

Evaluation of three pumpkin species: correlation with physicochemical, antioxidant properties and classification using SPME-GC–MS and E-nose methods

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Revised: 11 May 2017 / Accepted: 14 June 2017 / Published online: 7 July 2017
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Abstract To ascertain the most discriminant variables for three pumpkin species principal component analysis (PCA) was performed. Twenty-four parameters (pH, conductivity, sucrose, glucose, total soluble solids, L^* , a^* , b^* , individual weight, edible rate, firmness, citric acid, fumaric acid, L-ascorbic acid, malic acid, PPO activity, POD activity, total flavonoids, vitamin E, total phenolics, DPPH, FRAP, β -carotene, and aroma) were considered. The studied pumpkin species were *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo*. Three pumpkin species were classified by PCA based on aroma, physicochemical and antioxidant properties because the sum of PC1 and PC2 were both greater than 85% (85.06 and 93.64% respectively). Results were validated by the PCA and showed that PPO activity, total flavonoid, sucrose, glucose, TSS, a^* , pH, malic acid, vitamin E, DPPH, FRAP and β -carotene, and aroma are highly useful parameters to classify pumpkin species.

Keywords Antioxidant capacity · E-nose · PCA · Physicochemical · Pumpkin

Abbreviations

DPPH 1,1-Diphenyl-2-picrylhydrazyl
E-nose Electronic nose

FRAP	Ferric reducing antioxidant power
FW	Fresh weight
SPME-GC–MS	Solid phase micro-extraction-gas chromatography–mass spectrometry
TSS	Total soluble solids
PCA	Principal component analysis
POD	Peroxidase
PPO	Polyphenol oxidase

Introduction

Cucurbita pepo, *Cucurbita maxima* and *Cucurbita moschata* belong to the genus *Cucurbita* and *Cucurbitaceae* family, and are cultivated in the world today, producing over 20 million tons annually (Kim et al. 2012). *C. pepo* (“zucchini”), *C. moschata* (“miben”), and *C. maxima* (“Jinli”) are compatibly cultivated in China. Some similar cucurbit varieties and the pumpkin (*Cucurbita pepo* L.) which initially were domesticated in Mexico, have turned into a main part of diet nowadays (Stevenson et al. 2007). Pumpkins (*Cucurbita maxima*) are planted broadly in temperate and subtropical regions over the world, which is an extremely general cultivar of *Cucurbitaceae* family. Pumpkins (*Cucurbitaceae*) can be used to produce jams, juices, alcoholic beverages and purees (Jiao et al. 2014). Pumpkins can be eaten fresh or in the form of processed foods such as jellies, syrups, purees, and jams since their pulp and seeds are good for human nutrition (Provesi et al. 2011). Pumpkins (*Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*) can be used for medicine as well as nutritional food in many countries. These varieties are planted throughout the world and have high yields (Kim et al. 2012). It is very popular with its sweet taste, soft texture, low-calorie count, and abundant nutrition. Pumpkins are especially valuable due to its

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abundant quantity of carotenoids in its pulp, including lutein, β -carotene, and violaxanthin (Biesiada et al. 2009). Also, lots of species of *Cucurbita maxima* are characterized because of its substantial content of ascorbic acid (Gonçalves et al. 2007; Biesiada et al. 2009). Organic acids and total soluble sugars mainly including fructose, glucose, and sucrose give pumpkins a tasty trait, however, volatile components endow different flavors.

Latest studies are concentrated on the protective properties of fruits and vegetables against illnesses, such as coronary heart disease or cancer. This is due to its high concentration of antioxidant vitamins, such as ascorbic acid and vitamin E, phenols compounds, and last but not least carotenoids (Jacobo-Valenzuela et al. 2011). The orange or red colored pumpkins, *Cucurbita maxima* L., are valuable sources of carotenoids among special orange pulps of vegetables or vegetable juices (Kreck et al. 2006). There is an increasing number of research about oxidation resistance, especially about carotenoids in pumpkins that have a high concentration of β -carotene, lutein, and α -carotene. The carotenoids can protect body organs and tissue from free radical damage, and also efficiently clear those singlet oxygen. Oxidative damages are always related to cancer, premature aging, cataracts, age-related macular degeneration, atherosclerosis, and a series of other degenerative diseases (Murkovic et al. 2002). The oil from pumpkin seeds has many benefits such as in avoiding of arthritis, reducing of bladder and urethral pressure, preventing prostate disease, retardating of the progression of hypertension, mitigating hypercholesterolemia and improving bladder compliance, lowering the levels of breast, colorectal, gastric, and lung cancers, providing antioxidant potential and alleviating diabetes by promoting hypoglycemic activity (Jiao et al. 2014; Rezig et al. 2012; Stevenson et al. 2007).

Phenolic compounds can strengthen the activity of other reducing agents, especially lipid solubility vitamins, and often function as a group of reducing agents. They act via different patterns, for example, they enhance the dissimilation of free radicals to compounds of a virtually lower reactivity, by inhibiting or enhancing the activity of enzymes, chelating metals, and 'scavenging' free radicals (Nawirska-Olszanska et al. 2013).

Pumpkin pulp is low-caloric, depending on its species, containing only 15–25 kcal in 100 g. Because of this pumpkins have an abundant dietary nutrition and they have become a favorable vegetable of slimming foods (Nawirska-Olszanska et al. 2013.) The organic acids of vegetables usually play a significant role in inhibiting natural microorganism and enhancing flavor. However, there is little investigation about the content of organic acids of different pumpkin species in the same planted region. In China, there is a higher consumption frequency of *Cucurbita* species, *C. maxima* and *C. moschata*, whereas

the consumption rate of *C. pepo* is much lower. Therefore, very few people study *C. pepo*. Some reference data about the analysis of nutritional components, vitamin E, fatty acids, the amino acid, and carotenoid contents are available. Until now, there was not much research on functional analysis of *C. pepo* cultivated in China, including the nutrients of the different parts amongst the pumpkin species. Pumpkins are planted in different areas and in environments that will show various nutrient composition (Applequist et al. 2006).

The purpose of the study is to determine and discuss of the physicochemical and antioxidant properties of three pumpkin species, and to obtain a sensory comparative description of pumpkins in China. In the future, pumpkins will be used to their full potential because of this study.

Materials and methods

Raw materials

Three pumpkin species (*Cucurbita pepo*-Zucchini, *Cucurbita moschata*-Miben and *Cucurbita maxima*-Jinli) were planted in a field at the Experimental Station of Jiangxi Science and Technology Normal University on 20 April, and harvested on 15 August, 15 September and 20 September 2014 respectively. The University is located at latitude of 28° 15' N and longitude of 115° 28' east and an altitude of 22 m. The rainfall of the area is bimodal (in April and August) type with an average annual rainfall of 1650 mm. The average annual temperature of Nanchang recorded by China Meteorological Agency is 17.5 °C with mean minimum and maximum temperatures of 3.5 and 35 °C, respectively. The mean relative humidity is 78.5%, varying from 50 to 95% and the soil type of the area is well-drained deep clay loam type. Harvest took place in the second week of August or September. After harvest, pumpkins were placed for two weeks at about 22 °C in the dark. Healthy fruits weighing between 1.8 and 2.5 kg were selected and stored at 4 °C. The grade of maturity was detected by the traditional parameters, fruit growing period life in the aspects of days after fertilization, fruit color, size and shape. Twenty pumpkin mature and healthy samples were chosen, cleaned and frozen in liquid nitrogen immediately, after that stored at –80 °C until experiment within 3 months for fresh fruit quality assessment. Samples of three species fruits from each sample (one fruit from each replicated plot) were taken for analysis.

Chemical reagent

1,1-Diphenyl-2-picrylhydrazyl hydrate (\cdot DPPH), Formic acid, Methanol, acetonitrile of HPLC-grade, 2,

6-dichlorophenolindophenol and Gallic acid, citric acid, malic acid, oxalic acid, acetic acid, tartaric acid, fumaric acid, The sugars (glucose, fructose, sucrose) as the external standards of HPLC-grade, L-ascorbic acid, and 1,3,5-tri (2-pyridyl)-2,4,6-triazine (TPTZ) reagents were purchased from Sigma-Aldrich Co. (Shanghai, China). Other chemicals were bought from Beijing Chemicals Co. (Beijing, China).

L-ascorbic acid

The contents of L-ascorbic acid were measured by 2, 6-dichlorophenolindophenol titration (Zhou et al. 2014). Briefly, 50 g pumpkin pulp was immediately homogenized in 50 mL of 0.02 g/mL oxalic acid solution and then centrifuged at 15,000g and 4 °C for 15 min. Total of 10 mL supernatant were titrated to a permanent pink color by 0.1% 2, 6-dichlorophenolindophenol titration. L-ascorbic acid concentration was calculated according to the titration volume of 2, 6-dichlorophenolindophenol and expressed as milligram per 100 g of fresh weight.

