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TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, AND ANTHOCYANIN PROFILE OF SPROUTED ONION POWDER

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The effect of sprouting of onions on total phenolic content, antioxidant activity, and anthocyanin profile of freeze dried powders produced from Indian onion cultivars was examined. Sprouting was carried out at an average light intensity of 64 Lumen, average relative humidity of 80.2%, and average temperature of 15.22 °C for three weeks. Two way analyses of data revealed significant ($P<0.05$) effect of sprouting and cultivar on functional composition of freeze dried powders. In all sprouted powder samples, there were significant ($P<0.05$) enhancements in total phenolic content, total flavonoid content, vitamin C content, antioxidant activity, and colour due to increase in anthocyanin contents. The anthocyanin profile depicted an increase in total anthocyanin concentration in powders developed from all four cultivars with the highest increase in powder from PRO-6 (23.87–27.15 mg kg^{-1}), followed by Punjab Naroya (9.97–12.92 mg kg^{-1}), commercial (7.15–8.43 mg kg^{-1}), and Punjab White cultivar (3.45–4.09 mg kg^{-1}).

Keywords: onion powder, sprouting, antioxidant activity, anthocyanin profile

Onion (*Allium cepa* L) is extremely important vegetable for daily cuisine in India, which is the 2nd largest producing country experiencing huge global demand (APEDA, 2016). The epidemiological studies have proved consumption of onion associated with a reduced risk of coronary heart disease, and flavonoids, such as quercetin, are believed to be a major contributor for it (Hamauzu et al., 2011). The phenolic components have been identified as the active components exhibiting anti-asthmatic activity, anti-fungal and anti-bacterial properties (Insani et al., 2016). In order to take advantage of the potential health benefits of onion, there is a need to develop onion powders that not only have desired functionality but also are stable over a longer storage time. Freeze drying is considered suitable for drying of plant derived products as it results in minimal compositional change (Febriyenti et al., 2014).

Onion crop undergoes a natural biological process of sprouting, which is characterized by increased activity of phenylalanine ammonia-lyase and peroxidase that are linked to phenolic metabolism during sprouting (Benkeblia, 2000). Sprouting in onion, however, is considered to be a major contributor (20–40%) of the total post-harvest storage losses (50–90%) (National Bank for Agriculture and Rural Development, 2016). Sprouting has received little attention, even though sprouted onion samples were found to contain higher concentration of phenolics (Albishi et al., 2013). It has also been reported in our previous work that sprouting in onion was an excellent method of enhancing nutritional and functional potential (Majid et al., 2016). Since, several above mentioned findings have proven sprouting to be beneficial in case of onion, but so far no study has been carried out for the development and characterization of powder from sprouted onions. This study was thus undertaken to determine the effect of sprouting on colour, total phenolic content, total flavonoid content, antioxidant activity, and anthocyanin profile of onion powder.

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1. Materials and methods

1.1. Chemicals, raw material, and storage conditions

The Folin-Ciocalteu reagent, gallic acid, sodium carbonate, 2,2-diphenyl picrylhydrazyl, acetone, methanol (HPLC grade) were purchased from Ranbaxy, New Delhi, and cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(6”-malonylglucoside), cyanidin 3-malonyl-laminaribioside, and cyanidin 3-(6”-malonilaminaribioside) from Acros Organics, New Jersey, USA.

Three Rabi season onion cultivars (Punjab Naroya, Punjab white, and PRO-6) were procured from the Department of Vegetable Science, P.A.U Ludhiana, India, sown in January and harvested in May. Onions from all cultivars were oval in shape, with Punjab White cultivar white and the other two red in colour with PRO-6 being dark red. The 150 onion bulbs of each cultivar were stored in jute bags under dark storage conditions till December in order to keep dormancy at an average temperature of 26.3 °C and average relative humidity of 64.3%. The sprouting of the onions was then carried out at an average light intensity of 64 Lumen, average relative humidity of 80.2%, and average temperature of 15.22 °C for three weeks, till onions attained the sprout length of 8 cm for the preparation of onion powder. One commercial cultivar bought from local market was also stored, utilized, and analysed under similar conditions.

