Quantitative analysis of flavonoids, sugars, phenylalanine and tryptophan in onion scales during storage under ambient conditions

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Abstract A comprehensive quantitative analysis of flavonoids, sugars, phenylalanine, and tryptophan have been carried out in different onion scales during storage at ambient temperature (20–23 °C) and relative humidity (60–80 %). Depending on the length of storage, dry matter content and composition shows variation inside the onion bulbs. Inner sprouts were observed on longitudinally cut bulbs after 2 months and visible sprouts appeared after 5 months of storage. The bulbs lost 20 to 30 % of their weight at the end of the storage. Higher dry matter content was observed in the inner scales. Significantly high content of quercetin in inner scales and high level of quercetin-3,4'-O-diglucoside and quercetin-4'-O-monoglucoside in outer scales was observed during a 7 months storage. During storage period, high content of fructose and glucose was observed in the middle scales while sucrose was high in the inner scales. There was no particular trend observed within analyzed amino acids. However, the content of phenylalanine was higher than tryptophan.

Keywords Onion · Scales · Flavonols · Sugars · Amino acids · Storage

Introduction

Onion (*Allium cepa* L.) is remarkably nutritious and commonly used flavoring vegetable in a great variety of food ingredients. Onions contain various types of phytonutrients, which are beneficial for onion plant and human being as well. Flavonoids are the major compounds in onion classified as polyphenolic compounds, which are responsible to protect the plants against UV light, fungal parasites, herbivores, pathogens and also prevent from oxidative cell injury (Cook and Samman 1996). In onions, quercetin-3, 4'-O-diglucoside and quercetin-4'-O-monoglucoside, are the two major components of flavonols that constitute up to 85 % of the total flavonoids content (Price and Rhodes 1997). Grzelak et al. (2009) confirmed that the content of quercetin glycosides in outer and middle scales of onion are $3,386.3 \pm 190$ and $1,017.4 \pm 188$ mg/100 g dry matter (DM), respectively. The thermal stability of onion, which depends on the ratios of flavonol glucosides is very important for food processing industries. The characterization of flavonoids in different onion cultivars on the basis of different scales and parts of onion have been carried out by many researchers (Price and Rhodes 1997; Marotti and Piccaglia 2002; Bonaccorsi et al. 2005). The change in the levels of flavonoids have been studied, in various onion cultivars and according to the cultivation method, storage conditions, handling at harvest, thermal and domestic processing (Price and Rhodes 1997; Lee et al. 2008). The amount of flavonoids in different parts of the onion bulb increases in the following order: inner layers < middle layers < outer scales

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(Bilyk et al. 1984; Prakash et al. 2007). Reason behind this development is different light exposure and varietal allocation of the enzymes participating in metabolism of onion bulbs (Hirota et al. 1999; Lee et al. 2008). It has been reported that synthesis of phenolic compound in plants is controlled by the phenylalanine ammonia-lyase (Benkeblia 2000). The phenylalanine ammonia-lyase catalyzed the deamination of L-phenylalanine to yield transcinnamic acid and ammonia.

Fresh onion contains 80 to 85 % moisture and up to 80 % of the dry matter is non- structural carbohydrates (Darbyshire and Henry 1979). Glucose, fructose, and sucrose and low molecular weight fructans are important non-structural carbohydrates of onion. During the storage period of onion, metabolic activity takes place, which results in the variation of sugar contents (Rutherford and Whittle 1982; Hurst et al. 1985; Benkeblia et al. 2002; Benkeblia and Varoquaux 2003). The phytochemical translocation is also affected by various pre- and post-storage conditions. After harvesting, storage is very important for fortification of phytonutrients and especially to the semi perishable crop such as onion because it gets deteriorated easily. Storage losses of onions are caused due to rotting, sprouting, and physiological weight loss. Physiological weight loss is higher in first month, thereafter; it reduces with a little exception from 60th to 75th days, the weight loss increases as a result of rotting and sprouting (Biswas et al. 2010).

Some of the previous works on the composition analysis of onions includes the flavonoids and sugar content in fresh and during postharvest in whole onion bulbs were reported by Bilyk et al. (1984); Price and Rhodes (1997); Prakash et al. (2007); Grzelak et al. (2009). Various onion scales have been analyzed for flavonoids, sugar content, non-structural carbohydrate and pyruvate in fresh onion (Slimestad and Vågen 2009). However, to the best of our knowledge the change in the concentration of flavonoids, sugars and amino acid in onion scales that may result from the metabolic activities occurring during storage is not reported yet. The aim of the present study is to gain knowledge of the distribution of phytochemical in onion scales during storage and which may be important in food application industry and for the retailers of onion. Moreover, during storage outer and inner scales of onion get affected more as compared to middle one due to environmental effects (temperature, light, disease etc) and sprouting respectively. The differences in the chemical composition revealed within the edible scales of onion bulbs might fit with different food applications of onion. For instance, as the storage time increases inner and outer layers get deteriorated in terms of quality (black spots form on the outer layer and sprouting at the inner) and become less attractive for food consumption. Although the outer and inner layer of onions is not suitable in terms of quality but the middle layer can be used for culinary practices and also in small food industries for making pickles and chutneys. Furthermore, for

analytical sampling of long stored onion, this knowledge has to be considered within research organizations and food industry. Therefore, it is important to study the change in chemical composition in scales during storage.

