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ORIGINAL ARTICLE

Effects of ultraviolet irradiation, pulsed electric field, hot water and ethanol vapours treatment on functional properties of mung bean sprouts

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Abstract The present investigation was conducted with the objective to study the effects of various treatments and storage conditions on ascorbic acid, total phenols, antioxidant activity and polyphenol oxidase activity of mung bean sprouts. The sprouts subjected to various treatments viz., pulsed electric field (PEF) (10,000 V for 10 s), hot water dip (HWD) (50 °C for 2 min), ethanol vapours (1 h) and UV-Irradiation (10 kJm^{-2} in laminar flow chamber for 1 h); and then stored at room (25±1 °C) and low (7±1 °C) temperature conditions. The sprouts were analyzed regularly at 24 h interval till end of shelf life. Different treatments given to sprouts resulted in differential effect on various parameters. The ascorbic acid, total phenols and antioxidant activity were highest in ethanol vapours treated sprouts. There was a general decrease in polyphenol oxidase activity by various treatments. During storage ascorbic acid, total phenols and antioxidant activity of sprouts first increased and then decreased significantly, however, for polyphenol oxidase activity a progressive increase with increase in storage period was observed. The trends were similar at room and low temperature storage conditions. Thus, it can be concluded that the ethanol vapours significantly improved the ascorbic acid content, total phenols and antioxidant activity of mung bean sprouts, both at room as well as low temperature conditions of storage.

Keywords Antioxidant activity · Ethanol vapours · Hot water dip · Mung bean sprouts · Pulsed electric field · UV-irradiation

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Introduction

Mung bean (*Vigna radiata* L. Wilczek) is a leguminous species/pulse crop, grown for its protein rich edible seeds. It is native to India and Pakistan and also cultivated in Iran, Vietnam, China and Phillipines. China is the leading country in the production of mung bean followed by Indonesia, Turkey and then India. In India, mung bean ranks third among the pulse crop, after chick pea and pigeonpea (Singh and Yadav 1978). With about 67% carbohydrate, 27% protein, 1.46% lipids, 132 mg calcium, 380 mg phosphorus and 2.91 mg niacin (moisture free basis), mung bean is a rich source of nutrients (Poehlman 1991). Protein in mung beans is comparatively rich in lysine, an amino acid deficient in cereal grains. 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 cereal and grain legumes (Khattak et

al. 2008). A multitude of chemical changes occur to mobilize the stored carbohydrate and protein reserve into the growing sprout; and simple carbohydrates, free amino acids and essential nutrients in available form can be used readily by the body. Therefore, sprouting improves the nutritional value of the seed sprouts significantly (Ghorpade and Kadam 1989) and decreases the content of phytic acid in the seeds, making legume sprouts more digestive, less flatulent and with enhanced nutritional quality. Mung bean sprouts are normally prepared after 3 to 8 days when length is 1.3 to 7.6 cm. Fresh Mung bean sprouts have crisp white hypocotyls and yellow or green cotyledons. Due to high moisture content and high metabolic activity, mung sprouts like other high moisture fruits and vegetables are highly perishable and most last one day at room temperature and 5 to 10 days at 0 °C at 95 to 100% RH. Symptoms of deterioration include; darkening of the root and cotyledons, development of dark streaks on the hypocotyls, and eventual development of sliminess, decay, and a musty odour (Lipton et al 1981). The rapid quality loss at relatively modest temperature emphasizes the critical need to enhance the shelf life and maintain the keeping quality during storage. It is reported that pre-exposure to short UV radiation slowed down respiration and ripening of fruits stored at room temperature, thus enhancing their shelf life (Baka et al. 1999; Siddiqui et al. 2001). High electric field exposure for short period was reported to suppress the respiration rate in some fruits and extended the freshness of sweet peppers (Kharel and Hasinaga 1996). It has been reported the effectiveness of hot water immersions in elimination of microorganisms in alfalfa and mung bean sprouts (Pao et al. 2008). It is also reported that ethanol vapours treatment given to broccoli suppressed respiration, transpiration, and chlorophyll breakdown, which resulted in prolonged shelf life (Corcuff et al. 1996). It is observed that perforated film packaging helped to maintain the quality of fresh sprouts by reducing water loss (DeEll and Vigneault 2000). Since there is no comprehensive information available in literature on these aspects and in order to explore the possibilities of these treatments to enhance the shelf life of mung bean sprouts, the present project work was planned to study the effects of various treatments on the functional properties (ascorbic acid, total phenols, antioxidant activity, etc.) and shelf life of mung bean sprouts during storage period.