β-carotene

β-Carotene was tested with HPLC technology with slight changes (Liu et al. 2013). 15 g of sample were mixed with 30 mL acetone, sonicated for 15 min and centrifuged at 10,000 rpm at 4 °C for 10 min, the extraction was treated three time to guarantee the most extraction. All the extracts were gathered and made up to final volume of 100 mL. Then, 50 mL of KOH (10%) dissolved methanol was mixed with the extracts for saponification at 45 °C water-bath for 50 min. After that all the extracts were added to 100 mL petroleum ether, and the organic layers were dehydrated and concentrated using an anhydrous sodium sulfate column and evaporated to dryness. The waterless residue was mixed and dissolved in hexane and filtered through a 0.45 μm membrane filter for analysis using the RF-10AXL HPLC system measured at 445 nm at 30 °C (Shimadzu Co., Ltd, Japan). A Sunfire™ C18 analytical column was used from Waters (4.6 × 250 mm i.d, 5 μm particle size). The mobile phase was using acetonitrile: methanol: methylene chloride (6:2:2, v/v/v), with isocratic flow at a rate of 1.0 mL/min. The concentration content was calculated on the basis of external standard β-carotene and shown as milligram β-carotene per 100 g of FW.

Total phenolics

Total phenolics were measured by the Folin–Ciocalteu method reported by Liu et al. (2013) with slight changes. 2.5 g pumpkin pulps were added to 30 mL methanol, the mixtures were placed in the 90 w, 55 °C ultrasonic cleaner

for 30 min, after that centrifuged at 9000 rpm for 10 min at 4 °C. The supernatant was gathered and dissolved to 50 mL with pure methanol. 400 μL of the extracted sample was added to 2 mL of Folin–Ciocalteu reagent (diluted tenfold using double distilled water in advance) and placed in the dark for 1 h at 25 °C. After that 1.8 mL sodium carbonate (7.5%) was added to the above mixture and reacted for 20 min, and the reaction mixtures were determined at 765 nm by a spectrophotometer immediately (UV-726, Shimadzu, Shanghai, China). The contents of total phenolic of extracts were showed as mg gallic acid equivalents mg (GAE)/100 g pumpkin pulp.

Total flavonoids

The contents of total flavonoids were determined on the basis of the aluminum chloride colorimetric method (Di Marco et al. 2014). Aliquots of 0.1 g of fruit pulp was added to 1 mL deionized water respectively. This solution (0.5 mL) was dissolved with 95% alcohol, 0.1 mL of 10% aluminum chloride hexahydrate (AlCl₃), 0.1 mL of 1 M potassium acetate (CH₃COOK), and 2.8 mL of deionized water. Then incubation at 25 °C for 50 min, the reaction mixture absorbance was determined at 415 nm using a spectrophotometer. Deionized water and quercetin was set as blank and standard respectively. Using a seven point standard curve (0–50 mg/l), the contents of total flavonoids in all pumpkin samples were determined in triplicate. The results were shown as milligram quercetin equivalents (QE)/100 g of fresh weight.

Reducing sugar (glucose, fructose), and sucrose

50 g of pumpkin pulp were added to 150 mL distilled water and blended using a JYL-B060 beater (Jiuyang Co., China), then the homogenate was placed at 4 °C for 2.5 h and centrifuged with 12,000 rpm for 25 min at 4 °C (GL-166-A, Shanghai Anting Scientific Equipment Factory, Shanghai, China), after that filtered through a 0.45 μm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China); the supernatant was gathered for the HPLC measurement of glucose, sucrose, and fructose (Liu et al. 2013). HPLC system (Knauer Co., Ltd, Germany) was composed of a K-501pump (Knauer Co., Ltd, Germany), linked to a refractive index detector (RI-2401, Knauer Co., Ltd., German), and a 20 μL sample injection. The column was YMC-Pack Polyamine II (4.6 × 250 mm i.d, 5 μm particle size). The mobile phase was acetonitrile: water (75:25) and the flow rate was 1.0 mL/min at 25 °C. Calculations were carried on using the external standard with HPLC-grade glucose, sucrose, and fructose.

Organic acids

Organic acids was measured also using HPLC technology, and extracted using the same method as sugars reported by Tyagi et al. (2014) with Some modification. All solvents were HPLC grade and filtered through a 0.45 μm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China) prior to analysis. The organic acids extracts were analyzed in a HPLC chromatography system equipped with RF-10AXL HPLC system (Shimadzu Co., Japan), including a SIL-20A autosampler (Shimadzu, Kyoto, Japan), two reciprocating pump (Shimadzu LC-10 ATVP), a RF-20AXS detector (Shimadzu, Kyoto, Japan) and a reversed-phase C_{18} column (4.6 \times 250 mm i.d, 5 μm particle size). The temperature of the oven was set to 55 $^{\circ}\text{C}$. The wavelengths used for the quantification of organic acids by the detector were: 210 nm for UV range. An isocratic elution was used with a mobile phase consisting of potassium dihydrogen phosphate buffer: methanol, and solvent A, 0.01 mol/L potassium dihydrogen phosphate buffered at pH 2.55 with orthophosphoric acid, solvent B, 100% methanol. The injection volume was 20 μL , the flow rate was 0.5 mL/min at room temperature, and the duration of a single run was 20 min. The organic acid compounds were quantified using an external standard. The pumpkin extracts and standard compounds were analyzed under the same conditions, and a 15 min equilibrium time was allowed between injections. All standard and sample solutions were injected in triplicate and the reported values are the means of three replicates. All data were shown as milligram per 100 g of FW.

Determination of antioxidant activity by DPPH radical-scavenging assay

A trolox aliquot was applied to make a 50–500 $\mu\text{mol/L}$ standard curve. The results were shown as TROLOX equivalents ($\mu\text{mol}/100$ g pumpkin pulp) and antioxidant activities were known as trolox equivalents antioxidant capacity (TEAC) (Dini et al. 2013). Antioxidant capacity was measured using the DPPH assay with some changes (Shahdadi et al. 2015). The pumpkin pulps were centrifuged with 12,000 rpm at 4 $^{\circ}\text{C}$ for 20 min. After that the supernatants were gathered for next step treatment. 0.4 mL of tenfold diluted supernatant was dissolved in 8.0 mL of a methanol solution of DPPH (0.14 mmol/L). The mixtures were placed in the dark for 60 min, and the absorbance was determined at 517 nm using a spectrophotometer (UV-726, T6, PG General, Beijing, China).

Antioxidant activity

The freshly planned FRAP solution was included 25 mL 0.3 mol/L acetate buffer (pH 3.6), 2.5 mL 10 mmol/L

TPTZ (soluble in 40 mmol/L HCl) and 2.5 mL 20 mmol/L ferric chloride (Impei et al. 2015). The pumpkin pulp was centrifuged at 12,000 rpm for 20 min at 4 $^{\circ}\text{C}$. After that the supernatants were collected for next step using. 0.4 mL of tenfold diluted supernatant was mixed with 8.0 mL of FRAP solution (0.14 mmol/L) and reacted for 15 min at 37 $^{\circ}\text{C}$. The absorbance at 593 nm was detected by controlling the ferric reducing ability by a spectrophotometer (UV-726, Shimadzu, Shanghai, China), and the blank is the FRAP solution.

Aroma compounds

SPME-GC-MS technology

Ten milliliter pumpkin pulps were added into 15 mL glass vials, and then 3.0 g NaCl was added. Pumpkin pulps were equilibrated 40 min by oscillation in a 45 $^{\circ}\text{C}$ glass vials (Liu et al. 2013). The SPME machine was provided with a coated with polydimethylsiloxane/divinylbenzene/carboxen fused silica fiber (Supelco Co., Bellefonte, PA). A micro-syringe was inserted into the headspace samples for 40 min at 45 $^{\circ}\text{C}$. Samples were at 250 $^{\circ}\text{C}$ for 3.5 min for desorption in the injection port of an Agilent GC-MS (7890A-5975C). The volatile aromas were detected with an Agilent J&W DB-5 column (30 m, 0.25 mm I.D., 0.25 μm film thickness). The injection port was controlled in splitless type. The procedure of oven temperature was from 45 to 140 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}/\text{min}$, then the temperature was increased to 220 $^{\circ}\text{C}$, at a rate of 10 $^{\circ}\text{C}/\text{min}$, and the holding time was 5 min, finally, the temperature was increased to 250 $^{\circ}\text{C}$, and the final holding time was 2 min. A carrier gas was Helium at a constant flow rate of 1.0 mL/min. The MS fragmentation was carried through electronic effect EI at 70 eV, a source temperature of 230 $^{\circ}\text{C}$ and scan mode was between 50 and 550 mass units. The retention times of an array of straight-chain alkanes (C6-C24) were applied to calculate the retention indices for all detected aroma compounds.

