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Sprouting enhances phytonutrients and antioxidants in onion seeds

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ABSTRACT

Consumption of onion seed sprouts is getting popular for its flavour and texture. However, there is limited information on changes in the phytonutrients during sprouting of onion seed. The objective of this study was to determine the temporal variations in the phytonutrient content during the sprouting of onion seeds and to determine the appropriate time of sprouting for better nutrition. Seeds of three onion varieties (Bhima Red, Bhima Raj and Bhima Kiran) were germinated for different periods (5, 10 and 15 days). Changes in phytonutrients and antioxidant potential were analyzed. The highest flavonoids, thiosulfinates, and DPPH activity were observed in 10 days old sprouts, which were 38.78%, 160.71%, and 97.45% higher than seeds. Pyruvic acid, total phenols and ABTS activity was maximum in 15 days old sprouts, which was 682.69%, 189.03%, and 79.38% higher than seeds respectively. The sugars increased initially but declined with sprout age and the lowest levels were recorded in 15 days old sprouts. Sprouting enhanced the health-promoting phytonutrients and antioxidant activity of onion seeds and sprouts harvested at 10 and 15 days had a higher quantity of health-promoting phytonutrients.

Keywords: Antioxidants, Onion seed sprouts, Phenol, Sugar, Superfoods, Thiosulfinates

With the increasing awareness about health, people are keenly showing interest in adding superfoods to their diet. Superfoods are mainly plant-based, nutrient-dense food, rich in antioxidants and vitamins. They are consumed as seeds, sprouts, whole or specific parts of a plant (Wolfe 2010). In comparison to seeds, sprouts have lower anti-nutritional compounds, high in fiber, essential amino acids, vitamins, polyphenols, and flavonoids (Dueñas *et al.* 2009; Pająk *et al.* 2014). The consumption of sprouts is increasing among health-conscious individuals intended to improve and maintain their health (Guo *et al.* 2012). Onion is an important vegetable grown throughout the world, used mainly as a flavouring agent in food preparations. It is a rich source of quercetin, polyphenols, sapogenins, saponins, flavonoids, organosulfur compounds, selenium, and vitamins (Lachman *et al.* 2003, Bahram-Parvar and Lim 2018). The medicinal values of onion are well documented, regular consumption of onion reduces cardiovascular diseases and lowers the risk of certain cancers (Bahram-Parvar and Lim 2018). Onion has hypolipidemic, anti-inflammatory, anti-diabetic, anti-microbial, anti-fungal and anti-parasitic properties (Bahram-Parvar and Lim 2018). Sprouts are the cheapest and most convenient source of nutrients and its production requires less time and limited resources (Silva *et al.* 2013).

The information on phytonutrients and antioxidant potential of fresh and processed onion products are widely available, but there are very few reports on nutritional qualities of onion seeds. There is limited information on the nutritional composition of onion seed sprouts. There are no reports available on the changes in the nutritional quality of onion sprout during germination. Our study aims to find the temporal changes in the phytonutrients during sprouting to explore the feasibility of consumption of onion seed sprout as a superfood.

MATERIALS AND METHODS

The study was carried out at the Seed Technology Laboratory of the ICAR-Directorate of Onion and Garlic Research, Pune during July and August 2020. The experiment was laid out by using completely randomized design (CRD) with three replications.

Plant material and growing conditions: Seeds of onion varieties- Bhima Red, Bhima Raj and Bhima Kiran were surface sterilized and in a 460 mm × 285 mm germination paper, 25-gram seeds were germinated for different periods - 5, 10 and 15 days by rolled towel method at 20 ± 1^0C, 80% RH.

