

Germinated Legumes (Mung Bean and Cowpea) as Potential Commodities for Preparing Complementary Baby Foods

A. Yasser^a, K. Rezaei^{b*}, M. Salami^c

^a Student of the Department of Food Science, Engineering and Technology, University of Tehran, Karaj, Iran.

^b Professor of the Department of Food Science, Engineering and Technology, University of Tehran, Karaj, Iran.

^c Assistant Professor of the Department of Food Science, Engineering and Technology, University of Tehran, Karaj, Iran.

Received: 27 September 2019

Accepted: 4 November 2019

ABSTRACT: Legumes and pulses have high nutritive values and functional properties that exert positive effects on health and nutrition. This study developed a novel complementary baby food using germinated mung bean and cowpea as sources of extra nutrients supplemented to the infants (aging 6-12 months). The carbohydrate contents of mung bean and cowpea showed 64.3 and 64.0% reduction, respectively, during 48 h of germination. However, phosphorous and zinc contents and antioxidant activities of mung bean and cowpea and the iron content of cowpea increased over the same period of germination. Five baby food formulations were prepared and evaluated according to a standard formula for a commercial baby food which was also used as the control. The finalized formula had higher protein (22.4%), calcium (6,100 mg kg⁻¹), phosphorus (5,133 mg kg⁻¹) and vitamin D (329 IU in 100 g) contents but lower contents of iron (55.5 mg kg⁻¹), vitamin C as ascorbic acid (0.1 mg in 100 g) and vitamin B₁₂ (1.2 mg in 100 g) than the control. A finalized formula with good appearance, flavor and taste as well as an overall general acceptance was obtained that can be used to fight mal-nutritional issues in certain developing countries.

Keywords: *Antioxidant Activities, Complementary Baby Food, Cowpea, Germination, Mung Bean.*

Introduction

Legumes have traditionally been consumed for more than 10,000 years among the people from different countries (Erbersdobler *et al.*, 2017). Legumes have been considered as sources of major nutrients and many health-promoting phytochemicals and provide higher micronutrient contents and wider varieties of essential compounds than other food sources (Ojiewo *et al.*, 2015). Moreover, considering their relatively lower costs and higher accessibility levels, legumes and pulses are potential solutions to fight hunger and malnutrition in developing countries. Studies

have shown that the nutritional qualities of cereals can be improved through supplementation with legumes (Kouris-Blazos, & Belski, 2016). Due to their high contents of dietary fiber, resistant starch, protein contents, oligosaccharides, vitamins, minerals and bioactive components (Erbersdobler *et al.*, 2017), legumes have been suggested as functional foods for the control of dyslipidemia (Saraf-Bank *et al.*, 2016). Cowpea (*Vigna unguiculata* L.) or black eye pea is a famous legume in Africa, South America, Asia and Europe. It's an important source of protein, vitamins and minerals such as calcium, potassium, phosphorus, zinc, and iron (Devi *et al.*,

*Corresponding Author: krezaee@ut.ac.ir

2015). It has been used for the preparation of different foods mostly in developing countries (Jayathilake *et al.*, 2018).

Mung bean (*Vigna radiata* L.) is a nutritious crop with grains having large amounts of protein, certain minerals and vitamins (Pataczek *et al.*, 2018). Several epidemiological studies have reported that the weekly replacement of meat-rich diets with legumes can positively affect longevity, diabetes, cardiovascular diseases and obesity-related issues (Saraf-Bank *et al.*, 2016). Furthermore, the nutritional values of the legumes can be improved through germination, which is a simple and inexpensive technique (Masood *et al.*, 2014). Germination is reportedly capable of utilizing the bioactive compounds in leguminous sprouts to supply energy and essential amino acids necessary for plant growth (Suryanti *et al.*, 2016). According to Masood *et al.* (2014), germinated seeds possess higher nutrient availability, better texture and organoleptic characteristics and also contain higher levels of health-promoting phytochemicals than their non-germinated counterparts. The concentrations of secondary metabolites such as phenolic compounds, vitamins and carotenoids have also been shown to increase during the germination (Gulati, 2010).

One of the issues impacting millions of people, mainly children, is insufficient protein intake (Fasuan *et al.*, 2017). Breast milk offers all nutrients a baby needs for the first six months of his/her life. However, once an infant reaches 6 months of age, semi-solid or solid foods should be introduced into his/her diet to help a proper health and growth condition for the infant (Ijarotimi & Keshinro, 2013). Improved supplementary foods can help children aging 6–23 months to enhance their normal growth and knowledge development (Tufa *et al.*, 2016). Generally, all children are breastfed during this period. However, as they get older, they need supplementary foods to

meet their daily requirements for energy and nutrients (Abiose *et al.*, 2015). Supplementing the children with foods other than milk is important in the development of their food behaviors (Kehinde Alawode *et al.*, 2017). Improving the quality and consumption of legumes is one of the effective methods to resolve malnutrition and micronutrient insufficiencies (Davis *et al.*, 2010). Germination is the most active and inexpensive method that can be suggested to improve the nutritional quality of legumes and for decreasing the flatulence (Fayyaz *et al.*, 2018) and also for reducing the activity of trypsin inhibitor and to lower phytic acid content in legumes (Jiang *et al.*, 2013).

In the current study, the impact of germination on the proximate compositions, bioactive compounds and antioxidative activities of mung bean and cow pea as two popular legumes was investigated. The germinated legumes were then utilized for the preparation of a complementary baby food (CBF) without the need for any cereals. Such formulation is new and has not yet been reported in published studies. Therefore, the effects of the addition of germinated legume were investigated to find about the nutritional and sensory behaviors of different formulas.

Materials and Methods

- Materials

Seeds of mung bean and cowpea were purchased from a local market in Karaj (Alborz, Iran). Folin-Ciocalteu reagent, gallic acid (GA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, acetone, boric acid, sulfuric acid, sodium hydroxide, methyl red, bromocresol green, and *n*-hexane were purchased from Merck Chemical Company (Darmstadt, Germany) and Indigo carmine and potassium permanganate from Scott Science (Kent, UK). Sugar, coconut oil, sodium bicarbonate and vanillin were purchased from a local

market in Karaj. Skim milk powder was obtained from Pegah Dairy Company (Tehran, Iran). The reference baby food (Cerelac™) was obtained from Nestle Iran (P.J.S. CO., Old Qazvin-Tehran road, Tehran, Iran). All chemicals and reagents were of analytical grade.

