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Characterization of physico-chemical and bio-chemical compositions
of selected four strawberry cultivars

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Summary

The physico-chemical and bio-chemical compositions of 'Hongyan', 'Tiangxiang', 'Tongzi I' and 'Zhangji' strawberries in China were analyzed. Their values were pH 3.42 to 3.73, titratable acidity 0.63 to 0.79 g/100 g FW, total soluble sugars 5.26 to 8.95 g/100 g fresh weight (FW), ascorbic acid 21.38 to 42.89 mg/100 g FW, total phenolics 235.12 to 444.73 mg/100 g FW, pectin 82.84 to 96.13 mg/100 g FW, total organic acids 874.30 to 1216.27 mg/100 g FW, individual phenolics other than anthocyanins 7.60 to 12.18 mg/100 g FW, free amino acids 13.35 to 32.66 mg/100 g FW, monomeric anthocyanins 4.47 to 47.19 mg/100 g FW, antioxidant capacity of DPPH 14.14 to 18.87 and FRAP 7.97 to 10.54 equal to mg/100 g ascorbic acid, polyphenol oxidase (PPO) activity 0 to 0.42 Abs/min, peroxidase (POD) activity 0.17 to 0.34 Abs/min and pectin methyl esterase (PME) activity 0.012 to 0.018 mL/min. 'Tongzi I' was most suitable for food processing due to the highest titration acidity, total phenolics, pectin, total organic acids, monomeric anthocyanins, antioxidant capacity of ·DPPH and FRAP with lower PPO, POD and PME activity.

Introduction

Strawberry which is cultivated almost all over the world is a soft and juicy, sweet-sour and palatable berry with bright-red color, and also an important commercial fruit with good processing potential. The common quality characteristics of strawberries for consumer acceptance are appearance (uniform bright red color, size and shape), firmness, and flavor perceived by the combination of taste and smell (aroma) senses (Gunnessa, Kravchukb, Nottinghamc, D'Arcyb and Gidleya, 2009). The attractive color of strawberry is mainly due to the presence of abundant anthocyanins. Polyphenol oxidase (PPO) and peroxidase (POD) are demonstrated to be the color-related enzymes and play a key role in color degradation due to enzymatic browning and decreasing anthocyanins. Pectin is a critical structure component of the cell wall (Riahi and Ramaswamy, 2013), which is easy to be de-esterified by pectin methyl esterase (PME) and converted into low-methoxy pectin or pectin acid, thereby influence the texture and firmness of strawberry. The sugars (fructose, glucose and sucrose) (Skupień and Oszmiański, 2004) and organic acids (Watson, Wright, McBurney, Taylor and Linforth, 2002) give strawberry its characteristic taste, while more than 360 volatile compounds distinguish its aroma (Gunnessa et al., 2009). According to Häkkinen et al. (2000), strawberries exhibit high antioxidant capacity compared to other fruits due to its high content of phenolic compounds. Ascorbic acid is another plentiful component in strawberry, which is known as a powerful reducing agent that plays a key role in human nutrition.

Antioxidant capacity of strawberry can participate in the prevention of cancer, cardiovascular and other chronic diseases (Oszmiajski and Wojdyio, 2009). At present, foods are assumed the status of being "functional", that is they should be capable of providing additional physiological benefit, such as preventing or delaying onset of chronic diseases, as well as meeting basic nutritional requirements (Skupień et al., 2004), so strawberry will be one of the most popular summer fruits, consumed fresh, conserved or in manufactured products along with the unique, highly desirable taste and flavor.

In the late 1980s, strawberry cultivars were introduced into China and great efforts were made to extend the use of strawberries, which accelerated the development of strawberry industry. According to the statistical data by the Ministry of Agriculture, China, the total planting area of strawberry in China increased up to 20 million hectares and the strawberry output totaled 2.442 million tons in 2015. In China, some strawberry cultivars come from European countries, USA and Japan, but most of the cultivars are self-bred. In general, the cultivars are classified into two categories for fresh consumption and food processing. Cultivars with large fruits, bright red color, good taste, moderately sweet/sour ratio and abundant aromatic compounds are suitable for fresh consumption, while high ascorbic acid and anthocyanins contents, fleshy hard and great acidity are in flavor of food processing. Though the planting areas are booming and the outputs of strawberry in China are increasing year by year, there is still lack of evaluation of the physico-chemical and biochemical compositions closely related to the quality of strawberries. The aim of the present work is to analyze and to compare physico-chemical and bio-chemical compositions of four strawberry cultivars in China, hoping to give useful information for the best use of the strawberry cultivars, for both technological research and processing practice.

Materials and methods

Plant materials
Strawberries ('Hongyan', 'Tiangxiang', 'Tongzi I', 'Zhangji') grown Tianyi Bio-engineering Company in Changping county (Beijing, China) were harvested at commercial maturity in March 18th, 2015. The berries were individually frozen by liquid nitrogen and packed in aluminum foil bags, then stored at -80 °C until analyzed within 4 months.

Weight of individual fruits
Average weight of individual fresh fruits was determined with a scale (EY-300A, Panasonic, Japan). 100 individual strawberries were used for average.

Measurement of the pH values
The pH value measurement was carried out by an Orion 868 pH meter (Thermo Orion, USA) with a combined pH electrode at 25 °C. The meter was calibrated with commercial buffer solutions at pH 6.8 and 4.0.

