Applied Biological Research 19 (3): 336-341; (2017) **SHORT COMMUNICATION**
DOI: 10.5958/0974-4517.2017.00048.9

COMPARISON OF ASCORBIC ACID, BETA-CAROTENE, CHLOROPHYLL AND ANTIOXIDANT ACTIVITY OF FRESH AND DRY WHEATGRASS (*Triticum aestivum* L.)

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(Received 15 February, 2017; accepted 3 September, 2017)

ABSTRACT

The study was conducted to compare ascorbic acid, β-carotene, chlorophyll and antioxidant activity of fresh and dry wheatgrass (*Triticum aestivum* L.) grown under indoor and outdoor cultivation. Three methods of drying *i.e.*, shade-, oven- and freeze-drying were employed. All the three methods significantly reduced ascorbic acid, β-carotene, chlorophyll and antioxidant activity by 73.4 to 83.5, 38.7 to 73.4, 17.6 to 60.0 and 27.6 to 48.5%, respectively. Out of the three drying methods, oven-drying showed maximum reduction in all the parameters studied, followed by shade drying while least was observed in freeze drying method. Better retention of ascorbic acid and β-carotene was in outdoor cultivation of wheatgrass; whereas, higher retention of chlorophyll and antioxidant activity was observed in indoor cultivated wheatgrass.

Key words: Freeze drying, indoor/outdoor cultivation, oven-drying, shade drying, wheatgrass

INTRODUCTION

Wheatgrass (*Triticum aestivum* L.) is a young wheat plant which is usually harvested before the plant forms its node. The plant is believed to be the power-house of various nutrients like vitamins, minerals, amino acids, bioactive compounds (saponins, tannins and flavonoids), active enzymes and chlorophyll (Padalia *et al.*, 2010). Pharmacological studies have revealed the presence of enzymatic and non-enzymatic compounds in the extract of fresh wheatgrass (Vidya and Padma, 2014). Wheatgrass is proven to be effective for the treatment of degenerative diseases like diabetes (Premkumari and Haripriya, 2010; Uma *et al.*, 2010), cardiovascular diseases (Kothari *et al.*, 2011), cancer (Mancinelli *et al.*, 2009) and hyperlipedemia (Kothari *et al.*, 2011). Wheatgrass has shown an inhibitory effect of oxidative DNA damage due to chlorophyll present in it (Falcioni *et al.*, 2002). One important characteristic of wheatgrass that accounts for its therapeutic property is its antioxidant property and nutrients like ascorbic acid, β-carotene and chlorophyll to some extent.

Vitamins like ascorbic acid and β-carotene are principal antioxidant vitamins that provide defense against free radicals and contribute to antioxidant activity of wheatgrass (Bonfili *et al.*, 2009). Chlorophyll and its derivatives also exhibit antioxidant property and hence, prevent oxidative damage (Aydos *et al.*, 2011). Processing techniques especially drying affect the nutrient components of wheatgrass. Wheatgrass must be dried at lower possible temperature, so as to prevent the loss of sensitive elements (Anonymous, 2009). The quality of dried powder of plants depends on the plant's inherent properties and drying techniques. Hot air drying is the most

commonly used method but it can lead to thermal damage of various compounds (Antal *et al.*, 2011). Ambient temperature and temperature below 50°C are best to retain the nutrient content. For drying of wheatgrass, spray-dried (Murphy and Sean, 2002) and freeze-dried (Sagliano and Sagliano, 1998) powder from wheatgrass juice is the most competent option but it is a costly affair. The attempts made to commercially preserve wheatgrass through vacuum drying processes indicate that the elevated temperatures to which the product was exposed during this processing resulted in the formation of products which were inconsistent in composition, poor in flavour and had less nutrients and viable enzymes (Sagliano and Sagliano, 1998). The present study was aimed to assess the effect of various drying methods on various bioactive compounds and antioxidant activity so as to identify the most appropriate method for drying of wheatgrass.

