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Effect of Germination Time and Type of Illumination on Proximate Composition of Chickpea Seed (*Cicer arietinum* L.)

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Abstract: Impact of germination time and type of illumination on proximate composition of chickpea seed was investigated. Germination time and type of illumination had highly significant influence ($p < 0.001$) on the level of moisture, protein, fat, fiber, ash and Nitrogen Free Extract (NFE) contents. Increase in germination time was associated with increase in moisture, protein, ash and fat contents and decrease in fiber and NFE contents. Moisture accumulation increased significantly ($p < 0.001$) with dark, fluorescent light and γ -irradiated seed sprouts, while green, blue and yellow lights have significant ($p < 0.001$) promotional effects on protein and fiber contents. Germination of γ -irradiated chickpea seed had significant ($p < 0.001$) promotional effect on ash and fat contents, while dark, fluorescent and yellow lights on NFE content. Interaction of the treatments (germination time X type of illumination) on all the parameters studied was also highly significant ($p < 0.001$).

Key words: Germination time, type of illumination, proximate composition

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a source of dietary protein in general and particularly for poor/vegetarian segments of the world population. It is also used as a protein supplement in the European countries (Viveros *et al.*, 2001). Chickpea is an ancient crop and has been grown and consumed in tropical, sub-tropical and temperate regions for centuries. It is valued for its nutritive seeds with high protein content. Chickpea is used exclusively as food in many countries (Muehlbauer and Singh, 1987; Malhotra *et al.*, 1987) and its traditional uses include boiling, roasting, canning or processing into humus (a traditional dish in the Middle East).

Sprouting (the practice of soaking then draining and leaving seeds until they begin to sprout) has been identified as an inexpensive and effective technology for improving the nutritional quality of cereals and grain legumes. It is reported to be associated with improvements in the nutritive value of seeds (Badshah *et al.*, 1991; Sattar *et al.*, 1995; Zanabria *et al.*, 2006; Khattak *et al.*, 2007a). As the seed imbibe water the enzymes are activated and the biochemical changes take place. Proteins break into amino acids. Water-soluble vitamins such as B complex and vitamin C are created. Fats and carbohydrates are converted into simple sugars. Weight increases as the seed absorbs water and minerals. At the same time there are reports that germination is effective in reducing phytic acid, flatulence causing oligosaccharides (namely stachyose and raffinose) and polyphenols thereby increasing protein digestibility and improving sensory properties (Lintschinger *et al.*, 1997; Zanabria *et al.*, 2006; Khattak *et al.*, 2007). It is reported that sprouting improved the protein/amino

acid digestibility by decreasing anti-nutritional factors and increasing the true/apparent protein/amino acid digestibility (Schulze *et al.*, 1997; Rubio *et al.*, 2002). According to Lorenz (1980) the practice of sprouting 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 (Mao *et al.*, 2005; Khattak *et al.*, 2007a).

The present research, being part of a study “Nutritional enhancement of chickpea seed through germination techniques”, was undertaken to investigate the impact of germination time and type of illumination on proximate composition of chickpea sprouts.

MATERIALS AND METHODS

The present research is a continuation of previous studies (Khattak *et al.*, 2007a, b). It was started in the Nuclear Institute for Food and Agriculture (NIFA), Tarnab, during March 2006. A brief account of methodology used was as follows:

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 un-soaked 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.

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.

Sprouting Chamber

Wooden chambers each with 91×91×60 cm (L×H×W) dimensions were used for germination of seeds. There were 5 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.

Gamma Irradiation Treatment

The seed samples were irradiated at a dose of 30 krad in Co-60 gamma radiation source (Isseldovatel, Konhpobba, USSR). Soaking and sprouting was then carried out in dark conditions.

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 am, 1 and 6 pm daily.

