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Effect of Storage Temperature on Vitamin C, Total Phenolics, UPLC Phenolic Acid Profile and Antioxidant Capacity of Eleven Potato (*Solanum tuberosum*) Varieties

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A B S T R A C T

Storage of potato tubers at low temperature affects their metabolism and may alter their phytochemical properties. There is a need to elucidate the changes in antioxidant compounds, activity and enzymes during storage of tubers. Eleven Indian potato varieties were evaluated for antioxidant parameters, after 0, 30, 60 and 90 days of storage at room temperature, 15 °C and 4 °C. Total phenolics (0.0786–0.1546 mg gallic acid equivalents·g^{-1} FW) and vitamin C content (0.0828–0.2416 mg·g^{-1} FW) varied among the varieties and were different with storage temperature; their levels fluctuated during storage but remained above the initial level until the last day of observation. Phenolic acid profiling by UPLC identified 12 compounds among which the most abundant was chlorogenic acid followed by gallic acid, sinapic acid and ellagic acid. Except para-coumaric acid which decreased at 4 °C, all the phenolic acids increased with storage. Caffeic acid, chlorogenic acid, protocatechuic acid and gallic acid mostly correlated with total phenolic content (r = 0.456, 0.482, 0.588 and 0.620, respectively). Antioxidant activity against both DPPH and ABTS radicals increased during the initial days of storage and then dropped to a level comparable or lower than the original value, irrespective of the storage temperature. Correlation study revealed that chlorogenic acid, gallic acid and ferulic acid mostly contributed to antioxidant activity. Activity of both antioxidant enzymes, superoxide dismutase and ascorbate peroxidase, increased initially but then decreased to values lower than the initial level and were not influenced by storage temperature. Correlation with antioxidant activity indicated that the enhancement of reactive oxygen scavenging species in cold stored tubers could result mainly from ascorbate peroxidase activity. Our results demonstrate that storage temperature adversely influences the metabolism and the content of antioxidant compounds in potato tubers, with subsequent increase on their antioxidant capacity.

Keywords: potato; cold storage; UPLC; phenolics; vitamin C; antioxidant

1. Introduction

The cultivated potato (*Solanum tuberosum* L.) is not only the third most important food crop and the most important non-grain food in the world, but also one of the most essential basic vegetables worldwide as well as in Indian subcontinent. Potato makes up a considerable proportion of the Indian diet (Kumar et al., 2013). India ranks third in terms of area and second in production of potato in the world, next to China. The majority of potato crop in India is harvested during February–March and that coincides with a steep rise in temperature in the Indo-Ganges plains which account for 87% of the production in the country. Consequently, potatoes are stored in cold storage to provide year-round supply to markets and consumers (Marwaha et al., 2010). However, when subjected

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to chilling temperatures (usually <10 °C) dormant potato tubers stored at cold temperature are still metabolically active especially for slow starch degradation and production of sucrose with subsequent accumulation of reducing sugars glucose and fructose (Burton, 1969) in a phenomenon known as cold-induced sweetening.

Many natural phytochemicals, such as phenolic compounds, anthocyanins, carotenoids and vitamins are related to antimicrobial, antimutagenic and antioxidant activities. Since high levels of antioxidants are naturally found in fruits and vegetables, there is considerable and growing interest in potential health benefits from consumption of antioxidants-rich vegetables and fruits (Külen et al., 2013). Significant antioxidant activity of potato tuber was extensively reported (Lachman et al., 2000; Reddivari et al., 2007) and potato tubers exhibited wide variation in their antioxidant activities (Hale, 2003; Campos et al., 2006; Navarre et al., 2011; Jang and Yoon, 2012). Various factors such as cultivation method, genetic differences, growing conditions, industrial processing and post-harvest storage conditions may affect the total phenolic content, Vitamin C content and antioxidant activity in plants (Lewis et al., 1998; Dale et al., 2003; Stushnoff et al., 2008; Blessington et al., 2010).

Potato tubers contain high amounts of antioxidant compounds, among which phenolics contribute to 58%–82% of the total antioxidant activity (Reddivari et al., 2007). Various reports mentioned a wide range of total phenolic content of potato depending on cultivars and growing conditions (Navarre et al., 2011; Jang and Yoon, 2012; Külen et al., 2013) including Indian cultivars (Singh et al., 2009; Kumar, 2011; Kumar et al., 2013). Diverse changes in the phenolic content and antioxidant activity of potatoes during low-temperature storage have been reported (Mqndy et al., 1966; Stushnoff et al., 2008; Blessington et al., 2010; Madiwale et al., 2011; Külen et al., 2013).

Phenolic acids and flavonoids are the most prominent polyphenols present in potato. Chlorogenic acid and caffeic acid are the two most prominent phenolic acids reported in potato, followed by protocatechuic acid, *trans*-cinnamic acid, para-coumaric acid, ferulic acid, vanillic acid, gallic acid, syringic acid, sinapic acid and salicylic acid (Lewis et al., 1998; Shakya and Navarre, 2006; Reddivari et al., 2007; Navarre et al., 2011). An increase in the chlorogenic acid, caffeic acid and sinapic acid contents was observed during storage of tubers for 90 days at 3 °C (Madiwale et al., 2011). But still the changes of other phenolic acids during cold storage of potato tubers remain unknown. Besides, the contribution of each phenolic acid to the antioxidant potential of potato tubers is unclear.

The most abundant vitamin in potato tubers, vitamin C, is important in the human diet as a nutrient substance and is also an effective potent antioxidant against oxidative stress (Liso et al., 1988; Navas and Gomezdiaz, 1995). However, the significant amount of vitamin C found in potato at harvest decreases during storage at low temperatures (Dale et al., 2003; Burgos et al., 2009; Abong et al., 2011; Cho et al., 2013; Külen et al., 2013). Although considerable amount of vitamin C was found in 10 prevalent Indian potato varieties at harvest (Kumar et al., 2013), there is no information on their changes during storage of tuber at low temperature.

Concerning the changes in antioxidant enzymes, an increase in catalase, superoxide dismutase (SOD) and ascorbate peroxidase (APX) was correlated with antioxidant activity during potato tuber aging or during sprouting (Bajji et al., 2007; Delaplace et al., 2009; Afify et al., 2012). However, little is known of their behavior during cold storage as well as the consequence of the changes in these enzyme activities on level of antioxidant compounds and antioxidant activity of potato tubers (Mizuno et al., 1998). It appears that effects of low-temperature storage on the antioxidant potential of potato tubers have not yet been fully explained (Külen et al., 2013). Consequently, there is a need to elucidate the changes in antioxidant activity, antioxidant compounds, as well as antioxidant enzymes during cold storage of tubers. Additionally, there is less information available on these parameters in Indian potato cultivars; hence the present work was planned.

2. Materials and methods

2.1. Plant materials

Tubers of 11 potato (*S. tuberosum* L.) Indian cultivated varieties were obtained from Main Vegetable Research Station, Anand Agricultural University, Anand. They included 'DSP 287', 'DSP 186', 'Kufri Surya', 'Kufri Chipsona-3', 'Kufri Sutlej', 'Kufri Sadabahar', 'Kufri Jyoti', 'Kufri Lauvkar', 'Kufri Himsona', 'Kufri Bahar' and 'Kufri Badshah'. They are yellow- and white-fleshed potato varieties, used for fresh market or potato industry. The plants were grown from November 2013 to February 2014 according to the agronomy package recommended by the research station, and after harvest, healthy and uniform potato tubers of the 11 cultivars were collected. The tubers were cured during 15 days at room temperature to allow starch sugar conversion and wound healing or suberization of skin lesions. The tubers were then separately packed in small plastic net bags and stored in different conditions i.e., (i) at room temperature (25–32 °C), (ii) in the incubator (15 °C) and (iii) in cold storage (4 °C). Samples were collected for analysis at 0, 30, 60 and 90 days of storage (DOS). However, samples at room temperature were collected up to 60 days only, because of decaying. For each sampling period, 3 tubers of each variety were randomly taken and thoroughly washed with tap water. The peel was removed and immediately the flesh was cut into small dices of approximately 0.5 cm^3 and pooled. The pooled sample was powdered with liquid nitrogen using a mortar and pestle.

2.2. Chemicals

For UPLC profiling, HPLC grade phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, USA) except protocatechuic acid which was procured from HWI Analytik GmbH (Ruelzheim, Germany). Solvents for UPLC (HPLC grade and LC–MS grade methanol) were obtained from Merck Specialties (Mumbai, India). Solvents for extraction and reagents for colorimetric tests of analytical grade were procured from Himedia Laboratories (Mumbai, India).

2.3. Vitamin C content

Vitamin C was extracted from potato powder using the protocol described by Külen et al. (2013) and estimated using the modified 2,4-dinitrophenylhydrazine (DNP) method as reported by Cho et al. (2013). The vitamin C content was expressed in $mg \cdot g^{-1}$ FW.

2.4. Total phenolic content

One gram of potato powder was extracted in 20 mL of 80% (v/v) methanol at 40 °C for 30 min and centrifuged at 5 000 × *g* for 15 min. The extraction volume was collected and evaporated to dryness in water bath at 80 °C. Phenolic compounds were dissolved in 5 mL of 50% (v/v) methanol and stored at –20 °C until analysis. Total phenolic content of the methanol extracts was determined according to a modified Folin–Ciocalteu colorimetric method (Singleton et al., 1999). The sample solution (0.5 mL) was allowed to react with 3 mL of freshly diluted 10-fold Folin–Ciocalteu reagent, followed by the addition of 3 mL of sodium carbonate solution (60 g·L^{-1}), and mixed by vortexing. After 90 min at room temperature, the absorbance reading was recorded at 725 nm against a blank of 50% (v/v) methanol solution in place of phenolic extract. The standard curve was prepared using 20–100 mg·L^{-1} solutions of gallic acid in 50% (v/v) methanol. Total phenolics values were expressed as milligrams of gallic acid equivalent (GEA) per g of fresh weight (mg GAE·g^{-1} FW).