Materials and methods

Chemicals and standard solutions

All solvents used in this study were of HPLC grade. Water was from J.T. Baker (Phillipsburg, NJ, USA), methanol from Duksan (Seoul, Korea), acetonitrile from Daejung (Gyeonggi-do, Korea) and chloroform from Burdick and Jackson (Ulsan, Korea). Trifluoroacetic acid (extra pure grade) was supplied by Alfa Aesar (Ward Hill, MA, USA). Tryptophan, phenylalanine, and quercetin used as standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). 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 controlled by HPLC and found to be better than 99 %. 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. Those of amino acids and sugars were prepared in water, with concentration 2.6 and 50 mg/ml, respectively. 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 Bioenergy Crop Research Center, National Institute of Crop Science, Rural Development Administration (Muan, Republic of Korea). Onions harvested in mid June 2011 were cured in the field for 10 days transported to our laboratory. The bulbs in a weight range 160–220 g with no visible defects were taken for this study. The bulbs used were not irradiated and stored in open cardboard boxes in one scale, each bulb was weighed and labeled to measure the weight lost during storage. Onions were kept in a dark place in a storage room equipped with an air conditioning system at temperature 21–23 °C. Humidity fluctuated according to weather conditions between 60 % and 80 %. This experiment was modeled by domestic storage conditions. Eight bulbs were randomly sampled for analysis at day 0 and then every 1 month until 7 months. The selected bulbs were cleaned and separated from the roots and outer dry scale. Further, these bulbs were subdivided into three different parts viz. outer scales (scales

1–2), middle scales (scales 3–4) and inner scales (scales 5–6, 7). Each individual different part were chopped into small pieces, and mixed thoroughly to obtain a representative sample from all eight bulbs.

Moisture content

The percentage of dry onion bulbs was determined by drying chopped samples of approximately 25 g in an oven with air circulation first at 80 °C for 24 h and then at 105 °C for 2 h. Every determination was made in triplicates.

Analysis of flavonoids

Flavonoids were extracted in triplicate according to a method described by Bonaccorsi et al. (2005) with slight modification. Approximately 10 g of a chopped sample were left overnight in 100 ml of methanol at 4 °C. Then methanol extract was separated and the residue was homogenized with a blender 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 and 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 45 °C to approximately 8 ml and made up to 10 ml with methanol. The extracts were stored at –20 °C if not used immediately.

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 Lichrospher 100 RP-18 (250 mm × 4.6 mm) column with particle size of 5 µm (Merck KGaA, Darmstadt, Germany) protected with a Phenomenex (USA) C18-type guard column. The column was maintained at 25 °C. The mobile was consisted of 0.1 % TFA in water (solvent A) and methanol (solvent B). A gradient elution program was 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 a respective calibration curves. Chromatographic analysis of each replicate sample was repeated twice, and the average peak areas were used in calculations.

Analysis of sugars

Glucose, fructose and sucrose content were determined according to Benkeblia et al. (2002) with some modifications. Samples of 5 g of chopped onion tissues were homogenized in

50 ml of water. The homogenate was heated for 30 min in a boiling water bath and after cooling, the homogenate was centrifuged (10,000 rpm, 40 min, 4 °C). The supernatant was collected and the residue was suspended in 50 ml of water, stirred for 30 min, and again centrifuged. The two supernatant phases were pooled and evaporated on a rotary evaporator to approximately 8 ml. The concentrated solution was transferred in a measuring flask for 10 ml and brought to the mark with water. The extracts were stored at –20 °C if not used immediately.

Twenty microliters of an extract were injected on a Zorbax Carbohydrate (150 × 4.6 mm) column from Agilent (Palo Alto, CA, USA) protected with an Agilent NH₂ pre-column. The sample was eluted with acetonitrile/water (75:25, v/v) as recommended by the manufacturer. The column temperature was maintained at 30 °C and the flow rate was 1 ml/min. 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. Chromatograms were integrated using the Shimadzu Class-VP software. Each injection was repeated two or three times.

Analysis of amino acids

Amino acids were extracted in triplicate according to Rhodes et al. (1986) with some modifications. Five g of chopped onion tissues were mixed with 25 ml of acetonitrile and 15 ml of water and left overnight at 4 °C. Then 60 ml of chloroform were added followed by homogenization with a blender. The homogenate was left for 2 h at 4 °C for phase separation. When the phase separation was accomplished, the aqueous layer was collected and centrifuged (10,000 rpm, 40 min, 4 °C). The supernatant was collected and the residue was suspended in 40 ml of acetonitrile:water (25:15, v/v), stirred for 30 min, and again centrifuged. The two supernatant phases were pooled and evaporated on a rotary evaporator to approximately 8 ml. The concentrated solution was transferred in a measuring flask for 10 ml and brought to the mark with water. The extracts were stored at –20 °C if not used immediately.

Chromatographic analysis was performed with the Agilent 1100 HPLC system described above at temperature 25 °C. A Chromolith HR RP-18e monolithic column (100 mm × 4.6 mm) from Merck KGaA (Darmstadt, Germany) was used. Amino acids were eluted with a linear gradient of 0–80 % of water in methanol over 20 min with the flow rate set at 0.8 ml/min. The sample size was 10 µl. Chromatograms were recorded at a wavelength of 210 nm. Quantification of phenylalanine and tryptophan was made through comparison with the respective calibration curves. Chromatographic analysis of each

replicate sample was repeated twice, and the average peak areas were used in calculations.