Light Exposure

Fluorescent tubes (40 W, Philips, Lahore, Pakistan) were used as a white light source. Respective colored bulb (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 were obtained by turning on the fixed number of light sources and by adjusting fixed distances between the lamps and the test materials. Germination under all types of illuminations was repeated four times.

Proximate Composition

Air oven method was (AOAC, 1984, method # 14.004) used to determine moisture content of the sprouted and un-sprouted chickpea samples. Protein content (%) was determined using Micro Kjeldahl (AOAC, 1984 method # 14.067). Crude fat was determined by soxhlet method using soxtec (labconco) apparatus (AOAC, 1984-method # 14.066).

For ash determination, 5 g samples were ignited in broad ashing dish that has been previously ignited, cooled in desiccator and weighed soon after reaching room temperature. The charred samples were placed in furnace at 550°C until light gray ash results, or to constant weight. The samples were then cooled in desiccator and weighed soon after reaching room temperature (AOAC, 1984, method # 14.006). Crude fiber was determined using AOAC method #7.070 (AOAC, 1984). Nitrogen Free Extracts (NFE) was measured by difference i. e, $100 - (\text{moisture} + \text{protein} + \text{Crude fat} + \text{ash}) = \text{percent nitrogen free extract}$. Determination of each of the trait was repeated three times and the values reported are on moisture free basis.

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

RESULTS AND DISCUSSION

Moisture contents of the sprouts were significantly ($p < 0.001$) influenced by germination time, type of illumination as well as their interaction (Table 1). Mean value for maximum moisture percent was noted in samples of 120 h germination, while in case of type of illumination, maximum mean values were noted for germination under dark, fluorescent light and germination of gamma irradiated seed. Lowest mean value for percent moisture was observed in samples germinated under red light (49.98%) followed by germination under blue (50.28%), yellow (50.35%) and green (50.37%) lights. Germination under dark after 120 h has the highest moisture content (62.57%) followed by germination under fluorescent light after 120 h (62.40%) and germination in dark after 96 h (62.00). The impact

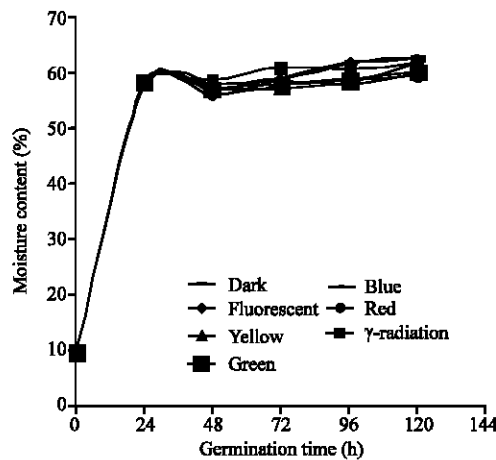


Fig. 1: Effect germination time and type of illumination on moisture content of chickpea

of time of germination was almost linear on moisture content under all types of illuminations up to 24 h, beyond which time the moisture of almost all samples leveled off with very little increase during the rest of germination time (Fig. 1).

Highly significant ($p < 0.001$) variations in protein content were observed due to time of germination, types of illuminations and their interaction. The average protein content of chickpea was 19.84% which increased to a maximum level of 21.97% after 96 h germination. The increase in protein content as a function of germination time was linear for all types of illuminations. Highest mean value for protein % was observed in germination under blue light (21.40%) followed by germination under red (21.38%) and green (21.31%) lights. Lowest mean protein content (21.10%) was observed in germination in dark condition. Maximum increase in protein concentration due to interaction of germination time and type of illumination was investigated in germination in yellow light after 96 h (22.67%) followed by germination in red light after 120 h (22.41%) and green light after 96 h (22.23%). In general, blue, green and red lights seem to have a promoting effect on protein concentration of chickpea sprouts (Fig. 2).