1.2. Preparation of freeze-dried onion powders

Raw and sprouted onion bulbs were rinsed with water, cut into quarters, and frozen at −20 °C. The frozen slices were freeze dried (Christ Alpha1-2 LD, UK) and powdered.

1.3. Colour measurement

The colour of raw and sprouted onion powders was determined using a colorimeter (Model CR-10, Konica Minolta Sensing, Inc. Japan). The results were expressed in triplicates as L^*, a^*, and b^*, and chroma and hue angle values were calculated.

1.4. Total phenolic, flavonoid, vitamin C, and DPPH radical scavenging activity

Total phenolic and flavonoid contents expressed as mg of gallic acid equivalents (mg GAE/100 g) and mg of quercetin equivalents per 100 g, respectively, DPPH radical scavenging activity and vitamin C content (measured spectrophotometrically, HACH DR 6000, Dusseldorf, Germany) were determined using the method of SHARMA and co-workers (2015).

1.5. High-performance liquid chromatography (HPLC) analysis for anthocyanins

From raw and sprouted onion powders (3 g) anthocyanins were extracted with 25 ml of methanol : formic acid : water (MFW; 50:5:45; v:v:v) and stabilized with 2 g l^{-1} of *tert*-butyl hydroquinone. The homogenate was shaken for 15 min and centrifuged. Two additional extractions were performed for each raw and sprouted samples with 15 and 10 ml of MFW, and the 50 ml of this final extract from each sample was diluted to 60 ml with MFW. An aliquot (50 μl) was then injected into HPLC/DAD. Analyses of the extracts were carried out by Agilent 1290 infinity LC system equipped with a quaternary pump (G4204A) and UV absorbance detector with diode array detection according to the method of PEREZ-GREGORIO and co-workers (2010) with some modifications. The separation was performed by using

Phenomenex Luna C18 100 Å column (150 × 4.6 mm) with a particle size of 5 µm. The mobile phase consisted of the following: (A) 0.5% formic acid in water, (B): 0–10 min, 20% B; 10–15 min, 20–80% B; 15–22 min, 80–20% B. Detection was set to 520 nm for anthocyanins with a flow rate of 0.8 ml min^{-1} at 30 °C, and the injection volume was 20 µl. Identification of anthocyanins was carried out by comparing their retention time and spectral characteristics with those of the reference standards.

1.6. Statistical analysis

A two way ANOVA was carried out to assess the significant main effects for cultivar and sprouting on various analysed parameters, followed by Duncan's test using Statistica.v.12. (StatSoft India Pvt. Ltd. New Delhi, India).

2. Results and discussion

2.1. Colour evaluation

Colour analysis revealed significant differences (P<0.05) in lightness (L^*) in both raw and sprouted onion powders. However, a-value significantly (P<0.05) varied in raw powders and sprouted Punjab White powder only. The b-value showed significant difference (P<0.05) in powders from raw and sprouted Punjab White and Punjab Naroya. In the present study, sprouting led to increase in a-value (green/red), b-value (yellow), and chroma with significant increase (P<0.05) in a-value in all powders except commercial (Table 1). The results are well supported by the studies reported on sprouted soy flour by Murugkar and Jha (2010). The increase in redness, greenness, and yellowness can be attributed to increased anthocyanin content with sprouting in all four onion cultivars (Majid et al., 2016).

2.2. Total phenolic content

Total phenolic content varied significantly (P<0.05) in raw as well as sprouted onion powders, and within cultivar, significant difference (P<0.05) was found between raw and sprouted powder samples. Results were well supported by previous studies, which showed increase in total phenol content in germinated rough rice powder (Moongngarm & Saetung, 2010). The increase in phenolic compounds occurs during sprouting when there is increased susceptibility for oxidation, and as a result of which γ-glutamyl transpeptidase produces more phenolic compounds (Tian et al., 2004).

