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ORIGINAL ARTICLE

Effect of different storage temperature on chemical composition of onion (*Allium cepa* L.) and its enzymes

Kavita Sharma[1] · **Yong Rok Lee**[2]

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Abstract Onion stored at 4, 10, and 25 °C for 9 months were analyzed for changes in quercetin and its glucosidase content, enzymes, pyruvic acid, and sugar content. During storage, concentration of quercetin and its glucosidase showed an irregular variation at all studied temperature but at 4 °C the rate was high as compared to 10 and 25 °C. The enzymatic activity of Q4'G glucosidase and Q4'glucosyltransferase increased progressively until six months at 4, 10 and 25 °C, but later it started to decrease. At 4 and 10 °C, peroxidase activity increased during the first five weeks then decreased, while at 25 °C peroxidase activity decreased progressively after two months storage. Fructose, glucose and sucrose showed a different although more regular pattern by decreasing progressively at 4, 10 °C. At 4 °C fructose and glucose accumulated in the initial 3 to 4 months of storage while sucrose was unchanged. However, at 10 and 25 °C, fructose and glucose concentration continuously decreased, while sucrose increased consistently. Onion pyruvic acid increased at 4 and 10 °C during the first six months, while at 25 °C the fluctuation was observed during the whole storage period. Overall, we conclude that storage at 4 °C maintained the quality of onions best, as evidenced by the positive changes.

Keywords *Allium cepa* · Flavonols · Sugars · Pungency · Peroxidase · Temperature

Highlights:
- Onions were stored at 4, 10, and 25 °C for 9 months.
- Flavonol and sugar content fluctuated at all temperature, but significant change was at 4 °C.
- Peroxidase activity was highest during 5th months at 10 °C.
- Depending upon storage temperature, pyruvic acid increased at 4 and 10 °C.
- Storage at 4 °C maintained the quality of onions best.

✉ Yong Rok Lee
yrlee@yu.ac.kr

[1] Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

[2] School of Chemical Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea

Introduction

Efficient storage of onion is important for extending the availability of onion to customers throughout the year without compromising the quality. Onion storage offers two advantages, firstly, it removes the bulbs from adverse climatic conditions, thus avoiding the chances of damage. Secondly, it provides the best conditions to regulate physiology and metabolic activity and predict the future usage pattern. Numerous factors such as temperature, humidity, light, that control the physiology and metabolic activity of onions, from the date of planting until they reach the customers (Mogren et al. 2006). Onions domestic storage is usually performed during long periods at room temperature or in a refrigerator. Post-harvest sprouting is a major physiological factor limiting their storage period (Sharma et al. 2014, 2015). A change in quality of stored onions is due to high catabolism of substrates, primarily carbohydrates, and other phytochemicals (Rutherford and Whittle 1982; Mogren et al. 2007a, b). Storage conditions play an important role in the physiology of onions, which ultimately affects the physicochemical and phytochemical properties of onion. The physiology of stored onion bulbs is greatly influenced by temperature and depending on cultivars, 10 °C to 25 °C temperature was found to be optimal for sprouting (Miedema 1994). For the last 50 years, researchers have been

continuously investigating the optimal temperature for storing onions with minimum weight loss from all causes. Large growers, retailers, and food companies follow different practices of pre and postharvest treatment to protect onion quality during storage. It includes storage at low temperatures, application of mitosis inhibitors, ethylene atmosphere etc. (Gubb and MacTavish 2002; Brewster 2008). Among the onions grown in Korea, Sunpower seems to have the highest storability (Nam et al. 2011). It has been reported that Sunpower bulbs begin to sprout after 170 days of ambient storage and only 5 % of bulbs sprout after 230 days of harvest (Nam et al. 2011). Storage of onion bulbs can affect chemical composition during storage, since many changes have been reported, e.g. changes in glucose and pyruvate content (Abayomi and Terry 2009), changes in flvonol and sugar contents (Sharma et al. 2014), soluble solids content, bulb fimness, increased pungency, pH and flvonols (Coolong et al. 2008), and antixidant activity (Gennaro et al. 2002). The fresh market demands onion varieties that are rich in reducing sugars and typically sweeter than storage/dehydration onions. Selections for sweet onions are primarily based on their pungency as determined by pyruvic acid content (Schwimmer and Weston 1916). Sweetness along with other health-enhancing parameters such as fructooligosaccharide (FOS) content, flavonols and antioxidant capacity (Vagen and Slimestad 2008). Sweet onion cultivars are usually characterised by low pyruvic acid content, low FOS content and greater amounts of glucose and fructose. Benkeblia and Selselet-Attou (1999)) reported the effect of low temperature on the compositional change of phenolics, oligosaccharides, and peroxidase during the dormancy break. Peroxidase enzyme play important role in the enzymatic browning of fruits and vegetables. The antioxidant activities of fruits and vegetables decrease by direct oxidation of phenolics and flavonoids catalyzed by POD enzymes (Espın and Wichers 2000). The outermost scales of onion bulbs change to brown dry skin during aging or storage, and the glucosides of quercetin are transformed to quercetin during the dry skin formation (Bilyk et al. 1984; Patil and Pike 1995). The transformation suggests that quercetin is included in the formation of brown compounds in the dry skin. The role of enzymes in the metabolism of onion bulb and its distribution in different tissues was studied by Hirota et al. (1999). Takahama et al. (2000) reported the concentration of quercetin and its glucosidase concentration in different section from top to bottom. The concentration of quercetin monoglucosidase was high at middle section and decreased gradually at the top. However, quercetin 4′-glucosyltransferase, which catalyzes the formation of QMG from quercetin, decreased from bottom to top, and no activity was detected at the top. Similarly, the activity Q4'G glucosidase, which catalyzes QMG to quercetin decreased gradually from bottom to top. On contrary, the concentration of quercetin was high at top of the onion bulb. However, the change in the activity of different enzymes with respect to bioactive components at different storage temperature is unclear. To the best of our knowledge, the effect of storage at different temperature on the chemical composition of the bulbs with respect to the corresponding enzymes has not been studied yet. It is important to simulatanously study the chemical composition and the enzymes and to find out if the enzymes are directly correlated with the chemical composition of onion. The three temperature used for the study are commonly used as conventional systems by the local growers (25 °C) and low temperature storage (4 to 10 °C) by the wholesale supplier. In this study, changes in concentration of quercetin flavonols, sugars and pyruvic acids with the enzymes, transferase, glucosidase and peroxidase during onion storage were monitored.

