Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 797–801
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Impact of germination time and type of illumination on carotenoid content, protein solubility and in vitro protein digestibility of chickpea (*Cicer arietinum* L.) sprouts

Amal Badshah Khattak *, Aurang Zeb, Nizakat Bibi

Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan

Received 7 September 2007; received in revised form 17 January 2008; accepted 23 January 2008

Abstract

Sprouts have been reported to be nutritionally superior to their respective seeds with higher levels of nutrients and lower amounts of antinutrients. Significant differences occur in these nutrients and antinutrients with germination under different types of illumination. This paper reports the impact of germination conditions on changes in β-carotene content, protein solubility and in vitro protein digestibility of chickpea sprouts. The influence of germination time, type of illumination and their interaction on β-carotene content, protein solubility and in vitro protein digestibility of chickpea sprouts was highly significant ($p < 0.01$). Highest value for β-carotene were observed for sprouts germinated under yellow light for 72 h (131.74 mg 100 g^{-1}) and lowest for blue light group after 120 h germination. Sprouts of irradiated group had overall higher content of β-carotene throughout germination period. Protein solubility was also higher for sprouts of irradiated group and green illumination group after 120 h germination. Sprouts of irradiated group had highest value for % in vitro protein digestibility after 96 h germination followed by the same group after 120 h germination. It is inferred from the study that irradiation of chickpea seed prior to germination improved the β-carotene content, protein solubility and in vitro protein digestibility of chickpea sprouts.

Keywords: Germination time; Type of illumination; β-Carotene; Protein solubility; In vitro protein digestibility

1. Introduction

Sprouting is reported to be associated with improvement in the nutritive value of seeds (Badshah, Zeb, & Sattar, 1991; Khattak, Zeb, Bibi, Khalil, & Khattak, 2007; Sattar, Shah, & Zeb, 1995). Several nutritive factors such as vitamin concentrations and bioavailability of trace elements and minerals are reported to increase during germination (El-Adawy, 2002; Khattak et al., 2007). At the same time there are indications that germination is effective in reducing phytic acid and flatulence causing oligosaccharides stachyose and raffinose, increasing protein digestibility and improving sensory properties (Khattak et al., 2007; Lintschinger et al., 1997). In addition, among the food legumes, chickpea is the most hypocholestrolemic agent; germinated chickpea was reported to be effective in controlling cholesterol level in rats (Geervani, 1991). In case of white kidney beans it improves the digestion of protein by decreasing the content of bean anti-nutritional factors and increasing the bean true protein digestibility (Schhulze et al., 1997). Fermentation and sprouting (Khetarpaul & Chauhan, 1990, 1991) have been reported to improve in vitro protein quality, starch digestibility and overall acceptability score by sensory panelists in germinated cowpea flour. Bishnoi and Khetarpaul (1994) found improvement in protein digestibility (in vitro) by the common methods of domestic processing and cooking including soaking, dehulling, ordinary cooking, pressure cooking and sprouting of legume grains. Yang, Basu, and Ooraikul, 2001 reported that upon germination the concentrations of

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

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.01.046

vitamins C and E, β-carotene, ferulic acid and vanillic acid steadily increased with increasing germination time.

Within animals, caroteinoid provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity (Britton, 1995; Ong & Tee, 1992). In human beings, caroteinoid can serve several important functions. The most widely studied and well-understood nutritional role for caroteinoids is their provitamin A activity. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain caroteinoid, notably β-carotene (Britton, Liaaen-Jensen, & Pfander, 1995). Dietary β-carotene is obtained from a number of fruits and vegetables, such as carrots, spinach, peaches, apricots, and sweet potatoes (Mangels, Holden, Beecher, Forman, & Lanza, 1993). Health benefits of caroteinoids that may be related to their anti-oxidative potential include enhancement of immune system function, protection from sunburn (Mathews-Roth, 1990), and inhibition of the development of certain types of cancers (Nishino, 1998).