Statistical analysis

Results presented in graphs are means $\pm$ standard deviations for 3 replicate samples. In chromatographic assays, each replicate solution was injected 2 times, and the averaged peak areas were used to calculate analyte concentrations. Differences between mean values were assessed by Student's *t* test with a significance level of $p < 0.05$. All statistical calculations were made using OriginPro 8.1 software (OriginLab; Northampton, MA, USA).

Results and discussion

The onion cultivar 'Sunpower' was chosen for this study because of its high storability. Storability of the onion can be defined as extending the availability of onion to the customer without compromising the quality or the capability of the bulb to retain firmness and integrity of the outer layer and to persist the sprouting and rooting. Among the onions grown in Korea, *cv.* Sunpower seems to have the highest storability and reported that 'Sunpower' bulbs begin to sprout after 170 days of ambient storage and only 5 % of bulbs sprout after 230 days after harvest (Nam et al. 2011). For comparison, 20 % of bulbs of the next most storable cultivars 'Magic Gold' and 'New Turbo' sprouted for the same period of time (Nam et al. 2011). Onset of sprouting is commonly preferred to characterize the storage life of onions because of its clear relationship to physiological processes in bulbs (Pak et al. 1995; Chope et al. 2007a; Yasin and Bufler 2007; Bufler 2009). In the present work, the development of inner sprouts was observed on longitudinally cut bulbs after 2 months of storage and visible sprouts appeared after 5 months in a good agreement with the above mentioned results of Nam et al. (2011).

Sprouting is an indication of the emergence from dormancy. During the dormancy, which supposedly started near harvest and varies from several weeks to months depends on the various cultivars (Miedema 1994; Yasin and Bufler 2007), many physiological processes are slowed down, although not ceased (Pak et al. 1995; Carter et al. 1999; Yasin and Bufler 2007; Brewster 2008). After the dormancy breaking, onion bulbs enter the regrowth phase that is accompanied by gradually increasing metabolic activity. Metabolic activities within the onion are responsible for the damage of tissue and thus, the chemical composition of onions may be expected to change in a certain relation to the dormancy duration. Many researcher reported that the biochemical composition to be regular functions of storage time, usually the change of a trend, in the neighborhood of the dormancy breaking date (necessary references are given below where appropriate).

On other hand, others reported the irregular dependencies correlated to the temperature rather than dormancy duration (Hurst et al. 1985; Salamal et al. 1990).

Weight loss and dry matter content

Bulb weight is an integral characteristic determined by a superposition of respiration and desiccation processes. During ambient storage bulb weight normally reduces (Ward 1976), but to increase the storability and quality of onion the bulb weight decelerated with the help of mitosis inhibitors, controlled atmosphere, low temperature (Hurst et al. 1985; Yoo and Pike 1996; Praeger et al. 2003). In present study, bulb weight was steadily decreasing (Table 1) and finally ended up with the loss of almost 30 % of the initial weight. The DM content another integral indicator that directly correlates to the nutritional value of crops. It also depends on respiration and other metabolic processes causing changes in the organic matter of the bulbs. In the present work the dry matter content increased regularly towards inner scales except for 3 and 4 month storage where slight inconsistency within the scales was observed and represented in Table 1. The outer scales have been found to have low dry matter content as compared to middle and inner scales. The total dry matter content decreased slowly during storage period. Significant differences in dry matter content were observed in onion scales.

The results were consistent with what was previously reported (Darbyshire and Henry 1979; Slimestad and Vågen 2009). On the contrary, (Kaack et al. 2004) found dry matter content to be constant during 8 months of storage at 5 °C and relative humidity of 75–80 %. It has been suggested that scales dry matter content is not good predictor of total dry matter content of the whole bulb (Lancaster and Kelly 1984). Chope et al. (2007b, 2012) have reported that field site and cultivar but not storage mostly affect onion dry matter (DM), although a DM loss can be prevented by the application of 1-methylcyclopropan, an ethylene-action inhibitor (Chope et al. 2007a).

Flavonoids content

The distribution of some flavonoids was determined in different scales of onions during 7 months storage. It was observed that outer scales were more enriched compared to middle and inner scales. A general increase in the total sum of flavonoids analyzed was seen, whereas fluctuations were observed in different onion scales. In each scales the content of quercetin-3,4'-*O*-diglucoside was higher than quercetin-4'-*O*-monoglucoside and these ratio maintained during the storage period. The outer fleshy scales contain higher water content and also highest concentration of flavonoids per dry weight. Table 1 shows stepwise increase in quercetin content in inner scales during storage whereas in middle and outer

Table 1 Variation in certain constituents of different layers of onions on storage