Materials and methods

Chemicals and standard solutions

All solvents used in this study were of HPLC grade. Trifluoroacetic acid (extra pure grade) was supplied by Alfa Aesar (Ward Hill, MA, USA). Quercetin used as standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and quercetin-3, 4′-*O*-diglucoside and quercetin-4′-*O*-monoglucoside were supplied by Polyphenols Laboratories AS (Sandnes, Norway). The purity of the flavonol standards was found to be >99 % and the saccharide standards sucrose (> 99.5 %), D-glucose (>99.5 %), and D-fructose (guaranteed reagent grade) were from Fluka (Buchs, Switzerland), Sigma-Aldrich, and Junsei Chemical Co. (Japan), respectively. The stock solutions of quercetin (1 mg/mL) and quercetin glucosides (4 mg/mL) were prepared in methanol. The saccharides were prepared in water, with concentration 50 mg/mL. All the solutions were stored at −20 °C. Calibration standards were obtained by appropriate dilution of the stock solutions.

Samples and storage conditions

Yellow onions (*Allium cepa L*. cv. Sunpower) were grown at the Mokpo Experiment Station of the National Institute of Crop Science of the Rural Development Administration (Muan, Republic of Korea). The onions harvested in July 2013 were cured in the field for 10 days, trimmed of leaves and roots and transported to the laboratory. Storage experiments were performed under temperature-controlled conditions at three different temperatures. The bulbs in a weight range of 200–220 g with no visible defects were taken for the study. Onion bulbs distributed in three batches of 70 kg each and were placed in a temperature-controlled chambers at 4 °C, 10 °C and 25 °C with 65–75 % relative humidity, respectively. These bulbs were kept in plastic trays in one layer,

each bulb weighed and labeled to measure weight lost during storage. In each experiment, eight bulbs were randomly sampled for analysis at day 0 and then at an interval of a month until the 9th months. The selected bulbs were cleaned by removing the outer dry skin, chopped into small pieces and were mixed thoroughly to obtain a representative sample.

Dry matter content

The dry matter (DM) content in onion bulbs was determined by drying chopped samples of approximately 30 g in an oven with air circulation first at 80 °C for 24 h and then at 105 °C for 2 h (Hansen 1999).

Analysis of flavonoids

Flavonoids were extracted in triplicates according to the method of (Bonaccorsi et al. 2005) with slight modifications. Approximately 10 g of a chopped sample was left overnight in 100 mL of methanol at 4 °C. Then the methanol extract was separated and the residue was homogenized with a blender in 100 mL of methanol for 3 min, followed by stirring on a magnetic stirrer for 1 h. The slurry was centrifuged at 10, 000 rpm for 40 min at 4 °C. The supernatant was removed, the residue was mixed with a new portion of methanol, and centrifugation was repeated. The combined methanolic fractions were evaporated on a rotary evaporator at 35 °C to approximately 8 mL and were diluted up to 10 mL with methanol. The extracts were stored at −20 °C till it has been analysed.

The HPLC analysis of the extracts was carried out using an Agilent 1100 chromatograph (Agilent, Palo Alto, CA, USA) equipped with a solvent delivery system, an auto-sampler, a DAD detector set at 360 nm, and a ChemStation data acquisition system. Flavonoids were separated on a Zorbax Eclipse XDB C-18 column (250 mm × 4.6 mm) with particle size of 5 μm (Agilent, Santa Clara CA, USA) protected with a Phenomenex (USA) C18-type guard column. The column was maintained at 25 °C. The mobile phase consisted of 0.1 % TFA in water (solvent A) and methanol (solvent B). A gradient elution program was set as follows: 0–10 min, 20 % B; 10–15 min, 20–80 % B; 15–22 min, 80–20 % B. The flow rate was 0.8 mL/min, and the injected volume was 10 μL. Quercetin flavonols were quantified through comparison with respective calibration curves.

Cell-free extracts

The cell free extract for the enzymatic activities were obtained by previously used method (Tsushida and Suzuki 1996) with some modification. To obtain the cell free extracts for enzyme and peroxidase, 10 g of chopped onion was homogenized with 10 mL of 0.1 M sodium phosphate (pH 7.0) in the presence of 1 g of polyvinylpyrrolidone. Homogenates were centrifuged at 20,000 rpm for 15 min at 4 °C. The supernatants obtained were used for enzymatic activities and peroxidase.

Glucosyltransferase and glucosidase activity

These activities were measured by previously reported methods (Tsushida and Suzuki 1996). The reaction mixture (100 μL) contained 2 μL of 5 mM quercetin dissolved in methanol, 20 μL of 40 mM UDP-glucose, 10 μL of 0.1 M ascorbic acid, 20 μL of cell-free extract and 58 μL of 0.1 M Tricine-NaOH (pH 8.0) (for measurement of quercetin 4′-glucosyltransferase which results in the increase of QMG). The mixture was incubated for 10 min at 37 °C and the reaction was terminated by adding 100 μL of methanol. After centrifugation at 4000 rpm for 5 min, formed QMG were quantified by HPLC. For the measurements of activities of Q4'G glucosidase (which results in the increase of quercetin), 2 μL of 9.2 mM Q4'G and 18 μL of 0.1 M ascorbic acid were added to 80 μL of cell-free extract and incubated for 1 h at 37 °C. The reactions were terminated by adding 100 μL of methanol. After centrifugation at 4000 * *g* for 5 min, quercetin were quantified by HPLC. Enzymatic activity was defined as nmole/gFW/min.