Extraction and determination of pectin
For extracting and determination of soluble pectin, the method described by Peng et al. (2008) was used. 5 g strawberry puree was mixed with 100 mL 95% ethanol and cooked in boiling water for

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30 min, this process was repeated for 3 times. The mixture was filtered using a bush funnel by vacuum, the residues were collected and mixed with 20 mL water and heated in 50 °C for 30 min to dissolve soluble pectin, then filtered using quantitative filter paper (Xinxing 202, Hangzhou special paper Co. Ltd.). The residues were washed 3 times again by distill water, the filtrate was transferred into a flask and was made up to 50 mL by adding distill water. 6 mL 0.0125 mol/L sodium tetraborate (dissolved in H_2SO_4) and 1 mL galacturonic acid solution or sample (diluted 10 times with water) were added into a glass tube with plug, and cooled in an ice cold bath. The cooled mixture was boiled for 5 min in boiling water bath and then cooled again immediately. 0.1 mL 0.15% n-phenylphenol (dissolved in 0.5% NaOH) was added into the above-mentioned tube and was mixed by shaking for 30 s on an oscillator. After 5 min reaction, samples were evaluated at 520 nm by spectrophotometer (UV-726, Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China). Results were expressed as milligrams of galacturonic acid per 100 g of FW.

Extraction and HPLC analysis of organic acids

The organic acids in strawberry were extracted as described in an earlier study with some modifications (LIU et al., 2014). 50 g strawberry purees were mixed with 100 mL distilled water and heated at 50 °C for 30 min, then centrifuged using 12000 rpm/min for 15 min at 4 °C, and filtered through two-layer cheese-cloth. The supernatant was collected. The analytical column was an Alltech AlltimaTM C18, (4.6×250 mm i.d, 5 μm particle size) from Waters. The organic acids were separated by the methods described by GAO et al (2014). The mobile phase was an isocratic solvent system consisting of 97% of 0.01 mol/L dipotassium phosphate (pH=2.55) and 3% methanol, the flow rate was 0.5 mL/min at 30 °C. The detection was carried out at 210 nm in absorbance mode. Results were expressed as milligrams per 100 g of FW.

Determination of titratable acidity

The titratable acidity (TA) was determined via titration with 0.1 M NaOH, set to reach pH 8.1 by an automatic titrimeter (751 GPD titrino, Metrohm, Switzerland). TA value was analyzed in triplicate and expressed as citric acid equivalents, since citric acid is the predominant acid found in strawberries (WATSON et al., 2002). The TA of strawberry was calculated using the following formula.

$$TA(\%) = \frac{C \times V_2 \times K}{V_1} \times \frac{V_0}{m} \times 2 \times 100\%$$

Where, C was NaOH concentration (0.1 M), m was the weight of strawberry for extraction (50 g), V_2 was the volume of NaOH used (mL), V_1 was the volume of sample used (50 mL organic acid extracts), V_0 was the volume of extracts (mL), K was conversion factor of citric acid (0.07). The result was expressed as g/100 g of citric acid equivalent FW.

Extraction and HPLC analysis of sugar

The sugars in strawberry were extracted by the same method as organic acids. The sugars were detected by the method described by WANG et al. (2006) and were performed on Asahipak NH2P-50 4E (4.6×250 mm i.d, 5 μm particle size) equipped with Asahipak NH2P-50G 4A (4.6×10 mm i.d, 5 μm particle size) as guard column. 50 mg/L calcium disodium salt of ethylene diamine tetraacetic acid (EDTA) was used as mobile phase for separating sucrose, glucose and fructose. In the separation systems, the solvent flow rate was 0.5 mL/min at 90 °C and aliquots of 60 μL were injected. It was detected by a differential refraction detector (RID-2301, Knauer Co. Ltd., German). The sugars were expressed as grams per 100 g of FW.

Extraction and HPLC analysis of ascorbic acid

For extracting and analyzing ascorbic acid in strawberry, the method of TIWARI et al. (2009) were used with some modifications. 50 g strawberry purees were mixed with 250 mL 2.5% metaphosphoric acid and incubated at 4 °C for 2 h, then centrifuged using 12000 rpm/min for 15 min at 4 °C, and filtered through two-layer cheese-cloth, the supernatant was collected. The ascorbic acids were performed on the same column as organic acids. The mobile phase was an isocratic solvent system consisting of 95% of 50 mM monopotassium phosphate (pH=3.0) and 5% acetonitrile, the flow rate being 1 mL/min. The analyses were conducted at ambient temperature. The detection was carried out at 245 nm in absorbance mode. Results were expressed as milligrams per 100 g of FW.

Extraction and HPLC analysis of monomeric anthocyanins

250 g frozen strawberries were homogenized with a juice extractor (JY-610, Joyoung Co., Ltd. Shandong, China) to obtain strawberry purees for further analysis. Anthocyanin was extracted as earlier described by CAO et al. (2012) with some modifications. 50 g strawberry purees were mixed with 100 mL 0.1% HCl in methanol and kept 30 min at 4 °C, then centrifuged at 12000 rpm/min for 10 min at 4 °C (Hitachi himac CR21G, Japan), the insoluble plant material was re-extracted with 100 mL solvent. The supernatants were collected and mixed for HPLC analysis. Chromatographic separation was performed on a Venusil C18 column (250 mm×4.6 mm i.d, 5 μm particle size) equipped with a 5 μm C18 guard column both from Agela (USA). The separation of anthocyanins was as described by GARC et al. (1997) with mobile phases consisting of (A) formic acid/water (5:95, v: v) and (B) methanol. The detection was carried out at 520 nm for anthocyanins at 30 °C with the flow rate at 1 mL/min. Anthocyanins were dissolved in 0.1% HCl. Results were expressed as milligrams per 100 g of FW.