Reports on impact of germination time on β-carotene, protein solubility and protein digestibility in chickpea are many; however, evidence on influence of type of illumination on these parameters is lacking. The present study was, therefore, aimed to investigate the impact of germination time and type of illumination on changes in β-carotene, protein solubility and in vitro digestibility of 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 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.

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 Co-60 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 coloured bulb (40 W, Philips, Lahore, Pakistan) were used as per illumination treatments. The trays were distributed under the light at a distance of 100 cm 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.

2.2. Analysis

2.2.1. β-Carotene

The β-carotene in the oil extracted from the sample was determined according to PORIM test method (1993) by accurately weighing the oil in a 25 ml volumetric flask. The test portion in each flask was dissolved with a few ml of solvent and diluted to the mark. Then it was transferred to a 1 cm cuvette and absorbance was determined at 446 nm against solvent used with the help of a Shimadzu (Kyoto, Japan) UV-160 spectrophotometer. In each treatment, the oil extraction was carried out in triplicate. All the replication were then separately subjected to β-carotene analysis.

2.2.2. Protein solubility

Protein solubility was determined using method as outlined by Persons, Hashimoto, Wedekind, & Baker, 1991. A brief account of which is as follow:

Half gram sample and 10 ml 0.5 N KOH solution was taken in a centrifuge tube. After shaking for half an hour, the samples were centrifuged at 2000 rpm for 15 min. Supernatant was used for protein determination.

$$\%\ \text{Protein solubility} = \frac{\text{Titration reading} \times N \times 0.014 \times 6.25 \times 100 \times 10}{\text{Wt of sample} \times (\%\ \text{Crude protein})}$$

Three samples were taken from each of the treatment for determination of protein solubility.

2.2.3. In vitro protein digestibility (IVPD)

In vitro protein digestibility was determined by the method as described by Khalil et al. (2007). A brief account of which is as below.

Take 100 mg sample of each chickpea variety and 4 mg of trypsin enzyme in a small beaker. Add 7.5 ml of 0.1 M phosphate buffer (pH 8.0) to the samples. Then incubate the samples at a temperature of 37 °C for 24 h with constant shaking. Terminate the reaction by adding 10% trichloro acetic acid (TCA) solution Soluble nitrogen separated by centrifugation at $1260\times g$ for 30 min and take the supernatant for the determination of soluble nitrogen by Kjeldahl method. In vitro protein digestibility determination for each of the treatment was repeated three times.

In vitro protein digestibility = Soluble protein × 100/ total protein.

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 (MSU, 1987).

4. Results and discussion

Data on effect of germination time and type of illumination on β-carotenes are compiled in Fig. 1. The initial content of β-carotenes in chickpea seed was 48.25 mg 100 g^{-1} which significantly increased ($p < 0.01$, Table 1) to 62.54 mg 100 g^{-1} with 24 h germination. On further increase in germination time, the β-carotenes content of sprouts of irradiated chickpea increased significantly, while that of sprouts under fluorescent, yellow and red illumination decreased significantly with 48 h germination. β-Carotenes content of sprouts under dark and blue illumination on 48 h germination remained nearly the same as for 24 h germination. Minimum value for β-carotenes (28.36 mg 100 g^{-1}) were noted in sprouts under fluorescent light and maximum in sprouts of irradiated chickpea seed (80.05 mg 100 g^{-1}) after 48 h germination. After 72 h germination, highest value for β-carotenes were observed in sprouts under yellow light (131.74 mg 100 g^{-1}) followed by sprouts under green illumination (91.63 mg 100 g^{-1}). Highest value for β-carotenes with 96 h germination was noted in sprouts under dark condition (86.27 mg 100 g^{-1}) while in the sprouts under rest of illuminations decreased significantly. The β-carotenes content after 120 h germination were highest in sprouts under fluorescent light followed by sprouts of irradiated seed. For all other sprouts, β-carotenes values reached to a minimum level after 120 h germination. It is evident from the results that both germination time and type of illumination have a highly significant impact on the β-carotenes content of chickpea sprouts. Our results are in agreement to that of Yang et al., (2001) who reported that upon germination the concentrations of vitamins C and E, β-carotene, ferulic acid and vanillic acid steadily increased with increasing germination time. Jirapa, Normah, Zamaliah, Asmah, and Mohamad (2001), while working on weaning food reported that vitamin A activity in 24 h germinated cowpea flour-weaning food was higher than in control cowpea flour-weaning food.