- *Seed germination*

Seed germination was carried out using a method similar to that reported by Abiose *et al.* (2015). For this purpose, the seeds were cleaned to remove dirt and stones and then soaked in water for 12 h and allowed to germinate inside a plastic plate on a wet paper in an incubator at 33.5 ± 2.0 °C. Two layers of wet paper towels were used to cover the seeds to prevent rapid moisture loss. Seeds were sprayed with water 2-3 times a day for the periods of 18, 24, and 48 h and then dried at 60 °C for 12 h. The seeds were then ground and placed in polyethylene bags and stored in a refrigerator (4 °C) until they were used in the formula and also for the analysis.

- *Chemical compositions*

Moisture, ash, fat, and protein contents of germinated mung bean (GM) and germinated cowpea (GC) and CBF were determined following the standard methods of AOAC (2010). Carbohydrate contents were determined through a method reported by Onwuka (2005). Total energy values (in kcal per 100 g of the samples) for the food formulations were determined according to the Atwater's conversion factors applied by Tufa *et al.* (2016) by using equation 1.

$$\text{Total energy} = \text{Carbohydrate content} \times 4 + \text{Protein content} \times 4 + \text{Fat content} \times 9 \quad (1)$$

where values of carbohydrate, protein and fat contents are used in grams per 100 g of sample.

Mineral (zinc, iron and calcium) contents of the germinated legumes and formulas

were determined according to Iqbal *et al.* (2006) applying atomic absorption spectrophotometry. Phosphorus levels in the germinated legumes and in the formula were also measured based on a spectrophotometric method using a UNICO S2100 spectrophotometer (plain field, New Jersey, USA) at 470 nm (Tausky & Shorr, 1953). Vitamin C was determined by indophenol titrimetric method from AOAC (2010) and reported as ascorbic acid. For this purpose, 5 g of each of CBF was accurately weighed and blended for 2-3 min. Then, 20 ml of 3%-metaphosphoric acid (prepared by adding 15 g metaphosphoric acid into 40 ml acetic acid and the volume was taken to 500 ml) was added and the mixture was filtered and its volume was taken to 100 ml using the 3%-metaphosphoric acid solution and analysis was done on the aliquots of this solution. Vitamin D and vitamin B12 were also determined following their corresponding AOAC methods (AOAC, 2010).

For vitamin D analysis, an HPLC system (model 2790) from Waters (Milford, MA) equipped with a photodiode array and a C₁₈ column (25 cm × 46 mm) was used. For vitamin B₁₂, the absorbance values for the samples were recorded at 445 nm using a UNICO S2100 instrument. Phytic acid contents in the formula were estimated using a method reported by Ijarotimi & Keshinro (2013) based on the empirical conversion factor of 3.55. For such purpose, 3.00 g of each sample was dissolved in 50 mL of 3% (w/v) trichloroacetic acid (in distilled water). After shaking the samples for 30 min, the samples were centrifuged at 3,500 rpm for 15 min. Phytate was precipitated as ferric phytate and to measure the iron, the precipitate was then dissolved in hot 3.2-N nitric acid solution and the absorbance values for the samples were recorded at 480 nm using a UNICO S2100 spectrophotometer. A standard curve was plotted using Fe(NO₃)₃ solutions at 0.05-

0.50 mg/mL concentrations and was used to obtain the iron level in the precipitate.

Tannin contents were determined applying an AOAC titration method (AOAC, 2010), where 3.00 g of the sample was extracted at ambient conditions (using water as solvent for 4 h) and then the filtrate was treated by Indigo Carmine solution and titrated with KMnO_4 solution until a golden yellow color was observed. A mixture of 25 ml Indigo Carmine solution and 750 ml distilled water was prepared and used as reagent blank. Tannin contents of the samples were determined applying equation 2 as follows:

$$T (\%) = + \frac{n(v - v^0) \times 0.004157 \times 250 \times 100}{g \times 25} \quad (2)$$

where T is the tannin content in % (w/w), v is the volume of 0.1 N KMnO_4 aqueous solution for titration of the sample in mL and v^0 is the volume of KMnO_4 solution used in the titration of the reagent blank.

- Determination of total phenolic content (TPC)

TPC values of the samples were determined according to the method described by Xu and Chang (2007). The ground samples were precisely weighed and placed in test tubes and 5.0 mL of a 50:50 (v/v) mixture of acetone/water was added as the extraction solvent and shaken at 300 rpm by an orbital shaker for 3 h at ambient conditions and placed in the dark for 12 h and then centrifuged using a Universal 320 benchtop centrifuge (Hettich, Germany) at 3,000 rpm for 10 min and supernatants were collected and pooled in new tubes. At this stage, again, 5.0 mL solvent was added to the residues and the extraction was repeated in accordance with the procedures presented above and two portions were combined and maintained in the dark at 4 °C for the determination of their TPC and antioxidant activities. GA was used as the external

reference and TPC was expressed as GA equivalent (GAE, mg GA/g sample).

- DPPH scavenging capacity

To obtain the DPPH scavenging capacities of the legume extracts, a DPPH solution was prepared at 0.2 mM in methanol and 0.2 mL of each extract was added to 3.8 mL of this solution (Xu & Chang, 2007). A UNICO S2100 SVIS spectrophotometer (New York, NY) was applied at 517 nm to measure the absorbance. A reagent blank was also prepared by combining 0.2 mL distilled water and 3.8 mL of DPPH solution. The percentage of DPPH inhibition of the samples were determined using equation 3 (Xu & Chang, 2007):

$$\text{Inhibition (\%)} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (reagent blank)}} \times 100 \quad (3)$$

- Formulations of complementary baby food (CBF)

Figure 1 shows the stages applied for the preparation of CBF from GM and GC. The flour samples were blended with 36% skim milk powder, 10% coconut oil, and 10% sucrose, according to the composition of the reference baby food (CerelacTM from Nestle Iran, P.J.S. CO., Old Qazvin-Tehran road, Tehran, Iran) containing rice, skim milk and sucrose originally fortified with minerals (iron and zinc) and vitamin C (as ascorbic acid). The compositions of six formulas prepared in the current study are shown in Table 1.