2.3. Total flavonoid content

Total flavonoid content was found to vary significantly (P<0.05) in raw onion powders, and was highest in PRO-6. Sprouted Punjab White and commercial and Punjab Naroya and PRO-6 had statistically similar content of total flavonoids. The significant increase (P<0.05) in total flavonoid content was observed between raw and sprouted onion powders within cultivar with the exception of commercial. The increase is attributed to the activation of phenylalanine ammonia-lyase and peroxidase enzymes in onion, which results in release of bound phenolic compounds involved in sprouting, thereby increasing the total flavonoid content (Alvarez-Jubete et al., 2010).

Table 1. Effect of sprouting on total phenol, flavonoid, and antioxidant contents and colour of freeze dried powders from four Indian onion cultivars

		Punjab White		Punjab Naroya		PRO-6		Commercial	
		Raw	Sprouted	Raw	Sprouted	Raw	Sprouted	Raw	Sprouted
Vit. C (mg/100 g)		5.82 ± 0.26^{aP}	6.14 ± 0.57^{AP}	5.79 ± 0.33^{aP}	6.21 ± 0.37^{AP}	4.36 ± 0.20^{bQ}	5.45 ± 0.45^{AP}	3.82 ± 0.36^{bP}	4.37 ± 0.39^{BP}
TPC (mg/100 g)		218.03 ± 1.46^{dQ}	306.01 ± 1.43^{CP}	310.04 ± 1.35^{aQ}	317.20 ± 1.37^{BP}	305.04 ± 3.32^{bQ}	328.08 ± 2.09^{AP}	276.07 ± 3.29^{cQ}	295.04 ± 8.57^{DP}
TFC (mg/100 g)		103.01 ± 1.77^{cQ}	115.00 ± 1.26^{BP}	109.00 ± 1.47^{bQ}	129.66 ± 1.91^{AP}	124.06 ± 3.42^{aP}	131.16 ± 3.51^{AQ}	109.00 ± 2.94^{bP}	114.00 ± 6.46^{BP}
DPPH (%)		20.71 ± 0.92^{cQ}	26.52 ± 1.42^{CP}	40.78 ± 0.58^{bQ}	51.93 ± 1.55^{BP}	46.11 ± 1.54^{aQ}	66.85 ± 3.33^{AP}	22.69 ± 1.85^{cQ}	28.54 ± 0.94^{CP}
Colour	L-value	81.50 ± 0.77^{aP}	73.27 ± 2.31^{ABQ}	76.92 ± 2.36^{aQ}	68.28 ± 2.19^{AP}	63.01 ± 2.26^{bQ}	70.46 ± 3.70^{BP}	60.53 ± 2.17^{bP}	61.33 ± 2.04^{CQ}
	a-value	1.22 ± 0.08^{dQ}	6.51 ± 0.49^{BP}	3.23 ± 0.15^{cQ}	7.80 ± 0.48^{AP}	4.71 ± 0.26^{bQ}	7.81 ± 0.36^{AP}	6.69 ± 0.71^{aP}	7.61 ± 0.11^{AP}
	b-value	24.71 ± 1.23^{aQ}	28.81 ± 2.11^{AP}	23.46 ± 0.81^{aP}	23.59 ± 1.97^{BP}	18.91 ± 2.01^{bP}	19.40 ± 2.14^{CP}	17.77 ± 2.03^{bP}	19.61 ± 1.11^{CP}
	Chroma	24.73 ± 1.22^{aQ}	29.53 ± 1.96^{AP}	23.68 ± 0.83^{aP}	24.85 ± 1.98^{BP}	19.49 ± 1.69^{bP}	20.91 ± 1.85^{CP}	18.99 ± 1.66^{bP}	21.04 ± 1.00^{CP}
	Hue angle(°h)	87.21 ± 2.07^{aP}	77.27 ± 1.92^{AQ}	82.18 ± 0.95^{abP}	71.63 ± 1.80^{BQ}	76.03 ± 2.91^{bcP}	68.09 ± 4.95^{BP}	69.55 ± 6.41^{cP}	68.80 ± 4.62^{BP}

Means in a row with same superscripts (a,b,c,d) for raw and (A,B,C,D) for sprouted are not significantly different ($P<0.05$). Means in a row with same superscripts (P,Q) within a particular cultivar are not significantly different ($P<0.05$).