Extraction and HPLC analysis of individual phenolic compounds other than anthocyanins

The extraction of soluble individual phenolic compounds other than anthocyanins was presented in a previous study with some modifications (CAO et al., 2011). 50 g strawberry purees were mixed with 100 mL ethyl acetate and stirred with a magnetic stirrer for 30 min, and the ethyl acetate phase was collected. The extractions were performed by repeated vigorous vortexing of samples with ethyl acetate (3×100 mL). The combined ethyl acetate extracts were evaporated to dryness at 40 °C with a rotary evaporator (SENCQ R-501, Shenshun Biotechnology Co., Shanghai, China), then dissolved in 10 mL methanol. The separation of individual phenolic compounds other than anthocyanins was as described by CAO et al. (2011) with some modifications. The mobile phases were (A) formic acid/acetonitrile (2.5:97.5, v: v) and (B) formic acid/water (2.5:97.5, v: v). The detection was carried out at 280 nm and 320 nm for phenolic compounds in absorbance mode with the flow rate being 1 mL/min at 30 °C, and aliquots of 20 μL were injected. The gradient elution was as follows: 0 to 5 min, 5% A; 5 to 15 min, 5-13% A; 15-20 min, 13% to 30% A; 20 to 25 min, 30% A; 25 to 28 min, 30% to 45% A; 28 to 32 min, 45% B; 32 to 35 min, 45% to 90% B; 35 to 40 min, 90% B; 40 to 45 min, 90% to 5% B. Results were expressed as milligrams per 100 g of FW.

Determination of total phenolics

Total phenolics were determined using Folin-Ciocalteau method described by CAO et al. (2012) with some modifications. 125 μL 100-folds diluted phenolic compounds extracts were mixed with 2 mL Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and set 1 hour at room temperature away from light.

Then 1.8 mL of sodium carbonate (105.99 g/mol) were added to the mixture and reacted for 15 min, the mixture was measured at 765 nm by spectrophotometer (UV-726 Shimadzu, Shanghai, China), immediately. Results were expressed as mg gallic acid/100 g FW.

Extraction and HPLC analysis of FAAs

The analysis of free amino acids (FAAs) in strawberry were extracted using the method as earlier described. 50 g strawberry purees were mixed with 100 mL of solvent (0.1% HCl in 95% ethanol) and stirred with a magnetic stirrer for 30 min and then centrifugation (12000 rpm/min for 15 min at 4 °C), filtered and collected supernatant, the insoluble plant material was re-extracted two times with 0.1% HCl in 80% ethanol. The residual ethanol was removed in a rotary evaporator at 40 °C. The volume of the extracts was accurately recorded. Chromatographic analysis method of FAA was proposed by Agela, it was performed on a Venusil-AA column (4.6×250 mm i.d, 5 μm particle size) equipped with a C_{18} guard column both from Agela, USA. The standards of 20 kinds of AAs and norleucine (Nle) were dissolved in HCl (0.1 mol/L). For derivatisation, 200 μL standards or samples were mixed with 20 μL Nle, 100 μL 1 mol/L triethylamine/acetonitrile (1.4:8.6, v/v), 100 μL 0.1 mol/L PITC/acetonitrile (1:80, v/v) and shaking for 30 s on an oscillator, after 1 h reaction away from light, 400 μL n-hexane was added and shook for 30 s, after 10 min setting, the underlayer solution was obtained for analysis. The mobile phase was consisted of (A) 0.1 mol/L sodium acetate (pH=6.5)/acetonitrile (93:7, v/v), (B) 80% acetonitrile (diluted with water), the flow rate was 1 mL/min at 40 °C with detected at 254 nm. Results were expressed as milligrams per 100 g of FW.

The sugars and organic acids were separated using a Knauer HPLC system (Knauer Co. Ltd., German) equipped with a pump (K-1001) connected a refractive index detector (RID, K-2301) or UV detector (K-2501) and a 20 μL loop. Ascorbic acid, anthocyanins, phenolic compounds and free amino acids were separated using a Shimadzu liquid chromatograph (Shimadzu Co. Japan) equipped with a prominence UV/Vis detector (SPD-20AV), an auto sampler (SIL-20A) and a column oven (CTO-20A). Prior to injection, all the samples were filtered through a 0.45-mm Millipore membrane filter. Quantification was performed based on external standards of known concentrations.

Determination of antioxidant activity

The antioxidant activity was studied through the evaluation of the free-radical scavenging-effect on DPPH radical according to procedure described by ODRIOZOLA-SERRANO et al. (2009) and ferric reducing/antioxidant power (FRAP) according to the method developed by BENZIE et al. (1996).

DPPH assay

The reaction started by adding 100 μL Vc solution or the diluted strawberry juice to the cuvette containing 4 mL 0.14 mol/L methanol solution of the free radical (DPPH). The mixture was set away from light for 15 min, and then the absorbance was measured at 765 nm with a UV-726 spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China).

FRAP assay

The freshly prepared Ferric ion reducing antioxidant power (FRAP) solution contained 25 mL 0.3 mol/L acetate buffer (pH 3.6) plus 2.5 mL 10 mmol/L tripyridyltriazine(TPTZ) (dissolved in 40 mmol/L HCl) and 2.5 mL 20 mmol/L ferric chloride ($FeCl_3·6H_2O$). 4 mL FRAP solution at 37 °C was mixed with 100 μL ascorbic acid solution or diluted strawberry juice. Ten minutes later, the ferric

reducing ability of strawberry juice was measured by monitoring the increase of absorbance at 593 nm with an UV-726 spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China) and the FRAP solution was used as blank.