Peroxidase (POD) activity

Activity of POD was determined according to the method of Gůnes and Bayindirh (1993). 10 g of chopped onion were mixed with 50 mL of phosphate buffer pH 7.0 and blended for 5 min in a Vorwerk blender at minimal speed. The homogenate was centrifuged for 15 min at 20 000 rpm and the supernatant was removed for POD assay. Activity was determined by measuring the colour development at 430 nm with a PUY Unicam spectrophotometer (SP 9000 model). POD activity was measured spectrophotometrically by mixing 1 mL of crude enzyme extract with the substrate soluction, which is mixture of 1 mL of guaiacol (0.5 ml/100 mL) and 1 mL of H_2O_2 (0.5 mL/100 mL) and 18 mL of phosphate buffer with pH 6.5. One unit of activity was defined as a change in absorbance of 0.001/min.

Analysis of sugars

Sugars were extracted in triplicates according to the method of Kahane et al. (2001) with slight modifications. Approximately 10 g of a chopped sample were mixed with 80 % (*v*/*v*) ethanol (100 mL) and refluxed for 1 h at 80 °C. The samples were filtered through Buchner filter and readjusted to 100 mL with 80 % (*v*/*v*) ethanol. Samples were then concentrated in a rotary evaporator under reduced pressure. These concentrated extracts were diluted with 10 mL of water (HPLC grade) and stored at −20 °C until needed. Twenty microliters of an extract

was injected into a Zorbax Carbohydrate (150 × 4.6 mm) column from Agilent (Palo Alto, CA, USA) protected with an Agilent NH_2 pre-column. The sample was eluted with acetonitrile/water (75:25, *v*/*v*) and column temperature was maintained at 30 °C with 1 mL/min flow rate. The analysis was carried out on a Shimadzu (Kyoto, Japan) 10A-VP series chromatograph with a Rheodyne 7725i manual injector (Rheodyne, Cotati, CA, USA) with a 20 μL sample loop and a refractive index detector calibrated against standard solutions (2–25 mg/mL) of respective sugars.

Pyruvic acid analysis

Pyruvic analysis was performed in triplicate according to the method of Abayomi et al. (1961) with slight modifications. Briefly, 10 g of chopped onion was homogenised for 3 min in 10 mL distilled water. The homogenate was centrifuged for 10 min at 20 000 rpm and the supernatant was removed for pyruvate assay. Supernatant of 1.5 mL were then diluted 10-fold in deionised water. An aliquot of 0.5 mL was added to 1 mL of 2,4-dinitrophenyl hydrazine (DNPH) (0.0125 %; *v*/*v*) in 2 mol/LHCl and 1.5 mL deionised water in a boiling tube. The reaction mixture was vortexed and kept for 10 min at 37 °C temperature and after cooling 5 mL of 0.6 mol/L NaOH was added, and the absorbance was measured at 420 nm with Shimadzu UV-1700 spectrophotometer. The calibration curve was made by preparing pyruvic acid solutions at concentrations 0.04–0.4 mmol/L in water and the pyruvic acid concentration were expressed in terms of (μmol/g freshweight (FW)).

Statistical analysis

All statistical calculations were made using OriginPro 8.1 software (OriginLab; Northampton, MA, USA). Results presented in tables are means ± standard deviations for 3 replicate samples. In chromatographic assays, each replicate solution was injected 2 or 3 times and the averaged peak areas were used to calculate analyte concentrations. The flavonoid, carbohydrate, pyruvic acid and enzymatic changes for long-term storage at different temperature were also determined by ANOVA followed by the Dunn-Sidak' s multiple range tests at $p = 0.05$..

Results and discussion

The onion cultivar 'Sunpower' was chosen for the study because of its high storability. In the present work, the development of inner sprouts was observed on longitudinally cut bulbs in different months at different storage temperatures. Visible sprouts appeared after 7 months at 4 °C and after 6 months at 10 °C, which is in agreement with previous results (Nam et al. 2011). At 25 °C, very less percentage of visible sprouting appeared and largely, the bulbs were not in good quality. After the dormancy break-in, which depends on temperature and other factors, onion bulbs enter the re-growth phase accompanied with a gradual increase in metabolic activity. Thus, the chemical composition of onions may be expected to change in relation to different storage temperatures. Many authors have indeed reported the biochemical parameters to be regular functions of the storage time, temperature and other parameters (necessary references are given below).

Weight loss and dry matter content

The dry matter of onion indicates that onion contains 91.60 % of volatile compounds, which largely includes water. Temperature and relative humidity are the most important factors that influence the dry matter (DM) percentage and moisture content. During storage, the weight loss in onions takes place due to desiccation, respiration, sprouting and all these processes are related to temperature, at higher temperature the weight loss is mainly due to desiccation (Ward 1976). Kamerbee (1962) noticed the weight loss of upto 6 to 14 % of the fresh weight due to respiration at 25 °C. In addition, Stow (1975)reported different levels of weight loss in onion stored at 30 °C with various levels of relative humidity. The increase in temperature results in the increase in activity of water and rate of reaction for the same moisture content, consequently affecting the quality of the onion. Yasin and Bufler (2007) reported that the most significant change in DM content occurred in sprouted leaves with the range of 16.5 % to 21.8 % after 4 weeks of storage. However, Kaack et al. (2004) found the dry matter content to be constant during 8 months storage at 5 °C with relative humidity of 75–80 %. The DM composition of the onion changes during storage, but it is independent of storage temperature between 4 °C and 37 °C (Darbyshire and Henry 1979). The present study shows no drastic change in DM during 9 months storage at three different temperatures. Relatively small DM fluctuations were observed at 10 °C and 25 °C (Table 1), corresponding to the dormancy breakage which indicated by onset of inner sprouting. However, we do not associate the DM changes to different storage temperature, but it is the physiological parameters such as dormancy and sprouting of onion which leads to change in DM. Table 1, also demonstrates the DM corrected for the weight loss, which provides additional clues on the mass balance of storage. Storage resulted in the degradation of one third of the organic matter of onions and the processes of conversion of organic substances to volatile compounds intensify during dormancy break and sprouting. The DM contents decreased relatively less throughout the storage period at 4 °C, 10 °C and 25 °C, but the weight loss was higher at 25 °C compared to 4 °C and 10 °C. The loss in the fresh

