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Research Article

Physico-chemical and Antioxidant Properties of Different Pumpkin Cultivars Grown in China

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Abstract: To obtain more detailed knowledge of the differences among major pumpkin species grown in China, physico-chemical and antioxidant properties of four pumpkin cultivars (Miben, Hongli, Lvli, Xihulu) were characterized and compared. Dry matter, total soluble solids, titratable acidity, fruit color, protein, fat, sugars, minerals, amino acids, β-carotene, L-ascorbic acid, total phenols and antioxidant activities (DPPH and FRAP) were measured in the studied cultivars. The results showed great differences in the composition and characteristics of the pumpkin cultivars. Miben exhibited the highest concentration of dry matter, fat, Total Soluble Solid (TSS), Titratable Acidity (TA), sucrose, β-carotene, K, P, Fe, Zn and aspartic acid. Hongli had the highest concentration protein, L-ascorbic acid, Na, Ca, Mg and all individual amino acids except for asparitic acid. Lvli exhibited significantly ($p<0.05$) higher antioxidant activities (DPPH and FRAP), which are highly related to total phenols content in pumpkin fruits ($r = 0.94$ and $r = 0.98$, respectively). Principal Component Analysis (PCA) allowed the four pumpkin cultivars to be differentiated clearly based on all these physico-chemical and antioxidant properties determined in the study.

Keywords: Antioxidant properties, cultivars, physico-chemical, principle component analysis, pumpkin

INTRODUCTION

Pumpkin is one of the most important vegetables belonging to the family Cucurbitaceae (Whitaker and Davis, 1962). The pumpkin species available mainly include *Cucurbita moschata*, *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita mixta* and *Cucurbita ficifolia* (Robinson and Decker-Walters, 1997). Three of these, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* represent economically important species cultivated worldwide and have high production (Taylor and Brant, 2002; Fu *et al*., 2006). Recognized for its attractive color, delicious taste and unique flavor, pumpkin is a good source of carbohydrates, amino acids, vitamins, minerals and other bioactive compounds. In view of the abundant nutrients, the consumption of pumpkin has been reported to have many positive health benefits including anti-oxidant property. Natural antioxidants like β-carotene (Shi *et al*., 2013), ascorbic acid (Biesiada *et al*., 1962), phenols (Nawirska-Olszanska *et al*., 2013) and polysaccharides (Košťálová *et al*., 2013) in pumpkin have been proved to possess antioxidant activity which can provide protection against free radical damage and subsequently reduce the risk of chronic diseases (Adams *et al*., 2011; Song *et al*., 2013).

It is known that varietal, geographical, seasonal and maturity differences greatly affect the composition and properties of vegetables. Recently, antioxidant activity of pumpkin fruit has been reported to be affected by planting date (Oloyede *et al*., 2014), maturity differences (Oloyede *et al*., 2012) as well as industrial and domestic processing conditions (Dini *et al*., 2013). Despite the studies mentioned above, the comprehensive compositional data regarding the physico-chemical and antioxidant properties of different pumpkin cultivars has not been characterized. This information is important for the food industry in the selection of cultivars with suitable processing properties and enhanced functional benefits. Additionally, most of the studies were undertaken using pumpkin cultivated in Nigeria, Poland, Brazil, Italy, Korea and Mexico (De Carvalho *et al*., 2012; Dini *et al*., 2013; Jacobo-Valenzuela *et al*., 2011; Kim *et al*.,

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2012; Nawirska-Olszanska *et al*., 2013; Oloyede *et al*., 2012). However, there are limited reports about different pumpkin species in China, which is a dominating producing country of pumpkin. Pumpkin cultivars such as Miben (*Cucurbita moschata*), Hongli (*Cucurbita maxima*), Lvli (*Cucurbita maxima*) and Xihulu (*Cucurbita pepo*) are the principal pumpkin varieties in the market in China. A more detailed knowledge of the composition of these species will be of great benefit for both technological research and processing practice.

Hence, this research mainly focused on the analysis and comparison of the physico-chemical and antioxidant properties of four pumpkin cultivars popular in China, aiming to give useful information for the best use of the pumpkins. The physico-chemical and antioxidant properties measured were also used as descriptors for Principle Component Analysis (PCA) in order to differentiate the analyzed pumpkin cultivars.

MATERIALS AND METHODS

Pumpkin cultivars: Four pumpkin cultivars including Miben, Hongli, Lvli and Xihulu were harvested at commercial maturity stage from gardens of the experimental station of the China Agriculture University located in Beijing and stored at 4°C before use. All of them were of good quality and free of serious mechanical damage. All analyses were performed in triplicate and data were reported as the mean±Standard Deviation (S.D.).

Determination of general parameters and color of pumpkin pulp: General parameters were determined in samples following official methods (AOAC, 2000). Dry matter was determined by oven drying at 70°C until a constant weight was obtained; Total Soluble Solids (TSS) contents were determined as °Brix at an ambient temperature (20±1°C) by WAY-2S digital Abbe Refraction meter (Shanghai Precision and Scientific Instrument Co., Shanghai, China). Titratable Acidity (TA) was measured by titration with 0.1 M NaOH solution to an endpoint of pH 8.1 and expressed as percentage of citric acid per 100 g of FW.

Color assessment was conducted at 25±2°C using a Color Difference Meter (SC-80, 229 Kang guang Co., Ltd, Beijing, China) in the reflectance mode (Liu *et al*., 2010). Color was expressed in L*, a* and b* values. Three measurements were performed and results were averaged. Hunter L* (lightness: 100 for white and 0 for black), a* (green-red) and b* (yellow-blue) values of the samples were measured. The hue angle (h°) was calculated as arctan (b*/a*) and chroma (C*) from $(a^{*2}+b^{*2})^{1/2}$.