S. No.	Parameter	Onion layer	Storage period (months) at 21–23 °C and 60–80 % relative humidity (mean ± SD)							
			0	1	2	3	4	5	6	7
1	Weight loss (%)	Total	0	1.96	1.3	2.9	6.9	10.2	14.3	21.31
2	Dry weight (%)	Outer	9.23	8.92	8.64	8.52	8.27	8.12	8.00	7.91
		Middle	9.49	9.01	8.92	8.60	8.45	8.62	8.34	8.12
		Inner	9.91	9.62	9.72	8.84	8.51	8.92	8.56	8.50
		Total	28.63	27.28	27.28	25.96	25.23	25.66	24.9	24.53
3	Quercetin ($\mu\text{mole/g DW}$)	Outer	0.15±0.02	0.21±0.04	0.18±0.02	0.15±0.01	0.16±0.01	0.15±0.01	0.14±0.02	0.20±0.04
		Middle	0.09±0.01	0.11±0.03	0.11±0.01	0.11±0.01	0.11±0.02	0.11±0.01	0.12±0.04	0.10±0.01
		Inner	0.53±0.07	0.49±0.06	0.82±0.09	1.06±0.08	1.14±0.13	1.13±0.10	1.28±0.01	1.21±0.09
		Total	0.77±0.10	0.81±0.13	1.11±0.12	1.32±0.10	1.41±0.16	1.39±0.12	1.54±0.07	1.52±0.14
4	Quercetin-4'-O- monoglucoside ($\mu\text{mole/g DW}$)	Outer	3.75±0.37	5.07±0.28	4.82±0.24	4.06±0.58	4.24±0.33	4.42±0.32	4.92±0.42	5.17±0.83
		Middle	1.79±0.24	2.25±0.27	2.04±0.28	1.96±0.40	1.82±0.54	1.95±0.47	2.00±0.35	2.31±0.42
		Inner	1.78±0.33	2.75±0.23	2.24±0.21	3.53±0.21	4.03±0.32	3.81±0.22	3.22±0.42	2.80±0.19
		Total	7.32±0.94	10.07±0.72	9.10±0.73	9.55±1.19	10.09±1.19	10.18±1.01	10.14±1.19	10.28±1.44
5	Quercetin-3, 4'-O -diglucoside ($\mu\text{mole/g DW}$)	Outer	6.69±0.40	5.63±0.60	5.54±0.42	5.60±0.90	5.82±0.72	5.71±0.54	9.33±0.53	10.02±0.38
		Middle	3.89±0.30	2.49±0.18	2.84±0.14	3.67±0.39	3.95±0.32	4.23±0.42	5.54±0.52	6.18±0.93
		Inner	2.39±0.49	4.34±0.37	4.76±0.38	4.82±0.42	4.62±0.42	4.53±0.34	4.22±0.30	4.15±0.30
		Total	12.97±1.19	12.47±1.05	13.14±0.94	14.09±1.71	14.39±1.46	14.47±1.20	19.09±1.35	20.35±1.61
6	Fructose (mmole/g DW)	Outer	1.86±0.09	2.02±0.07	1.72±0.05	1.40±0.24	1.04±0.09	1.12±0.09	1.34±0.09	0.86±0.07
		Middle	1.95±0.30	2.32±0.17	2.13±0.24	1.89±0.26	1.85±0.23	1.86±0.21	1.80±0.11	1.07±0.06
		Inner	1.76±0.06	1.80±0.04	1.56±0.13	1.17±0.06	1.16±0.05	1.14±0.04	1.13±0.06	0.46±0.07
		Total	5.57±0.45	6.14±0.28	5.41±0.42	4.46±0.56	4.05±0.37	4.12±0.34	4.27±0.26	2.39±0.20
7	Glucose (mmole/g DW)	Outer	2.36±0.35	2.57±0.21	2.43±0.46	2.55±0.29	2.30±0.40	2.24±0.42	2.11±0.44	1.18±0.14
		Middle	2.71±0.40	2.85±0.33	2.87±0.32	2.89±0.25	2.45±0.42	2.42±0.43	2.32±0.42	1.57±0.19
		Inner	2.52±0.09	1.89±0.02	1.78±0.13	2.24±0.06	2.14±0.22	2.01±0.32	1.90±0.06	0.98±0.17
		Total	7.59±0.84	7.31±0.56	7.08±0.91	7.68±0.60	6.89±1.04	6.67±1.17	6.33±0.92	3.37±0.5
8	Sucrose (mmole/g DW)	Outer	0.13±0.04	0.14±0.05	0.32±0.04	0.46±0.04	0.26±0.05	0.21±0.04	0.22±0.04	0.14±0.02
		Middle	0.21±0.03	0.44±0.04	0.56±0.06	0.62±0.08	0.42±0.04	0.46±0.05	0.45±0.08	0.20±0.05
		Inner	0.49±0.04	0.45±0.04	0.63±0.07	0.78±0.03	0.52±0.01	0.63±0.07	0.55±0.09	0.27±0.05
		Total	0.83±0.11	1.03±0.13	1.15±0.17	1.86±0.15	1.20±0.10	1.30±0.16	1.22±0.21	0.61±0.12
9	Phenylalanine ($\mu\text{mole/g DW}$)	Outer	4.61±1.03	2.20±0.18	3.42±0.20	5.97±0.70	5.05±0.24	5.52±0.32	6.24±0.12	4.28±0.59
		Middle	4.28±0.67	4.66±0.21	5.31±0.36	6.48±1.02	6.28±0.42	6.82±0.42	7.24±0.31	4.68±0.72
		Inner	5.91±0.90	5.49±0.96	5.47±1.01	5.69±0.89	5.23±1.04	5.23±0.92	5.11±0.80	4.95±0.59
		Total	14.80±2.60	12.35±1.35	14.2±1.57	18.14±2.61	16.56±1.70	17.57±1.66	18.59±1.23	13.91±1.90
10	Tryptophan ($\mu\text{mole/g DW}$)	Outer	2.05±0.40	1.46±0.26	3.92±0.42	4.45±0.19	1.04±0.24	4.92±0.23	5.24±0.77	2.74±0.18
		Middle	2.47±0.48	3.72±0.85	4.21±0.42	5.24±0.60	4.92±0.12	5.32±0.39	5.54±0.44	3.21±0.04
		Inner	4.79±0.88	4.44±0.58	4.63±0.72	3.18±0.45	2.78±0.56	3.92±0.42	4.96±0.43	3.56±0.14
		Total	9.31±1.76	9.62±1.69	12.76±1.56	12.87±1.24	8.74±0.92	14.16±1.04	15.74±1.64	9.51±0.36

