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Chemical compositional characterization of some apple cultivars

Jihong Wu^a, Haiyan Gao^b, Lei Zhao^a, Xiaojun Liao^a, Fang Chen^a, Zhenfu Wang^a, Xiaosong Hu^{a,*}

^a College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, People's Republic of China ^b School of Life Science, Shanghai University, Letter Box 303, No.17, Qinghua Donglu, Haidian District, Beijing 100083, People's Republic of China

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Abstract

Eight commercially harvested apple cultivars were analysed by gas chromatography and high-performance liquid chromatography, in particular the composition and level of sugars, organic acids, amino acids, phenolic compounds and fatty acids. The results showed great quantitative differences in the composition of the apple cultivars, particularly in their phenolic contents. Fructose was the most dominant sugar in the different apple cultivars, followed by glucose and sucrose, while malic acid was the principal organic acid. The C16:0, C18:0, C18:1, C18:2 and C18:3 fatty acids were the most abundant fatty acids, and the C18 family accounted for more than 70% of the total fatty acids content. Asparagine and serine were the principal amino acids. Chlorogenic acid and protocatechuic acid were the dominating phenolic compounds. The results provide important information on how to make the best use of the apple cultivars investigated, for both technological research and processing practice.

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1. Introduction

Apples are one of the most frequently consumed fruits. In China, commercial apple production in recent years amounted to 24 million t/year, of which most (${\sim}70\%$) were used for direct consumption, and the others $(\sim 30\%)$ were processed to produce juice concentrates. Apples constitute an important part of the human diet, as they are a source of monosaccharides, minerals, dietary fibre, and various biologically active compounds, such as vitamin C, and certain phenolic compounds which are known to act as natural antioxidants. Some researchers also consider polyphenols to be antimutagenic and anticarcinogenic compounds [\(Lee & Mattick, 1989; Miller & Rice-Evans, 1997\)](#page-5-0). Along with sugars and organic acids, phenolics determine the quality of apples [\(Dolenc & Stampar, 1997; Fuleki, Pelayo,](#page-5-0) [& Palabay, 1994\)](#page-5-0). They have important roles in providing

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taste characteristics, such as flavour, bitterness and astringency, and also colour [\(Bengoechea, Sancho, & Bartolome,](#page-5-0) [1997; Miller & Rice-Evans, 1997](#page-5-0)). The concentration of these phenolic compounds, which is strongly dependent on the variety of apples and their maturity, is closely associated with the nutritional and sensory qualities of the fruits. Low concentrations may protect apples from oxidative deterioration, for instance during juice production. High concentrations of phenolics and their oxidation products may cause discolouration of fruit products and haze formation in juices, as a result of the interaction of proanthocyanidins (condensed tannins) with proteins, carbohydrates or minerals [\(Ayaz, Kadioglu, & Reunanen,](#page-5-0) [1997\)](#page-5-0). The susceptibility of apples to browning depends on the relative concentrations of different groups of phenolic compounds. The most reactive are catechins and both chlorogenic and caffeic acids [\(Amiot, Tacchini, Aubert, &](#page-5-0) [Nicolas, 1992; Simon, Ilzarbe, & Hernandez, 1992\)](#page-5-0). Phenolic compounds, and particularly flavanols, appear to be important for the classification of different apple cultivars

Corresponding author. Tel.: +86 10 62737434; fax: +86 10 62737434. E-mail address: huxiaos@hotmail.com (X. Hu).

into groups, with respect to their uses. For cider production, apples with a high phenolic content are preferred, while for naturally cloudy juices a low level of phenolics is required. Apples for direct consumption should be rich in biologically active compounds, such as ascorbic acid, and phenolic compounds, particularly flavanols, including catechins and proanthocyanidins. Furthermore, free amino acids and fatty acids, which are nutritive components of many fruit and vegetables also play important roles in human health and maintaining fruit quality [\(Schieber, Kel](#page-5-0)[ler, & Carle, 2001\)](#page-5-0). The roles of fatty acids and amino acids in fruit aroma have been reported in previous research. It has been reported that tyrosine and phenylalanine were substrates for the formation of volatile components [\(Jie](#page-5-0) [& ji Zhong, 2002](#page-5-0)).