Determination of enzyme activity
Extraction and determination of PPO and POD

The extraction of PPO and POD was described by GARCIA-PALAZON et al. (2004). 100 g strawberries were mixed with 20 mL extraction solution and 4% polyvinyl polyvinylpyrrolidone (PVPP) and pulped for 2 min, then extracted for 4 h at 4 °C. The mixture was centrifuged at 12000 rpm/min for 10 min at 4 °C, and the supernatant was collected and analyzed for enzyme activity.

The extraction solution was 0.2 M phosphate buffer (pH=6.5) for PPO and POD. PPO and POD activity were determined by a spectrophotometric method (GARCIA-PALAZON et al., 2004) with some modifications. The reaction mixture for PPO was 0.5 mL extract and 2.5 mL 0.07 M catechol (o-diphenol) in 0.5 M sodium phosphate buffer (pH=6.5) solution. The reaction mixture for POD contained 2.2 mL 1.0% (v/v) guaiacol (dissolved in 0.2 M phosphate buffer, pH 6.5), 0.2 mL of 1.5% H_2O_2, and 0.8 mL extract. The increase in absorbance at 420 nm for PPO and 470 nm for POD, respectively, was monitored at intervals of $0.1 \, s^{-1}$ immediately after incubation with a Cary 50 spectrophotometer (Varian Co. Ltd., California, USA), which was equipped with a peltier thermostatted cell holder, a water pump (Varian Co. Ltd., California, USA) to keep temperature at 30±0.1 °C and an in-built electromagnetic stirring to mix up the substrate and the extracts. Prior to measurement, a pre-equilibrium at 30 °C of the substrate solution as well as the extracts by the peltier thermostatted cell holder was obtained. The slope of the very first linear part of the reaction curve was taken as PPO and POD absolute activity (Abs/min).

Extraction and determination of PME

PME was extracts by a previous method described by ZHI et al., 2008. The composition of the extraction buffer was as follows: 0.2 M Tris-6 M HCl buffer (pH 8.0) (containing 0.1 M NaCl). The ratio of buffer volume (mL) to strawberry puree (g) was 2:1.The mixture was incubated at 4 °C for 12 h, then centrifuged using 12000 rpm/min at 4 °C for 15 min, filtered and collected supernatant as enzyme extracts for further analysis. The PME activity was detected by pH Automatic Potentiometric Titrator (751 GPD titrino, Metrohm, Switzerland). 5 mL PME extracts were added into 40 mL 0.1 g/L pectin (pH=7.5), and automatically added 0.05 M NaOH to keep the pH value at 7.5, the reaction temperature was 30 °C. The volume of NaOH was recorded every 30 s. Slope of liner (volume-time) was obtained for calculating the PME activity using the formula.

$$PME \; activity = \frac{Slope \times C_{NaOH}}{V_{sample}}$$

Statistical Analysis

All the experiments were performed in triplicate. The data were analyzed using the Statistical Program for Social Sciences (SPSS 12.0) software for analysis of variance, Duncan's test. The significance was established at p ≤ 0.05.

Results and discussion

Basic physico-chemical characteristics

As shown in Tab. 1, the average weight of individual fruits of 'Hongyan' berries was the highest and of 'Tianxiang' berries was the smallest. Sugar content is an important taste attribute for straw-

Tab. 1: Basic physico-chemical characteristics of selected four cultivars of strawberries.

Characteristics[A]	'Hongyan'	'Tianxiang'	'Tongzi I'	'Zhangji'
Average weight of individual fruit (g)	25.79±3.812[b]	19.78±2.00[a]	22.91±2.61[ab]	22.045±1.91[a]
pH	3.70±0.015[c]	3.42±0.015[a]	3.52±0.021[b]	3.73±0.012[c]
TA (g/100 g FW expressed as citric acid)	0.65±0.013[a]	0.73±0.015[b]	0.79±0.023[c]	0.63±0.031[a]
Sucrose (g/100 g FW)	0.046±0.0027[b]	0.059±0.0029[c]	0.015±0.0015[a]	0.75±0.042[d]
Glucose (g/100 g FW)	4.25±0.23[d]	3.25±0.18[c]	2.36±0.11[a]	2.73±0.16[b]
Fructose (g/100 g FW)	4.65±0.25[d]	3.75±0.16[c]	2.88±0.19[a]	3.13±0.14[b]
Total soluble sugars (g/100 g FW)	8.94±0.48[d]	7.06±0.34 [c]	5.25±0.31 [a]	6.61±0.33[b]
TSS/TA ratio	13.80±0.82[d]	9.69±0.32[b]	8.28±0.52[a]	10.46±0.88[c]
Ascorbic acid (mg/100 g FW)	42.89±0.65[c]	30.92±0.79[b]	22.76±0.88[a]	21.38±0.73[a]
TP (mg gallic acid/100 g FW)	366.53±6.53[c]	313.39±8.54[b]	444.73±4.58[d]	235.12±4.86[a]
Pectin(mg galacturonicacid/100 g FW)	95.51±1.85[c]	92.19±3.54[b]	96.13±5.28[c]	82.84±2.66[a]

[A] Data were presented as the means ±standard deviations (n=6). a-d: values with different superscript letters within one row were significantly different (p≤0.05).

berries and is highly correlated with consumer acceptance (CRESPO et al., 2010). 'Hongyan' berries were the richest in sugars and had approximate 1.5 times reducing sugars as 'Tongzi I' and 'Zhangji'. SKUPIEŃ et al. (2004) determined similar reducing sugars content (5.74~9.09 g/100 g FW) in different strawberry cultivars. However, the sucrose in this cultivars was remarkable lower than 3.16~7.25 g/ 100 g FW as was reported by SKUPIEŃ et al. (2004). This great difference resulted from various cultivars, seasons, origins, agricultural practices, as well as the differences in the extraction procedures used between this and other studies; the greater solubility of sucrose in methanol as compared to fructose and glucose has already been pointed out by others (CRESPO et al., 2010; GINÉ et al., 2009).