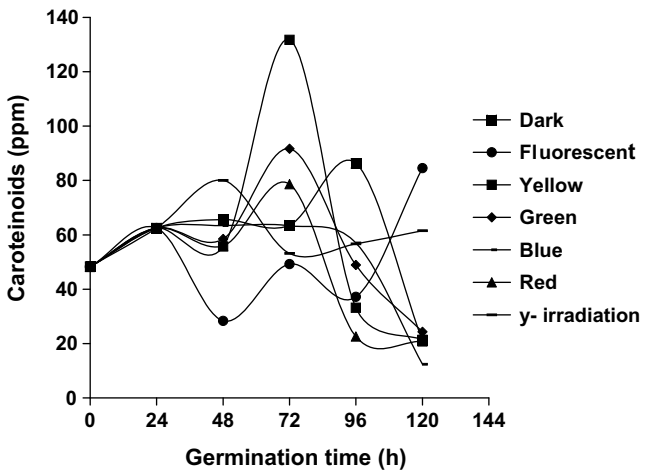

Fig. 1. Effect of germination time and type of illumination on carotenoid content (ppm) of chickpea sprouts.

Protein solubility and digestibility are important criteria for evaluation of protein quality. The data on protein solubility are compiled in Fig. 2. The impact of germination time and type of illumination have a highly significant effect ($p < 0.01$) on protein solubility of chickpea sprouts (Table 1). The protein solubility of control chickpea was 22.63% and that increased to 41.37% with 24 h germination. The maximum protein solubility% was observed for sprouts under green illumination (55.83%) followed by sprouts under dark and irradiated seed sprouts (51.8 and 51.77, respectively) after 48 h germination. The protein solubility increased as the germination time increased and the maximum value were recorded for sprouts of irradiated seed after 120 h germination (70.70%) followed by sprouts under green illumination. In general, with increase in germination time, the protein solubility% increased significantly; however, the increase was higher in sprouts under dark, green illumination and irradiated seed.

In vitro protein digestibility as influenced by germination time and type of illumination is depicted in Fig. 3. Both germination time and type of illumination have a highly significant effect ($p < 0.01$) on in vitro protein digestibility (Table 1). The digestibility of protein increased as the germination time increased. The in vitro protein digestibility of un-germinated seed was 34.18% and this increased to a level of 47.49% with 24 h germination. Upon further increase in germination time, the in vitro digestibility increased consequently and the maximum value was noted for sprouts under fluorescent illumination after 48 h germi-

Table 1
Analysis of variance table showing mean sum of the squares and F values in parenthesis

Source of variation	Degrees of freedom	In vitro protein digestibility	Protein solubility	β-carotene
Light type	6	109.0^{***} (25.08)	248.6^{***} (47.0)	373.7^{***} (79.4)
Germination time	5	5724.8^{***} (1316.8)	5127.3^{***} (868.4)	4124.3^{***} (876.2)
Light type X germination time	30	485.9^{***} (111.8)	29.3^{***} (5.5)	1258.4^{***} (267.4)
Error	84	4.4	5.3	4.7
Total	125			

$^{***}p < 0.01$.