Determination of minerals in pumpkin pulp: An Model contrAA 700 high-resolution continuum source Atomic Absorption Spectrometer (Analytik Jena, Jena, Germany) was used for the determination of K, Ca, Na, P, Mg, Fe and Zn in pumpkin pulp according to the method previously reported by Subramanian *et al*. (2012). (2.0 g) of pumpkin pulp were transferred into a silica crucible and kept in a muffle furnace for ashing at 450°C for 3 h and then 5 mL of 6 mol/L HCl was added to the crucible. Then, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 0.1 mol/L HNO_3 solution and made up to 50 mL. Air-acetylene flame was used for determination of metal content. The instrument was operated with the following conditions in flame mode: acetylene 1.8 L/min, air 15 L/min, the inert argon gas flow and the temperature parameters were followed as recommended by manufacturer.

Determination of crude protein, fat and sugars (sucrose, glucose and fructose): Crude protein and fat were determined using the routine chemical analytical methods of the AOAC (2000). Sucrose, glucose and fructose were determined by HPLC according to the method reported by Zhou *et al*. (2014) with some modifications. Fifty gram of pumpkin pulp was dissolved in 100 mL distilled water and homogenized using a JYL-B060 beater (Jiuyang Co., China), thereafter the homogenate was incubated at room temperature for 2 h and centrifuged at 12,000 rpm for 15 min at 4°C (GL-166-A, Shanghai Anting Scientific Equipment Factory, Shanghai, China). The supernatant was collected and passed through a 0.45 μm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China) before use. HPLC system (Knauer Co., Ltd, German) was equipped with a K-501 pump (Knauer Co., Ltd., Germany), connected to a refractive index detector (RI-2401, Knauer Co., Ltd., German) and a 20 μL injection loop. The analytical column was an YMC-Pack Polyamine II (4.6×250 mm i.d., 5 μm particle size). The mobile phase was acetonitrile: water (75:25) with isocratic flow at a rate of 1.0 mL/min at 25°C. Quantification was carried out using HPLC-grade sucrose, glucose and fructose as external standard.

Determination of amino acids: Amino acids were analyzed using a Hitachi 835-50 auto amino acid analyzer, (Tokyo, Japan) equipped with an ion-exchange resin column 2619 (2.6 mm in inner diameter and 150 mm long). Samples were filtered with 0.45 μm syringe filters (Milford, USA) and the supernatant was injected into the amino acid analyzer for the determination of the amino acid composition. The amino acids were separated using sodium citric acid buffer at pH 2.2, a flow rate of 0.225 mL/min, a column temperature of 85°C and post column reaction with ninhydrin (0.3 mL/min ninhydrin flow rate), followed by photometric detection at 570 nm. Individual amino

acids were quantified on the basis of amino acid standard (AAS18, Sigma Chemical Co., USA).

Determination of β-carotene: β-carotene was determined using HPLC method as described previously by Hymavathi and and Khader (2005) with some modifications. Twenty gram of pumpkin pulp were added to 30 mL acetone, sonicated for 15 min and centrifuged at 9000 rpm at 4°C for 15 min, the procedure was repeated twice to ensure the maximum extraction. The extracts were collected and made up to final volume of 100 mL. After that, 50 mL of methanolic KOH (10%) was added to the extracts for saponification at 45°C water-bath for 1 h. Then the extracts were transferred to 100 mL petroleum ether and the organic layer was dried by passing through an anhydrous sodium sulphate column and evaporated to dryness. The dried residue was dissolved in hexane and filtered through a 0.45 μm membrane filter for analysis with the RF-10AXLHPLC system (Shimadzu Co., Ltd., Japan) carried out at 445 nm at 30°C The analytical column was a Sunfire™ C18 (4.6×250 mm i.d., 5 μm particle size) from Waters. The mobile phase was acetonitrile: methanol: methylene chloride (6:2:2, v/v/v), with isocratic flow at a rate of 1.0 mL/min. The concentration was calculated using β-carotene as external standard and expressed as milligram β-carotene per 100 g of Fresh Weight (FW).

Determination of L-ascorbic acid: L-ascorbic acid was determined using HPLC method as described by Liu *et al.* (2013) with some modifications. Thirty gram of pumpkin pulp were mixed with 100 mL 2.5% metaphosphoric acid and homogenized with a JYL-B060 beater (Jiuyang Co., China), thereafter the homogenate was incubated at 4°C for 2 h and centrifuged at 9000 rpm/min for 15 min at 4°C. HPLC system (RF-10AXL, Shimadzu Co., Japan) was equipped with a prominence UV-visible Detector (SPD-20AV), a system controller (CBM-20A), an auto sampler (SIL-20A), two pumps (LC-20AT) and a Column Oven (CTO-20A). The analytical column was a Sunfire™ C18 (4.6×250 mm i.d., 5 μm particle size) from Waters. The mobile phase was distilled water adjusted to pH 2.2 with 1 mol/L metaphosphoric acid. A 20 μL sample was analyzed with isocratic flow at a rate of 0.6 mL/min at 30°C. UV chromatograms were recorded at 254 nm in absorbance mode. The concentration of L-ascorbic was calculated using L-ascorbic acid as external standard and expressed as milligram ascorbic acid per 100 g of Fresh Weight (FW).

