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Characterization of Volatile and Non-Volatile Compounds and Classification of Different Cultivars of Chinese *Ziziphus jujuba* Mill. Fruits

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CHARACTERIZATION OF VOLATILE AND NON-VOLATILE COMPOUNDS AND CLASSIFICATION OF DIFFERENT CULTIVARS OF CHINESE *ZIZIPHUS JUJUBA* MILL. FRUITS

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ABSTRACT

Jujube (*Ziziphus jujuba* Mill.) is a desirable fruit that is widely grown in China. Also, it has been used as an herbal medicine and a functional food simultaneously for a very long time. In this study, 15 cultivars of jujube that were collected from a same farm in Shanxi Province, China, were analyzed in terms of their non-volatile components, including reducing sugars, organic acids, fatty acids, amino acids, minerals and antioxidants, and volatile compounds, in an effort to investigate their nutritional values, and the similarity between the cultivars so as to classify the cultivars based on their chemical composition.

The results showed that, in generally speaking, there were significant differences in the chemical compositions among the cultivars ($p<0.05$). The content of glucose varied from 85.87 to 1004.95 mg/100g FW; malic acid and citric acid were main organic acids, of which the contents ranged from 120.15–508.67 mg/100g FW and 29.40–180.69 mg/100g FW, respectively. Jujube fruits contained a variety of polyunsaturated fatty acids, including linoleic acid, linolenic acid, eicosapentaenoic acid, arachidonic acid, and docosahexaenoic acid. In addition, the fruits were rich of lauric acid (967.20–4035.78 μg/kg DW), palmitic acid (685.68–1936.91 μg/kg DW), myristoleic acid (1718.96–5862.64 μg/kg DW), oleic acid (427.87–2864.98 μg/kg DW), linoleic acid (533.34–7330.05 μg/kg DW). Besides, iron (52.72–125.16 mg/kg DW), calcium (162.29–287.53 mg/kg DW) and magnesium (511.77–699.77 mg/kg DW) were also determined as the main minerals in the fruit. By using the hierarchical cluster analysis and principal

component analysis, the 15 cultivars, based on the contents of reducing sugars, were classified into 6 groups, including group A (PZ and DB), group B (NP and LZ), group C (YZ, LB, XZ, HP, BJ and JB), group D (YL, JS, JD), group E (BZ) and group F (PB). Except the group E and group F, the other groups can be differientated from each other.

Antioxidants including cAMP, ascorbic acid, triterpenes, and the total phenolic content, total flavonoid content, as well as the antioxidant capacity (i.e., FRAP, DPPH, ABTS, HRSA) were also analyzed in this study. According to the results, the content of cAMP was in a range of 66.33 to 2716.88 μg/100g FW; the content of ascorbic acid ranged from 317.9 to 679.6 mg/100g FW. In addition, jujube contained a low content of triterpenes (6.66 to 18.19 mg/100g FW). The total phenolic content was determined in a range from 330.74 to 571.44 mg gallic acid /100g FW, while the total flavonoids content varied from 43.14 to 154.09 mg rutin/100g FW. The range of antioxidant capacity such as DPPH, ABTS, FRAP and HRSA were determined to range from 0.603 to 1.842 mmol Trolox/100g FW, 2.276 to 2.786 mmol Trolox/100g FW, 1.228 to 3.823 mmol Trolox/100g FW, and 1.353 to 3.560 mmol Trolox/100g FW, respectively. All the 15 cultivars were classified into five clusters based on hierarchical cluster analysis. As a result, the cultivars of NP, JS, YZ were categorized in the same cluster, which contained relatively high contents of antioxidant components and strong antioxidant capacity.

Solid phase micro extraction method (SPME) was used to extract the volatile compounds of jujube, which were further identified by GC–MS. The identified volatiles included aldehydes, alcohols, acids, ketones and esters. Among them, hexanal (276.5 to 1314 μg/100g FW), (*E*)-2-hexanal (145.1 to 1876 μg/100g FW), nonanal (188.2 to 1047

μg/100g FW), and n-decanoic acid (58.42 to 1268 μg/100g FW) were found to be the main volatile compounds in fresh jujube. Based on the contents of the volatile components, the jujube fruits were classified into five clusters, including cluster 1 (LB, HP, LZ, NP, JS, PZ, and YL), cluster 2 (BJ, DB), cluster 3 (PB, BZ, JD and XZ), cluster 4 (JB) and cluster 5 (YZ). Cluster 1, cluster 2 and cluster 3 were found to be crossed over together in the two-dimension plot, which means they could not be discriminated from each other based on contents of volatile compounds. However, the cluster 4 and cluster 5 could be separated very well from each other and from the other clusters. Moreover, two extraction methods, SDE and SPME, were compared in regards of their efficiency of extracting volatile compounds from the dried jujube fruits. (*E*)-2-Hexenal and hexanal were found to be the major aldehyde compounds in the SDE extract, while nonanal and benzaldehyde were major aldehyde compounds extracted by the SPME method.

DEDICATION

To my parents, for their support, encouragement, and endless love they gave to me.

To my husband, for his support and love, making me to be better and better.

To my daughter, for her love and making my life more colorful.

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CHAPTER ONE

INTRODUCTION

1. Jujube fruit

Ziziphus belongs to the *Rhamnaceae* family that includes some species like *Ziziphus jujuba* Mill., *Ziziphus lotus*, *Ziziphus mauritiana*, *Ziziphus celata*, *Ziziphus oenoplia* and other species. They are widely grown in Asia, Europe, Africa, and Australia.

Jujube fruits in China belong to the *Ziziphus jujuba* Mill.. They are widely distributed in Xinjiang, Ningxia, Shanxi, Shaanxi, Hebei, Henan, Hunan, Zhejiang, and Shandong provinces. Most of these districts are located in the northern part of China. In 2009, the jujube production in Hebei province accounted for 24.22% of all the jujube yield in China, followed by 20.44% in Xinjiang province, 19.63% in Shandong province, 12.31% in Shaanxi province, 11.68% in Shanxi province, and 7.75% in Henan province (*1*). Besides, based on the data from the Chinese Jujube Market Competition and Development (*2*), the yield of jujube in China were as much as 7.4 million tons in 2014. In addition, Chinese jujubes are also planted in California, Louisiana, Kansas, Georgia and other states in United States (*3*). Jujube trees normally have a height of 20 – 30 feet based on their plantation location and local environmental condition. The tree wood is very hard, and the leaves are 2.5 cm to 5.5 cm long, 2 cm to 4 cm wide (*4*). In China, some cultivars are planted in arid or semiarid locations, because jujube trees can survive with 200 mm of rain precipitation a year. Some saline – alkaline regions also can be used as plantation of jujube (*5*). Its flower blooming period is between May to July and the flowers are yellow with 4 – 6.4 mm in diameter (*6*). Flowering would last more than 30

days, and the ripening stage of jujube fruit is between August to October. Moreover, it is worthy of mention that intercropping is one of useful methods in improving the agricultural ecosystem, which has also been used for jujube in an effort to improve the comprehensive utilization of the limited cultivated land. According to the study, jujube/cotton system did not affect the production of jujube fruits significantly, but increased the productivity of cotton. Jujube/maize system could enhance the water and light uses (*7*). However, intercropping jujube with wheat inhibited the root length density of these two crops, resulting in the decreased yields of both crops (*8*). In order to clarify the mechanism of intercropping the jujube and its effect on other crops, more studies in terms of the intercrop species and methods need to be done by the researchers.

Jujube has long history been used as a herbal medicine in ancient China, which is considered as a functional food due to its rich amounts of nutrients, including sugars, fatty acids, amino acids, minerals, vitamins, polyphenols and other antioxidants (*9*). Also, the fruit can be processed into different kinds of foods, such as dried jujube, jujube jelly, etc. (*10*). Among them, the most popular product in the market is red dates which is produced by natural exposure to the sunshine, or dried by the oven.

According to USDA national nutrient database (2016), jujube fruits, based on 100 g of fresh weight, approximately contain 77.86 g of water, 20.23 g of carbohydrates, 1.20 g of protein, and 0.20 g of total lipids. In another report, Chinese jujube contained 64.7–81.8% of water, and 13.2 – 22.9 % of sugar per 100 g of the fresh jujube (*11*). In comparison, Spanish jujube had a water content between 78.3% to 82.1% (*12*). The basic information of the compositions of jujube on dry basis are listed in **Table 1.1** for details.

Jujube fruits are believed to have a lot of health benefits. The essential oil extracted from the seeds of jujube was reported to possess anti-inflammatory activity (*13*); the polysaccharides from jujube have hepatoprotective activity (*14*) and immune-biological activity (*15, 16*); betulinic acid from sour jujube fruits was reported to be able to inhibit the breast cancer cells (*17*). Besides, the jujube fruits were found to have antioxidant (*18*) capacity and the bark of *Ziziphus mauritiana* performed anti-obesity (*19*) activity.

2. Nutritional Compounds of Jujube

2.1 Sugars

Carbohydrates are one of the main components in fruits. They are precursors of many chemicals contributing to browning colors, aromas, flavors, etc. They also play important roles on human health and growth, such as providing the energy and recognizing the cells. Carbohydrates can be separated into different groups based on their chemical structures, and molecular weights which include monosaccharide, oligosaccharide and polysaccharide. Glucose, fructose and sucrose are the major sugars in fruits such as papaya (*20*), berries, peach, apple, watermelon, and cherry, (*21*) etc. In jujube fruits, the major sugars include glucose, fructose and sucrose, as same as those in the aforementioned fruits.

Carbohydrates are soluble in polar solvents such as water and 80% of methanol, so water is a common solvent for extraction of monosaccharide or oligosaccharide. In contrast, polysaccharide determination involves acid hydrolysis at high temperature (*22*, *23*) and enzymatic hydrolysis.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are two widely used instruments for sugar measurement. GC with the flame ionization detector (FID) and mass spectrometer (MS) detector are usually used to determine monosaccharide. HPLC with the evaporated light scatting detector (ELSD) (*21*), refractive index (RI) (*24*) and near-infrared spectroscopy (*25*) can be used for direct detection of sugars. Other detectors like UV-Vis and fluorescence detector (FD) can be used after sugar derivatization. 1-Phenyl-3-methyl-5-pyrazolone (PMP) (*26*-*30*) is a popular chemical reagent to derivatize sugars at 70 °C, resulting in products that can be measured at 250 nm by the UV detector. Chemical benzamidine was also reported to be able to derivatize the reducing sugars that could be detected by HPLC with the fluorescence detector (FD) at the excitation wavelength at 288 nm and emission wavelength at 470 nm (*31*). Another method called high performance anion exchange chromatography-pulsed amperomeric detection (PAD) is also often used for measurement of sugars, such as glucose, maltose, isomaltose, maltotriose, maltotetraose and maltopentaose in wheat flour (*32*).

Ziziphus jujuba Mill. is a good source of sugars including sucrose, fructose, glucose, rhamnose and sugar alcohols such as sorbitol (*9, 11*). The sugar content in jujube fruits varies significantly. It was reported the three sugars (i.e., sucrose, fructose and glucose) of the *Z. jujuba cv*. Lingwuchangzao were affected by growing stages after its flowering. Particularly, the fructose and glucose increased significantly in the last two stages (i.e., 89 days and 115 days after flowering), but the sucrose could not be detected until the last two stages (*33*).

2.2 Free Amino Acids

Twenty amino acids are commonly required for syntheses of human proteins. However, human beings can only synthesize some of them and the rest should be obtained from food. Therefore, the amino acids are classified into two types: essential amino acids that cannot be synthesized de novo by human beings, and non-essential amino acids. The former includes lysine, phenylalanine, methionine, threonine, tryptophan, valine, histidine, leucine and isoleucine, while the latter includes alanine, cysteine, glutamic acid, glutamine, arginine, asparagine, proline, serine, aspartic acid, and tyrosine. Amino acids as nutrients have special functions for human bodies. They are involved in urine cycle and TCA cycle for body health and energy supply. Amino acids contribute to growth, body composition, immune system, antioxidant defense in lipid oxidation, are necessary for DNA and RNA synthesis, and regulate the cell signaling, etc.(*34*).

In plant, amino acids also have special functions. They are the precursors of some volatile compounds. For instance, the amino acids can be degraded to aldehydes by different pathways by amino acid transaminase, amino acid decarboxylase, and aldehyde synthase to form many volatile compounds (*35*).

Peptides are composed by different amino acids, which may possess bioactivities. Many antioxidant peptides were found to contain 5–16 amino acids, and their composition and sequence could affect the antioxidant capacity (*34, 36*). For fermentation food, composition and sequence of the amino acids in peptides can affect the food taste. Proline is a major amino acid which contributes to the bitter taste, other

amino acids including glycine, valine, leucine, phelalanine, alanine and tyrosine also make the peptide to be tested in bitterness (*37*). Some amino acids (e.g., alanine, cysteine and s-methyl cysteine) can protect iron materials from corrosion in acid solution such as 1.0 M HCl solution (*38*). Branch amino acids (e.g., valine, leucine and isoleucine) were reported to be able to improve the life quality of some people who were suffered from liver cirrhosis (*39*).

In order to analyze the amino acids by HPLC–DAD and/or FD, chemical derivatization is a necessary step. o-Phthalaldehyde 3-mercaptopropionic acid (*40*) and o-phthaldehyde (*41*) are often used to derivatize the amino acids for the DAD, while 9-fluorenylethyl chloroformate is commonly used for the FD under the excitation wavelength at 266 nm and the emission wavelength at 305 nm, and 6-aminoquinolyl-N-hydroxysuccinymidyl carbamate is used for the fluorescence detector (excitation at 250 nm, emission at 395 nm) (*42, 43*). According to the report of Horanni (*44*), 9-fluorenylmethyloxycarbonyl chloride is also used to derivatize the amino acids in tea and tea products, which were detected by HPLC–UV at 262 nm. Some other chemicals such as ninhydrin (*45*), dansyl chloride (*46*), dabsyl chloride, 1-fluoro-2,4-dinitrobenzene, phenylisothiocyanate and diethyl 2 (ethoxymethylidene) propanedioate were also used as common derivative agents for amino acids analysis (*47*). Ion-exchange chromatography is another instrument used for amino acid detection, but it could not separate all the amino acids and takes longer time than the HPLC methods (*48*). Gas chromatography is another option (*49*), but the amino acids should be derivatized to volatile amino acid derivatives before they are introduced into GC for which FID (*50*) and MS (*51, 52*) are

often used to analyze the derivatives. NIR was also used to analyze 16 amino acids, most of them exhibited good calibration curves except proline, histidine and arginine in Chinese rice wine (*53*).

Chemical profiles of free amino acids of 46 jujube fruits, including different jujube cultivars or the same cultivar in different regions, were determined by ultra high performance liquid chromatography (UHPLC) with triple quadrupole mass spectrometry (MS) (*54*). All of the aforementioned amino acids were detected except Thr in some samples. Lin et al. (*55*) used the UPLC tandem mass spectrometry to determine the free amino acids in jujube samples, which were derived by 4-chloro-3,5-dinitrobenzotrifluoride. Trp and Cys-Cys were not detected in those samples. The Tyr was only detected in the species of Junzao and Hupingzao. Proline was the most abundant free amino acid in the samples. Choi (*56*) et al. collected jujube fruits at eight stages after flowering from 10 days to 115 days, and analyzed their free amino acids by the ion exchange chromatography. According to the results, no Met and Trp were found in all the stages and the most abundant amino acids was Asn which contributed to 78.3% in 52 days after the flowering.

2.3 Fatty Acids

Fatty acids have three categories based on the numbers of double bonds, which include saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). High intake of saturated fatty acids during breakfast with a short term can induce insulin resistance in bodies (*57*). It was reported that palmitic and stearic acids were the inducer. The saturated fatty acids can also activate the inflammatory signaling,

decrease the mitochondria function (*58*) and alternate the gene expression (*59*). PUFA can affect the blood pressure, and prevent cardiovascular disease. In addition, PUFA also have antioxidant, anti-inflammatory (*60*) and anticancer properties (*61*). The major sources of PUFA are vegetable oils from olive, canola, soybean, marine fish such as salmon, tuna, etc. (*62*). Linoleic and linolenic acids are essential fatty acids for human beings. Linolenic acid is the precursor of DHA and EPA. These omega-3-polyunsaturated fatty acids play an important role in human health especially for babies' and older adults' brain functions (*63*). For instance, they have a positive effectiveness on cognitive functioning (*64*).

GC is a common instrument used to identify fatty acid derivatives. Long chain fatty acids need to be methylated before they are analyzed by GC–FID or GC–MS. Acetyl-chloride and methanol are the reagents used to separate positional and geometrical isomers of FAs (*65*). Zeng et al. (*66*) analyzed fatty acids methyl esters (FAME) by GC–MS to test different capillary ionic liquid columns in different polarity. The authors found the polyunsaturated fatty acid methyl esters had higher retention time in the higher polarity column; saturated fatty acid methyl esters in higher polarity column needed lower eluted temperature. Bromke et al. (*67*) extracted fatty acids from plant labeled by ^{13}C arobidopsis. The extract without methylation was injected into a LC–MS system through a C18 reverse phase (RP) column at 60 °C. Compared with GC–FID, LC–MS had higher sensitivity and selectivity, but the ionization efficiency of the analytes can affect the quantification. In another study, HPLC–UV was used to identify lipids and FAMEs at 205 nm, with a reverse phase column with different mobile phases. Mobile

phase that was consisted of methanol and a mixture solution of 2-propanol and hexane (5:4, v:v) was used to simultaneously determine the amount of triacylglycerides, diacylglycerides, and monoacylglycerides, while acetonitrile was used as a mobile phase to analyze the FAMEs (*68*). Trans fatty acids in oils was determined by middle infrared spectroscopy, which is a very sensitive instrument with coefficient-correlation more than 0.98 (*69*). Short chain volatile fatty acids, such as acetic acid, propionic acid, butyric acid, pentanoic acid, and hexanoic acid, can be detected by head space solid phase micro-extraction (HS–SPME) with GC–FID (*70*).

Zhao et al. (*71*) used HPLC–ELSD and pressurized liquid extraction to determine the fatty acids in *Ziziphus jujuba* Mill. var. spinose. The results showed that lauric acid, palmitoleic acid, and linoleic acid were the major compounds of fatty acids. The contents of these three fatty acids were 110.43 μg/mL, 126.15 μg/mL and 146.25 μg/mL, respectively. Different cultivars of *Ziziphus jujuba* Mill. in India had a lower content of C20 saturated fatty acid, from 0.13 mg/100g to 1.17 mg/100g, while the C20 unsaturated fatty acids were from 0.25 mg/100g to 2.00 mg/ 100g. Besides, C8, C10, C12 saturated fatty acids were the other major compounds of the fatty acids in the jujube (*72*). In four promising jujube fruits of Turkey, oleic acid and linoleic acid were the major PUFA, which accounted for 68.54% to 72.44% of total amounts of unsaturated fatty acids. Linoleic acid had the highest amount among all the fatty acids (*73*).

2.4 Minerals

Minerals are one group of food components. They are very important for the function of body and nutrient balance (*74*). Iron is important for oxygen transportation,

the formation of hemoglobin and myoglobin, and for the blood cells; calcium is the main component of human's bones, teeth, and often serve as the enzyme activator; phosphorus is the critical element of ATP. In this context, it is very important for people to have enough minerals from daily diet (*75, 76*). However, some minerals can also cause some adverse effects. High intake of sodium is associated with cardiovascular disease, the inbalanced ratio of sodium to potassium is related to blood pressure (*77*).

Inductively coupled plasma-optical emission spectrometry (ICP-OES) is an efficient instrument to determine the mineral content. It can simultaneously analyze the micro-minerals (e. g., manganese, zinc, iron, copper, magnesium, selenium, iodine, chromium) and macro-minerals (e. g., chloride, sodium, potassium, calcium, phosphorus) in foods (*78, 79*). Potassium is the most abundant mineral in some fruits such as apple, banana, bael, avocado, fig, cherry, apricot, etc. (*80*). Atomic absorption spectrophotometry (AAS) was also used to determine iron, phosphorus, zinc, copper, magnesium, manganese, and calcium in different cultivars of blackberry (*81*). It was found that potassium (129.80 mg/100g FW) was the most abundant mineral in all blackberry cultivars. However, lower content of potassium (51.24-90.92 mg/100g FW,), calcium (1.14-7.25 mg/100g FW), and phosphorus (0.7-12.21 mg/100g FW) in blackberry, strawberry, blueberry, raspberry, and sweet cherry that were grown in Brazil were reported (*82*). Date palm (*Phoenix dactylifera* L.) is a kind fruit which looks similar as jujube, Mohamed et al. (*83*) analyzed six cultivars of data palm in Sudan, and found that their calcium content was from 222.2 mg/100g to 293.04 mg/100g and the content of magnesium was from 66.3 to 120.88 mg/100g. Potassium was determined in the highest

amount in a range of 691.67 mg/100g to 1088.40 mg/100g. The minerals of three cultivars of mulberry were analyzed (*84*), the most abundant minerals was potassium with the range from 239 to 350 mg/100g FW, which is lower than that of the date, but higher than that in blackberry.