Apart from the sugars, acids within the fruit are part of the soluble solids pool and are also important contributors to strawberry taste and flavor (CRESPO et al., 2010). There were significant differences of pH and TA among the four cultivars. The pH value was 3.42~3.73 in the four cultivars strawberries. 'Tongzi I' had the highest TA content (0.79 g citric acid/100 g) and 'Zhangji' the lowest (0.63 g citric acid/100 g). The TA content of the cultivars studied was in concurrent with the content of total organic acids in Tab. 3.

The ratio of total soluble solids/titration acidity is related to flavor quality for various fruits, especially for strawberry flavor and determines the optimum time for strawberry harvesting, because it is considered to be an index of quality. However, PERKINS-VEAZIE et al. (1995) reported that the ratio of total soluble sugars to organic acid is a major parameter of strawberry taste and may be more important for quality and perceived sweetness by a sensory panel than total soluble solids alone. 'Hongyan' had the highest total soluble sugars/titration acidity ratio than other cultivars.

The ascorbic acid is also regarded as an important health-related indicator of fruit quality and its content in fruits causes by a balance between synthesis and degradation. The in situ synthesis occurs in strawberries from D-galacturonic acid, a component of cell wall pectins during fruit ripening and the final concentration in the fruits depends on the expression of the gene *GalUR* as well as the availability of the substrate D-galacturonic acid (AGIUS et al., 2003). In the present study, the content of ascorbic acid ranged from 21.38 mg/100 g in 'Zhangji' to 42.89 mg/100 g in 'Hongyan'. MASNY et al. (1999) estimated similar levels of ascorbic acid in different strawberry cultivars ranging from 25.7 to 53.2 mg/100 g, but SKUPIEŃ et al. (2004) reported a higher level ranging from 54 to 87 mg/100 g. The ascorbic acid in 'Hongyan' is significantly higher (up to 2-folds) compared to 'Zhangji', suggesting advantages of the former in terms

of better resistance to oxidizing agents during processing.

The total phenolics content in this study was from 235.12 mg/100 g in 'Zhangji' to 444.73 in 'Tongzi I', which was similar to 230~340 mg/100 g as reported by AABY et al. (2005), the difference in total phenolics in the studied cultivars could be attributed to the wide range of anthocyanins and other phenolic compounds content as discussed in the following. Pectin ranged from 82.84 to 96.13 mg/100 g and was least in 'Zhangji'.

Pectin is one of the most complex natural plant biopolymers, which are identified as critical structure components of the cell wall (SARA et al., 2012), since they provide cell-to-cell adhesion and mechanical strength (RIAHI et al., 2013). In this study it was shown that cultivars with higher pectin content were more suitable for processing.

Organic acids

The contents of organic acids in the studied cultivars were shown in Tab. 2. Citric acid was the most abundant acid, contributing 73.5~84.7% in total organic acids. Previous studies also found citric acid to be a dominating acid in different strawberry cultivars. The amount of citric acid was 0.64~0.93 g/100 g in this study, similar to 0.6~1.7 g/ 100 g in fully ripe berries (STURM, KORON and STAMPAR, 2003). The following two major organic acids were malic acid and oxalic acid, which contributed 9.5 to 21.7% and 4.5 to 7.9% in total organic acids, respectively. However, SKUPIEŃ et al. (2004) reported that malic acid is the most abundant acid and constituted 56% in total organic acid in Elsanta berries. The minor organic acids in this work were tartaric acid (0.82~1.35 mg/100 g), acetic acid (0.11~0.15 mg/100 g) and fumaric acid (0.19~0.73 mg/100 g). STURM et al. (2003) reported that tartaric acid was 0.05~0.18 g/100g and fumaric acid was 0.4~1.7 mg/100 g. Lactic acid and succinic acid were not observed in four cultivars, but SKUPIEŃ et al. (2004) detected 0.12~0.25 mg/100 g succinic acid in strawberries. There was a significant difference in total organic acids among the four cultivars. The summation of total organic acids determined by HPLC was higher than TA estimated by titration method for each cultivar in this study. 'Tongzi I' showed the highest acidity and 'Zhangji' the lowest regardless of these methods.

Individual phenolic compounds other than anthocyanins

Quantitative results of individual phenolic compounds other than anthocyanins in four cultivars were presented in Tab. 3. The

Tab. 2: Organic acids (mg/100 g FW) in selected four cultivars of strawberries.

Organic acids[A]	'Hongyan'	'Tianxiang'	'Tongzi I'	'Zhangji'
Oxalic acid	82.61±0.80[c]	54.08 ±0.55[b]	89.75±0.25[d]	39.48±0.13[a]
Tartaric acid	1.35±0.058[b]	1.30±0.02[b]	0.99±0.019[a]	0.82±0.013[a]
Malic acid	104.60±1.34[b]	93.08±0.37[a]	195.98±2.69[c]	189.80±15.54[c]
Acetic acid	0.11±0.0066[b]	0.14±0.0046[c]	0.11±0.0075[a]	0.15±0.0029[c]
Citric acid	851.81±25.38[b]	826.51±37.63[b]	930.19±47.96[c]	644.47±20.10[a]
Fumaric acid	0.31±0.011[b]	0.73±0.0029[d]	0.43±0.0010[c]	0.19±0.0026[a]
Total	1040.80±37.47[c]	975.30±38.59[b]	1216.27±50.94[d]	874.30±35.80[a]

[A] Data were presented as the means ±standard deviations (n=6). a-d: values with different superscript letters within one row were significantly different (p≤0.05).