E-nose method

Samples were conducted with the HERACLES Flash Electronic nose (Alpha M.O.S Company, France) made up a Heracles host machine and a headspace auto-sampler unit. The HERACLES is a programmed temperature gas chromatograph using a syringe or valve inlets to a flash evaporator. The sample is provided to an adsorbent trap to concentrate the sample for delivery to twin capillary columns and flame ionization detectors (FID) at the same time. Programme control and the original data analysis were finished using Alpha Soft (V11, Alpha M.O.S). The parameters are below: (1) Injector: injection time 1500 ms,

injector temperature 200 °C, injection volume 2000 µL; (2) Temperature program: initial temperature 40 °C, final temperature 200 °C, heating rate 2.0 °C/s; (3) Detector temperature: 220 °C; (4) Trap temperature: 250 °C, purge time 15 s. (5) Acquisition time: 84 s. 1000 µL of the sample was placed in a 10 mL glass with a pierceable disk device in the cap. Sample was agitated with the auto-sample unit at 40 (±0.2)°C before sample injection (Qin et al. 2013). All samples were detected in seven times and the mean were used for results statistical analysis.

PPO activity assay

PPO was obtained according to the steps reported by Hu et al. (2013) and Sampedro et al. (2014) with slight changes. Samples (20 g) were dissolved in 20 mL 4% polyvinyl pyrrolidone (PVPP) and 0.2 M phosphate buffer (pH = 6.5) and blended for 5 min, afterwards reacted for 1.5 h at 4 °C. The mixtures were centrifuged at 9000 rpm/min for 20 min at 4 °C, and the supernatants were gathered and measured for PPO enzyme activity. PPO mixtures were collected 0.5 mL and was added to 0.2 M sodium phosphate buffer (pH = 6.5) solution containing 2.5 mL 0.07 M catechol. The extracted mixtures were placed at 30 °C water bath in preparing for measuring the absorbance using a Cary 50 spectrophotometer (Varian Co., Ltd, Santa Clara, CA, USA). Enzyme activity of PPO activity (Abs/min) was calculated in accordance with the reaction curve of Δ_{A410} .

Peroxidase activity

The extraction of POD was similar with the method of PPO. 0.2 mL of 1.5% H₂O₂, 0.1 mL extract, and 3.0 mL 1.0% (v/v) guaiacol (dissolved in 0.2 M phosphate buffer, pH 6.5) was included in the reaction mixture of POD (Sampedro et al. 2014). The mixture was placed at 30 °C water bath for 20 s before detecting the absorbance. Enzyme activity of POD (Abs/min) was referred to accordance with the slope from the linear portion of the reaction curve of Δ_{A470} .

Measurement of other physicochemical parameters

Determination of color was carried through with a Color Difference Meter (SC-80, 229 Kang guang Co., Ltd, Beijing, China) in the reflectance pattern for pumpkin pulp at 25 °C. Color of the pumpkins was evaluated by measuring the L^* (100 = white; 0 = black), a^* (+, red; -, green) and b^* (+, yellow; -, blue) values using a Color Difference Meter (SC-80, 229 Kang Guang Co., Ltd, Beijing, China). The L^* value indicates lightness, the a^* and b^* values are the chromaticity coordinates (a^* , from green to red; b^* , from blue to yellow),

C^* value indicates for chroma, and h_{ab} for hue angle). The reported values are the means of three replicates

The calculation of C^* and h_{ab} equation were as follows:

$$h_{ab} = \arctan(b^*/a^*) \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

The hardness of pumpkin was evaluated by measuring the penetration force using a puncture test. Pulp hardness was analyzed by a TMS-Pro texture analyzer (Food Technology Corporation, USA) using a 5 mm diameter stainless steel puncture probe. The parameters of the test were set to compress a sample to a depth of 30% height from the contact point, the pre-test and the test speed was 50, 30 mm/min respectively and the initial force was 0.5 N (Zhou et al. 2014). To obtain an overall estimation of hardness, determination were conducted at bottom, middle, and top portions of pumpkin. Every samples was measures in eight times with a scale (EY-300A, Panasonic, Japan) and the mean were used. The force in Newton (N) was used to shown hardness. Pumpkins were cleaned, removed seeds and peeled, afterwards the edible rate was shown as weight of pulp/weight of entire fruit. 30 pumpkins were applied in the determination of the edible rate and fruit weight. A WAY-2S digital Abbe Refraction meter (Shanghai Precision and Scientific Instrument Co., Ltd, Shanghai, China) was used to measure Total soluble sugars as Brix at 25 ± 1 °C. An Orion 868 pH meter (Thermo Orion, USA) was applied with pH value measurement at 25 ± 1 °C. The conductivity measurements were made with a conductivity meter (OK-104, Radelkis, Hungary).

Data analysis

All parameters were measured triplicate, and data were shown as the mean ± standard deviation (SD). The software Origin 8.6 was used to calculate statistical data, run analysis of variance (ANOVA) for the whole experiment. Significant difference was set for $p < 0.05$. PCA was carried through the results of physic-chemical properties of the analytical pumpkin species by SPSS 12.0. PCA score plot was used to show whether pumpkin species could be grouped in different classes.

Results and discussion

Antioxidant compounds and antioxidant activity

The determined content of L-ascorbic acid, total flavonoid, β-carotene, and total phenols in the three pumpkin species was related to antioxidant activities. The total flavonoid and β-carotene content indicated significant variations

Table 1 Antioxidant activities and related compounds β -carotene, L-ascorbic acid, total phenols content in pumpkin vegetable of three species

Species	L-Ascorbic acid (mg/100 g FW)	Vitamin E (mg/100 g FW)	Total phenols (mg GAE/100 g FW)	Total flavonoid (mg QE/100 g FW)	β -Carotene (mg/100 g FW)	Antioxidant activities TEAC mmol/L trolox	
						DPPH	FRAP
<i>C. maxima</i>	13.8 \pm 0.23 ^a	1.74 \pm 0.08 ^a	436.16 \pm 2.89 ^a	8.23 \pm 0.03 ^a	1.67 \pm 0.06 ^a	3059.78 \pm 11.24 ^a	358.36 \pm 7.34 ^a
<i>C. pepo</i>	8.3 \pm 0.12 ^b	0.39 \pm 0.01 ^b	50.17 \pm 1.27 ^b	0.51 \pm 0.01 ^c	0.13 \pm 0.01 ^c	237.25 \pm 5.35 ^c	78.98 \pm 1.04 ^c
<i>C. moschata</i>	7.9 \pm 0.17 ^b	0.64 \pm 0.05 ^b	453.72 \pm 5.61 ^a	5.36 \pm 0.07 ^b	0.58 \pm 0.13 ^b	2514.09 \pm 3.57 ^b	298.42 \pm 4.78 ^b

All data were the mean \pm SD, n = 3. Means with different letters within a column are significantly different at $p < 0.05$

($p < 0.05$) in all of the species (Table 1). *C. maxima* showed maximum content of total flavonoid and β -carotene content of 8.23 \pm 0.03 mg QE/100 g FW, 1.67 \pm 0.06 mg/100 g FW, followed by *C. moschata* (5.36 \pm 0.07 mg QE/100 g FW, 0.58 \pm 0.13 mg/100 g FW), and *C. pepo* (0.51 \pm 0.01 mg QE/100 g FW, 0.13 \pm 0.01 mg/100 g FW). In previous report, the content of β -carotene in pumpkins planted in Austria among three different species (*C. maxima*, *Cucurbita pepo* and *C. moschata*) has a significant difference between 0.06 and 7.4 mg/100 g FW (Murkovic et al. 2002) and 1.246 mg/100 g FW in blanched pumpkins (Dutta et al. 2006). Murkovic et al. (2002) reported β -carotene, lutein and α -carotene content in the different species of *C. pepo* L, *C. maxima* L, and *C. moschata* L. The content of β -carotene ranged from 0.06 to 7.4 mg/100 g, from 0 to 17 mg/100 g for lutein, and from 0 to 7.5 mg/100 g for α -carotene. Previous research showed that the content of α - and β -carotene were both lower, and were discovered to change along with the species within a wide range (β -carotene from 3.1 to 7.0 mg and α -carotene from 1.0 to 5.9 mg 100 g⁻¹ fresh weight (Kim et al. 2012; Murkovic et al. 2002). Therefore, it will be useful to research the difference and content of carotenoids and its isomers or related pigments in the three pumpkin species. Moreover, the diversity and range of content and carotenoid types in pumpkins provide methods to maintain the nutritional value.