2.5. Ultraperformance Liquid Chromatography (UPLC) profiling of phenolic acids

A Waters Acquity UPLC™ H Class System (Waters Corp., Milford, MA) equipped with a Quaternary Solvent Manager and a Sample Manager FTN was coupled to a UV Photodiode array detector (PDA). Analyses were performed on a bridged ethylene hybrid (BEH) C18 analytical column (1.7 µm, 2.1 mm × 50 mm, Waters Corp., Milford, MA). The column and the auto sampler were held at 50 °C and 5 °C, respectively. Potato methanol extracts were filtered through a 0.2 µm Nylon membrane (EMD Millipore, Billerica, USA) then 2 µL were injected and the elution was completed in 8 min with a sequence of linear gradients and isocratic flow rate of 0.45 mL · min^{-1}. The mobile phase consisted of solvent A (100% water + 0.1% formic acid) and solvent B (95% methanol, 5% water, 0.1% formic acid). The program began with isocratic elution with 100% A, which was held for 0.5 min, followed by a 4.5 min linear gradient to 100% B, followed by a 1 min hold at 100% B. The column was returned to starting conditions over 0.2 min, and allowed to reequilibrate for 1.8 min. The PDA spectra was measured over the wavelength range of 200–800 nm and phenolic acids were detected at 280 nm in steps of 2 nm, with a data acquisition rate of 20 points·s^{-1}. Empower 3 software was used for chromatographic data gathering and in order to correct shifts in retention times, identification and confirmation of phenolic compounds were based on comparing retention times with standards and also ultraviolet absorption spectrum data. A stock solution of each standard i.e. gallic acid (Gal), protocatechuic acid (Pro), chlorogenic acid (Chl), caffeic acid (Caf), vanillic acid (Van), syringic acid (Syr), para-coumaric acid (*p*-Cou), ferulic acid (Fer), sinapic acid (Sin), salicylic acid (Sal), ellagic acid (Ell) and *trans*-cinnamic (Cin) was prepared by dissolving 10 mg standard into 1 mL of 50% (v/v) methanol. Calibration curves for the standards were obtained at concentrations ranging from 10 to 100 µg·g^{-1}. The content of each phenolic acid was calculated against its standard curve and expressed in µg·g^{-1} FW.

2.6. Antioxidant activity

The antioxidant activity of methanol extract was evaluated using a modified 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay and a modified 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard. For DPPH-antioxidant activity, 0.15 mL of the methanol extract was reacted with 2.85 mL of 0.1 mmol·L^{-1} DPPH solution prepared in 50% (v/v) methanol for 30 min and the developed yellow color was recorded at 517 nm. Antioxidant activity was calculated using a Trolox standard curve ranging from 5 to 25 µg Trolox and expressed as mg of Trolox equivalents per g of fresh potato sample (mg TE·g^{-1} FW). For ABTS-antioxidant activity, ABTS radical cation ($ABTS^{+}$) was produced by reacting volume to volume 7 mmol·L^{-1} ABTS solution with 2.45 mmol·L^{-1} ammonium persulfate solution, and the mixture was allowed to stand in dark at room temperature for 16 h before use. The obtained ABTS solution was diluted with 50% (v/v) methanol until an absorbance of (0.7 ± 0.02) at 734 nm was obtained. For sample analysis 2.85 mL of diluted ABTS solution was added to 0.15 mL of methanol extract. The resulting solution was mixed thoroughly and allowed to react for 30 min, and then absorbance was recorded at 734 nm. Trolox standard curve was constructed and the antioxidant activity of the samples was expressed as milligrams of Trolox equivalents per g of fresh potato (mg TE·g^{-1} FW).

2.7. Extraction of antioxidant enzymes

For antioxidant enzyme activities, 1 g of potato tuber powder was homogenized in 1.5 mL extraction buffer containing 50 mmol·L^{-1} sodium phosphate buffer (pH 7.6), 10 mmol·L^{-1} sodium metabisulfite, 1 mmol·L^{-1} ascorbic acid, 1 mmol·L^{-1} ethylene diamine tetraacetic acid (EDTA), 20% (w/v) sorbitol, and 2% (w/v) polyvinyl polypyrrolidone (PVP), and centrifuged at 12 000 × *g* for 20 min at 4 °C (Bajji et al., 2007). The supernatant was collected and the protein content was determined according to Lowry et al. (1951). Enzymes activities were measured immediately in fresh extracts.

2.8. Superoxide dismutase activity

The activity of SOD was measured based on the inhibition of nitroblue tetrazolium photoreduction in the presence of riboflavin (Dhindsa et al., 1981). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of nitroblue tetrazolium in 1 min in the presence of riboflavin and light, as monitored at 560 nm and was expressed as specific activity in units per mg protein (U·mg^{-1} protein).

2.9. Ascorbate peroxidase activity

The activity was measured based on the decrease of the absorbance at 290 nm of ascorbate in the presence of H_2O_2 and APX enzyme according to Nakano and Asada (1981). The enzyme was assayed in 3 mL reaction mixture containing 50 mmol·L^{-1} sodium phosphate buffer (pH 7.0), 0.1 mmol·L^{-1} H_2O_2, 0.5 mmol·L^{-1} ascorbic acid, 0.1 mmol·L^{-1} EDTA and 0.1 mL enzyme extract. The hydrogen peroxide dependent

oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($\varepsilon = 2.8$ mmol·L^{-1}·cm^{-1}) every 30 s during 2 min. Blank was carried out without substrate (H_2O_2). Unit activity (U) of APX was defined as ΔOD·min^{-1}·g^{-1} FW and the specific activity was expressed as U·mg^{-1} protein.

2.10. *Statistical analysis*

From the powder obtained with pooled samples, analyses were performed in 3 replications. Data obtained were analyzed using a completed randomized design in factorial arrangement, and Analysis of Variance (ANOVA) was carried out at 1% and 5% levels of significance to determine the significance of difference between the storage temperatures for each of the parameters. The differences were considered as significant if $P < 0.05$, and highly significant if $P < 0.01$. Pearson's correlation at 1% and 5% levels of significance was also performed between various parameters. The software SPSS Statistics 17.0.3 was used.

3. Results

3.1. *Vitamin C content*

The Vitamin C content at initial day of observation varied from 0.0828 mg·g^{-1} FW (K. Sadabahar) to 0.2416 mg·g^{-1} FW (K. Lauvkar). During storage, there was a highly significant difference in changes of Vitamin C content between room temperature and incubator, as well as between incubator and cold storage, but the difference between room temperature

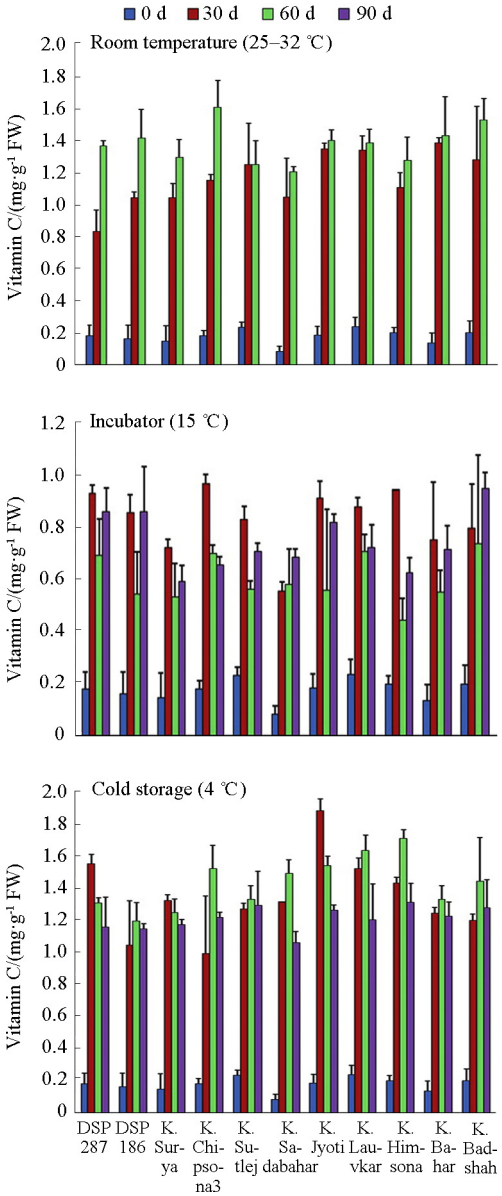

Fig. 1 Changes in vitamin C content of potato varieties stored at three different temperatures

Error bars represent standard error of means.

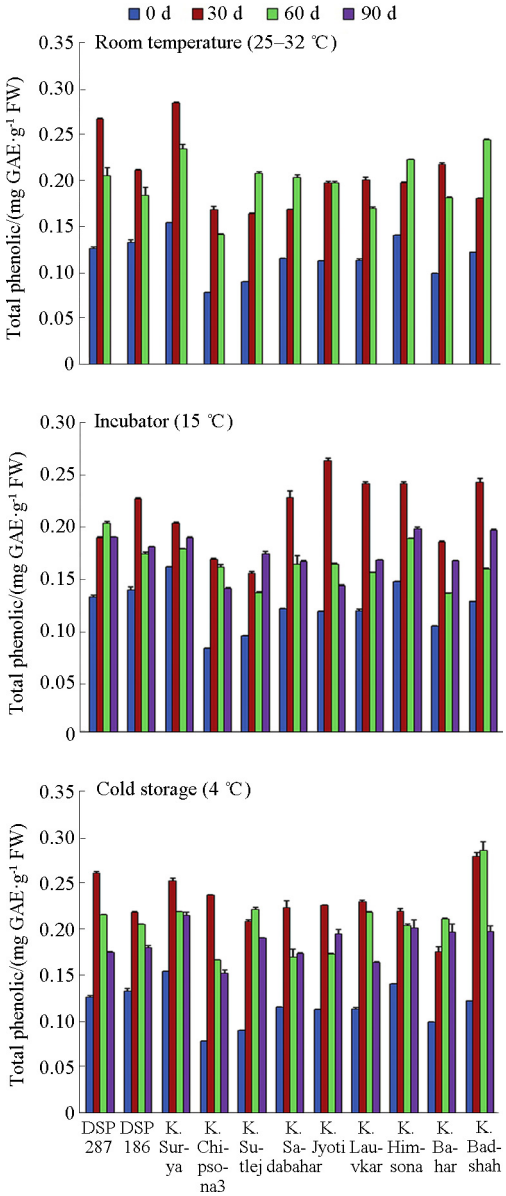

Fig. 2 Changes in total phenolic content of potato varieties stored at three different temperatures

Error bars represent standard error of means.

and cold storage was not significant. At room temperature, Vitamin C content increased over the course of the entire storage period with a jump between 0 and 30 DOS. At 15 °C, the trend was marked with various fluctuations of increase followed by decrease, but Vitamin C levels at 90 DOS were higher than initial levels. At 4 °C, after a drastic increase observed at 30 DOS, fluctuations were also recorded, with final levels higher than initial (Fig. 1). Basically, between the first and the last days of storage, the increase in Vitamin C was 5.4- to 14.7-fold at room temperature, 3.1- to 8.4-fold at 15 °C and 5.0- to 12.9-fold at 4 °C.

