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Effects of ethanol vapours, hot water dip and ultraviolet irradiation treatments on nutritional quality of chickpea sprouts

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ABSTRACT

The present investigation was conducted to study effects of various treatments on ascorbic acid, antioxidant activity, phytic acid, polyphenols and organoleptic quality of chickpea sprouts. Chickpea seeds were washed, soaked and allowed to sprout. The sprouts were subjected to various treatments *viz.*, ethanol vapour (30 min), hot water dip (50^0 C for 2 min) and UV-Irradiation (10 kJm^{-2} for 1 h). The chickpea sprouts were then packed in disposable glasses, wrapped with perforated cling films and stored at room (25 ± 3^0 C) and low (7 ± 0.5^0 C) temperature. The sprouts were analyzed at 24 h interval until the spoilage. Ascorbic acid and antioxidant activity of chickpea sprouts first showed the elevation and then reduced, however, polyphenols and phytic acid decreased progressively with storage period. Ascorbic acid and antioxidant activity of chickpea sprouts were highest in ethanol vapours treatment whereas maximum reduction of antinutritional factor was observed with UV- irradiation treatment. The sprouts showed acceptability upto 48 h at room temperature and 120 h at low temperature storage conditions.

Keywords: Antioxidant activity, ascorbic acid, chickpea sprouts, ethanol vapours, hot water dip, phytic acid, UV-irradiation.

Legumes contain complex carbohydrates, vitamins, proteins, minerals and worldwide known for its nutritional quality (Wang *et al*. 2010). Sprouting has proven to be an efficient method for improving the nutritional quality of legumes (Khattak *et al*. 2008) and reducing the antinutritional factors (Ghavidel and Prakash, 2007). It also increases the vitamin concentrations and bioavailability of minerals (El-Adawy, 2002).

There is a developing interest for chickpea (*Cicer arietinum* L.) because of its nutritional content. Chickpea seed contains elevated amount of carbohydrate (59.09%) and protein (17.80%). It contains (9%) moisture, (6.48%) fibre and (5.80%) fat (Masood *et al*. 2014). Sprouts of chickpea are gaining popularity due to its nutritional quality. The shelf life of sprouts is shorter due to the presence of high moisture content and high metabolic activity which diverts the need to enhance its shelf life during storage period. It is reported that pre-treatment of UV-C irradiation to fresh cut mango results in maintaining its nutritional content, preventing decay and extending shelf life upto 15 days (George, 2015).

Goyal and Siddiqui, 2014 reported that nutritional and keeping quality of mung bean sprouts was enhanced by ethanol vapour and hot water dip treatment. The shelf life of germinated beans is restricted to two days due to its perishable nature. Generally regarded as safe (GRAS) pre-treatments, low temperature storage conditions, modified atmosphere packaging enhanced the keeping quality of sprouts (Day 1990 and Goyal *et al*. 2014).

Keeping in view the dietetic importance of chickpea and its sprouts, the investigation was undertaken to study the effect of various pre-treatments on nutritional quality of chickpea sprouts during storage.

MATERIALS AND METHODS

Seeds were procured from local market, CCS HAU, Hisar, Haryana, India. Chickpea seeds were cleaned, washed in hypochlorite solution and soaked in 4–5 volumes of water for 10 h under ambient laboratory conditions. After soaking, seed were allowed to germinate in sprout maker (Novelle Plast, Delhi) for 24 h at 30 ± 1^0C.

Treatment and storage conditions: Sprouted chickpea were divided into 4 lots of equal amount and subjected to various treatments *viz*., hot water dip (HWD) (50° C for 2 min), ethanol vapours (in a glass chamber saturated with ethanol vapours) for 30 min., and UV irradiation (10 kJm^{-2} for 1 h in laminar flow chamber). Untreated sprouts were used as control. The sprouts from each treatment were filled in disposable plastic glasses (~200 ml volume) and wrapped with (2%) perforated cling films. Wet white filter paper was placed along the inner sides of plastic glasses to maintain high humidity inside the disposable glasses. There was ~100 g sprouts per pack and the packs were stored in dark at room (30 ± 3^0C) and low (7 ± 0.5^0C) temperature conditions maintained in B.O.D. incubator. The sampling for various parameters was done regularly at 24 h interval until the spoilage.

Chemical analysis

Ascorbic acid: It was assessed by titration method as described by AOAC (1995). Extraction of sample was done by macerating 2 g of sprouts with (3%) metaphosphoric acid and final volume was made up to 10 ml with metaphosphoric acid. The extract was filtered with whatman no. 1 filter paper and 10 ml of aliquot was titrated with 2,6-

dichloroindophenol dye till the end point (pink colour). The results were expressed in terms of mg ascorbic acid per 100 g sprouts.