Minerals in jujube fruits commonly include potassium, phosphorus, calcium, iron, sodium, zinc, copper, selenium, etc. San et al. analyzed four genotypes of jujube in Turkey, and found that potassium was in the highest content with a range from 314.67 mg/100 g dry weight to 420.00 mg/100 g dry weight, and the content of calcium was from 79.33 mg/100 g dry weight to 121.33 mg/100 g dry weight (*85*). *Ziziphus mauritiana* in Zimbabwe was found to contain very high content of potassium, from 1865.0 mg to 2441 mg /100g dry weight (*86*). *Ziziphus spina-christi* in Khartoum contained more minerals in fruit pulp than in the seeds (*87*). In Spain, because of the effect of Mediterranean soils, the content of iron in jujube was relatively low (10.2-17.6 mg/kg dry weight), but the content of zinc (4.0-5.1 mg/kg dry weight) was higher than that of copper and manganese (*12*). In Mexico, *Ziziphus sonorensis* had high levels of copper (0.53 mg/100 g dry weight), iron (10 mg/100 g dry weight) and zinc (4.2 mg/100 g dry weight) in their edible portion, and the seeds had similar content of copper (0.54 mg/100 g dry weight) and zinc (4.5 mg/100 g dry weight) compared to *Ziziphus jujuba* Mill. (*88*). Besides, minerals of five Chinese jujubes cultivars (*Z. jujuba cv.* Jinsixiaozao, *Z. jujuba cv.* Yazao, *Z. jujuba cv.* Jianzao, *Z. jujuba cv.* Junzao, *Z. jujuba cv.* sanbianhong) were analyzed (*9*). The results showed that 100 g of fresh weight (FW) of jujube fruit contained the following minerals in different levels: potassium (79.2 to 458 mg),

phosphorus (59.5 to 110 mg), calcium (45.6 to 118 mg), manganese (24.6 to 51.2 mg), iron (4.68 to 7.9 mg), sodium (3.22 to 7.61 mg), zinc (0.35 to 0.63 mg), and copper (0.19 to 0.42 mg).

2.5 Organic Acids

Organic acids are very important for fruit quality in terms of taste acidity, color, texture, and flavor (*89*). In many fruits, such as melon, peach (*90*), pomegranate (*91*), strawberry (*89*), malic acids and citric acids are the most common and major acids. It is well known that the environmental factors can significantly affect the contents of organic acids in fruits (*92, 93*). They are normally bound with metals in tissues. Some low molecular organic acids, such as citrate, succinate, malate, and fumarate, are involved in krebs cycle to provide energy for human bodies (*94*). Besides, some organic acids such as tartaric acid, ascorbic acid, and malic acid can be used as antioxidant.

Organic acids could be monitored by HPLC-UV-Vis at 210 nm (*95*) or 225 nm (*92*), or detected by HPLC-MS (*90*), and capillary zone electrophoresis (*96*). Other two analytic techniques, i.e., NIR and MIR (mid infrared), were also reported to detect the malic acids and citric acid in passion fruits (*97*).

Organic acid and their salts can be used as antimicrobial agents in food products to preserve foods (*98*) so as to extend their shelf lives (*99, 100*). Oxalic acid was used to treat the vegetables rocket and baby spinach during postharvest storage for decreasing the yellowing of the leaves and preserving the quality (*101*). Banana (*102*) and litchi (*103*) were treated by oxalic acid, which could inhibit the browning reaction during postharvest storage. Besides, pork treated by fumaric acid had less foodborne pathogens (e.g.,

Escherichia coli O157: H7, *Staphylococcus aureus*, *Salmonella Typhimurium* and *Listeria monocytogenes*) than the samples without the treatment under the same storage conditions. In addition, the treatment could prolong the pork shelf life up to 6 days or 4-5 days when it was stored at 4 or 10 °C, respectively (*104*).

Jujube fruits are rich of organic acids. Gao et al. (*11*) analyzed organic acids in 10 jujube cultivars, including malic acid, citric acid and succinic acid. The authors found malic acid (294.0-740.3 mg/100g FW) was the major organic acid, while succinic acid could not be detected in two cultivars, i.e., Zaowangzao and Junchangyihao. In addition, the same group researchers analyzed the effects of different drying methods on the contents of organic acids. They found that the contents of malic acid and citric acid decreased during drying process, while the content of succinic acid in freeze drying samples was higher than those obtained by other methods (*105*). Degradation of ascorbic acid is often used as a marker to estimate the fruit shelf life. Its contents in Spain jujube fruits that were processed by different drying methods, including convective drying, freeze drying and vacuum microwave drying, were measured by HPLC with tunable absorbance detector at 250 nm wavelength. The results showed that, after drying under high temperature and a long time, the ascorbic acid content decreased by almost 70%, although its content were still more than 2000 mg/100g dry weight in all the treated samples (*106*). In the fresh Spain jujube cultivars, the content of ascorbic acid were from 387 mg to 555 mg/100g FW, nearly equivalent to 1935 to 2775 mg/100g dry weight based on 80% water content (*107*). According to a previous report, the peach (2183 mg/100g FW) and grape (1095 mg/100g FW) had higher malic acid than jujube fruits,

while melon, orange and lemon had high content of citric acid (1005 mg/100g FW, 2049 mg/100g FW and 5149 mg/100g FW respectively) (*90*).

3. Bioactivity Compounds

Bioactive compounds in plants normally belong to the secondary metabolites, such as phenolic compounds, terpenes and alkaloids. These compounds protect plants against environmental challenges, UV light, microorganisms (*108, 109*), and help them to survive (*110*).

Phenolic compounds include phenolic acids, flavonoids, lignin, tannins, etc. They can be separated into two groups: hydroxybenzoic acids and hydroxycinnamic acids, which are derived from benzoic acid and cinnamic acid, respectively (*111*). Their structures contain at least one aromatic ring with at least one hydroxyl group. Flavonoids contain flavanol, flavone, flavonol, flavanone, isoflavone and anthocyanidin, forming a large polyphenol group, which have a benzo-γ-pyrone structure. In human diet, soy isoflavones, flavonols and flavones are the major groups of flavonoids (*112*). These phenolic compounds possess some bioactivities, such as anticancer, antioxidants, chelating metal ions, reducing the risk of heart disease (*113-115*), etc..

Based on previous research, phenolic acids in *Ziziphus jujuba* Mill. often include *p*-coumaric acid, cinnamic acid, caffeic acid, chlorogenic acid, ferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, and vanillic acid (*11, 105, 116-118*). Total phenolic content (TPC) is often determined by the Folin-Ciocalteu method, based on the principle that the phenolic acid can reduce the reagent, and produce a blue color product which can be detected at the absorbance of 765 nm wavelength.

Ziziphus mauritiana Lamk. is grown in India, which is another specie of *Ziziphus*, but not as same as the *Ziziphus jujuba* Mill. that is grown in China. The total flavonoid contents (TFC) of 12 commercial cultivars of *Ziziphus mauritiana* Lamk. were measured in a range of 8.36 to 21.97 mg catechin equivalent/100 g dry weight (*119*). *Ziziphus jujuba* Mill. cv. Changhong were collected at 72, 80, 88 days after petal fall, and their TFCs decreased from 0.25 to 0.18 mg/g FW (*120*). Siriamornpun et al. measured the content of total flavonoids in the pulp and seeds of green and ripe jujube of different cultivars, and found the TFC values in the green tissues were higher than in the ripen tissues (*121*).

Choi et al. found procyanidin dimer B2, epicatechin, quercetin-3-robinobioside, quercetin-3-rutinoside, kaempferol-glucosyl-rhamnoside, quercetin-3-galactoside, in different stages of Korean Boen-daechu jujube (*56*). Zozio et al. also identified some flavonoids in the *Ziziphus mauritiana* Lamk., and obtained a similar result as Choi did. Some other compounds were also identified, including gallocatechin, catechin, myricitin dirhamnoside, myricitin rhamnoside, quercetin dirhamnoside, and other kaempherol derivatives (*122*).

Terpenes contain at least one five carbon isoprene structure unit, according to the number of unit, these chemicals can be separated into different groups, including hemiterpene, monoterpenes, sesquiterpenes, diterpenes, sesterpenes, triterpenes, and tetraterpenes (*109*). In plants, terpenes are synthesized in cytosols and plastids by different pathways. Terpenes have various functions such as hormones, electron carriers, etc. (*123*). They can react with nitrate, hydroxyl radicals (*124*), and had anti-

inflammatory function (*125*). Most terpenes are volatile compounds, which can be detected by gas chromatograph. For example, limonene and linalool were found in jujube fruit (*12*).

Jujube fruits are rich of bioactive compounds. Compared with other popular fruits such as pomegranate, sweetsop and guava, jujube fruits have higher antioxidant capacity (*11*). Zhao et al. measured the antioxidant capacity of ethanolic extracts of seven cultivars of Chinese jujubes (*126*) by three methods, including phosphomolybdenum assay, superoxide radical scavenging activity, and hydroxyl radical scavenging activity. All the extracts showed strong antioxidant activities, though there were significant differences among cultivars. Phenolic compounds existed in different forms, including free, esterified, glycosidic and insoluble bound. Wang et al. measured the antioxidant capacity of different forms of phenolic compounds in different tissues of jujube (peel, pulp and seed) (*118*). In all tissues of jujube, the glycosidic and insoluble-bound phenolic acids were determined to have very high antioxidant activities, while the free form of phenolic acids in all three tissues showed the lowest antioxidant activity.

4. Volatile Compounds

Volatile compounds provide the major contribution to the food flavor, which can be detected by gas chromatography, commonly connected with flame ionization detector (FID) or mass spectrometry (MS) detector. The volatile compounds include low molecular esters, organic acids, fatty acids, alcohol, aldehydes, lactones, terpenes and terpenoids, etc. They are often extracted by organic solvents like hexane, pentane, dichloromethane, or the mixture of these solvents. Besides, many methods can be used to

extract the volatile compounds, such as liquid-liquid extraction (LLE), simultaneous distillation and extraction (SDE), solid phase micro-extraction (SPME), stir bar sorptive extraction (SBSE), static and dynamic headspace techniques, solid phase dynamic extraction, dispersive liquid-liquid micro-extraction (DLLME), etc. (*127-129*).

4.1 Solvent Extraction

Liquid-liquid extraction (LLE) is commonly used to extract target compounds from a liquid sample, for example, to extract volatile compounds in wine. However, this method has some inevitable disadvantages. It needs a high volume of solvent, which will cause environmental pollution and money cost; it is usually time-consuming and not convenient; after the extraction, the solvent needs to be evaporated to concentrate the volatile compounds, possibly resulting in the loss of volatile compounds (*130*). In order to improve the efficiency of liquid-liquid extraction method and overcome the aforementioned defects, a new method was introduced by Rezaee et al. (*131*) in 2006, which is called dispersive liquid-liquid microextraction. Sample solution was injected into disperser solvent and extraction solvent with a syringe, and mixed well to form a cloudy solution. Through the centrifuge, the dispersed fine particles would be condensed in the bottom which was used for GC analysis. **Figure 1.1** shows the principal steps of dispersive liquid-liquid microextraction method (*132*).

Simultaneous distillation and extraction method (SDE) is another solvent extraction which is normally used to extract semi-volatile compounds from fruits (*133-135*), tea (*136-138*), meat (*139, 140*), etc.. An apparatus was designed by Likens and Nickerson in 1964 in order to analyze hop oil. **Figure 1.2** shows the apparatus. The

samples can be aqueous solution or solid mixed with water, which were placed in the flask which is connected to one arm, and the organic solvent was in a flask connected to another arm. Volatile compounds in the steam were extracted by the solvent steam, water and solvent with volatile compounds condensed in the separator and returned to their own flasks. If the organic solvent has lower density than water, the solvent should be on the left, otherwise on the right. If not, the solution would return to the wrong flask and the extraction could not be continuous (*141*). This method needs less time and less volume of solvent than the LLE. However, same as LLE, the SDE method still needs to concentrate the volatile compounds before the GC analysis. Thus, the loss of volatile is inevitable too.

4.2 Solvent Selection

The LLE method often uses the organic solvents with low boiling point, and not miscible with the liquid sample. Since most samples are water phase, so the non-polar solvents are normally chosen as the extraction solvents, including dichloromethane, hexane, pentane, chloroform, etc.. However, both pentane and hexane are strong non-polar and inflammable, which can affect the extraction efficiency of volatile compounds. Chloroform is toxic and has a high boiling point, not good to do concentration; Dichloromethane is the best choice for volatile compounds extraction through liquid-liquid extraction method as well as the SDE method, because it is not dissolved in water, and has a relatively lower boiling point, and a low toxicity.

Dispersive liquid liquid method needs an extraction solvent and a disperser. The extraction solvent must have higher density than water, and low solubility in water. For disperser solvent, the miscibility in both extraction solvent and water is essential. In this

case, chloroform, tetrachloroethane, dichloroethane, carbon tetrachloride, dichloromethane and tetrachloroethylene can be used as the extraction solvent. Acetone, acetonitrile, 2-propanol, methanol, and ethanol are normally used as the disperser solvent (*142*). According to optimization of these solvents, chloroform was widely used as the extraction solvent; and acetonitrile and methanol were disperser (*143, 144*).

4.3 Solvent Free Extraction

Solid phase micro-extraction (SPME) is now frequently used for volatile compound analysis in environment samples, plant samples, food samples and medicine samples. In 1990, Pawliszyn and Arthur invented the method with obvious advantages, such as high selectivity, free of solvent, inexpensive, short time consuming, less operation steps, and no concentration needed (*145*). This method can be used for volatile analytes in soil and wastewater (*146-148*), fruits (*149, 150*), flowers (*151*), and leaves (*152, 153*); food analysis (*154*) include wine (*155, 156*), meat (*157*) and clinic application (*158, 159*) such as tests of blood, organs, urine, etc. **Figure 1.3** shows the device of SPME. The fused silica fiber coated with adsorbent serves as the stationary phase, where the chemicals will be extracted (*160*). SPME method can be used as two types: head space SPME (HS-SPME) and direct immersion SPME (DI-SPME). **Figure 1.4** shows the extraction steps of SPME method. For the HS-SPME method, the extraction fiber will be exposed to the gas phase to adsorb the chemicals, the fibers will not contact the sample, no matter it is a solid sample or liquid sample; for the DI-SPME method, the fiber will be immersed into the liquid sample. HS-SPME is widely used to extract volatile compounds from food samples

SPME also has some inconveniences, such as fragile fibers, and competitive absorption among the chemicals (*161*). In order to make the fiber has longer shelf life, stainless-steel wire was used as an alternative material of fiber, and the corresponding coating was also developed. Normally the coatings were polyacrylate (PA), divinylbenzene (DVB), polydimethylsiloxane (PDMS), carboxen (CAR), templated resin (TPR) and carbowax (CW). These coating materials can be used in single, or combination to improve their extraction efficiency. SPME technique has developed rapidly in recent period, and some novel coating materials have also been introduced, such as ionic liquids, graphene, carbon nanomaterials including single-walled carbon nanotubes and multi-walled carbon nanotubes, polymeric ionic liquids, molecular imprinted polymers, metal-organic frameworks (*162-164*).

Another method called stir bar sorptive extraction (SBSE) was first introduced by Baltussen (*165*) et al. A stir bar coated with a large amount of PDMS can be used in aqueous phase and gas phase. **Figure 1.5** shows the extraction mode of SBSE which was cited from Prieto (*166*) et al. After the extraction, the stir bar needs to be gently rinsed by distilled water in order to remove some interfering compounds, especially non-volatile compounds. Before injected into chromatography instrument, desorption is needed, which normally adopts either the thermal desorption or the liquid desorption. The former that is connected with GC is achieved by a thermal desorption unit, of which the temperature is from 150 °C to 300 °C for a longer desorption time compared with the SPME method. This desorption method is primarily used for thermal stable volatile and

semi-volatile chemicals (*166*). Liquid desorption is performed by non-polar solvent to rinse the volatile compounds in the vial, which can be analyzed by GC or HPLC (*167*).

Stir bar sorptive extraction (SPSE) is also widely used in environmental analysis such as pesticides in water samples. In food analysis, SBSE is used to extract trace off-flavor compounds. In addition, SBSE also can be used in clinic analysis, extract chemicals from urine, serum, plasma samples (*168*). Some factors could affect the extraction efficiency, such as stir speed, the volume of sample, the amount of adsorbent, polarity of sample, pH as well as ionic strength (*169*). This extraction method is also a solvent free method, and has high recovery for trace chemicals. Besides, the stir bar can be reused after desorption. However, compared to the SPME method, SBSE method needs more extraction and desorption time, and the thermal desorption unit is expensive.

Since jujube has a special flavor, it is often used as a desirable flavoring agent in food industry. Wang et al. compared the effect of different extraction methods on jujube (*Ziziphus jujube*) Mill. aromas, including LLE, SDE, ultrasound-assisted solvent extraction (UAE) and head space solid-phase micro-extraction (HS-SPME). A total of 92 volatile compounds were identified, of which the SDE method yielded a higher percentage of esters. In comparison, HS-SPME was more efficient in extraction of low molecular weight volatile chemicals, while LLE and UAE were more efficient in extraction of a wider range of polarity of the aroma compounds (*170*). It was reported that volatile compounds in four cultivars (i.e., *Grande de Albatera, GAL, MSI, PSI* and *Datil, DAT*) of *Ziziphus jujuba* Mill. in Spain included aldehydes, terpenes, esters, ketones and hydrocarbons (*12*). Most volatile compounds in jujube brandy wine were

identified as esters and acids (*171*), while z-ocimene and 1,1-dimethyl-3-methylene-2-ethenyl-cyclohexane acetate were found in the leaves (*172*).

Generally speaking, the objectives of this dissertation are:

1) to characterize the non-volatile compounds including sugars, organic acids, amino acids, fatty acids, minerals, and antioxidants in 15 cultivars of jujube fruits (such as *Ziziphus jujuba* Mill. cv. Huping, *Ziziphus jujuba* Mill. cv Lizao, *Ziziphus jujuba* Mill. cv. Junzao, *Ziziphus jujuba* Mill. cv. Yuanling, etc.) collected in Shanxi province, China;
2) to characterize the volatile compounds, and find out the major volatile compounds which can represent the characteristic aromas of jujube fruits;
3) to classify different cultivars of the aforementioned jujube fruits, based on the measurements of the aforementioned chemical profiles.

Through these studies, it is expected to help us to know the jujube fruit better in light of its nutrients and non-nutrient chemicals, and so as to utilize the fruit more efficient as a nutrient food source.