3.2. *Total phenolic content*

Initially the lowest (0.0786 mg GAE·g^{-1} FW) and the highest (0.1546 mg GAE·g^{-1} FW) total phenolic content (TPC) were recorded in K. Chipsona-3 and K. Surya, respectively. The potato varieties have shown highly significant differences in TPC changes during storage. Moreover, the difference in changes of TPC between room temperature and incubator as well as room temperature and cold storage was not significant, but a highly significant difference was obtained between incubator and cold storage. At room temperature, the TPC initially increased, and then a decrease was observed, except in K. Sutlej, K. Sadabahar, K. Himsona, and K. Badshah which showed a continued increase. In incubator and cold storage, TPC fluctuated during the whole storage period but until the last day of observation TPC was higher than at day 0, suggesting an overall increase during low temperature storage of potatoes (Fig. 2). In general, DSP 287, K. Surya and K. Badshah showed higher TPC, and between the first and the last days of storage, TPC increased from 1.4- to 2.3-fold at room temperature, 1.2- to 1.9-fold at 15 °C and 1.4- to 2.1-fold at 4 °C.

3.3. *Phenolic acid profiling*

Phenolic acids from potato tuber samples were analyzed by UPLC. From the chromatograms obtained, a total of 12 phenolic acids were identified and quantified (Fig. 3).

Gallic acid initially ranged from 4.4167 to 17.5277 μg·g^{-1} FW. Its content increased with storage at all the 3 temperatures (up to 45.7059 μg·g^{-1} FW), but highest increases were observed at room temperature. In addition, a drop of Gal content was observed after 30 or 60 DOS in some varieties at 15 °C and 4 °C. Generally, K. Badshah and K. Himsona have shown higher Gal contents, while the lowest contents were obtained from K. Chipsona-3 (Fig. 4).

The initial content of Pro was recorded between 0.0261 and 0.5677 μg·g^{-1} FW. It considerably increased with storage with no critical difference noted between the 3 temperatures. The highest value of 1.0486 μg·g^{-1} FW was obtained from DSP 287 at 4 °C. For some varieties, a drop was observed after 30 or 60 DOS, but the content at 90 days was always superior to the initial content. The varieties DSP 287 and K. Badshah have shown higher content of Pro, whereas lowest contents were recorded from K. Sutlej and K. Sadabahar (Fig. 5).

Chlorogenic acid at initial period of storage was determined between 5.9781 and 28.8757 μg·g^{-1} FW. Its content increased with storage at all the 3 temperatures, but the highest increases were observed at room temperature, with a peak value of 62.5058 μg·g^{-1} FW measured from K. Badshah. A decline was observed after 30 or 60 days at 15 °C and 4 °C in a few varieties. K. Badshah has shown the highest contents (except at 15 °C) and the lowest contents were obtained from K. Chipsona-3 (Fig. 6).

At first, Caf content was between 0.3219 and 1.5419 μg·g^{-1} FW. Except for K. Badshah and DSP 287 at 15 °C where a continuous increase was observed during storage, Caf content rose considerably from 0 to 30 DOS with the highest increase obtained at 4 °C, followed by a drop up to 90 DOS, but it still remained higher than the initial amount. Variety DSP 287 has exhibited the highest Caf content (6.383 μg·g^{-1} FW) recorded at 60 DOS at 4 °C (Fig. 7).

Initially, vanillic acid content ranged from 0.1878 to 0.8066 μg·g^{-1} FW. It increased with storage at all the 3 temperatures although the pattern of increase was not standard in all the varieties; the content at 90 DOS was higher than the

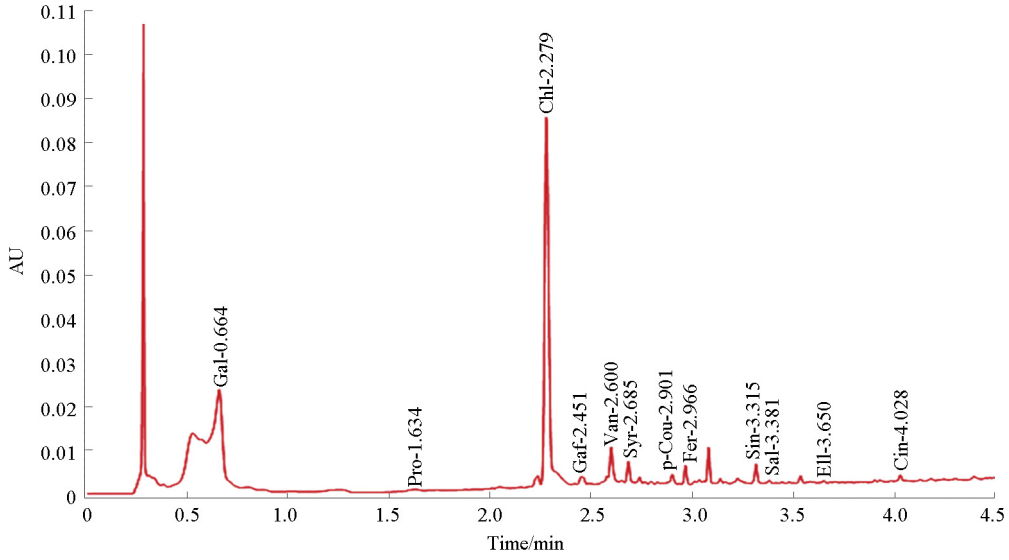

Fig. 3 Sample UPLC chromatogram at 280 nm from variety DSP 287 at initial day of storage

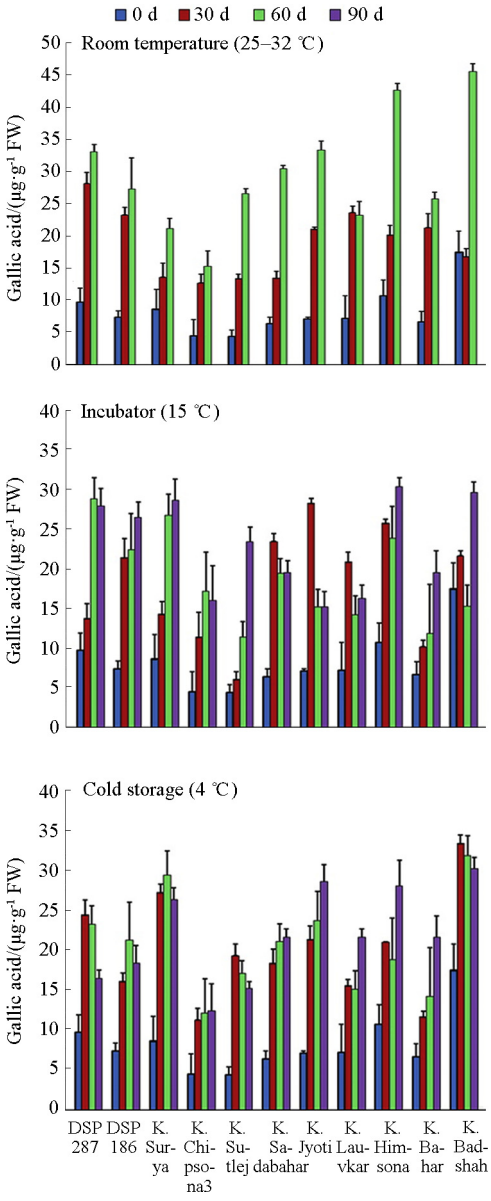

Fig. 4 Changes in gallic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, gallic acid content was calculated according to peak area. Error bars represent standard error of means.

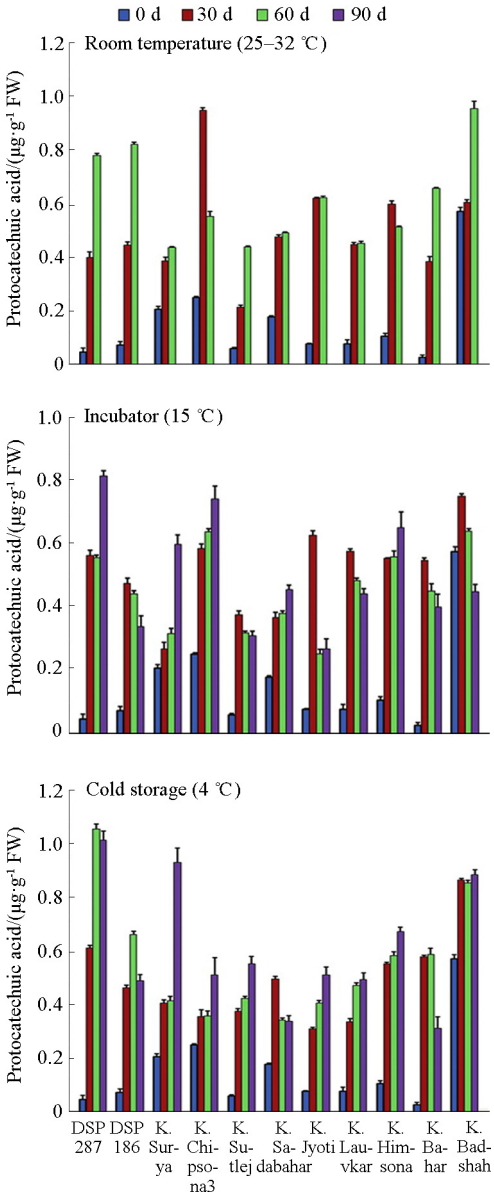

Fig. 5 Changes in protocatechuic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, protocatechuic acid content was calculated according to peak area. Error bars represent standard error of means.

initial value. The highest vanillic acid content (3.0723 μg·g^{-1} FW) was measured from K. Himsona at room temperature. Generally, K. Badshah, K. Himsona and DSP 287 maintained high levels at all the 3 storage temperatures (Fig. 8).