All the factors (sprouting time, light type and sprouting time \times light type) have a highly significant effect ($p < 0.001$) on ether extract (fat %). As for moisture and protein content, there was a linear increase in mean values of fat % (4.24 to 6.03%) with increase in time of germination. Highest mean value for fat % was observed in irradiated chickpea sprouts (6.09%), while blue light ranked 2nd

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

Source of variation	df	Moisture	Ash	Protein	Fat	Fiber	NFE
Sprouting time	5	8502.837*** (28586.0)	2.868*** (97.6)	13.024** (5.5)	9.378*** (105.1)	17.321*** (32.4)	20.372* (3.7)
Error	12	0.297	0.029	2.381	0.089	0.535	5.515
Light type	6	7.067*** (16.9)	0.166*** (68.4)	0.370*** (7.2)	1.920*** (53.3)	0.049* (2.2)	3.664*** (6.5)
Sprouting time \times light type	30	2.391*** (5.7)	0.029*** (11.9)	0.332*** (6.5)	0.504*** (13.9)	0.032** (2.0)	1.245** (2.2)
Error	72	0.418	0.002	0.051	0.036	0.016	0.564
Total	125						

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

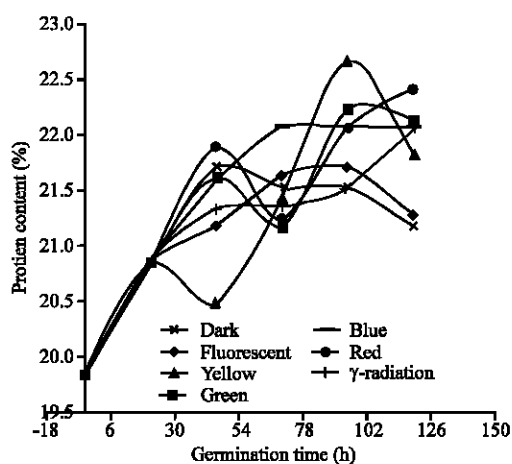


Fig. 2: Effect of germination time and illumination on protein content of chickpea

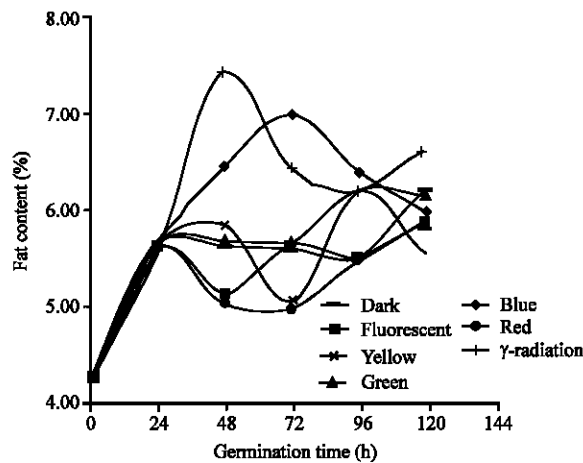


Fig. 3: Effect of germination time and type of illumination on fat content of chickpea

in this respect. Sprouts under red light have the lowest fat % (5.19%). Forty eight hours sprouting of irradiated seed has the maximum content of fat percent (7.42%) followed by 72 h germination under blue light (6.98%). Highest values for fat% in germination in dark, fluorescent and red light conditions was observed after 120 h, while in blue, green, yellow and gamma irradiation were 96, 72, 96 and 48 h, respectively (Fig. 3). Germination of irradiated seed, blue light and to some extent green light have promotional effect on fat content of chickpea sprouts as maximum fat content were observed in these sprouts.

Fiber content of chickpea sprout decreased significantly ($p < 0.001$) with the advancement of germination time (Table 1). The average content of fiber in chickpea was 7.90%. The mean value decreased to 5.55% after 120 h germination. While, on the other hand, mean values of fiber content were statistically the same for all the illumination types except for the fluorescent light which has significantly (5.96%) lowest mean value for fiber content. There was a linear decrease in fiber content in sprouts of all types of illuminations with the increase in germination time. The control have minimum and 120 h sprouts maximum fiber content for sprouts under all conditions (Fig. 4).

