Bangladesh J. Bot. **45**(1): 85-92, 2016 (March)

EFFECTS OF POSTHARVEST STORAGE CONDITIONS ON PHYSICO-CHEMICAL ANALYSIS AND ANTIOXIDANT CAPACITY OF *BRASSICA RAPA* L.

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Key words: Brassica rapa, Red turnip, Physico-chemical, Antioxidant analysis, Phenolic and flavonoid contents

Abstract

Brassica rapa L. (red turnip) was studied regarding its juice yield, moisture contents, total dissolved solids (TDS), conductance, pH, total soluble solids (TSS), ash contents, density, viscosity, refractive index, titratable acidity and sugar contents under different temperatures 25 ± 2°C (room temperature) and 10 ± 1°C (refrigerator) at different time of intervals (1^{st}, 5^{th} and 10^{th} day). Antioxidants such as total phenolic contents (TPC), total flavonoid contents (TFC), and ferric reducing power assay (RPA) and phosphomolybdenum assay (PA) were carried out. TPC and TFC increased by the time at both temperatures, and comparatively higher at room temperature. Antioxidant activities significantly correlated with TPC at both temperatures. Antioxidant activity increased with time as well as increasing temperatures. These findings revealed that red turnip has stable antioxidant contents, which have no effect by temperature and storage but texture and physical appearance were affected.

Introduction

Vegetables and fruits are thought to provide the status like functional foods. They are capable to fulfill physiological needs as well as delivering health benefits (Kaur and Kapoor 2002). A higher consumption of fruits and vegetables supply a large amount of nutrients like minerals, vitamins (vitamin A, vitamin C, vitamin B6, niacin and thiamin) and dietary fibers. Antioxidant compounds including phenolics, carotenoids and flavonoids are important constituents that are responsible in preventing degenerative diseases as well as lowering risk of heart diseases and cancer (Kevers *et al*. 2007). Phenolic constituents possess antioxidant activity with regard to their ability to act as reducing agents, metal chelators, hydrogen donors and singlet oxygen quenchers (Kaur and Kapoor 2002). Vegetables and fruits, rich in flavonoids are associated with preventing heart diseases, cancer and oxidative DNA damage (Malin *et al*. 2003).

Pakistan has good agro-climatic conditions due to which a large variety of vegetables are cultivated, known for their good nutritional values. Turnip (*Brassica rapa* L.) is considered to be an important root vegetable, widely grown in Pakistan, and commonly used in many parts of the world in different ways according to culture of region. Turnips are of different varieties e.g. white, yellow and red turnip. Turnip is a good source of nutrients i.e. fibers, minerals (Ca, Mg, Fe and Zn), proteins, riboflavin, thiamine and vitamin C (Azam *et al*. 2013). Turnip contains a high amount of glucosinolates, phenylpropanoids, flavonoids, phenolics and organic acids too (Fernandes *et al*. 2007). They are found to be effective in control and prevention of many forms of cancer prevalent of lungs, stomach, pancreas, breast and bladder, and also helpful in reducing risk of suffering certain diseases including obesity, hypertension, diabetes and cardiovascular diseases (Azam *et al*. 2013).

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Antioxidant properties of vegetables can be influenced by various external factors including the cultural system or the storage temperatures. It is of great interest to investigate the variations in antioxidant contents during storage at different temperatures. The aim of this study was to evaluate the effect of storage at different temperatures (25 and 10°C) on TPC, TFC and antioxidant capacity as well as physico-chemical changes in the juice of *Brassica rapa* L. (red turnip).

Materials and Methods

Fresh red turnips collected from a local market of Karachi, were washed to remove dirt and dried with cloth then stored at room temperature (25 ± 2°C) as well as in refrigerator (10 ± 1°C). Analysis was performed on 1st, 5th and 10th day. After peeling juice of weighed turnips biomass was extracted by using multiple juice extractor (NJ-506, Nova, China). Juice was then filtered by common cotton cloth, weighed and juice yield was calculated then subjected to physico-chemical and antioxidant analysis.

Moisture contents were determined according to the A.O.A.C. official method (1990). Approximately 3 g of juice sample was weighed in dried petri dish. Petri dish was kept in oven (Binder E28 # 05-86486, Germany) at 105 ± 3 °C for 1 hr and dish was placed in a desiccator to cool then the sample was weighed further.