Table 1 Changes in the Dry matter (DM) content and corrected DM content for weight loss in onion bulbs kept in storage chamber at 4 °C, 10 °C and 25 °C

Storage time (Months)	DM % at 4 °C	Weight loss at 4 °C	DM content corrected for weight loss at 4 °C	DM % at 10 °C	Weight loss at 10 °C	DM content corrected for weight loss at 10 °C	DM % at 25 °C	Weight loss at 25 °C	DM content corrected for weight loss at 25 °C
0	8.27	0	8.27	8.27	0	8.27	8.27	0	8.27
1	8.92	1.66	8.77	8.73	0.93	8.65	8.42	1.79	8.27
2	9.03	2.48	8.80	9.01	2.31	8.80	8.60	3.71	8.28
3	8.49	3.08	8.22	8.10	2.79	7.88	8.26	8.39	7.56
4	7.88	4.09	7.56	7.93	0.78	7.87	8.19	8.61	7.49
5	7.48	5.30	7.08	8.16	4.31	7.80	7.83	13.58	6.76
6	7.39	5.54	6.98	7.39	5.57	6.98	7.39	15.4	6.24
7	7.89	5.53	7.45	7.18	8.14	6.59	7.18	22.90	5.53
8	7.75	9.88	6.98	7.34	9.64	6.64	7.82	26.32	5.78
9	7.33	11.66	6.48	7.05	13.86	6.07	8.03	28.96	5.70

weight increased with increase in temperature and weight loss at 25 °C was approximately thrice as much as at 4 °C.

Quercetin glucosides and their enzymes

Quercetin (in forms of aglycone, mono, diglucosides, isorhamnetin) was measured during the 9 months storage at three different temperatures. The effect of storage conditions on the changes in quercetin content in various onion varieties has been studied previously (Price et al. 1997; Mogren et al. 2007a, 2007b). However, only few reports are available on the curing and storage of onion at different temperatures (Downes et al. 2009; Chope et al. 2012). During the initial period of storage at 4 °C and 10 °C, the concentration of quercetin glucosidase increased, and gradually decreased in the later months, due to dormancy and the initiation of internal sprouts (Table 2). The enzymatic activity of both the enzymes (quercetin-4'G glucosidase and quercetin 4′-glucosyltransferase) increased upto six months with some fluctuation and later after six months it started to decrease at all studied temperature. The activity of quercetin-4'G glucosidase was very less as compared to quercetin 4′-glucosyltransferase at all the temperatures (Table 3). At 25 °C the total flavonoids increased continuously till 5th months, followed by a gradual decrease during 6th and 7th months. Interestingly, after 7 months, the concentration of total flavanoids increased again. We observed sprouting at all storage temperatures, but the time and rate of sprouting was different for all temperatures. Storage at 10 °C and 25 °C showed no significant increase in total flavonol and the possible reasons could be the morphogenesis (sprouting) and external decay (microbial, fungal attack) of bulbs at 10 °C and 25 °C respectively. The total concentration of the flavonoids was higher as compared to the fresh sample during the storage at all temperatures. The reason could be the decrease of surgars and the fall of sugars may provide substrates for the synthesis of flavonoids. However, little is known about the effects of long-storage on the retention of flavonols in whole onions. Some authors suggest that phytochemicals accumalations in vegetables depends on the initial levels within tissue. So, cultivars with low levels of compounds at harvest would have a higher phenolics accumulation in response to a biotic stress (Cantos et al. 2003). In addition, reports have shown that low temperature positively effect the biosynthesis of phenolics compounds and induce flavonoid accumulation (Cisneros-Zevallos 2003). At all storage temperatures, the concentration of quercetin diglucoside (QDG) and quercetin monoglucoside (QMG) changed with similar rate. The ratio of QDG: QMG increased slowly during the storage period with fluctuation during the 4th or 5th month depending on the temperature and internal sprouting. These findings indicate that QDG increase at the expense of the QMG, which coincides with previous results (Starke and Herrmann 1976). The constant ratio of QDG and QMG throughout the storage was reported by (Mogren et al. 2007a, 2007b). Over all, the behavior of the total flavonoids during storage fluctuates with its value higher during the storage as compared to fresh sample, except during dormancy break and sprouting. The reason for fluctuation can be the distribution of different glucosidaes in different part of onion as well as the other physiological process which change as per the storage temperature.

