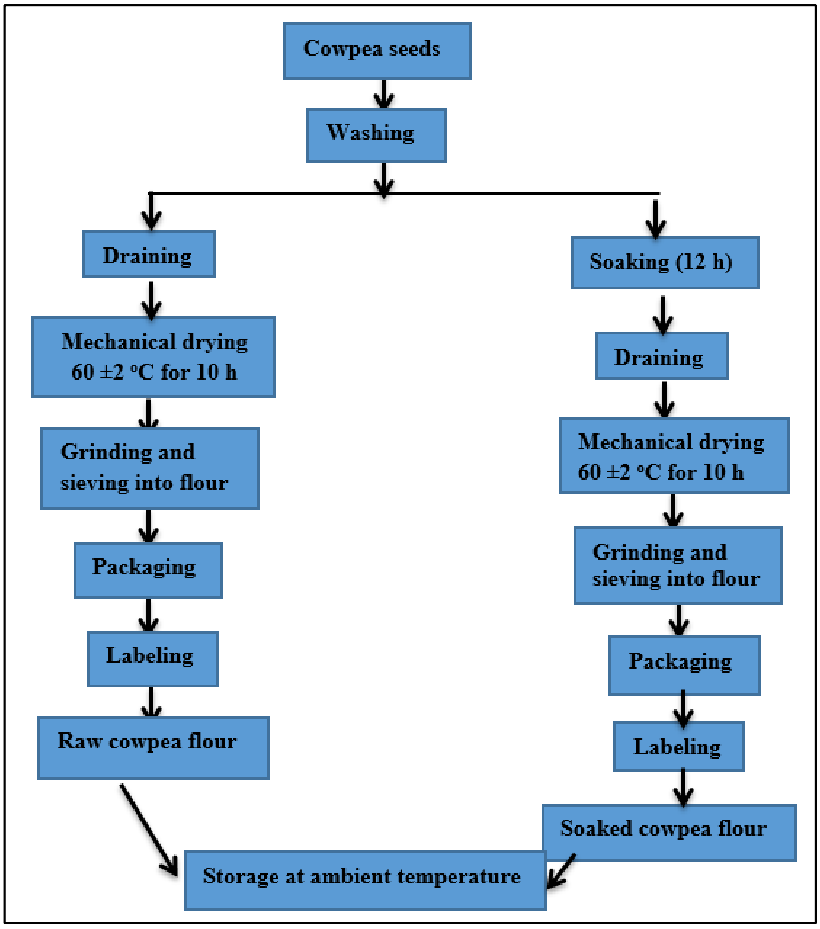

Fig 1: Flow sheet for preparation of raw and soaked cowpea seeds flour

Preparation of roasted and germinated cowpea flour

The cowpea seeds (4 kg) were washed and soaked in warm water (1:3) overnight (12 hours). After soaking cowpea seeds (3 kg) were divided into three lots of 1 kg each, drained into a mesh bowl followed by spreading into trays, covered with muslin cloth and germinated for 24, 36 and 84 hours, respectively under ambient temperature. Water was sprinkled daily to provide moisture during sprouting. The sprouts were thoroughly washed and plumules, radicles were carefully removed. The germinated seeds were dried in a mechanical dehydrator at 60 ± 2°C for 10 hours. The dried germinated seeds were grounded in a mixer cum grinder and sieved into a mesh having a sieve size of 36 mm to flour. The cowpea flour was packed in a tightly closed container (PET jars) for further investigation. Other seeds (1 kg) after washing and drained were dehydrated in a mechanical dehydrator at 60 ± 2 °C for 10 hours followed by roasting in a nonstick pan for 2-3 minutes till light brown colour. The roasted seeds were cooled at ambient condition followed by ground into flour in a mixer cum grinder (Havells, Model MX-1155) and sieved through a mesh of sieve size of 36 mm to uniform flour. The flour was kept in a PET jar container, sealed properly and kept at room temperature for conducting subsequent experiments. Below (Fig. 2) is a flow sheet for the preparation steps followed for the preparation of roasted and germinated cowpea seeds flour.