Materials and methods

Plant material

Mung beans variety Muskan was procured from Pulse Section, Dept. of Plant Breeding, CCS HAU, Hisar, Haryana, India. Mung seeds were cleaned, washed and soaked in 4–5 volumes of water (22–25 °C) for 12 h under ambient laboratory conditions. At the end of the period, the water was drained and the seed samples were allowed to germinate in sprout maker (Novelle Plast, Delhi) for 24 h at 25±1 °C.

Treatments and storage conditions

Sprouted mung beans were divided into 5 lots of equal amount for treatments and then subjected to the various treatments viz. Pulsed Electric Field (PEF) [by PEF generator designed by Ambala Associates, Ambala (Haryana), 50 Hz, 10,000 V pulses for 10 s], Hot water dip (HWD) (50 °C for 2 min), Ethanol vapours (In a glass chamber saturated with ethanol vapourss for 1 h), and UV irradiation (10 kJm^{-2} in laminar flow chamber for 1 h). Untreated sprouts were used as control.

The sprouts from each treatment were packaged in thermocol cups (~200 ml volume) and wrapped with 2% perforated cling films. Water soaked filter paper was placed along the inner sides of plastic cup to maintain high humidity inside. There was ~100 g sprouts per pack and the packs were stored in dark at room (25±1 °C) and low (7± 1 °C) temperature conditions maintained in B.O.D. incubator. The sampling for various parameters was done regularly at 24 h interval up to 72 h at room and 120 h at low temperature conditions.

Chemicals

The chemicals used for investigation were of analytical grade reagents (A.R.) from standard suppliers like B.D.H., C.D.H., S.D. fine chemicals and Sisco Research lab, India.

Chemical analysis

Ascorbic acid, total phenols and antioxidant activity

Three replicates of each treated samples were used for analysis. Ascorbic acid was analyzed as per the procedure suggested by AOAC (1995). Ascorbic acid was extracted by macerating 2 g of sprouts with 3% metaphosphoric acid (MPA) and total volume was made up to 10 ml with acid. The extract was filtered and 5 ml of aliquot was titrated against 2,6–dichlorophenol indophenol dye till the appearance of pink color. The results were expressed in terms of mg ascorbic acid per 100 g sprouts.

Total phenols were estimated by the method of Amorium et al. (1997) using Folin – Ciocalteau reagent. Total phenols were expressed as tannic acid mg/100 g sample.

Antioxidant activity was assessed using free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) dye, as per the procedure described by Shimada et al. (1992). 500 mg of sprouts were macerated in 10 ml methanol and centrifuged at 4000 rpm and filtered. 0.1 ml of supernatant was mixed with 3 ml of dye (25 mg/L methanol) and incubated for 30 min at 25–30 °C in water bath. The inhibition percentage of the absorbance at 517 nm of DPPH solution added with sample was calculated using the following equation.

$$\text{Antioxidant activity}(\%\ \text{scavenging of DPPH}) = (\text{Abs}_{t=0\ \text{min}} - \text{Abs}_{t=30\ \text{min}}) \times 100/\text{Abs}_{t=0\ \text{min}}$$

(Where Abs: Absorbance)

Polyphenol oxidase (PPO) activity

Polyphenol oxidase was assayed by the method described by Kaul and Farooq (1994). Two g sprouts were homogenized in 10 ml of cold 0.2 M Tris–HCl buffer (pH=7.5) and centrifuged at 15,000 rpm for 20 min at 4 °C in a refrigerated centrifuge. One ml of enzyme extract (supernatant) was incubated with 4 ml of 0.05 M pyrocatechol solution for 30 min at 37 °C. The reaction was terminated by adding 1 ml of chilled 10% TCA and the optical density was read at 430 nm against the reagent blank. The total enzyme activity was measured in units. One unit of enzyme was represented as increase in O. D. by 0.1 under the standard conditions.