The content of total of phenols in the three pumpkin species are also shown in Table 1. There was no significant differences in total phenols ($p > 0.05$) between *C. maxima* and *C. moschata* (436.16 \pm 2.89 mg GAE/100 g, 453.72 \pm 5.61 mg GAE/100 g). Their content of total phenols was higher ($p < 0.05$) than that of *C. pepo* (50.17 \pm 1.27 mg GAE/100 g). The content of total phenols in a ripe pumpkin's pulp, planted in Nigeria, was 23.7 mg/100 g, as reported by Oloyede, was lower than our results (Oloyede et al. 2012).

The content of Vitamin E and L-ascorbic acid were significantly different amongst the species ($p < 0.05$), which was shown in Table 1. The content of L-ascorbic acid and Vitamin E between *C. pepo* and *C. moschata* were

not significantly different ($p > 0.05$), however, the content of L-ascorbic acid and Vitamin E were significantly lower than *C. maxima* (13.8 \pm 0.23 mg/100 g FW, 1.74 \pm 0.08 mg/100 g FW) ($p < 0.05$). This result was similar to our previous findings reported by Zhou in which the content of L-ascorbic acid was 9.47 mg/100 g FW in *C. maxima* in China (Zhou et al. 2014). Roura et al. (2007) reported that the content of L-ascorbic acid 22.87 mg/100 g FW in mature fruit of *Cucurbita moschata* Duch planted in Argentina, compared to our results was much higher. The phenomenon occurred could be credited to different species and measurement methods.

Antioxidant activity data (DPPH and FRAP) are shown in Table 1, the DPPH radical-scavenging activity from the three pumpkin species was significantly different ($p < 0.05$). The wide range was from 237.25 to 3059.78 equal to mmol/L Trolox TEAC. The DPPH radical-scavenging activity from *C. maxima* was 1.22 and 12.91 times greater than that from *C. moschata* and *C. pepo* which approximately conformed to the content of flavonoid, phenolic compounds, β -carotene contents. The research of Jiao et al. (2014) also showed that free radical scavenging activity is closely related to β -carotene concentration, and the similar results in our study were shown that the antioxidant activity was (78.98–358.36 equal to mmol/L Trolox TEAC), with *C. maxima* having the highest and *C. pepo* having the lowest using the FRAP method.

The content of Vitamin E and L-ascorbic acid were as low as scores for FRAP and DPPH in the three pumpkin species, the relationships between the content of L-ascorbic acid and Vitamin E, and the antioxidant activity had not yet been discovered in recent research. Nevertheless, in our previous study, the content of ascorbic acid and phenolic were strongly related to reducing power and DPPH radical-scavenging activity in *C. maxima* pulp extracts (Zhou et al. 2014). The content of total phenols was much higher than the Vitamin E and L-ascorbic acid content in this research. Thus probably masking the effect resulting from the difference. To brief, it would be hard to tell the rate of contribution of these compounds related to antioxidant activity, so the group influence of bioactive compounds

could be endowed with the antioxidant potential of pumpkin.

Acids and sugars

The content of sugar in pumpkins had normally been based of total sugars, TSS or individual sugar. As indicated in Table 2, the content of total sugar (7.76 g/100 g FW) was higher in *C. maxima*, which was in accordance with the much higher content of TSS (7.2 ± 0.02 °Brix) ($p < 0.05$). The total sugars mainly contained fructose, sucrose, and glucose in the three pumpkin species, which was made up the main TSS. The content of sucrose was the highest in the three pumpkin species and fructose the major reducing sugar. The content of total sugars (about 7.76 g/100 g) was the highest ($p < 0.05$) in *C. maxima* pumpkins, mostly due to sucrose's contribution. Meanwhile, *C. maxima* pumpkins showed the highest content in reducing sugars. The content of reducing sugars in the three species was 2.16, 1.08, and 1.77 g/100 g respectively, and *C. moschata* pumpkins' was much higher. *C. pepo* pumpkins have the lowest concentrations of sucrose, glucose and fructose, 1.02, 0.51, and 0.65 g/100 g, respectively. Compared to our results, lower values of total soluble solids (TSS) in *C. moschata* pumpkins was 4.4 ± 0.01 °Brix and higher total soluble solids (10.5 °Brix) were reported by Conti for field grown organic winter squash (*Cucurbita moschata* Duch.) in Italy (Conti et al. 2015). The content of sugars in pumpkin's pulp was very different relative to the different varieties

which was reported by Zinash and WorknehTS (2013). The higher the content of total soluble solids (TSS), the higher the ion concentration, and the stronger the conductivity. *C. maxima* exhibited the highest TSS content of 7.2 ± 0.02 °Brix, followed by *C. moschata* (4.4 ± 0.01 °Brix) and *C. pepo* (2.6 ± 0.01 °Brix).

Changes in conductivities of vegetable and fruits samples were studied and shown to be affected by a number of factors, for example, field strength, soluble solids, melting of fats, and cell structure changes (Leizeron and Shimoni 2005). The variation trend of TSS was closely related to the content of conductivity in the three pumpkin varieties. Compared to grape, orange and tangerine, all the three pumpkin had highest conductivity. *C. maxima* showed the highest conductivity of 7158 ± 11.01 μS/cm, followed by *C. moschata* (7005 ± 21.09 μS/cm) and *C. pepo* (6109 ± 12.32 μS/cm). The conductivity of fruits at 20 °C was 1.23 mS/cm in the case of grapefruit juice samples, 1.2 mS/cm in the case of lemon and orange juice samples and 1.5 mS/cm in the case of tangerine juice samples, respectively (Leizeron and Shimoni 2005).

Acidity has usually been expressed through using organic acids or pH. *C. pepo* pumpkins revealed the highest acidity in spite of two different means in our research. The organic acids and pH in the three pumpkin species were significantly different ($p < 0.05$), and the pH value ranged from 5.91 to 6.88. *C. maxima* pumpkins had the highest pH (6.88) and *C. pepo* pumpkins had the lowest (5.91). The content of three organic acids in all species were

Table 2 Physico-chemical characteristics of three pumpkin species (sugar content, total soluble solids, pH, titratable acidity, organic acids content and color parameters, individual weight, edible rate and firmness)

	<i>C. maxima</i>	<i>C. pepo</i>	<i>C. moschata</i>
Sucrose (g/100 g FW)	4.02 ± 0.03^a	1.02 ± 0.02^c	2.16 ± 0.05^b
Glucose (g/100 g FW)	1.29 ± 0.01^a	0.51 ± 0.01^c	1.08 ± 0.02^b
Fructose (g/100 g FW)	2.45 ± 0.06^a	0.65 ± 0.02^c	1.77 ± 0.02^b
Total sugar (g/100 g FW)	7.76 ± 0.04^a	2.18 ± 0.03^c	5.01 ± 0.02^b
TSS(°Brix)	7.2 ± 0.02^a	2.6 ± 0.01^c	4.4 ± 0.01^b
pH	6.88 ± 0.01^a	5.91 ± 0.01^c	6.09 ± 0.01^b
Citric acid (mg/100 g FW)	0.18 ± 0.02^b	0.32 ± 0.01^a	0.04 ± 0.01^c
Malic acid (mg/100 g FW)	0.16 ± 0.01^c	0.28 ± 0.02^a	0.22 ± 0.01^b
Fumaric acid (mg/100 g FW)	0.02 ± 0.00^b	0.05 ± 0.01^a	0.01 ± 0.00^c
Conductivity (μS/cm)	7158 ± 11.01^a	6109 ± 12.32^b	7005 ± 21.09^a
L^*	36.08 ± 0.24^a	32.67 ± 0.53^c	35.58 ± 0.12^b
a^*	3.30 ± 0.22^a	-0.13 ± 0.02^c	0.52 ± 0.23^b
b^*	17.60 ± 0.42^a	1.68 ± 0.09^c	11.24 ± 0.21^b
C^*	17.91 ± 0.34^a	1.69 ± 0.11^c	11.25 ± 0.37^b
h_{ab}	79.37 ± 1.09^b	-85.58 ± 0.66^c	87.37 ± 0.47^a
Individual weight (g)	1560 ± 23.19^b	245 ± 14.65^c	2020 ± 18.39^a
Edible rate (%)	82.98 ± 2.65^a	79.35 ± 4.56^b	83.21 ± 2.14^a
Firmness (N)	38.53 ± 1.08^b	26.75 ± 1.23^c	56.35 ± 2.15^a

All data were the mean \pm SD, n = 3. Means with different letters within a column are significantly different at $p < 0.05$

demonstrated in Table 2. The highest content of organic acid among the three pumpkin species was malic acid ranging from 0.16 to 0.28 mg/100 g, the next being citric acid ranged from 0.04 to 0.32 mg/100 g. Nawirska-Olszanska et al. (2013) reported that malic acid, fumaric acid, and citric acid were the main organic acids in different pumpkin species. Their contents varied, depending on a cultivar to a large degree and also reported that the contents were decreasing in both *C. maxima* and *C. pepo* species that were in storage and increasing in *C. moschata* species after three months. The content of organic acids of pumpkin pulp from different species planted in the same region were previously discussed by authors (Kafkas et al. 2006; Kim et al. 2012). In this study, the content of fumaric acid had a lower concentration (less than 0.05 mg/100 g) in all three pumpkin species. As for the *C. moschata* pumpkins, malic acid is the main organic acids, with a content of 0.22 mg/100 g, the content of citric acid and fumaric acid were both relatively low, almost nonexistent. Citric acid and malic acid mainly existed in *C. maxima* and *C. pepo*, with a content of (0.18, 0.16 mg/100 g) and (0.32, 0.28 mg/100 g) respectively.