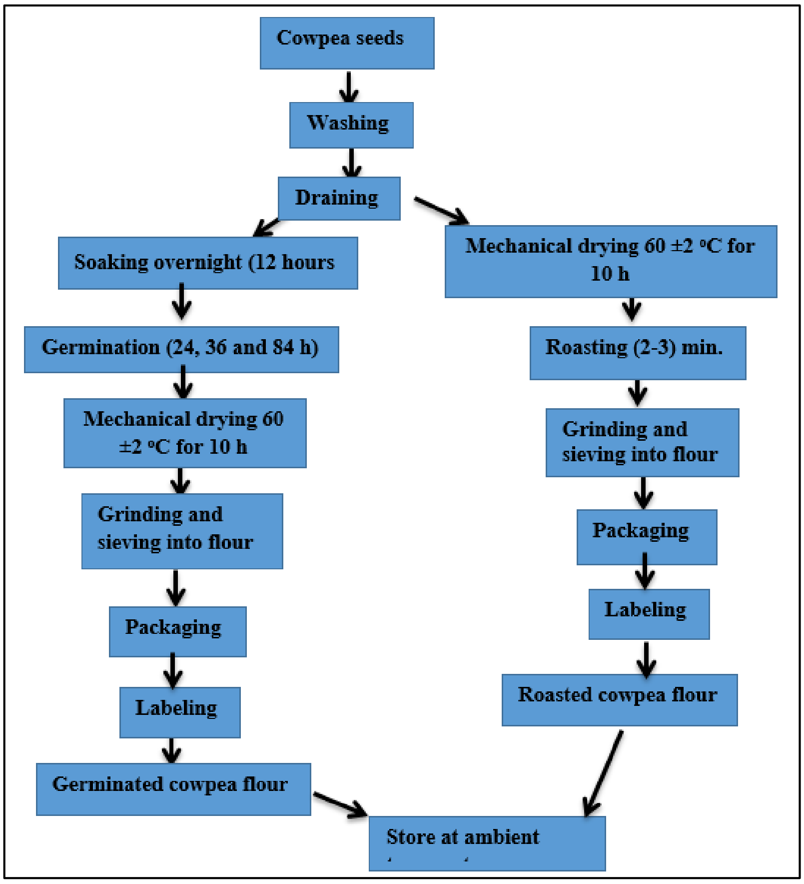

Fig 2: Flow sheet for preparation of roasted and germinated cowpea seeds flour

Chemical analysis

The moisture content (%), ash (%), protein (%) and minerals (mg/100 g) were determined as per the method suggested by AOAC (2012) [7]. Crude fibre (%) was analyzed as per (AOAC, 2010) [6], and Crude fat (%) was determined using the (AOAC, 2009) [5] method. Ranganna's (2009) [26] procedure was employed in scrutinizing β-carotene (mg/100 g), total carbohydrates (%) and total energy (Kcal/100 g) were calculated by the differential method as per AOAC (2006) [4] method. Ascorbic acid was determined as per (AOAC, 2004) [3].

Results and Discussion

Data in Table 1revealed the chemical characteristics of raw, soaked, roasted and germinated cowpea seed flour denoted by T_1, T_2, T_3 and T_4, respectively. The highest moisture content was recorded in soaked and germinated seed flourT_4 (11.21 ± 1.73%) while the lowest moisture content was analyzed in roasted seed flour T3 (7.13 ± 0.07%). Soaking and germination significant affect the moisture content of seed flour (Table 1). A similar trend of moisture has been reported by Devi *et al.* (2015) [12]. The increase in moisture content of the soaked and germinated sample is due to the absorption of water by the seed's coat during sprouting. Soaking and germination had a significant increase in the crude protein content of treatment T_4 with the highest crude protein (29.24 ± 0.62%) and the lowest protein (22.13 ± 0.40%) in treatment T_3. A significant ($p \leq 0.05$) increase in protein was noticed in soaked and germinated seed flour. Mehta *et al.* (2007) [21] and Devi *et al.* (2015) [12] analyzed the same trend in germinated cowpea flour. The apparent increase in protein content may be attributed to loss in dry matter, particularly carbohydrates through respiration during sprouting (Uppal and Bains, 2012) [31] another reason is due to the increase of proteolytic enzymes during germination.

