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ORIGINAL ARTICLE

Quantification of flavonoids, total phenols and antioxidant properties of onion skin: a comparative study of fifteen Indian cultivars

Narashans Alok Sagar[1] · Sunil Pareek[1] · Gustavo A. Gonzalez-Aguilar[2]

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Abstract Onion waste disposal issue could be solved by using onion skin as food ingredient. Therefore, the aim of present study is the estimation of flavonoid concentration, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of onion skin of fifteen Indian cultivars. Flavonoid quantification was achieved by high performance liquid chromatography, which showed highest concentration of quercetin, quercetin 3-β-D-glucoside, luteolin and kaempferol in cv. 'NHRDF Red' (11,885.025 mg/kg), 'Hissar-2' (1432.875 mg/kg), 'Pusa Riddhi' (1669.925 mg/kg) and 'Bhima Shakti' (709.975 mg/kg), respectively in dry weight. Highest TPC and TFC were found in cv. 'NHRDF Red' while lowest were measured in cv. 'Bhima Shubhra'. DPPH assay (%), ABTS assay (%) and FRAP assay (μmol gallic acid/g) were showed maximum antioxidant capacity for cv. 'NHRDF Red' whereas least obtained for cv. 'Bhima Shubhra'. Skin of cv. 'Hissar-2' and 'NHRDF Red' are the best source of flavonoids and natural antioxidants.

Keywords Onion skin powder (OSP) · Total phenolic content · Flavonoids · Antioxidant activity

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✉ Sunil Pareek
sunil_ciah@yahoo.co.in

[1] Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Plot No. 97, Sector 56, HSIIDC Industrial Estate, Kundli, Sonepat, Haryana 131028, India

[2] Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera Gustavo Enrique Astiazarán Rosas No. 46, Col. La Victoria, 83304 Hermosillo, Sonora, Mexico

Introduction

Onion (*Allium cepa* L.) is the most important horticultural crop and cultivated worldwide. Globally, 3,92,536 tons of onion is produced from 24,08,498 ha land area. China is the main onion producer (2,20,813 hg/ha) followed by India (1,71,723 hg/ha) (FAO 2018), with production continuously increasing. Onion processing generates copious waste, mainly skin (Gawlik-Dziki et al. 2015). For example, the European Union (mainly UK, The Netherlands and Spain), annually discard 500,000 tons of onion waste, which has created an environmental issue in European Union countries (Gawlik-Dziki et al. 2015). Most skin waste comes from industries that perform peeling. This wasted skin is not suitable as fodder or fertilizers because of its peculiar aroma and growth of white rot (*Sclerotium cepivorum*) (Roldán et al. 2008).

Many investigations have explored onion skin as a rich source of fructooligosaccharides, dietary fibers, polyphenols and antioxidants (Downes et al. 2009; Sagar et al. 2018). Cultivar, environment, agronomic practices, maturation stage and storage duration affect the chemical composition of onion (Rodríguez et al. 2009). It has been shown that waste onion skin contains significantly higher flavonoid concentration as compared to the edible part (Duan et al. 2015). There are two main flavonoids subgroups found in onion; anthocyanins and quercetin and its derivatives, which contribute yellow to purple colour to onion skins (Benítez et al. 2011). Primary onion flavonoids are quercetin, quercetin diglucoside, quercetin aglycone, quercetin 4′-glucoside and kaempferol. Quercetin aglycone is found in higher amount in skin (Downes et al. 2009). Furthermore, onion skin has various health benefits in terms of anti-carcinogenic, hypocholesterolemic, and having anti-asthmatic effect (Moreno et al. 2006; Hassan et al.

2014). Quercetin is the key flavonoid behind all the health benefits because of its antioxidant activity (Bonaccorsi et al. 2008).

Recycling, re-using, bioconversion of waste and by-products utilization are keys to a sustainable environment. Hence, full characterization of biomass with proper understanding is important for its utilization in an effective way by obtaining '5-stage universal recovery process' which helps to utilised bioactive compounds in effective way (Galanakis 2015). There are many conventional and non-conventional processing techniques which can be utilized for better extraction of bioactive components (Galanakis and Schieber 2014). Additionally, several emerging technologies such as electro-osmotic dewatering, pulsed electric field, electro-osmotic dewatering, high voltage electrical discharge ultrasound-assisted extraction etc. are taken into consideration to recover bioactive materials from agricultural waste (Galanakis 2013). Stabilisation and processing of onion wastes could solve two problems: first, an environment solution related to the disposal of huge onion waste, second, utilisation of stabilised onion by-products as natural antioxidant-rich food materials (Roldán et al. 2008). Therefore, the present study was taken for flavonoid quantification, total phenols and antioxidant capacities of onion skin of fifteen Indian cultivars.

Materials and methods

Plant material

Fifteen popular, commercially available and process-able onion cultivars ('Agri Found Dark Red, ADR', 'Agri Found Light Red, ALR', 'Arka Kirtiman', 'Bhima Kiran', 'Bhima Shakti', 'Bhima Shubhra', 'Hissar-2', 'Hissar-3', 'NHRDF Red', 'Phursungi Local', 'Pusa Madhavi', 'Pusa Red', 'Pusa Riddhi', 'Sukh Sagar', 'Udaipur Local') were procured from All India Coordinated Research Project on Onion and Garlic, Division of Vegetable Sciences, Indian Agricultural Research Institute, New Delhi, India.