Total dissolved solids (TDS), conductance and pH of juice were measured by using TDS meter (S 518860, Korea), conductivity meter (Jenway 4510, England) and pH meter (Jenway 3510, England), respectively. Total soluble solids (TSS) of juice were determined using refractometer (KRÜSS DR 6200, Germany), and expressed as brix invert sugar, brix fructose, brix sucrose and brix glucose.

Ash contents were determined according to the A.O.A.C. official method (1990). Approximately 5 g of juice sample was weighed in dried silica crucible. Sample was heated slowly on burner to dryness and was placed in muffle furnace (Nabertherm GmbH Bahnhofstraße 20, 28865 Lilienthal, Germany) at 550 ºC for 8 - 12 hrs. Ash was weighed and then calculated the ash contents.

Density of juice was determined using R.D. bottle. Viscosity of juice samples was measured using Ostwald viscometer. Refractive index of juice was determined using refractometer (KRÜSS DR 6200, Germany).

Titratable acidity was measured according to the A.O.A.C. official method (1990). Twenty ml juice sample was titrated after adding 100 ml distilled water with 0.1N NaOH solution in the presence of phenolphthalein as an indicator, and titratable acidity was expressed as % citric acid.

Sugar contents in juice were determined using colorimetric method described by Okoye and Ugwu (2008). 1 ml of juice was mixed with 1 ml of 5% phenol, and mixed gently then 5 ml concentrated sulphuric acid and 9 ml distilled water were added to bring the volume up to 20 ml. It was allowed to stand for 10 min, shaken well and immersed in a water bath at 30°C for 20 min. A yellow orange colour developed and the absorbance was recorded at 490 nm using spectrophotometer (Jenway 6300, England). Total sugar contents were measured by comparing absorbance with those of standards with known concentrations of glucose solution (2 - 20 ppm), and expressed as g of glucose per 100 ml of juice.

Ten ml of juice was extracted with 25 ml methanol containing 1% HCl, shaken and stood for 1 hr. Centrifuged the juice extract for 10 min at 3500 rpm. Supernatant was used for antioxidant analysis.

Total phenolic contents (TPC) of juice were determined with some modifications according to Folin-Ciocalteu colorimetric method (Velioglu *et al*. 1998). Juice sample (0.1 ml) was mixed with 0.75 ml of Folin-Ciocalteu reagent (1 : 10 dilution with distilled water), and allowed to stand at

22 ºC for 5 min. After that, 0.75 ml of sodium carbonate (60 g/l) solution was added to the mixture and shaken vigorously. Then, mixture was incubated at 22 ºC for 90 min and absorbance was measured at 725 nm using a UV-visible spectrophotometer (Jenway 6300, England). TPC was determined by comparing absorbance with those of standards with known gallic acid concentrations (50 - 500 mg/l concentrations, r^2 = 0.982), and expressed as mg of gallic acid equivalent (GAE) per 1000 ml of juice.

Total flavonoid contents (TFC) of juice were determined according to the method reported previously (Zhishen *et al*. 1999). One ml juice extract was mixed with 4 ml distilled water. At zero time, 0.3 ml of 5% w/v $NaNO_2$ was added. After 5 min. 0.3 ml of 10% w/v Al $(NO_3)_3$ was added. After 6 min 2 ml of 1 M NaOH was added. After that, 2.4 ml distilled water was added to make the volume up to 10 ml. The mixture was shaken vigorously and the absorbance was recorded at 510 nm. TFC were determined using catechin as a standard for calibration curve (20 - 200 mg/l, r^2 = 0.993), and expressed as mg of catechin equivalent (CAE) per 1000 ml of juice.

Antioxidant capacity was carried out using reducing power assay (RPA) according to the method of Jayanthi and Lalitha (2011). 0.1 ml of juice extract was mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide. This mixture was kept at 50 ^oC on water bath for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The 2.5 ml upper layer of solution was mixed with 2.5 ml distilled water then freshly prepared 0.5 ml ferric chloride solution was added. The absorbance of mixture was recorded at 700 nm. Antioxidant capacity with respect to ascorbic acid (50 - 700 mg/l) in juice was determined using calibration curve (r^2 = 0.981).