MATERIALS AND METHODS

Preparation of samples for analysis

Wheat cv. 'PBW-621' was obtained from Wheat Section, Department of Plant Breeding & Genetics, PAU, Punjab (India) in October, 2015. Two methods of cultivation *i.e.* indoor and outdoor methods were adopted. For indoor cultivation, plastic trays of 40 × 23 cm were used. Outdoor cultivation was done on soil bed of 90 × 60 cm. Both cultivation methods were followed simultaneously in October, using a sandy loam soil obtained from the experimental field of PAU, Ludhiana. Prior to germination, wheat grains were soaked in distilled water for 12 h and allowed to germinate in an incubator at 29°C for 12 h. Germinated wheat grains were broadcast densely but enough space for each seed were maintained so as to prevent overlapping. Wheatgrass was grown in environmental temperature (27 - 30°C and relative humidity 55 - 60%). Seeds were covered with a layer of soil followed by jute sac and sprinkled with water for both indoor and outdoor cultivation experiments. Sac was removed after 2nd day. Water was sprinkled everyday so as to make it moist. Wheatgrass was harvested on 7th day when it attained 17 - 18 and 13 - 14 cm for indoor and outdoor cultivation, respectively. Wheatgrass of 374 and 410 g weight were harvested from 500 g wheat grain from indoor and outdoor cultivation, respectively. The harvested materials were divided into three equal amounts (124 g each for indoor and 136 g each for outdoor) and were shade dried under fan until constant weight was obtained, oven-dried (50°C for 8 h) and freeze-dried (-40°C for 72 h, using Labconco, Freezone benchtop freeze dry system) followed by grinding into a fine powder before analysis.

Biochemical analysis

Ascorbic acid was determined by spectrophotometric method (AOAC, 2000) and β-carotene by column chromatography (Ranganna, 2002). The standard method of chlorophyll estimation given by Thimmaiah (1999) was used. The antioxidant activity was determined by using the DPPH assay (Dehshahri *et al.*, 2012). The antiradical activity was evaluated by extracting the wheat grass with methanol (99.9%, 20 mL) twice. The extracts were pooled and centrifuged (10000 rpm, 15 min). The process involved mixing the DPPH solution (0.1 nM, 2.9 mL) with 100 µL of extract followed by homogenization. After incubation (30 min) in dark at room temperature (29°C), the remaining DPPH radicals were quantified by measuring the absorbance at 517 nm using double beam spectrophotometer (Elico SL 244). The DPPH scavenging effect was measured by the formula:

$$\text{Per cent inhibition} = [(A_B - A_A)/A_B \times 100]$$

Where, A_B and A_A were the absorbance of control solution and the standard sample, respectively.

Statistically analysis

All the experiments were conducted thrice. Mean and standard deviations for various parameters were computed. Analysis of variance was employed using computer software CPCS 1 to assess the

difference in parameters as influenced by drying methods. Microsoft excel statistical analysis tool pack was used for statistical analysis. Critical difference at 5% was calculated for comparison among the parameters. Percent reduction of sample after drying was also computed.

RESULTS AND DISCUSSION

The content of ascorbic acid, β-carotene, chlorophyll and antioxidant activity in fresh and dried wheatgrass grown under indoor and outdoor cultivation is presented in Table 1. The percent reduction in above parameters is given in Fig. 1 to 4. The results revealed that freeze-drying to be superior over shade- and oven-drying for retaining the ascorbic acid in wheatgrass powder. The mechanism of degradation of ascorbic acid is influenced by the temperature, salt and sugar concentration, pH, enzymes, metal catalyst and presence of oxygen (Kiremire *et al.*, 2010). In present study, the reduction of ascorbic acid was slightly higher in oven-dried wheatgrass as compared to shade-dried wheatgrass which may be attributed to exposure to heat during oven-drying.