Onion bulbs contain glucosyltransferases that transform QMG to QDG (Q4'G 3-glucosyltransferase) and quercetin to QMG (quercetin 4′-glucosyltransferase). They also contain glucosidases that transform QDG to QMG (Q3, 4'G 3-glucosidase) and QMG to quercetin (Q4'G glucosidase) but the activity for the respective enzymes is very less (Tsushida and Suzuki 1996). In the present study, the enzyme activities of quercetin 4′-glucosyltransferase and Q4'G glucosidase was determined, and it was found that the activities of both the

Table 2 Flavonols content (μmol/g FW) in onions kept in the storage chamber for 9 months at 4 °C, 10 °C and 25 °C

	0	1	2	3	4	5	6	7	8	9
4 °C										
Q	0.05 ± 0.00 ^{ab}	0.04 ± 0.00^{a}	0.04 ± 0.02^{a}	0.05 ± 0.00^{ab}	0.06 ± 0.02^{b}	0.06 ± 0.00^{b}	0.03 ± 0.0^{a}2	0.04 ± 0.00^{abc}	0.04 ± 0.16^{a}	0.05 ± 0.16^{a}
QMG	0.16 ± 0.01 ^{ab}	0.41 ± 0.06^{a}	0.32 ± 0.03^{a}	0.38 ± 0.05^{a}	0.36 ± 0.08^{a}	0.30 ± 0.00^{a}	0.36 ± 0.04^{a}	0.34 ± 0.01^{a}	0.28 ± 0.02^{a}	0.39 ± 0.02^{a}
QDG	0.23 ± 0.02 ^{a}	0.63 ± 0.07 ^{b}	0.47 ± 0.03^{ab}	0.58 ± 0.04^{b}	0.44 ± 0.09^{a}	0.53 ± 0.00^{b}	0.64 ± 0.03^{abc}	0.62 ± 0.04^{ab}	0.52 ± 0.02^{a}	0.59 ± 0.00^{a}
IMG	0.03 ± 0.00 ^{b}	0.11 ± 0.01^{ab}	0.09 ± 0.00^{b}	0.11 ± 0.01^{a}	0.11 ± 0.00^{b}	0.10 ± 0.01^{abc}	0.09 ± 0.00^{a}	0.10 ± 0.00^{a}	0.08 ± 0.01^{a}	0.11 ± 0.00^{a}
10 °C										
Q	0.05 ± 0.00 ^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.10 ± 0.00^{a}	0.01 ± 0.02^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
QMG	0.16 ± 0.01^{abc}	0.19 ± 0.03^{a}	0.21 ± 0.02^{a}	0.12 ± 0.01^{b}	0.15 ± 0.02^{a}	0.19 ± 0.02^{a}	0.26 ± 0.03^{a}	0.20 ± 0.04^{a}	0.15 ± 0.01^{a}	0.15 ± 0.02^{a}
QDG	0.23 ± 0.02^{a}	0.29 ± 0.02^{bc}	0.31 ± 0.03^{b}	0.20 ± 0.03^{b}	0.28 ± 0.01^{ab}	0.31 ± 0.02^{b}	0.34 ± 0.02^{ab}	0.29 ± 0.03^{b}	0.25 ± 0.03^{b}	0.30 ± 0.00^{a}
IMG	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{abc}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.08 ± 1.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}
25 °C										
Q	0.05 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{abc}	0.01 ± 0.00^{a}	0.01 ± 0.00^{abc}	0.01 ± 0.00^{b}
QMG	0.16 ± 0.01^{b}	0.13 ± 0.03^{a}	0.16 ± 0.05^{a}	0.15 ± 0.02^{a}	0.14 ± 0.01^{a}	0.15 ± 0.02^{a}	0.16 ± 0.02^{a}	0.10 ± 0.04^{a}	0.14 ± 0.01^{ab}	0.14 ± 0.01^{a}
QDG	0.23 ± 0.02^{b}	0.25 ± 0.04^{b}	0.33 ± 0.08^{b}	0.33 ± 0.02^{b}	0.35 ± 0.02^{ab}	0.35 ± 0.02^{b}	0.44 ± 0.02^{a}	0.29 ± 0.04^{b}	0.34 ± 0.02^{a}	0.36 ± 0.01^{b}
IMG	0.03 ± 0.01^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.04 ± 0.02^{ab}	0.03 ± 0.02^{ab}	0.05 ± 0.03^{a}	0.03 ± 0.01^{ab}	0.04 ± 0.03^{abc}	0.03 ± 0.01^{a}

Calibration curve equations for flavonols content

flavonols	Equation	r^2
Q	x = (y- 0.0253)/0.00002038	0.9904
QMG	x = (y-0.0174)/0.00006383	0.999
QDG	x = (y- 0.05204)/0.0000117	0.9935
IMG	x = (y- 0.06046)/0.00003633	0.9965

At the 0.05 level, the mean difference values with different letters of superscript are significantly different from each other and the interaction between temperature and storage time is significant

Abbreviations: Q = quercetin aglycone; QMG = quercetin-4′-*O*-monoglucoside; QDG = quercetin-3,4′-*O*-diglucoside; IMG = isorhamnetin-3-glucoside

Table 3 Changes in quercetin Q4'G glucosidase and 4′-glucosyltransferase (nmole/gFW/min) in onions kept in the storage chamber for 9 months at 4 °C, 10 °C, 25 °C