Individual weight, color, pulp firmness, and edible rate

The color of fruits and vegetables always directly affect the consumer acceptability. As indicated in the Table 2, the value of L^* , b^* , C^* and h_{ab} were significantly different ($p < 0.05$) in the three pumpkins. A much lower value for a^* and L^* were observed in the pulp of the *C. pepo*, while the values amongst the b^* , C^* and h_{ab} species were significantly higher ($p < 0.05$) in *C. moschata*. The yellowish-red color of the pumpkin's pulp is mainly due to the contribution of carotenoids. The close correlation is not only between total carotenoids and color value " a^* ", but also between lutein and color value " b^* " as reported by Conti et al. (2015) and Itle and Kabelka (2009). There were significant differences ($p < 0.05$) in the mean individual weight amongst the three pumpkin species, and the highest was in *C. moschata*, followed by was *C. maxima*, and *C. pepo* (Table 2). Similarly, there were also significant differences ($p < 0.05$) in firmness and edible rate, the value of edible rate and firmness was highest in *C. moschata*, and lowest in *C. pepo*. As far as edible rate, there were no significant differences between *C. moschata* and *C. maxima* ($p > 0.05$).

Aroma compounds

The aroma compounds of the three pumpkins planted in China were obtained by dynamic headspace extraction, and after that, analyzed using GC-MS. The volatile aroma

compounds were discussed and analyzed using comparisons of retention indices on DB-5 columns with libraries and their mass spectra and issued data as indicated in Table 3. In total, ninety-three aroma compounds were identified in the three pumpkin varieties. As shown in Table 3, the content of alcohols was much higher where there was an abundance of aroma compounds in *C. maxima*, followed by aldehydes. In this study, the most important volatile compound in *C. maxima* pulp seemed to be alcohols, aldehydes, and ketones, because these compounds had the highest percentage of peak area in chromatogram. In detail, 1-hexanol, 1-octen-3-ol, 3-hexen-1-ol and 1-butanol, 3-methyl- had higher percentage of peak area, which was 13.022, 10.285, 5.874 and 2.093% respectively. The percentage of aldehydes (hexanal and propanal) was 9.488% and 5.150% respectively. The percentage of 7-Octen-2-one was 2.038%. Propanal has a floral scent, hexanal is green, 3-hexen-1-ol smells herbal and green, 1-hexanol smells floral and green and 1-Butanol with fruity, which gives a green flavor to *C. maxima* pulp (Perry et al. 2009; Kourkoutas et al. 2006).

Together thirty-one flavor volatile aroma compounds were identified in *Cucurbita pepo* pulp. The most important volatile compounds in *Cucurbita pepo* pulp were alcohols, esters, and alkenes. Specifically, ethanol, 1-hexanol, and 1-butanol, 3-methyl had higher percentages of peak area, being 23.613, 4.782 and 0.749% respectively. Two important alcohols, 1-hexanol and 1-butanol, 3-methyl existed in both *C. maxima* and *Cucurbita pepo* pulp. The results were in agreement with results of other author (Leffingwell et al. 2015). Then, the percentages of Ethyl ether and Ethyl acetate were 15.431 and 12.850% respectively. The percentages of styrene was 6.041%. Acetic acid and Furan, 2-ethyl- had much higher percentages of compounds in *Cucurbita pepo* pulp.

A total of forty flavor volatile compounds were identified in *Cucurbita moschata* pulp. The most important volatile compound in *Cucurbita moschata* pulp seemed to be alcohols, esters, and alkenes. In detail, eucalyptol, ethanol, and 2-heptanol had higher percentages of peak area, which was 29.791, 6.954 and 1.021% respectively. The next most important compounds were esters (Ethyl Acetate and Ethyl ether), which was 24.148 and 11.087% respectively. The percentage of 1R- α -Pinene was 1.0922%.

Three important compounds, Ethyl acetate, Ethyl ether and Ethanol existed in both *Cucurbita moschata* and *Cucurbita pepo* pulp. Ethyl acetate has a with pineapple flavor and ethanol with ethereal, gives a pure and fresh flavor to *Cucurbita moschata* and *Cucurbita pepo* pulp (Verzera et al. 2010). There were only four kinds of common compounds in three pumpkin varieties, (1-hexanol, 2-ethyl-), eucalyptol, (propanoic acid, 2-methyl-, 3-hydroxy-2, 4,

Table 3 Volatile flavor compounds in pumpkin vegetable of three species

Number	RT (min)	Compounds	Relative area percent (%)		
			<i>Cucurbita mixma</i>	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>
1	3.7615	Ethanol	–	23.613 ± 0.03	6.954 ± 0.01
2	3.9274	Propanal	5.150 ± 0.02	–	–
3	3.9859	Ethyl ether	–	15.431 ± 0.04	11.087 ± 0.01
4	4.8252	Acetic acid	–	2.289 ± 0.01	–
5	4.9862	Ethyl acetate	–	12.850 ± 0.03	24.148 ± 0.03
6	5.7278	Butanoic acid, 3-hydroxy-, ethyl ester, (±)-	–	–	0.490 ± 0.00
7	5.762	Benzene	–	0.752 ± 0.01	–
8	6.1914	Isopropoxycarbamic acid, ethyl ester	–	0.757 ± 0.00	–
9	6.4695	Furan, 2-ethyl-	–	1.578 ± 0.01	–
10	7.2404	1-Butanol, 3-methyl-	2.093 ± 0.01	0.750 ± 0.01	–
11	7.2794	Vinyl ether	–	–	0.345 ± 0.01
12	7.6601	3-Pentanone, 2-methyl-	–	0.448 ± 0.01	–
13	8.148	Benzene, [(methoxymethoxy)methyl]-	–	–	0.272 ± 0.01
14	8.1724	3-Pentanol, 2-methyl-	–	1.334 ± 0.02	–
15	8.9335	Hexanal	9.488 ± 0.03	–	–
16	9.075	1-Octanamine	–	–	0.103 ± 0.00
17	9.7679	1H-Indole, 5-methyl-2-phenyl-	0.615 ± 0.02	–	–
18	10.6901	3-Hexen-1-ol	5.874 ± 0.02	–	0.364 ± 0.01
19	10.856	1-Propene, 3,3'-thiobis-	–	0.405 ± 0.01	–
20	11.0951	1-Hexanol	13.022 ± 0.05	4.782 ± 0.02	–
21	11.6269	Z,Z-2,5-Pentadecadien-1-ol	1.831 ± ±0.02	–	–
22	11.9002	Styrene	–	6.041 ± 0.02	–
23	12.027	Hexanal	0.232 ± 0.01	–	–
24	12.0953	2-Heptanol	–	–	1.021 ± 0.02
25	12.1588	Quinoline-5,8-dione-6-ol, 7-[[[(4-cyclohexylbutyl)amino]methyl]-	1.183 ± 0.00	–	–
26	12.6906	Oxalic acid, diisohexyl ester	–	1.334 ± 0.01	–
27	12.7716	Benzene, methoxy-	1.300 ± 0.02	–	–
28	13.2663	1R-.alpha.-Pinene	–	–	1.092 ± 0.02
29	13.5445	2,2'-Thiodisuccinic acid	0.290 ± 0.02	–	–
30	13.7738	Camphene	–	–	0.558 ± 0.02
31	14.3598	cis-1-butyl-2-methylcyclopropane	0.629 ± 0.02	–	–
32	14.613	1-Octen-3-ol	10.285 ± 0.02	–	0.524 ± 0.02
33	14.6618	6-Oxa-bicyclo[3.1.0]hexan-3-ol	–	0.650 ± 0.02	–
34	14.8912	7-Octen-2-one	2.038 ± 0.02	–	–
35	14.9009	5-Hepten-2-one, 6-methyl-	–	–	1.951 ± 0.02
36	15.1059	7H-Dibenzo[b,g]carbazole, 7-methyl-	–	1.639 ± 0.02	–
37	15.4084	Cyclohexane, ethylidene-	1.571 ± 0.02	–	–
38	15.4377	Octanal	–	–	0.100 ± 0.02
39	15.5225	.alpha.-Phellandrene	–	–	0.241 ± 0.02
40	15.6914	Benzene, 1-methoxy-2-methyl-	1.398 ± 0.02	–	–
41	15.9842	(S)-3-Ethyl-4-methylpentanol	–	1.526 ± 0.02	–
42	16.194	1-Hexanol, 2-ethyl-	0.597 ± 0.02	0.603 ± 0.02	0.320 ± 0.00
43	16.3013	Cyclopentane, 1-methyl-3-(2-methylpropyl)-	2.661 ± 0.00	–	–
44	16.4038	Eucalyptol	0.809 ± 0.01	0.875 ± 0.01	29.791 ± 0.05
45	17.2869	Benzenemethanol,.alpha.-(1-aminoethyl)-, (R*,R*)-	0.211 ± 0.00	–	–
46	17.5016	1-Octanol	–	0.604 ± 0.00	–