Chemicals and reagents

High performance liquid chromatography (HPLC)-grade methanol and trifluoroacetic acid were obtained from Thermo Fisher Scientific (Waltham, MA, US). All phenolic standards (quercetin, quercetin 3-β-D-glucoside, kaempferol, luteolin) with ≥ 97.0% purity were purchased from Sigma-Aldrich Ltd. (New Delhi, India). DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), Folin-Ciocalteu's (FC) reagent, ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), aluminium chloride and all other chemicals were purchased from Himedia Laboratories (Mumbai, India).

Sample preparation

Skin of the cultivars was collected (100 g each) after curing at open field (20 days). Skins were washed with chlorinated water (0.5%) for complete removal of dust and other impurities. Skins were washed again with distilled water and left open in a porous tray for 10 min to remove excess water, then kept in deep freeze (Vestfrost Solutions, Denmark) at − 40 °C for 24 h. Samples were freeze-dried using lyophilizer (Mini Lyodel, Delvac Pumps, Chennai, India) with plate temperature at − 50 °C at 0.039 mbar pressure and the drying process was continued up to 48 h. Freeze-dried skin was grounded using mixer-grinder (3053 Colt, Usha International Ltd, India) for the formation of onion skin powder (OSP) (see supplementary material Fig. S1) and stored at − 30 °C in air tight plastic containers for further use.

Extracts preparation

Extraction was carried out according to Jang et al. (2013) with minor changes. One g OSP of each cultivar was mixed separately with 25 mL of methanol (1:25, 25 times diluted) into beakers. All beakers with samples were left overnight at 5 °C temperature. All the samples were sonicated for 10 min using a sonicator (QSonica, Newtown, US) and vortexed for 5 min. This step was repeated two times. Centrifugation was carried out for 10 min on 5000*g* at 5 °C temperature using a refrigerated centrifuge (3-18KS, Sigma, Hossein Shakeri, Germany). This step was repeated 4 times and the supernatants were collected in capped tubes and stored at − 25 °C for further use.

Quantification of flavonoids

Flavonoid quantification was carried out in an HPLC (Prominence UFLC, Shimadzu, Kyoto, Japan) equipped with an auto-sampler and photodiode array (PDA) detector. A Zorbax Eclipse plus C18 column (reverse phase) (100 mm × 4.6 mm, 5 µm, Agilent, CA, US) was utilized to separate flavonoids. Calibration curves from the standards were used for quantification and the results are presented in mg/kg dry weight (dw) (Sharma et al. 2014). Optimized chromatographic parameters for the detection of flavonoids are presented in Table 1.

Total phenolic content

Total phenolic content (TPC) of OSP was measured by using method of Sun et al. (2007) with minor changes.

Table 1 Chromatographic conditions for HPLC to quantify the flavonoids

Chromatographic parameters			
Mobile phase	A (trifluoroacetic acid in water 0.1% (v/v)): B (methanol)		
Gradients (elution)	Time (min)	Solvent	Concentration (%)
	10	B	20
	20	B	80
	30	B	20
Injection volume	20 µL (25 times diluted)		
Flow rate	1 mL/min		
Run time	32 min		
Scanning	200–400 nm		
Oven temperature	40 °C		
Detection wavelength	365 nm		

Extracts (20 µL) of OSP were mixed with 0.5 mL FC reagent (10-fold) and 5 mL of distilled water was added to the test tubes. Test tubes were incubated for 10 min, then, 0.5 mL of sodium carbonate (2%) was poured into each test tube and incubated for 45 min. Absorbance was measured at 760 nm using UV–Vis Spectrophotometer (UV-2600, Shimazdu, Kyoto, Japan). Gallic acid was used as standard and the concentration was expressed as mg gallic acid equivalents (GAE/g dw).

Total flavonoid content

Total flavonoid content (TFC) was estimated as per the method of Benítez et al. (2011) with minor modifications. OSP extract was taken in quantity of 0.5 mL in test tube from different samples. Methanol (80%, 1.5 mL) was added into each test tube. Further, 0.1 mL aluminium chloride (10%) and 0.1 mL of potassium acetate (1 M) was poured. Distilled water (2.8 mL) was poured and incubated for 30 min at room temperature (30 °C). The absorbance was measured at 410 nm via UV–Vis Spectrophotometer (UV-2600, Shimazdu, Kyoto, Japan). Quercetin was used as standard and the results were expressed as mg quercetin/g dw.

Assay of antioxidant potential

DPPH scavenging assay

DPPH radical scavenging assay was performed as per the method of Duan et al. (2015) with minor changes. OSP extract in 2.0 mL quantity was mixed with 2.0 mL DPPH (0.4 mM) and shaken vigorously. Mixtures were kept in the dark for 1 h at room temperature. The absorbance was measured with UV–Vis Spectrophotometer (UV-2600, Shimazdu, Kyoto, Japan) at 517 nm. Gallic acid was used as standard. The percentage inhibition of DPPH activity was determined by using following formula:

$$\text{DPPH radical scavenging activity}\,(\%) = (A_c - A_s)/A_c \times 100$$

where A_c = reference/control absorbance, A_s = sample absorbance.