Table 1. 1 Basic Composition of Different Species of Jujube, % of Dry Weight

	Ziziphus jujuba Mill. cv. (China)					*Ziziphus spina-christi* L. Willd (Sudan)
	Jinsixiaozao	Yazao	Jianzao	Junzao	Sanbianhong	
Carbohydrates	81.62±3.12	80.86±3.55	84.85±1.83	82.17±1.94	85.63±0.96	74.31±2.52
Lipids	0.37±0.01	1.02±0.05	0.39±0.02	0.71±0.07	0.65±0.03	2.55±0.02
Protein	5.01±0.05	6.86±0.02	4.75±0.03	6.43±0.02	6.60±0.04	4.34±0.12
Moisture	18.99±1.23	20.98±1.12	17.38±1.21	21.09±1.39	22.52±1.43	10.53±1.02
Ash	2.26±0.03	2.78±0.05	2.41±0.09	3.01±0.06	2.56±0.02	5.16±0.05

Data was represented as mean value ± standard deviation (data of *Ziziphus jujuba* Mill. was cited from Li *et al.*, 2007 (*9*); data of Z*iziphus spina-christi* L. was cited from Salih *et al.*, 2015 (*173*))

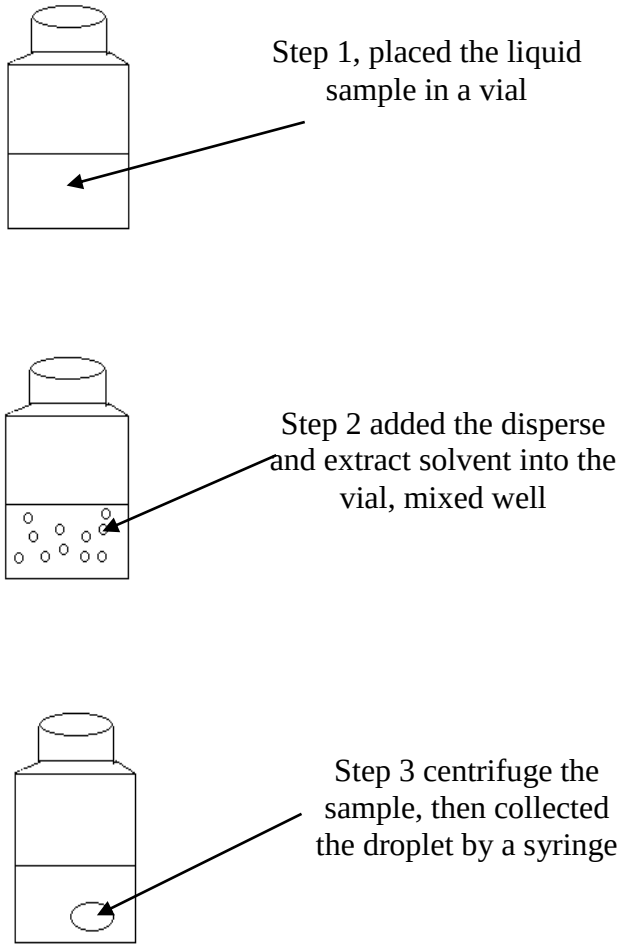

Figure 1. 1 The Principal Steps of Dispersive Liquid-Liquid Extraction

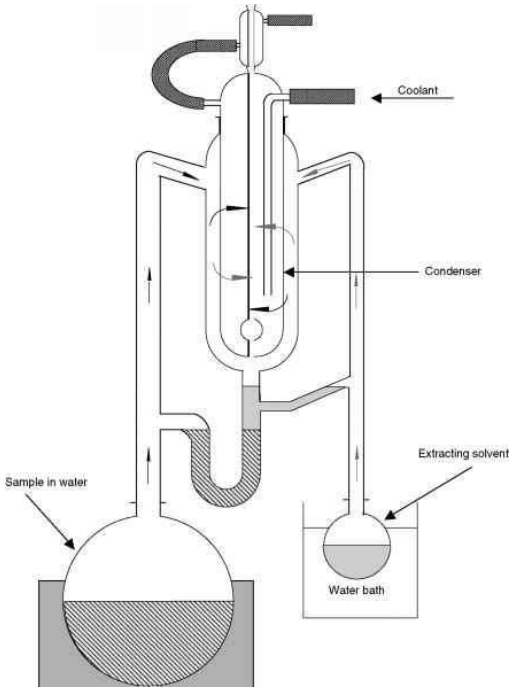

Figure 1. 2 SDE Apparatus
(Cited from the website: https://www.fitness-vip.com/muscle-foods/simultaneous-distillation-extraction.html)

Figure 1. 3 The Device for SPME

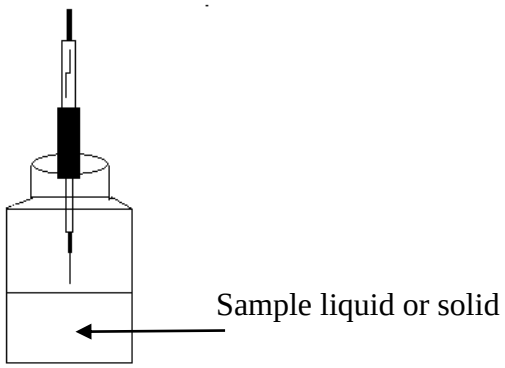

Figure 1. 4 Head Space Extraction by SPME Device

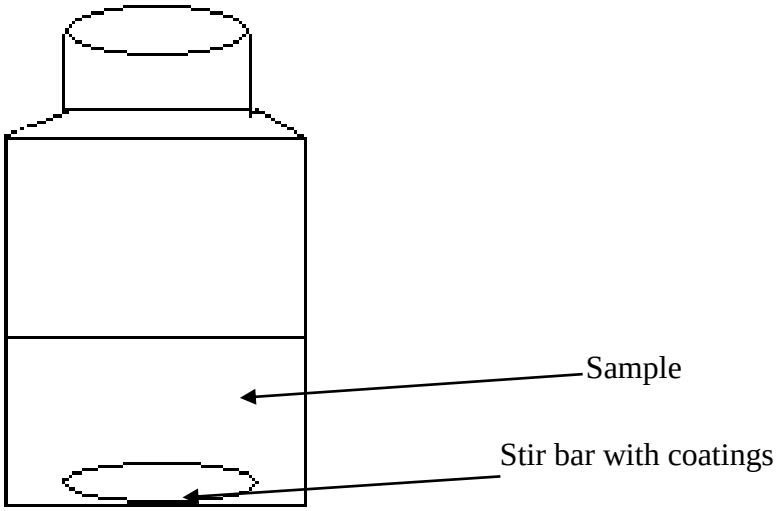

Figure 1. 5 SBSE Extraction

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CHAPTER TWO

ANALYSES OF REDUCING SUGARS, ORGANIC ACIDS, FREE AMINO ACIDS, FREE FATTY ACIDS AND MINERALS IN 15 CULTIVARS OF JUJUBE (Ziziphus jujuba Mill.) FRUITS

Abstract

Ziziphus jujuba Mill. has a long history of being used as an edible fruit and in Chinese medicine. In this study, the chemical profiles in terms of reducing sugars, organic acids, free amino acids, free fatty acids, and minerals were analyzed from 15 cultivars of the jujube fruit. Reducing sugars, organic acids, and free amino acids were measured by HPLC-UV; free fatty acids were analyzed by GC-MS; and minerals were detected by ICP-OES. The contents of the aforementioned components were significantly different among the cultivars (p<0.05). Glucose (85.87–1004.95 mg/100g FW), malic acids (120.15–508.67 mg/100g FW), citric acid (29.40–180.67 mg/100g FW), lauric acid (967.20–4035.78 μg/kg DW), palmitic acid (685.68–1936.91 μg/kg DW), myristoleic acid (1718.96–5862.64 μg/kg DW), oleic acid (427.87– 2864.98 μg/kg DW), linoleic acid (533.34–7330.05 μg/kg DW), iron (52.72–125.16 mg/kg DW), calcium (162.29–287.53 mg/kg DW) and magnesium (511.77–699.77 mg/kg DW) were the major compounds in the fruits. In addition, the fruits contained some health benefiting polyunsaturated fatty acids such as linoleic acid, linolenic acid, eicosapentaenoic acid, arachidonic acid, and docosahexaenoic acid. Principal component analysis (PCA) and hierarchical cluster (HA) were used to classify these cultivars of jujube based on the chemical profiles mentioned above. According to the PCA analysis, and the induced ellipses of constant distance calculation in 95% confidence interval, the classification of the jujube fruits based on the

content of reducing sugars is more reasonable and reliable than other parameters. In this classification, 15 cultivars were categorized into 6 groups, which were all significantly different from each other except the *Ziziphus jujube* Mill. *cv*. PB and BZ. All the results have given us more insights of the nutritional values of these jujube fruits to facilitate the potential product development of jujube fruits as both functional foods and nutraceutical products.

1. Introduction

Ziziphus jujuba Mill. is a common fruit, which has been cultivated in Asia for more than thousands of years. It is reported that at least 700 cultivars of the fruits have been found in China (*1*), which are distributed in different regions, including Henan, Shanxi, Shaanxi, Shandong, Xinjiang, Hebei, Gansu provinces, etc., in the Peoples' Republic of China. These jujube fruits have been found to have quite different chemical profiles because of the influence of various environmental conditions in terms of locations, climates, soils, precipitation, etc..

Primary metabolites such as carbohydrates, proteins, lipids, amino acids play important roles in growth and developments (*2*) of plants, animals and humans. Sugars and organic acids are well known to contribute to the flavor and taste of fruits. Sugars can be measured by different methods. For instance, Li (*3*) et al. used oxime-trimethylsilyl to derivative sugars of five cultivars and analyzed the sugar derivatives by GC-MS. Gao (*4*) et al. measured the sugars by the HPLC-RI detector. In these two studies, fructose, glucose and sucrose were found to be the main sugars in jujube fruits; while rhamnose could not be found in most cultivars. In addition, Guo et al. (*5*) found that the sugars in the jujube fruits increased as their maturity increased. However, the reports about other reducing sugars in jujube fruits are very limited. Besides, organic acids including malic acid, succinic acid, and citric acid were also analyzed. It was found that malic acid (294.0–740.3 mg/100 g fresh weight) had the highest concentration among these three organic acids (*4*).

Free amino acids are important for human health. The contents of free amino acids in different foods have been reported, such as tea (*6*), soybean (*7*), apple (*8*), strawberry (*9*), grapes (*10*), rice wine (*11*), cereals (*12*), etc.. Lin (*13*) et al. measured 20 free amino acids in Chinese jujubes by using the 4-chloro-3, 5-dinitrobenzotrifluoride derivatization technique. In Korea, amino acids in the pulp and seed of three varieties of jujube were analyzed. It was reported that proline and asparagine were the main amino acids in the jujube pulp (*14*). The same research group found that tryptophan and methionine could not be detected in any maturity stage of jujube (*15*). In comparison, it was reported that proline, aspartic and glutamic acids were the major amino acids in jujubes in Tunisia. Such kind of discrepancies in chemical profiles of jujubes were attributed to the environmental conditions. For example, it has been found that water deficit resulted in the decrease of asparagine in jujube (*16*).

Fatty acids are classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) based on the number of double bounds in their chemical structures. They are also a source of energy as same as carbohydrates and proteins. Unsaturated fatty acids are well known for their health benefits in light of their anticancer, anti-inflammatory, antibacterial properties, etc. (*17*). Jujube fruits contain a quite different varieties of fatty acids. It was reported that lauric acid, capric acid, palmitic acid, palmitoleic acid, oleic acid, and linoleic acid were the main fatty acids in six varieties of Spain jujubes (*18*). Zhao (*19*) et al. reported that lauric acid, palmitoleic acid, oleic acid and linoleic acid were the major compounds in the seed of *Ziziphus jujuba* Mill. *var. spinose* (a traditional Chinese medicine). In Turkey, the

content of fatty acids in four promising jujubes were determined. Oleic acid, linoleic acid, palmitic acid and palmitoleic acid were reported as the major compounds (*20*). In four ecotypes of Tunisian jujube, it was reported that the compound with the highest amount was oleic acid that accounted for 32.37%–50.68% of total fatty acids, and the other two major compounds were the linoleic acid and palmitic acid (*21*). Based on all these reports, the major unsaturated fatty acids were suggested to be the oleic acid and linoleic acid. However, information of identification of different species of jujube based on the content of different fatty acids is very limited.

Minerals often serve as important cofactors of enzymes and/or involve in biological reactions in human bodies and other biological organisms. For example, iron exists in hemoglobin that is used to transport oxygen; calcium is important for growth of bones; selenium is involved in antioxidant capacity, sodium and potassium are necessary for sugars absorption. Mineral deficit could cause some diseases such as anemia, but too much mineral absorption can also be harmful for humans. In this case, it is important to measure the content of minerals in human's diet including cereals, vegetables, meats, seafood, fruits, etc. It was reported that Russian mulberry and black mulberry cultivated in Xinjiang province in China had high contents of calcium (124 mg/100g FW and 113 mg/100g FW) and iron (11.4 mg/100g FW and 11.9 mg/100g FW) (*22*). In contrast, five cultivars of Chinese jujubes have been found to contain low concentration of copper (0.19–0.42mg/100g) (*3*). Interestingly, it was reported that the amount of minerals, such as potassium, calcium, magnesium and iron, in four genotypes of jujube in Turkey, were higher in the leaves than in the fruits (*23*).

In this study, nutritional compounds of 15 cultivars of jujube collected from northern area of China were analyzed. 1-Phenyl-3-methyl-5-pyrazolone (PMP) derivatization was applied to identify the composition of reducing sugars; organic acids, particularly those that are involved in the TCA cycle, as well as lactic acid and acetic acid, were analyzed by HPLC; 16 free amino acids and the composition of free fatty acids were analyzed by HPLC-UV and GC-MS, respectively; 12 minerals including heavy metals were analyzed by ICP-OES.

In general, the aim of this research was to obtain a relatively comprehensive chemical profiles, and identify the differences among the investigated different jujube cultivars, in an effort to set up the nutritional database to facilitate the potential development of jujube foods and relevant nutraceutical products

2. Materials and Methods

2.1 Jujube Sample Collection

Fifteen cultivars of jujube samples were collected from the same farm in Shanxi province, China in October, 2015. The fruits were carefully picked up without any visible scratch of the skins and broken part, and in the similar shape and size. Then, the samples were transported to the lab directly frozen at -80 °C. All the fruits were peeled to remove the seeds to just keep the pulp for analysis. The cultivars include *Ziziphus jujuba* Mill. *cv.* Banzao (BZ), *Ziziphus jujuba* Mill. *cv.* Dabailing (DB), *Ziziphus jujuba* Mill. *cv.* Cang county Jinsixiaozao (JS), *Ziziphus jujuba* Mill. *cv.* Huping (HP), *Ziziphus jujuba* Mill. *cv.* Lingbao (LB), *Ziziphus jujuba* Mill. *cv.* Yuanling (YL), *Ziziphus jujuba* Mill. *cv.* Jidan (JD), *Ziziphus jujuba* Mill. *cv.* Lizao (LZ), *Ziziphus jujuba* Mill. *cv.* Baode Youzao (YZ),

Ziziphus jujuba Mill. *cv.* Bin county Jinzao (BJ), *Ziziphus jujuba* Mill. *cv.* Junzao (JB), *Ziziphus jujuba* Mill. *cv.* Pingshun Junzao (PB), *Ziziphus jujuba* Mill. *cv.* Xiangzao (XZ), *Ziziphus jujuba* Mill. *cv.* Pozao (PZ), *Ziziphus jujuba* Mill. *cv.* Neihuangbianhesuan (NP), which are summarized and listed in **Table 2.1**.

2.2 Chemicals

Chemical standards including oxalic acid, malic acid, citric acid, fumaric acid, succinic acid, lactic acid, tartaric acid, acetic acid, rhamnose, mannose, glucose, galactose, xylose, and arabinose were bought from J. T. Baker (J.T. Baker Chemicals, PA, USA); amino acids chemicals including aspartic acid (Asp), threonine (Thr), alanine (Ala), arginine (Arg), cysteine (Cys), glutamic acid (Glu), glycine (Gly), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), serine (Ser), histidine (His), tyrosine (Tyr), valine (Val) were bought from Sigma-Aldrich company (Sigma, St. Louis, MO., USA); 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from the Sigma-Aldrich company; HPLC grade methanol and acetonitrile were bought from Fisher Scientific (Fisher Scientific, Pittsburgh, PA, USA).

2.3 Reducing Sugars Analysis

Half of one gram of a sample was placed into a 50 mL test tube, added with 10 mL of water and shaken well. Then the tube was kept in an 80°C water bath for 1 hour before the samples were centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and diluted to 25 mL. One mL of the diluted solution was taken and dried by purge of nitrogen gas. The dried sample was reacted with 0.5 mol/L of 1-phenyl-3-

methyl-5-pyrazolone (PMP) and 0.3 mol/L of NaOH in a 70°C water bath for 60 minutes. After the sample was cooled down to room temperature, 0.3 mL of 0.3 mol/L HCl was added. Then, 1 mL of chloroform was added to extract the excessive amount of PMP. The water phase was filtered by 0.22 μm membrane prior to the HPLC analysis.

Thermo U3000 (Thermo Scientific, USA) series HPLC-UV was used to detect the sugars, for which a Thermo Hypersil Gold C18 column (4.6×250 mm, 5 μm) was used to separate the different sugars under an isocratic condition. Mobile phase was a mixture of 0.1 mol/L phosphate solution: acetonitrile (82:18, V:V) at pH 7.0. Flow rate was 1 mL/min, the temperature of column was controlled as 25°C, injection volume was 15 μL, and the detected wavelength was 245 nm.

2.4 Organic Acids Analysis

An amount of 1 gram of jujube pulp sample was mixed with 30 mL of deionized water and extracted under ultrasonic for 30 minutes at room temperature. The solution was centrifuged at 5000 rpm for 10 minutes to collect the supernatant. The residue was re-extracted for three times using the same procedure and under the same condition. After four times of the extraction, the supernatants were combined together, and then evaporated at 65ºC. The supernatant was filtered through 0.45 μm nylon filters before the HPLC analysis.

Organic acids were analyzed by the same Thermo U3000 (Thermo Scientific, USA) series HPLC-UV with the same aforementioned reverse phase Hypersil Gold column (4.6×250 mm, 5μm) and detected at 210 nm. Mobile phase was 0.5% NH4H2PO4 (pH 2.6), the flow rate was 0.8 mL/min. The concentration (expressed in mg

per 100g of fresh weight) of different compounds was calculated based on the external standard method.

2.5 Free Amino Acids Analysis

2.5.1 Sample Preparation

An amount of 5 grams of the sample pulp of each cultivars of jujube fruits was placed in a 50 mL test tubes with 40 mL of 0.01 M HCl solution, which were mixed by vortex for 5 minutes. Then, the samples were extracted under ultrasonication for another 5 minutes. The solution was diluted to 50 mL and kept in the dark for 2 hours. Then, the samples were centrifuged at 5000 rpm for 10 minutes at 4°C before 1 mL of the supernatant was pipetted into another test tube for derivatization.

Derivatization process was based on the Gonzalez-Castro (*24*) and Zheng's methods (*25*) with some modifications. In order to remove proteins from the sample, 1 mL of 5% sulfosalicylic acid was added to the solution, mixed well and then kept in darkness for 1 hour. Then the mixture was centrifuged at 15000 rpm for 15 minutes at 4°C. The supernatant was diluted into 2 mL before 500 μL of the diluted supernatant was dried at 65 °C. The dried sample was dissolved in 250 μL of a mixture of methanol: water: trimethylamine (2:2:1, V:V:V) and dried at 65°C in vacuum oven. The dried sample was dissolved in 250 μL of methanol: water: trimethylamine: PITC (7:1:1:1, V:V:V:V) and kept at room temperature for 20 minutes. The excess reagent was evaporated at 65°C, and the dried sample was dissolved in 150 μL of the mobile phase A (see the details of the mobile phase in section 2.5.2), and filtered by 0.45 μm Nylon membrane before the HPLC analysis.

2.5.2 Free Amino Acids Analysis by HPLC- UV-Vis

Free amino acids were analyzed by the same aforementioned HPLC-UV system with the same HPLC column, for which a mixture of 0.1 mol/L sodium acetate: acetonitrile (93:7, V:V) was used as the mobile phase A, and a mixture of acetonitrile : water (80:20, V:V) was adopted as the mobile phase B. Flow rate was 1.0 mL/min, temperature of column was 40°C, injection volume was 10 μL, and detected wavelength was 254 nm. Mobile phase was set in a gradient program. At the beginning, the mobile phase B was 0%, after 14 minutes, mobile phase B increased to 15%, then increased to 34 % at 29 minute, from 30 minute, mobile phase B was 100% until 37 minutes, then changed to 0% from 37.1 minutes to 45 minutes.

2.6 Free Fatty Acids Analysis

2.6.1 Preparation of Fatty Acid Methyl Esters

Preparation and extraction of fatty acids methyl esters (FAME) was based on the Lepage and Roy's report (*26*). One gram of the dried jujube pulp was ground and extracted by 10 mL chloroform-methanol mixture (2:1, V:V) for 90 minutes with moderate shaking. After filtration, the liquid solution was removed into a separated funnel. Saturated sodium chloride was added into the solution in order to separate methanol and chloroform. The extraction process was repeated three times. The lower phase of all three extractions were collected, pooled together, and evaporated for analysis.

Derivatization was performed by an acidic catalysis method, by which 10% sulfuric acid-methanol solution was added into the sample, incubated in a 100 °C water bath for 60 minutes for the methylation reaction. The formed fatty acid methyl esters

were dried by mild purge of nitrogen gas. Then hexane was added to dilute the sample to 1 mL for GC analysis.

2.6.2 Analysis of Fatty Acid Methyl Esters by GC-MS

Thermo GC-MS was used to analyze the fatty acid methyl esters (FAMEs). A Thermo TR-5 capillary column (30 m ×0.25 mm×0.25 μm) was used as the GC column, and the ultra-high purity (UHP) helium was the carrier gas. The flow rate was 1.2 mL/min. Injection volume was 1 μL. The inlet temperature was 290 °C. The oven temperature began at 50 °C, hold for 3 minutes, and then increased to 200 °C at the rate of 10 °C/min and hold for another 2 minutes, then to 225 °C at the rate of 1.0 °C, the temperature was up to 250 °C, hold for final 5 minutes. In regards of the mass spectrometer, its ion source temperature was 280 °C, transfer line temperature was 280°C, full scan range was 40 *m/z* to 500 *m/z*.

2.7 Minerals Analysis

The pulps of jujube fruits were dried by the conventional oven at 65 °C. Then, 1 g of the dried sample was placed in the digestion tube, mixed with 10 mL HNO_3 and 2 mL $HClO_4$, which was kept at room temperature overnight. Then, the tube was moved into the digestion oven at 280 °C until the solution became clear. After that, distill water was added to make the final volume at 50 mL. The mineral contents in the jujube samples were measured by an ICP-OES (Perking Elmer, USA). The operation power of the instrument was 1.20 kW; plasma flow rate was 15.0 L/min; auxiliary flow rate was 1.50 L/min; nebulizer pressure was 200 kPa.

2.8 Statistics

All the data were calculated in triplicates; the evaluation of significant different level was performed by one-way analysis of variance (ANOVA) by JMP software. Tukey test was used to evaluate the significant level ($p<0.05$). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted by the Metlab software.

3. Results and Discussion

3.1 Reducing Sugars Analysis

Sugars are produced by photosynthesis in plant leaves, where carbon dioxide in the air react with water under light to form sugars. These sugars can support the plant growth and development of flowers, fruit, new leaves and shoot. Previous researchers have demonstrated that the environmental condition was a major factor to affect the sugar synthesis.

In many previous studies, sucrose, fructose and glucose were reported as the major sugars (*3-5, 27*) in jujubes, but the information about other reducing sugars is limited. It was first reported by Honda (*28*) that reducing sugars could react with the PMP reagent to form sugar-PMP derivatives, which have strong UV absorbance. Two molecules of PMP were able to react with one molecule of glucose. In this study, the PMP derivative method was used to analyze the reducing sugars in jujube. The concentration of reducing sugars is listed in **Table 2.1**. Among the cultivars, the concentrations of the reducing sugars were significantly different. Mannose and xylose were detected only in some cultivars. In contrast, the content of rhamnose was from 1.42 to 5.48 mg/100g FW. Even though rhamnose was detected in all the cultivars, its content

was lower than that in a previous report (*4*). In addition, galactose was not detected in the cultivar YL. The content of glucose was in a range from 85.87 to 1004.95 mg/100g FW, for which the cultivar YZ contained the lowest content of glucose while the cultivar PZ contained the highest amount. Arabinose was also measured in jujube, of which its content varied from 2.30 to 16.74 mg/100g FW.