Tab. 3: Individual phenolic compounds (mg/100 g FW) in selected four cultivars of strawberries.

Individual Phenolic Compounds[A]	'Hongyan'	'Tianxiang'	'Tongzi I'	'Zhangji'
Catechin	1.95±0.054[b]	2.61±0.17[c]	4.31±0.29[d]	1.85±0.076[a]
Ellagic acid	3.35±0.23[b]	3.42±0.054[b]	3.93±0.15[c]	2.77±0.082[a]
β-hydroxybenzoic	1.06±0.046[c]	0.31±0.022[a]	0.75±0.039[b]	0.88±0.044[b]
ϱ-coumaric acid	0.63±0.075[c]	0.41±0.026[b]	0.66±0.022[c]	0.35±0.016[a]
Ferulic acid	0.55±0.037[b]	0.43±0.030[a]	0.67±0.043[c]	0.41±0.029[a]
Caffeic acid	0.15±0.0012[b]	0.14±0.00091[b]	0.13±0.00068[a]	0.14±0.0016[b]
Kaempferol	0.65±0.019[b]	0.44±0.029[a]	0.68±0.023[b]	0.44±0.014[a]
Quercetin	0.62±0.036[d]	0.47±0.030[b]	0.53±0.023[c]	0.39±0.0051[a]
Myricetin	0.59±0.036[c]	0.38±0.010[a]	0.53±0.041[b]	0.35±0.039[a]
Total	9.55±0.73[c]	8.61±0.35[b]	12.18±0.85[d]	7.59±0.38[a]

[A] Data were presented as the means ±standard deviations (n=6). a-d: values with different superscript letters within one row were significantly different (p≤0.05)

summation of individual phenolic compounds other than anthocyanins significantly reduced in the order of 'Tongzi I', 'Hongyan', 'Tianxiang' and 'Zhangji', closely corresponding to the decreasing order of TP in Tab. 1. The summation of the phenolic compounds other than anthocyanins was considerably lower than 45.20~84.62 mg/100 g (calculated as total phenolic compounds without hydrolysis) estimated by SKUPIEŃ et al. (2004) and 42.1~54.4 mg/100 g proposed by HIKKINEN et al. (1999). This was possibly due to that the achenes were not broken and discarded after centrifugation process, since there were abundant phenolic compounds in achenes, especially ellagic acid and ellagic acid glycosides (AABY et al., 2005). Strawberries contained 1% achenes on a fresh weight basis, however, they contributed to about 11% of total phenolics (AABY et al., 2005). Catechin varied from 1.85 to 4.31 mg/100 g depending on the cultivars in this study. AABY et al. (2005) reported 6.2~9.0 mg/100 g catechin in strawberries.

Ellagic acid, ϱ-coumaic acid and p-hydroxybenzoic were three predominant soluble phenolic acids. Ellagic acid was the highest, followed by ϱ-coumaric acid and p-hydroxybenzoic in four cultivars. Studies carried out by ODRIOZOLA-SERRANO et al. (2009) and HÄKKINEN et al. (1999) also showed that ellagic acid and ϱ-coumaric acid are predominant phenolic acids in strawberries. The free ellagic acid in strawberries (without achenes) was 2.77 to 3.94 mg/100 g, which fell in the range of 0.54 to 5.91 mg/100 g in different strawberry cultivars detected by SIMIRGIOTIS et al. (2009), but the range increased up to 60.00 to 151.38 mg/100 g after acid hydrolysis in strawberries since ellagic acid is a hydrolytic product of ellagitannins. In this work, we quantified the soluble phenolic acids

without acid or alkali hydrolysis. The ϱ-coumaric acid content was the highest (0.66 mg/100 g) in 'Tongzi I' and lowest (0.41 mg/100 g) in 'Tianxiang', which was lower than in previous studies. Mattila et al. (2006) showed it was 4.4 to 6.3 mg/100 g ϱ-coumaric acid in different cultivar strawberries in Finland, and SKUPIEŃ et al. (2004) detected 15.46 to 42.22 mg/100 g ϱ-coumaric acid in fresh strawberries of six cultivars grown in northwest Poland. Reasons for the high variation may lie in differences in the analytical methodology used and in the natural variation of sample material. The p-hydroxybenzoic (0.31 to 1.06 mg/100 g) was significantly different in four cultivars, and was lower than in the strawberry proposed by MATTILA et al. (2006). The p-hydroxybenzoic in 'Hongyan' was 3.4-folds in 'Tianxiang'. Other two soluble phenolic acids in strawberries were ferulic acid and caffeic acid. There were 0.41 to 0.67 mg/100 g ferulic acid and 0.13 to 0.15 mg/100 g caffeic acid. Ferulic acid in 'Tongzi I' was the highest and in 'Zhangji' the lowest. Apart from 'Zhangji', the other three cultivars showed no difference in caffeic acid content. The content of ferulic acid and caffeic acid in this study was not in agreement with the study of MATTILA et al. (2006) who reported of 0.25 to 0.32 mg/100 g ferulic acid and 0.14 to 0.42 mg/100 g caffeic acid in strawberries from Finland.