ABTS scavenging assay

The ABTS assay was determined from the method of Duan et al. (2015) with some modifications. Mixture containing 15 mL ABTS (7 mM) and 15 mL potassium persulfate (2.45 mM) was kept in the dark for 16 h at room temperature to get the green–blue free radical $ABTS^{\cdot+}$. The mixture was then diluted with ethanol to get 0.7–0.8 absorbance at 732 nm. Samples (0.1 mL) were mixed with ABTS (2.9 mL) working solution. After 10 min of reaction, absorbance was measured at 732 nm. Gallic acid was used as positive control. The calculation for the percentage of ABTS radical scavenging effect was as follow:

$$\text{ABTS scavenging activity}\,(\%) = (A_c - A_s)/A_c \times 100$$

where A_c = absorbance of reference/control, A_s = sample absorbance.

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was carried out as per the method of Benzie and Strain (1996) with some changes. The FRAP reagent was prepared by mixing 2.5 mL of TPTZ solution (10 mM/L) in hydrochloric acid (40 mM/L) and 25 mL of sodium acetate buffer (0.3 mol/L, 3.6 pH) mixed with 2.5 mL of $FeCl_3$ solution (20 mM). Freshly prepared FRAP solution (2.8 mL) was mixed with 0.2 mL of OSP extracts. Mixtures were incubated for 15 min at 35 °C in water bath and the absorbance was noted at 593 nm. Gallic acid was used as positive control and the FRAP values were expressed as µM gallic acid/g dw.

Statistical analysis

All analyses were carried out in triplicate and the results are presented as mean ± standard deviation on dw basis. One-way analysis of variance (ANOVA) with Duncan's test (post hoc) was applied for data analysis using IBM® SPSS statistics (version 20).

Results and discussion

Quantification of flavonoids

Quantification of quercetin 3-β-D-glucoside, quercetin, luteolin and kaempferol was carried out for sonication assisted OSP extracts (Fig. 1). There are many conventional and unconventional methods like solvent extraction, percolation, mecaration, ultrasound assisted extraction, microwave-assisted extraction, pulse electric field and high pressure with temperature are available which enhance extraction and quantification of flavonoid (Deng et al. 2015). However, innovative (unconventional) extraction technique like Pressurized hot water extraction is far better than other techniques for recovery of flavonoids from the samples (Kovačević et al. 2018). Additionally, half factorial design (two-level) can also be utilised for better extraction level of bioactive compounds (Wong et al. 2015). In the present study, the concentration of flavonoids ranged from 56.175 to 1432.875 mg/kg for quercetin 3-β-D-glucoside, 88.40 mg/kg to 11,885.025 mg/kg for quercetin, 45.475 mg/kg to 1669.925 mg/kg for luteolin and from 124.225 to 709.975 mg/kg for kaempferol. We determined that on dw basis, quercetin 3-β-D-glucoside, quercetin, luteolin and kaempferol were found at highest concentration in cv. 'Hissar-2'(1432.875 mg/kg), 'NHRDF Red'(11,885.025 mg/kg), 'Pusa Riddhi' (1669.925 mg/kg) and 'Bhima Shakti' (709.975 mg/kg), respectively. In contrast, lowest concentration was confirmed in cv. 'Bhima Shubhra' and 'Udaipur Local'. Highest cumulative concentration (mg/kg) of flavonoids was foundin cv. 'Hissar-2' followed by 'NHRDF Red', 'Pusa Riddhi', 'Sukh Sagar', 'ADR', 'Bhima Shakti' and 'Phursungi Local'. Quantified values of flavonoids are summarised in Supplementary material (Table S1).

OSP of white cultivars 'Bhima Shubhra' and 'Udaipur Local' had the lowest concentration of flavonoids, 315.875 mg/kg and 339.375 mg/kg, respectively. Previous papers report that white cultivars contain least or negligible quantities of flavonoids (Zhang et al. 2016). It is well explained that onion is a rich source of flavonoids as compared to other edible crops. Among 62 edible tropical plants, onions contained highest amount of quercetin (1497.5 mg/kg), kaempferol (832.0 mg/kg) and luteolin (391.0 mg/kg) in dw (Miean and Mohamed 2001). In a study of different skin-coloured onions, it was observed that red-skinned onions had higher concentration of quercetin (83,477 mg/kg), as compared to yellow-skinned onions (34,430 mg/kg) (Nuutila et al. 2003). This data is at par with most of the cultivars reported herein, because cv. 'NHRDF Red', 'Hissar-2' and 'Pusa Riddhi' had red-coloured skin, which were reported as having maximum concentration of quercetin. Moreover, Prakash et al. (2007) found quercetin and kaempferol in concentration of 5110 mg/kg dw and 481 mg/kg dw, respectively, in outer layers of red onions. Ko et al. (2011) analysed the flavonol concentration in onion skin and obtained 17,630 ± 0.87 mg/kg of quercetin and 880 ± 0.23 mg/kg of kaempferol. Total amount of quercetin and its derivatives were highest (66,330 mg/kg dw) in scaly leaf of red onions, while flavonoids were not detected in the inner layer of yellow and chartreuse coloured onions. The scaly leaf of commercial chartreuse onion had maximum concentration (32,810 ± 1.63 mg/kg) of quercetin (Kwak et al. 2017).