Preparation of pumpkin antioxidant extracts: The preparation of pumpkin antioxidant extracts was done following the method validated by Hertog *et al*. (1992) with some modifications. Twenty gram of pumpkin pulp were mixed in 40 mL 70% (v/v) methanol, thereafter the homogenate was heated to reflux at 80°C for 2 h with regular swirling. The extracts was cooled and centrifuged at 5000 rpm at 4°C for 15 min. The supernatants were recovered and made up to final volume of 50 mL with 70% (v/v) methanol.

Determination of total phenols: The concentration of total phenols in the pumpkin extracts was measured using a modified colorimetric Folin-Ciocalteu method (Budini *et al*., 1980). A 400 μL amount of pumpkin extracts solutions or gallic acid standard solutions was mixed with 2 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water). After reacting for 5 min at room temperature, 1.8 mL of 7.5% Na_2CO_3 solution were added. The mixture was let to incubate for 60 min at room temperature in dark. After that, the absorbances were measured at 765 nm using a spectrophotometer (UV-726, Shimadzu, Shanghai, China). The results were expressed as milligram Gallic Acid Equivalents (GAE) per 100 g FW.

Determination of antioxidant activity by DPPH radical-scavenging assay: The effect of pumpkin extracts on DPPH radical was estimated according to the method reported previously by Blois (1958). A 200 μL amount of pumpkin extracts solutions was mixed with 4.0 mL of a 0.14 mmol/L of DPPH solution in methanol and shaken vigorously. The mixture was let to incubate for 45 min at room temperature in dark. After that, the absorbances were measured at 517 nm using a spectrophotometer (UV-726, Shimadzu, Shanghai, China). A control reaction was prepared as above without the pumpkin extract. Trolox was used as a benchmark and the radical-scavenging activity of pumpkin extracts was expressed as milligram VE equivalents per 100 g of FW.

Determination of antioxidant activity by Ferric Reducing/Antioxidant Power (FRAP) assay: FRAP assay of pumpkin extracts was performed according to Benzie and Strain (1996) with some modifications. The fresh prepared FRAP solution contained 25 mL 0.3 mmol/L acetate buffer (pH 3.6), 2.5 mL 10 mmol/L TPTZ solution (dissolved in 40 mmol/L HCl) and 2.5 mL 20 mmol/L ferric chloride ($FeCl_3 \cdot 6H_2O$). The FRAP solution was kept at 37°C before use. One hundred μL pumpkin extract solutions were mixed with 4 mL of FRAP solution and then placed at 37°C for 10 min. The ferric reducing ability of pumpkin extracts was measured by monitoring the absorbance at 593 nm using a spectrophotometer (UV-726, Shimadzu, Shanghai, China). Trolox was used as a benchmark and the radical-scavenging activity of pumpkin extracts was expressed as milligram VE equivalents per 100 g of FW.

Statistical analysis: Data were reported as the mean±Standard Deviation (S.D.) of three replicates.

The results were compared by one-way Analysis of for all experimental runs. Significance of differences was defined at $p<0.05$. Correlations between parameters were examined using the Pearson correlation. Principal Component Analysis (PCA) was carried out using all physico-chemical and antioxidant data measured as descriptors. PCA score plot was used to determine whether various pumpkin cultivars could be grouped into different classes. All analyses were performed using Statistical Program for Social Sciences (SPSS 12.0, Chicago, IL, USA) software.

RESULTS AND DISCUSSION

General parameters and color: The results for dry matter, TSS, TA and color measurement (L*, a*, b*, h°, C*) of investigated pumpkin cultivars were presented in Table 1. Significant differences ($p<0.05$) were revealed among the pumpkin cultivars for dry matter, TSS and TA. The highest dry matter content (16.81%) and TSS content (11.43 °Brix) were found in Miben while the lowest in Xihulu (4.91% and 3.10°Brix). The pumpkin cultivars showed a higher amount of dry matter and TSS content compared to those reported for pumpkin cultivated in India (Kulkarni and Joshi, 2013) and Korea (Kim *et al.*, 2012) except for Xihulu. In terms of TA, mean values ranged from 0.36% (Hongli) to 0.68% (Miben) and no significant differences were found between Lvli and Xihulu. Similar results were reported by Sharma and Ramana Rao (2013) on a pumpkin cultivar (*Cucurbita maxima*) in India (0.38-0.64%).

The appearance and consumer acceptability of fruits and vegetables are directly affected by color. As showed in Table 1, there were wide variations in lightness (L*), redness (a*), yellowness (b*), hue angle (h°) and chroma (C*) of pumpkin cultivars. Xihulu exhibited a higher value for L* and lowest value for a*, b*, h° and C* compared to other cultivars, indicating that Xihulu has a light green color. Besides, Lvli showed a significant ($p<0.05$) higher value for L* and b* than Miben and Hongli, whereas lower value for a*, Variance (ANOVA) and Tukey's test was carried out which is attributed to the unique greenish color of Lvli. As for Miben and Hongli, there was no significant ($p<0.05$) difference in terms of most color parameters (a*, b*, h° and C*), which meant that these two cultivars are very similar to each other in color.