3.2 Organic Acids Analysis

Organic acids contribute to the acidity of fruits and are important to the fruit texture and flavor. Malic acid, citric acid, and succinic acid have been found in jujube (*4*, *27*). In this study, oxalic acid, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, fumaric acid and succinic acid were detected and are shown in **Table 2.2.** The contents of organic acids had significant difference in different cultivars. Particularly, lactic acid and succinic acid were not detected in some cultivars. The predominant organic acid is malic acid, its amount (120.15 to 508.67 mg/100g FW) is the highest among the organic acids in all cultivars. *Ziziphus jujuba* Mill. *cv.* JB contained 278.23 mg/100g FW, which was similar as JB (294.0 mg/100g FW) which was grown in Yulin county in China (*4*); Strawberry was reported to contain around 200 mg/100g FW of malic acid (*29*), which was similar to most cultivars of jujube fruits; grapes (1095 mg/100g FW) and peaches (2183 mg/100g FW) were reported to contain higher malic acid than jujube, while lemons (228 mg/100g FW) and oranges (131 mg/100g FW) have similar contents (*30*) of malic acid as jujube. *Ziziphus jujuba* Mill. *cv.* JS contained the lowest amount of oxalic acid (12.06 mg/100g FW), while the cultivar LB contained the highest amount (62.66 mg/100g FW). The content of tartaric acid varied from 14.67 mg/100g FW to 73.71

mg/100g FW. However, lactic acid in the DB, LB, PZ, HP cultivars were not detected. In contrast, its amount in the other cultivars had significant changes from 8.41 mg/100g FW to 31.47 mg/100g FW. Acetic acid in the LB and JD cultivars were less than 3.00 mg/100g FW, but the value was above 10 mg/100g FW in other cultivars. Particularly, its highest content (111.23 mg/100g FW) was found in the cultivar XZ. The content of citric acid (29.40 to 180.67 mg/kg FW) was higher than other acids in most cultivars. This result is similar to that reported by Gao (*4*) et al. Fumaric acid was detected in all cultivars. Its lowest content was only 0.55 mg/100g FW in JB, but the highest content was 162.58 mg/100g FW in PB. Succinic acid was not detected in some cultivars (i.e., BZ, DB, LB, HP, BJ, JS). In cultivar YL, the amount of succinic acid was only 2.27 mg/100g FW, while it was 163.21 mg/100g FW in the cultivar NP.

3.3 Free Amino Acids Analysis

In this study, 16 amino acids were detected, and their data are shown in **Table 2.3**. Only *Ziziphus jujuba* Mill. *cv.* HP contained all the 16 amino acids. It was reported that the branch chain amino acids, including valine, leucine and isoleucine could improve the insulin resistance, (*31, 32*). It was found that there were significant differences ($p<0.05$) in amounts of branch chain amino acids in the studied 15 cultivars of jujubes. For example, the content of valine varied from 0.30 mg/100g FW to 3.05 mg/100g FW, but in the *Ziziphus jujuba* Mill. *cv.* JD, NP and XZ, it was not detected. Isoleucine was not detected in the LZ, NP, PB, XZ and YL cultivars, but it was found in other cultivars from 0.40 mg/100g FW to 4.75 mg/100g FW. However, leucine was only detected in the HP and JB cultivars. In addition, other essential amino acids, such as histidine, lysine,

methionine, phenylalanine and threonine, were also analyzed in this study. Lysine and histidine were found in all the cultivars except two cultivars, including LZ and XZ. On the contrary, most cultivars did not contain methionine, phenylalanine and threonine. Besides, the amount of these amino acids in the cultivars were significantly different (p<0.05). Arginine is important for children because it is a critical factor to affect growth hormone in children (*33*). The highest content of arginine (31.90 mg/100g FW) was detected in the *Ziziphus jujube* Mill. *cv.* JD, while the lowest was in the *Ziziphus jujuba* Mill. *cv.* JS (0.32 mg/100g FW). Aspartic acid (0.51 mg/100g FW to 15.03 mg/100g FW) and serine (12.78 mg/100g FW to 71.04 mg/100g FW) were detected in all the cultivars. In a previous study, 20 amino acids were detected from four cultivars of dry jujube powder under different stored periods. It was reported that no tryptophan was detected and the content of amino acids decreased as the storage time increased (*13*). In Korea, jujube fruit in eight growth stages were collected for amino acids analysis, but neither tryptophan nor methionine was detected in any stages by the authors (*15*).

3.4 Free Fatty Acids Analysis

Fatty acids (FAs) can provide energy for human beings via β-oxidation. Linoleic acid and linolenic acid are the essential fatty acids which play an important role in fatty acids synthesis. **Table 2.4** and **Table 2.5** list the data of fatty acids in 15 cultivars of jujube fruits. It is obvious that lauric acid (C12:0) and palmitic acid (C16:0) were the major saturated fatty acids, while myristoleic acid (C14:1), oleic acid (C18:1) and linoleic acid (C18:2) were the major unsaturated fatty acids (UFAs) in these jujube fruits. These findings were as same as the results from San (*20*) et al. and Guil-Guerrero (*18*) et al..

The content of lauric acid was very low in the seeds of *Ziziphus jujuba* Mill. *var. spinose* from previous study, while linoleic acid, oleic acid and docosanoic acid were relatively higher (*19*).

Table 2.6 shows the percentage of individual unsaturated fatty acids in total unsaturated fatty acids in these 15 cultivars. Myristoleic acid (C14:1) was the main unsaturated fatty acid, from 31.81% to 46.62% of the total amount of unsaturated fatty acids. *Ziziphus jujuba* Mill. *cv*. DB, JB, LB, and PZ contained more oleic acid (C18:1) than other cultivars, which were in a range from 10.78% to 24.60%. In other cultivars, although oleic acid was lower than 10%, they were still higher than many other fatty acids, such as linolenic acid, arachidonic acid, and docosahexaenoic acid, in the same cultivar. Besides, these 15 cultivars of Jujube fruits contained a variety of polyunsaturated fatty acids (PUFAs), including linoleic acid (C18:2), linolenic acid (C18:3), eicosapentaenoic acid (C20:5), arachidonic acid (C20:4), docosahexaenoic acid (C22:6) and others. For instance, *Ziziphus jujuba* Mill. *cv*. BJ, BZ, HP, JD, JS, LZ, NP, PB, XZ, YL, YZ contained linoleic acid (C18:2) from 10.13 % to 51.44% of the total amount of unsaturated fatty acids, while *Ziziphus jujuba* Mill. *cv*. DB, JB, LB, and PZ had less than 10%. Although PUFAs in jujube fruits had, in most cases, less than 5% of the total amount of UFAs, these PUFAs are commonly considered to be health benefiting, e.g., for the brain development.

3.5 Minerals Analysis

Contents of minerals of 16 jujube cultivars are shown in **Table 2.7**. The data indicated that calcium, magnesium, and aluminum were the major minerals in these

jujube fruits. Lead was only detected in *Ziziphus jujuba* Mill. *cv.* BZ, DB, HP, LB, LZ, NP, and PB, of which the content was in a range from 0.23 to 1.30 mg/kg DW. The contents of nickel (2.19 to 2.95 mg/kg DW), aluminum (137.59 to 167.48mg/kg DW), boron (15.93 to 35.87 mg/kg DW), titanium (1.90 to 3.09 mg/kg DW), and chromium (3.66 to 9.91 mg/kg DW) were not significantly different among most cultivars ($p<0.05$). The detected content of boron in our cultivars was less than that in the jujubes which were planted in Turkey, ranging from 4.63 to 6.53 mg/100g (*23*).

Iron is an essential mineral for humans. The iron content in these jujube cultivars was from 52.72 to 125.16 mg/kg DW. Most cultivars contained similar amount of iron as other five cultivars of jujube fruits grown in Jinan in China (*3*). However, *Ziziphus jujuba* Mill. *cv.* BZ, PZ, YL contained more iron (85.64 to 125.16 mg/kg DW) than those five cultivars of jujube (4.68 to 7.90 mg/100g DW). The content of iron in cultivars JS (64.02 mg/kg) and JB (83.82 mg/kg) in this study was higher than that in the same cultivars (4.68 and 7.90 mg/100g DW) reported in a previous study (*3*). Nevertheless, the content of iron in all the cultivars in this study was higher than the amount of iron (0.67 to 1.43 mg/100g DW) in Turkey cultivars (*23*). Yet, the average content of iron (131.9 μg/g) of jujube fruit which was grown in Xinjiang province (*34*) was higher than jujube in Shanxi province in this study. This difference is generally attributed to the different climate and soil conditions.

Copper is a co-factor of many antioxidants. In this study, the content of copper was determined in a range of 3.81 to 8.12 mg/kg DW. This value was higher than the

amount in the Li's report (0.19 to 0.42 mg/100g), but similar as that in the jujubes planted in Bayikuleng region (4.62 to 8.28 μg/g) in Xinjiang province (*34*).

Zinc is important for many enzymatic reactions. *Ziziphus jujuba* Mill. *cv.* LB contained the lowest content of zinc (11.15 mg/ kg DW), while *Ziziphus jujuba* Mill. *cv.* JD had the highest value (17.58 mg/kg). The content of zinc in the cultivars in this study was higher than that in the Li's report (0.35 mg/100g to 0.63 mg/100g) (*3*) and San's report (0.53mg/100g to 1.27 mg/100g) (*23*). However, our result was in agreement with the value of zinc in jujubes which were grown in the Xinjiang province (*34*)

Calcium is well known to be essential for growth of human bones and teeth. **Table 2.7** shows that the lowest content of calcium was 162.29 mg/kg DW found in the *Ziziphus jujuba* Mill. *cv.* YL, while the highest content was 301.81 mg/kg DW in the *Ziziphus jujuba* Mill. *cv.* DB. However, its content in all the cultivars was found to be lower than that in both Li' report (45.6 to 118 mg/100g) (*3*) and San's report (79.33 to 121.33 mg/100g) (*23*).

Based on the data shown in **Table 2.7**, magnesium was obviously a primary mineral because its content was significantly higher than that of all the other minerals. *Ziziphus jujuba* Mill. *cv.* HP had the lowest content (511.77 mg/kg DW), and *Ziziphus jujuba* Mill. *cv.* DB contained the highest content (699.77 mg/kg DW). The value was also higher than that of jujube (15.77 to 20.77 mg/100g) found in Turkey (*23*).

The content of manganese of jujube in this study varied from 4.79 to 10.68 mg/kg DW. All of them were higher than the jujube fruits in Turkey (0.10 mg/100g to 0.2 mg/100g), and similar with the jujube in the Xinjiang province in China (*34*).

Overall, the contents of minerals in different cultivars were significantly different, but regarding the same mineral, most of the cultivars did not have significant difference. The possible reason of this is that all of the cultivars were grown and collected from the same farm so the environmental factors including climate and soil did not affect the mineral absorbance a lot. In this context, these differences are considered to be caused, in a large degree, by the genotype of jujube, which needs more investigations.

3.6 PCA and HCA analysis

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to classify the cultivars into different subgroups, and explore the significant difference among the groups. All the ellipses of the constant distance were calculated with 95% confidence interval. The classifications of HCA were based on the distance equals to 39. **Figure 2.1** was the dendrogram of all the cultivars based on the contents of reducing sugars. According to the mean values of the components, the studied jujube cultivars were categorized into six groups. *Ziziphus jujuba* Mill. *cv*. BZ, PZ, DB, NP were in the same group; *Ziziphus jujuba* Mill. *cv*. HP, JD, JS, BJ, PB were in the same group; *Ziziphus jujuba* Mill. *cv.* YZ, LB were clustered together and *Ziziphus jujuba* Mill. *cv.* XZ and JB were clustered together into another group; the rest two cultivars YL and LZ were separated in two different groups. **Figure 2.2** was the score plot of principal component analysis for reducing sugars. After reducing the variable dimensions, first two PCs (i.e., PC 1 and PC 2) can explain 85.6% of total data variability. As shown in the **Figure 2.2**, there was no overlap among the ellipses, which means the clusters have

significant difference among them, except the Group E and F that had crossed parts in their sections.

Different contents of organic acids in different cultivars result in another type of categories based on the HCA **(Figure 2.3)**. As a result, *Ziziphus jujuba* Mill. *cv*. LB, HP, PZ, DB were clustered in the first group. The second group contains *Ziziphus jujuba* Mill. *cv*. PB and JS; *Ziziphus jujuba* Mill. *cv*. BJ, JB, YZ are in the third group; *Ziziphus jujuba* Mill. *cv.* NP and JD are connected in the same group which is named as the forth group; *Ziziphus jujuba* Mill. *cv.* BZ, and XZ, as well as LZ, are in the fifth group; and *Ziziphus jujuba* Mill. *cv.* JB was categorized in a single group by itself. In addition, the PCA of the organic acids in the 15 cultivars of jujube fruits is plotted in **Figure 2.4**, where its PC1 and PC2 can only explain 55.6% of all data variance. Group A including the *Ziziphus jujuba* Mill. *cv.* DB, PZ, LB, HP is significantly different from the other groups; Group D which contains the YL, BZ, and JD cultivars is significantly different the Group B which is consisted of the XZ and LZ cultivars. However, both of them are overlapped with the Group C (*Ziziphus jujuba* Mill. *cv.* PB, JS, BJ). Group E contains two cultivars (i.e., NP and YZ), it has no overlapping with other groups except the Group D. Moreover, the Group F has no crossed section with any other groups.

Figure 2.5 shows the classification of all the cultivars based on their amounts of free amino acids. The dendrogram of the HCA suggests only the *Ziziphus jujuba* Mill. *cv.* LZ only in the first group; *Ziziphus jujuba* Mill. *cv.* PZ, XZ, LB, DB and NP are classified in the second group; the third group includes the *Ziziphus jujuba* Mill. *cv.* YL and BZ; *Ziziphus jujuba* Mill. *cv.* JB and HP are categorized in the fourth group; *Ziziphus*

jujuba Mill. *cv.* PB is placed in the group five; *Ziziphus jujuba* Mill. *cv.* YZ, JD, BJ, JS are connected in the sixth group. According to the corresponding PCA that is plotted in **Figure 2.6**, PC1 and PC2 can only explain 50.5% of data variance. As shown in **Figure 2.6**, the Group E (*Ziziphus jujuba* Mill. *cv.* NP) is not connected with all the other groups; Group F (*Ziziphus jujuba* Mill. *cv.* JS) and Group D (*Ziziphus jujuba* Mill. *cv.* BZ and YL) are almost embraced in the Group A, so these two groups have no significant differences with the Group A. In addition, both Group B and Group C also have a partial crossed sections with the Group A, although the Group B and Group C are separated and have significant difference.

Similarly, fifteen cultivars of jujube were classified into six groups according to the mean values of their concentrations of different fatty acids. This result is shown in **Figure 2.7**. After the PCA (**Figure 2.8**), group D (*Ziziphus jujuba* Mill. *cv.* PB) is only overlapped with the group A (*Ziziphus jujuba* Mill. *cv.* PZ, JB and JS), the latter is also, in a very small degree, overlapped with group E (*Ziziphus jujuba* Mill. *cv.* JD, BJ, LZ). However, the group C, E and F have severe overlapping regions among them, which means these three groups do not have significant difference. Group B is overlapped with group E and group C, but not the others.

According to the mean values of minerals, all the cultivars were classified into 6 different groups. As shown in **Figure 2.9**, the first group includes the *Ziziphus jujuba* Mill. *cv.* YL, NP, and LZ; the second group contains the *Ziziphus jujuba* Mill. *cv.* BJ, JS, and PB; the third group contains the *Ziziphus jujuba* Mill. *cv.* PZ and JB; the *Ziziphus jujuba* Mill. *cv.* BZ is in the fourth group by itself; the *Ziziphus jujuba* Mill. *cv.* XZ, HP,

and LB are classified in the fifth group; the last group contains the *Ziziphus jujuba* Mill. *cv.* DB, JD and YZ.

PC1 and PC 2 in the **Figure 2.10** can explain 47.2% of mineral variances. Based on this PCA, the Group F (*Ziziphus jujuba* Mill. *cv.* JD, YZ) has obvious overlapping regions with the Group A (*Ziziphus jujuba* Mill. *cv.* NP, LZ, YL), Group B (*Ziziphus jujuba* Mill. *cv.* BJ, PB, JS, BJ) and Group D (*Ziziphus jujuba* Mill. *cv.* BZ, JB, PZ), which means the Group F does not have a significant difference with these three groups. Group D also has crossed sections with the Group E (*Ziziphus jujuba* Mill. *cv.* DB), Group C (*Ziziphus jujuba* Mill. *cv.* HP, XZ, LB) and Group F. However, the Group E, Group F and Group C were completely separated, indicating they have a significant difference. Similarly, Group B and Group D are considered to be significantly different.

Considering the results of PCA and HCA of the reducing sugars, organic acids, free amino acids, fatty acids and minerals, it is clear that different jujube cultivars can be categorized into different clusters. However, only PCA analysis of reducing sugars might be more reliable since it could explain more than 80% of data variance, while the others could not. If all of these components are statistically analyzed together, the classification is changed again, which are shown in **Figure 2.11** and **Figure 2.12**. The latter shows that the Group C (including the PB and NP cultivars), Group D (including the YZ, BJ, JS, and JD), and Group E (PZ, DB and LZ) are overlapped together, so these three groups do not have a significant difference. Group A (HP and LB) only has a very small overlapping with the Group B (BZ, JB, and XZ), but has no overlapping with the other groups. Group

F only crossed with Group D. Unfortunately, PC 1 and PC 2 in **Figure 2.12** can only explain 31.3% of data variance based on all the chemical profiles in this chapter.

In summary, except the reducing sugars, the PC1 and PC2 values in the aforementioned PCA analyses based on the other compounds could not represent more than 80% of total data variance, which has significantly affected the quality and reliability of the classifications. In this case, the classified subgroups from the PCA and HCA based on the concentrations of organic acids, free amino acids, fatty acids and minerals might not be accurate and convincing enough because the information of variables was not sufficient. Nevertheless, the classification according to the reducing sugars was more reliable in comparison with other components.

4. Conclusion

In this study, reducing sugars, organic acids, free amino acids, fatty acids and minerals of 15 cultivars of jujube fruits were analyzed. Among the cultivars, the contents of reducing sugars, organic acids, and free amino acids were significantly different ($p<0.05$). The components of fatty acids and minerals did not show the significant differences among some cultivars. Based on the data, only *Ziziphus jujube* Mill. *cv.* HP contained all the free amino acids. According to the PCA, only principal components of reducing sugars can explain more than 80% of data variance if two-dimensional plot is used, while the other components such as organic acids, minerals, free amino acids and free fatty acids could not, which means the classification of the jujube fruits based on the PCA of reducing sugars might be more reliable compared to the other food components. Nevertheless, the obtained comprehensive data of the components in the jujube fruits

have provided us some insights of the nutritional values of the jujube. In addition, it is a useful attempt to classify different jujube cultivars by PCA and HCA based on the measured data of jujube components, although it seems that only the reducing sugars might be the more reliable variable for classification in an effort to improve the processing quality, and avoid adulteration. Finally, these systematical analyses of jujube fruits can help us for better utilization of the jujube as a functional food.