Total phenolic content

TPC of OSP ranged from 14.55 ± 0.41 mg GAE/g dw to 288.74 ± 1.27 mg GAE/g dw for cv. 'Bhima Shubhra' and 'NHRDF Red', respectively (Table 2). A significant difference ($P \leq 0.05$) was found between cv. 'ADR', 'Bhima Shubhra', 'Hissar-2', 'Phurgungi Local', 'Pusa Red', 'Pusa Riddhi', 'Sukh Sagar' and 'Udaipur Local', while no significant difference was obtained between cv. 'Arka Kirtiman' and 'Hissar-2', 'Hissar-3' and 'NHRDF Red', 'Bhima Shakti' and 'Pusa Madhavi', 'ALR' and 'Bhima Kiran' at ($P \leq 0.05$) level. In previous studies, large variation in TPC of red onion skin has been reported. An approximately similar TPC content (384.7 ± 5.0 mg GAE/g) was obtained in the extract of red onion skin (Singh et al. 2009). Another study revealed that TPC content of red onion skin was found between the ranges of 63.62 ± 2.03 to 208.42 ± 4.34 mg GAE/g, which varied according to solvent used to extract them. However, ethanolic extract had higher TPC content (Skerget et al. 2009). Generally, Ethanol and methanol are preferred for extraction of bioactive compounds for food industries as they do not possess toxic effects on food products and human as well. Study revealed that alcohol (ethanol or methanol) as solvents increase the level of extraction than other organic solvents (Galanakis et al. 2013). Extraction of total phenols in hydroethanolic mixture showed better recovery of total phenols (73.3 mg/g) at 40 °C for 8 h (Tsakona et al. 2012). Other report a reported significantly higher TPC (79.82 mg