The blends were prepared in duplicate and the results of all analyses were reported as means of three replicates. Sensory evaluation of CBF samples was carried out at the Department of Food Science, Engineering and Technology, University of Tehran (Karaj, Iran) using 11 individuals from the staff and students of the Department as the panelists evaluating the samples based on a five-point hedonic scale

with 1 assigned as the lowest score and 5 as the highest score (Onoja, *et al.*, 2014).

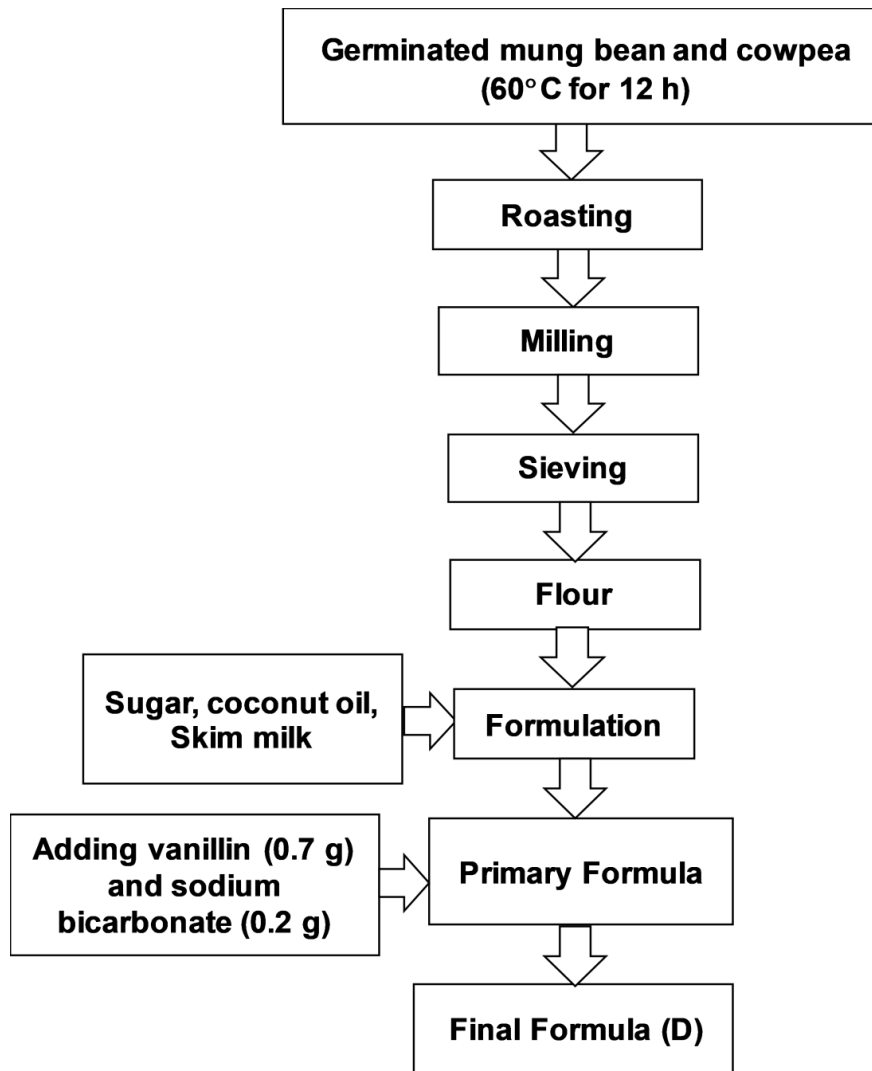


Fig. 1. Flow diagram for the preparation of a baby food from germinated mung bean and cowpea. The mineral contents are formulated in the figures.

Table 1. Different formulations for the complementary baby foods prepared from germinated mung bean and cowpea (values in grams per 100 grams of the formula)

Formulas	Skim milk	Sugar	Coconut oil	Germinated mung bean	Germinated cowpea
A	36	10	10	28	16
B	36	10	10	16	28
C	36	10	10	22	22
D	36	10	10	12	32
E	36	10	10	32	12

- Bulk density (BD), water absorption capacity (WAC) and swelling index (SI)

BD was determined following a method by Onwuka (2005) using eqn. 4.

$$\text{Bulk density (g.mL}^{-1}\text{)} = \frac{\text{Weight of sample}}{\text{Volume of sample after tapping}} \quad (4)$$

WAC was determined following the method reported by Onwuka (2005) applying eqn. 5.

$$\text{WAC(\%)} = \frac{\text{Volume of water added} - \text{Free water}}{\text{Weight of sample} \times \text{Density of water}} \times 100 \quad (5)$$

SI was also determined according to Onwuka (2005).

- Statistical analysis

The data from this study was analyzed using the SAS software (version 9.3; SAS Institute, Cary, NC). A one-way analysis of variance (ANOVA) with Duncan's multiple range test was conducted to assess the significance of the differences (at $p < 0.05$) among the chemical compositions of legumes at different germination stages. To analyze the data from the CBF samples, ANOVA was conducted by SPSS (Version 22.0, IBM Corp., Armonk, New York).

Results and Discussion

- Chemical compositions

Table 2 compares the chemical compositions of mung bean and cowpea at different germination stages (0-48 h). According to Table 2, the moisture contents of mung bean samples were below 10% at different stages of germination. The mean moisture content (7.4%) of mung bean was higher at 24 h of germination than those at 18 and 48 h of germination (5.5 and 6.9%, respectively). The moisture content of cowpea increased significantly after 48 h of germination (6.5%) relative to that of the raw seed flour (control, 4.0%). Moisture

content is an important parameter for food samples. Moisture contents below 10% inhibits the bacterial activity and therefore result in extending the shelf life of the processed flours (Desalegn *et al.*, 2015).