Fat content decreased significantly with increasing germination time in T_4 from (4.98 ± 0.07 to 4.35 ±0.17%) soaked and germinated seed flour as compared to roasted T_3 (6.73 ± 0.17%) and raw T_1 (5.94 ± 0.07%). A 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) [22]. An increase in crude fibre was also significant with increasing germination time in T_4 (soaked and germinated seed flour from (9.88 ± 0.27 to11.54 ± 0.43%) as compared to the roasted sample T_3 (9.34 ± 0.25%). Result obtained was that of Uppal and Bains (2012) [31] where a 20 to 24% increase has been reported after sprouting cowpea. A significant increase in fibre during the sprouting process of chickpea was also reported by Sood *et al.* (2002) [30] due tothe disappearance of starch. High significant decrease in total carbohydrates was observed in the soaked and germinated seed flour as per increasing germination periodin treatment T_4 from (53.49 ± 3.88to 51.53 ± 0.33%) as compared with raw T_1 (55.57 ± 3.88%). Uppal and Bains (2012) [31] reported a 5.6% decrease and Jirapa *et al.* (2001) [16] reported a 2.34% decrease in carbohydrate content after 24 h of sprouting in cowpea. This may be due to sprouting, carbohydrate was used as a source of energy for embryonic growth which could explain the changes in carbohydrates content after sprouting. Ascorbic acid increased significantly with an increasing sprouting time in treatment T_4 from (10.25 ± 0.06 to11.98 ± 0.51 mg/100 g) i.e. soaked and germinated seed flour and minimum (3.27 ± 0.52mg/100 g) in T_3. Soaking and germination increase ascorbic acid significantly ($p \leq 0.05$) as compared to other treatments. The increase in ascorbic acid may be due to enzymatic hydrolysis of starch by amylases and diastases that increase the availability of glucose for the biosynthesis of ascorbic (Desai *et al.*, 2010) [13]. The increase of ascorbic acid in different sprouted pulse seed flour has been reported by Shah *et al.* (2011) [28].

The data in Table 1reflects the β-carotene content present in cowpea seed flour of different treatments A significant ($p \leq 0.05$) decrease in β-carotene was found in soaked and germinated from 2.14 ± 0.02 to 2.01 ± 0.13% seed flour and lowest in roasted seed flour (1.19 ± 0.13%). The lowest β-carotene content in T_3 may be due to oxidative degradation in thermal processing that results from the conversion of trans-cis isomerization of all transform. A similar trend has been analyzed by Khyade and Jagtap (2016) [17] in germinated cowpea, black gram chickpea and yellow mustard. The highest ash content (3.67 ± 0.98%) is reflected in treatment T_4 and the lowest in treatment T_1 (2.78 ± 0.07%). The effect of treatment shows higher significance ($p \leq 0.05$) on soaked and germinated seed flour as compared to raw in ash content. Similar results were reported by Ranhotra *et al.* (1977) [25]. An increase in ash content may be apparent due to the loss of starch (Lorenz, 1980) [19]. The total energy of 408.58 ± 4.12Kcal/100 g was found to be maximum in treatment T_3`and minimum in treatment T_4 (369.26 ± 2.81 Kcal/100 g). Significant low energy in soaked and germinated seed flour (T_4) is due to the low energy value of sprouted seed flour for fat and total carbohydrates (Uppal and Bains, 2012) [31] in which energy depends on them during calculation (differential method). Seed sprouting involves energy use, which is provided by the breakdown of starch to sugars and lipids to free fatty acids resulting in a shift in nutrient profile.