	0	1	2	3	4	5	6	7	8	9
4 °C										
Q4'G glucosidase	2.38 ± 0.20^{ab}	2.88 ± 0.23^{b}	2.90 ± 0.12^{a}	3.00 ± 0.24^{a}	3.13 ± 0.22^{b}	3.48 ± 0.19^{ab}	3.80 ± 0.12^{ab}	3.12 ± 0.22^{ab}	2.98 ± 0.19^{a}	2.45 ± 0.23^{abc}
Q4'glucos-yltransferase	13.60 ± 0.40^{a}	14.20 ± 0.06^{b}	15.48 ± 0.04^{b}	16.01 ± 0.18^{a}	16.92 ± 0.48^{a}	17.56 ± 0.52^{a}	18.20 ± 0.47^{ab}	16.20 ± 0.32^{a}	15.80 ± 0.35^{a}	15.20 ± 0.34^{ab}
10 °C										
Q4'G glucosidase	2.38 ± 0.20^{ab}	2.48 ± 0.20^{ab}	2.59 ± 0.29^{ab}	2.98 ± 0.25^{ab}	3.03 ± 0.26^{a}	3.28 ± 0.20^{a}	3.40 ± 0.15^{a}	3.04 ± 0.22^{a}	2.78 ± 0.12^{a}	2.05 ± 0.29^{a}
Q4'glucos-yltransferase	13.60 ± 0.40^{b}	14.80 ± 0.45^{a}	16.48 ± 0.14^{b}	17.01 ± 0.49^{a}	18.92 ± 0.37^{b}	18.56 ± 0.11^{a}	18.90 ± 0.45^{ab}	18.20 ± 0.25^{a}	16.80 ± 0.32^{a}	15.52 ± 0.23^{a}
25 °C										
Q4'G glucosidase	2.38 ± 0.20^{a}	2.28 ± 0.17^{ac}	2.39 ± 0.23^{bc}	2.18 ± 0.12^{ab}	3.03 ± 0.25^{ab}	2.98 ± 0.26^{b}	3.28 ± 0.18^{ab}	2.29 ± 0.19^{a}	2.28 ± 0.21^{ab}	2.15 ± 0.22^{ab}
Q4'glucos-yltransferase	13.60 ± 0.40^{a}	14.62 ± 0.22^{b}	15.52 ± 0.03^{a}	14.55 ± 0.06^{a}	16.80 ± 0.44^{a}	16.20 ± 0.38^{a}	16.70 ± 0.38^{a}	16.00 ± 0.38^{a}	15.80 ± 0.33^{a}	15.20 ± 0.48^{ab}

Calibration curve equations for quercetin-4'G glucosidase and quercetin 4′-glucosyltransferase

Enzymes	Equation	r^2
quercetin-4'G glucosidase	x = (y- 0.0211)/0.000204	0.9995
quercetin 4′-glucosyltransferase	x = (y- 0.052037)/0.000363	0.9955

At the 0.05 level, the mean difference values with different letters of superscript are significantly different from each other and the interaction between temperature and storage time is significant

enzymes were higher at 4 °C as compared to 10 °C and 25 °C (Table 3). At 4 °C and 10 °C the quercetin 4′-glucosyltransferase activity increased consistently up to 6 months and then decreased thereafter, whereas at 25 °C the increase and decrease was observed between the initial time and the end of storage. These findings indicate that enzymatic activity was not the only reason for the increase in the concentration of total flavonoids (Q + OMG + QDG), but other physiological factors such as dormancy break and sprouting could be factors for controlling the total flavonoids. Hirota et al. (1999) studied the distribution of flavonols and enzymes responsible for the metabolism in onion and reported that the activities of glucosytransferase and glucosidase, were highest in the abaxial epidermis enriched with quercetin, QMG and QDG. Takahama et al. (2000) reported the concentration of flavonal and its enzymes in the different sections of onion bulbs from top to bottom and suggested that the concentration of quercetin was increased from bottom to top with low activities of glucosidase and transfrease at the top. However, QMG and QDG were higher at the bottom and the activity of glucosidase and transfrease decreased from bottom to top of the onion bulb. No activity of quercetin 4′-glucosyltransferase was observed at the top of the onion bulb. In the present study, we analyzed whole onion bulbs and found that the activity was higher at 4 °C which is correlating with total flavonoids, suggesting that these enzymes are more active with high metabolic rate in terms of dormancy break and sprouting.

Sugar content

In onion bulbs, the non-structural carbohydrates consist of glucose, fructose, sucrose and fructooligosaccharides (Salamal et al. 1990; Kaack et al. 2004;). During the 9 months storage at all three temperatures, the sugar content varied continuously. At 4 °C and 10 °C, the pattern for change in sugar content was almost similar, however, at 25 °C the change in sugar content was completely different. Initially during the 1st month, sugar content increased and then consistent reduction was observed in the following months. At all three different temperatures, the content of sucrose was higher during the storage period as compared to the fresh onion. At 4 °C and 10 °C, fructose and glucose reduced to half from 1st to 9 months storage (Table 4). At 25 °C, the glucose content increased during 2nd to 6th months and then decreased after 6th months, while the fructose decreased consistently after 2nd months (Table 4). These findings are in contrast with a previous study (Rutherford and Whittle 1982), which showed the total sugar content remained remarkably constant throughout the storage, when stored at 4 °C. Sprouting and fluctuation in sugar content in onion have been correlated in previous studies (Hurst et al. 1985; Pak et al. 1995; Benkeblia et al. 2002). In the present investigation, visible sprouting was found at different time intervals. The inner and visible sprouting could be the reason for the continuous decrease in fructose and glucose contents at 4 °C and 10 °C. While at 25 °C the pattern for the sugar content was different and very less sprouted bulbs were found till the end of the storage. Benkeblia and Varoquaux (2003) reported a decrease in sugar content as an indication of internal sprouting and loss of quality of onion. On the contrary, Hurst et al. (1985) and Pak et al. (1995) reported a sharp increase in glucose accumulation is related to internal sprouting. Similar fluctuations at the initial stage of storage were reported by Benkeblia et al. (2004) after the 4 week cold treatment at 0 °C, and subsequent transfer to 20 °C in the dark. Rouge Amposta were stored at 4 °C, 10 °C, and 20 °C and a peak of soluble sugars was observed between the 6th and 10th week of storage, the position of the peak was stable at the three investigated temperatures (Benkeblia et al. 2002). They related this peak to the intensification of fructan hydrolysis before the initiation of sprouting. However, in the present study, a different pattern of change in sugar content was observed at all temperatures used. An increase in the concentration of glucose and fructose has been associated with the onset of sprouting (Benkeblia and Selselet-Attou 1999). However, in the present study, the concentration of fructose and glucose decreased during the inner sprouting at 4 °C and 10 °C and the possible reasons for this decrease might be the sprouting after the 4 months storage. Chope et al. (2007a) suggested that the decrease in sugar concentration is correlated with an increase in sprout length, and sugars are metabolized to provide energy for the growing sprout. Nevertheless, the variation in glucose and fructose levels in onion is not clearly elucidated, as it depends on numerous factors, particular cultivar, sugar content and the dormancy release time, which affect largely the metabolism of sugars during the breakage of dormancy and the onset of sprouting.