Antioxidant activity: It was analyzed using free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) dye as per the procedure suggested by Shimada *et al.* (1992). Extraction of sample was done by macerating 500 mg of sprouts in 10 ml of methanol, centrifuged at 4000 rpm and filtered with whatman no.1 filter paper. 100 µl of supernatant was added to 3 ml of dye and incubated for 30 min at 25-30^{0}C. The inhibition percentage of DPPH solution at 517 nm with sample was calculated using the following equation:

Antioxidant activity (% scavenging of DPPH) =

$(Abs_{t=0\ min} - Abs_{t=30\ min}) \times 100/Abs_{t=0\ min}$

(Where, Abs: Absorbance)

Antinutritional Factor

Phytic acid: It was determined by the method of Haug and Lantzsch (1983). Finely ground (one gram) sample was extracted with 0.2 N HCl for 3 h with continuous shaking and filtered through Whatmann No.1 filter paper. 500 µl of extract was added to the tube containing 1.0 ml of ferric ammonium sulphate solution and the tube was heated in a boiling water bath for 30 min. The contents of the tube were then mixed and centrifuged for 30 min. at 3000 rpm. One ml supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. Phytic acid concentration at 519 nm was calculated from the standard curve prepared using graded concentrations of sodium phytate.

Polyphenols: It was estimated by the method of Swain and Hills (1959). Defatted sample (500 mg) was refluxed with methanol containing (1%) HCl for 3h. The extract was concentrated by evaporating methanol on a boiling water bath and its volume was made to 25 ml with methanolic-HCl. To the 1.5 ml of the extract 8.5 ml of water and 0.5 ml Folin-Denis reagent was added. After three minutes, one ml saturated sodium carbonate solution was added with vigorous shaking. After one hour, the absorbance was read at 725 nm using methanolic- HCl as blank. The corresponding concentration of polyphenols was calculated from the standard curve prepared using graded concentrations of tannic acid.

Overall acceptability: Sensory evaluation of treated sprouts and control samples was carried out using 9-point hedonic scale. A trained panel of 10 judges checked the variation present in samples for various organoleptic qualities and acceptability of samples was decided on the basis of that.

Statistical analysis: There were three replicates for each treatment and the data was subjected to analysis of variance (ANOVA) technique and analyzed according to two factorial completely randomized designs (CRD) at 5% level of significance.

RESULTS AND DISCUSSION

Ascorbic acid: Data regarding the ascorbic acid content of chickpea sprouts at low and room temperature storage is presented in Table 1. Initial value of ascorbic acid of chickpea sprout was 14.7 mg/100 g which significantly increased to 29.6 mg/100 g after 48 h and thereafter decreased

Table 1. Effect of different treatments on ascorbic acid (mg/100g) of chickpea sprouts during storage

Treatments	Period of storage (h)						
	0	24	48	72	96	120	Mean
				Room Temperature			
Control	17.2	20.0	23.2	20.6	-	-	20.2
HWD	14.7	25.5	27.2	23.7	-	-	22.8
Ethanol	15.4	27.2	29.6	26.2	-	-	24.6
UV	16.9	24.0	26.6	22.2	-	-	22.4
Mean	16.1	24.2	26.6	23.2	-	-	
C.D. at 5%		Treatments (T) = 0.44 ;		Storage (S) = 0.44 ;	TxS = 0.88		
				Low Temperature			
Control	18.4	19.3	21.7	23.4	24.3	21.9	21.5
HWD	15.8	23.0	26.4	29.4	28.1	24.8	24.6
Ethanol	16.7	23.3	28.1	31.5	28.5	26.4	25.8
UV	17.9	20.9	23.0	24.3	25.6	22.6	22.4
Mean	17.2	21.7	24.8	27.2	26.6	23.9	
C.D. at 5%		Treatments (T) = 0.29 ;		Storage (S) = 0.35 ;	TxS = 0.70		

HWD= Hot water dip; Observation were not recorded due to spoilage of samples significantly to 20.6 mg/100 g during storage at room temperature conditions. At low temperature storage conditions, ascorbic acid content significantly increased from 15.8 mg/100g to 31.5 mg/100 g upto 72 h and thereafter reduced to 21.9 mg/100 g upto 120 h storage period. All the treatments resulted in significant reduction in ascorbic acid content at 0-day, however, helped in retention of higher ascorbic acid during storage over untreated sprouts. Among the various treatments and at both the storage temperatures, maximum retention of ascorbic acid was obtained in ethanol vapour treatment followed by hot water dip treatment, while it was minimum for UV treated sprouts. The results of the present investigation are in accordance with the earlier reports. It has been reported that ascorbic acid content increased in oranges (Ansari and Feridoon 2007) and in Kumquat (Fortunella japonica Lour. fruit Schirra *et al.* 2008) just after the hot water dip treatment but then decreased significantly during storage. Similarly Goyal *et al*. (2014) reported that hot water dip and ethanol treatments improved the ascorbic acid content of mung bean sprouts as compared to control.