Table 3 continued

Number	RT (min)	Compounds	Relative area percent (%)		
			<i>Cucuibita mixma</i>	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>
47	17.6549	Cyclopentanecarboxylic acid, 3-methyl-4-methylene-, methyl ester, trans-	1.674 ± 0.02	–	–
48	18.0529	Benzene, 1,2,4,5-tetramethyl-	0.214 ± 0.00	–	–
49	18.1505	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	0.267 ± 0.01	–	–
50	18.3165	4-Nonanone, 7-ethyl-	–	0.349 ± 0.01	–
51	18.3945	3-Carene	–	–	1.003 ± 0.02
52	18.5165	Nonanal	4.253 ± 0.01	–	–
53	18.9459	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-	–	–	0.131 ± 0.01
54	19.1313	Benzene, 1,2,3,4-tetramethyl-	0.716 ± 0.02	–	–
55	19.1459	Benzene, 1-ethyl-2,3-dimethyl-	–	0.154 ± 0.02	–
56	19.4875	Octane, 1-methoxy-	–	1.234 ± 0.02	–
57	19.9315	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-	–	–	0.242 ± 0.02
58	20.0437	Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans(-)-	–	–	0.203 ± 0.00
59	20.0437	3-Nonen-1-ol, (Z)-	0.764 ± 0.01	–	–
60	20.2047	2-Dodecenal, (E)-	1.404 ± 0.01	–	–
61	20.273	Isoborneol	–	–	0.103 ± 0.00
62	20.4584	1-Nonanol	2.055 ± 0.01	–	–
63	20.517	Borneol	–	–	1.574 ± 0.02
64	20.5219	1,4-Pentadiene, 2,3,3-trimethyl-	–	0.311 ± 0.02	–
65	20.639	Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-	–	–	0.133 ± 0.01
66	20.678	Benzyl alcohol, p-hydroxy-.alpha.-(methylamino)methyl]-	–	0.144 ± 0.00	–
67	20.8	8-Azabicyclo[4.3.1]decan-10-one, 8-methyl-	0.470 ± 0.01	–	–
68	20.8293	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	–	–	0.367 ± 0.01
69	21.1903	1R-.alpha.-Pinene	–	–	0.647 ± 0.01
70	21.2196	1-Dodecanamine	0.367 ± 0.02	–	0.070 ± 0.00
71	22.0735	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	0.277 ± 0.01	–	0.258 ± 0.01
72	22.5126	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	0.159 ± 0.02	–	–
73	23.0055	1-Undecyn-4-ol	–	0.205 ± 0.00	–
74	23.2397	2,6-Octadienal, 3,7-dimethyl-, (E)-	–	–	0.483 ± 0.02
75	23.6641	Phenol, 4-(2-aminopropyl)-, (.±.)	0.209 ± 0.02	–	–
76	23.6739	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	–	–	0.121 ± 0.02
77	23.8105	Tridecane	0.148 ± 0.02	–	–
78	25.0987	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	–	–	0.134 ± 0.02
79	25.4793	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	0.323 ± 0.00	0.275 ± 0.01	0.189 ± 0.01
80	26.2551	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.574 ± 0.02	–	–
81	26.4698	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	–	–	0.292 ± 0.02
82	27.0016	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	0.197 ± 0.02	–	–
84	27.1138	Butylated hydroxytoluene	0.557 ± 0.02	1.067 ± 0.00	0.620 ± 0.02
85	27.3383	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	–	0.194 ± 0.01	1.656 ± 0.01
86	27.4212	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.588 ± 0.02	–	0.186 ± 0.01
87	27.5139	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	–	–	1.608 ± 0.02
88	27.7237	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	–	–	1.161 ± 0.03
89	27.7872	2-Isobenzazol, 1,3-dioxo-2-methyl-5,6-methylenedioxy-	0.129 ± 0.02	–	–
90	27.9677	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	–	–	0.894 ± 0.02

Table 3 continued

Number	RT (min)	Compounds	Relative area percent (%)		
			<i>Cucurbita mixma</i>	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>
91	28.9338	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	–	0.130 ± 0.01	0.084 ± 0.01
92	29.2802	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-	–	0.134 ± 0.02	–
93	30.1	2-Hexene, 2,5,5-trimethyl-	0.149 ± 0.01	–	–

4-trimethylpentyl ester), and butylated hydroxytoluene respectively.

Discrimination of pumpkin species using E-nose

The comprehensive analysis of the volatile compounds using E-nose were measured among samples which were amongst the same batch using GC–MS to obtain more detailed information about the volatile compounds and aroma descriptors involved in differentiating the pumpkin varieties. The Principal Component Analysis (PCA) was applied to each sample. The PCA is a dimension-reduced, data-processing method which reduces input data multi-dimensions to a lower dimension in order to capture the closed, relevant set of input data information (Qin et al. 2013). As reported by López de Lerma (2013), it would be easier to process lower-dimension data if the relationships between samples and many variables were simplified. In order to differentiate the pumpkin samples in an efficient manner, the PCA method was used on account of an abundance of sensor data information. In detail, the first two principal components accounted for approximately 85% of the total variance (54.38% of the total variance for PC1 and 30.68% for PC2). Projections for the samples in the space formed by the principal components, PC1 and PC2, are shown in Fig. 1; the variables that contribute the most to the first two principal components are also indicated. PC1 showed that the three varieties that were clearly distinguished from the other samples were grouped in the positive area of PC2, whereas the others were grouped in the negative area. The practicability of electronic nose for the classification of mechanical grade and the different fermentation times among black tea samples are also reported by Laureati et al. (2010).

In the meantime, the volatile aroma compounds were studied using SPMC-GC–MS and electronic nose responses amongst the three species of Chinese pumpkin. The results demonstrated that the three pumpkin species planted in China have different volatile compositions. The ninety-three volatile aroma compounds in the three pumpkin species that were identified in all three species and that were different in the composition of volatile aroma components, suggests that the aroma components of pumpkin

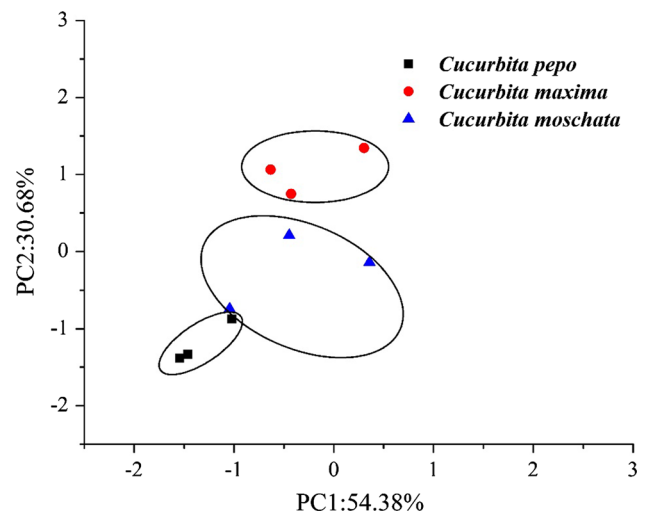


Fig. 1 Score plot of the first two principal components of volatile compounds in the three pumpkin species samples analyzed by electronic nose

samples were differentiated clearly among species. Thus, it can be seen that the E-nose technology is able to identify pumpkins from different quality species. From the results, the volatile aroma compounds analysis, using E-nose technology responses, with the combination of the multivariate decrease-dimension analysis and SPME-GC–MS methods together would be a promising tool for comprehensive quality identification for pumpkin beverages (Cheng et al. 2015).