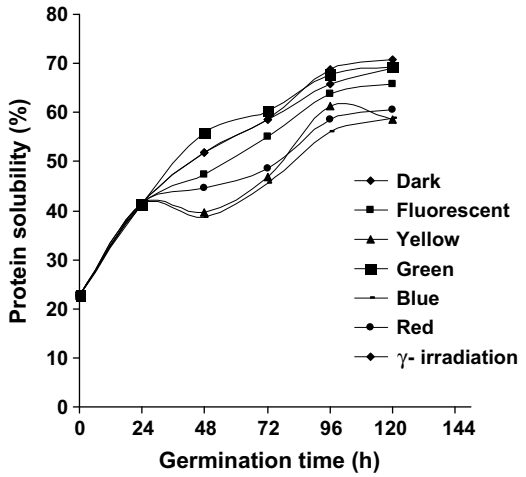

Fig. 2. Effect of germination time and type of illumination on protein solubility of chickpea.

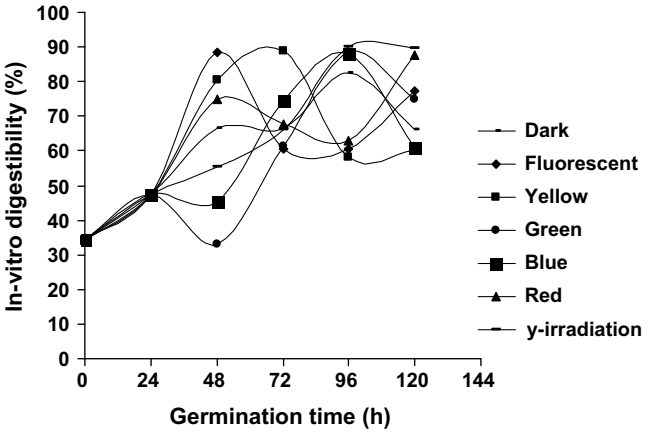

Fig. 3. Impact of germination time and type of illumination on in vitro protein digestibility of chickpea sprouts.

nation. After 72 h germination, highest value for in vitro digestibility was recorded in sprouts under yellow illumination (89.01%). In general, highest value for in vitro protein digestibility was found in sprouts of irradiated chickpea seed after 96 h germination (90.03%) followed by the same group after 120 h germination (89.82%).

Fermentation and sprouting (Khetarpaul & Chauhan, 1990, 1991) have been reported to increase the protein digestibility of millet and hence confirm our results. Jirapa et al. (2001) also found improvement in in vitro protein quality, starch digestibility and overall acceptability score by sensory panelist in germinated cowpea flour. Bishnoi and Khetarpaul (1994) found improvement in protein digestibility (in vitro) by the common methods of domestic processing and cooking including soaking, dehulling, ordinary cooking, pressure cooking and sprouting of legume grains. All these studies confirm our findings.

Although, reports on effect of germination on carotenoids, protein solubility and digestibility are numerous, however, evidence on impact of type of illumination on these parameters is lacking. The significant effect of type of illumination on β-carotene, protein solubility and digestibility might be attributed to differences in wavelength or energy level of light sources applied in this experiment. It is previously reported (Khattak et al., 2007; Khattak, Zeb, Bibi, & Khattak, 2008) that type of illumination have pronounced effect on ascorbic acid biosynthesis, phytic acid, polyphenols content and proximate composition of chickpea sprouts.

5. Conclusions

It is inferred from this experiment that germination time and type of illumination have significant effect on β-carotene, protein solubility and protein digestibility. Irradiation of chickpea seed has a significantly promotional effect on biosynthesis of β-carotene, protein solubility and protein digestibility.

References

Badshah, A., Zeb, Anurang, & Sattar, A. (1991). Effect of soaking, germination and autoclaving on selected nutrients of rapeseed. *Pakistan Journal of Scientific and Industrial Research, 34*, 446–448.

Bishnoi, S., & Khetarpaul, N. (1994). Protein digestibility of vegetables and field peas (*Pisum sativum*). Varietal differences and effect of domestic processing and cooking methods. *Plant Foods for Human Nutrition, 46*(1), 71–76.