The total antioxidant activity of juice sample was measured by phosphomolybdenum assay (PA) with some modifications (Prieto *et al*. 1999). Briefly, 0.1 ml of sample was mixed with 4 ml of reagent solution (4 mM ammoniummolybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid) in a vial. Another vial contained blank solution (4 ml of reagent solution). Both vials were capped and placed on water bath for incubation at 95 °C for 90 min. After that, cooled the samples up to room temperature; then absorbance was measured at 695 nm against blank. The antioxidant activity was determined using standard curve of ascorbic acid, plotted at different concentrations (100 - 1000 mg/l).

Samples were prepared and analyzed in three replicates. Results were expressed as mean ± S.E.M. All data were statistically analyzed by ANOVA using the software SPSS 21 with the Duncan's multiple range test (DMRT) to evaluate differences between storage conditions at level of significance ($p \leq 0.05$).

Results and Discussion

The results of physico-chemical analysis of red turnip juice are given in Table 1. Results showed that total juice decreased with respect to time at both temperatures (25 and 10 °C), which was due to the loss in weight of turnip. There was rapid decreased in juice yield (59.88 - 20.74%) at high temperature. Rate of weight loss was low at 10 °C, so the juice yield was higher (42.28 - 22.79%) as compared to turnip stored at 25 °C (23.69 - 20.74%). Possibly, main cause for higher weight loss of turnip could be higher rate of transpiration at room temperature in comparison to lower temperature.

Moisture contents also decreased with the time, which were also cause of decrease in juice yield. Moisture contents were low at room temperature (93.87 - 92.73%) as compared to refrigerator (94.98 - 93.42%) due to higher rate of transpiration at room temperature.

TDS increased day by day (11.08 - 19.49 g/l and 14.20 - 18.27 g/l) at both temperatures as moisture contents decreased. This showed that TDS is temperature dependent parameter. Normally, process of transpiration is to be slow at low temperature. This is the reason that TDS

Table 1. Physico-chemical analysis of *Brassica rapa* L. (red turnip) at various stored stages.

Sl. No.	Parameters	Units	25 ± 2°C (Room temperature)			10 ± 1°C (Refrigerator)	
			1st day	5th day	10th day	5th day	10th day
			Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
1	Juice yield	%	59.88 ± 0.53^{e}	23.69 ± 0.15^{c}	20.74 ± 0.89^{a}	42.28 ± 0.10^{d}	22.79 ± 0.41^{b}
2	Moisture contents	%	97.02 ± 0.24^{e}	93.87 ± 0.09^{c}	92.73 ± 0.13^{a}	94.98 ± 0.04^{d}	93.42 ± 0.03^{b}
3	TDS	g/l	11.08 ± 0.06^{a}	15.96 ± 0.05^{c}	19.49 ± 0.04^{e}	14.20 ± 0.11^{b}	18.27 ± 0.02^{d}
4	Conductance	mS	18.46 ± 0.01^{a}	26.20 ± 0.20^{c}	32.60 ± 0.10^{e}	23.73 ± 0.12^{b}	30.37 ± 0.15^{d}
5	pH	-	6.17 ± 0.001^{e}	5.95 ± 0.001^{b}	5.92 ± 0.004^{a}	6.00 ± 0.001^{d}	5.98 ± 0.003^{c}
6	Invert sugar	°Brix	1.96 ± 0.01^{a}	5.45 ± 0.06^{c}	7.30 ± 0.05^{e}	4.16 ± 0.04^{b}	6.14 ± 0.02^{d}
7	Fructose	°Brix	2.02 ± 0.01^{a}	5.49 ± 0.02^{c}	7.24 ± 0.02^{e}	4.24 ± 0.02^{b}	6.16 ± 0.03^{d}
8	Sucrose	°Brix	1.98 ± 0.04^{a}	5.37 ± 0.04^{c}	7.09 ± 0.03^{e}	4.08 ± 0.02^{b}	6.08 ± 0.02^{d}
9	Glucose	°Brix	1.98 ± 0.02^{a}	5.41 ± 0.04^{c}	7.15 ± 0.03^{e}	4.15 ± 0.05^{b}	6.12 ± 0.04^{d}
10	Ash contents	%	1.20 ± 0.02^{a}	2.10 ± 0.02^{c}	2.85 ± 0.25^{d}	1.76 ± 0.01^{b}	2.62 ± 0.01^{c}
11	Density	g/ml	1.02 ± 0.001^{a}	1.03 ± 0.000^{c}	1.05 ± 0.000^{e}	1.03 ± 0.000^{b}	1.04 ± 0.000^{d}
12	Viscosity	Milipoise	0.073 ± 0.00^{a}	0.094 ± 0.00^{b}	0.133 ± 0.00^{c}	0.098 ± 0.00^{b}	0.132 ± 0.00^{c}
13	Refractive index	-	1.336 ± 0.00^{a}	1.341 ± 0.00^{c}	1.346 ± 0.00^{e}	1.339 ± 0.00^{b}	1.342 ± 0.00^{d}
14	Titratable acidity	% citric acid	0.20 ± 0.01^{a}	0.29 ± 0.05	0.36 ± 0.01^{c}	0.37 ± 0.05^{c}	0.39 ± 0.00^{c}
15	Sugar contents	g glucose/100ml	0.51 ± 0.04^{a}	0.75 ± 0.04^{b}	1.28 ± 0.03^{c}	1.32 ± 0.05^{d}	1.78 ± 0.03^{e}