At day 0, syringic acid was measured between 0.0513 and 0.2461 μg·g^{-1} FW. At room temperature and at 15 °C it increased over the course of the entire storage period with the highest at 1.0582 μg·g^{-1} FW obtained from K. Badshah at room temperature. At 4 °C the increase observed in initial phase was followed by a decrease after 60 DOS in most varieties. However, the content at 90 DOS always remained greater than the initial content. Overall, K. Badshah, K. Himsona and DSP 287 maintained high levels in all the 3 storage temperatures (Fig. 9).

The original content of para-coumaric acid ranged between 0.0786 and 0.2357 μg·g^{-1} FW. All over the storage period it increased at room temperature and at 15 °C with a peak value of 2.1617 μg·g^{-1} FW recorded at 90 DOS in K. Bahar stored at 15 °C. At 4 °C para-coumaric acid trend varied among the varieties, but at 90 DOS, its content was lower than at initial (Fig. 10).

The initial content of ferulic acid was obtained between 0.0637 and 0.3228 μg·g^{-1} FW. At room temperature, a drastic in-

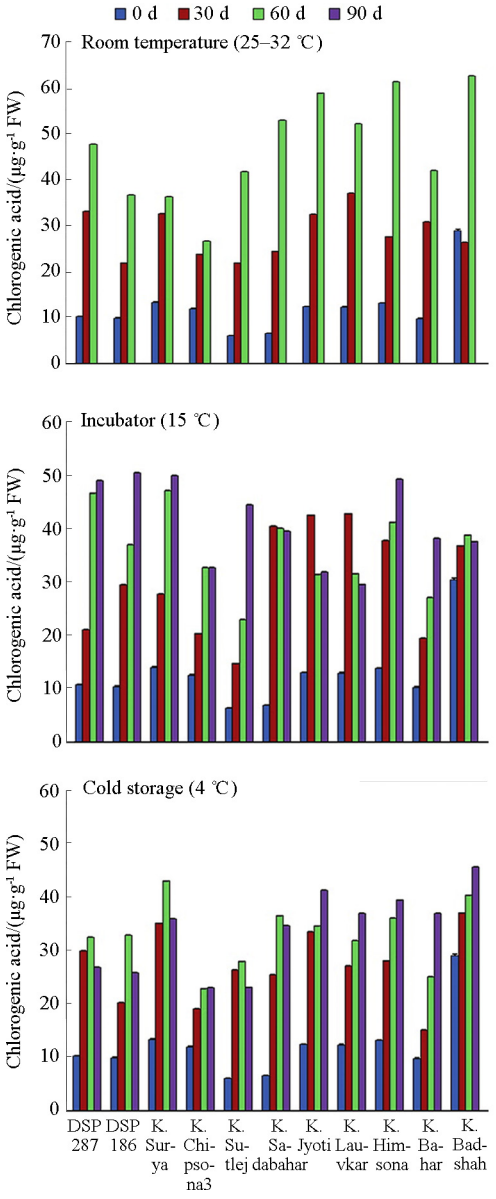

Fig. 6 Changes in chlorogenic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, chlorogenic acid content was calculated according to peak area. Error bars represent standard error of means.

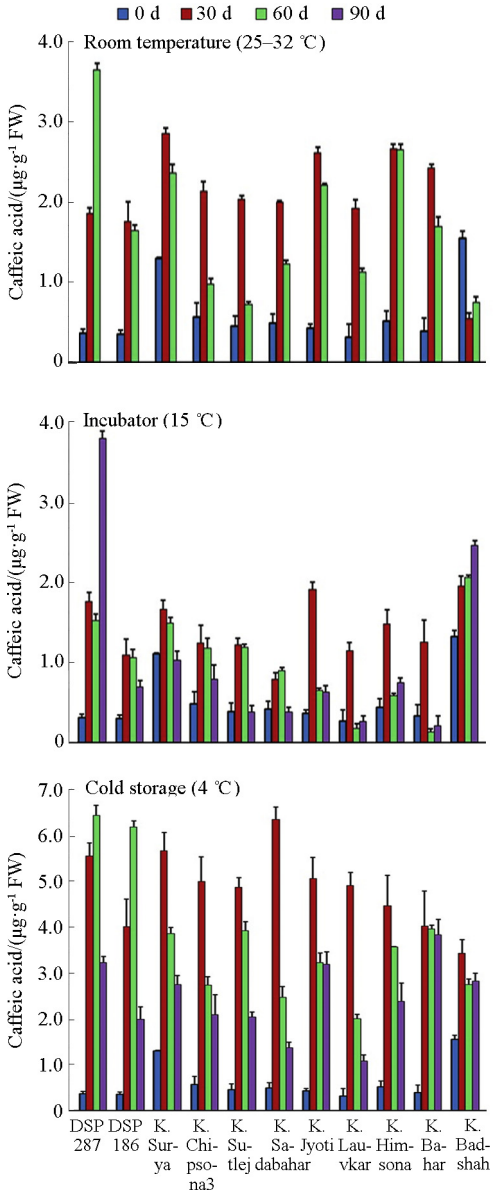

Fig. 7 Changes in caffeic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and caffeic acid content was calculated according to peak area. Error bars represent standard error of means.

crease was observed in all the varieties, with a peak value of 3.0097 μg·g^{-1} FW obtained from DSP 287. In incubator and in cold storage, the trend of changes in ferulic acid varied among the varieties, but in general, the content at 90 DOS was greater than initial content, except in K. Chipsona-3 at 4 °C and 15 °C where a decline was observed (Fig. 11).

The initial sinapic acid content ranged from 0.3629 to 9.5207 μg·g^{-1} FW. Disregarding the atypical trend observed in K. Jyoti at 15 °C, the content of sinapic acid drastically increased during storage at all the 3 temperatures with a strong increase at 90 DOS observed in most varieties. The highest value (46.2783 μg·g^{-1} FW) was obtained from K. Surya at 4 °C. In general, K. Sadabahar, K. Badshah and K. Sutlej have exhibited high sinapic acid contents, while low values were obtained from K. Himsona (Fig. 12).

The content of salicylic acid at initial period ranged between 0.0227 and 0.1706 μg·g^{-1} FW. During storage at all the 3 temperatures, its content varied in different patterns among the varieties: an increase of Sal content was observed in some varieties, while others showed a decrease. The highest value (0.8627 μg·g^{-1} FW) was measured from K. Sutlej at 30 DOS at 15 °C. Overall, throughout the storage period, K. Badshah has

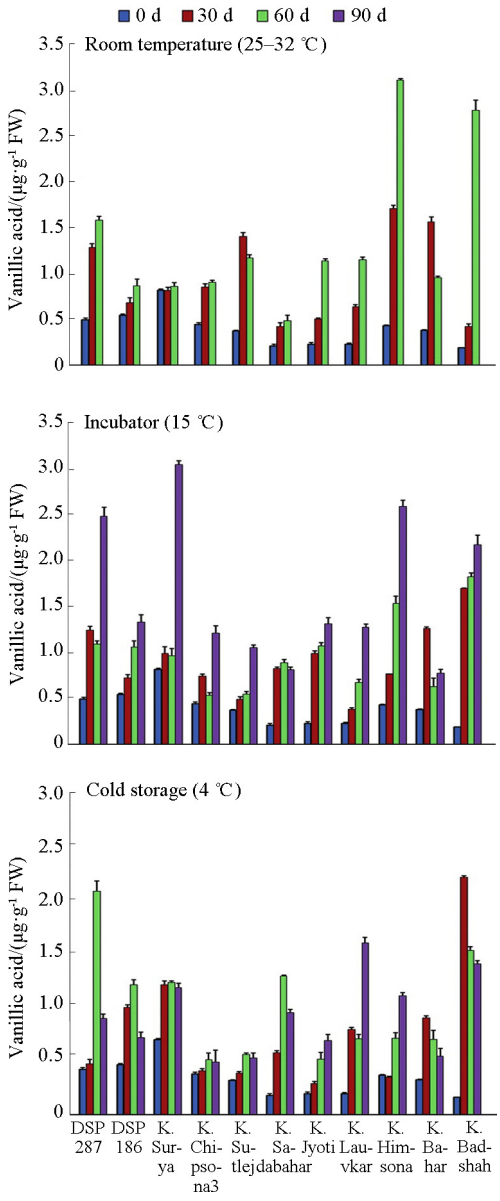

Fig. 8 Changes in vanillic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and vanillic acid content was calculated according to peak area. Error bars represent standard error of means.

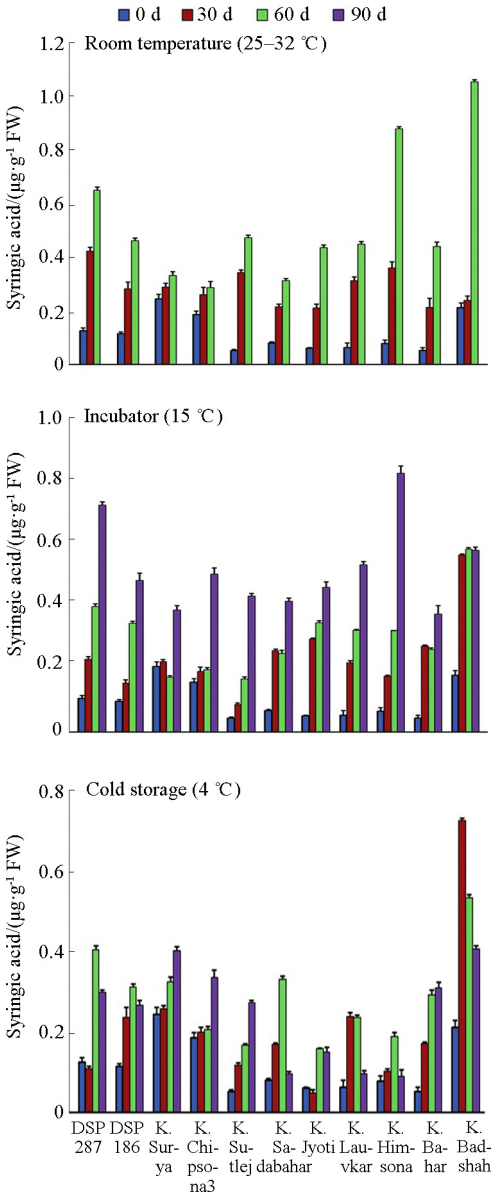

Fig. 9 Changes in syringic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and syringic acid content was calculated according to peak area. Error bars represent standard error of means.

maintained high contents while low contents were recorded from K. Jyoti and K. Himsona (Fig. 13).