The increase in the protein levels are due to the reduction in the dry matter content, particularly that of carbohydrates, through the respiration during the germination (Masood *et al.*, 2014). Nonogaki *et al.* (2010) also reported that the dry weight is reduced and crude protein content is increased during the germination. On the other hand, Kaushik *et al.* (2010); Joshi, & Varma, (2016); Veluppillai *et al.* (2009) reported an increase in the crude protein during the germination of rice seed. However, Masood *et al.* (2014) reported a decline in the protein content and concurrent increase in the amino acid content during the germination of mung bean due to the increase in the protease activity. The differences in the protein contents of germinated grains can vary by the cultivar as well as the germination conditions (Masood *et al.*, 2014). In the current study, the fat contents of mung bean and cowpea decreased after slight increase during the germination (Table 2). Such changes are in agreement with the results of Devi *et al.* (2015) for the germination of cowpea, mung bean and chickpea. While the ash content of cowpea decreased during the germination (Table 2), the relative ash content of mung bean did not change significantly. Phosphorus and calcium are among the important minerals in the foods. During the germination of mung bean in the current study, the phosphorus content indicated a slight increase from ~0.32% to ~0.45% (Figure 2).

The slope of such increase in the cowpea was somewhat smaller. Calcium did not indicate any major changes in either seed. These results corresponded well with those reported by Omenna *et al.* (2016) for the germination of cowpea seed. Zinc contents

of mung bean and cowpea increased during the first 18 h of germination (Figures 3a and 3b, respectively) and then started to decline afterwards. However, for mung bean, it continued to increase again during 24-48 h of germination. These results are consistent with those reported by Omenna *et al.* (2016) for the germination of cowpea seed. The iron content of mung bean decreased continuously during the 48 h of germination. However, that of cowpea decreased during the first 24 h and then increased afterwards (Figure 3b). According to Omenna *et al.* (2016), the iron levels in germinated cowpea increased relative to that of the raw seed. Similarly, Devi *et al.* (2015) reported that

the iron content of germinated cowpea was slightly higher than that of the un-germinated seed. If required, plants can receive certain minerals from water and also release un-necessary minerals into the water (Ertop & Bektaş, 2018).

- Total phenolic content

Changes in the TPC values of mung bean and cowpea during the germination are shown in Figure 4a, which shows higher values of TPC for mung bean compared to those for cowpea. The TPC value for mung bean indicated a slight increase up to 18 h of germination followed by a decrease during the germination up to 48 h. However, for

Table 2. Chemical compositions of mung bean and cowpea during 48 h of germination

Germination Time (h)	Mung bean (% w/w) [†]				
	Moisture	Carbohydrate	Protein	Ash	Fat
0 [‡]	6.9±0.3 ^a	67.0 ±2.3 ^a	22.9±0.0 ^c	2.4 ±2.1 ^a	0.8±0.0 ^c
18	5.5±0.9 ^b	65.6±1.5 ^{ab}	24.0±0.2 ^a	3.5±0.1 ^a	1.0±0.0 ^a
24	7.4±0.3 ^a	64.9±0.3 ^{ab}	23.0±0.1 ^b	3.0±0.0 ^a	1.0±0.0 ^a
48	6.9±0.3 ^a	64.3±0.5 ^b	24.0±0.2 ^a	3.5±0.0 ^a	0.9±0.0 ^b
Germination Time (h)	Cowpea (% w/w) [†]				
	Moisture	Carbohydrate	Protein	Ash	Fat
0 [‡]	4.0±0.1 ^d	67.0 ±0.1 ^a	23.0± 0.0 ^c	3.9±0.0 ^a	1.0 ±0.0 ^c
18	4.9±0.0 ^c	65.8±0.0 ^b	23.9±0.0 ^b	3.7±0.0 ^b	1.7±0.0 ^a
24	8.0±0.0 ^a	62.5±0.1 ^d	24.0±0.1 ^b	3.0 ±0.0 ^c	1.5±0.0 ^b
48	6.5±0.3 ^b	64.0±0.4 ^c	24.5±0.1 ^a	3.0±0.1 ^c	1.4±0.0 ^b

[†] Values are expressed as means ± standard deviations in percent (w/w) of three replicates.

[‡] Control (raw seed flour).

^{a, b, c, d} In each column, different letters indicate significant differences among the means (p < 0.05).

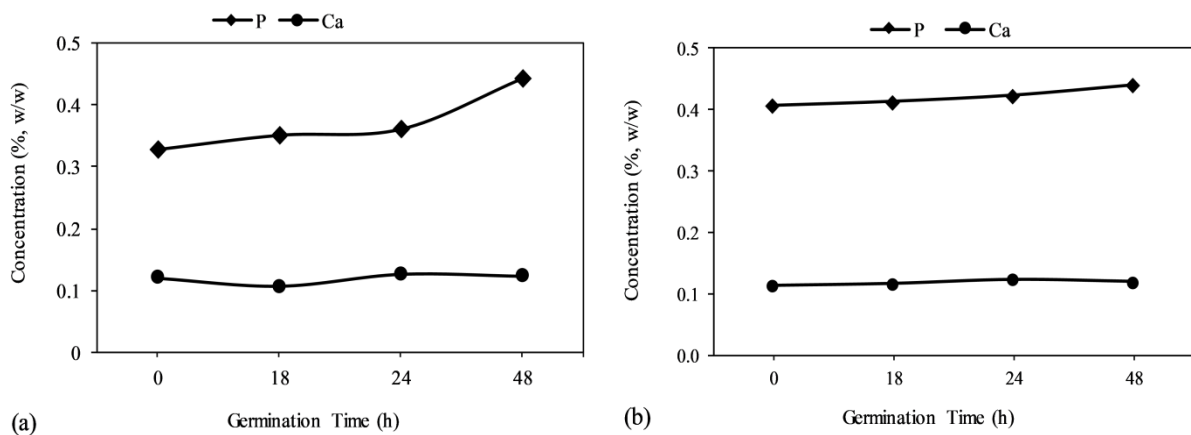


Fig. 2. Phosphorus and calcium contents of germinated mung bean (a) and cowpea (b) during the germination for 48 h.

cowpea, changes in TPC were positive during the entire germination period of 48 h. According to Fouad & Rehab (2015), the increase in the TPC could be related to the biosynthesis and bioaccumulation of specific phenolic compounds as a protective mechanism against environmental stresses. During the germination, phenolic compounds can fluctuate depending on the type of seeds and germination conditions (Lopez-Amoros *et al.*, 2006).