Table 1: The ascorbic acid and β-carotene contents (on dry matter basis) of shade-, oven- and freeze-dried wheatgrass during indoor and outdoor cultivation

Parameters	Fresh wheatgrass	Method of drying			$CD_{0.05}$ (between drying methods)
		Shade	Oven	Freeze	
Ascorbic acid (mg 100 g^{-1})					
Indoor	12.0 ± 1.0	2.30 ± 0.1	1.98 ± 0.1	3.18 ± 0.4	0.9
Outdoor	9.1 ± 0.8	2.08 ± 0.3	1.87 ± 0.2	2.22 ± 0.2	ns
β-carotene (µg 100 g^{-1})					
Indoor	501.0 ± 1.7	193.6 ± 36.6	133.1 ± 22.7	230.3 ± 36.6	72.1
Outdoor	557.0 ± 1.6	278.3 ± 49.4	223.9 ± 65.8	341.6 ± 39.9	ns
Chlorophyll (g 100 g^{-1})					
Indoor	4.4 ± 0.2	2.30 ± 0.2	2.1 ± 0.9	3.6 ± 0.9	0.6
Outdoor	15.7 ± 0.1	6.30 ± 0.6	7.3 ± 0.3	9.8 ± 0.2	0.9
Antioxidant activity (%)					
Indoor	78.4 ± 1.4	50.10 ± 1.9	48.9 ± 1.1	53.8 ± 1.8	ns
Outdoor	65.7 ± 2.7	43.20 ± 0.9	33.8 ± 2.7	47.4 ± 1.7	6.6

Values are mean ± SD; ns = non-significant; **= Significant at 5%

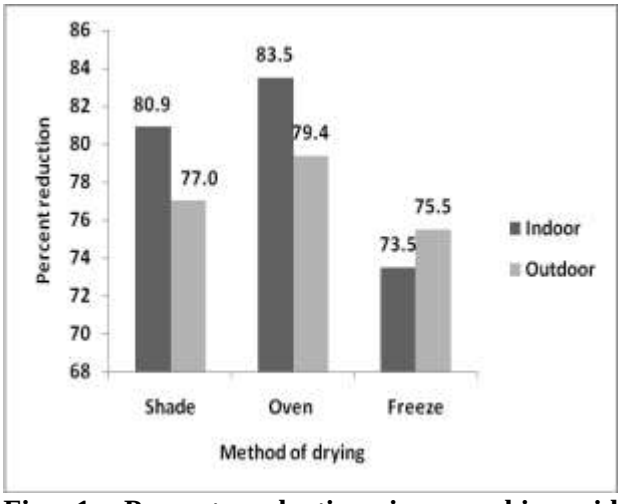

Fig. 1: Percent reduction in ascorbic acid content of wheatgrass after drying

The vitamin may also be lost by enzymatic degradation (Maharaj and Sankat, 1990). The prolonged handling process prior to drying may be enhanced due to the oxidative loss, which may be one of the reasons for lesser retention of ascorbic acid in dried wheatgrass observed in present study.

Highest β-carotene content was observed in freeze-dried wheatgrass in both indoor and outdoor cultivation. The finding is in agreement with Chen *et al.* (2007) who found that lyophilization of mango fruits resulted in more carotenoid content than oven-dried fruit. Zhou *et al.* (2007) also reported that freeze-dried loquat flower had highest β-carotene content while oven-dried had lowest value. Nawirska *et al.* (2009) found