scales it is fairly constant. The total quercetin content increased monotonously except a slight drop at the 5th and 7th month storage but the overall trend has been a positive one. The total lowest and highest quercetin level was 0.78 $\mu\text{mole/g DW}$ and 1.55 $\mu\text{mole/g DW}$ in fresh and after 6th month of storage respectively. It can be seen that, during storage quercetin content increased in the order middle < outer < inner. Table 1 shows no increase in quercetin-4'-O-

monoglucoside among scales during storage. The outer scales was found to contain higher content of quercetin-4'-O-monoglucoside compared to middle and inner scales and it increased to 5.07 $\mu\text{mole/g DW}$ after 1 month storage, then decreased to 4.06 $\mu\text{mole/g DW}$ during 3rd month. After 3 month storage quercetin-4'-O-monoglucoside shows a gradual increase. The middle scales contain nearly constant quercetin-4'-O-monoglucoside during the storage period.

The inner scales showed slow increase during 4 month storage and then decreased slowly. The highest content of quercetin-4'-*O*-monoglucoside in inner layer was 4.03 $\mu\text{mole/g DW}$ during 4 month storage whereas lowest was observed in fresh sample, 1.78 $\mu\text{mole/g DW}$. During storage, a slow increase in quercetin-3,4'-*O*-diglucoside content was observed in outer and the middle scales (Table 1). The highest concentrations, 10.01 $\mu\text{mole/g DW}$ and 6.18 $\mu\text{mole/g DW}$, were recorded for outer and middle scales respectively after 6 month storage. The concentration of quercetin-3,4'-*O*-diglucoside was fairly constant in inner scales and highest was observed at 4th month of storage.

During 7th month storage at ambient temperature, the total quercetin is increased. However, there was inconsistency observed in different scales. During 5 month storage at 5, 24 and 30 °C (Patil et al. 1995) found an inconsistent pattern of quercetin content due to sprouting. Relation between flavonoids and dormancy breakage are not yet clear and not been studied here. Further, decrease in phenolic content was observed after dormancy break (Benkeblia and Selselet-Attou 1999). Our results are in contrast with Price et al. (1997), where they noticed continuous decrease in quercetin glucosides during the storage of 168 days. Previously it was reported Benkeblia et al. (2004) total phenolic content of inner buds for control sample (stored at 18 °C) increased during 5th week and then decreased progressively during last 3 weeks of storage. On the contrary, Gennaro et al. (2002) reported that for flavonoids in red onion tends to decrease after 6 weeks storage especially at 20 °C and 30 °C. The total phenolic content in onion tissue was increased during the 6th week storage and after that started to decrease at 20 °C while reverse observed at 4 °C (Benkeblia 2000).

Sugars content

Carbohydrates account for a major portion of the dry weight of onion bulbs and include glucose, fructose, sucrose, and fructooligosaccharides (fructans) with the degree of polymerization 3 to 12 (Darbyshire and Henry 1979; Suzuki and Cutcliffe 1989; Salamal et al. 1990; Kaack et al. 2004).

Carbohydrate also play important role in the physiology of onion with source-to-sink transition. The source for onion bulb is the carbohydrate (Fructans) produced from sucrose and fructose and accumulated in scales during bulbing and sinks where it been consumed for the purpose of regrowth phase in onion (Pak et al. 1995; Benkeblia et al. 2005). The source to sink transition is responsible for the metabolic activity in onion. Fructans may be translocated to the sink tissues directly through the phloem, or be hydrolyzed first, then transported and resynthesized (Benkeblia et al. 2005; Yasin and Bufler 2007). Reallocation and conversion of fructans accompanied by the production and consumption of sucrose, fructose, and glucose (a by-product of fructan

synthesis (Vijn et al. 1998)) in combination with own metabolic transformations of mono- and disaccharides involved in respiration, osmotic regulation and other processes result in different time-dependent profiles of sugars in different parts of the bulb (Rutherford and Whittle 1982; Salamal et al. 1990; Pak et al. 1995; Hansen 1999; Yasin and Bufler 2007). There are conflicting reports on the fate of sucrose and the monocarbohydrates in the whole bulbs during storage, as follows from a comprehensive summary compiled by Chope et al. (2007b).