Table 2. 1 Contents of Reducing Sugars in 15 Cultivars of Jujube Fruits, mg/100g FW

Name of *Ziziphus jujuba* Mill. cultivars	Abbreviation	Rhamnose	Mannose	Glucose	Galactose	Xylose	Arabinose
Bin county Jinzao	BJ	1.68±0.13 efg	n.d.	232.96±6.67 fg	1.32±0.05 efg	n.d.	3.28±0.07 g
Banzao	BZ	2.06±0.11 def	n.d.	303.64±7.55 e	2.59±0.08 d	n.d.	5.96±0.16 d
Dabailing	DB	4.88±0.17 b	n.d.	752.93±17.32 b	4.08±0.15 b	n.d.	6.94±0.15 c
Hupingzao	HP	2.20±0.08 de	n.d.	268.16±4.36 ef	0.99±0.07 gh	n.d.	3.16±0.06 g
Junzao	JB	2.08±0.04 def	0.60±0.05 c	286.35±6.85 e	1.14±0.06 fgh	26.66±1.29 c	3.29±0.08 g
Beijingjidanzao	JD	1.97±0.13 defg	n.d.	200.56±7.04 gh	1.61±0.03 e	n.d.	2.86±0.04 gh
Cang county Jinsixiaozao	JS	1.63±0.11 efg	n.d.	172.66±6.00 h	1.29±0.03 efg	n.d.	4.19±0.10 f
Lingbaozao	LB	1.58±0.10 fg	0.42±0.09 c	195.77±5.11 gh	1.02±0.04 gh	36.27±1.66 a	2.84±0.05 gh
Lizao	LZ	5.48±0.08 a	1.32±0.11 a	739.94±10.11 b	3.50±0.10 c	n.d.	16.76±0.29 a
Neihuangbianhesuan	NP	4.29±0.14 c	1.06±0.05 b	641.64±12.63 c	2.76±0.06 d	n.d.	8.34±0.23 b
Pingshun Junzao	PB	2.35±0.07 d	n.d.	359.79±5.03 d	1.54±0.05 ef	n.d.	4.96±0.12 e
Pozao	PZ	4.29±0.15 c	n.d.	1004.95±20.04 a	5.05±0.16 a	n.d.	6.10±0.15 d
Xiangzao	XZ	2.06±0.10 def	0.47±0.06 c	205.43±4.61 gh	1.19±0.07 fg	32.51±1.20 b	3.21±0.08 g
Yuanlingzao	YL	1.95±0.11 defg	n.d.	187.16±1.79 gh	n.d.	n.d.	2.98±0.08 gh
Baode Youzao	YZ	1.42±0.09 g	n.d.	85.87±3.22 i	0.78±0.02 h	28.29±1.01 c	2.30±0.06 h

Data are presented in mean value ± standard error, (n=3)

Different letters followed the data in the same column means significant difference (p<0.05)

n.d. means not detected

Table 2. 2 Contents of Organic acids in 15 Cultivars of Jujube Fruits, mg/100g FW

	oxalic acid	tartaric acid	malic acid	lactic acid	acetic acid	citric acid	fumaric acid	succinic acid
BJ	19.26±0.68 h	25.59±0.64 h	222.05±1.07 ef	21.35±0.59 d	11.02±0.15 i	121.37±1.73 d	121.41±1.70 c	n.d.
BZ	21.04±0.15 h	42.12±0.90 e	258.18±2.67 d	15.01±0.39 e	36.79±1.10 e	67.78±0.51 g	1.48±0.03 h	n.d.
DB	50.26±0.60 c	36.26±0.44 f	177.45±2.63 g	n.d.	54.72±0.79 c	100.54±1.46 e	1.40±0.02 h	n.d.
HP	50.30±1.80 c	36.76±1.60 f	253.97±3.27 d	n.d.	15.16±0.27 hi	180.67±3.10 a	1.01±0.02 h	n.d.
JB	30.41±0.72 ef	41.55±0.51 e	278.72±2.59 c	25.00±0.41 b	180.22±1.90 a	159.91±1.75 b	0.55±0.01 h	80.71±1.04 c
JD	30.26±0.41 ef	38.80±0.36 ef	230.85±2.04 e	11.80±0.14 f	2.93±0.03 j	51.47±0.75 h	52.26±0.71 f	35.78±0.38 f
JS	12.06±0.81 i	41.29±1.08 e	232.68±2.85 e	14.72±0.37 e	26.00±0.84 g	88.22±1.13 f	88.24±1.13 d	n.d.
LB	62.66±1.21 a	56.08±0.66 c	120.15±1.90 i	n.d.	2.74±0.01 j	69.10±1.07 g	2.30±0.05 h	n.d.
LZ	26.91±0.55 fg	17.36±0.53 i	188.32±2.11 g	15.46±0.41 e	40.30±0.63 e	42.88±1.11 i	0.85±0.01 h	95.30±1.04 b
NP	36.63±1.03 d	60.73±1.12 b	508.67±4.31 a	8.41±0.16 g	31.46±0.45 f	129.01±1.42 cd	129.75±1.23 b	163.21±1.83 a
PB	25.35±0.31 g	73.71±1.38 a	218.95±1.52 ef	23.89±0.34 bc	29.20±0.35 fg	161.14±2.06 b	162.58±0.99 a	57.72±0.30 d
PZ	543.66±8.23 b	14.67±0.54 i	161.60±2.27 h	n.d.	16.76±0.24 h	130.59±1.98 c	1.277±0.02 h	7.06±0.15 g
XZ	28.95±0.51 fg	31.83±0.85 g	156.62±1.84 h	22.48±0.48 cd	111.23±1.46 b	29.40±0.58 j	1.30±0.04 h	43.69±0.52 e
YL	32.88±0.89 e	46.78±0.96 d	206.56±2.55 f	31.47±0.64 a	16.13±0.45 h	34.23±0.41 j	34.23±0.41 g	2.27±0.03 h
YZ	32.57±0.46 e	48.53±0.89 d	463.58±5.33 b	8.72±0.16 g	49.26±1.01 d	63.41±1.28 g	64.18±1.26 e	57.92±0.85 d

Data are presented in mean value ± standard error, (n=3)

Different letters followed the data in the same column means significant difference (p<0.05)

n.d. means not detected

Table 2. 3 Contents of Free Amino Acids in 15 Cultivars of Jujube Fruits, mg/100g FW

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Asp	4.83±0.15 de	2.05±0.17 gh	4.17±0.17 ef	3.81±0.14 ef	6.42±0.10 c	3.77±0.11 ef	3.34±0.10 f	3.05±0.16 fg	1.34±0.08 hi	15.03±0.71 a	12.16±0.26 b	6.76±0.19 c	0.51±0.04 i	1.77±0.09 h	5.70±0.14 cd
Thr	2.01±0.05 b	n.d.	n.d.	25.36±0.38 a	n.d.	n.d.	0.99±0.12 c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ser	21.94±0.33 fgh	22.59±1.12 fg	12.7±0.85 j	16.74±0.52 hij	46.6±1.19 c	28.1±0.83 de	18.0±0.38 ghi	21.98±0.41 fg	23.81±0.98 ef	71.04±2.44 a	58.00±1.34 b	30.48±0.57 d	14.20±0.29 ij	15.04±0.24 ij	41.85±0.98 c
Glu	21.96±0.47 d	18.38±0.74 e	4.59±0.29 g	23.06±0.51 d	23.73±0.53 d	28.38±0.35 bc	n.d.	18.47±0.71 e	13.02±0.53 f	52.54±1.16 a	30.99±0.83 b	22.30±0.36 d	10.61±0.31 f	18.49±0.34 e	27.84±0.70 c
Gly	1.08±0.06 cd	1.52±0.08 b	0.99±0.05 d	1.04±0.15 d	1.44±0.04 bc	2.19±0.08 a	1.19±0.05 bcd	0.82±0.04 d	n.d.	n.d.	2.08±0.13 a	1.10±0.09 cd	0.19±0.08 e	1.17±0.05 bcd	1.17±0.07 bcd
Ala	3.98±0.14 d	4.72±0.28 bcd	4.10±0.17 cd	4.43±0.13 bcd	3.19±0.11 e	6.80±0.21 a	4.14±0.13 cd	4.76±0.12 bc	n.d.	n.d.	7.08±0.18 a	4.11±0.11 cd	4.17±0.10 cd	4.27±0.07 cd	5.04±0.15 b
Cys	4.71±0.11 e	3.11±0.19 f	2.10±0.09 gh	2.79±0.08 fg	1.16±0.04 i	9.51±0.32 a	6.21±0.24 d	2.09±0.13 gh	n.d.	2.81±0.06 fg	8.44±0.19 b	2.73±0.04 fg	1.74±0.06 hi	5.84±0.14 d	7.35±0.17 c
Val	0.74±0.04 ef	1.71±0.09 c	0.30±0.01 gh	2.95±0.09 a	1.99±0.07 c	n.d.	1.16±0.08 d	0.41±0.01 g	1.80±0.09 c	n.d.	0.53±0.04 fg	0.98±0.07 de	n.d.	2.34±0.05 b	3.05±0.11 a
Met	n.d.	n.d.	0.14±0.04 de	1.18±0.05 b	0.79±0.04 c	n.d.	n.d.	0.34±0.04 d	3.15±0.14 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ile	3.71±0.08 b	1.29±0.09 d	0.91±0.05 de	2.36±0.12 c	1.03±0.05 d	4.55±0.12 a	4.75±0.15 a	0.62±0.03 ef	n.d.	n.d.	n.d.	0.40±0.04 f	n.d.	n.d.	2.51±0.10 c
Leu	n.d.	n.d.	n.d.	1.46±0.06 b	2.21±0.05 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tyr	n.d.	1.81±0.10 b	n.d.	2.98±0.08 a	3.07±0.12 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phe	n.d.	n.d.	n.d.	11.53±0.32 a	0	n.d.	n.d.	n.d.	9.60±0.41 b	8.41±0.17 c	n.d.	n.d.	n.d.	n.d.	n.d.
Lys	0.82±0.04 d	1.81±0.10 b	0.12±0.02 f	3.26±0.09 a	1.45±0.05 c	0.82±0.02 d	0.53±0.03 e	0.11±0.01 f	n.d.	1.76±0.05 b	0.80±0.03 d	0.11±0.02 f	n.d.	1.84±0.04 b	1.82±0.06 b
His	2.33±0.07 cd	2.58±0.15 c	1.10±0.04 gh	2.68±0.09 c	1.97±0.09 def	4.45±0.11 a	1.80±0.03 ef	0.73±0.04 h	n.d.	2.16±0.06 de	1.89±0.04 ef	1.12±0.05 g	n.d.	3.23±0.05 b	1.66±0.06 f
Arg	11.91±0.43 e	4.35±0.21 g	12.11±0.44 de	14.19±0.41 cd	1.88±0.07 hi	31.90±1.13 a	0.32±0.02 i	8.31±0.25 f	15.86±0.61 bc	n.d.	3.68±0.13 gh	1.17±0.05 i	4.54±0.13 g	7.77±0.23 f	17.09±0.40 b

Data are presented in mean value ± standard error, (n=3)

Different letters followed the data in the same row means significant difference (p<0.05); n.d. means not detected

Table 2. 4 Contents of Saturated Fatty Acids in 15 Cultivars of Jujube Fruits, μg/kg DW

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
C10 ^{m}:0^{n}	138.20±1.31 f	134.68±6.79 f	254.17±8.58 e	148.34±11.12 f	729.38±19.27 c	399.35±11.50 d	952.88±47.21 a	247.40±11.11 e	105.01±4.02 f	928.76±14.93 a	241.21±3.94 e	841.41±16.70 b	273.53±17.15 e	771.65±10.49 bc	245.13±6.78 e
C12:0	1209.73±104.23 ef	1453.63±39.36 def	1740.49±71.22 cd	967.20±22.37 f	3735.65±50.33 a	2074.80±70.00 c	3972.41±37.47 a	1181.79±53.26 ef	1529.71±30.93 de	2788.26±314.84 b	4035.78±27.05 a	3143.07±94.61 b	2758.26±45.65 b	2798.43±22.71 b	1629.23±50.12 cde
C14:0	718.17±35.79 de	772.37±3.39 cde	757.14±20.07 cde	482.31±5.36 f	1233.65±63.74 b	632.84±27.51 e	1167.82±29.99 b	716.33±5.93 de	885.67±17.22 c	850.32±28.70 cd	1138.66±30.56 b	1475.22±37.49 a	668.11±10.38 e	771.65±14.12 cde	637.95±20.26 e
C15:0	114.16±3.93 cde	196.85±19.83 b	58.57±4.14 g	74.79±1.82 fg	217.89±3.88 b	72.85±4.27 fg	146.78±3.54 c	107.86±8.57 def	74.87±2.33 fg	66.92±5.61 g	77.88±1.06 efg	260.17±7.99 a	134.41±9.00 cd	65.72±1.08 g	87.69±4.07 efg
C16:0	1266.95±24.42 cd	848.88±15.38 gh	685.68±9.17 i	1048.15±29.81 f	984.31±25.79 fg	1936.91±31.87 a	1077.59±46.87 ef	855.69±22.64 gh	1216.91±20.74 de	1848.51±41.44 ab	1772.05±21.44 b	1402.95±21.05 c	1350.81±15.91 cd	823.10±35.09 hj	934.88±46.13 fgh
C17:0	175.68±16.77 b	10.80±0.37 f	108.18±1.46 d	157.85±4.29 bc	209.55±2.05 a	137.24±9.71 cd	178.16±5.01 b	125.77±6.82 d	109.95±7.81 d	127.63±2.38 cd	12.48±0.98 f	13.99±0.88 f	11.47±0.50 f	54.07±1.39 e	12.31±0.89 f
C18:0	267.24±11.27 f	965.72±4.74 a	221.67±3.67 f	272.26±1.54 f	618.79±8.44 d	794.80±20.96 c	270.69±17.49 f	216.86±15.85 f	569.07±15.30 de	542.67±19.22 de	890.92±31.41 ab	863.45±20.38 bc	532.21±12.86 e	278.74±5.06 f	828.20±17.26 bc
C20:0	240.20±20.76 bcdefg	221.24±13.71 efg	273.81±5.09 abcde	199.36±9.12 g	237.97±4.21 cdefg	306.34±2.25 a	308.04±7.20 a	226.51±13.85 defg	291.79±11.75 abc	265.63±20.22 abcdef	263.58±10.78 abcdef	297.94±5.63 ab	208.23±9.75 fg	230.97±6.45 defg	283.08±6.40 abcd
C21:0	127.61±3.03 bc	96.24±0.80 d	122.29±9.60 bc	98.18±1.14 d	118.09±0.45 c	147.12±1.62 a	127.58±2.63 bc	121.18±0.47 bc	144.04±1.93 a	118.81±1.37 bc	124.03±2.80 bc	134.23±0.79 ab	116.85±3.12 c	118.37±0.70 bc	115.89±1.85 c
C22:0	605.44±6.37 bc	464.39±3.76 f	124.64±12.55 g	456.59±5.44 f	563.70±3.21 de	707.76±6.52 a	624.71±1.52 b	61.99±1.81 h	91.04±3.35 h	566.54±4.76 de	580.44±6.06 cd	622.05±7.30 b	544.82±5.57 e	566.93±0.91 de	567.18±8.06 de
C23:0	633.99±1.53 bcd	481.02±1.48 gh	637.50±22.61 bcd	431.73±11.17 h	521.49±10.89 fg	719.35±9.11 a	653.45±14.66 bc	605.62±5.79 cde	669.89±28.97 ab	596.63±1.87 cde	608.05±8.93 bcde	622.60±10.74 bcde	562.30±1.79 ef	587.93±6.19 de	586.16±9.02 de
C24:0	696.36±17.67 cde	804.73±38.01 bc	450.06±1.08 hi	491.74±11.51 gh	1059.59±57.84 a	612.03±10.83 efg	780.46±6.48 bcd	355.77±7.33 ij	533.49±11.65 fgh	467.96±18.73 hi	840.06±26.45 b	831.33±22.77 b	542.11±9.25 fgh	293.96±21.92 j	660.51±39.73def

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Total SFA	6193.72±170.27	6450.56±50.64	5434.2±65.96	4828.4947.15	10230.06±51.20	8541.39±163.20	10260.58±150.14	4822.76±52.85	6221.45±69.04	9168.64±403.87	10585.42±82.48	10508.4±184.72	7703.12±56.74	7361.52±8.38	6588.22±123.57

Data are presented in mean value ± standard error, m is the number of carbons, n is the number of double bounds
Different letters followed the data in the same row means significant difference (p<0.05)

Table 2. 5 Contents of Unsaturated Fatty Acids in 15 Cultivars of Jujube Fruits, μg/kg DW

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
C14:1	3581.12±121.93 f	2978.97±46.77 g	3934.53±57.96 ef	2572.28±21.65 g	5219.02±114.04 bc	4335.61±109.52 de	5862.64±107.44 a	4014.99±85.47 ef	3577.49±119.66 f	4737.74±100.92 cd	5620.77±84.74 ab	5449.56±68.24 ab	1718.96±79.26 h	3645.14±154.47 f	4587.18±98.68 d
C15:1	107.01±8.32 cd	94.15±2.32 d	139.88±5.86 bc	115.33±10.03 bcd	144.83±6.86 bc	119.67±1.72 bcd	129.88±9.46 bcd	115.95±5.20 bcd	152.01±14.52 bc	154.97±8.39 b	562.76±10.44 a	151.03±13.14 bc	148.64±5.54 bc	122.31±11.51 bcd	108.72±6.43 cd
C16:1	200.86±9.76 g	157.62±3.14 g	423.89±15.55 de	193.83±7.43 g	453.92±16.75 de	555.45±15.99 cd	1163.22±67.04 a	343.66±5.05 ef	272.35±3.29 fg	679.64±19.27 c	664.64±10.10 c	937.46±28.25 b	200.36±8.00 g	1235.17±48.85 a	350.77±21.61 ef
C17:1	154.51±10.72 def	178.50±3.85 bcde	152.94±1.55 def	143.62±8.45 ef	218.85±13.80 ab	178.86±9.03 bcde	163.22±3.20 cdef	130.16±2.79 f	160.39±10.04 def	140.08±0.89 ef	205.63±17.34 abc	187.02±8.69 abcd	227.40±3.41 a	143.31±12.73 ef	182.56±2.71 bcde
C18:1	608.87±21.31 ef	505.15±2.22 ef	2039.28±69.64 c	424.87±4.09 f	2864.98±42.15 a	705.69±23.21 e	704.60±33.32 e	1085.14±41.20 d	622.22±5.11 ef	586.25±12.20 ef	1176.07±100.01 d	2552.94±33.69 b	582.50±16.27 ef	578.48±27.54 ef	660.51±27.53 e
C18:2	4742.77±74.08 d	6158.15±65.97 b	631.55±23.65 i	1512.04±62.27 h	597.24±37.53	4013.66±99.06 e	7330.05±65.43 a	533.34±21.81 i	2133.98±76.19 g	4659.92±54.61 d	2137.68±49.71 g	720.06±15.07 i	547.46±8.84 i	2471.19±94.21 f	5272.31±48.02 c
C18:3	149.36±2.62 abc	110.20±1.51 e	157.15±3.72 ab	114.04±3.00 e	143.63±4.09 bcd	167.80±1.13 a	145.98±3.04 bcd	136.01±1.42 cd	168.11±2.95 a	135.41±3.92 cd	146.15±8.57 bcd	141.13±5.76 bcd	128.84±0.87 de	136.74±0.95 cd	135.90±1.85 cd
C20:1	224.89±7.15 abc	170.35±4.55 d	231.55±3.90 ab	168.49±7.16 d	177.60±9.31 cd	259.51±2.52 a	258.62±6.05 a	199.85±11.10 bcd	249.34±14.53 a	224.12±16.17 abc	222.54±8.88 abc	250.74±4.61 a	179.11±9.34 cd	191.08±9.81 bcd	231.80±10.37 ab
C20:2	82.11±0.75 fg	98.62±4.96 def	645.24±1.57 a	61.74±4.45 g	116.17±0.23 cd	113.82±3.96 d	215.52±2.64 b	101.44±4.69 def	114.28±9.08 d	86.64±2.75 ef	108.55±4.27 d	137.29±3.74 c	106.82±2.81 de	116.43±0.82 cd	109.86±5.54 d
C20:3	50.93±2.50 e	165.61±4.49 a	55.24±1.96 e	51.45±0.74 e	118.94±5.37 c	57.24±2.35 e	52.33±1.50 e	58.24±3.15 e	53.02±0.57 e	49.29±2.26 e	98.69±2.82 d	92.80±3.24 d	103.07±5.07 cd	49.34±4.10 e	137.95±6.24 b
C20:4	210.58±2.06 cde	151.84±1.51 h	221.82±2.62 bc	157.77±1.13 h	187.73±0.45 fg	234.80±2.35 ab	215.52±6.06 cd	201.42±4.54 def	237.68±1.11 fg	190.92±1.37 fg	198.51±2.43 efg	220.23±2.73 c	186.76±1.79 g	191.60±1.38 fg	191.28±3.69 fg
C20:5	231.05±4.83 bc	242.27±14.19 b	148.69±10.10 d	131.13±3.18 de	299.97±1.2.35 a	204.87±9.82 bc	252.30±2.50 ab	209.13±1.34 bc	133.60±3.74 de	141.63±12.58 de	212.51±17.46 bc	210.79±6.99 bc	299.75±13.57 a	90.28±5.33 e	177.94±19.58 cd

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
C22:1	69.82±3.03 defg	52.57±1.11 gh	90.48±3.62 bc	51.02±1.87 h	69.50±0.89 defg	70.50±1.16 def	105.75±8.11 b	579.92±0.93 a	90.34±3.38 bc	81.97±2.74 cd	68.68±5.12 defgh	90.86±2.13 bc	60.92±3.28 efgh	77.16±3.96 cde	58.27±1.64 fgh
C22:2	571.67±9.08 cd	421.003±2.22 g	652.38±9.34 ab	475.03±3.51 fg	570.92±4.01 cd	669.92±11.72 ab	651.15±16.00 ab	556.35±4.58 cde	689.85±23.29 a	567.57±20.07 cd	608.04±36.99 bc	668.44±8.92 ab	448.42±9.815 efg	532.28±7.10 def	541.03±15.99 cdef
C24:1	51.27±2.98 c	137.04±7.32 b	117.26±6.29 b	51.35±1.93 c	109.14±8.09 b	53.33±2.34 c	134.48±7.17 b	119.86±6.35 b	70.21±3.40 c	52.92±2.37 c	69.88±3.48 c	129.44±7.34 b	54.75±1.62 c	45.67±4.81 c	225.13±7.75 a
C22:6	223.17±3.57 def	347.59±16.24 ab	183.92±8.80 efg	124.89±2.55 gh	352.25±16.66 ab	165.20±5.67 fg	291.38±6.52 bc	223.80±7.78 def	130.75±10.06 gh	156.16±10.64 g	269.91±7.06 cd	262.66±11.86 cd	370.09±13.51 a	80.84±2.28 h	247.18±31.73 cde
Total UFA	11260.01±153.88	11969.69±23.34	9825.80±57.24	6348.87±49.49	11644.70±116.63	11905.94±76.80	17677.05±209.59	8612.61±168.65	8855.64±210.95	12645.24±160.48	12371.02±189.93	12202.45±76.82	5403.87±42.40	9707.77±108.70	13218.39±139.88