Biochemical analysis: Thiosulfinates was estimated as per Han *et al.* (1995) with some modifications. In brief, one-gram sample was crushed in 15 mL distilled water and

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centrifuged at 10000 RPM for 10 minutes and the supernatant was used for analysis. The reaction mixture containing 1.2 mL of 2 mM L-cysteine and 800 µL of the extract was incubated at 37^{0}C for 15 minutes, subsequently, 3 mL HEPES buffer (50 mM, pH 7.6) and one mL 1.5 mM DTNB was added. The absorbance was recorded at 412 nm against a HEPES Buffer as blank. Total pyruvic acid was estimated as per Anthon and Barrett (2003) with modifications. Total flavonoid content was estimated calorimetrically as per Zhishen *et al.* (1999) with some modifications suggested by Gorrepati *et al.* (2020). The total phenol content of the seeds and sprouts was estimated as per Singleton and Rossi (1965) using the Folin-Ciocalteau (FC) reagent. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay was done as per Re *et al.* (1999). The DPPH radical scavenging assay was carried out as per Brand-Williams *et al*. (1995). Sucrose and fructose was estimated as per (Percheron *et al.*1963). Reducing sugars was estimated by Nelson- Somogyi method (Nelson 1944). Total sugar was estimated by the phenol- sulfuric acid method (DuBois *et al.* 1956).

Statistical analysis: The data on the changes in the phytonutrients in onion seeds and sprouts was analysed with analysis of variance (ANOVA) for a factorial design using PROC GLM in the statistical analysis software (SAS Institute, USA). The means were separated by Fishers least significant difference test (if the ANOVA indicated significant differences; P≤0.05).

RESULTS AND DISCUSSION

Onions are a major source of two phytonutrients flavonoids and derivatives of sulfur-containing alk(en)yl cysteine sulphoxides (ACSOs) (Lachman *et al*. 2003). The two flavonoid subgroups found in onions are the anthocyanins and flavanols, which impart colour to flesh and scales in the onion. There are more than 50 sulfur-containing compounds in onions of which thiosulfinates are the major group (Griffiths *et al.* 2002). In the present study, we studied the changes in the phytonutrients in onion seeds and sprouts harvested at different intervals. The values of flavonoids, thiosulfinates, total phenol, antioxidant activity, pyruvic acid, and sugars for seeds would indicate the amount synthesized while the seed was attached to the parent plant and for sprouts, values indicate synthesis or catabolism (Cevallos-Casals and Cisneros-Zevallos 2010).

Physical changes during germination: Seed germination is a process initiated by the imbibition of water followed by restarting of the metabolic and synthetic machinery. The germination, seedling length, and seedling weight increased with the sprout age. On the 5th day, almost 90% of the seeds germinated (Table 1). The average germination at 15 days was 95.56%. Zaghloul *et al.* (2013) also reported an increase in germination with time and the average germination at 15 days was 96.50%. A significant difference in sprouting capacity at different periods has been reported previously (Devi *et al*. 2015). In comparison to the 5 days sprouts, 15 days sprouts recorded 230.76% and 120% higher seedling length and seedling weight (Table 1). Devi *et al.* (2015) also reported an increase in sprout length and seedling weight with an increase in sprout age of cowpea.

Changes in thiosulfinates and pyruvic acid: The anti-inflammatory properties of onion is attributed to the thiosulfinates. Allinase derived pyruvic acid is the widely used indicator of pungency in Allium crops (Anthon and Barrett 2003). In the present study, the highest thiosulfinate and pyruvic acid was recorded in 10 and 15 days sprouts respectively. In comparison to the seeds, sprouts had 160.71% and remarkably 682.69% higher thiosulfinates and pyruvic acid respectively (Table 1). Among the varieties, highest thiosulfinates and total phenol was recorded in Bhima Red

Table 1 Physical and compositional changes in seeds and sprouts of three onion varieties harvested at different intervals

Treatment	G (%)	SL (cm)	FW (mg)	TS (µmol/g DW)	TPA (µmol/g DW)
Variety (V)					
Bhima Red	91.78 (74.83$^{a\wedge}$)	8.16^a	10.30^b	11.40^a	1.84^c
Bhima Raj	92.22 (74.50 ^{a})	8.07^a	15.50^a	10.90^b	2.99^a
Bhima Kiran	91.33 (73.76^a)	8.15^a	12.70^b	10.40^c	2.77^b
LSD (*p≤0.05*)	NS	NS	2.52**	0.42**	0.35**
Days (D)					
0	-	-	3.20^d	5.60^d	0.52^d
5	89.11 (70.89 ^{b})	3.51^c	10.00^c	10.00^c	2.16^c
10	90.67 (73.62^b)	9.24^b	16.00^b	14.60^a	3.40^b
15	95.56 (78.56^a)	11.61^a	22.00^a	13.30^b	4.07^a
LSD (P≤0.05)	4.55**	0.54	2.91**	0.48**	0.409**
Interaction (V × D)					
LSD (P≤0.05)	NS	NS	NS	0.83**	0.708**