The original content of ellagic acid was measured from 0.3020 to 0.7164 μg·g^{-1} FW. It then gradually increased in all the varieties at all the 3 storage temperatures, with the highest value (8.1215 μg·g^{-1} FW) obtained from K. Chipsona-3 at 90 DOS in incubator. In general, higher increases of ellagic acid were recorded at 15 °C, and K. Chipsona-3, K. Himsona and K. Bahar maintained high contents, whereas K. Badshah maintained low values (Fig. 14). To our knowledge, this is the first report of ellagic acid in potato content and its changes during storage of potato tubers.

In the beginning of observation, cinnamic acid content was quantified between 0.0213 and 0.0393 μg·g^{-1} FW. Except in K. Chipsona-3 at room temperature, it gradually increased in all the varieties at all the 3 storage temperatures, with the highest value (0.3092 μg·g^{-1} FW) obtained from K. Himsona at 90 DOS in incubator. In general, K. Chipsona-3 and K. Himsona showed high contents, whereas K. Sadabahar and K. Badshah exhibited low values (Fig. 15).

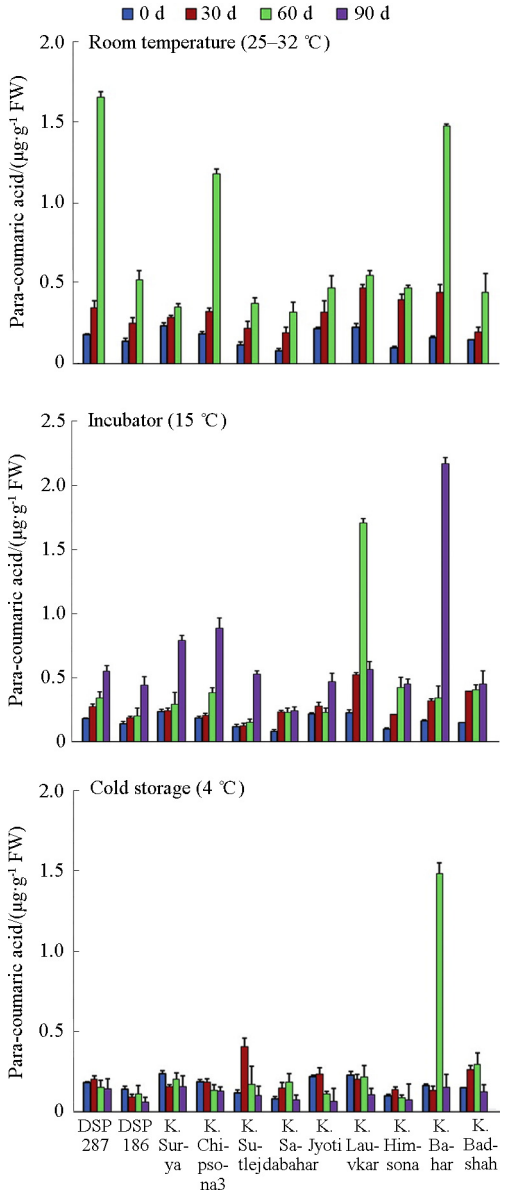

Fig. 10 Changes in para-coumaric acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and para-coumaric acid content was calculated according to peak area. Error bars represent standard error of means.

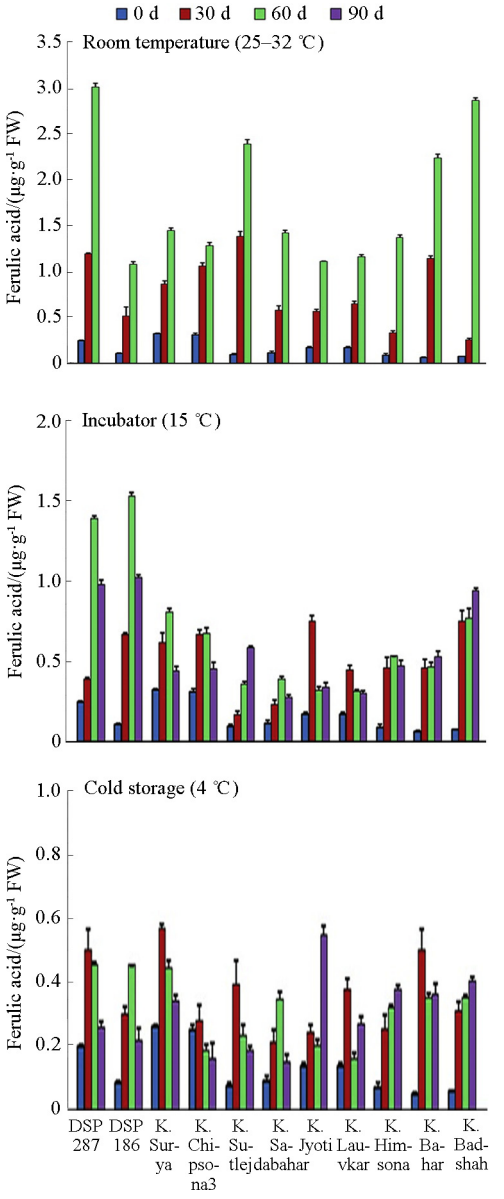

Fig. 11 Changes in ferulic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and ferulic acid content was calculated according to peak area. Error bars represent standard error of means.

To sum up, the most abundant phenolic acid was Chlorogenic acid and the lowest was Cinnamic acid. Considering the first and the last days of observation, except para-Coumaric acid, which decreased at 4 °C, all the phenolic acids increased with storage, but Sinapic acid and Ferulic acid appeared to be most enhanced. In fact, before storage, the phenolic acid content in decreasing order was as follows: Chlorogenic, Gallic, Sinapic, Ellagic, Caffeic, Vanillic, para-Coumaric, Ferulic, Syringic, Protocatechuic, Salicylic and Cinnamic. But after storage, the order was rearranged into Chlorogenic, Sinapic, Gallic, Ellagic, Caffeic, Vanillic, Ferulic, para-Coumaric, Syringic, Protocatechuic, Salicylic and Cinnamic. The observed dissimilar changes of phenolic acids during storage can be explained by the fact that synthesis and degradation of each phenolic acid are governed by genes and enzymes of its specific pathway. Then the given metabolic pathway of each phenolic acid could respond differently to low temperature stress, independent of the other pathways. The more the enzymes and genes of a synthesis pathway are sensitive to low temperature, the more its resulting phenolic acid content will increase. Besides, changes in TPC were also observed during storage. Thus, this general increase in the phenolic acids could

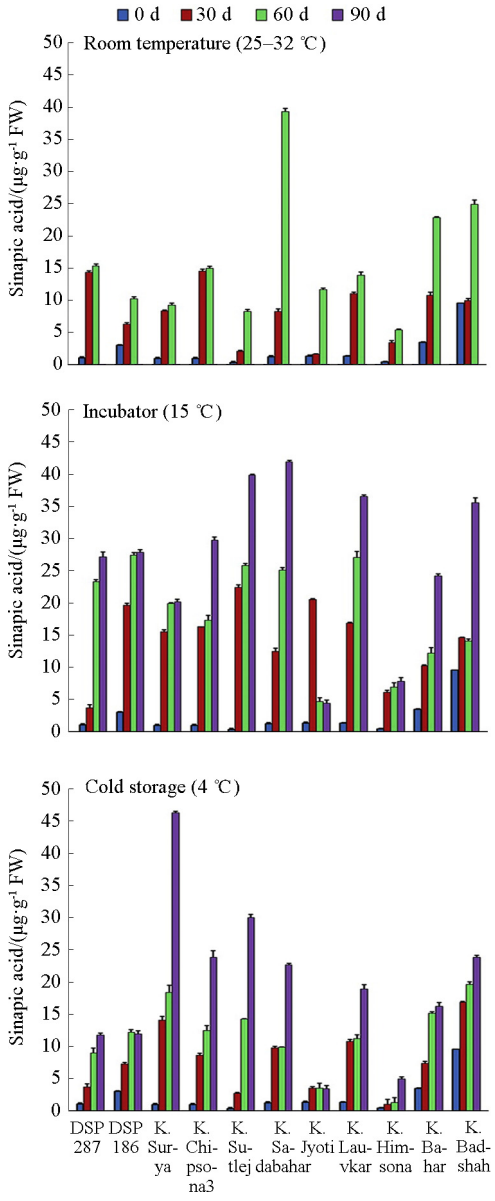

Fig. 12 Changes in sinapic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, sinapic acid content was calculated according to peak area. Error bars represent standard error of means.