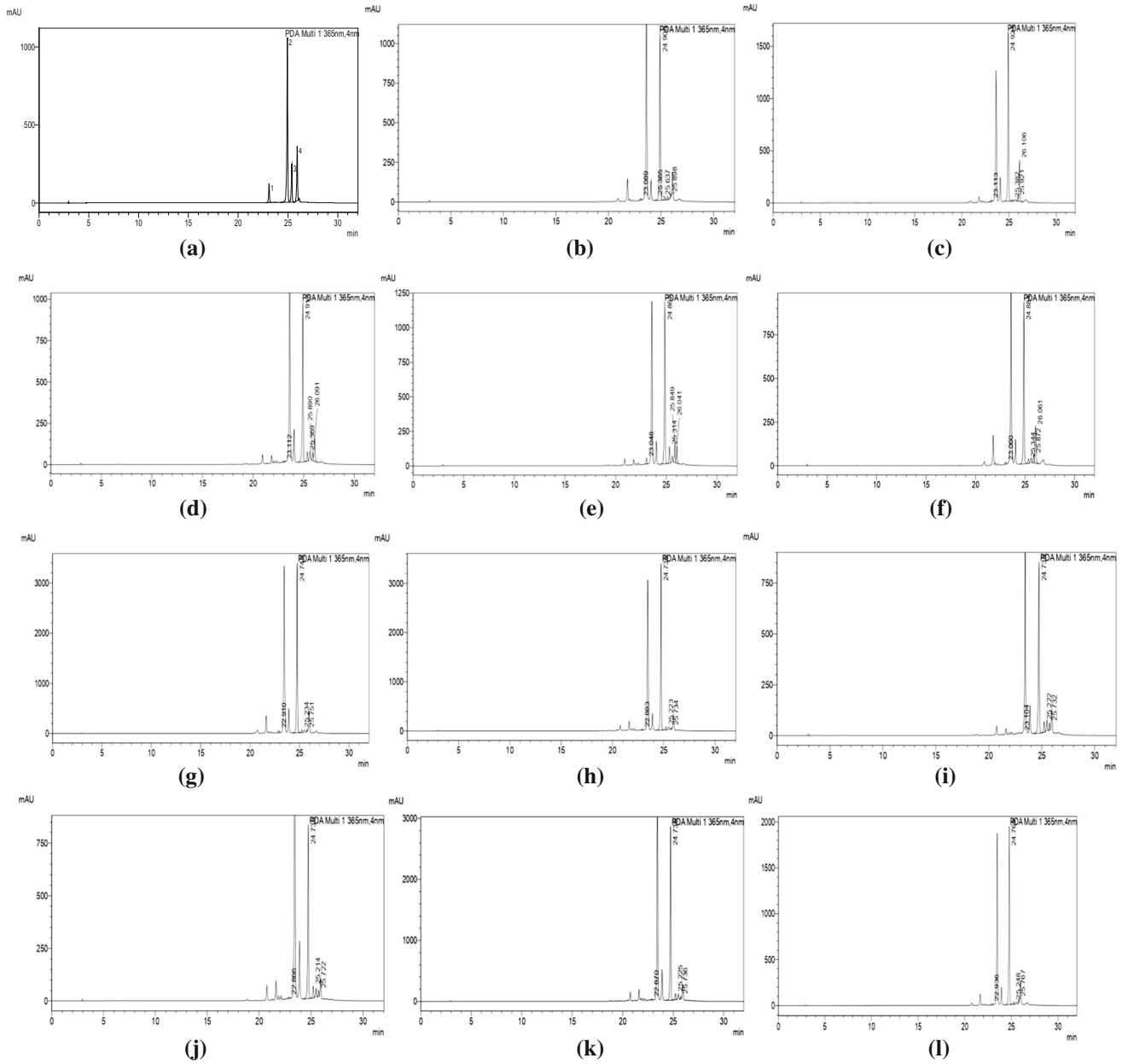

Fig. 1 HPLC chromatograms of flavonoids quantified in the extracts of onion skin powder: **a** Standard, **b** Arka Kirtiman, **c** Agri Found Dark Red, **d** Agri Found Light Red, **e** Bhima Shubhra, **f** Bhima Kiran, **g** Hissar-2, **h** NHRDF Red, **i** Pusa Madhavi, **j** Pusa Red, **k** Pusa Riddhi, **l** Sukh Sagar Where 1: Quercetin 3-β-D-glucoside, 2: Quercetin, 3: Luteolin and 4: Kaempferol

GAE/g) in red onion skin compared to its edible part (2.075 mg GAE/g) (Nuutila et al. 2003). A previous study on red and white skin of onion reported higher amount of TPC in red skin (74.1 mg GAE/g) compared to white (4.6 mg GAE/g) (Prakash et al. 2007). Moreover, Duan et al. (2015) obtained 113.56 ± 0.86 (mg GAE/g) concentration of TPC in the extract of onion peel. In addition to this, Sharma et al. (2014) also investigated onion bulb of 18 Korean cultivars and obtained concentration ranges between 2952.67 ± 105.40 to 5546 ± 52.68 in μg GAE/g DW for TPC. The polyphenols as natural antioxidants can replace synthetic antioxidants in food products and cosmetic industry. Galanakis et al. (2018a) compared olive wastewater polyphenols with α-tocopherol and ascorbic acid for antioxidant potential and found that phenols from wastewater showed higher antioxidant activity (as UV-filter) than α-tocopherol and ascorbic acid. The phenolic compounds recovered from agricultural waste possess various health benefits and can be utilised as natural food additives in the food products (Galanakis 2018b).

Table 2 Total phenolic and total flavonoids content in extracts of onion skin powder of fifteen cultivars

Cultivar	Total phenolic content (mg GAE/g dw)	Total flavonoid content (mg QE/g dw)
Agri Found Dark Red	265.10 ± 0.81^{i}	92.31 ± 1.93^{h}
Agri Found Light Red	241.12 ± 1.34^{d}	83.67 ± 0.37^{e}
Arka Kirtiman	262.69 ± 0.69^{h}	96.46 ± 1.38^{i}
Bhima Kiran	239.91 ± 0.64^{d}	81.48 ± 0.58^{d}
Bhima Shakti	243.68 ± 1.25^{e}	75.36 ± 1.02^{c}
Bhima Shubhra	14.55 ± 0.41^{a}	1.31 ± 0.32^{a}
Hissar-2	262.78 ± 1.52^{h}	98.09 ± 0.94^{j}
Hissar-3	288.74 ± 1.27^{k}	160.18 ± 0.84^{l}
NHRDF Red	289.04 ± 1.31^{k}	168.77 ± 0.87^{m}
Phursungi Local	231.73 ± 1.23^{c}	66.58 ± 0.55^{b}
Pusa Madhavi	245.58 ± 1.04^{e}	66.88 ± 0.24^{b}
Pusa Red	251.71 ± 1.21^{f}	89.62 ± 0.70^{g}
Pusa Riddhi	269.74 ± 1.35^{j}	150.94 ± 0.17^{k}
Sukh Sagar	254.41 ± 1.96^{g}	88.11 ± 0.19^{f}
Udaipur Local	19.74 ± 0.45^{b}	2.01 ± 0.02^{a}

Values are mean ± standard deviation (n = 3). Values with different superscripts $^{a–m}$ in same column are significantly different ($P < 0.05$) by Duncan's multiple range test

Total flavonoid content

TFC of extracts of OSP is presented in Table 2. The highest and lowest TFC was measured in cv. 'NHRDF Red' (168.77 ± 0.87 mg QE/g dw) and 'Bhima Shubhra' (1.31 ± 0.32 mg QE/g dw), respectively. All cultivars were significantly different ($P \leq 0.05$), except 'Bhima Shubhra', 'Udaipur Local', 'Phursungi Local' and 'Pusa Madhavi'. According to our data, coloured cultivars had maximum concentration of TFC, as compared to white cultivars. Same pattern of higher TFC was reported in red cultivar (111.10 ± 5.98), and lowest in white cultivar in FW, according to a study of three coloured onions (Zhang et al. 2016).