Analysis of minerals in pumpkin fruits: The concentration of minerals found in pumpkin pulp was presented in Table 2. All pumpkin cultivars exhibited high levels of K, Ca, Mg and P content but low Na content. It was reported that a high K^+ and low Na^+ diet could reduce blood pressure of hypertensive rats (Sugden *et al*., 1987). Huge amounts of Ca, Mg and P made pumpkin a good source of minerals. However, wide variations in the concentrations of minerals were also found among different pumpkin cultivars as showed in Table 2. Highest levels of K (392.25 mg/100 g) and P (54.14 mg/100 g) were found in Miben, followed by Hongli, whereas highest Na (1.05 mg/100 g), Ca (39.41 mg/100 g) and Mg (27.84 mg/100 g) were found in Hongli. The concentration of Fe showed significant ($p<0.05$) differences among the cultivars. Highest Fe content was found in Miben (0.95 mg/100 g), followed by Hongli (0.67 mg/100 g), Lvli (0.52 mg/100 g) and Xihulu (0.34 mg/100 g). The level of Zn in Miben, Hongli and Lvli was found to be very similar, with higher values than Xihulu. The mineral contents obtained in this study were generally higher when compared with pumpkin cultivars in South America in a previous research by Mahabir and Verma (2012). This might attribute to the different types of soil conditions and the various climatic and weather conditions of the different geographic locations.

Crude protein, fat and sugars (sucrose, glucose and fructose) analysis: Protein, fat and sugars are the most common constituents in food. Table 3 showed the concentration of these three constituents in the studied pumpkin cultivars. In terms of crude protein content, a

Table 1: Dry matter, total soluble solids, titratable acidity and color parameters of pumpkin fruits of four investigated cultivars

				Color parameters				
Cultivar	Dry matter (%)	TSS (°brix)	TA (%)	L*	a*	b*	h°	C*
Miben	16.81±0.09^{a}	11.43±0.31^{a}	0.68±0.03^{a}	62.97±0.61^{d}	31.46±0.22^{a}	107.00±0.98^{b}	73.61±0.25^{b}	111.53±0.88^{a}
Hongli	12.52±0.03^{b}	7.73±0.15^{b}	0.36±0.00^{c}	65.40±1.04^{c}	31.41±0.89^{a}	110.77±1.60^{ab}	74.16±0.61^{b}	115.14±1.35^{a}
Lvli	11.40±0.05^{c}	5.17±0.12^{c}	0.57±0.01^{b}	72.14±0.80^{b}	17.70±0.29^{b}	112.63±2.60^{a}	81.07±0.08^{a}	114.01±2.62^{a}
Xihulu	4.91±0.04^{d}	3.10±0.00^{d}	0.57±0.01^{b}	83.22±0.72^{a}	-4.20±0.47^{c}	23.44±1.39^{c}	-79.85±0.55^{c}	23.81±1.45^{b}

All data were expressed as the means±S.D., n = 3; Means with different superscript letters within a column are significantly different at $p<0.05$

Table 2: Mineral content in pumpkin fruits of four investigated cultivars

Cultivars	K (mg/100 g FW)	Na (mg/100 g FW)	Ca (mg/100 g FW)	Mg (mg/100 g FW)	P (mg/100 g FW)	Fe (mg/100 g FW)	Zn (mg/100 g FW)
Miben	392.25±2.91^{a}	1.05±0.06^{d}	13.45±0.08^{d}	19.13±0.08^{c}	54.14±0.12^{a}	0.95±0.04^{a}	0.42±0.03^{a}
Hongli	234.86±2.21^{b}	2.20±0.06^{a}	39.41±0.04^{a}	27.84±0.04^{a}	52.32±0.24^{b}	0.67±0.01^{b}	0.38±0.01^{a}
Lvli	150.59±0.78^{d}	1.22±0.04^{c}	21.87±0.01^{c}	21.92±0.07^{b}	38.38±0.04^{c}	0.52±0.03^{c}	0.37±0.03^{a}
Xihulu	183.01±0.88^{c}	1.97±0.02^{b}	27.98±1.10^{b}	18.29±0.06^{d}	31.33±0.10^{d}	0.34±0.01^{d}	0.26±0.04^{b}

All data were expressed as the means±S.D., n = 3; Means with different superscript letters within a column are significantly different at $p<0.05$

Table 3: Crude protein, crude fat and sugars in pumpkin fruit of four investigated cultivars

			Sugars (%)		
Cultivar	Crude protein (%)	Crude fat (%)	Sucrose	Glucose	Fructose
Miben	1.45±0.04^{b}	0.50±0.03^{a}	5.21±0.04^{a}	0.46±0.02^{b}	0.88±0.02^{bc}
Hongli	1.66±0.03^{a}	0.41±0.04^{a}	0.89±0.03^{b}	0.40±0.00^{b}	0.75±0.05^{c}
Lvli	0.99±0.04^{d}	0.41±0.05^{a}	0.61±0.03^{c}	0.64±0.02^{a}	1.09±0.09^{a}
Xihulu	1.19±0.02^{c}	0.31±0.03^{b}	0.04±0.00^{d}	0.57±0.06^{a}	0.95±0.08^{ab}

All data were expressed as the means±S.D., n = 3; Means with different superscript letters within a column are significantly different at p<0.05