** Significant at P≤0.01; ^Values in the parenthesis are arcsine transformed values; Values with the same letters in a column are not significantly different at P≤0.05; G%: germination percentage; SL: Seedling length cm seedling^{-1}; FW: Fresh weight mg seedling^{-1} or seed; TS: Thiosulfinates; TPA: Total pyruvic acid; DW: Dry weight; NS: non-significant.

and Bhima Raj respectively. There was significant (P≤0.01) interaction between variety and sprout age for thiosulfinates and pyruvic acid. Onion seeds have a negligible quality of alliinase enzyme, and upon germination a rapid increase in alliinase activity has been reported previously, reaching a maximum at 15-20 days (Lancaster and Boland 1990). Increase in the content of thiosulfinates and pyruvic acid suggests that there is substantial biosynthesis of flavour precursors ACSO and expression of metabolic enzymes involved sulphur metabolism.

Enhancement of antioxidants in sprouted seeds: Antioxidants have a vital role in human health as they inhibit or slow down the oxidation process. The reactivation of metabolism during the imbibition of seeds generates ROS. To quench the ROS produced, the production of antioxidant compounds such as flavonoids, phenols, and ascorbic acids are increased during germination (Kumar *et al.* 2019; Yalamalle and Tomar 2019). Flavonoids are a diverse group of polyphenol compounds with having antioxidants activity. In the present study, sprouting enhanced the total flavonoids by 38.78% (Table 2). Among varieties, the highest total flavonoids were recorded in Bhima Raj. Sprouts harvest at 10 days had the highest total flavonoids content. There was significant (P≤0.01) interaction between variety and sprout age for total flavonoids. Enhancement of flavonoids in sprouts have been reported previously by Kim *et al.* (2007) in buckwheat. Onion is one of the richest sources of flavonols- quercetin, the average quercetin content in onion bulbs is 5-10 times higher than other vegetables (Lachman *et al*. 2003). Zujko *et al.* (2016) studied the flavonoid content of 10 species and the flavonoid content which ranged from 70 mg QE/ 100 g DW to 280 mg QE/100 g DW, in the present study the average flavonoid content of 10 days sprouts was 294.13 mg QE/100 g DW (Table 2).

Consumption of foods rich in flavonoids have several health benefits like- reduced atherosclerosis and cholesterol levels in the body. Thus, onion seed sprouts can be an important dietary source of flavonoids.

Germination enhances the phenol content in seeds due to the activation of endogenous enzymes and other complex biochemical systems (Dueñas *et al.* 2009). Sprouts had 189.03% higher total phenol content in comparison to the seeds (Table 2). Among varieties, highest total phenol was recorded in Bhima Red. Sprouts harvest at 15 days had the highest total phenol content. There was significant (P≤0.01) interaction between variety and sprout age for total phenol. The results are in agreement with Pająk *et al.* (2014) wherein germination enhanced phenols content in sprouts. Kaur *et al.* (2009) studied the phenol content in 10 onion cultivars which ranged from 42.95 GAE mg/100 g to 129.62 GAE mg/100 gram FW (fresh weight), whereas in the present study the average phenol content in 15 days sprouts was 284.44 GAE mg/100 DW, even if we assume 90% water content in the bulbs studied, the total phenol content of onion sprouts are higher compared to the onion bulbs. The methanolic extracts of the seeds and sprouts was assessed for total antioxidant capacity using ABTS and DPPH radicals. Sprouting enhanced the antioxidant activity, in comparison to the seeds, sprouts recorded 79.38% and 97.45% higher ABTS and DPPH activity respectively (Table 2). Among the varieties, highest ABTS activity was recorded in variety, Bhima Raj. Highest ABTS and DPPH activity was recorded in sprouts harvested at 15 and 10 days respectively. There

Table 2 Changes in total flavonoid, total phenols, antioxidants and sugars in seeds and sprouts of three onion varieties harvested at different intervals