No comprehensive data have been reported on the chemical composition of different cultivars cultivated and processed in China. As China is the biggest cider producer and exporter, detailed research on raw materials is necessary, to ensure good product quality. Therefore, this research is focused on analysis and comparison of the chemical compositions, such as monosaccharides, organic acids, amino acids, phenolic compounds and fatty acids of different apple cultivars. A more detailed knowledge of the variability of the compositions of different cultivars will be of benefit in the future selection of apple genotypes with improved nutritional quality and suitable processing characteristics for the manufacture of apple juice concentrate ([Edgar, Allen, & Roy, 1996](#page-5-0)).

2. Materials and methods

2.1. Sample preparation

Eight apple cultivars (Delicious, Golden Delicious, Ralls, Fuji, QinGuan, Jonagold, Granny Smith and Orin) grown in Shandong province of China were used for this study. The apples were harvested at commercial maturity. Fruits were loosely packed inside conventional modular bulk containers with polyliners and stored at $0^{\circ}C$, 80– 90% relative humidity. The humidity inside the polyliner was approximately 95%. The air was exchanged with fans four times daily to remove ethylene.

2.2. Chemical analysis

High-performance liquid chromatography (HPLC) was used for separation, identification and quantification of individual compounds in apple juice. The HPLC system consisted of Thermo Separation Products (TSP) equipment with a model K-1001pump and K-1500 solvent mixer. Solute elution was monitored using a variable wavelength UV detector (model K-2501, KNAUER CO., Berlin, Germany) and RI detector (model K-2301, KNAUER, Germany).

For sugar (glucose, fructose, sucrose) and organic acid (citric, fumaric and shikimic) determination, we used the modified HPLC method of [Dolenc and Stampar \(1997\).](#page-5-0)

Samples were prepared by extracting juice from cored fruit flesh using a commercial blender. Five to eight apples from each variety were pooled, and then filtered through filter paper. The fruit juice (5 ml) was diluted to 100 ml with redistilled water, centrifuged in a refrigerated centrifuge at 0° C and at 3000g for 10 min, and then filtered through a $0.45 \mu m$ Millipore filter. Analiquot (20 ml) of the resultant supernatant was used for HPLC analysis.

Sugar analyses were performed isocratically on a PronotSIL, 120-10-Amino column $(10.0 \text{ µm}, 250 \times 4.6 \text{ mm})$ i.d., KNAUER, Germany) attached to a retractive index (RI) detector (model K-2301, KNAUER, Germany). The analysis was carried out at 30 \degree C at a flow rate of 1.5 ml/ min with acetonitrite/water CH_3CN/H_2O (85:15) as the mobile phase. Sugars present in each sample were identified and quantified using external standards. The reproducibility of the chromatographic separation of the components was determined by making five injections of the standard solutions and apple samples. The results expressed as relative standard deviation (RSD%) are as follows: 0.67 for sucrose, 0.23 for glucose, and 0.20 for fructose.

Organic acids were determined by HPLC analysis using a ProntoSIL, $120-10-C_{18}$ $(10.0 \,\mu \text{m}, 250 \times 4.6 \,\text{mm}$ i.d., KNAUER, Germany) column, with 0.01 M K₂HPO₄. $3H₂O$, pH 2.6 as mobile phase, with a flow rate of 0.5 ml/ min, at 30° C.

Organic acids were identified and quantified by comparison of their retention time and peak area with standard solutions of known organic acids, using a UV detector with wavelength set 210 nm (model K-2501, KNAUER, Germany). The results of the reproducibility study of chromatographic separation for organic acids, expressed as RSD%, are as follows: 0.013 for tartaric acid, 0.044 for quinic, 0.84 for malic acid, 0.004 for shikimic acid, and 0.041 for succinic acid.

The total soluble solids (TSS), expressed as $\%$, was determined in the juice of each sample using an Atago digital refractometer at 21 $\mathrm{^{\circ}C}$.

As for phenolic acid analysis, 10 ml juice was extracted twice with 10 ml of ethyl acetate: fractions were pooled and evaporated to dryness, and the residue was dissolved in 1.0 ml of methanol (HPLC grade). The resultant solution was filtered through a $0.45 \mu m$ membrane filter prior to HPLC analysis [\(Spanos & Wrolstad, 1992](#page-5-0)).

Fatty acid analysis was performed using a Hewlett-Packard 5890 Gas Chromatography with flame ionization detector. Single aliquots of lipid extract (approximately $0.5 \mu L$) were injected in splitless mode onto a DB-23 capillary column $(60 \times 0.25 \text{ mm} \text{ i.d., } 0.25 \text{ µm} \text{ film thickness});$ Agilent, USA. The injector and detector temperatures were set at 250 °C and 270 °C, respectively. The oven was set at an initial temperature of 130° C for 1 min, which was increased to 215 °C at a rate of 6 °C/min, maintained for 2 min, then raised to 230 °C at a rate of 40 °C/min, and held at $230 \degree$ C for 40 min. The flow rate was 32 cm/s , helium used as carrier gas, and the electronic pressure control set in the constant flow mode. A calibration mixture of

fatty acid standards (Sigma) was used for identification and quantification of the fatty acids.

