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Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts

Amal Badshah Khattak [a,*], Aurang Zeb [a], Nizakat Bibi [a], Shahid Akbar Khalil [a], Mohammad Saeed Khattak [b]

[a] *Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan*
[b] *Department of Agricultural Chemistry, Faculty of Agriculture, Gomal University D.I. Khan, Pakistan*

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Abstract

Influence of germination time and type of illumination on phytic acid and polyphenols of chickpea was investigated. With blue light illumination, a significant decrease ($p < 0.01$) in phytic acid content was observed, while all other illuminations had no effect on this parameter. Germination time up to 48 h significantly reduced ($p < 0.01$) the phytic acid content from 1.01% to 0.6% while beyond that time it increased significantly ($p < 0.01$) reaching the maximum value of 0.9% after 120 h which is still significantly lower than control (1.01%). A similar trend was observed for methanol extractable polyphenols as for phytic acid. It decreased significantly ($p < 0.01$) with 24 h and 48 h germination time while after that it increased significantly ($p < 0.01$) and the maximum value was noted with 120 h germination. Red light significantly diminished methanol extractable phenols as compared to dark, fluorescent, yellow and irradiation illuminations. Effect of germination time and type of illumination was highly significant ($p < 0.01$) for water and acidic methanol extractable phenols. Acidic methanol extractable phenols significantly increased ($p < 0.01$) from 0.055% to 0.14% within the first 24 h germination while beyond that it decreased significantly reaching the minimum value as for control. Germination under dark and irradiated chickpea seed enhanced the methanol extractable phenols content followed by fluorescent and yellow illuminations. Lowest values for methanol extractable phenols were noted for red followed by green and blue illuminations. Water extractable polyphenols decreased significantly with the increase in germination time. Significantly higher water extractable polyphenols content were noted under blue light germinating samples followed by irradiated samples and lower values for germination under yellow light.
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1. Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop due to its protein content and wide adaptability as a food grain. It is a source of dietary protein in general and particularly for vegetarian segments of the Indian-subcontinent population. It is also used as a protein supplement in the European countries (Viveros, Brenes, Elices, Arija, & Canales, 2001). Green chickpea is commonly used as a vegetable while dry seed is consumed as such, in the form of dhal, and in the form of fried products from its flour. Apart from being a valuable source of protein, consumption of legumes has also been linked to reduced risk of diabetes and obesity (Geil & Anderson, 1992; Venkateswaran, Pari, & Saravanan, 2002), coronary heart disease (Anderson et al., 1984; Bazzano et al., 2001), colon cancer (Hangen & Bennink, 2002; Hughes, Ganthavorn, & Wilson-Sanders, 1997) and gastrointestinal disorders, (Bourdon et al., 2001; Kolonel et al., 2000). Consumption of legumes may also have a protective effect against prostate cancer in humans (Kolonel et al., 2000). The phenolic compounds present in these legumes are known to exhibit strong antioxidant, anti-mutagenic, and anti-genotoxic activities (Badshah, Zeb, & Sattar, 1991).

* Corresponding author. Tel.: +92 91 2964060–2; fax: +92 91 2964059.
E-mail address: amalbad2000@yahoo.co.uk (A.B. Khattak).

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On the other hand, anti-nutritional properties of phytic acid and polyphenols have been a concern for the nutritionists. Phytic acid binds minerals, thereby rendering them unavailable for metabolism. Phenolic compounds or their oxidized products form complexes with essential amino acids, enzymes and other proteins, thus lowering their nutritional value (Shahidi & Naczk, 1992) and protein digestibility (Mitaru, Reichert, & Blair, 1984). These anti-nutrients can be fully or partially removed by processing (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987; Ibrahim, Habiba, Shatta, & Embaby, 2002; Singh, 1988).

Sprouting is the practice of soaking and leaving seeds until they germinate and begin to sprout. This practice is reported to be associated with improvements in the nutritive value of seeds (Badshah et al., 1991; Khattak et al., 2006; Sattar, Badshah, & Zeb, 1995; Zanabria, Katarzyna, De Jong, Birgit, & Robert, 2006). At the same time there are indications that germination is effective in reducing phytic acid and flatulence causing oligosaccharides (namely stachyose and raffinose), thereby increasing protein digestibility and improving sensory properties (Lintschinger et al., 1997; Zanabria et al., 2006). In case of white kidney beans, faba beans and chickpeas; sprouting improved the protein/amino acid digestibility by decreasing anti-nutritional factors and increasing the true/apparent protein/amino acid digestibility (Rubio, Muzquiz, Burbano, Cuadrado, & Pedrosa, 2002; Schulze et al., 1997). According to Lorenz (1980) the practice of sprouting of cereal grains has become popular in the western world. They can be used in many different foods including breakfast items, salads, soups, casseroles, pasta, and baked products. It has been recently reported that germination under different type of illumination has significant effect on biosynthesis of ascorbic acid and sprout yield of soybean and chickpea (Khattak et al., 2006; Mao, Dong, & Zhu, 2005).