In present case the variation in sugar content in onion scales during the storage period is observed. This variation suggests that sugar content highly depends on the physiological and metabolic activity within the onion. In Table 1, outer scales contain higher fructose content with respect to inner ones. In outer scales, fructose content increased slightly from 1.86 to 2.03 mmole/g DW during 1 month storage, and then content decreased from 2.03 to 1.04 mmole/g DW during 4 months storage. After 7 month storage, fructose content reached to 0.86 mmole/g DW. The middle scales contain higher percentage of fructose as compared to the outer and inner scales. In the middle scale, fructose content changed from 1.95 to 2.32 mmole/g DW after 1 month and remained in steady state during the 6 months. At the end of the experiment, it decreased to 1.07 mmole/g DW. Distribution of fructose in inner scales during storage period follows similar pattern similar to that of the middle scales. In outer scales (Table 1), the glucose content increased slightly from 2.36 to 2.57 mmole/g DW after 1 month and showed a slight decreasing pattern till the end of the storage (1.18 mmole/g DW). The distribution of glucose is higher in the middle scales throughout the storage period as compared to outer and inner scales. In middle scales glucose content increased slightly from 2.70 to 2.89 mmole/g DW after 3 months storage and then gradual decrement was observed till end of the storage period (1.57 mmole/g DW). Inner scales follow similar trend for glucose as in middle scales. However, the concentration of glucose is less in the inner scales.

After prolong storage period and when sprouting starts, a considerable increase in the amount of sugars such as fructose and glucose takes place. There are conflicting reports on the variation of glucose and fructose in onion during the storage period. In terms of total sugars, the present results are in agreement with the report of Benkeblia et al. (2002) and in contrast with the finding of Hurst et al. (1985). The levels of fructose have also been reported to be increased during 1 month storage and later it decreased (Benkeblia et al. 2002; Benkeblia and Varoquaux 2003). On contrary, Downes et al. (2010) reported the decrease of fructose during initial storage and sudden increase after 12 week of storage. Previous work Benkeblia and Selselet-Attou (1999) showed that increase in the concentration of glucose and fructose is associated with onset of sprouting. Present results are not in

agreement with a previous study by (Rutherford and Whittle 1982), showing that the total sugar content remain remarkably constant before sprouting. The present results confirm the claim of (Slimestad and Vågen 2009) about the distribution of fructose and glucose in different scales of onion. The increase in fructose content and occasionally glucose may be due to the hydrolysis of fructans.

The distribution pattern for sucrose in scales was different from glucose and fructose (Table 1). The outer scales contain less sucrose with respect to the middle and inner scales. During 3 months, sucrose content increased stepwise in outer and middle scales and after that gradual decrease noticed with increase in storage period. The inner scales were found to contain high percentage of sucrose. The content of sucrose peaked after 2 months of storage and reached to 0.78 mmole/g DW. Afterwards, it started to decrease till the end of 7 month storage. The present results for distribution of sucrose in scale are coinciding with report of (Ogata 1961; Slimestad and Vågen 2009) in which they observed the high content of sucrose in inner scales. However, no change in sucrose content during 16 month storage was reported by (Yasin and Bufler 2007). In the present study, the variation in sucrose content is characterized by the alternate increase or decrease in its concentration, which is not correlated with the trends of glucose and fructose. Our repeated experiments on this point concludes sucrose is deviated from glucose and fructose. Further, the metabolism of sucrose into glucose and fructose catalysed by the enzyme invertase is largely independent of temperature and depends on the physiology of onion (Benkeblia et al. 2004). Also, a similar behavior has been reported by Chope et al. 2007a, where the trend of fructose and glucose correlative and the sucrose level varying differently.

Amino acid content

There is only few reports on free amino acids (FAA) in onions except for S-derivatives of cysteine and glutathione studied intensively (Griffiths et al. 2002 and refs therein). Kuon and Bernhard (1963) measured FAA in four cultivars of American onions using an obsolete now paper chromatography technique and reported very less variation between the cultivars, with total FAA concentration ranging from 0.47 to 0.56 mg/g fresh weight (FW). On the contrary Matikkala and Virtanen (1967) and Hansen (2001) found a ten-fold higher concentration of FAA in onions grown in Scandinavia. The variation in both the result can be explained on the basis of different analytical methods employed rather than distinction in onion cultivars. The fate of FAA in onions during storage is even more obscure question. Hansen (2001) seems to be the only researcher who investigated the problem and monitored changes in the contents of 16 amino acids in typical Denmark onions ‘Hudiro’ and ‘Hyton’ during 48 weeks of a cold

storage. The total amount of FAA was relatively constant (~60 mg/g DW) during the whole trial while the contents of individual amino acids changed according different trajectories depending on their biochemical functions.

Concerning particular amino acids in the present study, the total phenylalanine content is higher than the tryptophan in all scales. As shown in Table 1, a fluctuation in the distribution of phenylalanine within the scales can be seen. During 2 month storage, phenylalanine was higher in inner scales than outer and middle scales. After 2 months, the concentration of phenylalanine was almost same in inner scales whereas, outer and middle scales showed increase with fluctuation till 6th month and after that its concentration started decreasing. The distribution of tryptophan in scales was similar to the distribution of phenylalanine. Throughout the experiment, tryptophan content was high in middle scales with respect to outer scales (Table 1). The content of tryptophan varied in all scales and the highest observed at 6th month storage. The variation was probably due to process of protein synthesis and degradation during maturation of onions.