Ash contents of the sprouts were significantly ($p < 0.001$) influenced by germination time, type of illumination as well as their interaction (Table 1). It increased significantly ($p < 0.001$) with the advancement of germination. The mean value of ash content of chickpea control was 3.76% which increased to 4.69% after 120 h germination. Highest mean value for ash content was observed in sprouts of irradiated chickpea seeds (4.44%) followed by red (4.37%) and green (4.36%) illuminations. In general, fiber content increased with the increase in germination time in sprouts under all types of lightings (Fig. 5).

The values for nitrogen free extract varied significantly ($p < 0.001$) with germination time and type of illumination. The mean value of control samples was 64.26%, which decreased to 61.88% after 120 h germination. Sprouts under green and blue lights have lower value for NFE while sprouts under all other lights have higher values (Fig. 6).

Although, reports on the effects of sprouting on nutrient contents in various cereals and legumes are well documented (Lintschinger *et al.*, 1997; Badshah *et al.*, 1991; Sattar *et al.*, 1995), evidence on the effect of sprouting under different illuminations on nutrient content is lacking. In fact fairly contrasting effects have been observed for the effects of sprouting under different types of illuminations in seeds of chickpea and soybean (Mao *et al.*, 2005; Khattak *et al.*, 2007a, b). Biochemical

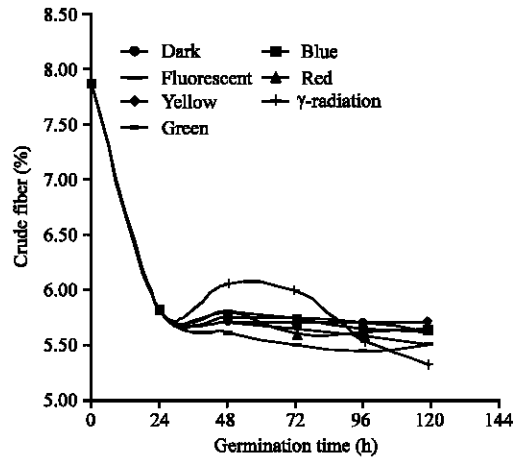


Fig. 4: Effect of germination time and type of illumination on crude fiber content of chickpea

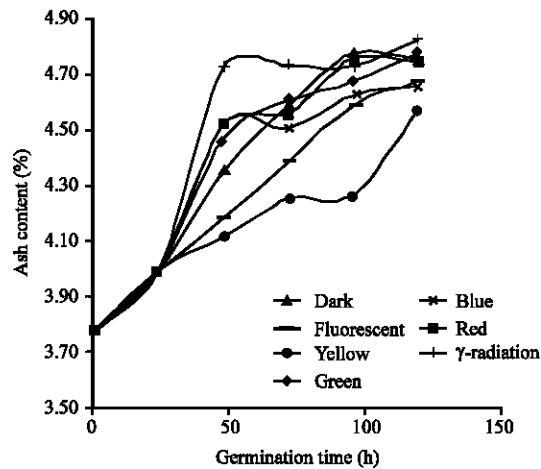


Fig. 5: Effect of germination time and type of illumination on ash content of chickpea