Another study revealed that skin of red onion had highest (20.22 ± 0.39) and yellow skin contained lowest (10.69 ± 0.40) content of total flavonoid in mg QE/g dw (Albishi et al. 2013). Moreover, Singh et al. (2009) confirmed that red onion skin possessed higher amount (165.20 ± 3.2) of TFC. In addition to this, skin and bulb of red onion was analysed, reporting a higher concentration of TFC in skin (163.14 ± 1.62 mg QE/g) than in bulb (13.76 ± 0.41 mg QE/g) (Skerget et al. 2009). Duan et al. (2015) also investigated onion peel and reported highest TFC value (49.69 ± 0.55 mg QE/g).

Apart from this, study of onion bulbs of six different cultivars showed TFC in range of 7.48 ± 4.16 to 9.92 ± 3.56 mg/100 g FW (Rodríguez et al. 2008). Onion bulbs of 18 Korean cultivars were reported having TFC value of 1.85 to 2.13 mg QE/g FW (Sharma et al. 2014). As per the literature and present study, it is clear that skin of onion contained higher amount of TFC than bulb. The antioxidant property of phenolics and flavonoids provides safety to the body from the diseases as well as protect from sunlight (UV-rays). Therefore, phenols and flavonols from agri-waste are the best replacement for synthetic chemicals as UV protectors in the cosmetic industry (Galanakis et al. 2018c). Study also suggested that polyphenols prevent oxidation of oils. Polyphenols concentration i.e. 500 mg/L and 1000 mg/L showed best result when mixed into different oils (refined and extra virgin olive oil) against oxidation. It revealed that polyphenols can be added in vegetable oils as natural preservative (Galanakis 2018).

Assay of antioxidant potential

The antioxidant potential of the extracts of OSP was studied through synthetic radicals (DPPH and ABTS), and FRAP assay. Values for antioxidant potential of fifteen Indian cultivars are given in Table 3.

DPPH scavenging assay revealed a range of 5.71 ± 0.47% to 94.17 ± 0.20% from lowest to highest activity of OSP of fifteen cultivars. It was found that cv. 'NHRDF Red' had maximum DPPH scavenging capacity and 'Bhima Shubhra' (white cultivar) possessed lowest scavenging activity among studied cultivars. A significant difference ($P \leq 0.05$) was observed in all fifteen cultivars. Dark colour of onion skin is responsible for higher antioxidant activity than light one. Prakash et al. (2007) also reported the same pattern of antioxidant activity (AOA) in outer skin of onion. Red onion skin showed highest AOA (84.1%), violet showed medium (73.9%)

Table 3 Antioxidant activity assay (DPPH, ABTS and FRAP) in extracts of onion skin powder of fifteen cultivars

Cultivar	DPPH scavenging activity (%)	ABTS antioxidant activity (%)	FRAP assay (μmol gallic acid/g)
Agri Found Dark Red	79.10 ± 0.14^{l}	82.15 ± 0.37^{l}	30.95 ± 0.18^{j}
Agri Found Light Red	59.33 ± 0.49^{e}	63.10 ± 0.67^{e}	14.98 ± 0.12^{d}
Arka Kirtiman	76.74 ± 0.47^{k}	79.10 ± 0.94^{k}	28.06 ± 0.81^{i}
Bhima Kiran	57.88 ± 0.30^{d}	60.86 ± 0.35^{d}	12.20 ± 0.32^{c}
Bhima Shakti	63.35 ± 0.47^{f}	66.80 ± 0.66^{f}	17.96 ± 0.35^{e}
Bhima Shubhra	5.71 ± 0.47^{a}	6.97 ± 0.08^{a}	0.12 ± 0.03^{a}
Hissar-2	75.65 ± 0.78^{j}	78.07 ± 0.77^{j}	27.26 ± 0.39^{h}
Hissar-3	89.99 ± 0.24^{n}	91.91 ± 0.29^{n}	34.82 ± 0.24^{l}
NHRDF Red	94.17 ± 0.20^{o}	96.04 ± 0.17^{o}	37.12 ± 0.49^{m}
Phursungi Local	55.45 ± 0.38^{c}	58.79 ± 0.35^{c}	10.06 ± 0.43^{b}
Pusa Madhavi	66.79 ± 0.54^{g}	69.66 ± 0.44^{g}	20.92 ± 0.37^{f}
Pusa Red	69.97 ± 0.12^{h}	73.18 ± 0.33^{h}	23.26 ± 0.57^{g}
Pusa Riddhi	81.81 ± 0.42^{m}	84.61 ± 0.42^{m}	32.63 ± 0.44^{k}
Sukh Sagar	73.07 ± 0.10^{i}	74.21 ± 0.65^{i}	23.19 ± 0.26^{g}
Udaipur Local	9.79 ± 0.41^{b}	10.89 ± 0.12^{b}	0.73 ± 0.23^{a}