Conclusion

We have determined the content of flavonoids, sugars and amino acids in different onion scales during 7 months storage at ambient condition. A comparison was made within the scales and the variation during storage. The data suggested that, throughout the storage, outer scales contain higher flavonoids content with respect to middle and inner scales. High content of glucose and fructose in the middle scales was observed whereas high content of sucrose is found in the inner scales. The dry matter content increased towards the inner scales of onion. During storage, the distribution of amino acid varied inconsistently in different scales. The study revealed that, there will be no trouble with flavonoids in layers infact its increased in all layers in stored onions, while in case of sugar, it starts to get decrease at some level. The type of experiment including its results may aware many researchers to carry out further more experiments and its application in controlling the quality on the basis of layers of onion and other crops in which layers has its importance.

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References

- Benkeblia N (2000) Phenylalanine ammonia-lyase, peroxidase, pyruvic acid and total phenolics variations in onion bulbs during long term storage. *Lebensm Wiss Technol* 33:112–116

- Benkeblia N, Selselet-Attou G (1999) Effects of low temperatures on the changes in oligosaccharides, phenolics and peroxidase in inner bud of onion *Allium cepa* L. during break of dormancy. *Acta Agric Scand B S P* 49:98–102
- Benkeblia N, Varoquaux P (2003) Effects of gamma-irradiation, temperature and storage time on the status of the glucose, fructose and sucrose in onion bulbs (*Allium cepa* L.). *Int Agrophysics* 17:1–5
- Benkeblia N, Varoquaux P, Shiomi N, Sakai H (2002) Storage technology of onion bulbs C.V. Rouge Amposta: effects of irradiation, maleic hydrazide and carbamate isopropyl, N- phenyl (CIP) on respiration rate and carbohydrates. *Int J Food Sci Tech* 37:169–175
- Benkeblia N, Onodera S, Yoshihira T, Kosaka S, Shiomi N (2004) Effect of temperature on soluble invertase activity, and glucose, fructose and sucrose status of onion bulbs (*Allium cepa*) in store. *Int J Food Sci Nutr* 55:325–331
- Benkeblia N, Ueno K, Onodera S, Shiomi N (2005) Variation of fructo-oligosaccharides and their metabolizing enzymes in onion bulb (*Allium cepa* L. cv. Tenshin) during long-term storage. *J Food Sci* 70(3):S208–S214
- Bilyk A, Cooper PL, Sapers GM (1984) Varietal differences in distribution of quercetin and kaempferol in onion (*Allium cepa* L.) tissue. *J Agric Food Chem* 32:274–276
- Biswas SK, Khair A, Sarker PK, Alom MS (2010) Yield and storability of onion (*Allium cepa* L.) as affected by varying levels of irrigation. *Bangladesh J Agric Res* 35(2):247–255
- Bonaccorsi P, Caristi C, Gargiulli C, Leuzzi U (2005) Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *J Agric Food Chem* 53:2733–2740
- Brewster JL (2008) Onions and Other Vegetable Alliums, 2nd edn. CAB International, Wallingford
- Bufler G (2009) Exogenous ethylene inhibits sprout growth in onion bulbs. *Ann Bot* 103:23–28
- Carter CE, Partis MD, Thomas B (1999) The expression of histone 2A in onion (*Allium cepa*) during the onset of dormancy, storage and emergence from dormancy. *New Phytol* 143:461–470
- Chope GA, Terry LA, White PJ (2007a) The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage. *Postharvest Bio Tech* 44:131–140
- Chope GA, Terry LA, White PJ (2007b) The effect of the transition between controlled atmosphere and regular atmosphere storage on bulbs of onion cultivars SS1, Carlos and Renate. *Postharvest Bio Tech* 44:228–239
- Chope GA, Cools K, Hammond JP, Thompson AJ, Terry LA (2012) Physiological, biochemical and transcriptional analysis of onion bulbs during storage. *Ann Bot* 109:819–831
- Cook NC, Samman S (1996) Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem* 7: 66–76
- Darbyshire B, Henry RJ (1979) The association of fructans with high percentage dry weight in onion cultivars suitable for dehydrating. *J Sci Food Agric* 30:1035–1038
- Downes K, Chope GA, Terry LA (2010) Postharvest application of ethylene and 1- methylcyclopropene either before or after curing affects onion (*Allium cepa* L.) bulb quality during long term cold storage. *Postharvest Bio Tech* 55:36–44
- Gennaro L, Leonardi C, Esposito F, Salucci M, Maiani G, Quaglia G, Fogliano V (2002) Flavonoid and carbohydrate contents in tropea red onions: effects of homelike peeling and storage. *J Agric Food Chem* 50:1904–1910
- Griffiths G, Trueman L, Crowther T, Thomas B, Smith B (2002) Onions-a global benefit to health. *Phytother Res* 16:603–615