Table 4: Amino acids in pumpkin fruit of four investigated cultivars

	Clutivar			
Amino acids (g/100 g FW)	Miben	Hongli	Lvli	Xihulu
Aspartic acid	0.26±0.03^{a}	0.14±0.01^{b}	0.06±0.02^{c}	0.12±0.01^{b}
Threonine	0.04±0.01^{ab}	0.05±0.01^{a}	0.03±0.01^{b}	0.02±0.00^{b}
Serine	0.05±0.01^{a}	0.05±0.01^{a}	0.03±0.00^{b}	0.03±0.01^{b}
Glutamic acid	0.18±0.02^{b}	0.24±0.04^{a}	0.07±0.01^{c}	0.18±0.02^{b}
Glycine	0.05±0.01^{a}	0.06±0.01^{a}	0.03±0.00^{b}	0.03±0.01^{b}
Alanine	0.07±0.02^{a}	0.09±0.02^{a}	0.04±0.01^{b}	0.04±0.01^{b}
Valine	0.05±0.01^{ab}	0.07±0.01^{a}	0.04±0.02^{ab}	0.04±0.01^{b}
Methionine	0.02±0.00^{a}	0.02±0.01^{ab}	0.01±0.01^{b}	0.01±0.01^{ab}
Isoleucine	0.04±0.01^{ab}	0.06±0.01^{a}	0.03±0.01^{b}	0.03±0.02^{b}
Leucine	0.07±0.02^{ab}	0.09±0.01^{a}	0.05±0.01^{b}	0.04±0.01^{b}
Tyrosine	0.03±0.01^{a}	0.03±0.01^{a}	0.02±0.00^{a}	0.02±0.01^{a}
Phenylalanine	0.04±0.01^{ab}	0.06±0.02^{a}	0.03±0.01^{bc}	0.02±0.00^{c}
Lysine	0.06±0.01^{ab}	0.08±0.02^{a}	0.04±0.01^{b}	0.04±0.01^{b}
Histidine	0.02±0.00^{a}	0.03±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
Arginine	0.05±0.01^{b}	0.08±0.02^{a}	0.04±0.01^{b}	0.04±0.01^{b}
Proline	0.04±0.01^{b}	0.05±0.00^{a}	0.03±0.01^{bc}	0.02±0.00^{c}
Total	1.07±0.03^{b}	1.20±0.02^{a}	0.55±0.01^{d}	0.70±0.02^{c}

All data were expressed as the means±S.D., n = 3; Means with different superscript letters within a column are significantly different at p<0.05

significant (p<0.05) variation was observed (0.99-1.66%). The highest amount of protein was observed for Hongli (1.66%), follwed by Miben (1.45%), while the lowest was in Lvli pumpkin (0.99%). As shown in Table 3, there were not very great variations in fat content among coutivars except for Xihulu, which had the lowest fat content valued 0.31%. Similar values of protein and fat were found for a pumpkin cultivar grown in India, 1.44 and 0.34%, respectively (Kulkarni and Joshi, 2013). In another literature, *Cucurbita pepo* and *Cucurbita moschata* were reported to have much lower protein and fat content when cultivated in Korea (Kim *et al*., 2012). This could be partially due to the differences in soil constitution, climatic conditions and agronomic practices of local area in different countries.

The sugars in four pumpkin cultivars were identified as sucrose, glucose and fructose in this study as showed in Table 3. Wide variations were found both between pumpkin cultivars and sugar types. Sucrose was observed to be considerably high in Miben (5.21%) while very low in Xihulu (0.04%). Huge differences in sucrose content could contribute partly to distinct mouth feel of pumpkin fruits since sucrose is the dominant sugar for sweetness. Fructose was found to be the major reducing sugar with approximate twice hihger values than glucose in four pumpkin cultivars. Lvli exhibited the highest concentrations of fructose and glucose, 1.09 and 0.64%, respectively. Hongli had the lowest concentrations of fructose and glucose, which were 0.57 and 0.40%, respectively. The result agreed with the findings of Zinash *et al*. (2013) who reported that sugar content of pumpkin fruit significantly varied from cultivar to cultivar.

Amino acids analysis: The amino acid compositions of pumpkin fruits were presented in Table 4. A significant variation in amino acid concentrations was found among the four pumpkin cultivars studied. The summation of amino acids significantly (p<0.05) reduced in the order of Hongli (1.20 g/100 g FW), Miben (1.07 g/100 g FW), Xihulu (0.70 g/100 g FW) and Lvli (0.55 g/100 g FW). The fruit of Hongli had the highest individual amino acid contents except for aspartic acid, which was measured in big concentration in the fruit of Miben. As showed in Table 4, glutamic acid and aspartic acid were found to be the dominant amino acids present in pumpkin fruit of all investigated cultivars, varied from 0.07 to 0.24 g/100 g FW and 0.06 to 0.26 g/100 g FW, respectively. Alanine, valine, leucine, lysine and arginine were also detected to be major amino acids with a large amount. In a previous study, total amino acids in mature fruit of *Cucurbita maxima* had been reported to be 10.77 mg/g FW, which was close to our results of Hongli and Miben (Sharma and Ramana Rao, 2013). They also found that amino acid composition was changed during the stage of growth and ripening. However, differences in the amino acid composition among different pumpkin cultivars had not been published before this research.

Table 5: Antioxidant activities and related compounds β-carotene, L-ascorbic acid, total phenols content in pumpkin fruit of four investigated cultivars

Clutivar	β-carotene (mg/100 g FW)	L-ascorbicacid (mg/100 g FW)	Total phenols (mg GAE/100 g W)	Antioxidant activities (mg VE/100 g FW)	
				DPPH	FRAP
Miben	1.12±0.03[a]	15.87±0.11[c]	21.78±0.16[b]	13.07±0.06[b]	34.04±0.15[b]
Hongli	0.77±0.03[b]	20.98±0.34[a]	13.80±0.17[c]	6.59±0.06[c]	26.16±0.45[c]
Lvli	0.66±0.04[c]	19.70±0.05[b]	26.96±0.04[a]	24.65±0.02[a]	41.09±0.07[a]
Xihulu	0.17±0.01[d]	14.49±0.11[d]	6.82±0.07[d]	3.65±0.05[d]	10.48±0.19[d]

All data were expressed as the means±S.D., n = 3; Means with different superscript letters within a column are significantly different at p<0.05

Antioxidant activity and related compounds (β-carotene, L-ascorbic acid and phenols): The antioxidant activity and related antioxidant compounds such as β-carotene, L-ascorbic acid and phenols were analyzed in four pumpkin cultivars.