2.4. DPPH radical scavenging activity

DPPH radical scavenging activity of raw and sprouted onion powders varied significantly ($P<0.05$) in Punjab Naroya and PRO-6. The significant ($P<0.05$) increase from raw to sprouted onion powder was consistent with results reported in freeze dried sesame sprouts powder (Shabbir et al., 2015). The increase in antioxidant activity occurs due to increased activity of phenylalanine ammonia-lyase, which is a key enzyme for synthesis of phenolic compounds that constitute the greatest share of antioxidant content (Yang et al., 2001).

2.5. Vitamin C content

Raw onion powders Punjab White and Punjab Naroya had statistically similar vitamin C contents, and similarly, powders of sprouted onion Punjab White, Punjab Naroya, and PRO-6. Vitamin C content was found to be higher in sprouted onion powders than raw onion powders with significantly ($P<0.05$) higher in sprouted PRO-6 onion powder. Since the only difference between raw and sprouted onion powders is process of sprouting, it is believed to be accountable for the increase in vitamin C content. Similar findings with sprouting have been reported in freeze dried soybean sprout powder (Kim et al., 2016). The increase in vitamin C content during sprouting occurs due to enhanced activity of L-galactono-γ-lactone dehydrogenase participating in the oxidation of L-galactono-1,4-lactone to ascorbic acid (Xu et al., 2005).

2.6. HPLC analysis of anthocyanins

The anthocyanin profile of raw and sprouted onion powders are presented in Table 2 with the representative HPLC chromatograms for raw and sprouted PRO-6 powder presented in Figures 1 and 2, respectively. Regarding anthocyanins, in our study, the most abundant anthocyanins identified were cyanidin derivatives, diglycosylated at C-3 and glycosylated at C-6 positions with quantitative differences among cultivars and due to sprouting. The concentrations of the peaks corresponding to cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(6”-malonylglucoside), cyanidin 3-malonyl- laminaribioside, and cyanidin 3-(6”-malonilaminaribioside) were statistically different between raw and sprouted powder samples. Anthocyanin composition as cultivar dependent characteristic has been reported earlier for onion cultivars (Perez-Gregorio et al., 2010), while the fate of the anthocyanins in onion powder after sprouting is reported here for the first time. From the current results, the influence of the sprouting on the quantity of anthocyanin present in the powder samples is clear. Lower anthocyanin contents were found in white cultivar (Punjab White) compared to coloured onion cultivars, which stays in agreement with the results reported in Portuguese red and white onion cultivars (Rodrigues et al., 2009). Anthocyanin profile with sprouting followed statistically significant ($P<0.05$) irregular trend of increase and decrease in specific anthocyanin components, and the increase or decrease in one specific anthocyanin component was compensated by the corresponding decrease or increase in other anthocyanin components, thereby increasing the total anthocyanin profile in all onion powders (Table 2). The significant ($P<0.05$) increase of the total levels of these pigments in sprouted powder samples is slightly more sensitive to the process of sprouting in onion powder from PRO-6 cultivar (Table 2). The results indicated that an increase of anthocyanin contents in powders was due to sprouting, and similar effect was observed in Sango sprouts, as well (Matera et al., 2012). It has been reported that the increase of anthocyanin content in sprouted counter parts was accompanied with the rise of transcription level of anthocyanin biosynthetic genes that

Table 2. Effect of sprouting on anthocyanin concentrations of freeze dried powders from four Indian onion cultivars