changes in the germinating seeds have also been reported to be significantly different in different varieties. Khalil *et al.* (2007) while working on Kabuli and desi type chickpea varieties, reported significant increase in moisture, protein, ash and there is no period after ether extract contents. and decrease in fiber and NFE contents. According to their investigations, changes in macro nutrients among desi and Kabuli types were significant. These findings are in agreement to our present investigations. El-Mahdy *et al.* (1985) studied the effect of germination on the nutritional quality of two varieties of lentil seeds. They reported changes in nutritional quality due to germination as well as genotypic differences. Effect of sprouting on inter-varietal differences in water-uptake and biochemical traits of legumes were also reported by Jood *et al.* (1997), Mulimani *et al.* (1996), Obizoba (1991) and Hoene *et al.* (1987). They noted differences in water uptake during 4 days germination of wheat, chickpeas and mung beans. Increases in the content of polyunsaturated fatty acids in wheat and of dietary fiber in wheat and mung beans were noted. Similarly Chung *et al.* (1998) reported that in barley (but not in canola), sprouting was associated with significant increase in crude fiber. These findings are contradictory to present results in chickpea. In canola, there were significant losses in lipid content (Badshah *et al.*, 1991). These results again confirm the existence of inter-specific differences in the

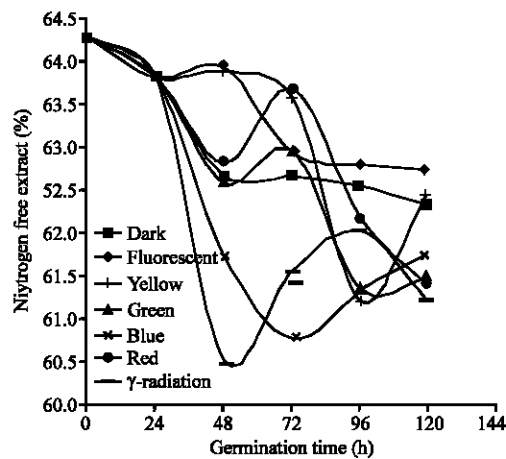


Fig. 6: Effect of germination time and type of illumination on nitrogen free extract (NFE) of chickpea

biochemical changed during sprouting. Jimenez *et al.* (1985) noted an increase in the content of protein and fiber in soybean seeds. Ether extract increased until the third day and then decreased on the fifth. Lorenz (1980) found that the increase in nutrients during sprouting is not true increases. They simply reflect the loss of dry matter, mainly in the form of carbohydrates (NFE), due to respiration during sprouting. As total carbohydrates decrease, the percent ratio of other nutrients increases. Parameswaran *et al.* (1994) noted increase in the percent protein in germinated grains of porso millet as a result of dry matter loss during germination (Mao *et al.*, 2005; Khattak *et al.*, 2007), while working on soybean and chickpea, respectively, reported significant effects of germination time and type of illumination on biosynthesis of ascorbic acid and sprout yield. The earlier one found a promotional effect of ultra violet illumination on biosynthesis of ascorbic acid while the same type of illumination depressed the sprout growth. According to Khattak *et al.* (2007), green illumination was effective in promoting synthesis of ascorbic acid and retarding the growth of sprouts. Khattak *et al.* (2007) reported in another study significant impact of illumination on degradation/synthesis of phytic acid and polyphenols. These reports as well as the present one indicate that lights with different wavelengths influence differently the enzyme system responsible for biosynthesis of different nutrients and hence result in differing concentration of the compound in the germinating seeds. In the present study, higher mean values of protein and fiber contents of sprouts under blue green and red illuminations, of moisture content under dark, fluorescent and gamma irradiated chickpea sprouts, of ash content of irradiated chickpea sprouts and that of NFE content under dark, red yellow illumination, can be attributed to the wavelengths influence. Hence, it can be inferred that different biochemical pathways are differently influenced by different wavelengths of light. The mechanism as to how the illumination types influenced the nutrients hydrolysis or biosynthesis is still to be investigated; however, the present findings suggest that different light types have differing effects on the biochemical reactions at various stages of the germinating chickpea seeds.

CONCLUSION

It is inferred from this study that germination time and type of illumination have highly significant ($p < 0.01$) effect on proximate composition of chickpea sprouts. Protein, moisture, fat and ash contents increased with the increase in germination time while the NFE and fiber content decreased. Red, green, blue and yellow lights have promotional effect on protein and fiber contents, irradiation on ash and fat content and dark, fluorescent and yellow on NFE content.

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