Concentration of β-carotene showed significant differences (p<0.05) among cultivars (Table 5). Miben exhibited the highest β-carotene content of 1.12 mg/100 FW, followed by Hongli (0.77 mg/100 FW), Lvli (0.66 mg/100 FW) and Xihulu (0.17 mg/100 FW). A previous study reported that the β-carotene content in different varieties of pumpkins cultivated in Austria ranged from 0.06 to 7.4 mg/100 g FW (Murkovic *et al*., 2002). Their result was in agreement with our study. Additionally, Dutta *et al*. (2006) found that blanched pumpkin had higher β-carotene content (12.46 μg/g FW) than the unbalanced one (10.94 μg/g FW). Provesi *et al*. (2011) found that cooking resulted in a slight degree of isomerisation of β-carotene while storage for 180 days did not significantly affect (p<0.05) the concentrations of major carotenoids. Thus, it will be of great importance to investigate the optimized processing technologies to reduce their losses of β-carotene during processing.

A significant (p<0.05) variation in the L-ascorbic acid content among the cultivars was showed in Table 5. The values of L-ascorbic acid ranged from 14.49 to 20.98 mg/100 g of FW. Hongli showed the highest concentration of L-ascorbic acid (20.98 mg/100 FW), followed by Lvli (19.70 mg/100 FW), Miben (15.87 mg/100 FW) and Xihulu (14.49 mg/100 FW). This result corroborated with the findings reported by Roura *et al*. (2007) that L-ascorbic acid content was 22.87 mg/100 g FW in full-ripe *Cucrubita moschata* Duch cultivated in Argentina. However, in contrast to our study, Zhou *et al*. (2014) obtained much lower L-ascorbic acid content of 9.47 mg/100 g FW in a local pumpkin cultivar in China. This could be attributed to the HPLC detection method we used which is more accurate than titration method.

As one of the most important antioxidant plant components, total phenols in four pumpkin cultivars were also analyzed in this study. As shown in Table 5, the concentration of phenols was dependent on cultivars and a significant (p<0.05) difference was observed. Among the cultivars, Lvli exhibited highest concentration of total phenols (26.96 GAE/100 g FW), followed by Miben (21.78 GAE/100 g FW) and Hongli (13.80 GAE/100 g FW), where as Xihulu showed the lowest level of total phenols (6.82 GAE/100 g FW). Oloyede *et al*. (2012) reported that value of total phenolics in a mature pumpkin fruit grown in Nigeria was 23.7 mg/100 g, which was close to our results.

Differences in the profile and contents of β-carotene, L-ascorbic acid and phenols in pumpkin could contribute to the differences in antioxidant activity. Two *in vitro* assys (DPPH, FRAP) were used as complementary methods to evaluate the potential antioxidant activity. As in the case of related antioxidant compounds, significant (p<0.05) differences were observed between different cultivars in the two assys. As shown in Table 5, the DPPH scavenging activity of pumpkin extracts had significantly different values with a range from 3.65 to 24.65 mg VE/100 g FW. Extracts from Lvli scavenged the most DPPH radical (24.65 mg VE/100 g FW), followed by Miben with a value of (13.07 mg VE/100 g FW). Extracts of Hongli and Xihulu had got very low scores for DPPH scavenging activity, 6.59 mg VE/100 g FW and 3.65 mg VE/100 g FW, respectively. Similar behavior was found when the antioxidant activity was measured with the FRAP assay (10.48-34.04 mg VE/100 g FW), with Lvli the highest and Xihulu the lowest.

Correlation analysis showed that the DPPH scavenging activity and Feric Reducing Antioxidant Power (FRAP) were closely corresponded to the concentration of total phenolic compounds (r = 0.94 and r = 0.98, respectively). In previous studies, a good correlation (r = 0.79) was observed between the percentage of reduced DPPH and the total phenol content of traditional apple cultivars from Southern Italy (Panzella *et al*., 2013). Teow *et al*. (2007) reported that the total phenolic contents highly (R^2 = 0.820) correlated with the DPPH values of sweet potatoes. It seems that the total phenols content can serve as a useful indicator for the antioxidant activities of pumpkin. In contrast, the result of β-carotene and L-ascorbic acid content in the studied samples didn't correlated significantly with their antioxidant capacity determined by DPPH and FRAP methods. However, these results didn't agree with previous studies reporting that vitamin C was one of the best predictors of antioxidant capacity of *Brassic a*ssessed by FRAP (R^2 = 0.832) (Kaulmann *et al*., 2014) and a high correlation (r = 0.857) between DPPH and carotenes in lettuce (Viacava *et al*., 2014). The differences were

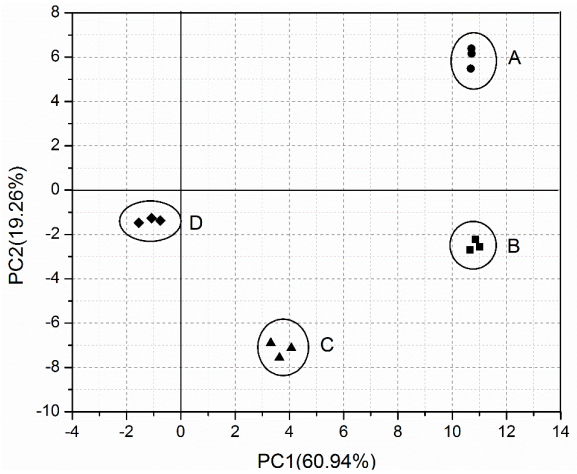

Fig. 1: Score plot of the first and second principal components resulting from a PCA analysis of four pumpkin cultivars; Class A included all the Hongli pumpkins; Class B-D represented the Miben, Lvli and Xihulu pumpkins, respectively

possibly due to the masking effect since total phenols content was far higher than β-carotene in this study.