Statistical analysis

All the data were analyzed and expressed as means. The data was subjected to analysis of variance (ANOVA) technique and analyzed according to two factorial completely randomized designs (CRD). Three replicates of each treated samples were used for analysis. The critical difference value at 5% level was used for making comparison among different treatments during storage.

Results and discussion

Ascorbic acid

Table 1 shows total vitamin C content of mung bean sprouts over a storage period of 48 h and 120 h when stored at room and low temperature, respectively. At room temperature, ascorbic acid of sprouts first increased (from 19.3 to 36.7 mg/100 g sprouts) up to 24 h and then decreased to 22.7 mg/100 g sprouts. All the treatments showed a significant increase in ascorbic acid content, except by PEF treatment, where no significant change in ascorbic acid content was observed. Throughout the storage period, maximum ascorbic acid content was maintained by ethanol vapours treatment, while it was least in control. Interaction between treatments and storage were found to be significant. At low temperature, similar trends were observed. There was no significant effect of various treatments on ascorbic acid content of sprouts, except ethanol vapours and HWD treatment, where significantly higher ascorbic acid content was observed during storage. Interaction between treatments and storage were found to be significant.

Ascorbic acid content increased in hot water treated sprouts due to higher rate of metabolic activity than control. The possible reason could be synthesis of ascorbic acid during initial period of storage. Utilization of ascorbic acid at later storage periods may have resulted in its decreased amounts. It has been reported that the levels of vitamin C in Valencia and local oranges of Siavarz after hot water treatment increased from 40.83 mg/100 ml (control) to 42 mg/100 ml (treated) but vitamin C subsequently decreased during storage (Ansari and Feridoon 2007). It is observed that ascorbic acid content increased from 18.19 to 19.66 mg/100 g just after hot water treatment in Kumquat (*Fortunella japonica* Lour.) fruit (Schirra et al. 2008).

Ascorbic acid content was higher in ethanol treated sprouts than in control. It could probably be due to inhibition of metabolic activity by ethanol vapours, so initially formed ascorbic acid did not get utilized during storage and was maintained at higher levels in treated sprouts. However, no detectable changes are reported in ascorbic acid content of tomato fruit treated with ethanol vapourss during storage (Atta-Aly et al. 1998).

Ascorbic acid content significantly increased in the sprouts after UV-irradiation treatment. Higher levels of ascorbic acid in apple fruits after the treatment with UV irradiation has been reported (Hagen et al. 2007). Contradictorily, it is reported that UV-C had a negative effect on the maintenance of ascorbic acid during storage of fresh cut mangoes (Gustavo et al. 2007).

Ascorbic acid was not significantly affected by application of PEF. The results of the present investigation are in agreement with the findings of other workers. It is studied that on treating fresh apple cider with PEF, treatment had significant effect in retaining the vitamin C content in the juice samples (Evrendilek et al. 2000).