Approximately 10 g from each sample were placed in tarred test tubes, heated at $100\degree C$ for 5 min in order to inactive the lipase, and then ground into pulp with 30 ml chloroform/methanol (1:2, v/v). Samples were extracted for 20 min and filtrated with 20 ml chloroform, and the resultant extracts combined and shaken for 15 min with 20 ml NaCl (0.76%). The supernatant was removed and the solvent evaporated under vacuum. The mass of crude lipid extracted from each specimen was then determined gravimetrically.

The crude lipid fraction was redissolved in 6 ml petroleum ether (90–120 range) saturated with methanol (6 ml). The substrates were re-extracted with 6 ml of the same solvent and concentrated. The polar lipid was obtained, quantified gravimetrically, and then dissolved in 1 ml methanol (GC grade) for storage at -20 °C for esterification.

Polar lipid $(300 \mu L)$ was re-dissolved in 2 ml methanol with 0.4 mol/l KOH and 2 ml benzene/ petroleum ether $(30-60 \text{ range})$ $(1:1, v/v)$, vibrated, and then allowed to rest for 15 min. Distilled water (16 ml) was added to the vials, the upper layer of the extracts withdrawn and evaporated under nitrogen, and then redissolved in 1 ml hexane for analysis by gas chromatography.

For amino acid analysis, juice samples from each apple sample (2 ml) were placed in 10 ml glass ampoules containing 1 ml of internal standard (α -amino butyric acid) and 9 ml of 6N HCl. The ampoules were frozen in liquid nitrogen, evacuated, sealed and then placed in an oven at 110° C for 20 h. After cooling the ampoules were centrifuged for 10 min to remove acid. Samples were redissolved in 50 ml 6N HCl and 10 ml aliquots removed for derivatization. Each sample was analyzed in duplicate.

Aliquots were dried in a centrifuge, and then derived with $2A + 2B$ reagent (Waters AccQ-Fluor regent box, include boracic acid buffer solution1, derivating agent power2A and dilution2B) at 56 °C for 15 min. The AccQ-Tag system (Waters, Milford, MA) was used for quantitative determination of the amino acid composition. An aliquot of each sample $(5 \mu L)$ was injected onto a column

(Waters AccQTag column; 4.6×150 mm) for analysis of individual amino acids. A Waters 2475 fluorescence detector was used, with a mobile phase consisting of special mobile phase/acetonitrile $(93:7, v/v)$ with a flow rate of 1.0 ml/min.

2.3. Standard materials

Standards for sugar, organic acid, fatty acid, amino acid and phenolic analysis were obtained from Sigma Co. Linearity of the response to UV and RI detection was tested for each compounds with five different concentrations prepared in twice distilled water and all correlation coefficients were in the required range.

2.4. Statistical analysis

The results were statistically evaluated by one way analysis of variance (ANOVA). Statistically, differences with Pvalues under 0.05 were considered significant and means were compared by 95% Tukey's HSD multiple range test.

3. Results

The composition of the apple cultivars investigated is presented in Table 1. The summer variety, Delicious, showed relatively low levels of soluble solids, compared to the cultivars harvested in September and October, the level of soluble solids differed considerably. For the production of apple juice concentrates of good quality, Ralls, Granny Smith and Jonagald are ideal cultivars because they have relatively high contents of soluble solids and acids.

Fructose and glucose were identified as the principal monosaccharides [\(Table 2](#page-3-0)). Fructose level (average 53.9 g/l) was almost always higher than glucose level. There have been numerous studies examining fruit sugar and the increasing levels of fructose, glucose and sucrose at advanced stages of fruit maturity ([Dolenc & Stampar,](#page-5-0) [1997; Miller & Rice-Evans, 1997](#page-5-0)). As shown in [Table 2](#page-3-0), the fructose content of QinGuan apple (59.9 g/l) is the highest of all. The quantification of glucose and fructose

Results as mean \pm SEM of triplicate measurements.