Principal component analysis: Principal Component Analysis (PCA) was used to examine the similarity among varietal pumpkin fruits using all physico-chemical and antioxidant data measured as descriptors. As presented in Fig. 1, the first two principal components took into account 80.20% of the total variation. The first Component (PC1) represented 60.94% and the second Component (PC2) represented 19.26%. Based on the scores on PC1 and PC2, four different classes were well separated, with Class A including all the Hongli pumpkins and Class B-D representing the Miben, Lvli and Xihulu pumpkins, respectively. PC1 was mainly correlated to dry matter (0.198), TSS (0.195), a* (0.203), β-carotene (0.197), K (0.202), P (0.203), Fe (0.196) and Zn (0.195). PC2 was mainly attributed by protein (0.254), Na (0.294), Ca (0.277) and glutamic acid (0.292), whereas inversely correlated with fructose (-0.325), total phenols (-0.223), DPPH (-0.271) and FRAP (-0.194). Miben and Hongli showed much higher scores on PC1 than Lvli and Xihulu as they both showed a more reddish color and had higher concentration of dry matter, TSS, β-carotene, P, Fe and Zn. The PC2 score of Lvli was found to be much lower than other cultivars, indicating that it had lower concentration of protein, Na, Ca, glutamic acid but higher contents of fructose, total Phenols and Higher Antioxidant activities (DPPH and FRAP).

CONCLUSION

In conclusion, different pumpkin cultivars investigated in this study presented different physico-chemical and antioxidant properties, which are important factors for appraising the nutritional quality and processing characteristic of pumpkin fruits. Among the four cultivars, Lvli cultivar showed the highest antioxidant activity (DPPH and FRAP), which were highly correlated (r = 0.94 and r = 0.98, respectively) with total phenols content in pumpkin fruit. According to the results, the quality of pumpkin fruit was affected by genotype to a large extent. The four pumpkin cultivars were well separated by Principal Component Analysis (PCA) and each cultivar presented a distinctive characteristic. These data will help in the selection of pumpkin cultivars to improve the processing efficiency and health benefits of pumpkin products. In addition, further studies are required to obtain a thorough evaluation of pumpkin fruit and novel technological treatments should be applied in order to reduce the nutritional losses during processing of pumpkin.

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REFERENCES

Adams, G.G., S. Imran, S. Wang, A. Mohammad, S. Kok, D.A. Gray, G.A. Channell, G.A. Morris and S.E. Harding, 2011. The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines. Food Res. Int., 44: 862-867.

AOAC, 2000. Official Methods of Analysis of AOAC International. In: Horwitz, W. (Ed.), 17th Edn., AOAC International, Office of the Federal Register, U.S. Government, Washington, D.C.

Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., 239: 70-76.

Biesiada, A., A. Nawirska, A. Kucharska and A. Sokó£-£êtowska, 1962. Chemical composition of pumpkin fruit depending on cultivar and storage. Ecol. Chem. Eng. A, 18: 9-18.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Budini, R., D. Tonelli and S. Girotti, 1980. Analysis of total phenols using the Prussian Blue method. J. Agr. Food Chem., 28: 1236-1238.

De Carvalho, L.M.J., P.B. Gomes, R.L.D.O. Godoy, S. Pacheco, P.H.F. do Monte, J.L.V. de Carvalho, M.R. Nutti, A.C.L. Neves, A.C.R.A. Vieira and S.R.R. Ramos, 2012. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): A preliminary study. Food Res. Int., 47: 337-340.

Dini, I., G.C. Tenore and A. Dini, 2013. Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. LWT-Food Sci. Technol., 53: 382-385.

Dutta, D., A. Dutta, U. Raychaudhuri and R. Chakraborty, 2006. Rheological characteristics and thermal degradation kinetics of beta-carotene in pumpkin puree. J. Food Eng., 76: 538-546.

Fu, C., H. Shi and Q. Li, 2006. A review on pharmacological activities and utilization technologies of pumpkin. Plant Food. Hum. Nutr., 61: 73-80.

Hertog, M.G.L., P.C.H. Hollman and D.P. Venema, 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agr. Food Chem., 40: 1591-1598.

Hymavathi, T.V. and V. Khader, 2005. Carotene, ascorbic acid and sugar content of vacuum dehydrated ripe mango powders stored in flexible packaging material. J. Food Compos. Anal., 18: 181-192.

Jacobo-Valenzuela, N., M.R. Maróstica-Junior, J.d.J. Zazueta-Morales and J.A. Gallegos-Infante, 2011. Physicochemical, technological properties, and health-benefits of *Cucurbita moschata* Duchense vs. Cehualca: A review. Food Res. Int., 44: 2587-2593.

Kaulmann, A., M.C. Jonville, Y.J. Schneider, L. Hoffmann and T. Bohn, 2014. Carotenoids, polyphenols and micronutrient profiles of Brassica oleraceae and plum varieties and their contribution to measures of total antioxidant capacity. Food Chem., 155: 240-50.