Anthocyanin component (mg kg^{-1})	Punjab White		Punjab Naroya		PRO-6		Commercial	
	Raw	Sprouted	Raw	Sprouted	Raw	Sprouted	Raw	Sprouted
Cyanidin 3-glucoside	$0.78±0.06^{dP}$	$0.53±0.05^{CQ}$	$3.16±0.16^{bP}$	$4.64±0.07^{AP}$	$4.35±0.83^{aP}$	$2.26±0.03^{BQ}$	$1.62±0.06^{cP}$	$0.49±0.03^{CQ}$
Cyanidin 3-laminaribioside	$0.19±0.02^{bQ}$	$0.36±0.04^{CP}$	$0.32±0.08^{bQ}$	$0.69±0.08^{CP}$	$2.14±0.06^{aQ}$	$4.73±0.78^{AP}$	$0.45±0.12^{bQ}$	$1.93±0.03^{BP}$
Cyanidin 3-(6”-malonyl-glucoside)	$0.59±0.08^{dP}$	$0.24±0.07^{DQ}$	$4.78±0.46^{bQ}$	$6.38±0.79^{BP}$	$11.64±0.51^{aQ}$	$13.68±0.44^{AP}$	$2.73±0.13^{cP}$	$2.56±0.14^{CP}$
Cyanidin 3-malonyl-laminar-ibioside	$1.63±0.05^{cQ}$	$2.27±0.12^{BP}$	$1.27±0.07^{dP}$	$0.56±0.03^{CQ}$	$5.11±0.10^{aP}$	$5.94±0.75^{AP}$	$2.11±0.08^{bQ}$	$2.83±0.09^{BP}$
Cyanidin 3-(6”-malonyl laminaribioside)	$0.26±0.03^{cQ}$	$0.68±0.08^{AP}$	$0.43±0.06^{bQ}$	$0.64±0.06^{ABP}$	$0.62±0.05^{aP}$	$0.54±0.08^{CP}$	$0.24±0.05^{cQ}$	$0.61±0.06^{ACP}$
Total anthocyanin component concentration	$3.45±0.09^{dQ}$	$4.09±0.11^{DP}$	$9.97±0.29^{bQ}$	$12.92±0.75^{BP}$	$23.87±0.51^{aQ}$	$27.15±1.25^{AP}$	$7.15±0.24^{cQ}$	$8.43±0.16^{CP}$

Means in a row with same superscripts (a,b,c,d) for raw and (A,B,C,D) for sprouted are not significantly different ($P<0.05$). Means in a row with same superscripts (P,Q) within a particular variety are not significantly different ($P<0.05$).

induce anthocyanin accumulation (Gonzalez et al., 2008). The anthocyanin biosynthesis via the flavonoid and upstream pathway of proanthocyanidins form anthocyanidins due to catalysis by anthocyanidin synthase which is more active in stored plant materials. The liberated anthocyanidins, besides acting as direct substrates for the anthocyanin synthesis, also get catalyzed by anthocyanidin reductase to enhance anthocyanidins. The anthocyanidins generated with cyanidin 3-glucoside as the starter compound undergo glycosylation and acylation to convert anthocyanidins to diverse anthocyanin molecules (Shi & Xie, 2014). Since the key enzyme, anthocyanidin synthase, involved in the anthocyanin biosynthesis occurs more in stored plant products, it is thus speculated that powder from sprouted onion cultivars might possess more anthocyanins compared to raw ones.