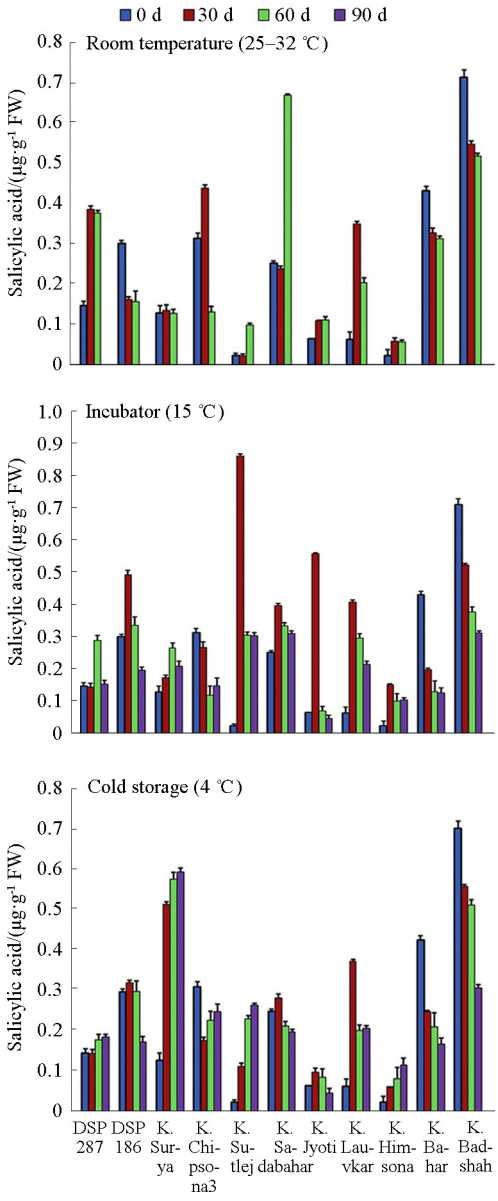

Fig. 13 Changes in salicylic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and salicylic acid content was calculated according to peak area. Error bars represent standard error of means.

explain the observed increase in TPC with storage (Madiwale et al., 2011).

3.4. *Antioxidant activity*

3.4.1. *DPPH-antioxidant activity*

The antioxidant activity measured by DPPH method initially ranged between 0.3116 (K. Bahar) and 1.0676 mg TE·g^{-1} FW (K. Badshah). During storage, the differences in changes of DPPH-antioxidant activity between the 3 storage temperatures were not significant. In all the 3 temperatures, antioxidant activity gradually increased up to 60 DOS, with a maximum value of 3.0471 mg TE·g^{-1} FW obtained from K. Badshah at room temperature. Then at 90 DOS, it dropped to a level comparable to the original value (Fig. 16).

3.4.2. *ABTS-antioxidant activity*

The initial antioxidant activity measured by ABTS method varied from 0.4074 (K. Lauvkar) to 1.6892 mg TE·g^{-1} FW (K. Badshah). During storage, there was no significant difference in changes of ABTS-antioxidant activity between the storage temperatures. In all the 3 temperatures, antioxidant

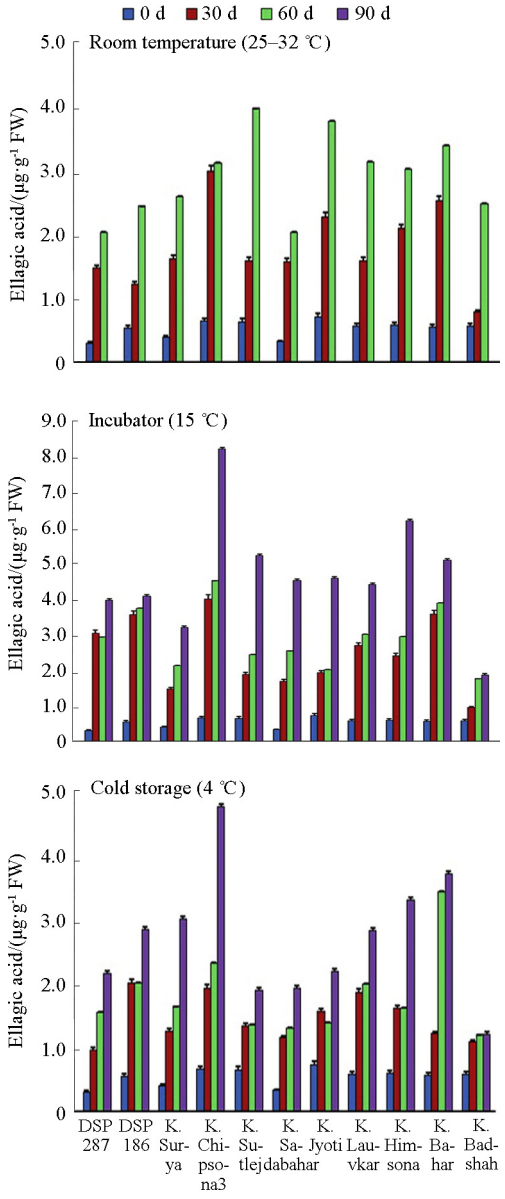

Fig. 14 Changes in ellagic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and ellagic acid content was calculated according to peak area. Error bars represent standard error of means.

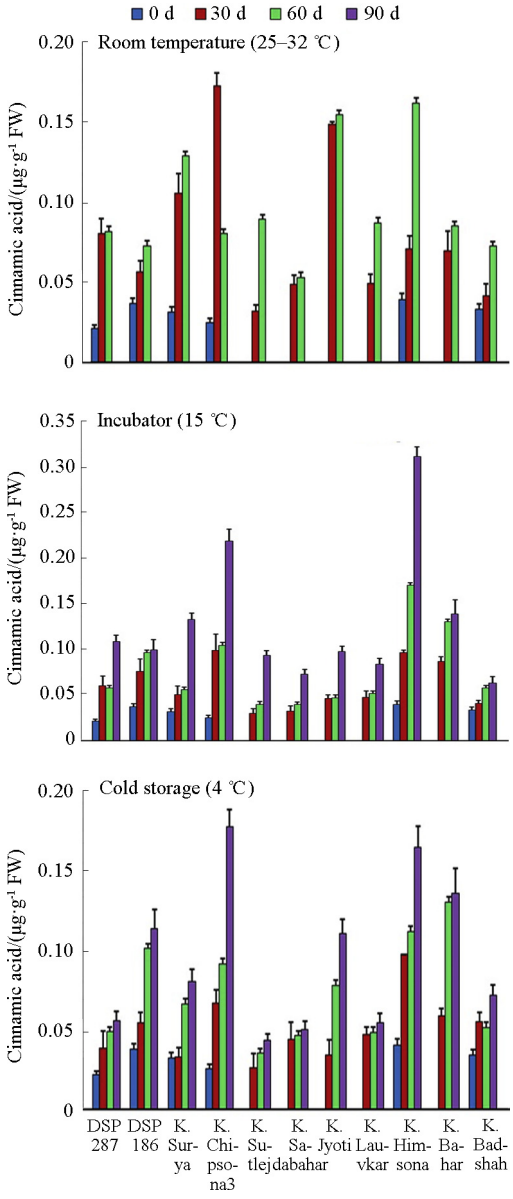

Fig. 15 Changes in cinnamic acid content of potato varieties stored at three different temperatures

Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times and UV spectra of standards, and cinnamic acid content was calculated according to peak area. Error bars represent standard error of means.

activity increased and peaked at 60 DOS, with a maximum value of 4.1993 mg TE·g^{-1} FW obtained from DSP 287 at 15 °C. Then it decreased at 90 DOS but remained higher than the original value, except in K. Surya and K. Badshah at 15 °C (Fig. 17).

Overall, ABTS-antioxidant activity was higher than DPPH-antioxidant activity but the variation of antioxidant activity measured by ABTS paralleled the DPPH method. Considering the first and the last days of observation, increase of antioxidant activity with storage was clearly revealed in almost all the varieties by ABTS method but varied among the varieties as per DPPH method.

3.5. *Antioxidant enzymes activities*

3.5.1. *Superoxide dismutase activity*

The activity of SOD at initial day was lowest in K. Lauvkar (90.06 U·mg^{-1} protein) and highest in DSP 287 (142.23 U·mg^{-1} protein) which was higher than other varieties. During storage, a significant difference in changes of SOD activity was found

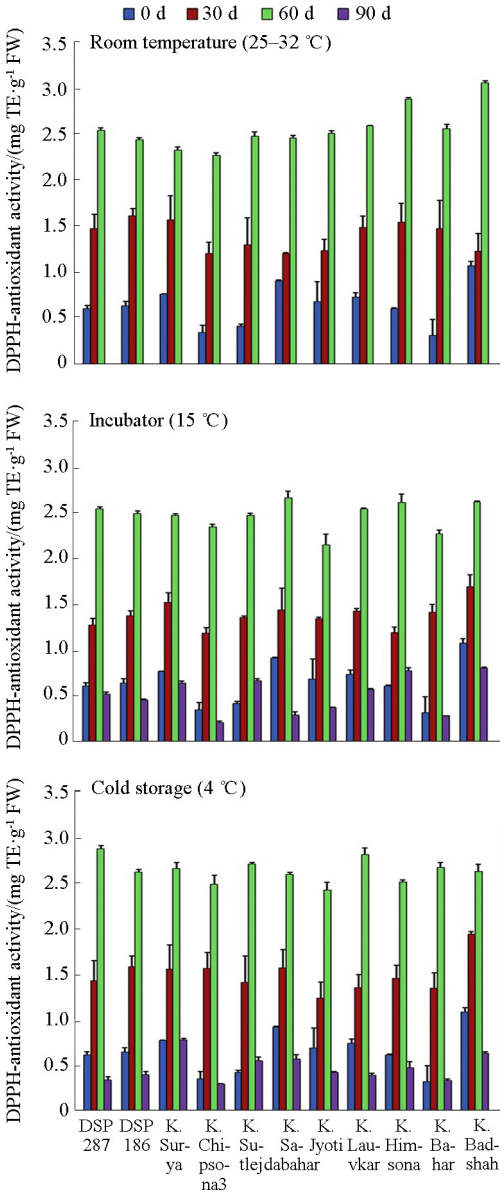

Fig. 16 Variation of antioxidant activity of potato varieties evaluated by DPPH assay

Antioxidant activity from potato tubers' methanol extract was measured with Trolox as standard. Error bars represent standard error of means.