The data for minerals in cowpea seeds of different treatments are appended in Table 2. The mineral content of different parameters increased with an increase in germination time except for iron. The highest amount of sodium was obtained in treatment T_4 (118.46 ± 2.45 mg/100 g) at maximum germination time while the lowest was in treatment T_1 (109.24 ± 3.31 mg/100 g) in raw cowpea seed flour. Soaking and germination increased significantly ($p \leq 0.05$) with an increase in the germination period. Soaking and germination of cowpea seed eliminate phytate and other antinutritional factors due to activation of phytase enzyme during germination and their instability on heat treatment thereby increasing minerals. A significant increase in Potassium was analyzed in treatment T_4 (1458.55 ± 2.12 mg/100 g. Phosphorous increases significantly (406.55 ± 0.21 mg/100 g) in treatment T_4. Calcium increased significantly in treatment T_4 (197.45 ± 1.54 mg/100 g). Dave *et al.* (2008) [11] observed a 24 to 62% increase in calcium content of cowpea after sprouting. An increase in calcium content may be attributed to the presence of calcium salts in water used during the sprouting process (Ranhotra *et al.*, 1977) [25].

There was a slight decrease in iron content after soaking and germination of cowpea seed flour in treatment T_4 from (8.15 ± 0.05 g). Bains *et al.* (2011) [8] also reported that soaking and different sprouting period's non-significant decrease in iron content *of mung* bean and cowpea. During soaking and germination most of the iron leach into the soaked water. The highest copper (12.67 ± 0.89 mg/100 g) was noticed in treatment T_4 while the lowest value of 8.95 ± 0.14mg/100 g was analyzed in treatment T_1. Treatment T_4 determined the maximum zinc content of 8.76 ± 0.23 mg/100 g and treatment T_1 (5.14 ± 0.02 mg/100 g) lowest zinc content. Soaking and germination increase significantly the manganese in cowpea seed flour in treatment T_4 (7.45 ± 3.13 mg/100 g) as compared to raw treatment T_1 (1.75 ± 0.01 mg/100 g). The highest magnesium was recorded in treatment T_4 (10.23 ± 0.91 mg/100 g) and the lowest in treatment T_1 (4.79 ± 0.22 mg/100 g).

Table 1: Chemical characteristics of different treatments of cowpea seeds flour

Parameters	Treatments					
	T_1	T_2	T_3	T_4		
Sprouting time (h)				**24**	**36**	**84**
Moisture (%)	10.82 ± 0.69^a	10.97 ± 0.23^a	7.13 ± 0.07^b	10.52± 0.69^a	10.93 ± 0.23^a	11.21 ± 0.07^b
Crude protein (%)	24.98 ± 0.68^b	25.54 ± 0.14^a	22.13 ± 0.40^c	27.62 ± 0.68^b	28.15 ± 0.14^a	29.24 ± 0.40^c
Crude fat (%)	5.94 ± 0.07^b	5.33 ± 0.08^c	6.73 ± 0.17^a	4.98 ± 0.07^a	4.33 ± 0.08^b	4.35 ± 0.17^b
Crude fibre (%)	9.72 ± 0.27^a	9.89 ± 0.11^a	9.34 ± 0.25^b	9.88 ± 0.27^b	11.05 ± 0.11^c	11.34 ± 0.25^a
Total carbohydrates (%)	55.57 ± 3.88^b	54.48 ± 0.09^c	61.17 ± 0.33^a	53.49 ± 3.88^a	53.11 ± 0.09^b	51.53 ± 0.33^a
Ascorbic acid (mg/100 g)	5.19 ± 0.06^a	4.06 ± 0.01^b	3.27 ± 0.52^c	10.25 ± 0.06^c	10.84 ± 0.01^b	11.98 ± 0.52^a
β-carotene (mg/100 g)	2.39 ± 0.02^a	2.17 ± 0.06^b	1.19 ± 0.13^c	2.14 ± 0.02^a	2.05 ± 0.06^b	2.01 ± 0.13^c
Ash (%)	2.78± 0.07^c	2.97 ± 0.02^b	2.94 ± 0.14^a	3.39± 0.07^c	3.48 ± 0.02^b	3.67 ± 0.14^a
Total energy (Kcal/100 g)	336.78 ± 2.81^b	332.86 ± 3.19^c	408.58 ± 4.12^a	369.26 ± 2.81^a	364.01 ± 3.19^b	362.23 ± 4.12^b