Antioxidant activity: Result regarding the antioxidant activity of chickpea sprouts at low and room temperature storage condition is presented in table 2. Initial value of ascorbic acid of chickpea sprout was (21.3%) scavenging of DPPH which significantly increased to 61.5 percent after 48 h and thereafter decreased significantly to 31.3 percent during storage at room temperature conditions. Similarly, at low temperature storage conditions, ascorbic acid content significantly increased from (22.2 to 71.3 %) scavenging of DPPH upto 72 h and thereafter reduced to (44.9%) upto 120 h storage period.

At both the storage temperatures, a significant increase in antioxidant activity over control was observed in all the treatments except in HWD treatment showing significantly lower antioxidant activity. Throughout the storage period, maximum activity was observed under ethanol vapour followed by UV treatment. The results of the present investigation are in accordance with the earlier reports. Goyal *et al*. 2014 reported that antioxidant activity in mungbean sprouts was decreased by heat treatment (50 ^{0}C for 2 min). They also observed the increase in antioxidant activity by ethanol vapours in sprouts and were attributed due to higher amounts of phenols and ascorbic acid content. Choi *et al*. (2015) studied the effect of UV-C irradiations with 245 nm on fresh-cut paprika and reported that ascorbic acid content was retained more by UV-C as compared to control. Similarly, Pataro *et al*. (2015) reported an increase in antioxidant activity in tomato and Annurca apples treated with UV-C and pulsed light irradiation.

Polyphenol and phytic acid: Data regarding polyphenol and phytic acid content of chickpea sprouts among various treatments at room and low temperature during storage is presented in table 3 & 4. Polyphenol and phytic acid contents of sprouts decreased progressively with the increase in storage duration. Polyphenol and phytic acid contents of sprouts decreased significantly from 552 to 215 mg/100 g and 473 to 185 mg/100 g respectively, at room temperature. Similarly at low temperature storage conditions, polyphenol and phytic acid contents of sprouts decreased significantly from 536 to 206 mg/100 g and 490 to 123 mg/100g respectively. Significant reduction in antinutritional content was observed in all the treatments with respect to control. The reduction in polyphenol and phytic acid was maximum in UV-treatment followed by hot water dip and ethanol vapour treatments. The results of present study are in accordance with the finding of Kala and Mohan, (2011) who reported decreased levels in tannins and phytic acid in overnight soaked seeds of *Mucuna pruriens* var. *Utilis* when treated with UV irradiation. Duodu

Table 2. Effect of different treatments on antioxidant activity (% scavenging of DPPH) of chickpea sprouts during storage

Treatments	Period of storage (h)						
	0	24	48	72	96	120	Mean
				Room Temperature			
Control	31.4	45.2	52.5	43.9	-	-	43.2
HWD	21.3	35.3	41.5	31.3	-	-	32.3
Ethanol	41.6	53.3	61.5	52.6	-	-	52.2
UV	37.5	49.4	57.4	48.7	-	-	48.3
Mean	33.0	45.8	53.2	44.1	-	-	
C.D. at 5%		Treatments (T) = 0.40;		Storage (S) = 0.40;		TxS = 0.82	
				Low Temperature			
Control	30.9	48.1	54.1	56.9	55.3	53.4	49.8
HWD	22.2	37.0	43.0	45.4	47.1	44.9	39.9
Ethanol	41.8	56.0	63.3	71.3	70.0	68.8	61.9
UV	36.0	49.0	58.0	61.2	59.4	56.9	53.4
Mean	32.7	47.5	54.6	58.7	58.0	56.0	
C.D. at 5%		Treatments (T) = 0.24;		Storage (S) =0.29 ;		TxS = 0.58	

HWD= Hot water dip; -Observation were not recorded due to spoilage of samples

Table 3. Effect of different treatments on polyphenol content (mg/100g) of chickpea sprouts during storage

Treatments	Period of storage (h)						
	0	24	48	72	96	120	Mean
				Room Temperature			
Control	608	537	385	267	-	-	449
HWD	576	470	342	236	-	-	406
Ethanol	593	490	376	250	-	-	427
UV	552	450	329	215	-	-	387
Mean	582	487	358	242	-	-	
C.D. at 5%		Treatments (T) = 12.4;		Storage (S) = 12.0 ;	TxS =24.4		
				Low Temperature			
Control	600	529	472	403	335	260	433
HWD	566	492	445	371	302	228	401
Ethanol	583	513	457	389	317	245	417
UV	536	478	419	354	273	206	378
Mean	571	503	448	379	306	234	
C.D. at 5%		Treatments (T) = 10.5 ;		Storage (S) = 12.4 ;	TxS =23		