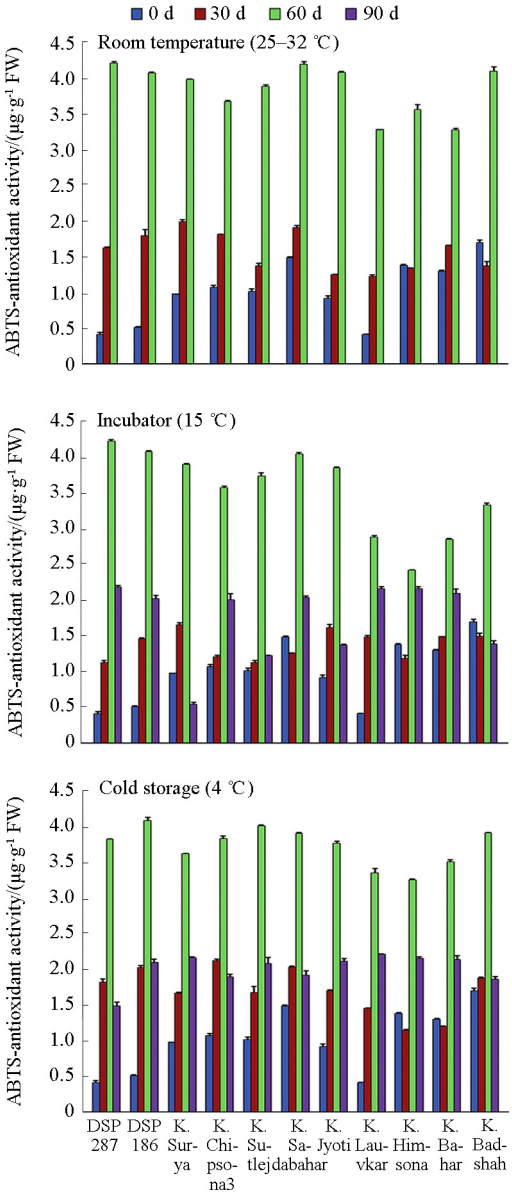

Fig. 17 Variation of antioxidant activity of potato varieties evaluated by ABTS assay

Antioxidant activity from potato tubers' methanol extract was measured with Trolox as standard. Error bars represent standard error of means.

between incubator and cold storage and between room temperature and incubator. For the storage period as a whole, the trend of change in SOD activity varied depending on the variety, but except for a few cases the values at 90 DOS were lower than initial values, indicating a decrease of the enzyme during storage. Varieties DSP 287 and K. Sutlej have shown higher activities (Fig. 18). The values of SOD activity obtained in this investigation are lower than the findings of Mizuno et al. (1998) which ranged between 125 and 280 U·mg^{-1} protein. However, the same authors observed similar trends in changes of SOD activity in potato tubers stored at 5 °C and 20 °C.

3.5.2. Ascorbate peroxidase activity

The activity of APX at initial day was lowest in K. Lauvkar (0.21 U·mg^{-1} protein) and highest in K. Sutlej (1.84 U·mg^{-1} protein), which was on par with K. Chipsona-3. During storage, there was no significant difference in changes of APX activity among the three temperatures. Initially, the enzyme activity rose drastically during storage and maximum activities were reached at 30 DOS for most of the varieties at room temperature and at 4 °C, or at 60 DOS for most of the samples stored at 15 °C. Then APX activity declined at 90 DOS and returned to values lower than basal level, except in DSP 287, K. Lauvkar and K. Himsona at 15 °C. The highest activities were ob-

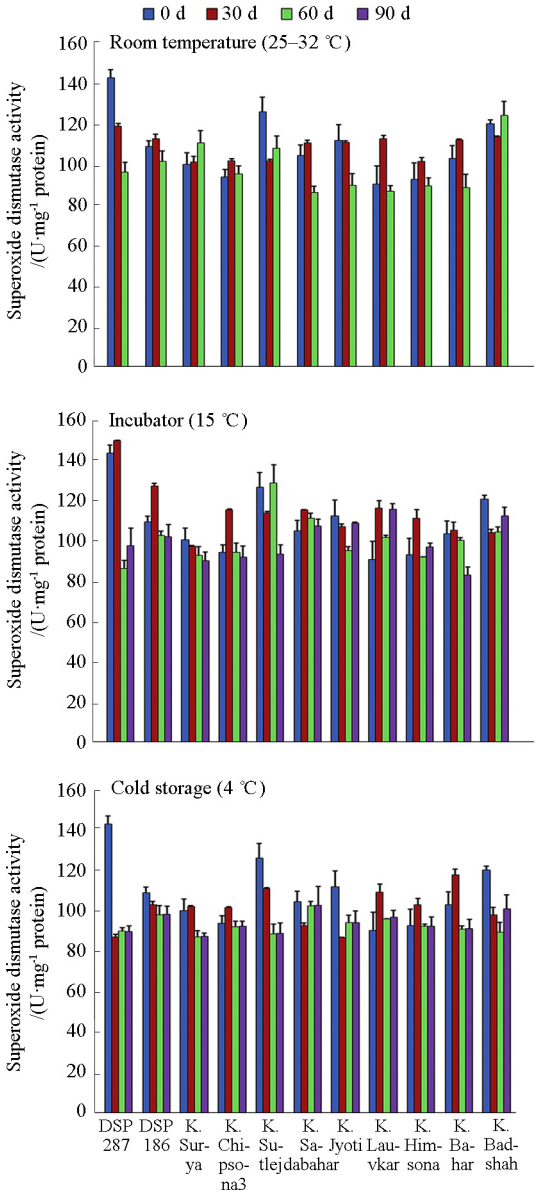

Fig. 18 Changes in activity of antioxidant enzyme superoxide dismutase of potato varieties

Enzyme was extracted in phosphate buffer and superoxide dismutase activity was assessed based on inhibition of nitroblue tetrazolium photoreduction. Error bars represent standard error of means.

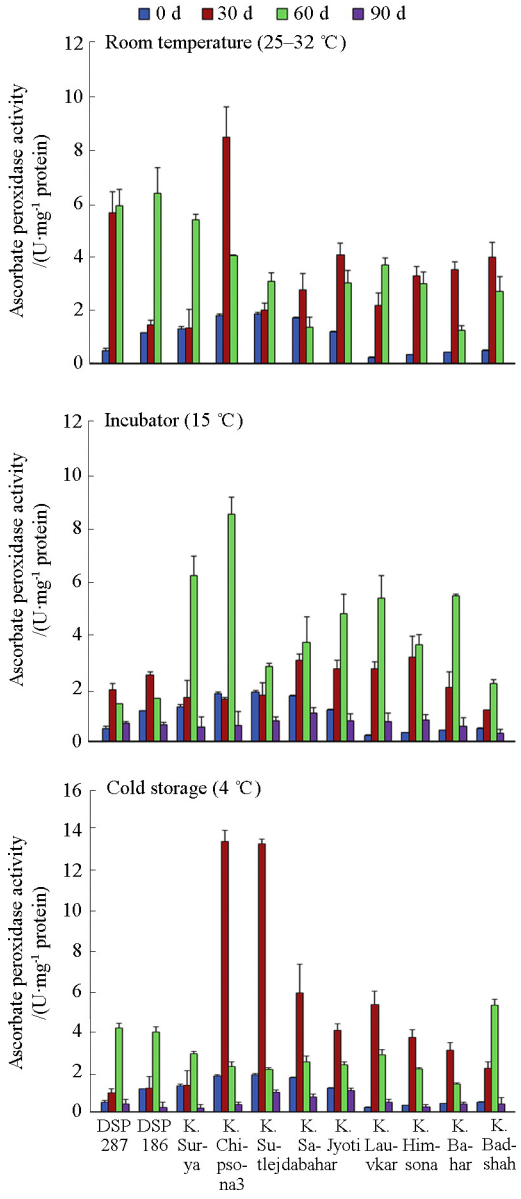

Fig. 19 Changes in activity of antioxidant enzyme ascorbate peroxidase of potato varieties

Enzyme was extracted in phosphate buffer and ascorbate peroxidase activity was measured by following the hydrogen peroxide dependent oxidation of ascorbate at 290 nm. Error bars represent standard error of means.

tained from K. Chipsona-3, with a peak value of 13.41 U·mg^{-1} protein measured at 4 °C (Fig. 19).

3.6. *Correlations between antioxidant parameters*

The Pearson correlation coefficients between antioxidant parameters each for 99 observations are presented in Table 1. It appeared that TPC had a strong positive correlation with Vitamin C content and Gal (r = 0.620 each), a moderate positive correlation with Pro, Chl and Caf (r = 0.456, 0.482 and 0.588, respectively) but a weak or no association with other phenolic acids. This suggests a strong contribution of Gal and a significant contribution of Pro, Chl and Caf in TPC estimation; and the changes in these phenolic acids could explain the changes in TPC. However, TPC had a weak positive correlation with DPPH, ABTS and APX. A weak positive correlation existed between Vitamin C content and DPPH (r = 0.371) and ABTS (r = 0.382). This demonstrates that as an antioxidant Vitamin C positively contributes to radical scavenging in potato tubers, but is not the exclusive key-component of the antioxidant capacity. Similar tendency is observed with TPC and antioxidant activities. Between ABTS-antioxidant activity and phenolic acids, a positive moderate relationship was found with Chl (r = 0.518), Fer (r = 0.467) and Gal (r = 0.412). A similar

Table 1 Correlation coefficients between antioxidant parameters in potato varieties during storage at different temperatures

	TPC	Vitamin C	Gal	Pro	Chl	Caf	Van	Syr	*p*-Cou	Fer	Sin	Sal	Ell	Cin	DPPH	ABTS	SOD
Vitamin C	0.620																
Gal	0.620*	0.457															
Pro	0.456**	0.486	0.538*														
Chl	0.482*	0.424	0.902	0.490													
Caf	0.588*	0.602	0.228	0.345	0.101**												
Van	0.338	0.189	0.638*	0.492	0.579	0.154											
Syr	0.244	0.097**	0.613	0.485	0.643	−0.072**	0.765										
p-Cou	−0.085**	0.092**	0.184	0.136*	0.273	−0.193	0.120*	0.321									
Fer	0.283	0.316	0.558	0.321**	0.560	0.008**	0.361	0.514	0.378								
Sin	0.075**	0.040**	0.317	0.306	0.415	−0.156	0.228	0.428	0.209	0.195							
Sal	0.211	0.001**	0.169	0.254	0.123*	0.019**	0.049**	0.122*	−0.088**	0.087**	0.470						
Ell	−0.069**	0.060**	0.211*	0.271	0.390	−0.239	0.249	0.467	0.347	0.217	0.486	−0.174					
Cin	0.100**	0.161	0.322	0.370	0.415	−0.041**	0.342	0.438	0.217**	0.191**	0.125*	−0.267	0.752				
DPPH	0.340	0.371	0.331	0.252	0.381	0.220	0.179	0.209	0.162	0.443	−0.011**	0.125*	−0.083**	−0.025**			
ABTS	0.186*	0.382	0.412	0.306	0.518	0.150*	0.199	0.312**	0.220	0.467	0.244	0.026**	0.200**	0.175	0.801		
SOD	−0.152	−0.299	−0.279	−0.246	−0.371	−0.246	−0.178	−0.147	−0.199	−0.083**	−0.170	0.125*	−0.197	−0.292	−0.164	−0.398	
APX	0.231	0.213	0.029**	0.128*	0.031*	0.268	−0.118*	−0.079**	0.103**	0.177	−0.185	−0.019**	−0.087**	−0.024**	0.466	0.304	0.035**

Note: * Correlation is significant at 5% level; ** correlation is significant at 1% level.

trend was observed with the DPPH-antioxidant activity. This implies a higher contribution of these phenolic acids to the antioxidant capacity.