Total phenolic content

Total phenols of mung bean sprouts under various treatments during storage are presented in Table 2. At room temperature, total phenols of sprouts first increased (33.3–79.0 mg/100 g sprouts) up to 24 h and then decreased to

Table 1 Effect of different treatments on ascorbic acid (mg/100 g) of mung bean sprouts during storage at room temperature and low temperature (T- Treatments; S- Storage)

Treatments	Period of storage (h)									
	Room temperature (25±1 °C)				Low temperature (7±1 °C)					
	0	24	48	Mean	24	48	72	96	120	Mean
Control	23.5	24.9	22.7	23.7	25.7	27.1	27.9	24.2	24.9	25.9
PEF	24.2	27.9	22.9	25.0	26.4	27.9	29.3	23.5	23.5	26.1
HWD	20.5	31.5	30.1	27.4	27.1	31.5	35.9	29.3	27.1	30.1
Ethanol	19.3	36.7	38.9	31.5	27.9	34.5	36.7	29.5	28.4	31.4
UV	23.5	31.5	30.1	28.4	25.7	27.1	30.1	22.7	19.8	25.0
Mean	22.1	30.5	28.9		26.5	29.6	31.9	25.8	24.7	
C.D. at 5%	T=2.2; S =1.7; T x S=3.8				T=1.1; S=1.2; T x S=2.7					

45.3 mg/100 g sprouts at 48 h. There was no significant effect of total phenols by PEF and UV treatments, an increase was observed by ethanol and a decrease by HWD treatment. Throughout the storage period, minimum total phenols were observed in HWD treatment, while it was highest in ethanol treatment. Similar trends were observed at low temperature. Total phenols under various treatments ranged from 35.6 to 47.3 mg/100 g sprouts during storage period of 120 h.

The PEF treatment on orange juice showed no detectable changes in phenolic content (Moreno et al. 2005). Hot water treatment caused a reduction in total phenols over control. It could be due to leaching of phenols in water during the treatment. During storage, phenols increased initially due to synthesis from sugars, and decreased later due to its participation or utilization in other metabolic processes. It is reported that after heat treatment, free phenolic acids like benezoic acids & cinnamic acids significantly increased while glycosides and ester bound fractions of phenolic acids decreased in citrus peel extracts (Xu et al. 2007). There was higher level of total phenolic contents after ethanol vapour treatment and during storage as compared to control. Action of ethanol vapours maintained higher levels of phenols due to inhibition of sprout growth. Similarly, a sharp increase is reported in total phenolic compounds during the first 5 days at 7.5 °C in strawberries treated with the ethanol vapours while untreated berries showed the lowest values (Zavala et al. 2005).

Total phenolic content increased in the sprouts after UV-irradiation treatment. It is well documented that UV-C irradiation has an effect on the phenylpropanoid metabolism. UV-C irradiation increased the activity of phenylalanine ammonia-lyase (PAL) (key enzyme in phenol biosynthesis) and thus the phenolic content. The effect of UV-C technology on the synthesis of antioxidant compounds and enzymes can vary depending on the hermetic doses, time of exposure and treated fruit. It was observed that phenols and flavonoids were increased in guava and banana after 30 min exposure to UV-C light, contrary to the decrement found in pineapple (Alothman et al. 2009).

The effects of UV-C irradiation on fresh-cut mangoes were studied and increased level for total phenols were reported (Gustavo et al. 2007). A similar effect was observed in UV-C treated strawberries (Nigro et al. 2000). Similarly, broccoli florets showed an increase in total phenols after UV-C irradiation treatment and during storage as compared to un-irradiated controls (Costa et al. 2006).

Table 2 Effect of different treatments on total phenolic content (mg/100 g) of mung bean sprouts during storage at room temperature and low temperature (T- Treatments; S- Storage)

Treatments	Period of storage (h)									
	Room temperature (25±1 °C)				Low temperature (7±1 °C)					
	0	24	48	Mean	24	48	72	96	120	Mean
Control	40.0	69.6	65.3	58.3	44.3	45.3	30.6	29.6	28.3	35.6
PEF	43.6	73.0	62.6	59.7	44.0	47.0	38.0	35.3	30.3	38.9
HWD	33.3	59.0	45.3	45.8	36.0	38.6	28.3	27.6	25.0	31.1
Ethanol	45.6	79.0	65.6	63.4	45.3	47.3	42.6	39.6	35.6	42.0
UV	40.6	74.3	69.3	61.4	44.3	45.6	36.6	33.0	30.3	37.9
Mean	40.6	71.0	61.6		42.9	44.8	35.2	33.0	29.9	
C.D. at 5%	T=2.2; S=1.7; T x S=3.8				T=1.1; S=1.2; T x S=2.6					