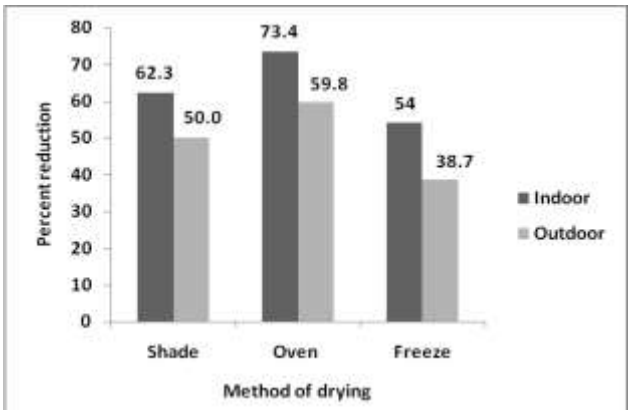

Fig. 2: Percent reduction in β-carotene content of wheatgrass after drying

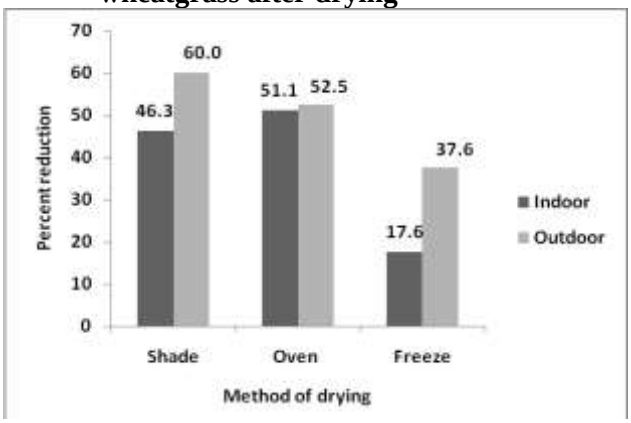

Fig. 3: Percent reduction in chlorophyll content of wheatgrass after drying

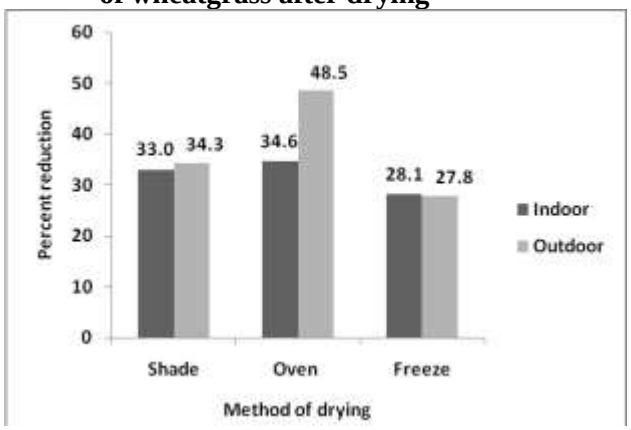

Fig. 4: Percent reduction in antioxidant activity of wheatgrass after drying

that freeze drying is the best method to preserve carotenoids in dried pumpkin slices. Decrease in β-carotene content in oven-dried wheatgrass may be attributed to auto-oxidation.

Fresh wheatgrass showed significantly ($p \leq 0.05$) higher chlorophyll content than wheatgrass dried by shade-, oven- and freeze-drying methods. The chlorophyll loss was 46.3, 51.1 and 17.6% on shade-, oven- and freeze-drying, respectively, under indoor cultivation. The corresponding reduction of chlorophyll for outdoor cultivation was 60.0, 52.5 and 37.6%. The highest chlorophyll retention was observed in freeze-dried wheatgrass, followed by shade-dried and oven-dried wheatgrass. Similar results were obtained by Zhou *et al.* (2011) where freeze drying of loquat flowers had highest chlorophyll, followed by microwave-drying and oven- drying and least with natural conventional drying. Dorta *et al.* (2012) and Panela *et al.* (2012) have concluded that freeze-drying increases the extraction of bioactive compounds of different products in comparison to air-drying. This is because freeze-drying is based on dehydration by sublimation of a frozen product. Schwartz and Lorenzo (1991) **re**ported that chlorophyll is sensitive to heat and its retention is affected by temperature and duration of heat. Increasing the drying air temperature particularly drying under natural conventional method enhanced the loss in colour of plants, concomitant with increase in pheophytin formation (Maharaj and Sankat, 1990). Erge *et al.* (2008) observed that chlorophyll content of green peas decreased with increase in drying temperature and showed faster degradation at 100°C than at 70°C.

Higher retention of antioxidant activity was observed in outdoor cultivated wheatgrass. Freeze-dried wheatgrass had highest retention of antioxidant activity in all the three drying methods. Decrease in antioxidant properties are often accompanied by the loss of bioactive phyto-chemicals (Roy *et al.*, 2007) which may be attributed to thermal degradation of phenolic compounds, degenerative enzymes and to loss of antioxidant enzyme activities in dried plants (Lim and Murtijaya, 2007). During dehydration or drying process, the structure collapses and leads to the release of more phenolic compounds, together with oxidative and hydrolytic enzymes which usually degrades the phenolic compounds (Toor and Savage, 2006).

Conclusion: The study highlighted an appreciable reduction of ascorbic acid, β-carotene, chlorophyll and antioxidant activity in wheatgrass after drying. Among all the drying methods, freeze-drying proved superior over shade-drying and oven-drying in terms of higher retention in the wheatgrass powder.

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