Pyruvic acid

Onion pungency change during storage of onion have been associated with dormancy breakage. Pyruvic acid changed significantly at all three different storage temperatures. The greatest increase was observed at 10 °C followed by 4 °C and 25 °C. The change in pyruvic acid content of onion showed a similar pattern at 4 °C and 10 °C for the first 7 months and increased to 32.5 and 42.5 μmol/g of fresh weight (FW), followed by a decrease of 22.5 and 32.5 μmol/g FW, respectively (Table 5). The change in the study is related with dormany and sprouting becacuse the the 1st sprouting was observed at temperature at 10 °C and later at 4 °C. However, at 25 °C very less percentage of sprouting was observed and which is correlating with the low pyruvic acid. The pyruvic acid concentration fluctuated with increase and decrease till 6 months, followed by an increase of 22.2 μmol/g

Table 4 Carbohydrate content (mmol/g FW) in onions kept in the storage chamber for 9 months at 4 °C, 10 °C and 25 °C

	0	1	2	3	4	5	6	7	8	9
4 °C										
Fructose	0.13 ± 0.01^{a}	0.15 ± 0.00^{a}	0.11 ± 0.02^{ab}	0.12 ± 0.01^{a}	0.10 ± 0.01^{b}	0.10 ± 0.01^{a}	0.06 ± 0.00^{a}	0.07 ± 0.00^{a}	0.07 ± 0.01^{b}	0.09 ± 0.01^{a}
Glucose	0.15 ± 0.01^{ab}	0.19 ± 0.02^{ab}	0.16 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.14 ± 0.00^{a}	0.14 ± 0.01^{b}	0.13 ± 0.01^{ab}	0.13 ± 0.01^{b}	0.12 ± 0.01^{a}	0.10 ± 0.01^{b}
Sucrose	0.01 ± 0.00^{ab}	0.03 ± 0.00^{a}	0.03 ± 0.00^{ab}	0.03 ± 0.04^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}
10 °C										
Fructose	0.13 ± 0.01^{a}	0.16 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.00^{a}	0.11 ± 0.00^{ab}	0.11 ± 0.01^{ab}	0.06 ± 0.02^{ab}	0.06 ± 0.00^{b}	0.05 ± 0.01^{a}	0.08 ± 0.01^{a}
Glucose	0.15 ± 0.01^{ab}	0.18 ± 0.03^{ab}	0.15 ± 0.01^{bc}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.00^{a}	0.15 ± 0.01^{a}	0.11 ± 0.0^{b}	0.12 ± 0.01^{b}	0.13 ± 0.01^{b}
Sucrose	0.01 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.01^{ab}	0.02 ± 0.01^{a}	0.02 ± 0.03^{a}	0.02 ± 0.03^{a}	0.01 ± 0.00^{a}
25 °C										
Fructose	0.13 ± 0.01^{a}	0.10 ± 0.01^{ab}	0.08 ± 0.01^{a}	0.08 ± 0.0^{a}	0.07 ± 0.00^{a}	0.06 ± 0.01^{ab}	0.06 ± 0.00^{ab}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}
Glucose	0.15 ± 0.01^{ab}	0.13 ± 0.02^{a}	0.15 ± 0.01^{a}	0.14 ± 0.00^{ab}	0.14 ± 0.00^{ab}	0.16 ± 0.01^{a}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.13 ± 0.01^{a}
Sucrose	0.01 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.01^{ab}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}

Calibration curve equations for carbohydrate content

Carbohydrate	Equation	r^2
Fructose	x = (y + 0.009)/0.000000122	0.9975
Glucose	x = (y + 0.02005)/0.000000193	0.9985
Sucrose	x = (y + 0.00946)/0.000000117	0.9995

At the 0.05 level, the mean difference values with different letters of superscript are significantly different from each other and the interaction between temperature and storage time is significant

Table 5 Pyruvic acid content (μmol/g FW) in onions kept in the storage chamber for 9 months at 4 °C, 10 °C, 25 °C

	0	1	2	3	4	5	6	7	8	9
4 °C										
Pyruvic acid	19.20 ± 2.24^{a}	20.81 ± 2.24^{b}	23.85 ± 1.81^{b}	24.76 ± 2.23^{a}	26.00 ± 1.20^{a}	28.42 ± 2.30^{a}	29.71 ± 3.30^{ab}	32.50 ± 2.52^{a}	27.10 ± 2.20^{a}	22.50 ± 2.10^{ab}
10 °C										
Pyruvic acid	19.20 ± 2.24^{b}	21.90 ± 1.35^{a}	24.60 ± 2.00^{b}	26.00 ± 2.63^{a}	29.00 ± 1.22^{b}	35.00 ± 3.20^{a}	40.80 ± 2.54^{ab}	42.50 ± 1.80^{a}	36.40 ± 1.55^{a}	32.50 ± 2.10^{ab}
25 °C										
Pyruvic acid	19.20 ± 2.24^{a}	19.70 ± 2.36^{a}	17.20 ± 2.10^{b}	18.40 ± 1.88^{a}	16.80 ± 2.10^{b}	19.40 ± 1.22^{a}	19.70 ± 1.18^{ab}	18.00 ± 2.20^{a}	22.20 ± 1.02^{a}	20.50 ± 1.01^{a}
Calibration curve equations for pyruvic acid										
Pyruvic acid	Equation	r^2								
Pyruvic acid	x = (y + 0.004)/0.0000661	0.999								