Data are presented in mean value ± standard error (n=3),

Different letters in the row means significant difference (p<0.05)

Table 2. 6 Percentage of Major Unsaturated Fatty Acids in Total Amount of Unsaturated Fatty Acids, %

	C14:1	C18:1	C18:2	C18:3	C20:4	C20:5	C22:2	C22:6
BJ	31.79±0.78	5.41±0.22	42.12±0.48	1.33±0.01	1.87±0.03	5.08±0.05	5.08±0.05	1.98±0.01
BZ	24.89±0.45	4.22±0.02	51.44±0.58	0.92±0.01	1.27±0.01	3.52±0.02	3.51±0.02	2.90±0.13
DB	40.04±0.57	20.75±0.71	6.42±0.20	1.60±0.04	2.25±0.03	6.64±0.13	6.64±0.13	1.87±0.08
HP	40.52±0.41	6.69±0.03	23.81±0.87	1.79±0.03	2.48±0.04	7.48±0.09	7.48±0.09	1.97±0.03
JB	44.81±0.61	24.60±0.11	5.12±0.31	1.23±0.04	1.61±0.01	4.90±0.02	4.90±0.02	3.02±0.16
JD	36.41±0.80	5.92±0.16	33.71±0.89	1.41±0.02	1.97±0.03	5.62±0.13	5.62±0.13	1.38±0.04
JS	33.16±0.26	3.99±0.19	41.47±0.12	0.83±0.03	1.21±0.04	3.68±0.10	3.68±0.10	1.65±0.06
LB	46.62±0.21	12.59±0.25	6.18±0.14	1.58±0.04	2.34±0.10	6.46±0.13	6.46±0.13	2.59±0.07
LZ	40.38±0.53	7.03±0.15	24.08±0.29	1.90±0.06	2.68±0.05	7.78±0.15	7.78±0.15	1.47±0.09
NP	37.46±0.42	4.63±0.04	36.86±0.64	1.07±0.05	1.51±0.03	4.49±0.08	4.48±0.08	1.23±0.08
PB	45.43±0.08	9.49±0.65	17.30±0.66	1.17±0.05	1.60±0.01	4.91±0.25	4.91±0.25	2.18±0.06
PZ	44.66±0.38	20.92±0.24	5.89±0.08	1.16±0.05	1.80±0.03	5.47±0.06	5.47±0.06	2.15±0.09
XZ	31.79±1.22	10.78±0.38	10.13±0.24	2.38±0.03	3.45±0.03	9.03±0.18	9.04±0.18	6.85±0.20
YL	37.53±1.25	5.95±0.22	25.48±1.10	1.41±0.01	1.97±0.02	5.48±0.02	5.48±0.02	0.83±0.03
YZ	34.70±0.70	4.99±0.19	39.88±0.24	1.03±0.02	1.44±0.04	4.09±0.10	4.09±0.10	1.86±0.23

Data are presented in mean value ± standard error, (n=3)

Table 2. 7 Contents of Minerals in 15 Cultivars of Jujube Fruits, mg/kg DW

	Mn	Fe	Ni	Cu	Zn	Al	B	Pb	Ca	Mg	Ti	Cr
BJ	5.08±0.17 h	69.16±4.16 cde	2.45±0.0 8 ab	7.08±0.3 4 ab	15.51±1.7 3 abcd	164.79±8.3 2 ab	25.92±1.4 1 bcd	n.d.	287.53±10.7 7 ab	623.43±20.7 5 b	2.45±0.0 1 abcd	3.85±0.0 7 e
BZ	8.33±0.11 b	125.16±5.2 6 a	2.57±0.2 1 ab	6.05±0.0 5 bcde	16.07±0.2 3 abc	144.75±1.4 5 def	16.32±0.2 7 ef	1.30±0.1 1 a	192.63±6.52 ghi	597.47±15.5 0 bc	2.09±0.0 4 cd	5.85±0.5 5 bc
DB	10.68±0.1 5 a	72.25±1.08 bcde	2.95±0.3 4 a	4.80±0.3 9 efg	17.13±0.0 7 ab	137.59±1.1 9 f	23.38±0.8 9 cde	0.54±0.0 2 c	301.81±4.13 a	699.77±9.42 a	1.95±0.0 2 d	4.63±0.2 1 bcde
HP	5.17±0.26 gh	67.04±0.94 cde	2.31±0.1 2 ab	4.86±0.0 4 efg	13.44±0.2 2 bcde	140.99±4.2 6 ef	20.89±1.0 2 def	0.91±0.0 1 b	197.19±2.84 gh	511.77±4.15 d	2.27±0.2 9 bcd	5.80±0.3 1 bcd
JB	7.66±0.14 bcd	83.82±2.97 bcd	2.41±0.0 4 ab	5.36±0.0 8 def	16.21±1.1 3 ab	144.87±3.8 6 def	20.80±0.5 7 def	n.d.	179.82±5.72 hi	614.03±14.3 4 b	2.14±0.1 1 cd	6.48±0.3 7 b
JD	7.23±0.08 cde	62.28±0.55 de	2.19±0.0 3 b	6.03±0.0 5 bcde	17.58±0.4 5 a	148.27±2.5 3 bcdef	25.24±2.7 5 bcd	n.d.	249.71±8.94 cde	647.22±11.3 3 ab	2.05±0.0 6 cd	3.84±0.1 4 e
JS	4.79±0.12 h	64.02±3.29 cde	2.35±0.1 4 ab	4.38±0.1 3 fg	12.31±0.2 2 de	167.48±4.0 6 a	22.69±3.4 9 cdef	n.d.	277.78±4.99 abc	614.45±13.0 7 b	2.66±0.2 8 abc	3.94±0.0 1 de
LB	6.88±0.53 de	63.41±3.98 de	2.33±0.0 4 ab	3.81±0.1 2 g	11.15±0.1 8 e	145.50±2.5 0 cdef	20.49±0.3 1 def	1.25±0.0 6 a	219.38±3.62 efg	534.24±11.1 5 cd	2.17±0.0 7 cd	4.11±0.4 8 cde
LZ	8.26±0.24 bc	73.85±6.31 bcde	2.50±0.0 7 ab	6.59±0.0 7 bcd	16.08±1.0 4 abc	148.14±1.1 9 bcdef	31.08±0.8 7 ab	1.05±0.0 9 ab	216.43±4.27 fg	624.51±3.35 b	2.51±0.1 4 abcd	5.30±0.4 0 bcde
NP	7.58±0.18 bcd	63.46±0.17 cde	2.43±0.1 3 ab	6.74±0.4 8 bc	15.54±0.7 7 abcd	161.99±1.5 8 abc	28.54±1.8 1 abc	0.82±0.1 2 b	236.04±6.33 def	697.16±26.2 0 a	2.82±0.0 6 ab	4.00±0.1 0 cde
PB	7.12±0.14 de	65.46±3.05 cde	2.49±0.1 2 ab	6.56±0.4 8 bcd	16.10±0.5 2 abc	157.96±3.3 3 abcd	23.68±0.6 2 cde	0.23±0.0 5 d	261.87±1.19 bcd	585.94±8.39 bc	2.91±0.0 9 ab	3.82±0.0 6 e
PZ	6.21±0.10 efg	94.24±11.0 6 b	2.36±0.1 7 ab	4.72±0.0 5 efg	13.60±0.2 6 bcde	142.39±1.5 9 def	21.09±0.8 9 def	n.d.	283.83±4.84 ab	634.57±13.8 7 ab	2.04±0.0 8 cd	9.91±0.9 4 a
XZ	5.80±0.15 fgh	76.88±2.38 bcd	2.38±0.0 5 ab	5.55±0.0 9 cdef	12.40±0.0 7 cde	145.17±1.1 1 cdef	15.93±0.4 0 f	n.d.	214.27±5.24 fg	541.37±14.3 7 cd	1.90±0.0 6 d	5.36±0.1 2 bcde
YL	6.93±0.03 de	85.64±2.29 bc	2.34±0.0 2 ab	8.12±0.3 0 a	13.72±0.8 3 bcde	156.35±1.6 0 abcde	35.87±0.4 4 a	n.d.	162.29±7.22 i	588.85±5.38 bc	3.09±0.0 4 a	4.71±0.3 1 bcde
YZ	6.43±0.05 ef	52.72±2.76 e	2.34±0.1 2 ab	6.01±0.3 4 bcde	16.18±0.6 2 ab	145.65±1.1 5 cdef	22.55±0.5 6 cdef	n.d.	266.61±5.01 bcd	632.60±4.34 ab	2.07±0.0 1 cd	3.66±0.0 5 e

Data are presented in mean value ± standard error, (n=3)

Different letters followed the data in the same column means significant difference (p<0.05); n.d. means not detected

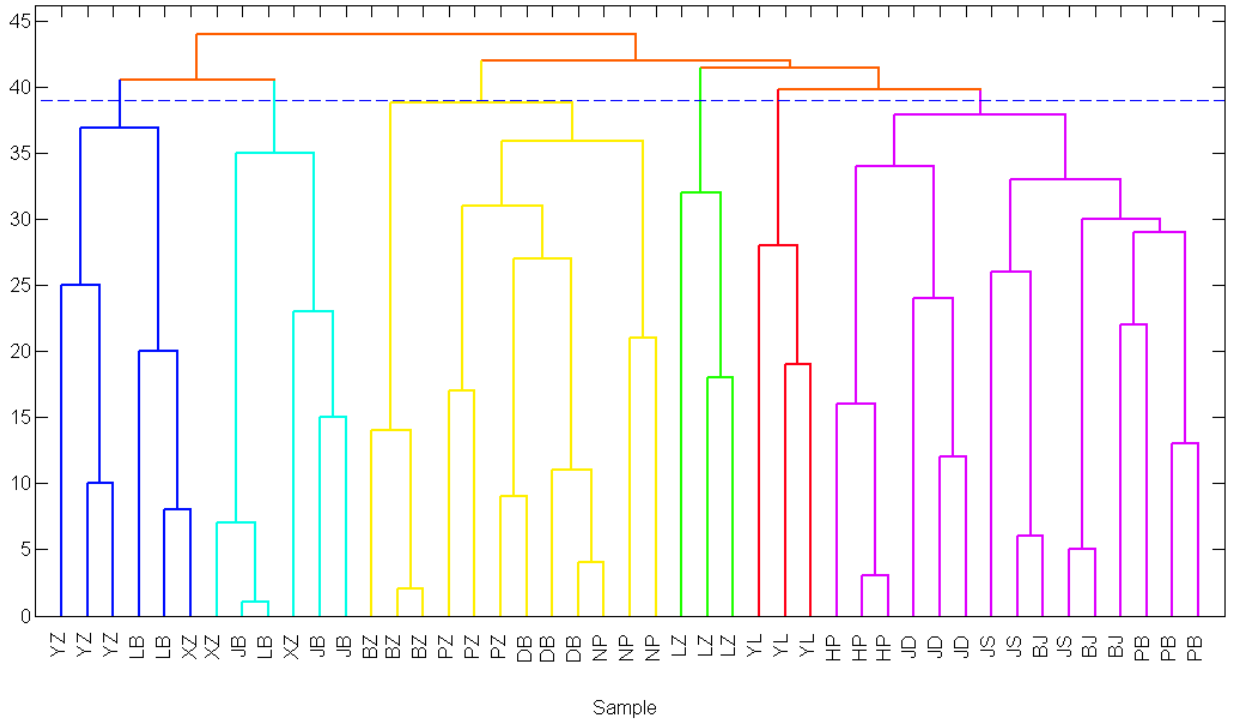

Figure 2. 1 Dendrogram of 15 Cultivars of Jujube Fruits Based on Reducing Sugars Analysis (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

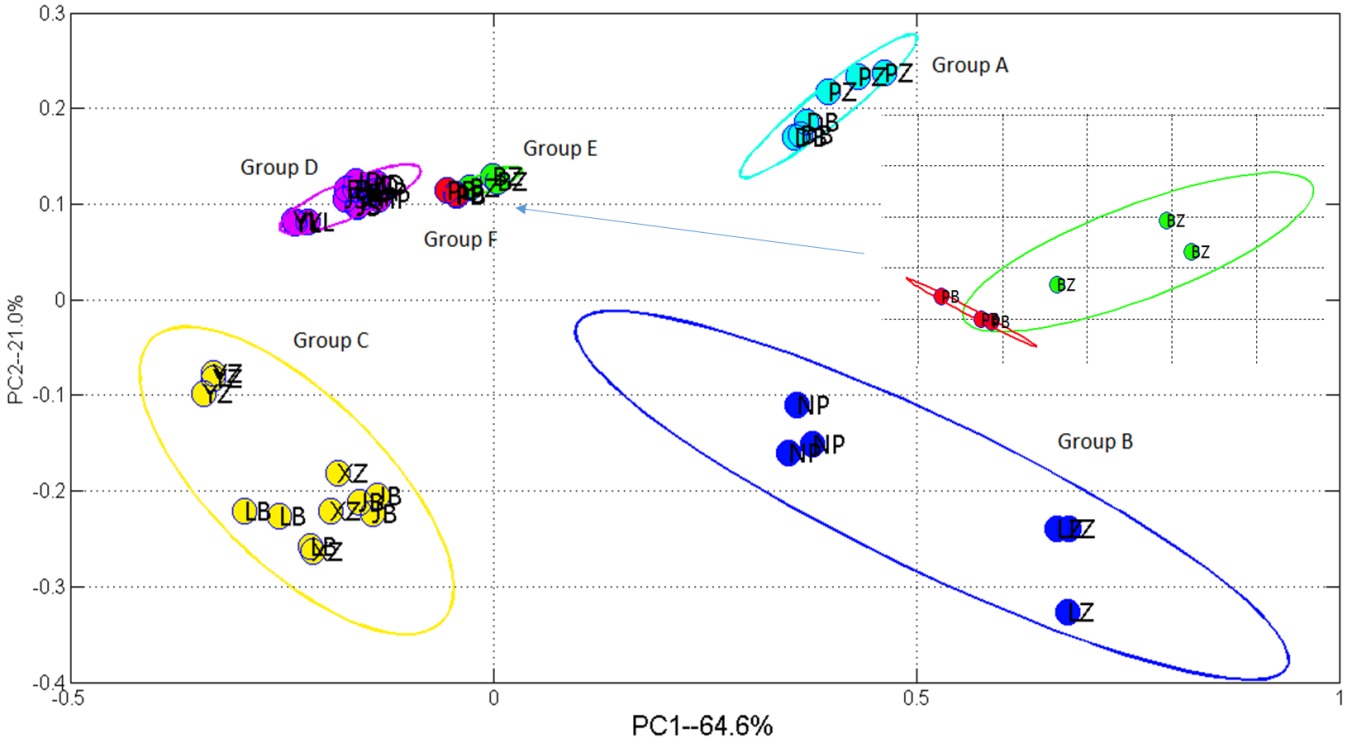

Figure 2. 2 PCA of Reducing Sugars in 15 Cultivars of Jujube Fruits

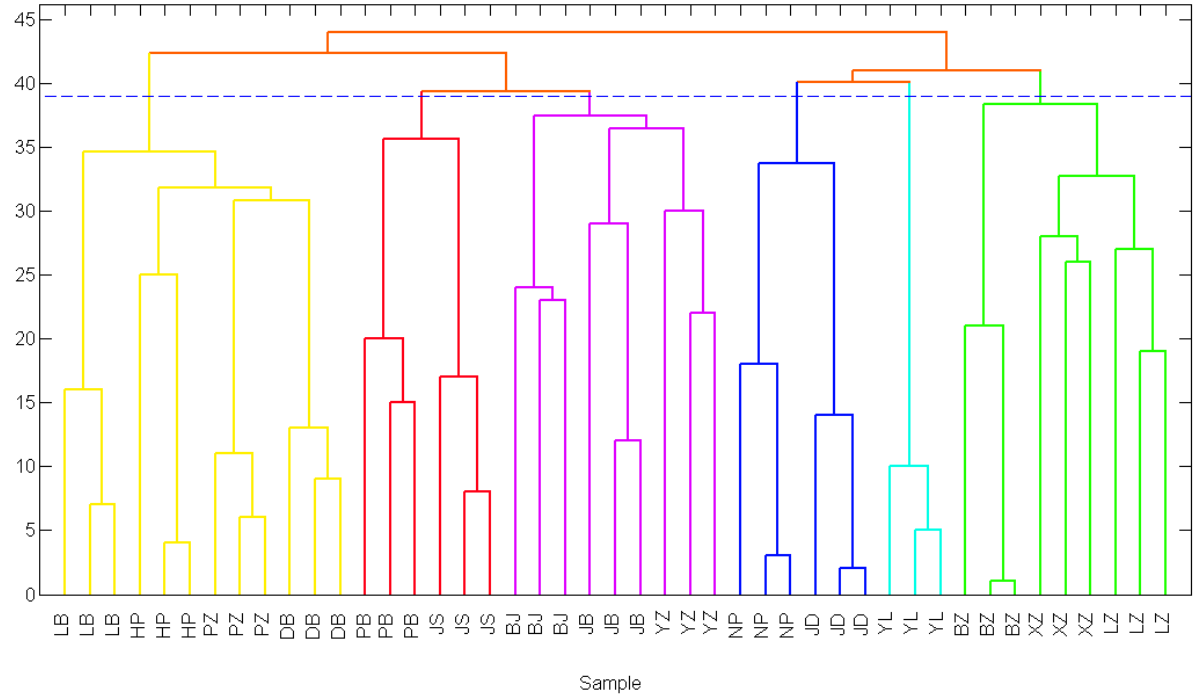

Figure 2. 3 Dendrogram of 15 Cultivars of Jujube Fruits Based on Organic Acids Analysis (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

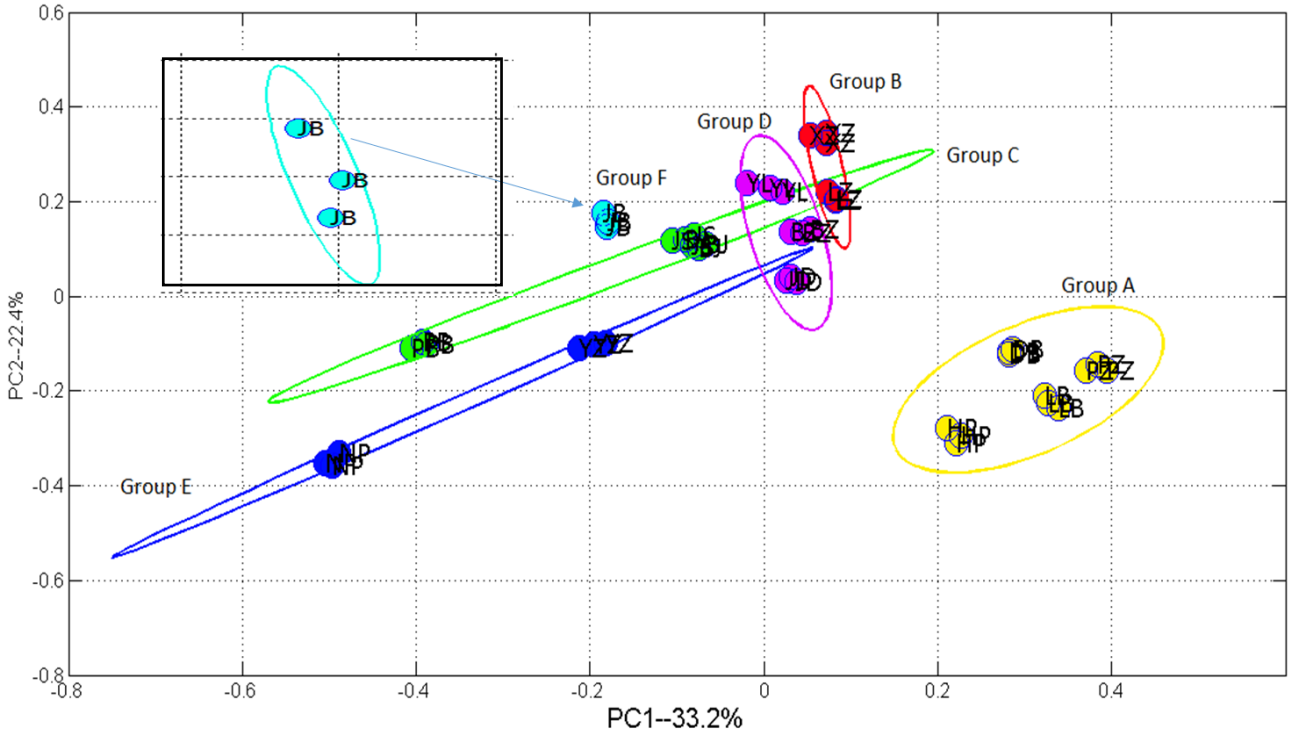

Figure 2. 4 PCA of Organic Acids in 15 Cultivars of Jujube Fruits

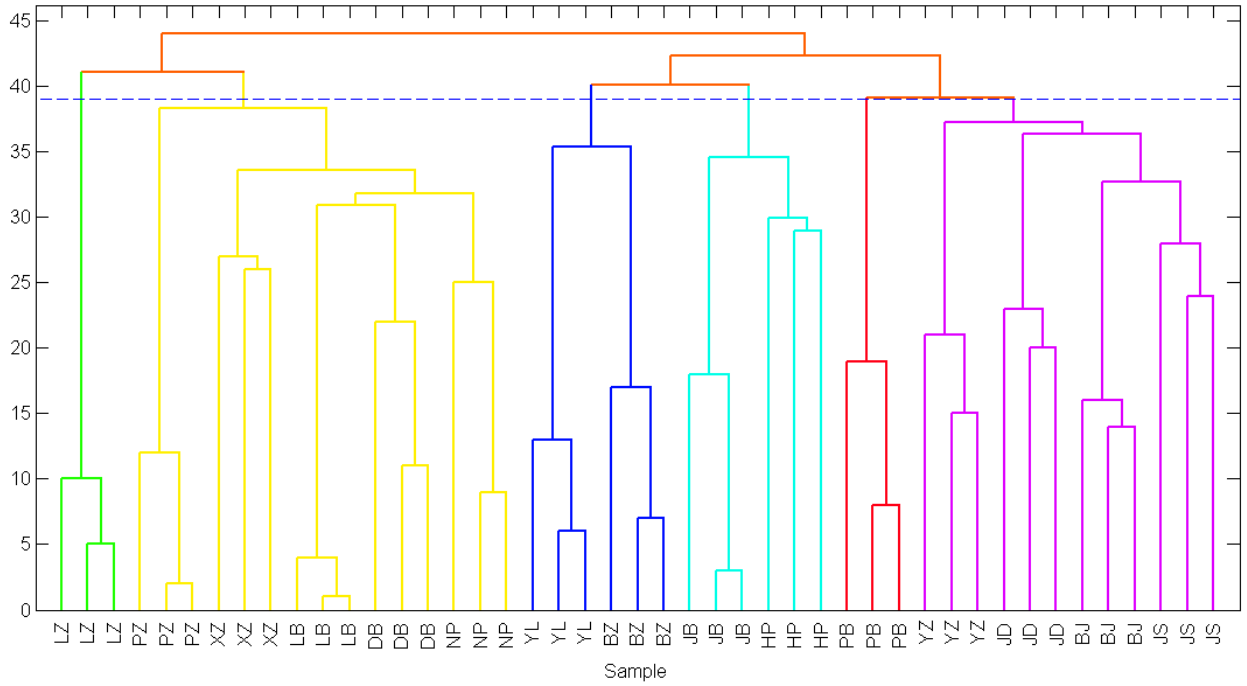

Figure 2. 5 Dendrogram of 15 Cultivars of Jujube Fruits Based o Amino Acids Analysis (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