The present research work was conducted to investigate the impact of germination time and type of illumination on total phenolic content and phytic acid in chickpea sprouts.

2. Materials and methods

Chickpea seeds of desi type variety NIFA-2005, developed at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar were cleaned from all impurities including broken and diseased seeds. Part of the unsoaked sample was ground in a stainless steel grinder to pass through a 40 mesh screen. The ground samples were kept in plastic bags, stored at 4 °C for chemical determinations.

2.1. Soaking of chickpea seeds

The seeds were soaked by submerging in tap water in glass containers for 24 h at room temperature. After pouring off the soaking water, the seeds were rinsed with water, spread evenly on a tray lined with absorbent paper and then placed in a controlled environment chamber at 28 °C.

2.1.1. Sprouting chamber

Wooden chambers each with $91 \times 91 \times 60$ cm ($L \times H \times W$) dimensions were used for germination of seeds. There were five chambers used for five types of illumination, i.e., fluorescent, yellow, blue, green and red and two for dark and gamma irradiated samples. The light source in the illuminated chambers was fitted on the ceiling of the chamber. The temperature of the chambers was maintained at 28 ± 3 °C.

2.1.2. Gamma irradiation treatment

The seed samples were irradiated at a dose of 3 krad in Co60 gamma radiation source (Isseldovatel, Konhpobba, USSR). Soaking and sprouting was then carried out in dark conditions.

2.1.3. Sprouting procedure

Sprouting was started in triplicate for each treatment (illumination i.e., dark, red, blue, tungsten, green and fluorescent and length of time i.e., 0, 24, 48, 72, and 96 h) in trays lined with absorbent paper (blotting paper). Seed/sprouts were washed twice a day to avoid microbial growth. Tap water was sprayed throughout the germination period at 9 a.m., 1 p.m. and 6 p.m. daily.

2.1.4. Light exposure

Fluorescent tubes (40 W, Philips, Lahore, Pakistan) were used as a white light source. Respective colored bulbs (40 W, Philips, Lahore, Pakistan) were used as per illumination treatments. The trays were distributed under the light so as to give uniform flux density to each tray. The same flux density was obtained by turning on the fixed number of light sources and by adjusting fixed distances between the lamps and the test materials.

2.2. Phytic acid

Phytic acid contents were determined by the method of Haug and Lantzsch (1983). The sample extract (with 0.2 N HCl) was heated with an acidic iron (III) solution of known iron content (0.2 g Ammonium iron (III) sulphate-12 H_2O was dissolved in 100 ml 2 N HCl and volume made up to 1000 ml with distilled water). Phytate phosphorous in the supernatant was measured as the decrease in absorbance of iron content using 2,2-bipyridine at 419 nm with a spectrophotometer (UV-120-02, Shimadzu, Kyoto, Japan).

2.3. Extraction and assay of total phenols (%)

A weighed amount of sample was extracted with methanol (6 ml/mg) for 30 min in a screw-capped test tube by shaking at a speed of 55 cycles/min in a shaker. The residue obtained after centrifugation (Model 50 A, Sakuma, Japan) at 906*g* for 10 min at room temperature (30 ± 2 °C), was re-extracted with methanol and the two methanol extracts were combined for methanol extractable

total phenols determination. The residue was further extracted twice with acidic methanol containing 1% (v/v) concentrated HCl and these were pooled together for the determination of acidic methanol extractable total phenols (Ramamurthi, Butler, Ranjit, & Lewisk, 1986). Aqueous extract for each sample was prepared by boiling 0.5 g sample in 50 ml distilled water for half an hour as reported by Bibi, Chaudry, Khan, Ali, and Sattar (2001). Total phenols were determined spectrophotometerically (by taking absorbance at 720 nm) using Folin Ciocalteau phenol reagent which consists of 2.5% sodium molybdate and 19% sodium tungstate (Senter, Robertson, & Meredith, 1989).