Regarding the flavonols, kaempferol, the main flavonol in four cultivars, was 0.44 to 0.68 mg/100 g, which was the lowest in 'Tingxiang' and the highest in 'Tongzi I'. Myricetin and quercetin ranged from 0.35 to 0.40 mg/100 g and 0.39 to 0.62 mg/100 g, respectively. The summations of kaempferol, myricetin and quercetin as total flavonols were significantly different in the four cultivars, which were 1.19 mg/100g in 'Zhangji' and 1.86 mg/100 g in 'Hongyan'.

The content of kaempferol, myricetin and quercetin in this study was similar to or slightly lower than previous studies (HÄKKINEN and TÖRRÖNEN, 2000; HÄKKINEN et al., 1999). HÄKKINEN et al. (2000; 1999) reported the contents of quercetin and kaempferol in strawberry were 0.52 to 0.7 and 1.18 to 1.8 mg/100 g, respectively. The content of phenolic compounds may vary according to the strawberry cultivars, geographical origin, harvesting seasons, growing condition as well as to different extraction/hydrolysis and analytical methods used (ODRIOZOLA-SERRANO et al., 2009).

Free amino acids

The free amino acids in four cultivars were summarized in Tab. 4, asparagine (Asn), glutamine (Gln), aspartic acid (Asp), glutamic acid (Glu) and alanine (Ala) were the five most prominent free amino acids. There were significant differences in the total free amino acids among four cultivars, which were 13.35~32.66 mg/100 g. There were few studies on the content of free amino acids in strawberry. Gallander (1979) reported that the total amino acids were 160-370 mg/100 mL in strawberry juice after hydrolyzing the protein, and the content of free amino acids was 79-87% of total amino acids in different cultivars. A small difference in the numbers of major amino acids in four cultivars was found. The most abundant free amino acids was Asn, contributing 44.96% ('Tianxiang') and 49.60% ('Tongzi I') of the total free amino acids. Asn in 'Tianxiang' was 14.68 mg/100 g and 2.3 times higher than in 'Zhangji'. The content of Asn varied from 218 to 75 mg/100 mL in early harvest and late harvest strawberry juice (GALLANDER, 1979). Other major Free amino acids greater than 1 mg/100 g in four cultivars were similar, and in the order were Serine (Ser), Glu, Asp, Gln and Ala in 'Hongyan', Gln, Ser, Glu and Asp in 'Tianxiang', Gln, Ser, Glu, Asp and Ala in 'Tongzi I', Ser and Glu in 'Zhangji'. The minor free amino acids lower than 1 mg/100 g in four cultivars exhibited significant differences. Cysteine (Cys) was not found in the studied four cultivars, methionine (Met) and isoleucine (Ile) were absent in 'Tongzi I' and 'Zhangji' while present less than 0.2 mg/100 g in 'Hongyan' and 'Tianxiang'.

Monomeric anthocyanins

Anthocyanins contributed well for the attractive color, affected consumer acceptance and preference, and also had great relations with antioxidant capacity. Strawberries are reported to owe two types of anthocyanidin pigments, derivatives of bright red pelargonidin and dark red cyanidin, and the major anthocyanins in strawberries were pelargonidin-3-glucoside (Pg-3-glu), pelargonidin-3-rutinoside (Pg-3-rut) and cyanidin-3-glucoside (Cy-3-glu) (DA SILVA et al., 2000). In this study, Pg-3-glu was predominant in four cultivars, contributing 67.46~89.41%. SKUPIEŃ et al. (2004) reported that Pg-3-glu contributes 78~92% as the predominant pigment in six cultivars. As shown in Fig. 1, 'Tongzi I' was the highest and 'Zhangji' the lowest in three major monomeric anthocyanins and total anthocyanins, respectively. In a previous study, the total anthocyanins were 20~60 mg/100 g in fresh strawberry (DA SILVA et al., 2000). The total anthocyanins and Pg-3-rut in 'Hongyan' and 'Tianxiang' were similar, Pg-3-glu in 'Tianxiang' (23.82 mg/100 g) was significantly higher than in 'Hongyan' (19.48 mg/100 g), but Cy-3-glu in 'Tianxiang' was significantly lower than in 'Hongyan'.

Antioxidant capacity

Differences in the profile and contents of these related antioxidant compounds reported above could contribute to the differences in

Tab. 4: Free amino acids (mg/100g FW) in selected four cultivars of strawberries.

FAAs[A]	'Hongyan'	'Tianxiang'	'Tongzi I'	'Zhangji'
Asp	1.37±0.033[b]	1.75±0.027[d]	1.44±0.0038[c]	0.89±0.018[a]
Glu	1.741±0.0951[c]	1.99±0.062[d]	1.46±0.048[b]	1.01±0.0057[a]
Asn	9.72±0.45[b]	14.68±0.23[d]	11.56±0.071[c]	6.41±0.073[a]
Gln	1.11±0.069 [b]	4.14±0.068 [d]	2.26±0.051 [c]	0.78±0.036[a]
Ser	2.35±0.016[c]	2.72±0.054[d]	1.97±0.084 [a]	1.15±0.037[a]
Gly	0.044±0.00031[b]	0.13±0.0065[d]	0.095±0.0024[c]	0.031±0.0034[a]
His	0.41±0.012[b]	0.92±0.039[c]	0.44±0.022[b]	0.33±0.034[a]
Arg	0.30±0.016[a]	0.59±0.058[c]	0.39±0.0034[b]	0.054±0.12[c]
Thr	0.31±0.025[a]	0.56±0.0080[c]	0.38±0.014[b]	0.26±0.023[a]
Ala	1.03±0.057[c]	0.31±0.033[a]	1.40±0.033[d]	0.59±0.027[b]
Pro	0.046±0.0012[b]	2.22±0.14[c]	0.011±0.0028[a]	0.014±0.0030[a]
Tyr	0.13±0.0075[b]	0.22±0.013[c]	0.096±0.0060[a]	0.078±0.0037[a]
Val	0.064±0.0024[c]	0.14±0.0095[d]	0.048±0.0031[b]	0.014±0.0035[a]
Met	0.030±0.0013[b]	0.18±0.011[c]	nd	nd
Ile	0.18±0.024[b]	0.15±0.0031[c]	nd	nd
Leu	0.037±0.0031[a]	0.37±0.018[b]	0.43±0.0066[c]	0.36±0.0052[b]
Phe	0.26±0.019[c]	0.044±0.0047[a]	0.10±0.00099[b]	0.10±0.0046[b]
Trp	0.33±0.0071[c]	0.27±0.0030[b]	0.33±0.015[c]	0.13±0.0055[a]
Lys	0.93±0.049[b]	0.92±0.045[b]	0.89±0.054[b]	0.73±0.058[a]
Total	20.39±0.66[b]	32.66±0.38[d]	23.31±0.32[c]	13.35±0.38[a]