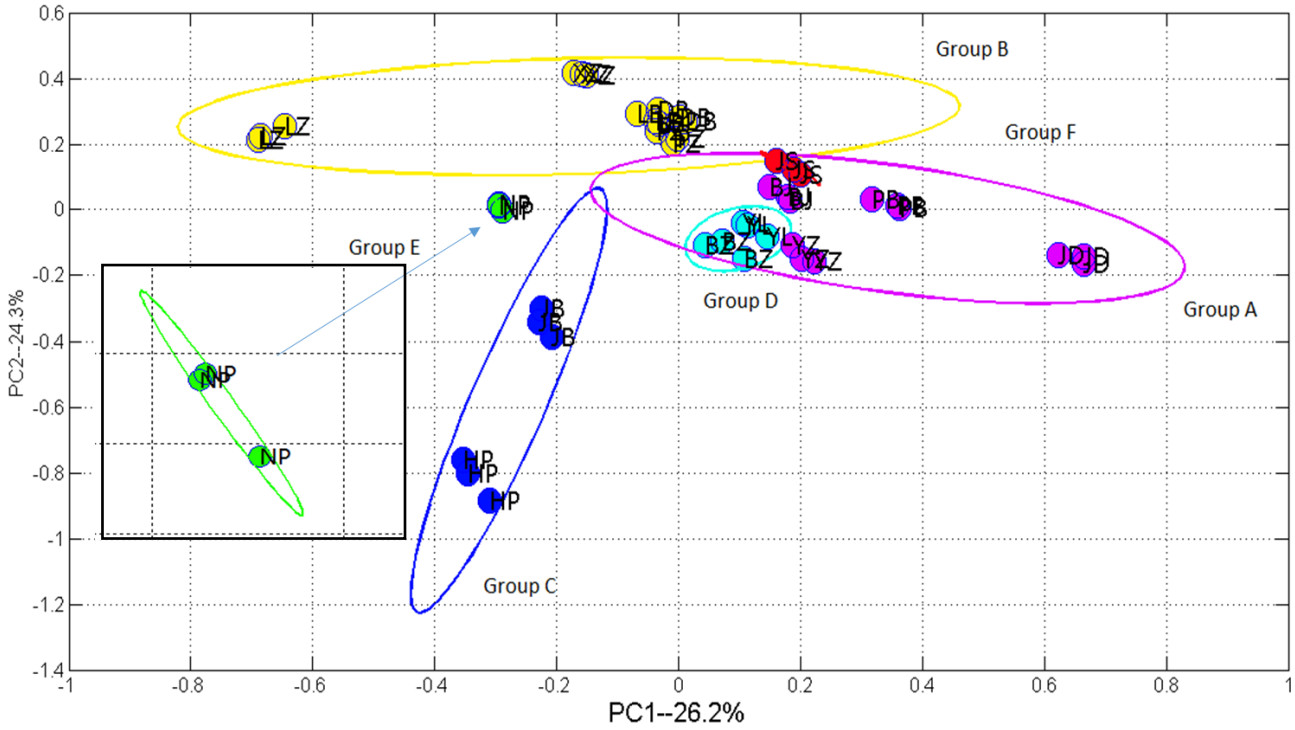

Figure 2. 6 PCA of Free Amino Acids in 15 Cultivars of Jujube Fruits

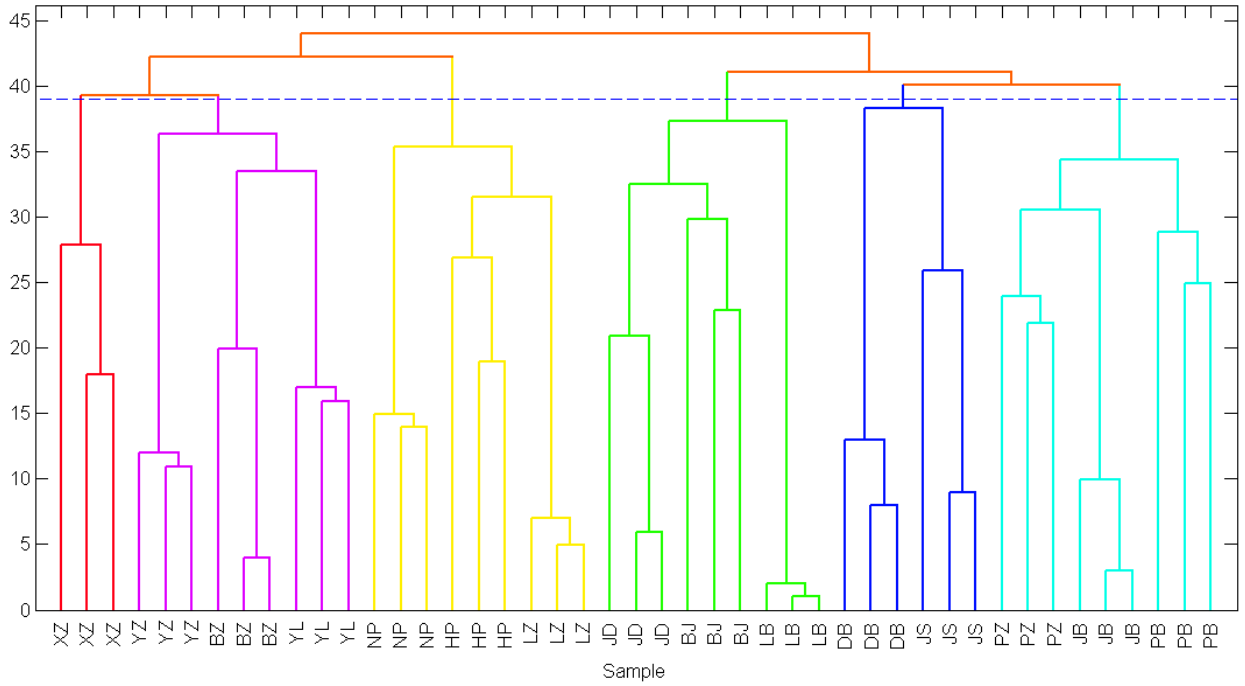

Figure 2. 7 Dendrogram of 15 Cultivars of Jujube Fruits Based on Free Fatty Acids Analysis (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

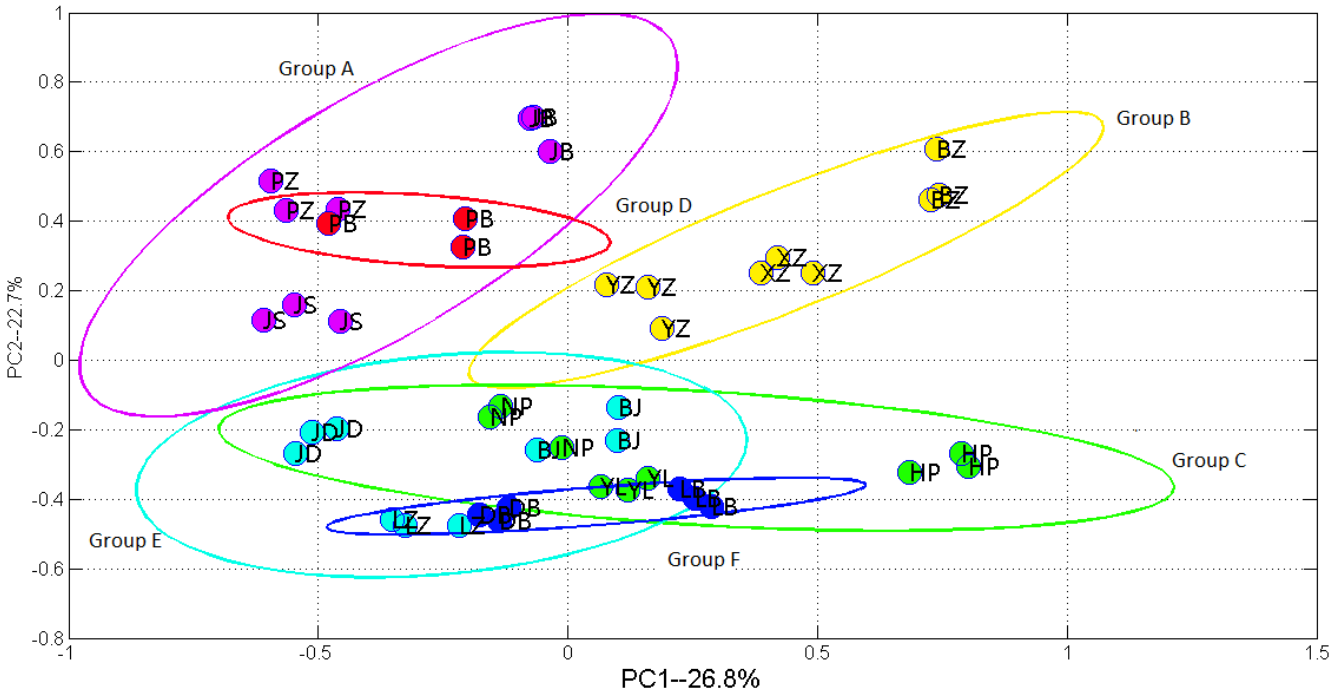

Figure 2. 8 PCA of Free Fatty Acids in 15 Cultivars of Jujube Fruits

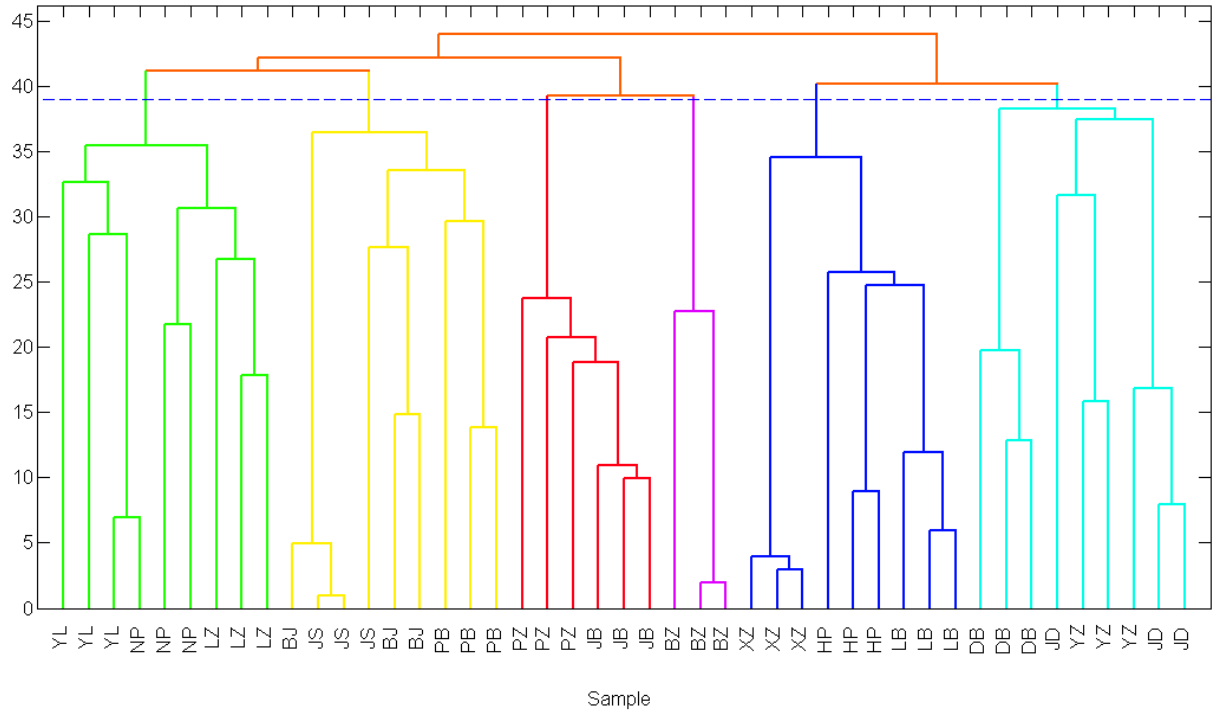

Figure 2. 9 Dendrogram of 15 Cultivars of Jujube Fruits Based on Minerals Analysis (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

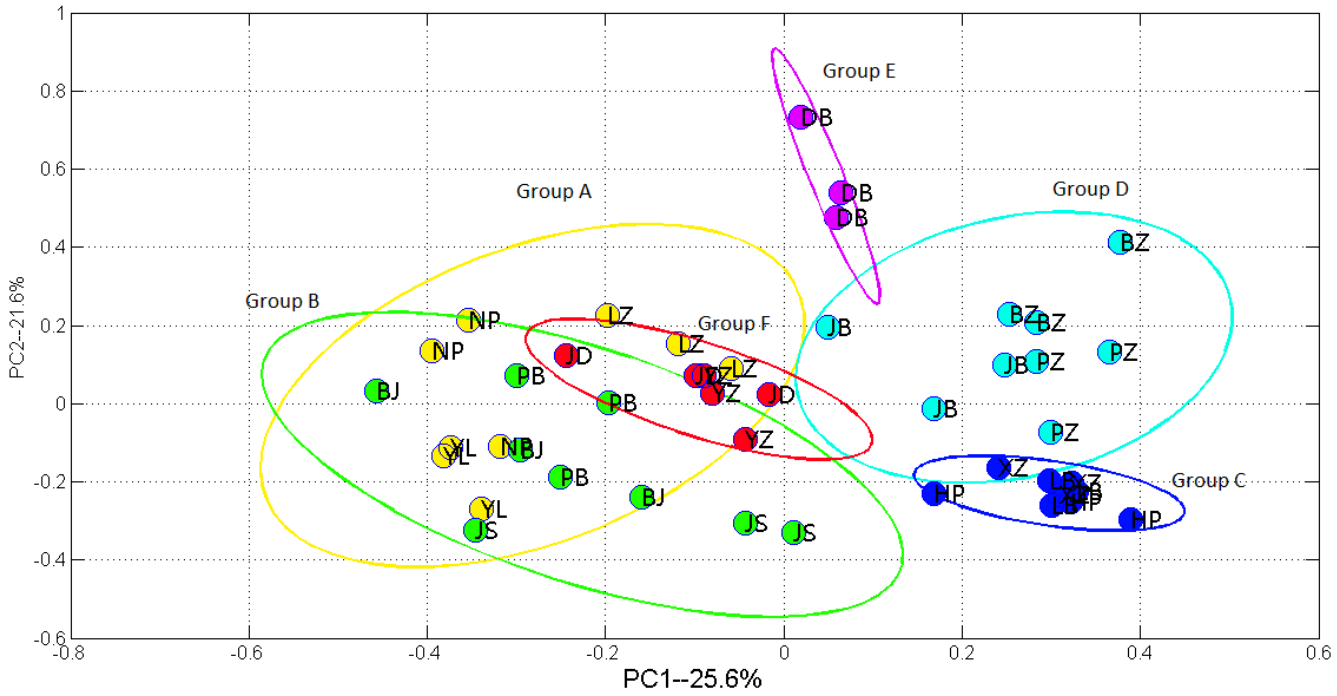

Figure 2. 10 PCA of Minerals in 15 Cultivars of Jujube Fruits

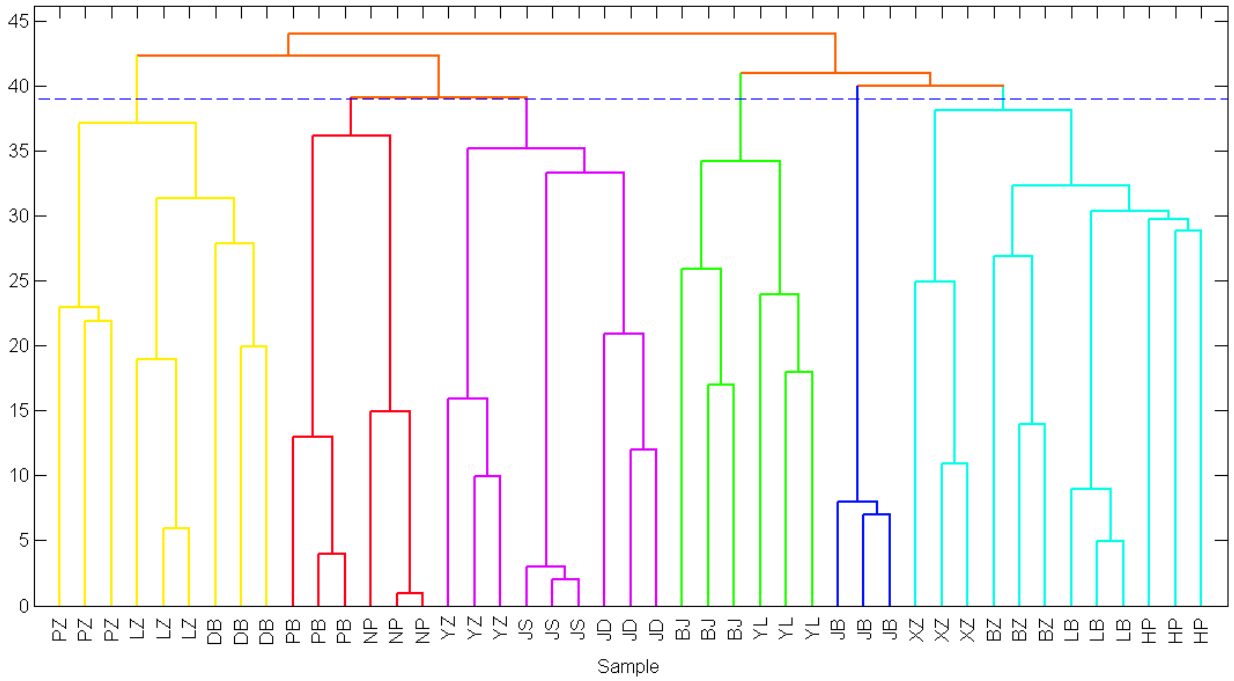

Figure 2. 11 Dendrogram of 15 Cultivars of Jujube Based on the Contents of Reducing Sugars, Organic Acids, Free Amino Acids, Free Fatty Acids and Minerals (Lines in the Same Color Mean the Cultivars were in the Same Cluster)

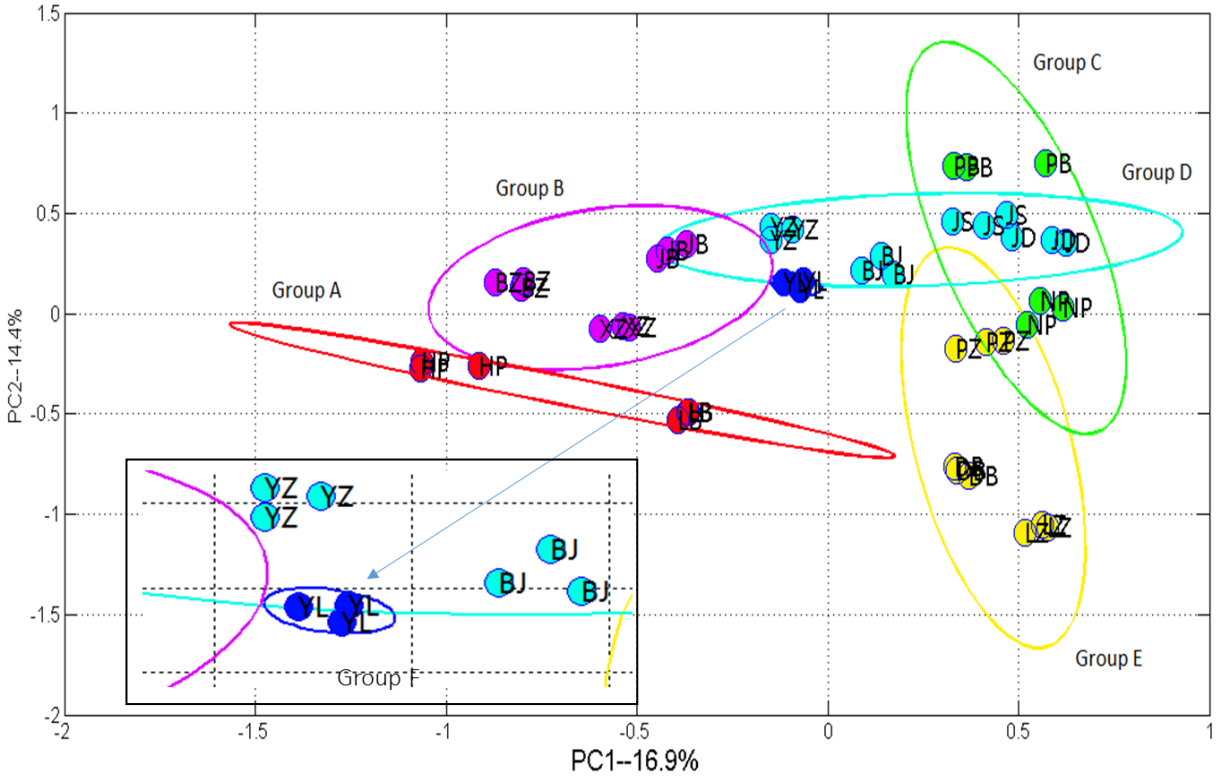

Figure 2. 12 PCA of All the Components Analyzed in Chapter Two

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CHAPTER THREE

ANTIOXIDANT COMPONENTS AND ANTIOXIDANT ACTIVITIES OF 15 CULTIVARS OF JUJUBE (*Ziziphus jujuba* Mill.)