At the 0.05 level, the mean difference values with different letters of superscript are significantly different from each other the interaction between temperature and storage time is significant

FW at 9 months of storage. Pyruvic acid is a reliable indicator of pungency, which is a stable product from the hydrolysis of *S*-alk(en)yl-l-cysteinesulphoxides (ACSOs). When the onion cells are disrupted by cutting and chopping, the enzyme alliinase hydrolyse the ACSOs to pyruvate and other stable products (Lancaster and Boland 1990). Pyruvic acid concentration increased with respect to the fresh sample during the storage at different temperatures, which is in agreement with previous findings (Hurst et al. 1985; Uddin and MacTavish 2003). Nevertheless, pyruvic acid content in onions depends on several factors such as dry matter, sugar content, cultivars, maturity and sulphur nutrition (Dhumal et al. 2007; Guo et al. 2007; Lee et al. 2009; Yoo et al. 2012). During storage, the pyruvic acid content also varied with changes in dry matter due to weight loss and dehydration of tissues, and this could be one of the possible reasons for the increase in pyruvic acid observed after long term storage. It was reported that onions exhibit an increase in pungency or pyruvic acid just before the bulbs sprout, due to the release of S-(1-propenyl)-L-cysteine sulfoxide (1-PeCSO) from γ -glutamylcysteinsulfoxide by γ -glutamyl peptidase (Schwimmer and Austin 1971; Lancaster and Shaw 1991). After dormancy breakage the flavor precursor decreased, and the pyruvic acid are stable product of the flavor precursor and is bulb pungency indicator (Randle and Bussard 1993). In this study, the increase was observed before sprouting at 4 °C and 10 °C. Yoo et al. (2012) reported a decrease in pungency at 30 °C storage with the feasible loss, while long-term storage at ambient cold temperature (approximately 5 °C) causes an increase in pungency.

Peroxidase activity

Peroxidase are known to be involved in flavonoid oxidation. At all temperatures, the peroxidase (POD) activity followed the similar pattern, but at different time scales. At 4 °C the POD activity increased upto 120 units/100 g FW during the initial 5 months and later at the end of storage it maintained to 100 units/100 g FW. The varying activity of peroxidase is not correlating with flavonoids which indicated that during the storage instead of the flavonoid oxidation, there was an accumulation. Similar trend was observed at 10 °C, but with higher rate. At 25 °C, POD activity increased slightly from 120 to 130 units/100 g FW during the 2nd months and decreased progressively from 130 to 100 units/100 g FW during the next 3 months and remained stable during the last 3 months of storage (Table 6). The first decrease in POD activity at either temperature coincided with the onset of bulb sprouting and low temperature was less significant for POD activity. It was reported previously that sprout initiation leads to increase the POD activity (Benkeblia 2000) and the increase in POD activity results in the oxidation of quercetin and formation of new compounds, which is the indication of sprouting (Takahama and Hirota 2000). Benkeblia and Selselet-Attou

Table 6 Peroxidase activity (units/100 g FW) in onions kept in the storage chamber for 9 months at 4 °C, 10 °C, 25 °C

	0	1	2	3	4	5	6	7	8	9
4 °C										
POD	88.0 ± 7.10^{ab}	90.0 ± 7.10^{a}	95.0 ± 7.10^{b}	98.2 ± 9.44^{ab}	101.0 ± 7.10^{ab}	120.0 ± 4.22^{b}	110.0 ± 8.70^{a}	81.7 ± 6.00^{a}	85.0 ± 5.44^{ab}	100.0 ± 6.22^{a}
10 °C										
POD	88.0 ± 7.10^{a}	98.0 ± 6.33^{ab}	105.0 ± 4.52^{ab}	120.0 ± 3.24^{a}	135.0 ± 2.58^{ab}	150.0 ± 7.92^{b}	120.0 ± 4.50^{b}	122.4 ± 6.20^{b}	124.5 ± 4.88^{a}	125.0 ± 5.66^{a}
25 °C										
POD	88.0 ± 7.10^{ab}	120.0 ± 8.10^{b}	130.0 ± 5.99^{a}	122.8 ± 6.22^{a}	108.9 ± 4.15^{abc}	100.0 ± 6.93^{a}	99.6 ± 5.7^{ab}	98.0 ± 2.94^{ab}	97.0 ± 2.44^{a}	98.0 ± 6.45^{b}

At the 0.05 level, the mean difference values with different letters of superscript are significantly different from each other the interaction between temperature and storage time is significant

(1999) associated the POD activity with total phenolics, while in the present study, the quercetin and its glucosidase and the POD activity did not show any correlation at all temperatures. It could be suggested that besides quercetin, there might be some other phenolic compounds that can be correlated with POD activity.

Conclusion

Fluctuation in the content of nutraceuticals is not an isolated phenomenon in storage studies. It is probably explained by superposition of a multitude of metabolic processes involving the target compound (resulting in both accumulation and consumption) that proceeds in the bulb with different rates. It can be hypothesized that sprouting and regrowth phase can vary at different temperatures. According to storage temperature and time, sprouting phase leads to slow decrease in the chemical composition and later the regrowth phase characterized with reduced DM, sugar, and pyruvic acid contents with relative increase in total flavonols content. The quercetin and it glucosides increased during the storage with some fluctuation, but the increase cannot be directly correlated with the enzymatic activies. The increase in the content of flavonoids and decrease in sugar content could be correlated with the other physiological factors such as dormancy break and sprouting. This physiological factors of onion are controlled by the enivornment in which it is stored. Browning of onion scales during aging is caused by the autoxidation of quercetin glucosides, after their deglucosylation later after storage the POD activity could enhance the quercetin oxidation. Presumably, a change in temperature is the most influencing factor during the sprouting phase and this hypothesis explains our findings..

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