- Grzelak K, Milala J, Krol B, Adamicki F, Badelek E (2009) Content of quercetin glycosides and fructooligosaccharides in onion stored in cold room. *Eur Food Res Technol* 288:1001–1007
- Hansen SL (1999) Content and composition of dry matter in onion (*Allium cepa* L.) as influenced by developmental stage at time of harvest and long- term storage. *Acta Agric Scand B S P* 49:103–109
- Hansen SL (2001) Content of free amino acid in onion (*Allium cepa* L.) as influenced by the stage of development at the harvest and long-term storage. *Acta Agric Scand B S P* 51:77–83
- Hirota S, Shimoda T, Takahama U (1999) Distribution of flavonols and enzymes participating in the metabolism in onion bulbs: Mechanism of accumulation of quercetin and its glucosides in the abaxial epidermis. *Food Sci Technol Res* 5: 384–387
- Hurst WC, Shewfelt RL, Schuler GA (1985) Shelf-life and quality changes in summer storage onions (*Allium cepa*). *J Food Sci* 50: 761–763
- Kaack K, Christensen LP, Hansen SL, Grevsen K (2004) Non-structural carbohydrates in processed soft fried onion (*Allium cepa* L.). *Eur Food Res Technol* 218:372–379
- Kuon J, Bernhard RA (1963) An examination of the free amino acids of the common onion (*Allium cepa*). *J Food Sci* 28:298–304
- Lancaster JE, Kelly KE (1984) Dry matter percentage of leaf blades and scales during the growth of onion (*Allium cepa* L.). *New Zeal J Agric Res* 27:83–87
- Lee SU, Lee JH, Choi SH, Lee JS, Ohnisi-Kameyama M, Kozukue N, Levin CE, Friedman M (2008) Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial products. *J Agric Food Chem* 56:8541–8548
- Marotti M, Piccaglia R (2002) Characterization of flavonoids in different cultivars of onion (*Allium cepa* L.). *J Food Sci* 67:1229–1232
- Matikkala EJ, Virtanen AI (1967) On the quantitative determination of the amino acids and γ -glutamylpeptides of onion. *Acta Chem Scand* 21:2891–2893
- Miedema P (1994) Bulb dormancy in onion. I. The effects of temperature and cultivar on sprouting and rooting. *J Hortic Sci* 69:29–39
- Nam E, Cho DY, Lee ET, Kim CW, Han T, Yoon MK, Kim S (2011) Bulb storability of red and yellow onion (*Allium cepa* L.) cultivars grown in Korea. *Korean J Breed Sci* 43:126–132
- Ogata K (1961) Physiological studies on the storage of onion bulb. *Bull Uni Osaka Prefect B* 11:99–119
- Pak C, Van der Plas LHW, De Boer AD (1995) Importance of dormancy and sink strength in sprouting of onions (*Allium cepa*) during storage. *Physiol Plant* 94:277–283
- Patil BS, Pike LM, Hamilton BK (1995) Changes in quercetin concentration in onion (*Allium cepa* L.) owing to location, growth stage and soil type. *New Phytol* 130:349–355
- Praeger U, Ernst MK, Weichmann J (2003) Effects of ultra low oxygen storage on postharvest quality of onion bulbs (*Allium cepa* L. var. cepa). *Eur J Hortic Sci* 68:14–19
- Prakash D, Singh BN, Upadhyay G (2007) Antioxidant and free radical scavenging activities of phenols from onion (*Allium Cepa*). *Food Chem* 102:1389–1393
- Price KR, Rhodes MJC (1997) Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*.) and changes in composition resulting from autolysis. *J Sci Food Agric* 74: 331–339
- Price KR, Bacon JR, Rhodes MJC (1997) Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J Agric Food Chem* 45:938–942
- Rhodes D, Deal L, Haworth P, Jamieson GC, Reuter CC, Ericson MC (1986) Amino acid metabolism of Lemna minor L. *Plant Physiol* 82: 1057–1062
- Rutherford PP, Whittle R (1982) The carbohydrate composition of onions during long term cold storage. *J Hortic Sci* 57:349–356
- Salamal AM, Hicks JR, Nock JF (1990) Sugar and organic acid changes in stored onion bulbs treated with maleic hydrazide. *Hortscience* 25: 1625–1628

- Slimestad R, Vågen IM (2009) Distribution of non-structural carbohydrates, sugars, flavonols and pyruvate in scales of onions, (*Allium cepa* L.). *J Food Agri Environ* 7:289–294
- Suzuki M, Cutcliffe JA (1989) Fructans in onion bulbs in relation to storage life. *Can J Plant Sci* 69:1327–1333
- Vijn I, Van Dijken A, Lüscher M, Bos A, Smeets E, Weisbeek P, Wiemken A, Smeekens S (1998) Cloning of sucrose: sucrose 1-fructosyltransferase from onion and synthesis of structurally defined fructan molecules from sucrose. *Plant Physiol* 117:1507–1513
- Ward CM (1976) The influence of temperature on weight loss from stored onion bulbs due to desiccation, respiration and sprouting. *Ann Appl Biol* 83:149–155
- Yasin HJ, Bufler G (2007) Dormancy and sprouting in onion (*Allium cepa* L.) bulbs. I. Changes in carbohydrate metabolism. *J Hortic Sci Biotech* 82:89–96
- Yoo KS, Pike LM (1996) Controlled-atmosphere storage suppresses leaf growth and flowering in onion bulbs. *Hortscience* 31:875