Enzyme activity

The oxidation of phenolic compounds is catalyzed mainly by PPO; this reaction is famous for enzymatic browning. POD plays a key role in the development of off-flavors and discoloration in raw and unbleached vegetables (Sampedro et al. 2014) and is another oxidoreductase enzyme involved in enzymatic browning. This is due to diphenols' one of many functions as a substrate reducer in its reaction (Chisari et al. 2008). As shown in Fig. 2, PPO activity was much higher in *C. maxima* and *C. moschata* and there was a significant difference ($p < 0.05$) between the two species. However, *C. pepo* had significantly lower

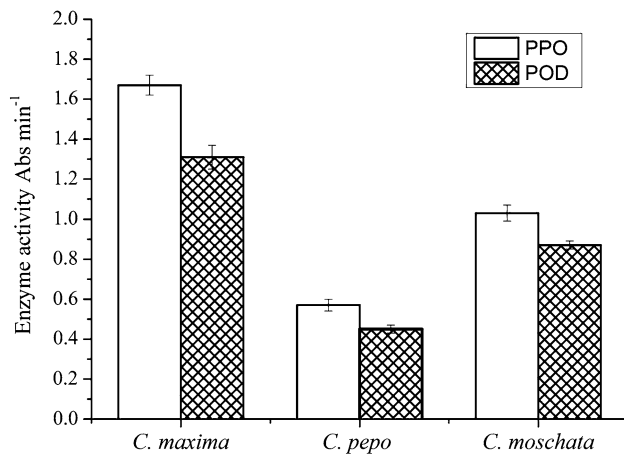


Fig. 2 PPO and POD activities in pumpkin fruit of three species

($p < 0.05$) PPO activity, and showed that the color of *C. pepo* was not easy to change or changed at a slower rate during storage. Since the best pH is between 5 and 7 for PPO activity, the low pH of the pulp puree caused the PPO activity to decrease. It is frequently acidized to a low pH, in order to prevent, inhibit, or reduce PPO activity (Guerrero-Beltrán et al. 2005). An acidifying agent, for example, fumaric, malic, or citric acid, could be used to minimize PPO activity by chelating copper or reducing pH in fruits and vegetables (Guerrero-Beltrán et al. 2005). The pH value and contents of organic acids were in sync with the PPO activities in this study, as indicated in Table 2. The content of malic, fumaric, and citric acid were significantly higher ($p < 0.05$) with a lower pH value in *C. pepo* pumpkin.

The tendency of POD activity was in accordance with PPO in three pumpkin species, and similarly, *C. pepo* showed a significantly lower ($p < 0.05$) POD activity. *C. maxima* and *C. moschata* exhibited a higher POD activity, but POD activity in *C. maxima* was much higher than that in *C. moschata* ($p < 0.05$). MacDonald and Schaschke (2000) reported POD was not only responsible for texture loss and the off-flavors, but also the change of color. This research implied that *C. maxima* and *C. moschata* were more susceptible to lose quality. Gonçalves et al. (2007) found that in the pumpkin (*Cucurbita maxima* L.), POD inactivation adhered to a first-order Arrhenius model, in which the activation energy and rate of the reaction at a reference temperature of 85 °C were $86.20 \pm 5.57 \text{ kJ mol}^{-1}$ and $0.27 \pm 0.01 \text{ min}^{-1}$, respectively. Furthermore, the activity of PPO and POD from both species as reported by Chisari et al. (2008), exhibited a lower mode along with a sugar concentration increase in medium, except for in POD extract from ‘Charentais’ that belonged to melons (*Cucumis melo* L.), which maintained its activity under a much higher D-glucose concentration condition (up to 5 M). *Cucurbita pepo* L. was suitable for processing due to lower PPO and

POD activity, and this is the reason for the importance of quality and color maintenance of the raw material before processing (Neves et al. 2012).

Principal component analysis

Principal component analysis (PCA), multivariate pattern recognition procedure, was put into use to analyze many possible factors in different species (Liu et al. 2013; Cheng et al. 2015). The result of dimension reduction process from the three pumpkin species was demonstrated in Fig. 3. The three pumpkin species could be clearly differentiated; ‘A’ represented *C. pepo* pumpkins, ‘B’ represented *C. moschata* pumpkins, and ‘C’ represented the *C. maxima* pumpkins. The percentages of the first two principal components were 93.64% in all of the variations and PC1 and PC2 accounted for 74.14, 19.50% respectively. All physicochemical and antioxidant properties discussed in this study, not including some individual factors, resulted in the clustering variety. Thus, the three pumpkin species had been separated using the PCA method. PC1 was mainly related to PPO activity (0.269), total flavonoid (0.267), sucrose (0.264), glucose (0.263), TSS (0.264), a^* (0.250), pH (0.250), malic acid (0.262), vitamin E (0.241), DPPH (0.237), FRAP (0.243) and β -carotene (0.255), whereas it inversely correlated with malic acid (-0.263). PC2 was mainly correlated to firmness (-0.491), citric acid (0.427), fumaric acid (0.342), L-ascorbic acid (0.305) and total phenols (-0.311). The PC1 scores of *C. maxima* were much higher than those of the other two pumpkin species and the same score of the much higher content of PPO activity, total flavonoid, sucrose, glucose, TSS, a^* , pH, malic acid, vitamin E, higher value of FRAP and DPPH (antioxidant activities) and β -carotene. The *C. maxima* had a lower content of malic acid. The higher scores of *C. pepo*

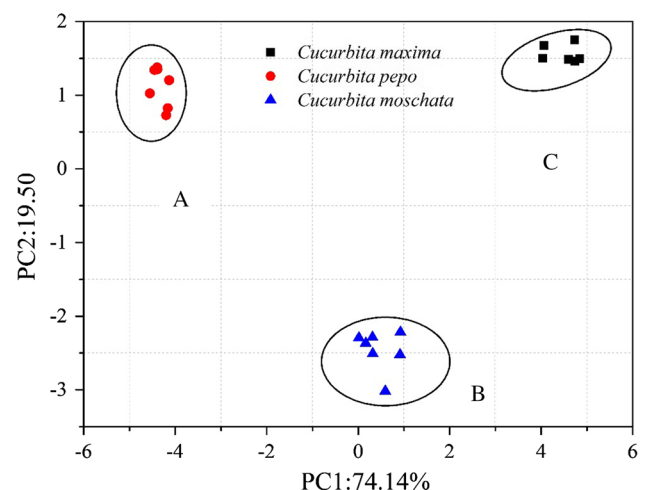


Fig. 3 Principal component analysis plot of data from different physicochemical and antioxidant properties of three pumpkin species

indicated that the contents of citric, fumaric, and L-ascorbic acid, lower firmness, and total phenols were higher in the PC2. The scores of clustering variety being lower for *C. moschata* pumpkins in both PC1 and PC2, demonstrated that the contents of glucose, sucrose, TSS and PPO activity, total flavonoid, antioxidant activity, total phenols, malic acid, and firmness were much lower.

Conclusion

The principal component analysis (PCA) has been proven to be a promising approach to classify different plant species that are determined by a numerous amount of quality properties. This study has been reported that the utilization of the traditional method gears toward determining twenty-four physicochemical parameters and antioxidant activities to obtain the quality index of three pumpkin species. *Cucurbita maxima* pumpkins showed a higher content of PPO activity, total flavonoids, sucrose, glucose, total soluble solids, a^* , pH, malic acid, vitamin E, DPPH and FRAP and β -carotene, and a lower content of malic acid. The content of citric acid, fumaric acid, L-ascorbic acid, lower firmness, and total phenolics were higher in *Cucurbita pepo* pumpkins. *Cucurbita moschata* pumpkins exhibited a much higher content of total flavonoids, sucrose, glucose, TSS and polyphenol oxidase activity, lower total phenolics, firmness, antioxidant abilities and malic acid. Ninety-three aroma components were identified using SPME-GC-MS and varied amongst the different pumpkin species. The three pumpkin species were classified correctly based of physicochemical parameters and antioxidant activities as well as aroma compounds which contributed to the principal component analysis. The results of PCA showed that the sum of PC1 and PC2 were both more than 85% (85.06 and 93.64% respectively). Aroma compounds using E-nose technology and quality parameters for the principal component analysis (PCA) could be suitable for use in food product applications, such as high pressure processing (HPP), pulsed electric fields (PEF), and ultrasound processing.

Acknowledgements This work was supported by the Natural Science Foundation of Jiangxi Province, China (Grant No. 20151BAB204039), Doctoral Research Startup Fund Project of Jiangxi Science and Technology Normal University (3000990310), and Quality engineering projects of Jiangxi province (Biological chemistry curriculums, 2015).