Values are mean ± standard deviation (n = 3). Values with different superscripts $^{a–o}$ in same column are significantly different ($P < 0.05$) by Duncan's multiple range test

while white skin had lowest AOA (23.40%). Moreover, red-skinned and yellow-skinned onions were investigated for DPPH radical activity, concluding that red skin had higher activity (74.7%), as compared to yellow skin (40.8%) (Nuutila et al. 2003). Presence of flavonoids is a key factor that affects the level of antioxidant activity, where quercetin plays a main role in it. In a previous study, red onion peel was analysed for DPPH assay. It showed 75.3 ± 4.5% antiradical power for DPPH assay (Singh et al. 2009). Another study was carried out for red onion peel and 47.17 ± 1.85% DPPH activity was recorded (Skerget et al. 2009). Likewise, a study showed DPPH scavenging activity in higher amount 93.45 ± 0.42% for onion peel (Duan et al. 2015).

$ABTS^{\cdot+}$ (radical-cation) is formed after the oxidation of ABTS. ABTS remains stable in the absence of antioxidants but turns activated when reacts with phenolics (H^{+}atom donor). Hence, blue/green colour gets gradually converted into uncoloured form of ABTS according to antioxidant capacity of phenolics. Inhibitory effect values of OSP extracts on ABTS radical is given in Table 3. The range of ABTS radical scavenging test was found between 6.97 ± 0.08% and 96.04 ± 0.17%, which shows that cv. 'Bhima Shubhra' had the lowest inhibitory effect on ABTS, whereas 'NHRDF Red' had highest. Analysis of variance showed that all the cultivars were significantly different ($P \leq 0.05$) from each other. A previous study was carried out for ABTS assay with different onion cultivars and reported that dark coloured onion had highest percentage of ABTS inhibition at 95.8 ± 0.3%, as compared to light coloured onion (72.7 ± 0.72%) (Manohar et al. 2017). Analysis of onion peel revealed that it had 59.08% ABTS scavenging activity (Duan et al. 2015). Moreover, Burri et al. (2017) measured a maximum ABTS inhibition by the skin of 'Donna' cultivar (dark) (63.4 ± 8.3%) and minimum by skin of cv. 'Hy Park' (43.4 ± 10.8%) (light).

FRAP assay is another important test to determine antioxidant activity of plant materials. The basis of this assay is the capacity of the antioxidants to reduce Fe^{3+} ions into Fe^{2+} in the presence of TPTZ, which produces a blue-coloured Fe^{2+}-TPTZ complex. The values of FRAP assay are summarised in Table 3. Highest value of FRAP test was observed in cv. 'NHRDF Red' (37.12 ± 0.49 μmol gallic acid/g) while lowest was found in 'Bhima Shubhra' (0.12 ± 0.03 μmol gallic acid/g) which is a white cultivar. Same pattern of test was detected in the skin of two varieties of onion, i.e. 'Donna' contained highest (43.6 ± 4.4), whereas 'Barito' had lowest (28.2 ± 1.8) value for FRAP assay (Burri et al. 2017). In another study, six onion cultivars were investigated for FRAP test, showing that red-coloured onion had higher FRAP value (33.18 ± 0.43 μmol TE/g dw), as compared to the white cultivar (12.58 ± 1.14 μmol TE/g dw) (Sharma et al. 2015). Moreover, Sharma et al. (2014) analysed 18 Korean onion cultivars and range of FRAP assay was obtained between 11.59 ± 0.99 and 27.22 ± 1.34 μmol TE/g dw. Previous investigations and present study showed that dark coloured onion skin showed higher antioxidant activities, while light or white coloured onion skin had lower antioxidant capacity.

Correlation analysis

The result of correlation analysis between TPC, TFC and antioxidant potential of OSP of fifteen cultivars is given in Fig. 2. According to the analysis, an excellent correlation was found between TPC and DPPH scavenging activity ($r^2 = 0.93$) while a positive correlation was obtained between TFC and DPPH activity ($r^2 = 0.84$) at $P > 0.05$. Similarly, a higher correlation was observed between TPC and ABTS activity ($r^2 = 0.94$) and a good correlation was found between TFC and ABTS activity ($r^2 = 0.84$). A better correlation was observed between TFC and FRAP