3. Statistical analysis

Statistical analysis was conducted for each of the measured traits by analysis of variance (ANOVA – using CRD factorial design) and the means were separated by Duncan multiple range test (DMR) using Mstat-C software.

4. Results

Influence of germination time and illumination was highly significant ($p < 0.01$) on phytic acid content as well as methanol, acidic methanol and water extractable polyphenols (Table 1). As can be noted from Fig. 1, phytic acid contents (average of all illuminations) decreased significantly ($p < 0.01$) from 1.01% to 0.60% up to 48 h sprouting but with longer sprouting time its value increased significantly. However, its content after 120 h germination time (0.9%) was still significantly lower than control (1.01%). On overall basis, germination under blue light was most effective in reducing the phytic acid level while effect of all other illuminations was non significant. Impact of interaction of germination time and illuminations on phytic acid was also highly significant. Highest phytic acid values were observed for 96 h germination under dark followed by 120 h germination under red light. Minimum phytic acid content was recorded for 48 h germination under blue and red light and 72 h under blue light condition.

The effect of germination time and illuminations on methanol extractable polyphenols is shown in Fig. 2. It decreased significantly ($p < 0.01$) with 24 h and 48 h germination times and increased significantly with further advancement of germination time, thereby, reaching the maximum value of 0.14% after 120 h germination time. Germination under red light was more effective in reducing methanol extractable polyphenols followed by germination under green and blue lights. Influence of germination time and type of illumination was highly significant too (Table 1).

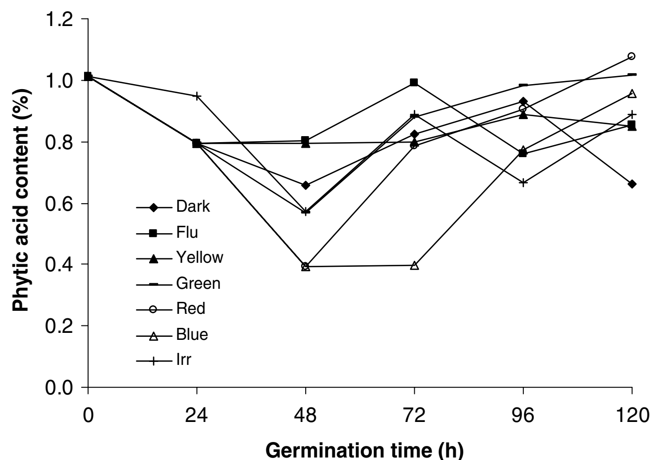

Fig. 1. Impact of germination time and illumination on phytic acid content of ckickpea.

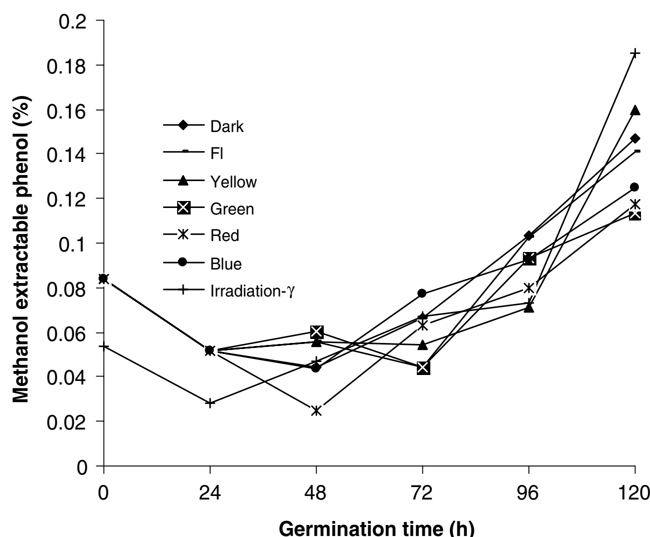

Fig. 2. Impact of germination time and illumination on methanol extractable phenol.

Table 1
Analysis of variance – showing mean sum of the squares (and F values in parentheses)

Source of variation	Degrees of freedom	Methanol extractable phenols	Acidic methanol extractable phenols	Water extractable phenols	Phytic acid
Time	5	0.026*** (1477.8375)	0.024*** (321.9020)	0.434*** (938.8520)	0.397 *** (63.6510)
Light source	6	0.00001*** (18.9881)	0.005*** (62.2729)	0.016*** (34.5619)	0.050*** (7.9471)
Time × light source	30	0.001*** (36.8998)	0.002*** (31.3640)	0.020*** (44.0252)	0.057*** (9.2140)
Error	84	0.00001	0.00001	0.00001	0.006
Total	125				

*** $p < 0.001$.

Maximum methanol extractable phenols (0.14%) were observed in 120 h germination of irradiated chickpea seed while minimum in 48 h germination under red light.