References

- Applequist WL, Avula B, Schaneberg BT, Wang YH, Khan IA (2006) Comparative fatty acid content of seeds of four *Cucurbita* species grown in a common (shared) garden. *J Food Compos Anal* 19(6–7):606–611
- Biesiada A, Nawirska A, Kucharska A, Sokół-Łętowska A (2009) The effect of nitrogen fertilization methods on yield and chemical composition of pumpkin (*Cucurbita maxima*) fruits before and after storage. *Veg Crops Res Bull* 70(1):203–211
- Cheng H, Chen J, Chen SG, Wu D, Liu DH, Ye XQ (2015) Characterization of aroma-active volatiles in three Chinese bayberry (*Myrica rubra*) cultivars using GC-MS-olfactometry and an electronic nose combined with principal component analysis. *Food Res Int* 72:8–15
- Chisari MR, Barbagallo N, Spagna G (2008) Characterization and role of polyphenol oxidase and peroxidase in browning of fresh-cut melon. *J Agric Food Chem* 56:132–138
- Conti S, Villari G, Amico E, Caruso G (2015) Effects of production system and transplanting time on yield, quality and antioxidant content of organic winter squash (*Cucurbita moschata* Duch.). *Sci Hortic Amst* 183:136–143
- Di Marco G, Gismondi A, Canuti L, Scimeca M, Volpe A, Canini A (2014) Tetracycline accumulates in *Iberis sempervirens* L. through apoplastic transport inducing oxidative stress and growth inhibition. *Plant Biol* 16(4):792–800
- Dini I, Tenore GC, Dini I (2013) Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. *LWT-Food Sci Technol* 53(1):382–385
- Dutta D, Dutta A, Raychaudhuri U, Chakraborty R (2006) Rheological characteristics and thermal degradation kinetics of beta-carotene in pumpkin puree. *J Food Eng* 76(4):538–546
- Gonçalves EM, Pinheiro J, Abreu M, Brandão TRS, Silva CLM (2007) Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima* L.) during blanching. *J Food Eng* 81(4):693–701
- Guerrero-Beltrán JA, Swanson BG, Barbosa-Cánovas GV (2005) Inhibition of polyphenol oxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid. *LWT-Food Sci Technol* 38(6):625–630
- Hu WZ, Jiang AI, Chen C, Tian MX, Liu CH, Jia L (2013) Effects of 2,5-norbornadiene(NBD) on physiological and biochemical responses of fresh-cut winter squash. *J Food Sci Eng* 3:123–130
- Impei S, Gismondi A, Canuti L, Canini A (2015) Metabolic and biological profile of autochthonous *Vitis vinifera* L. ecotypes. *Food Funct* 6(5):1526–1538
- Itle RA, Kabelka EA (2009) Correlation between $L^*a^*b^*$ color space values and carotenoid content in pumpkins and squash (*Cucurbita* spp.). *HortScience* 44(3):633–637
- Jacobo-Valenzuela N, Maróstica-Junior MR, Zazueta-Morales JDJ, Gallegos-Infante JA (2011) Physicochemical, technological properties, and health-benefits of *Cucurbita moschata* Duchense vs Cehualca. *Food Res Int* 44(9):2587–2593
- Jiao J, Li ZG, Gai QY, Li XJ, Wei FY, Fu YJ, Ma W (2014) Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. *Food Chem* 147:17–24
- Kafkas E, Koşar M, Türemiş N, Başer KHC (2006) Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chem* 97(4):732–736
- Kim MY, Kim EJ, Kim YN, Choi C, Lee BH (2012) Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. *Nutr Res Pract* 6(1):21–27
- Kourkoutas D, Elmore JS, Mottram DS (2006) Comparison of the volatile compositions and flavour properties of cantaloupe, Galia and honeydew muskmelons. *Food Chem* 97(1):95–102
- Kreck M, Kürbel P, Ludwig M, Paschold PJ, Dietrich H (2006) Identification and quantification of carotenoids in pumpkin species (*Cucurbita maxima* L.) and their juices by liquid

- chromatography with ultraviolet-diode array detection. *J Appl Bot Food Qual* 80:93–99
- Laureati M, Buratti S, Bassoli A, Borgonovo G, Pagliarini E (2010) Discrimination and characterisation of three species of *Perilla frutescens* by means of sensory descriptors and electronic nose and tongue analysis. *Food Res Int* 43(4):959–964
- Leffingwell JC, Alford ED, Leffingwell D (2015) Identification of the volatile constituents of raw pumpkin (*Cucurbita pepo* L.) by dynamic headspace analyses. *Leffingwell Rep* 9(1):1–14
- Leizeron S, Shimoni E (2005) Effect of ultrahigh-temperature continuous ohmic heating treatment on fresh orange juice. *J Agric Food Chem* 53(9):3519–3524
- Liu FX, Fu SF, Bi XF, Chen F, Liao XJ, Hu XS, Wu JH (2013) Physico-chemical and antioxidant properties of four mango (*Mangifera indica* L.) species in China. *Food Chem* 138(1):396–405
- LópezdeLerma N, Moreno J, Peinado RA (2013) Determination of the optimum sun-drying time for *Vitis vinifera* L. cv. Tempranillo Grapes by E-nose analysis and characterization of their volatile composition. *Food Bioprocess Tech* 7(3):732–740
- MacDonald L, Schaschke CJ (2000) Combined effect of high pressure, temperature and holding time on polyphenol oxidase and peroxidase activity in banana (*Musa acuminata*). *J Sci Food Agric* 80:719–724
- Murkovic M, Mülleler U, Neunteufl H (2002) Carotenoid content in different varieties of pumpkins. *J Food Compos Anal* 15(6):633–638
- Nawirska-Olszanska A, Kita A, Biesiada A, Sokol-Letowska A, Kucharska AZ (2013) Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 species. *Food Chem* 139(1–4):155–161
- Neves FIG, Vieira MC, Silva CLM (2012) Inactivation kinetics of peroxidase in zucchini (*Cucurbita pepo* L.) by heat and UV-C radiation. *Innov Food Sci Emerg* 13:158–162
- Oloyede FM, Agbaje GO, Obuotor EM, Obisesan IO (2012) Nutritional and antioxidant profiles of pumpkin (*Cucurbita pepo* Linn.) immature and mature fruits as influenced by NPK fertilizer. *Food Chem* 135(2):460–463
- Perry L, Wang Y, Lin J (2009) Analysis of honeydew melon (*Cucumis melovar. inodorus*) flavour and GC–MS/MS identification of (E, Z)-2,6-nonadienyl acetate. *Flavour Frag J* 24:341–347
- Provesi JG, Dias CO, Amante ER (2011) Changes in carotenoids during processing and storage of pumpkin puree. *Food Chem* 128(1):195–202
- Qin ZH, Pang XL, Chen D, Cheng H, Hu XS, Wu JH (2013) Evaluation of Chinese tea by the electronic nose and gas chromatography–mass spectrometry: correlation with sensory properties and classification according to grade level. *Food Res Int* 53(2):864–874
- Rezig L, Chouaibi M, Msaada K, Hamdi S (2012) Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Ind Crop Prod* 37(1):82–87
- Roura SI, Del Valle CE, Aguero L, Davidovich LA (2007) Changes in apparent viscosity and vitamin retention during thermal treatment of butternut squash (*Cucurbita moschata* DUCH) pulp: effect of ripening stage. *J Food Qual* 30:538–551
- Sampedro F, Phillips J, Fan X (2014) Use of response surface methodology to study the combined effects of UV-C and thermal processing on vegetable oxidative enzymes. *LWT-Food Sci Technol* 55(1):189–196
- Shahdadi F, Mirzaei HO, Daraei GA (2015) Study of phenolic compound and antioxidant activity of date fruit as a function of ripening stages and drying process. *J Food Sci Tech* 52(3):1814–1819
- Stevenson DG, Eller J, Wang LP, Jane J, Wang T, Inglett GE (2007) Oil and tocopherol content and composition of pumpkin seed oil in 12 species. *J Agric Food Chem* 55:4005–4103
- Tyagi G, Jangir DK, Singh P, Mehrotra R, Ganesan R, Gopal ESR (2014) Rapid determination of main constituents of packed juices by reverse phase-high performance liquid chromatography: an insight into commercial fruit drinks. *J Food Sci Tech* 51(3):476–484
- Verzera A, Dima G, Tripodi G, Ziino M, Lanza CM, Mazzaglia A (2010) Fast quantitative determination of aroma volatile constituents in melon fruits by headspace–solid-phase microextraction and gas chromatography–mass spectrometry. *Food Anal Method* 4(2):141–149
- Zhou CL, Liu W, Zhao J, Yuan C, Song Y, Chen D, Ni YY, Li QH (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, WorknehTS Woldetsadik K (2013) Effect of accessions on the chemical quality of fresh pumpkin. *Afr J Biotechnol* 12(51):7092–7098