Treatment	TF (QE mg/ 100g DW)	TP (GAE mg/100g DW)	ABTS (AEAC µmol/g DW)	DPPH (AEAC µmol/g DW)	Sucrose (mmol/g DW)	Fructose (mmol/g DW)	Reducing sugar (mg/g DW)	Total sugar (mg/g DW)
Variety (V)								
Bhima Red	233.80^{b}	228.04^{a}	1.44^{b}	4.35^{a}	1.33$^{a\wedge}$	2.20^{a}	79.79^{a}	183.11^{a}
Bhima Raj	259.80^{a}	210.19^{b}	1.58^{a}	4.39^{a}	1.19^{b}	1.94^{b}	72.69^{b}	134.45^{c}
Bhima Kiran	217.91^{c}	201.84^{c}	1.44^{b}	4.40^{a}	1.21^{b}	2.13^{a}	70.17^{b}	146.70^{b}
LSD (P≤0.05)	7.10**	4.46**	0.03**	NS	0.08**	0.09**	3.90**	6.03**
Days (D)								
0	211.94^{c}	98.41^{d}	0.97^{d}	2.75^{c}	1.34^{a}	2.30^{ab}	17.09^{d}	133.62^{c}
5	230.08^{b}	214.66^{c}	1.53^{c}	4.23^{b}	1.37^{a}	2.37^{a}	86.32^{b}	200.67^{a}
10	294.13^{a}	255.92^{b}	1.71^{b}	5.43^{a}	1.25^{b}	2.25^{b}	142.52^{a}	187.53^{b}
15	212.53^{c}	284.44^{a}	1.74^{a}	5.10^{a}	1.02^{c}	1.45^{c}	50.94^{c}	97.21^{d}
LSD (*P≤0.05*)	8.20**	5.15**	0.03**	0.10**	0.09**	0.10**	4.50**	6.97**
Interaction (V × D)								
LSD (P≤0.05)	14.20**	8.91**	0.06**	0.17**	0.15**	0.17**	7.79**	12.07**

** Significant at P≤0.01; Values with the same letters in a column are not significantly different at P≤0.05; TF: Total flavonoids; TP: Total phenols (GAE-gallic acid equivalent); ABTS: (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)); DPPH: (2, 2, 1-diphenyl-1-picrylhydrazyl), AEAC: Ascorbic acid equivalent; DW: Dry weight; NS: non-significant was significant (P≤ 0.01) interaction between variety and sprout age for ABTS and DPPH activity. Enhancement of antioxidant activity in sprouts is reported previously (Pająk *et al.* 2014). Guo *et al.* (2012) reported 3883% and 487% enhancement in ascorbic acid and total antioxidant capacity (TAC) respectively in mung bean sprouts.

Changes in sugars during seed germination: Glyoxylate cycle operates in germinating seed which converts stored lipids into metabolically more active intermediates by gluconeogenesis which are subsequently used in metabolism or converted into transportable sucrose for growing seedlings (Taylor 2020). In the present study, the sugars (sucrose, fructose, reducing sugars and total sugars) increased in 5 days and 10 days old sprouts and declined in 15 days old sprouts (Table 2). Among the varieties highest sugars was recorded in variety Bhima Red. There was significant (P ≤ 0.01) interaction between variety and sprout age for sugars. The developing sprouts contingent on the stored reserve, since onion seed being rich in lipid (26%) (Yalcin and Kavuncuoglu 2014), the sugar increase may be due to gluconeogenesis and utilized by the developing sprouts in the later stage. The results are also in agreement with previous studies by Silva *et al.* (2013) in soybean.

Sprouting of onion seeds enhanced the flavonoids, thiosufinates, phenols, pyruvic acid and antioxidant activity. Highest levels of health-promoting phytonutrients were observed in 10 and 15 days old sprouts. There was a considerable variation among the varieties for the phytonutrients, which may be due to different maternal environment during seed development and/or varietal difference. Large scale screening will help in identifying the varieties rich in health-promoting phytonutrients. Onion sprout has an excellent potential to replace spring onion and onion as a salad, or as a dressing agent and in other food preparation since it is a beneficial source of health-promoting foods.

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