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *FASEB Journal, 9*, 1551–1558.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (1995). Carotenoids today and challenges for the future. In G. Britton, S. Liaaen-Jensen, & H. Pfander, (Eds.), Carotenoids: Vol. 1A. Isolation and Analysis. Basel: Birkhäuser.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition, 57*, 83–97.

Geervani, P. (1991). Utilization of chickpea in India and scope for novel and alternative uses. In Uses of Tropical Grain Legumes: Proceedings of Consultants' Meeting 27-30 March, 1989. (pp. 47–54). Andhra Pradesh, India, Patancheru: ICRISAT Center.

Jirapa, P., Normah, H., Zamaliah, M. M., Asmah, R., & Mohamad, K. (2001). Nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in home prepared powdered weaning foods. *Plant Foods for Human Nutrition, 56*(3), 203–216.

Khalil, A. W., Zeb, A., Mahood, F., Tariq, S., Khattak, A. B., & Shah, H. (2007). Comparative sprouit quality characteristics of desi and kabuli type chickpea cultivars (*Cicer arietinum* L.). *LWT-Food Science and Technology, 40*, 937–945.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, S. A., & Khattak, M. S. (2007). Influence of germination techniques on phytic acid and polyphenols contents of chickpea (*Cicer arietinum* L.) sprouts. *Food Chemistry, 104*, 1074–1079.

Khattak, A. B., Zeb, A., Bibi, N., & Khattak, M. S. (2008). Effect of germination time and type of illumination on proximate composition of chickpea seed (*Cicer arietinum* L.). *American Journal of Food Technology, 3*(1), 24–32.

Khetarpaul, N., & Chauhan, M. (1991). Effect of natural fermentation on antinutrients and in vitro digestibility of starch and protein in pearl millet flour. *Journal of the Science of Food and Agriculture, 55*, 189–195.

Khetarpaul, N., & Chauhan, B. M. (1990). Improvement in HCl-extractability of minerals from pearl millet by natural fermentation. *Food Chemistry, 37*, 69–75.

Lintschinger, J., Fuchs, N., Moser, H., Jager, R., Hlebeina, T., Markolin, G., & Gossler, W. (1997). Uptake of various trace elements during germination of wheat buckwheat and quinoa. *Plant Foods for Human Nutrition, 50*, 223–237.

Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., & Lanza, E. (1993). Carotenoid content of fruits and vegetables: An evaluation of analytic data. *Journal of American Dietitian Association, 93*, 284–296.

Mathews-Roth, M. M. (1990). Plasma concentration of carotenoids after large doses of β-carotene. *American Journal of Clinical Nutrition, 52*(3), 500–501.

MSU, 1987. Mstat Version 4.C/EM, Michigan State University.

Nishino, H. (1998). Cancer prevention by carotenoids. *Mutation Research, 402*, 59–163.

Ong, A. S. H., & Tee, E. S. (1992). Natural sources of carotenoid from plants and oils. *Methods in Enzymology, 213*, 142–167.

Persons, C. M., Hashimoto, K., Wedekind, K. J., & Baker, D. H. (1991). Determination of protein solubility by the conventional KOH procedure. *Journal of Animal Science, 69*, 2918–2924.

PORIM test method (1993). *Methods for test for palm oil products. Section # 2*. Kulampur, Malaysia: PORIM Publication.

Sattar, A., Shah, A. B., & Zeb, A. (1995). Biosynthesis of ascorbic acid in germinating rapeseed cultivars. *Plant Food for Human Nutrition, 47*, 63–70.

Schhulze, H., Save, L. F. H., Verstegen, M. W., Van der Poel, A. F., Tamminga, S., & Groot Nibbelin, S. (1997). Nutritional evaluation of biological treated white kidney beans (*Phaseolus vulgaris* L.) in pigs: Ileal and amino acid digestibility. *Journal of Animal Science, 75*, 3187–3194.

Yang, F., Basu, T. K., & Ooraikul, B. (2001). Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Science and Nutrition, 52*(4), 319–330.