[A] Data were presented as the means ±standard deviations (n=6). a-d: values with different superscript letters within one row were significantly different (p≤0.05). nd, not detected.
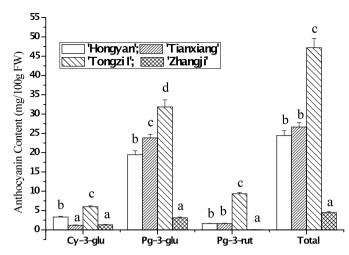
Fig. 1: Major monomeric anthocyanins (mg/100 g FW) in selected four cultivars of strawberries.

predominantly located in the chloroplast thylakoid membranes, and its phenolic substrates are mainly located in the vacuoles, but upon any cell-damaging treatment, the enzyme and substrates may come into contact, leading to rapid oxidation of phenols and contributing significantly to quality loss (JIANG et al., 2004). The results in this study implied that it was easy to turn brown in 'Hongyan' due to its highest PPO activity during the storage and processing, and there was no browning catalyzed by PPO in 'Tianxiang'.

POD activity was similar in 'Hongyan', 'Tianxiang' and 'Tongzi I', but was significantly higher in 'Zhangji'. The involvement of POD in browning was reported by many researchers (JIANG et al., 2004; CHISARI et al., 2007), although it is limited by the availability of electron acceptor compounds such as superoxide radicals, hydrogen peroxide, and lipid peroxides. POD is also responsible for the off-flavors and texture loss as well as color changing. Higher POD activity in 'Zhangji' led to easier quality loss during storage or processing than other cultivars.

PME is an enzyme found in all species of higher plants. PME catalyzes the de-esterification of the methyl group of pectin and converts it into low-methoxy pectin or pectic acid. PME activity likely plays an important role in strawberry fruit softening and viscosity of products decreasing (RIAHI et al., 2013). The PME activity was low in four cultivars, it was the least in 'Tianxiang' and 'Tongzi I', and the highest in 'Zhangji'.

antioxidant activity. The antioxidant capacity of DPPH and FRAP in four cultivars was shown in Fig. 2, there were significant differences in the antioxidant capacity of ·DPPH and FRAP among four cultivars. 'Tongzi I' had the highest antioxidant capacity of ·DPPH and FRAP, while 'Zhangji' was the lowest, which closely corresponded to the content of anthocyanins and other phenolic compounds. 'Tianxiang' was the second and 'Hongyan' the third in the antioxidant capacity of ·DPPH, but the two cultivars exhibited similar antioxidant capacity of FRAP. The antioxidant capacity of ·DPPH and FRAP was not consistent with the ascorbic acid content, since the antioxidant capacity of 'Hongyan' was lower than 'Tongzi I', while its ascorbic acid content was higher. KALT et al. (1999) found that phenolics and anthocyanins were both strongly correlated to the antioxidant capacity of fruits, whereas the ascorbic acid content and antioxidant capacity were inversely correlated. In general, the antioxidant potential of strawberry could be due to synergistic actions of complicated bioactive compounds, and it would be difficult to define the contribution of these bioactive compounds to antioxidant activity.
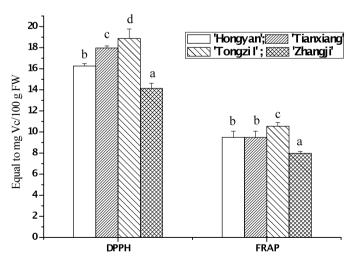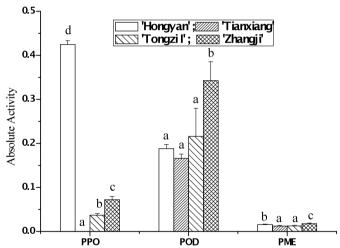

Fig. 3: The activity of PPO, POD and PME in selected four cultivars of strawberries.

Conclusions

The present study demonstrated that different strawberry cultivars presented different physico-chemical and biochemical compositions, which are important factors for appraising the characterization of strawberry cultivars with respect to their nutritional value and potential use for different products. 'Tongzi I' was most suitable for food processing due to the highest titration acidity, total phenolics, pectin, total organic acids, monomeric anthocyanins, antioxidant capacity of ·DPPH and FRAP with lower PPO, POD and PME activity.

Fig. 2: ·DPPH and FRAP values in selected four cultivars of strawberries.

Enzyme activity

As shown in Fig. 3, there were significant differences in PPO activity in four cultivars, which decreased in the order of 'Hongyan', 'Zhangji' and 'Tongzi', and it was not observed in 'Tianxiang'. In plants, PPO is

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