4. Discussion

The Vitamin C content values at initial storage day are consistent with the data of Kumar et al. (2013) obtained on similar Indian potato varieties, Burgos et al. (2009) from Andean varieties, Abong et al. (2011) from Kenyan cultivars and other previous reports (Dale et al., 2003; Love et al., 2003). But lower levels of Vitamin C were found in Korean cultivars (Cho et al., 2013) while higher levels were obtained from Colorado-grown specialty potato (Külen et al., 2013). Vitamin C content of potato can be affected by genotype, harvest date, and growing conditions (Love et al., 2003; Burgos et al., 2009; Cho et al., 2013). Several reports have mentioned a considerable decrease in Vitamin C level after harvest in potatoes stored under room temperature, farmer conditions for long duration (6 months and more) or during cooking (Mishra, 1985; Woolfe, 1987; Zee et al., 1991; Casañas et al., 2003; Pinhero et al., 2009). On the other hand, when potatoes were cold stored for long period, although Vitamin C decreased, significant amount was still retained (Külen et al., 2013). Similar observations were made in this study for the 90 DOS observation period. Many factors, such as genotype, temperature, light and diseases can alter Vitamin C metabolism in stored tubers (Külen et al., 2013). The changes in Vitamin C content of these potato tubers can result from alterations in expression of genes and activity of enzymes in the pathway of Vitamin C metabolism during storage. Vitamin C might be synthesized as a response to the stress caused by the storage temperature and then used as antioxidant compound in response to oxidative stress caused by low temperature storage. Moreover, the expression of these genes and the activity of the subsequent enzymes could be temperature-dependent.

The values of TPC obtained in this study are comparable to the range reported by Kumar (2011) and Navarre et al. (2011). Besides, Madiwale et al. (2011) and Külen et al. (2013) also obtained similar contents in white and yellow-fleshed potato clones, but TPC in purple-fleshed clones was much higher. Also, our findings are lower than the values of Singh et al. (2009), Jang and Yoon (2012) and Kumar et al. (2013). The phenolic content primarily depends upon the genotype, and variations reported by different authors may also be due to differences in the growing location, method of extraction, and sample preparation, as vigorous extraction methods can lead to an increase in the phenolic content (Rumbaoa et al., 2009; Madiwale et al., 2011). Fluctuations of TPC in cold stored potatoes with higher levels obtained after storage are also documented in recently published works by Kumar (2011), Madiwale et al. (2011), Külen et al. (2013). In plants as well as in potato tubers, environmental stresses such as low-temperature storage, strong light, wounding, or pathogen attacks have been shown to induce the generation of phenolic compounds via the phenylpropanoid pathway by activation of phenylalanine ammonia-lyase (PAL) (Dixon and Paiva, 1995; Jiang and Joyce, 2003). The decreasing trend also observed may be attributed to degradation of the polyphenolic compounds. It is difficult to generalize cold storage effects on TPC in potato due to variations among potato genotypes. Further detailed studies are necessary to completely elucidate cold storage effect on potato TPC (Külen et al., 2013).

The contents of Gal, Pro, Van, *p*-Cou, Syr, Sin and Sal obtained here are greater than the data reported by Lewis et al. (1998). The initial contents of Chl recorded in this study are in agreement with the values reported by Madiwale et al. (2011) in cultivars Atlantic and Yukon Gold, but are lower than those reported by Dao and Friedman (1992), Lewis et al. (1998) and Reddivari et al. (2007). This wide range of disparity can be attributed to inter-varietal differences (Stushnoff et al., 2008; Burgos et al., 2009; Navarre et al., 2011). Besides, the increase in Chl when potato tubers were cold-stored corroborates the findings of Madiwale et al. (2011). Reddivari et al. (2007) and Navarre et al. (2011) have obtained higher Caf content, and Madiwale et al. (2011) have also reported an increase in Caf in cold-stored potatoes after 90 DOS. The results of Fer content obtained here are higher than the values reported by Lewis et al. (1998) but are similar to the findings of Madiwale et al. (2011), the latter have also reported an increase in Fer and Sin with storage, as observed in this study. The initial values of Cin content obtained here are kin to the findings of Lewis et al. (1998).

The values of DPPH-antioxidant activity obtained here fall within the range reported by Madiwale et al. (2011) and Külen et al. (2013) in white- and yellow-fleshed potatoes, but are greater than the results reported by Hale (2003) and lower than those of Madiwale et al. (2011) and Külen et al. (2013) measured in purple-fleshed potatoes. This is because the radical scavenging capacity of purple-fleshed potatoes used by those authors is higher than the capacity of white- and yellow-fleshed potatoes evaluated in this study (Brown, 2005; Stushnoff et al., 2008; Blessington et al., 2010; Jang and Yoon, 2012). Elsewhere, changes in antioxidant activity observed over the course of the entire storage period in this study are on par with the conclusions of Rosenthal and Jansky (2008) and Külen et al. (2013) but do not agree with the findings of Madiwale et al. (2011), who observed a constant increase in antioxidant activity during storage. Madiwale et al. (2011) and Külen et al. (2013) reported similar data of ABTS-antioxidant activity in white- and yellow-fleshed potatoes, but higher values were obtained in purple-fleshed potatoes by these authors, as well as by Campos et al. (2006) earlier. As observed with DPPH method, Rosenthal and Jansky (2008) and Külen et al. (2013) reported similar trend of variation of antioxidant activity during storage, but Madiwale et al. (2011) reported a constant increase of antioxidant activity. Previous reports also mentioned a few differences between DPPH- and ABTS-antioxidant activities (Awika et al., 2003; Floegel et al., 2011; Madiwale et al., 2011; Külen et al., 2013). Increase of antioxidant activity during storage can be attributed to an increased level of total phenolics, phenolic acids, Vitamin C and other compounds like anthocyanins, carotenoids and flavonoids when tubers are stored.

Similar trends in APX activity in store potato tubers were also presented by Mizuno et al. (1998), although the values obtained here are little higher than those reported by these authors. Thus, when potatoes were stored at low temperature, antioxidant enzymes such as SOD and APX were induced in the first month of storage, and then their activities dwindled after longer storage periods. This suggests that reactive oxidant species are more readily generated during initial months of potato tuber storage, and their level later decreased (Mizuno et al., 1998).

The results on TPC correlation are in contrast with the conclusions of Navarre et al. (2011) and Külen et al. (2013) which found a strong correlation between TPC and both antioxidant activities. These results can be justified by the contribution of some of the non-phenolic compounds such as vitamins and minerals to the antioxidant activity (Gliszczynska-Swiglo, 2006; Shenkin, 2006). In the present study, a very strong positive linear relation ($r = 0.801$) was found between DPPH- and ABTS-antioxidant activities. Different coefficients were obtained by Madiwale et al. (2011) ($r = 0.960$) and Külen et al. (2013) ($r = 0.655$). Although a high variability exists among the various methods of antioxidant activity evaluation, these two methods remain the more accurate and less prone to variability (Thaipong et al., 2006; Dudoné et al., 2009). Except with Sal content, SOD negatively correlated all the other parameters, with a negative moderate relationship ($r = –0.398$) between SOD and ABTS, while APX positively correlated with most of the parameters, with a positive moderate relationship between APX and DPPH ($r = 0.466$) or ABTS ($r = 0.304$). This suggests that the enhancement of reactive oxygen scavenging species in tubers could result mainly from APX activity, while dismutation of oxygen radicals by SOD might be the primary step of defense during low temperature treatment (Mizuno et al., 1998).

5. Conclusion

Potatoes are gaining more and more place in our diet not only because of their high dietary value, but also because of the various health benefits their consumption offers. Antioxidant capacity of tubers' phytonutrients is among the most attractive benefits of potato consumption. This work has revealed the antioxidant profile of 11 Indian potato varieties. It was difficult to group the 11 on the basis of all the antioxidant parameters as they responded differently to each parameter. Nevertheless, it could be screened out that DSP 287, K. Himsona, K. Surya, K. Jyoti and K. Badshah were high antioxidant capacity varieties, and to a less extent K. Lauvkar and K. Sadabahar also exhibited some considerable antioxidant capacity, whereas K. Chipsona-3 and K. Bahar belonged to low antioxidant capacity group. Moreover, Vitamin C, phenolics and precisely Chl, Gal and Fer among the phenolic acids are the key compounds involved in antioxidant activity of potato tubers. As after harvest potatoes are usually stored before consumption, it is important to understand how storage condition affects its antioxidant compounds. This study has demonstrated that storage elevates antioxidant compounds and antioxidant capacity of potato tubers. Still various aspects of the mechanism explaining how storage alters these food properties are not clear. Therefore, effects of cold storage on other antioxidant enzymes such as catalase, phenylalanine ammonia-lyase and the expression of their genes should be investigated. It would be also interesting to study changes occurring during cooking of these potato tubers.

Acknowledgments

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