HWD= Hot water dip; -Observation were not recorded due to spoilage of samples

Table 4. Effect of different treatments on phytic acid (mg/100g) of chickpea sprouts during storage

Treatments	Period of storage (h)						
	0	24	48	72	96	120	Mean
				Room Temperature			
Control	518	429	327	227	-	-	375
HWD	483	389	293	204	-	-	342
Ethanol	504	406	310	216	-	-	359
UV	473	375	279	185	-	-	328
Mean	495	400	302	208	-	-	
C.D. at 5%		Treatments (T) = 13;		Storage (S) = 12.5 ;	TxS =NS		
				Low Temperature			
Control	545	470	405	335	257	179	433
HWD	515	435	369	307	230	149	401
Ethanol	530	453	387	315	242	162	417
UV	490	437	352	281	201	123	378
Mean	571	503	448	379	307	235	
C.D. at 5%		Treatments (T) = 9.8 ;		Storage (S) = 11 ;	TxS = 21		

HWD= Hot water dip; -Observation were not recorded due to spoilage of samples

Table 5. Effect of different treatments on overall acceptability (9 point hedonic) of chickpea sprouts during storage

Treatments	Period of storage (h)						
	0	24	48	72	96	120	Mean
				Room Temperature			
Control	8.0	6.9	5.9	4.4	-	-	6.3
HWD	8.5	7.4	6.6	5.1	-	-	6.9
Ethanol	8.0	6.8	5.8	4.2	-	-	6.2
UV	8.0	6.9	6.1	4.4	-	-	6.4
Mean	8.1	7.0	6.1	4.5	-	-	
C.D. at 5%		Treatments (T) = 0.50;		Storage (S) = 0.50;	TxS =1.00		
				Low Temperature			
Control	8.0	7.5	7.2	6.4	6.0	5.4	6.8
HWD	8.5	8.1	8.0	7.3	6.9	6.0	7.5
Ethanol	8.0	7.6	7.1	6.2	6.0	5.3	6.7
UV	8.0	7.5	7.2	6.4	6.0	5.5	6.8
Mean	8.1	7.9	7.4	6.7	6.2	5.6	
C.D. at 5%		Treatments (T) =0.40 ;		Storage (S) = 0.48 ;	TxS = 0.90		

HWD= Hot water dip; -Observation were not recorded due to spoilage of samples *et al*. (1999) suggested that degradation of phytic acid by radiation is due to cleavage of phytic acid itself.

Organoleptic score: Data regarding on organoleptic score of chickpea sprouts among various treatments at room and low temperature during storage is presented in table 5. There was a decrease in overall acceptability score with the advancement in storage period and became unacceptability at 72 h of storage at room temperature and 120 h of storage at low temperature. There was no significant effect of various treatments on the sprouting. However, under both the storage temperatures, overall acceptability of chickpea sprouts significantly increased by HWD and ethanol treatment, while it was decreased significantly by UV irradiation treatment. The results of present study are in accordance with the findings of Goyal and Siddiqui (2014), who reported that mung bean sprouts remained acceptable upto 48 h and 120 h at room and low temperature storage conditions, respectively. They studied that keeping quality of mung bean sprouts was enhanced by ethanol vapour and HWD treatments, both at room and low temperature storage conditions. Similarly, Bai *et al.(* 2004) and Hu *et al.* (2010) studied that the shelf life heat pre-treated apple slices and fresh cut eggplant respectively, were improved by ethanol vapour treatment.

CONCLUSION

Based on these results, it can be concluded that different treatments resulted in significant improvement of nutritional quality of chickpea sprouts during storage. The ascorbic acid content and antioxidant activity were highest in ethanol vapours treated sprouts. Polyphenols and phytic acid content of sprouts decreased progressively with the increase in storage period and maximum reduction was observed in UV treated sprouts. Ethanol vapour significantly improved the ascorbic acid content and antioxidant activity of chickpea sprouts and UV radiation resulted significance reduction of antinutritional compound. Keeping quality of chickpea sprouts can be maintained well upto 48 h at room temperature and 120 h at low temperature as against 24 and 96 h under control conditions by subjecting the sprouts to hot water dip treatment of 50^0 C for 2 min. This treatment could also represent a promising alternative to conventional methods for fruits and vegetables also.

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