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contents in the present work agree with the previously reported ranges for apple [\(Amiot et al., 1992; Ackerman,](#page-5-0) [Fischer, & Amado, 1992; Dolenc & Stampar, 1997](#page-5-0)). The sucrose content of Ralls apple (30.0 g/l) is higher than that of other apples. The sugar profile of fruit pulp is an important component of chemical composition tables and provides valuable information regarding the authenticity of fruit juices. It also has an effect on the sensory properties and nutritional value of fruit products. However, in order to evaluate the quality of apple fruit, other aspects of chemical composition should be determined.

The major organic acids were tartaric, quinic, malic, shkimic, citric and succinic acids (Table 3). The predominant organic acid in the tested apples was malic acid (average 4.61 mg/l). The total organic acid content of Granny Smith apple was the highest, followed by that of Ralls apple ([Table 1\)](#page-2-0). Sugar: acid ratios are commonly held to determine the sensory quality of fruit. In this study, Qin-Guan apple had a higher sugar:acid ratio than the others.

The functional and biological properties of flavanols are strongly dependent on their structure and degree of polymerisation. The most important flavanol compounds in

Results as mean \pm SEM of triplicate measurements.

apples, i.e., epicatechin, catechin and chlorogenic acid, were determined by HPLC ([Table 4\)](#page-3-0). The results confirmed earlier reports that chlorogenic acid is the main phenolic compound in apples ([Amiot et al., 1992; Simon et al.,](#page-5-0) [1992\)](#page-5-0). Only in Granny Smith, Delicious and Orin, was the epicatechin content higher than that of chlorogenic acid. Low catechin and chlorogenic acid contents in Granny Smith and Orin apples indicated that they are good varieties for the production of light-coloured juices and other products.

Palmitic acid and linoleic acid were the dominant fatty acids, constituting 70–80% of the total fatty acids in the fruit (Table 5). The major amino acids in the eight apple cultivars were alanine, aspartate, serine, and glutamine. Jonagold apple fruit contained the highest amount of total amino acid (1568 g/l), followed by Orin (1365 g/l) and Ralls (1098 g/l) (Table 6). Lysine and sulphur-containing

Table 5

Fatty acids content in apple fruits of eight cultivars (mg/l)

amino acids are usually limiting in proteins of vegetal origin, while sulphur-containing amino acids were found in apple cultivars in our experiment.

4. Discussion

Quality is usually estimated from the relative values of several characteristics considered together ([Kader, 2000\)](#page-5-0), Quality control of apple and apple juice starts in the field with the selection of the proper time to harvest and cultivars to select for maximum quality. As shown in [Table 1](#page-2-0), summer variety, such as Delicious showed relatively low levels of soluble solids compared to the late cultivars, (apples harvested in September and October). For the production of apple juice concentrates with good quality, Ralls, Granny Smith and Jonagold are ideal cultivars because they had relatively high contents of soluble solids

Constituent	Cultivar									
	Delicious	Golden Delicious	Ralls	Fuji	OinGuan	Granny Smith	Jonagold	Orin	Average	
Palmitic (C16:0)	0.0427	0.0659	0.0644	0.0516	0.0563	0.0625	0.0609	0.0634	0.058	
Palmitoleic (C16:1)	0.0004	0.0003	0.0003	0.0004	0.0004	0.0003	0.0005	0.0003	0.00036	
Margaric (C17:0)	0.0044	0.0029	0.0039	0.0021	0.0019	0.0025	0.0020	0.0024	0.0028	
Stearic (C18:0)	0.0160	0.0132	0.0202	0.0134	0.0137	0.0128	0.0220	0.0148	0.016	
Oleic $(C18:1)$	0.088	0.0158	0.0292	0.0124	0.0216	0.0116	0.0287	0.0102	0.027	
Linoleic $(C18:2)$	0.143	0.197	0.181	0.140	0.166	0.140	0.188	0.147	0.16	
α -Linolenic (C18:3)	0.0135	0.0102	0.0247	0.0253	0.0162	0.0515	0.0197	0.0469	0.026	
Arachidic $(C20:0)$	0.0078	0.0046	0.0073	0.0053	0.0058	0.0052	0.0079	0.0052	0.006	
Eicosenoic $(C20:1)$	0.0074	0.0023	0.0028	0.0032	0.0032	0.0016	0.0059	0.0022	0.0036	
Arachidonic (C20:4)	0.0074	0.0023	0.0037	0.0032	0.0032	0.0023	0.0059	0.0022	0.0068	
Docosanoic $(C22:0)$	0.0072	0.0048	0.0055	0.0074	0.0044	0.0103	0.0089	0.0061	0.0038	
Docosahexenoic (C22:6)	0.0011	0.0051	0.0029	0.0032	0.0029	0.0023	0.0017	0.0017	0.0026	
Docosapentaenoic $(C22:5)$	0.0049	0.0061	0.0070	0.0032	0.0045	0.0010	0.0018	0.0030	0.0039	
Tetracosanoic (C24:1)	0.0131	0.0163	0.0135	0.0140	0.0172	0.0150	0.0141	0.0143	0.015	
Total	0.292	0.347	0.367	0.284	0.318	0.319	0.368	0.367	0.33	