T_1: Raw seed flour, T_2: Soaked seed flour, T_3: Roasted seed flour, T_4: Soaked + germinated seed flour. The data presented in the table are the average pooled values (mean ± SD). The values with the same lower case superscript in the same row are non-significant at a $p \leq 0.05$ level of significance

Table 2: Mineral content of different treatments of cowpea seeds flour

Parameters	Treatments					
	T_1	T_2	T_3	T_4		
Sprouting time (h)				**24**	**36**	**84**
Sodium (mg/100 g)	109.24 ± 3.31^c	110.19 ± 5.21^b	112.56 ± 5.00^a	103.24 ± 3.31^c	110.32 ± 5.21^b	118.46 ± 5.00^a
Potassium (mg/100 g), (%)	1275.34 ± 1.34^c	1297.62 ± 0.08^b	1307.21 ± 0.51^a	1396.27 ± 1.34^c	1409.56 ± 0.08^b	1458.55 ± 0.51^a
Phosphorous (mg/ 100 g)	301.54 ± 0.15^c	318.63 ± 0.09^b	325.96 ± 0.56^a	398.25 ± 0.15^c	400.02 ± 0.09^b	412.55 ± 0.56^a
Calcium (mg/100 g)	174.21 ± 0.07^c	175.87 ± 3.17^b	176.76 ± 0.32^a	192.79 ± 0.07^c	195.87 ± 3.17^b	186.76 ± 0.32^a
Iron (mg/100 g)	4.25 ± 0.05^c	5.32 ± 0.12^b	6.19 ± 0.15^a	8.15 ± 0.05^c	7.32 ± 0.12^b	6.19 ± 0.15^a
Copper (mg /100 g)	8.95 ± 0.14^c	9.11 ± 0.03^b	10.31 ± 1.42^a	11.25 ± 0.14^c	11.54 ± 0.03^b	12.67 ± 1.42^a
Zinc (mg/100 g)	5.14 ± 0.02^c	5.97 ± 0.01^b	6.76 ± 1.23^a	7.14 ± 0.02^c	7.97 ± 0.01^b	8.76 ± 1.23^a
Manganese (mg/100 g)	1.75 ± 0.01^c	2.16 ± 0.08^b	3.74 ± 3.13^a	7.04 ± 0.01^c	7.19 ± 0.08^b	7.45 ± 3.13^a
Magnesium (mg/100 g)	4.79 ± 0.22^c	5.67 ± 0.09^b	6.13 ± 0.91^a	9.25 ± 0.22^c	9.49 ± 0.09^b	10.23 ± 0.91^a

T_1: Raw seed flour, T_2: Soaked seed flour, T_3: Roasted seed flour, T_4: Soaked + germinated seed flour. The data presented in the table are the average pooled values (mean ± SD). The values with the same lower case superscript in the same row are non-significant at a $p \leq 0.05$ level of significance

Conclusion

Soaking and germination decrease significantly crude fat, carbohydrates, β-carotene and total energy in cowpea seed flour while increase significantly moisture, crude protein, crude fibre, ascorbic acid and ash and minerals. The reduction of fat, carbohydrates and total energy is good news for overweight and obese people who wants to reduce weight. A significant increase in vitamin C, protein, fibre, ash and minerals was observed after the soaking and germination of cowpea seed flour. The present study concluded that soaking and germination of cowpea seed is an effective method for removing anti-nutritional factors which can be affordable for a large population in low-income countries. Therefore, this method is recommended to public health to combat Protein Energy Malnutrition by incorporating the flour in complementary food formulations.

Acknowledgement

The authors are grateful to Dr. Yashwant Singh Parmar of the University College of Horticulture and Forestry in Himachal Pradesh, India, for providing all of the necessary facilities and support for this study. Further, we thank the Director General of Tanzania Agricultural Research Institute (TARI) Dr. Geofrey Mkamilo for his encouragement, support and permission for conducting this research.

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