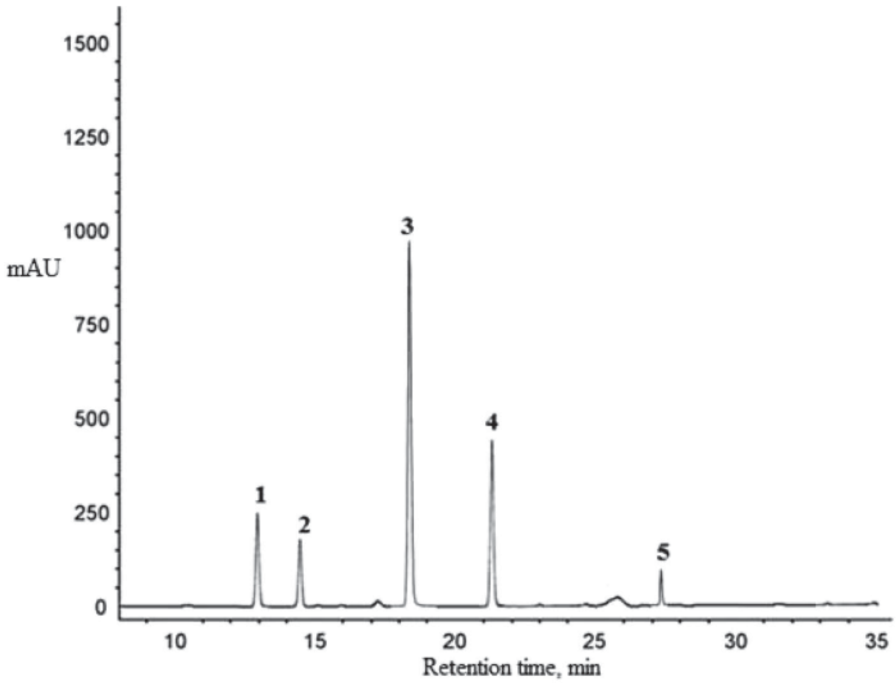

Fig. 1. The typical representation of HPLC chromatograms obtained for PRO-6 Raw onion powder (1: cyanidin 3-glucoside; 2: cyanidin 3-laminaribioside; 3: cyanidin 3-(6"-malonyl glucoside); 4: cyanidin 3-(6"-malonyl laminaribioside), 5: cyanidin 3-malonyl-laminaribioside)

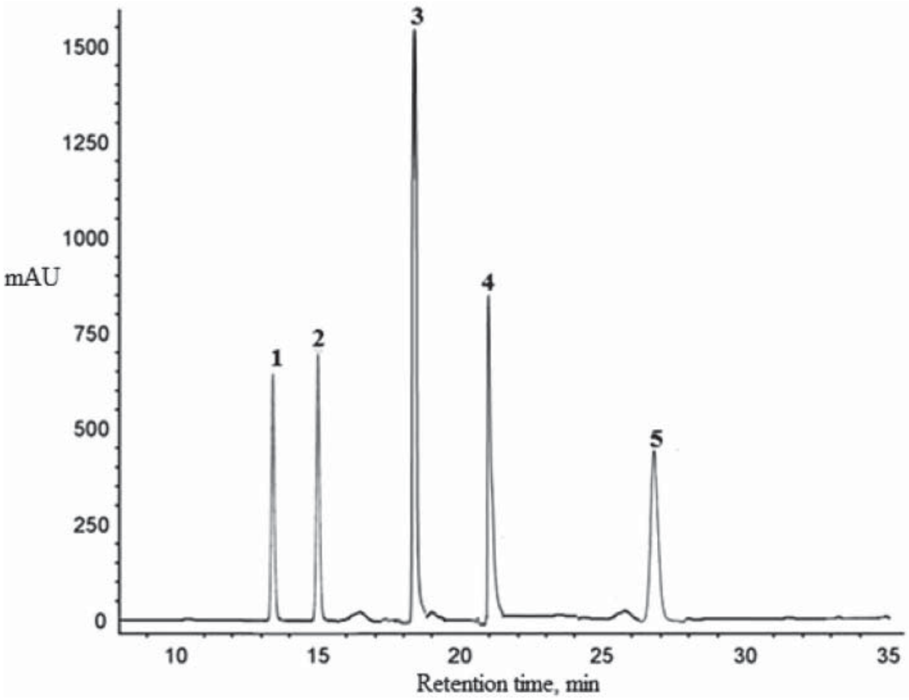

Fig. 2. The typical representation of HPLC chromatograms obtained for PRO-6 sprouted onion powder (1: cyanidin 3-glucoside; 2: cyanidin 3-laminaribioside; 3: cyanidin-3-(6”-malonyl glucoside); 4: cyanidin 3-(6”-malonyl laminaribioside), 5: cyanidin 3-malonyl-laminaribioside)

3. Conclusions

Onion powders produced from sprouted onion cultivars were found to have improved colour, higher contents of total phenols and total flavonoids, antioxidant activity, anthocyanins and vitamin C contents, which unveil their potential of use as phyto additive for culinary purposes. Utilization of sprouted onion powders in various food applications with enhanced nutritional and functional potential may contribute to alleviate the post harvest losses and environmental concerns arising from disposal of huge quantities of sprouted onions. The study thus concluded that development of value-added product (powder) from sprouted onions demands due attention as it could be advantageous to food industry by supporting its contribution to health-promoting effects in the present functional food market.

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