Table 6

Amino acids content in apple fruits of eight cultivars (g/l)

Amino acid	Cultivar										
	Delicious	Golden Delicious	Ralls	Fuji	OinGuan	Granny Smith	Jonagold	Orin	Average		
Asparagine	172	126	159	146	192	126	82.0	142	143.08		
Serine	87	122	66.6	118	91.7	109	97.2	117	101.12		
Glutamine	31.1	88.1	103	56.8	45.3	108	83.5	121	79.63		
Glycine	1.04	2.58	41.4	1.59	2.69	12.4	1.98	2.5	8.27		
Histidine	4.44	7.66	7.5	9.76	17.1	17.6	10.6	25.8	12.56		
Arginine	18.7	26.7	41.9	17.9	18.4	29.4	30.2	34.2	27.18		
Proline	28.6	45.5	43.5	22.9	23.7	34.1	39.8	40.7	34.85		
Alanine	335	432	507	568	347	419	393	376	422.13		
Cysteine	5.7	4.94	6.08	6.27	6.55	9.53	5.39	8.07	6.57		
Tyrosine	0.36	0.823	0.644	0.589	1.13	0.929	2.12	1.58	1.02		
Threonine	33.9	30.2	59.7	57.6	80.9	21.1	31.2	81.5	49.51		
Valine	3.26	3.09	0.172	3.46	9.77	3.16	5.42	5.31	4.21		
Methionine	1.14	1.9	2.96	0.589	1.59	2.8	0.818	4.33	2.02		
Lysine	0.431		1.32	0.95	0.688	0.549	0.904	0.665	0.81		
Isoleucine	20.1	6.39	22.9	6.42	6.07	4.18	12.1	3.82	10.25		
Leucine	35.1	12.9	32.6	30.8	33.2	35.6	31.5	31.9	30.45		
Phenylalanine	1.03	2.54	2.05	1.66	1.65	1.6	2.71	2.59	1.98		
Total amino acids	779	914	1100	1050	879	935	1570	1370	1070		

and acids. Granny Smith had the high levels of total acids and the highest sugar/acid ratio of the eight apple cultivars, followed by Ralls. These two cultivars are suitable for producing apple juice concentrate. Delicious, Fuji and Jonagold are good for direct consumption.

As far as the essential fatty acids are concerned, eight apple cultivars were rich in linoleic acid. The α -linolenic acid content of Qin Guan apple was relatively low, but that of Orin apple was high. This is of nutritional interest, since diets based on meat, starch sources, and fruits and vegetables are generally low in omega-3 fatty acids. The amounts of linoleic and α -linolenic acids in the plants are therefore noteworthy, as they are both essential fatty acids for humans and must be obtained through the diet. The ratio of saturated to unsaturated fatty acids indicates an excess of saturated fatty acids in each apple variety, the optimal value being 30/70 or less Rogez, Buxant, Mignolet, and Souza (2004).

Low flavanol content in Delicious (1.97 mg/l), Granny Smith (6.88 mg/l) , Jonagold (12.1 mg/l) and Orin (6.36 mg/l) [\(Table 4](#page-3-0)) and high levels of soluble solids ([Table 1\)](#page-2-0) in these apples make them good cultivars for the production of light-coloured juice (Edgar et al., 1996). The level of total phenolics in eight tested cultivars ranged from 26.2 mg/l to 88.2 mg/l.

5. Conclusions

Sugars and organic acids are important for quality evaluation of apple fruits. Additionally, phenolic content is also one of the most important factors for appraising the characterization of apple cultivars, with respect to their nutritional value and potential use for different products.

In the eight apple cultivars investigated, those appropriate for direct consumption are Golden Delicious, Fuji, Ralls and QinGuan which contain high levels of biologically active catechins and procyanidins. For the production of light-coloured juices, the appropriate cultivars are Granny Smith and Orin, with their relatively low catechin and chlorogenic acid contents. In consideration of the total quality demanded in apple juice, using different apple cultivars to produce mixed apple juice may be appropriate.

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