- Antioxidant activity

Changes in the antioxidant activities of mung bean and cowpea seeds during the germination are shown in Figure 4b based on the DPPH scavenging activities. For cowpea, a sharp increase followed by a decrease was observed in the antioxidant activity at 18 h of germination. The level of

radical scavenging activity for cowpea at the 18th h of germination was 44.5%, which is higher than that in the control (19.3%) and also at other germination stages (Figure 4b). The increase in the antioxidant activity is usually due to the enzymatic activities that occur during the seed germination (Fouad & Rehab, 2015). Vitamin C and tocopherols that are produced during the germination can contribute to the antioxidant activities of the seeds (Fouad & Rehab, 2015). On the contrary, the antioxidant activity for mung bean decreased during the germination for 24 h, after which it started to increase for the rest of germination time (Figure 4b). The initial decrease in the antioxidant activity for mung bean might be due to the utilization of stored phenolic compounds by polyphenol oxidase or other enzymes (Fouad & Rehab, 2015). Wong *et al.* (2006) reported that the

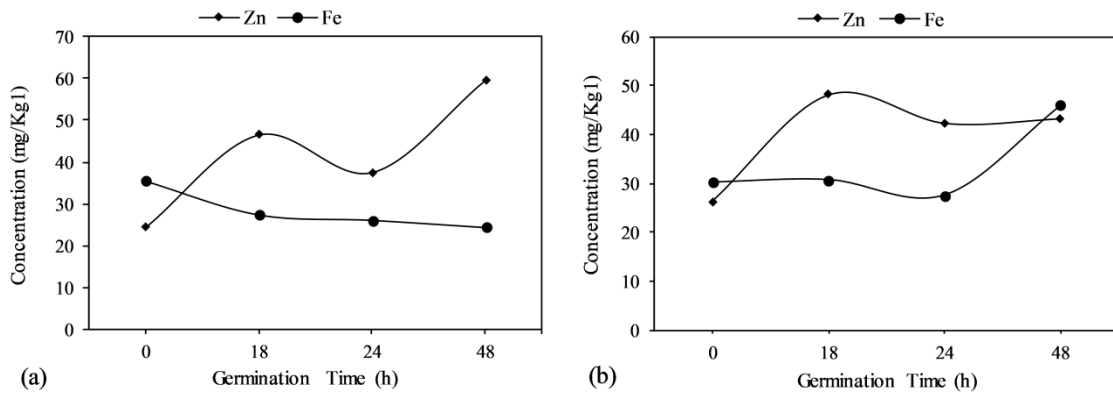


Fig. 3. Zinc and iron contents of germinated mung bean (a) and cowpea (b) during the germination for 48 h.

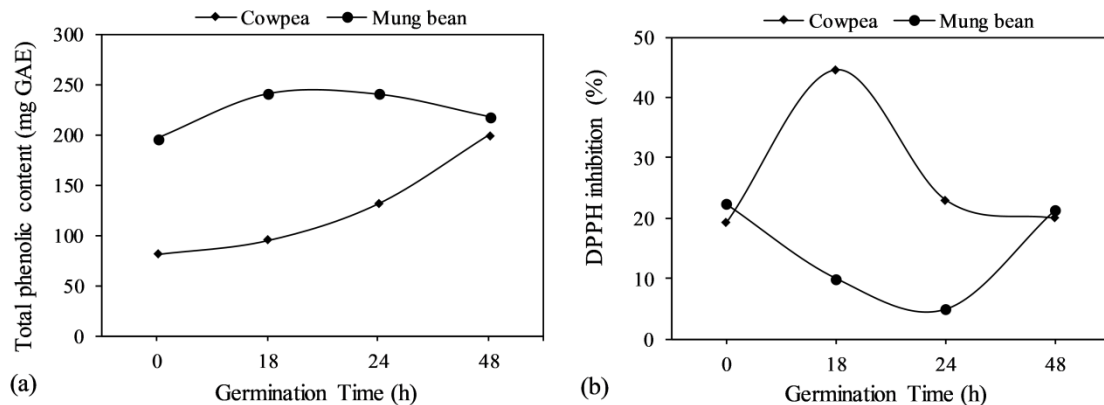


Fig. 4. Total phenolic contents (a) and antioxidant activities (b) of germinated mung bean (a) and cowpea (b) during the germination for 48 h.

low values of DPPH scavenging activity in the plant samples can be related to the accumulation of a number of compounds that are inactive against DPPH radicals. Other factors, such as type of endogenous enzymes used for the hydrolysis of protein, degree of hydrolysis, and the aromatic and hydrophobic amino acid residues within the peptide sequence improve the antioxidant activities of the compounds (Saadi *et al.*, 2015; Sefatie, *et al.*, 2013).

- Sensory evaluation of CBF

Table 3 shows the sensory evaluation of CBF samples prepared from GM and GC. The samples showed no significant differences ($p < 0.05$) in odor, color, and texture as compared to the control formula. By contrast, the samples showed significant differences in overall acceptance, flavor, and taste as compared to the control formula. Sample D was the most acceptable formula by the evaluators and was further selected for the analysis of chemical and functional properties. A total of 0.7 g of vanillin (as flavoring agent) and 0.2 g of sodium bicarbonate (for flour treatment) were added to formula D according to the Iranian standard for CBFs (Anon, 2003).

- Nutritional value of CBF

Table 4 shows proximate composition, energy value, minerals, vitamins, anti-nutritional contents and functional properties of CBF prepared from GM and GC. The moisture content of the formula prepared from GM and GC seed flour (formula D) is 5.7% (w/w), which is higher than that of the control formula (1.7%), but it is within the range (5–10%) reported by Achidi *et al.* (2016). Such level of moisture content (5.7%) can be due to the types of drying methods used for the preparation of the ingredients (Tufa *et al.*, 2016) and the high moisture of GM and GC seed flours. Formula D has 22.4% protein, which is higher than that of the control (15.4%) and

also within the limits of the FAO/WHO Codex Alimentarius standards (14.52–37.70 g in 100 g, FAO/WHO, 1994). Some studies reported that the protein contents of legumes can increase during the germination (Masood *et al.*, 2014; Fayyaz *et al.*, 2018).