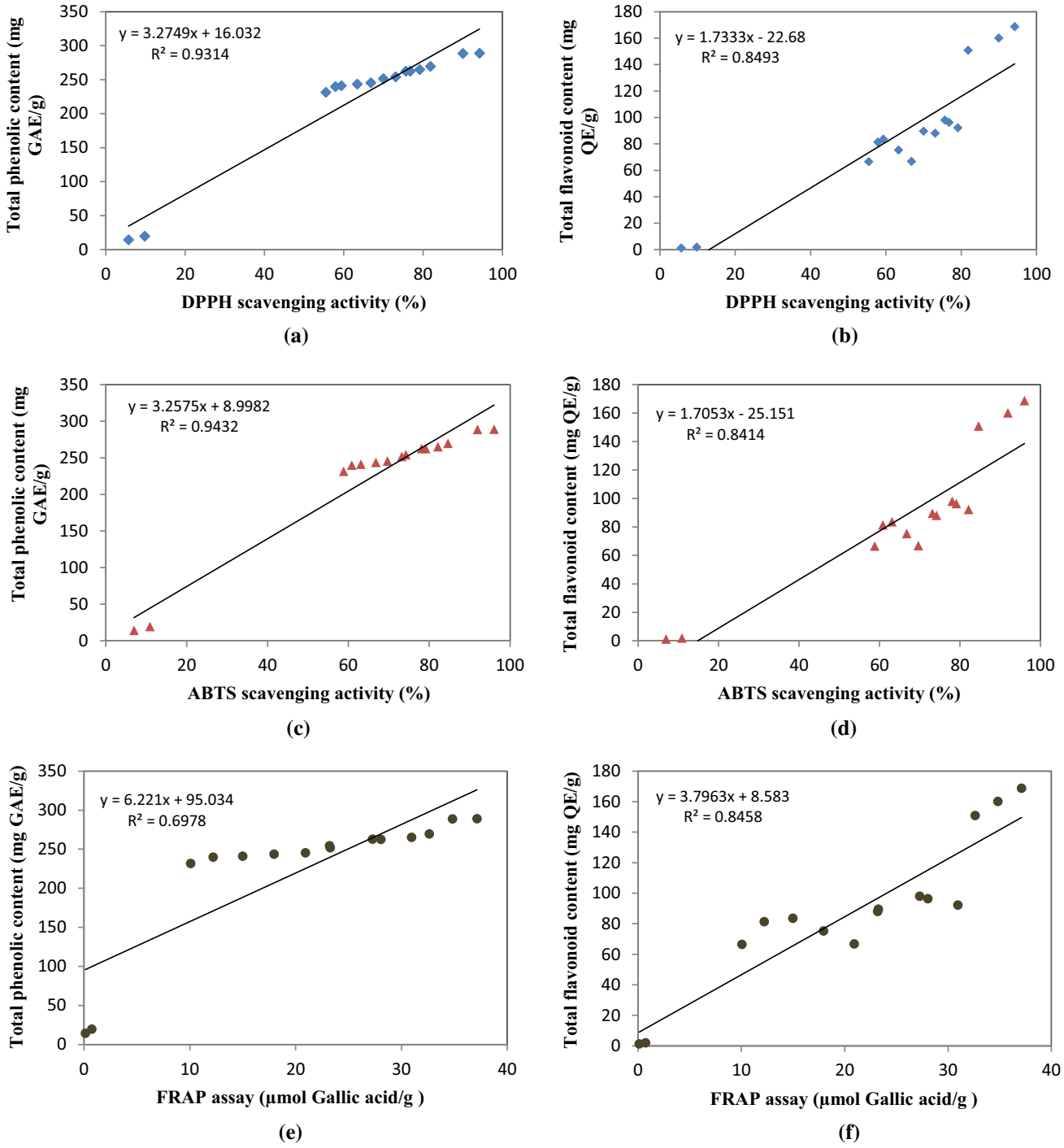

Fig. 2 **a** Correlation between TPC and DPPH assay (%). **b** Correlation between TFC and DPPH assay (%). **c** Correlation between TPC and ABTS scavenging activity (%). **d** Correlation between TFC and ABTS scavenging test (%). **e** Correlation between TPC and FRAP assay. **f** Correlation between TFC and FRAP assay

assay (r^2 = 0.84), as compared to correlation between TPC and FRAP assay (r^2 = 0.69).

The correlation between TPC, TFC and antioxidant activities showed that cultivars having higher flavonoids (polyphenols) exert higher antioxidant potential. Present study agrees with previous investigation that reported very good correlation between total phenols, total flavonoid content and antioxidant activities (Manohar et al. 2017). This study revealed that onion skin had higher capacity of antioxidant activities than bulb. Therefore, dark coloured onion skin can be utilized in food and nutraceuticals industries for product enrichment as a good source of natural antioxidants.

Conclusion

Outer skins of fifteen onion cultivars were utilized to make onion skin powder (OSP). Quantification of flavonoids, TPC and TFC were carried out after the extraction of OSP. Apart from this, antioxidant capacity of OSP extracts were also investigated in terms of DPPH assay, ABTS assay and FRAP test. As per the present study, maximum flavonoid concentration was estimated in cv. 'Hissar-2' and lowest in 'Bhima Shubhra'. Quercetin was recorded as the chief flavonol among all flavonoids. TPC and TFC were highest in cv. 'NHRDF Red' followed by 'Hissar-3', 'Pusa Riddhi' and 'ADR', whereas the were lowest in 'Bhima Shubhra' and 'Udaipur Local' cultivars. Antioxidant activities by DPPH assay, ABTS assay and FRAP assay showed maximum in cv. 'NHRDF Red'. The present investigation determined that cv. 'Hissar-2' and 'NHRDF Red' contained higher flavonoid concentration and over all antioxidant capacity, as compared to other cultivars. Therefore, the outer skin of these cultivars may be used in food fortification or nutraceutical preparations for better health products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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