The behaviour of acidic methanol extractable polyphenols towards germination time and type of illumination was altogether different from that of phytic acid and methanol extractable polyphenols (Fig. 3). It increased tremendously with 24 h germination under all types of illumination and then decreased continuously on further increase in germination time, reaching the same level as for control after 120 h germination. Type of illumination significantly ($p < 0.01$) influenced the acidic methanol extractable polyphenols. Average values for acidic methanol extractable polyphenols were significantly higher ($p < 0.01$) for germination of irradiated samples and germination under dark condition followed by germination under fluorescent and yellow illumination. Germination under red light significantly reduced acidic methanol extractable polyphenols followed by green and blue illuminations. The effect of interaction of germination time with illumination type was highly significant ($p < 0.01$) on acidic methanol extractable polyphenols. Highest value for this parameter was observed in 48 h germination under fluorescent light and lowest values were assayed in germination under red light for 48 h and 120 h.

Effect of germination conditions on water extractable phenols is shown in Fig. 4. The water extractable polyphenols decreased significantly ($p < 0.01$) as the germination time increased and the lowest values were noted in 120 h sprouts. Impact of type of illumination on water extractable polyphenols was highly significant ($p < 0.01$). Highest mean values were noted in germination under blue light followed by irradiation while lowest values in sprouts under dark followed by sprout under red light. The effect of interaction of germination time and type of illumination on water extractable polyphenols was also highly significant. Highest values in this case were recorded in control samples and the lowest values for 120 h germination under fluorescent light.

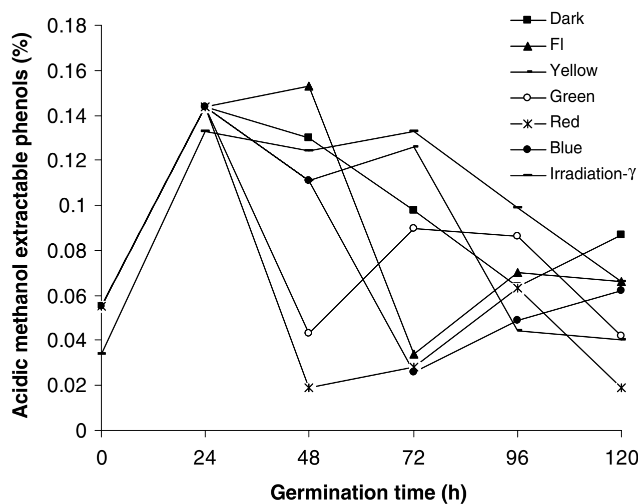

Fig. 3. Impact of germination time and illumination on acidic methanol extractable total phenol.

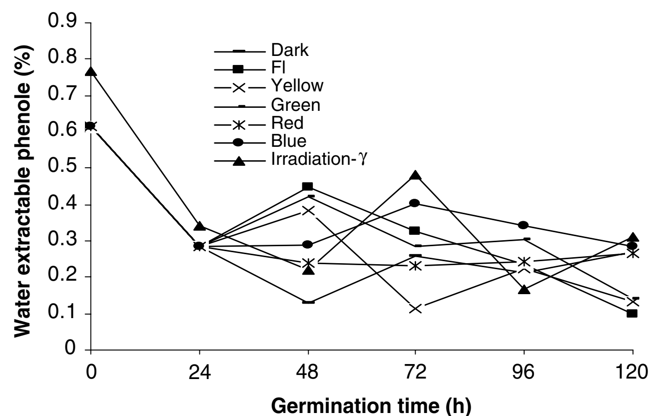

Fig. 4. Impact of germination time and illumination on water extractable total phenol.