The fat content of 10.5% (w/w) present in formula D is slightly above that of the control (10%), both of which below the recommended level by Codex (14.5%–41.1%). Formula D has a carbohydrate content of 57.0%, which is lower than that in the control formula (69.6%). These results meet the carbohydrate level recommended by WHO/FAO (2004) for CBF (≥ 65 g in 100 g). Total ash contents (4.4 and 3.5% in formula D and in the control, respectively) satisfy the recommended ash content levels by WHO/FAO (2004) for CBF (< 5 g in 100 g). The energy levels of formula D and the control formula are within 412–429 kcal in 100 g, which is close to the recommended energy level required by WHO/FAO (2004) for CBF (400–425 kcal in 100 g). Table 4 also presents the mineral and vitamin levels for both the CBF prepared from germinated mung bean (GM) and cowpea (GC) flour (formula D) and the control formula. Calcium level in formula D is 6,100 mg.kg^{-1} , which is higher than that of the control formula (5,500 mg.kg^{-1}). A calcium level of 265 mg or higher is recommended by the Iranian standard for CBF (Iranian Standard certification for complementary food, 2003) in 100 g of the dry food. Minimum required calcium level recommended by the Codex Alimentarius Standards (FAO/WHO, 1994) is 436 mg in 100 g. The iron contents are 56 mg.kg^{-1} in formula D and 97 mg.kg^{-1} in the control. The higher level of iron in the control formula is due to its fortification with iron by the manufacturer. Both formula D and the control have iron concentrations above the minimum amount (4.8 mg in 100 g) recommended by the Codex Alimentarius Standards (FAO/WHO, 1994), and close to the values for Iranian standard certification

for CBF (2003). The phosphorus concentration in formula D is 5133 mg.kg⁻¹, which is considerably higher than that of the control formula and also higher than that recommended by Iranian standards for the CBF (Anon, 2003) (183 mg in 100 g). Vitamin D contents in formula D and in the control are 329 and 280 IU in 100 g, respectively (Table 4). The vitamin D content in formula D is higher than the recommended nutrient intake (200 IU) for infants based on WHO/FAO (2004). Vitamin D is highly demanded for skeletal growth in the infants and also for the preservation of bone health (WHO/FAO, 2004). The vitamin C content as ascorbic acid in formula D is 0.05 mg per 100 g, which was less than that for the control sample (0.5 mg in 100 g). The control sample used in the current study was already enriched by the manufacturer with vitamin C. Vitamin B₁₂ were found at 1.2 and 1.5 mg in 100 g for formula D and control, respectively. These values are close to the

recommended nutrient intake of FAO/WHO (2004) and Iranian standard certification for complementary food (2003) for infants and children based on processed cereal.

- *Functional properties of CBF*

Table 4 also shows the functional properties of formula D. WAC is at 100%, which is significantly lower than that of the control (199%). In general, WAC can affect the consistency of the foods. In our case, it should be decreased by decreasing the viscosity of starchy components (or by using germinated seeds) to prepare a proper CBF (Usman et al., 2016). The bulk densities of both formula D and the control are at 0.80 g.ml⁻¹. Germination is a suitable method for preparing low-bulk CBF (Desalegn et al., 2015). The swelling indices of formula D and the control are 3.2 and 3.4, respectively. CBFs do not require high swelling indices that may otherwise result in a lower nutrient density (Fasuan et al., 2017).

Table 3. Sensory evaluation of different complementary baby foods (CBF) prepared from germinated mung bean and cowpea

CBF	Parameters [†]		
	Odor	Color	Taste
Control [‡]	4.3 ± 0.9 ^a	4.5 ± 0.5 ^a	3.9 ± 1.2 ^a
A	3.8 ± 0.9 ^a	4.4 ± 1.2 ^a	3.5 ± 0.7 ^{ab}
B	3.9 ± 1.1 ^a	4.0 ± 1.2 ^a	3.7 ± 1.0 ^{ab}
C	3.5 ± 1.3 ^a	4.0 ± 0.5 ^a	3.1 ± 0.4 ^b
D	4.0 ± 1.0 ^a	4.1 ± 0.7 ^a	4.2 ± 0.7 ^a
E	3.5 ± 1.1 ^a	3.9 ± 1.1 ^a	3.8 ± 0.6 ^{ab}
CBF	Texture	Flavor	Acceptability
Control [‡]	4.5 ± 0.8 ^a	3.7 ± 0.6 ^{ab}	4.3 ± 0.7 ^a
A	3.7 ± 0.5 ^a	3.3 ± 0.6 ^{bc}	3.9 ± 0.6 ^{ab}
B	3.6 ± 0.8 ^a	3.9 ± 1.1 ^{ab}	3.8 ± 0.8 ^{ab}
C	2.8 ± 0.7 ^a	2.9 ± 0.7 ^c	2.8 ± 0.6 ^c
D	4.7 ± 1.5 ^a	4.2 ± 0.6 ^a	4.8 ± 0.6 ^a
E	3.5 ± 1.2 ^a	3.6 ± 0.5 ^{abc}	3.4 ± 1.1 ^{cb}

[†] Values are expressed as means ± standard deviations of three replicates.

[‡] Control = the commercial baby food used as the reference in the current study.

^{a, b, c, d} the same letters in each column indicate that different formulas do not differ significantly with each other (p>0.05).