Table 3 Effect of different treatments on antioxidant activity (% scavenging of DPPH) of mung bean sprouts during storage at room temperature and low temperature (T- Treatments; S- Storage)

Treatments	Period of storage (h)									
	Room temperature (25±1 °C)				Low temperature (7±1 °C)					
	0	24	48	Mean	24	48	72	96	120	Mean
Control	38.2	55.6	40.3	44.7	56.9	65.9	62.5	61.8	59.0	61.2
PEF	43.1	57.6	40.9	47.2	59.7	74.3	74.3	72.2	69.4	69.9
HWD	29.2	38.9	31.21	33.1	37.5	58.3	56.9	55.5	56.2	52.8
Ethanol	49.9	66.7	51.4	56.0	63.8	80.5	79.2	73.6	73.6	74.1
UV	42.4	59.0	41.7	47.7	55.1	75.9	63.2	60.4	69.7	64.8
Mean	40.6	55.6	38.9		54.6	69.0	67.2	64.7	63.6	
C.D. at 5%	T=3.2; S=2.5; T x S=4.7				T=1.8; S=1.9; T x S=4.3					

Total antioxidant activity

The germination process modifies the antioxidant activity, measured by its free radical scavenging capacity. Antioxidant activity of mung bean sprouts under various treatments during storage is presented in Table 3. At room temperature, antioxidant activity of sprouts increased up to 24 h and then decreased at 48 h of storage. There was no significant effect of PEF and UV treatments on antioxidant activities, however, an increase was observed by ethanol vapours and a decrease by HWD treatment. Throughout the storage period, maximum antioxidant activity was maintained by ethanol vapours treatment.

At low temperature storage conditions also, similar trends were observed. Antioxidant activity under various treatments increased initially up to 96 h and then slightly decreased at 120 h. There was no significant effect of UV treatment on antioxidant activity, however, an increase was observed by PEF and ethanol treatments and a decrease by HWD treatment. Since there was no significant effect of PEF on total phenolics, thus, the antioxidant activity also did not show any significant change. It is reported that there was no significant effect on antioxidant activity when orange juice was treated with pulsed electric field (Moreno et al. 2005). Similarly, no differences between PEF-processed and unprocessed orange juice were reported (Elez-Martinez et al. 2006). In hot water treated sprouts, antioxidant activity was found to be lower as compared to control. It could probably be due to loss of phenols in water during the hot water dip treatment. These phenols, polyphenols and other bioactive compounds are responsible for antioxidant activity in the sprouts. The hot water dip treatment caused lower levels of antioxidants in fresh-cut red sweet peppers after treatment and also during storage (Raffo et al. 2008). However an increase in total antioxidant activity in heat treated pomegranate (dips at 45 °C for 4 min) was reported (Mirdehghan et al. 2006).

Higher levels of antioxidant activity were observed in ethanol vapours treated sprouts as compared to untreated control. This could probably due to the observed higher amounts of phenols and ascorbic acid content in ethanol treated sprouts. Ethanol treatment inhibited the growth of sprouts and reduced the consumption of acids and phenols as compared to control, thereby maintaining their higher levels in the sprouts. Similarly, it was studied that there was a significant increase in oxygen radical absorbance capacity (ORAC) values in strawberries treated with ethanol vapours (Zavala et al. 2005).