5. Discussion

Phytic acid, which is negatively correlated with in vitro protein digestibility, varies among legume species and among the varieties within a single legume (Chitra, Vimala, Singh, & Geervani, 1995). Reduction in phytic acid contents of cereals and legume seeds with sprouting has been frequently reported (Harmuth-Hoene et al., 1987; Ibrahim et al., 2002). This has been attributed to an increase of phytase activities. In fact, this enzyme makes the phytates soluble and releases soluble protein and minerals (Camacho, Sierra, Campos, Guzman, & Marcus, 1992). Nestares, Barrionuevo, Urbano, and Lopez-Frias (1999) recommended sprouting before cooking for both homes and industries, which produces food products for nutritionally vulnerable persons with high Ca and P requirements. The low nutritional quality of legume protein has partly been attributed to the presence of multiphysiological and toxic factors such as trypsin inhibitors, hemagglutinine, cyanogenic glycosides, saponine, flatulence factors, phytates and polyphenoles (Barroga, Laurena, & Mendoza, 1985). El-Adawy (2002) reported a decrease in all anti-nutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity during soaking in 0.5% sodium bicarbonate. Sprouting has also been reported to be more effective than other pretreatments in reducing phytic acid contents (Bakr, 1996). Phytates in chickpea seeds are more prone to hydrolysis during sprouting than in other legumes (Chitra, Singh, & Rao, 1996), however the present finding regarding the influence of germination time as well as type of illumination on this anti-nutrient has not been previously reported.

Barroga et al. (1985) reported a 23–36% reduction in mungbean polyphenols during germination with maximum polyphenol removal occurring after 48 h, which is in agreement with our findings for chickpeas. Paramjyothi and Anjali (2005) reported significant decrease in polyphenol content of chickpea on soaking. However, a significant

increase in methanol extractable polyphenol content of germinated seeds after 72 h may be due to renewed synthesis of polyphenols or degradation of high molecular weight insoluble polymers into smaller molecular weight that react with the reagent (Satwadhar, Kadam, & Salunkhe, 1981).

The biochemical reactions taking place during seed sprouting are influenced by the type of light as already reported. It was noted that different wavelength lights influence differently the enzyme system responsible for biosynthesis of ascorbic acid, and hence result in differing concentration of the compound in the germinating seeds (Mao et al., 2005). The same authors also concluded that biosynthesis of ascorbic acid was promoted by ultraviolet light, whereas sprout growth was more pronounced with red illumination. This indicates that different biochemical pathways are differently influenced by different wavelengths of light. A similar observation was made during the present study, in which the lowest phytic acid contents (at 48 h sprouting) were observed with blue and red lights, the lowest total phenol value (at 48 h sprouting) was noted with red illumination, the lowest acidic methanol extractable phenols (at 48 h and 120 h sprouting) again with red illumination and the lowest value for water extractable phenols (at 120 h sprouting) was noted with fluorescent light.

Although it still remains to be established as to how the illumination type influences the polyphenol hydrolysis or biosynthesis, the present findings suggest that different light types have differing effects on the biochemical reactions at various stages of the germinating chickpea seeds. Hence, by using different sprouting conditions, sprouts with different functionalities can be produced. For example, green light can be used for producing sprouts with high polyphenols (and consequently high anti-oxidant potentials) while sprouts with low polyphenols and low phytic acid contents (and hence higher protein digestibility) can be produced under red and blue lights for 48 h.

The range of wavelengths (400–700 nm) of visible light is centrally located in the electromagnetic spectrum. Light is a stream of minute packets of energy-photons in the energy range of around 2–3 electron volts (eV). As the energy of the light increases, the wavelength decreases (URL, 1995–2000). Among the tested visible illumination types, the blue and the green illuminations have the shortest wavelengths (475 nm and 510 nm, respectively). The significant effect of blue and green illuminations on phytic acid or polyphenols contents may be attributed to their shorter wavelengths and higher energy ranges (2.55 eV and 2.45 eV, respectively) as compared to other illuminations used in this experiment. However, a scientific cause and effect relationship between the wave length (radiant energy) and the reduction of phytic acid during germination remains to be established.

6. Conclusions

Germination for 48 h and blue illumination were most beneficial in diminishing phytic acid content to a maximum level in chickpea. The same level of germination was also sufficient to reduce the methanol extractable polyphenol to a lowest level while these types of polyphenol were more vulnerable to red illumination. Red light was also effective in keeping the acidic methanol extractable polyphenols to a minimum level. The acidic methanol extractable polyphenols increased twofold within 24 h germination while after that they decreased and reached the value almost equal to control after 120 h germination, thus counteracting the germination effect on these particular polyphenols. However, red light was highly effective in lowering the level of these polyphenols. Water extractable polyphenols were removed to a maximum level after 120 h germination. However, in this case, fluorescent and yellow illuminations were more effective in keeping this type of polyphenols to a minimum level.

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