Table 4. Proximate compositions, energy values, minerals, vitamins, functional properties and anti-nutritional contents of the selected complementary baby food (formula D) prepared from germinated mung bean and cowpea

Parameter	Composition [†]	
	Control Formula [‡]	Formula D [‡]
Moisture (% w/w)	1.7± 0.5	5.7± 0.9
Carbohydrate (% w/w)	69.6± 0.0	57.0± 0.9
Protein (% w/w)	15.4± 0.5	22.4± 0.3
Fat (% w/w)	10.0± 0.6	10.5± 0.1
Ash (% w/w)	3.5± 0.1	4.4± 0.1
Energy (kcal per 100 g)	429± 2	412± 3
Calcium (mg.kg ⁻¹)	5500±0	6100±755
Iron (mg.kg ⁻¹)	97±0	56±7
Phosphorus (mg.kg ⁻¹)	4400±3	5133± 208
Vitamin C (mg per 100 g)	0.50±0.02	0.05± 0.02
Vitamin D (IU per 100 g) [§]	280 [§]	329±0
Vitamin B ₁₂ (mg per 100 g)	1.50± 0.01	1.20± 0.01
Tannin content (%)	0.3± 0.0	0.2± 0.0
Phytic acid (mg.g ⁻¹)	1.2± 0.0	1.2± 0.0
WAC (%) [¶]	199 ± 2	100 ± 0
BD (g.mL ⁻¹) [¶]	0.8 ± 0.2	0.8 ± 0.5
SI [¶]	3.4 ± 0.1	3.2 ± 0.2

[†] Values are expressed as means ± standard deviations of three replicates.

[‡] Control =commercial complementary baby food, Formula D= prepared formula from germinated mung bean and cowpea according to Table 1.

[§] International Unit (IU) = 0.025 µg for cholecalciferol/ergocalciferol. For the control formula, vitamin D value is reported from the product label.

[¶] WAC: Water absorption capacity, BD: Bulk density, SI: Swelling index.

- Anti-nutritional factors of CBF

Table 4 also shows the levels of tannins and phytic acid, which are considered as anti-nutrient components in mung bean and cowpea. The tannin contents are 0.2% (w/w) in formula D and 0.3% in the control. Phytic acid contents both in formula D and in the control are at 1.2 mg.g⁻¹ level. Germination decreases the concentration of anti-nutritional factors in the grains and as a consequence improves their nutritional qualities (Ertop & Bektaş, 2018; Fayyaz *et al.*, 2018).

Conclusion

Germination resulted in some changes in the nutritional attributes of mung bean and cowpea. It did not, however, increase the crude fat contents and carbohydrate levels of

the seeds. The phosphorus and zinc contents of both seeds increased during the 48 h of germination, whereas the calcium content did not show a major change during such period. The iron content of cowpea showed a major increase within 24-48 h of germination. Germination also resulted in a gradual increase in its TPC value of both mung bean and cowpea. Results of the current study can be applied for designing such functional foods as baby foods and to prevent malnutrition and cardiovascular diseases. This study revealed that enriched flours can be formulated by utilizing GM and GC and skim milk. CBF prepared in the current study possesses high contents of calcium, phosphorus, vitamin D, and protein. Using a CBF similar to what prepared here can be a good approach to

fight malnutrition, particularly micronutrient deficiency diseases in children.

Acknowledgement

This project is part of a Ph.D. dissertation at the University of Tehran, Iran. The authors would like to acknowledge the support provided by the Ministry of Higher Education and Scientific Research of Iraq (through the Indigenous Scholarship Program) and the Research Council of the University of Tehran (Tehran, Iran).

References

- Abiose, S. H., Ikuje, A. V. & Abiodun, F. I. (2015). Nutritional quality assessment of complementary foods produced from fermented and malted quality protein maize fortified with soybean flour. *Polish Journal of Food and Nutrition Sciences*, 65(1), 49-56.
- Achidi, A. U., Tiencheu, B., Tenyang, N., Womeni, H. M., Moyeh, M. N., Ebini, L. T. & Fossi, T. (2016). Quality evaluation of nine instant weaning foods formulated from cereal, legume, tuber, vegetable and crayfish. *International Journal of Food Science and Nutrition Engineering*, 6(2), 21-31.
- Anon. (2003). Iranian standard complementary food for infants and children based on processed cereal. Certification No. 2285 4th revision? Tehran, Iran.
- AOAC. (2010). Official Methods of Analysis. Association of Official Analytical Chemists, 18th ed, 3rd rev., Washington, D.C.
- Davis, J., Sonesson, U., Baumgartner, D. U. & Nemecek, T. (2010). Environmental impact of four meals with different protein sources: case studies in Spain and Sweden. *Food Research International*, 43(7), 1874-1884.
- Desalegn, B. B., Abegaz, K. & Kinfe, E. (2015). Effect of blending ratio and processing technique on physicochemical composition, functional properties and sensory acceptability of quality protein maize (QPM) based complementary food. *International Journal of Food Science and Nutrition Engineering*, 5(3), 121-129.
- Devi, C. B., Kushwaha, A. & Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea (*Vigna unguiculata*). *Journal of Food Science and Technology*, 52(10), 6821-6827.
- Erbersdobler, H. F., Barth, C. A. & Jahreis, G. (2017). Legumes in human nutrition. Nutrient content and protein quality of pulses. *Ernahrungs Umschau*, 64(9), 134-139.
- Ertop, M. H. & Bektaş, M. (2018). Enhancement of Bioavailable Micronutrients and Reduction of Antinutrients in Foods with Some Processes. *Food and Health*, 4(3), 159-165.
- FAO/WHO. (1994). Codex Alimentarius Standards for Foods for Special Dietary Uses (including foods for infants and children), Vol. 4. Joint FAO/WHO Food Standards Program Rome: WHO, Codex Alimentarius Commission; Rome, Italy.
- Fasuan, T. O., Fawale, S. O., Enwerem, D. E., Uche, N. & Ayodele, E. A. (2017). Physicochemical, functional and economic analysis of complementary food from cereal, oilseed and animal polypeptide. *International Food Research Journal*, 24(1), 275-283.
- Fayyaz, N., Mohebbi, M. & Milani, E. (2018). Effect of germination on nutrients, mineral, phytic acid and enzyme activity of mung bean. *Acta Medica Mediterranea*, 34, 597-605.
- Fouad, A. A. & Rehab, F. M. (2015). Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris Medik.*) sprouts. *Acta Scientiarum Polonorum. Technologia Alimentaria*, 14(3) 233-246.
- Gulati, J. K. (2010). Child malnutrition: trends and issues. *The Anthropologist*, 12(2), 131-140.
- Ijarotimi, O. S. & Keshinro, O. O. (2013). Determination of nutrient composition and protein quality of potential complementary foods formulated from the combination of fermented popcorn, African locust and bambara groundnut seed flour. *Polish Journal of Food and Nutrition Sciences*, 63(3), 155-166.
- Iqbal, A., Khalil, I. A., Ateeq, N. & Khan, M. S. (2006). Nutritional quality of important food legumes. *Food Chemistry*, 97(2), 331-335.
- Jayatilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B. C., Nammi, S. & Liyanage, R. (2018). Cowpea: an overview on its nutritional facts and health

benefits. *Journal of the Science of Food and Agriculture*, 98(13), 4793-4806.