Table 4 Effect of different treatments on poly phenol oxidase activity (units)* of mung bean sprouts during storage at room temperature and low temperature (T- Treatments; S- Storage)

Treatments	Period of storage (h)									
	Room temperature (25±1 °C)				Low temperature (7±1 °C)					
	0	24	48	Mean	24	48	72	96	120	Mean
Control	26	36	42	35	22	31	52	55	60	44.0
PEF	23	34	39	32	22	32	54	56	60	44.8
HWD	23	35	48	35	23	31	51	54	61	44.0
Ethanol	26	34	35	32	20	22	32	36	41	30.2
UV	22	33	39	31	23	32	52	54	64	45
Mean	24	35	41		22	29	48	51	57	
C.D. at 5%	T=0.8; S=0.6; T x S=1.4				T=0.7; S=0.8; T x S=1.7					

* 1 unit=0.01 O.D. at 430 nm

Total free phenolic content and health relevant functionality of Indian wild legume grains were studied and it was found that sprouting+oil-frying treatment showed significantly higher total free phenolic content, antioxidant and type II diabetes relevant functionality, than open-pan roasting or soaking+cooking (Vadivel et al. 2011). It was studied that when mature-green tomato fruit (Lycopersicon esculentum cv. Zhenfen 202) were exposed to 20 or 40 kJ/m^2 dose of UV-B irradiation and stored in the dark at 14 °C, 95% RH for up to 37 days, it promoted the accumulation of total phenolics and total flavonoids, and enhanced antioxidant capacity during storage, though it could reduce the ascorbic acid content (Liu et al. 2011).

Antioxidant activity increased slightly in UV treated sprouts as compared to controls. Antioxidant activity depends upon the content of phenols and ascorbic acid present. In the present investigation, an increase in phenols and ascorbic acid contents has been observed in UV-irradiated sprouts, thereby resulting in increased antioxidant activity in treated sprouts. It was studied that antioxidant activity increased in fresh-cut mangoes treated with UV-C irradiation (Zavala et al. 2005). Similarly, it was reported that UV-C irradiation increased the phenols and flavonoid content and thus high radical quencher activity (Cao et al. 1996).

Polyphenol oxidase (PPO) activity

PPO activity of mung bean sprouts under various treatments during storage is presented in Table 4. At room temperature, there was a gradual increase in PPO activity with increasing storage period. All the treatments significantly decreased the PPO activity of sprouts, except HWD treatment, where no significant change was observed. The effect of various treatments on PPO activity was evident right from the time of treatment (0-day of storage). Throughout the storage period, lowest PPO activity was observed by UV-irradiation treatment followed by PEF and ethanol vapour treatments. At low temperature storage conditions all the treatments significantly decreased the PPO activity of sprouts, except PEF treatment, where no significant effect was observed. However, throughout the storage period, minimum PPO activity was maintained by ethanol vapours treatment. The decreased activity could be due to inactivation of enzyme or reduced activity of enzyme by PEF. It was reported that the mushroom PPO activity decreased up to a 60% of the original activity after the PEF treatment (Ho et al. 1997).

Conclusions

Based on our results, it can be concluded that different treatments given to sprouts resulted in differential effect on various parameters. The ascorbic acid, total phenols and antioxidant activity were highest in ethanol vapours treated sprouts. There was a general decrease in polyphenol oxidase activity by various treatments. All the treatments showed a significant increase in ascorbic acid content except PEF at room temperature. At low temperature storage conditions, only ethanol vapours and hot water treatments resulted in increased ascorbic acid contents. Minimum total phenols were observed in HWD treatment, while it was highest in ethanol treatment. Minimum total phenols were observed in HWD treatment, while it was highest in ethanol vapours treatment. At room temperature storage conditions, there was no significant effect of PEF and UV treatments on antioxidant activities, however, an increase was observed by ethanol vapours and a decrease by HWD treatment. At low temperature, there was no significant effect of UV treatment on antioxidant activity, however, an increase was observed by PEF and ethanol treatments and a decrease by HWD treatment. All treatments showed a decrease in PPO activity except HWD in which no change was observed at room temperature. However, at low temperature storage conditions, all the treatments except PEF significantly reduced PPO activity. Thus, it can be concluded from the present study that the ethanol vapours significantly improved the ascorbic acid content, total phenols and antioxidant activity of mung bean sprouts.

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