S.E.M. = Standard error of the mean of triplicate analysis. Means with different superscript lowercase letters (a - e) in the same row are significantly different ($p \leq 0.05$) were analyzed by ANOVA using DMRT.

showed decline in refrigerator than room temperature. Conductance of juice was gradually increased with or without cold storage due to increase in minerals and TDS. It was higher at 25 °C (26.20 - 32.60 mS) than at 10 °C (23.73 - 30.37 mS). pH of juice was decreased day by day. pH in refrigerator (6.00 - 5.98) was comparatively higher than room temperature (5.95 - 5.92). TSS increased gradually at both temperatures. Increase in TSS during storage may be related with conversion of pectin substances, starch, hemi-cellulose or other polysaccharides into soluble sugars (Singh and Singh 2010). These transformations are fast with the rate of transpiration. That is why TSS was higher at room temperature as compared to refrigerator.

Ash contents of juice of fresh red turnip (1.20%) were in agreement with reported literature (Incedayi *et al*. 2008). Ash contents of juice were increased with the time at both temperatures as moisture contents decreased, which showed the presence of mineral contents in turnip. They were significantly higher at room temperature (2.10 - 2.85%) as compared to low temperature (1.76 - 2.62%).

Density has long been used for the appraisal of TSS, dry matter contents, maturity, quality and defects found in vegetables (Abbott *et al*. 1997). Density and viscosity were increased with the time as moisture contents decreased. Density is also temperature dependent parameter. It was slightly higher at room temperature (1.03 - 1.05 g/ml) than in refrigerator (1.03 - 1.04 g/ml). It was observed that density was being strongly affected by TSS. Similar trend was observed in viscosity. It was also higher at room temperature (0.094 - 0.133 milipoise). Temperature influence on viscosity and it is directly related to TSS. As TSS increases, viscosity also increases (Saravacos 1970). Significant change ($p \leq 0.05$) in refractive index was observed. It was increased day by day on both temperatures with viscosity and density. At room temperature, refractive index was higher (1.341 - 1.346) than in refrigerator (1.339 - 1.342). Thicker or dense juices refract more. TDS of a juice is the cause to exhibit a refractive index in direct relation (Harrill 1998).

Titratable acidity of juice increased with the time. It was comparatively lower at room temperature (0.29 - 0.36 %) than in refrigerator (0.37 - 0.39 %). Acidity increased due to increase of acids in juice.

The storage time interfered to sugars accumulation. They also increased day by day. Total sugar contents were higher in refrigerator (1.32 - 1.78 g/100 ml) than at room temperature (0.75 - 1.28 g/100 ml). Previously, it was reported that mostly root vegetables accumulate sugar contents at low temperature (Shattuck *et al*. 1989). The accumulation of sugar contents may be due to the conversion of organic acids into sugars and breakdown of starch components into glucose and sucrose units (Selvaraj *et al*. 1989).

Temperature is an important factor that significantly affects the quality, composition and antioxidant activity of fresh vegetables and fruits. Temperature variations may transmute the mechanism of action of some antioxidants or affect them in another way. Temperature can affect some particular reactions in which antioxidants take part and help to prevent from lipid peroxidation (mainly the reactions in which lipid radicals are involved in oxidative stress) (Rèblová 2012). The results for TPC, TFC and total antioxidant activities for red turnip are presented in Table 2.