Kim, M.Y., E.J. Kim, Y. N. Kim, C. Choi and B.H. Lee, 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts. Nutr. Res. Pract., 6: 21-7.

Košťálová, Z., Z. Hromádková, A. Ebringerová, M. Polovka, T.E. Michaelsen and B.S. Paulsen, 2013. Polysaccharides from the Styrian oil-pumpkin with antioxidant and complement-fixing activity. Ind. Crop. Prod., 41: 127-133.

Kulkarni, A.S. and D.C. Joshi, 2013. Studies on selected physico-chemical properties of pumpkin. Asian J. Dairy Food Res., 32: 126-129.

Liu, F., X. Cao, H. Wang and X. Liao, 2010. Changes of tomato powder qualities during storage. Powder Technol., 204: 159-166.

Liu, F.X., S.F. Fu, X. F. Bi, F. Chen, X.J. Liao, X.S. Hu and J.H. Wu, 2013. Physico-chemical and antioxidant properties of four mango (*Mangifera indica* L.) cultivars in China. Food Chem., 138: 396-405.

Mahabir, V. and V. Verma, 2012. Application of atomic absorption spectroscopy in food sciences: (A study on cucurbita maxima). APCBEE Proc., 2: 135-140.

Murkovic, M., U. Mülleder and H. Neunteufl, 2002. Carotenoid content in different varieties of pumpkins. J. Food Compos. Anal., 15: 633-638.

Nawirska-Olszanska, A., A. Kita, A. Biesiada, A. Sokol-Letowska and A.Z. Kucharska, 2013. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. Food Chem., 139: 155-161.

Oloyede, F.M., G.O. Agbaje, E.M. Obuotor and I.O. Obisesan, 2012. Nutritional and antioxidant profiles of pumpkin (*Cucurbita pepo* Linn.) immature and mature fruits as influenced by NPK fertilizer. Food Chem., 135: 460-463.

Oloyede, F.M., O.C. Adebooye and E.M. Obuotor, 2014. Planting date and fertilizer affect antioxidants in pumpkin fruit. Sci. Hortic., 168: 46-50.

Panzella, L., M. Petriccione, P. Rega, M. Scortichini and A. Napolitano, 2013. A reappraisal of traditional apple cultivars from Southern Italy as a rich source of phenols with superior antioxidant activity. Food Chem., 140: 672-679.

Provesi, J.G., C.O. Dias and E.R. Amante, 2011. Changes in carotenoids during processing and storage of pumpkin puree. Food Chem., 128: 195-202.

Robinson, R.W. and D.S. Decker-Walters, 1997. Cucurbits. CAB International, New York.

Roura, S.I., C.E. Del Valle, L. Aguero and L.A. Davidovich, 2007. Changes in apparent viscosity and vitamin c retention during thermal treatment of butternut squash (*Cucurbita moschata* DUCH) pulp: Effect of ripening stage. J. Food Qual., 30: 538-551.

Sharma, S. and T.V. Ramana Rao, 2013. Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. Int. Food Res. J., 20: 2309-2316.

Shi, X., H. Wu, J. Shi, S.J. Xue, D. Wang, W. Wang, A. Cheng, Z. Gong, X. Chen and C. Wang, 2013. Effect of modifier on the composition and antioxidant activity of carotenoid extracts from pumpkin (*Cucurbita maxima*) by supercritical CO2. LWT-Food Sci. Technol., 51: 433-440.

Song, Y., Y. Yang, Y. Zhang, L. Duan, C. Zhou, Y. Ni, X. Liao, Q. Li and X. Hu, 2013. Effect of acetylation on antioxidant and cytoprotective activity of polysaccharides isolated from pumpkin (*Cucurbita pepo*, lady godiva). Carbohyd. Polym., 98: 686-691.

Subramanian, R., S. Gayathri, C. Rathnavel and V. Raj, 2012. Analysis of mineral and heavy metals in some medicinal plants collected from local market. Asian Pacific J. Trop. Biomed., 2: S74-S78.

Sugden, A.L., J.A. Straw and B.L. Bean, 1987. Effects of a high K+/low Na+ diet on blood pressure in young spontaneously hypertensive rats. Clin. Sci., 72: 313-319.

Taylor, M.J. and J. Brant, 2002. Trends in World Cucurbit Production, 1991 to 2001. In: Maynard, D.N. (Eds.), Cucurbitaceae. ASHS Press, Alexandria, VA, pp: 373-379.

Teow, C.C., V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem., 103: 829-838.

Viacava, G.E., G. Gonzalez-Aguilar and S.I. Roura, 2014. Determination of phytochemicals and antioxidant activity in butterhead lettuce related to leaf age and position. J. Food Biochem., 38: 352-362.

Whitaker, T.W. and G.N. Davis, 1962. Cucurbits. Interscience Publisher Inc., New York.

Zhou, C.L., W. Liu, J. Zhao, C. Yuan, Y. Song, D. Chen, Y.Y. Ni and Q.H. Li, 2014. The effect of high hydrostatic pressure on the microbiological quality and physical-chemical characteristics of Pumpkin (*Cucurbita maxima* Duch.) during refrigerated storage. Innov. Food Sci. Emerg., 21: 24-34.

Zinash, A., T.S. Workneh and K. Woldetsadik, 2013. Effect of accessions on the chemical quality of fresh pumpkin. Afr. J. Biotechnol., 12: 7092-7098.