Jiang, S., Cai, W. & Xu, B. (2013). Food quality improvement of soy milk made from short-time germinated soybeans. *Foods*, 2(2), 198-212.

Joshi, P. & Varma, K. (2016). Effect of germination and dehulling on the nutritive value of soybean. *Nutrition & Food Science*, 46(4), 595-603.

Kaushik, G., Satya, S. & Naik, S. N. (2010). Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterranean Journal of Nutrition and Metabolism*, 3(1), 39-46.

Kehinde Alawode, E., Idowu, M. A., Adeola, A. A., Oke, E. K. & Omoniyi, S. A. (2017). Some quality attributes of complementary food produced from flour blends of orange flesh sweetpotato, sorghum, and soybean. *Croatian journal of food science and technology*, 9(2), 122-129.

Kouris-Blazos, A. & Belski, R. (2016). Health benefits of legumes and pulses with a focus on Australian sweet lupins. *Asia Pacific journal of clinical nutrition*, 25(1), 1-17.

Lopez-Amoros, M. L., Hernandez, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, 19(4), 277-283.

Masood, T., Shah, H. U., & Zeb, A. (2014). Effect of sprouting time on proximate composition and ascorbic acid level of mung bean (*Vigna radiata* L.) and chickpea (*Cicer Arietinum* L.) seeds. *The Journal of Animal & Plant Sciences*, 24(3), 850-859.

Nonogaki, H., Bassel, G. W., & Bewley, J. D. (2010). Germination—still a mystery. *Plant Science*, 179(6), 574-581.

Ojiewo, C., Keatinge, D. J., Hughes, J., Tenkouano, A., Nair, R., Varshney, R., .. & Silim, S. (2015). The Role of Vegetables and Legumes in Assuring Food, Nutrition, and Income Security for Vulnerable Groups in Sub-Saharan Africa. *World Medical & Health Policy*, 7(3), 187-210. .

Olunike, A. A. (2014). Utilization of legumes in the Tropics. *J. Biol. Agric. Healthcare*, 4(12), 77-84.

Omenna, E. C., Olanipekun, O. T., & Kolade, R. O. (2016). Effect of boiling, pressure cooking and germination on the nutritional and antinutrients content of cowpea (*Vigna unguiculata*). *ISABB Journal of Food and Agricultural Sciences*, 6(1), 1-8.

Onoja, U. S., Akubor, P. I., Gernar, D. I., and Chinmma, C. E. (2014). Evaluation of complementary food formulated from local staples and fortified with calcium, iron and zinc. *Journal of Nutrition and Food Sciences*, 4(6), 1-6.

Onwuka, G. I. (2005). Food analysis and instrumentation: theory and practice. *Naphthalic prints, Surulere, Lagos, Nigeria*, 219-230.

Saadi, S., Saari, N., Anwar, F., Abdul Hamid, A., & Ghazali, H. M. (2015). Recent advances in food biopeptides: Production, biological functionalities and therapeutic applications. *Biotechnology advances*, 33(1), 80-116.

Pataczek, L., Zahir, Z. A., Ahmad, M., Rani, S., Nair, R., Schafleitner, R., ... & Hilger, T. (2018). Beans with benefits—the role of Mungbean (*Vigna radiata*) in a changing environment. *American Journal of Plant Sciences*, 9(7), 1577-1560.

Saraf-Bank, S., Esmailzadeh, A., Faghihmani, E., & Azadbakht, L. (2016). Effects of legume-enriched diet on cardiometabolic risk factors among individuals at risk for diabetes: a crossover study. *Journal of the American College of Nutrition*, 35(1), 31-40. *Nutrition*, 35(1), 31-40.

Sefatie, R.S, Fatoumata T., Eric K., Shi Y.H., & Guo-wei, L. (2013). In vitro antioxidant activities of protein hydrolysate from germinated black soybean (*Glycine max* L.). *Advance Journal of Food Science and Technology*, 5(4), 453-459.

Suryanti, V., Marliyana, S. D., & Putri, H. E. (2016). Effect of germination on antioxidant activity, total phenolics, β -carotene, ascorbic acid and [alpha]-tocopherol contents of lead tree sprouts (*Leucaena leucocephala* (Imk.) de Wit). *International Food Research Journal*, 23(1), 167.

Taussky, H.H., Shorr, E. (1953). A micro colorimetry method for determination of inorganic phosphorous. *The Journal of Biological Chemistry*, 202, 675-685.

Tufa, M.A., Urga, K., Weledesemayat, G.T., & Mitiku, B.G., (2016) Development and Nutritional Assessment of Complementary Foods from Fermented Cereals and Soybean. *Journal of Food Science and Nutrition*, 2: 014.

Usman, M. A., Bolade, M. K., & James, S. (2016). Functional properties of weaning food blends from selected sorghum (*Sorghum bicolor* (L.) Moench) varieties and soybean (*Glycine max*). *African Journal of Food Science*, 10(8), 112-121.

Velupillai, S., Nithyanantharajah, K., Vasantharuba, S., Balakumar, S., & Arasaratnam, V. (2009). Biochemical changes associated with germinating rice grains and germination improvement. *Rice Science*, 16(3), 240-242.

WHO/FAO. (2004). Human vitamin and mineral requirements. Report of a joint FAO /WHO consultation. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). Rome, Italy.

Wong, S. P., Leong, L. P. & Koh, J. H. W. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry*, 99(4), 775-783.

Xu, B. J. & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science*, 72(2), S159-S166.