Phenolic compounds are considered as primary antioxidants, which has potential to function as free-radical scavengers or terminators. The position and the degree of hydroxylation in phenolic compounds play an important role for determination of their antioxidant activity (White and Xing 1997). TPC was significantly increased day by day in the red turnip juice during storage at both temperatures. When comparing the storage temperatures, TPC accumulated to a significantly higher level at room temperature (520.33 - 680.22 mg/1000 ml) as compared to in refrigerator (508.67 - 635.83 mg/1000 ml). It is possible that turnip accumulate phenolic contents, which are stable at higher temperature and increase by storage.

Table 2. Antioxidant properties of *Brassica rapa* L. (red turnip) at various stored stages.

Sl. No.	Antioxidant analysis	Units	25 ± 2°C (Room temperature)			10 ± 1°C (Refrigerator)	
			1st day	5th day	10th day	5th day	10th day
			Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
1	TPC	mg GAE/ 1000 ml	277.67 ± 5.40^{a}	520.33 ± 11.20^{b}	680.22 ± 7.70^{c}	508.67 ± 56.60^{b}	635.83 ± 35.90^{c}
2	TFC	mg CAE/ 1000 ml	28.35 ± 0.30^{a}	47.95 ± 0.90^{b}	81.26 ± 0.70^{d}	62.02 ± 12.30^{c}	71.40 ± 1.80^{cd}
3	RPA	mg ascorbic acid/1000 ml	297.50 ± 9.20^{a}	550.67 ± 16.16^{b}	594.08 ± 16.50^{cd}	632.92 ± 39.40^{d}	567.80 ± 18.50^{bc}
4	PA	,,	1087.33 ± 49.00^{a}	3158.16 ± 360.00^{c}	3720.00 ± 94.20^{d}	2473.33 ± 165.00^{b}	3657.50 ± 261.00^{d}

S.E.M. = Standard error of the mean of triplicate analysis. Means with different superscript lowercase letters (a - d) in the same row are significantly different ($p \leq 0.05$) were analyzed by ANOVA using DMRT.

Flavonoids show antioxidant activity and induce various beneficial effects on human health regarding nutrition and disease prevention. The action mechanism of flavonoids has to be express, and completed through chelating or scavenging process (Kessler *et al.* 2003). TFC was also increased gradually with the time at both temperatures. TFC on 5th day was significantly higher in refrigerator (62.02 mg/1000 ml) as compared to the room temperature (47.95 mg/1000 ml). At the end of storage, TFC was higher at room temperature (81.26 mg/1000 ml). It has been reported that specific classes of polyphenolic constituents including flavonoids, were not effected in same manner and they presented a different evolution pattern throughout the storage period (Dourtoglou *et al.* 2006).

Antioxidant activity through RPA shows the conversion of Fe^{+3} to Fe^{+2}. Compounds having reducing power indicate that they have the potential to donate electrons, and have ability to reduce the oxidized intermediates, which are formed as a result of lipid peroxidation processes. Antioxidant activity of red turnip juice through RPA increased day by day at room temperature (297.50 - 594.08 mg/1000 ml) (Table 2) whereas increased to 5th day (632.92 mg/1000 ml) and decreased at 10th day (567.80 mg/1000 ml) in refrigerator.

PA is utilized for spectrophotometric quantification of total antioxidant activity. The presence of antioxidant compounds in the sample, promote the reduction of molybdenum [Mo (VI)] to form green complex of phosphate [Mo (V)]. Antioxidant activity through PA also increased during storage at both room temperature (1087.33 - 3720.00 mg/1000 ml) and in refrigerator (2473.33 - 3657.50 mg/1000 ml) (Table 2). This good antioxidant activity might be associated with the presence of certain phytochemicals such as phenolics, bioflavonoids and flavonoids in turnip. It could be suggested that as storage time increase, breakdown of tannins (complex polyphenols) into simple phenols take place due to the metabolic processes, which are responsible for antioxidant activity as reported by Mathiventhan and Sivakanesan (2013). The breakdown could be slow due to slow metabolic process at low temperature. Previous reports also showed that some vegetables and fruits did not lose its antioxidant activity during storage (Kevers *et al.* 2007).

Results showed that TPC, TFC and antioxidant activity of red turnip juice increased day by day at both temperatures (25 ± 2 and 10 ± 1 °C), and comparatively higher at 25 °C (room temperature).

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(Manuscript received on 10 May, 2015; revised on 27 May, 2015)