Abstract

Jujube fruits are considered as a promising antioxidant source due to their rich amounts of inherent antioxidant components. In this study, cAMP, ascorbic acid, triterpenes, total phenolic compounds content and total flavonoids content in jujube fruits were measured. The results showed that the content of cAMP varied from 66.33 to 2716.88 μg/100g FW; the content of ascorbic acid were from 317.9 to 679.6 mg/100g FW; the content of triterpenes was from 6.66 to 18.19 mg/100g FW; total phenolic content was from 330.74 to 571.44 mg gallic acid/100g FW; the range of total flavonoids content was from 43.14 to 154.09 mg rutin/100g FW. In addition, the antioxidant activities of jujubes were analyzed, resulting in the antioxidant capacity such as DPPH, ABTS, FRAP and HRSA in a range from 0.603 to 1.842 mmol Trolox/100g FW, 2.276 to 2.786 mmol Trolox/100g FW, 1.228 to 3.823 mmol Trolox/100g FW, 1.353 to 3.560 mmol Trolox/100g FW, respectively. Based on the contents of their antioxidant components and antioxidant activities, hierarchical cluster analysis and principal component analysis were used to classify the 15 cultivars of jujube, which were categorized into five major clusters. Particularly, the cultivar NP, JS, YZ that were in the same cluster contained relatively high contents of antioxidant components and stronger antioxidant capacity.

1. Introduction

Antioxidants is ‘any substances that, when present at low concentrations compared to those of an oxidizable substrate, significantly delay or prevent the oxidation of the substrates’ (*1*). Antioxidants are commonly used to avoid and/or delay the oxidation of lipids, that can maintain the quality of products and expand the shelf life (*2*). Also, they play an important role in avoiding diseases which are caused by oxidative damage, such as cancer, cardiovascular, rheumatoid arthritis, Alzheimer’s disease (*3*). Natural antioxidants in food include vitamin C, vitamin E, β-carotene, some free amino acids, flavonoids, phenolic compounds, etc. (*4, 5*). There are many methods to measure the antioxidant capacities, including oxygen radical absorbance capacity, total radical-trapping antioxidant parameter, total oxidant scavenging capacity, chemiluminescence, photochemiluminescence, croton or β-carotene bleaching by LOO*, low density lipoprotein oxidation, ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, thiobarbituric acid reactive substance assay, Folin-Ciocalteu assay, phycoerythyrin assay, etc. (*6, 7*).

Phenolic compounds are secondary metabolites, and widely distributed in plants. In our diet, vegetables, fruits, cereals and some beverages such as tea and juice are the major source of polyphenols (*8*). These phenolic compounds have antioxidant capacities, such as serving as free radical scavengers, superoxide radical scavengers, and hydrogen donators (*9*).

Ascorbic acid is an essential vitamin for human beings, which is often obtained from our diets, particularly from fruits and their products. It is an enzyme co-factor in

hydroxylation reactions, for instance, it is involved in synthesis of collagen. It also contributes to protect membranes against lipid oxidation. The major pathway in plant to produce ascorbic acid is known as D-mannose/L-galactose pathway, where L-GalL dehydrogenase (GLDH) in the final step converts L-galactono-lactone into L-ascorbate (*10*). As an antioxidant, ascorbic acid is found to scavenge superoxide, peroxyl radical, hydrogen peroxide, and hydroxyl radical (*4*).

Jujube fruits have a lot of health benefits, mainly due to its rich amounts of antioxidants, including ascorbic acid, cAMP, phenolic compounds, triterpenes, polysaccharides, tocopherol, and carotene. Compared with other common fruits such as pomegranate, sweetsop and guava, jujube fruits were reported to have higher antioxidant capacity (*11*). Total phenolic content (TPC), total flavonoid content (TFC) and other antioxidant activities in different jujube species have been reported (*12-16*). Wojdylo (*17*) et al. measured antioxidant capacities of dried jujube, which were dried by different methods, including convective drying, vacuum-microwave drying, freeze drying, and combined convective and vacuum-microwave together. The results showed that the sample processed by the freezing drying contained the highest vitamin C content from 2160 mg/100g DW to 3558 mg/100g DW, as well as the highest value of DPPH and ABTS. Drying methods including air drying, sun drying and microwave drying, decreased the content of antioxidants, but sample under air drying at 50°C was reported to be able to maintain cAMP in a relatively high level. Zhao et al. (*18*) reported the antioxidant capacity of ethanolic extracts of seven cultivars of Chinese jujubes by three antioxidant methods, including phosphomolybdenum assay, superoxide radical

scavenging activity, and hydroxyl radical scavenging activity. All the extracts showed strong antioxidant activities, while there were significant differences among the cultivars. On the other hand, it was found that, in different maturity stages of the jujube fruit, TPC and TFC were decreased with the increased maturity, as well as the antioxidant capacity (*19*).

In this chapter, 15 cultivars of jujube that were collected from the same farm in Shanxi Province, China, were measured in terms of their antioxidants and the antioxidant capacity. These cultivars of jujube are most widely planted in the northern area of China, such as Shanxi, Henan, Hebei Provinces, etc., and sold in the national market. Also, hierarchal cluster analysis (HCA) and principal component analysis (PCA) are applied to classify the jujube cultivars in an effort to help us to know the similarity of different cultivars, and help customers to choose proper health benefiting jujube products.

2. Materials and Methods

2.1 Sample Collection

Different cultivars of the jujube fruits were collected from a farm in Shanxi Province, China, in October 2015. The samples were transported to the lab and directly frozen at -80 °C, after the hand-pick. The cultivars include *Ziziphus jujuba* Mill. *cv.* Banzao (BZ), *Ziziphus jujuba* Mill. *cv.* Dabailing (DB), *Ziziphus jujuba* Mill. *cv.* Cang county Jinsixiaozao (JS), *Ziziphus jujuba* Mill. *cv.* Huping (HP), *Ziziphus jujuba* Mill. *cv.* Lingbao (LB), *Ziziphus jujuba* Mill. *cv.* Yuanling (YL), *Ziziphus jujuba* Mill. *cv.* Jidan (JD), *Ziziphus jujuba* Mill. *cv.* Lizao (LZ), *Ziziphus jujuba* Mill. *cv.* Baode Youzao (YZ), *Ziziphus jujuba* Mill. *cv.* Bin county Jinzao (BJ), *Ziziphus jujuba* Mill. *cv.* Junzao (JB),

Ziziphus jujuba Mill. *cv.* Pingshun Junzao (PB), *Ziziphus jujuba* Mill. *cv.* Xiangzao (XZ), *Ziziphus jujuba* Mill. *cv.* Pozao (PZ), *Ziziphus jujuba* Mill. *cv.* Neihuangbianhesuan (NP) (see **Table 2.1**).

2.2 Chemicals

Gallic acid, ursolic acid, ascorbic acid, trolox, Folin-Ciocalteu phenol reagent, vanillin, cyclic AMP, rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferrous chloride, and 2,4,6-tris (2-pyridyl)-s-triazine were purchased from Sigma-Aldrich Company (Sigma, St. Louis, MO., USA). Aluminum chloride, sodium nitrate, sodium hydroxide, sodium carbonate, perchloric acid, 2,6-dichlorophenol indophenol, acetic acid, and potassium persulphate were purchased from Fisher Scientific (Fisher Scientific, Pittsburgh, PA, USA); HPLC grade methanol were bought from Fisher Scientific (Fisher Scientific, Pittsburgh, PA, USA).

2.3 Analysis of Cyclic Adenosine Monophosphate (cAMP)

Jujube fruits were under freeze drying at first, and then ground into powder. One gram of the sample mixed with 20 mL of distilled water was put into a flask under ultra-sonication for 30 minutes. After filtration, the filtrate was diluted by distilled water to the final volume at 25 mL. The concentration of standard cAMP solution was prepared in water from 1 to 15 μg/mL. The extracts and the standard solutions were filtered through 0.45 μm filter membranes before the HPLC injection. Thermo Scientific U3000 series HPLC system with UV-Vis detector was used for the cAMP analysis. A Golden Hypersil

C18 column (4.6×250 mm, 5 μm) was used for the chemical separation. Mobile phase A was 20 mmol KH_2PO_4 in water, and mobile phase B was methanol. Isocratic mobile phase in a concentration (A:B, 80:20) was used at a flow rate of 1 mL/min, injection volume was 15 μL. The concentration of cAMP was calculated according to the external standard curve and expressed as μg/100g fresh weight.

2.4 Determination of Ascorbic Acid Content

Titration method was used to determine the content of ascorbic acid, based on the reduction of 2, 6-dichlorophenol indophenol dye. The procedures were based on the Rekha's method with a little modification (*20*). Three grams of fresh sample was homogenized with 25 mL of 4% oxalic acid solution in a 50 mL volumetric flask. After filtration, the same oxalic acid solution was added up to 50 mL. Standard ascorbic acid was dissolved in 4% oxalic acid solution to prepare its concentration at 0.5 mg/mL. Five mL of the extracts or the standard ascorbic acid was titrated by 0.01 M 2,6-dichlorophenol indophenol until the endpoint was turned into pink, while the 4% oxalic acid solution was used as the blank. The concentration of ascorbic acid was expressed as mg/100g fresh weight.

2.5 Determination of Triterpenes Content

The content of triterpenes in jujube fruits was measured by the vanillin-perchloric acid method. One gram of the freeze dried sample was steeped in 28 mL of 80% ethanol solution for 20 minutes before it was ultra-sonicated at 60 °C for 22min. Then, it was extracted three times by 80% of ethanol, and filtered. The filtrate was combined together

and adjusted to the final volume at 100 mL. Furthermore, an aliquot of 0.3 mL of the above diluted filtrate was dried by nitrogen, re-dissolved in a mixture which contained 0.2 mL of the freshly prepared 0.5 % vanillin-acetic acid solution and 0.8 mL of 70% of perchloric acid in 60 °C water bath for 15 minutes. After being cooled down to room temperature, the absorbance was measured at 550 nm. Ursolic acid was used as the standard to prepare an external standard curve, of which the concentrations were made in 7 levels from 0.004 mg/mL to 0.040 mg/mL. The triterpenes content was expressed as mg ursolic acid equivalent /100g fresh weight.

2.6 Sample Preparation for Determination of Phenolic Compounds Contents

Five grams of the fresh sample were mixed with 50 mL of 80% methanol solution, under ultra-sonication for 2 hours, then centrifuged at 5000 rpm at 4 °C for 15 minutes. The residue was re-extracted by 80% of methanol for three times, followed by the collection of all the supernatant together, which were evaporated at 45 °C under vacuum, then diluted with 5 mL of methanol. All the extractions were kept in the dark and stored at -20 °C.

2.7 Determination of Total Phenolic Content

Folin-Ciocalteu method was used for the determination of total phenolic content (TPC) (*21*). At first, an aliquot of 100 μL of the sample extracts was diluted with 5.9 mL of distilled water, then 500 μL of the Folin-Ciocalteu reagent was added into the extract before 1.5 mL of 20% sodium carbonate solution was added. The mixture was kept in darkness at room temperature for 2 hours, then measured at 760 nm. Methanol was used

as the blank, and gallic acid was used as a standard to construct an external standard curve within the concentration range from 100 mg/L and 1000 mg/L. The total phenolic content was expressed as mg gallic acid equivalent/100g fresh weight.

2.8 Determination of Total Flavonoids Content

Total flavonoids content (TFC) was measured at 510 nm by a colorimetric method. The mixture of 1 mL of the extracted solution and 4 mL of distilled water was mixed well, then 3 mL of 5% $NaNO_2$ was added. Five minutes later, 0.3 mL of 10% $AlCl_3$ was added in the mixture. After another one minute, 2 mL of 1 M NaOH was added. Rutin was used as the standard, methanol was used as the blank. The TFC was expressed as mg rutin equivalent /100g fresh weight.

2.9 Determination of Antioxidant Capacity

2.9.1 Free radical scavenging capacity by DPPH assay

Sample solution in a volume of 0.1 mL was mixed with 3.9 mL of 6×10^{-5} mol/L DPPH solution, kept in the dark at room temperature for 30 min. Its absorbance was measured at 515 nm. The scavenger capacity of DPPH was calculated based on the following equation:

$$\% \text{ scavenging capacity} = 100 \times (A_0\text{-}A)/A_0:$$

Where A_0 is the absorbance of the blank solution, and A is the absorbance of the solution with sample.

Trolox was used as the standard. The scavenging capacity was expressed as mmol Trolox equivalent /100g fresh weight.

2.9.2 Free radical scavenging capacity by ABTS assay

ABTS assay was conducted with a modified procedure as described in a previous study (*22*). In this study, 7 mM of the $ABTS^{+}$• solution was mixed with 140 mM of potassium persulphate, kept in the dark for 12-16 hours, then diluted by ethanol to make the absorbance at 734 nm at 0.70 ± 0.02. An aliquot of 40 μL of the sample solution was placed into a test tube, then added with 4.0 mL of the diluted $ABTS^{+}$• solution, mixed well, then kept in the dark at room temperature for 10 minutes. The scavenging capacity was calculated by the following equation:

$$\% \text{ scavenging} = 100 \times (A_0\text{-}A)/A_0:$$

Where A_0 is the absorbance of blank solution, and A is the absorbance of solution with sample. The results were expressed as mmol Trolox equivalent / 100 g fresh weight.

2.9.3 Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to a previous study reported by Benzie and Strain (*23*). Extract of the sample for phenolic compounds was diluted to a suitable concentration, when 0.1 mL of diluted extracts was mixed with 3.0 mL of the FRAP reagent which contained 10 mM TPTZ in 40 mM HCl, 0.3 M acetic acid buffer (pH 3.6) and 20 mM/L $FeCl_3$ solution (1:10:1, V:V:V). The reagent was freshly prepared. The mixture was kept in water bath at 37 °C for 4 min. The absorbance was measured at 593 nm. Trolox was used as the standard, and the results were expressed as mmol Trolox equivalent / 100 g fresh weight.

2.9.4 Hydroxyl radical scavenging activity (HRSA)

Two milliliters of the diluted sample was placed in the test tube, then 0.6 mL of $FeSO_4$ solution (8mM/L) was added before 0.5 mL of H_2O_2 solution (20 mM/L) was added. The mixture was vigorously shaken before 1.0 mL of salicylic acid (3mM/L) was added. The mixture was kept in 37 °C water bath for 30 minutes, then centrifuged at 10000 rpm for 10 minutes. Scavenging rate was calculated based on the following equation:

$$\text{Scavenging \%} = [1-(A_1-A_2)/A_0] \times 100$$

Where A_0 is the absorbance without the sample, A_1 is the absorbance with the sample, and A_2 is the absorbance without the salicylic acid.

Trolox was used as standard equivalent, and the final result was expressed as mmol Trolox /100g FW.

2.10 Statistics

All the data were analyzed in triplicate (n=3). The one-way ANOVA data analysis was conducted by JMP Pro 12.2.0 software. Significant difference level was compared by the Tukey's test ($p<0.05$). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed by JMP Pro 12.2.0 software.

3. Results and Discussion

3.1 cAMP Content Analysis

cAMP is also named 3',5'-cyclic adenosine monophosphate. As a second messenger, it is very important in intracellular signal transfer (*24*). In this study, the content of cAMP in 15 cultivars of jujube were measured, which is listed in **Table 3.1**.

The contents of cAMP among the aforementioned 15 jujube cultivars varied from 66.33 μg/100g FW to 2716.88 μg/100g FW, which had significant differences (p<0.05). The cultivars including BJ, HP, and YL contained less than 100 μg/100g FW of cAMP, and cultivar JS, LZ, PB and NP contained more than 1000 μg/100g FW of cAMP. The amount of cAMP in other cultivars were from 226.71 to 998.63 per 100 g FW. Particularly, the cultivar NP possessed the highest amount of cAMP, although its amount in this study was much lower than that (17.38 to 193.93 μg/g FW) in the report by Kou et al. (25).

3.2 Ascorbic Acid Analysis

Ascorbic acid in jujube was measured by the titration method. As shown in **Table 3.1**, the contents in different cultivars had significant difference (p<0.05). The ascorbic acid content ranged from 317.9 mg/100g FW to 679.6 mg/100g FW. Particularly, *Z. jujuba* Mill. *cv.* PZ contained the highest amount of ascorbic acid, while its content in BJ, DB, JS, JD, YL, YZ and NP were more than 500 mg/100g FW and its content in BZ, JB, LZ, PB and XZ were less than 400 mg/g FW.

L-galactose pathway is a major pathway involved in the ascorbic acid synthesis in plants. In addition, GDP-D-mannose pyrophosphorylase, GDP-mannose 3',5'-epimerase, GDP-L-galactose phosphorylase, and L-galactono-1,4-lactone dehydrogenase play important roles in accumulation of ascorbic acid in development stages (*26, 27*). Although no experiments in regards of these enzymes were conducted in this study, the difference of the ascorbic acid content in different cultivars might result from the different activities of these enzymes in different cultivars.

Compared to the other reported studies, the result of ascorbic acid in this study (317.9 mg/100g FW to 679.6 mg/100g FW) was in agreement with Kou's report (1.671 mg/g FW to 4.247 mg/g FW) (*25*) and Gao's (225.1 mg/100g to 387.9 mg/100g) (*11*). An Indian jujube that is named *Ziziphus mauritiana* Lamk. contained less ascorbic acid content (19.54 mg/100g to 99.49 mg/100g) than the jujubes in this study (*22*). In addition, the ascorbic acid content in four cultivars of Spanish jujube was analyzed by HPLC, which revealed that their contents were in a range from 387 mg/100g FW to 555 mg/100 FW, very close to the data shown in this study (*17*).

The content of ascorbic acid can be affected by the maturity and ripening stages. Based on a previous study in Iran, jujubes in a full maturity (red) had the highest content of ascorbic acid (637.56 mg/100g FW) while jujubes in fully ripe (dehydrated brown) stage only had 223.85 mg/100g FW (*28*). During the postharvest period, the content of ascorbic acid decreased when the storage time increased, even though some treatments, such as nitric oxide fumigation or 1-methylcyclopropene, can reduce the loss, but these treatments (and chemicals) could not stop the decrease of ascorbic acid in jujubes (*29, 30*). In this study, it was also found that the ascorbic acid content had positive correlations with the antioxidant capacity: DPPH ($r=0.7648$, $p<0.01$), FRAP ($r=0.6420$, $p<0.01$), HRSA ($r=0.5266$, $p<0.01$), and had a negative correlation with ABTS ($r=-0.1324$). (**Table 3.3**)

3.3 Total Triterpenes Content Analysis

Triterpenes are the second metabolites, of which the functions are protecting the plants from the invasive attacks by insects and/or microbials, as well as for the healthy

growth of the plants. Based on previous reports, triterpenic acids were found in jujube fruits, including ursolic acid, ursonic acid, betulinic acid, pleanonic acid, ceanothic acid, etc. (*31*). In this study, content of triterpenes in different cultivars were measured, which are shown in **Table 3.1**. Among the15 cultivars, *Ziziphus jujuba* Mill. *cv.* XZ contained the highest content of triterpenes in 18.19 mg ursolic equivalent /100g FW. On the contrary, the cultivar HP contained the lowest content in 6.66 mg ursolic equivalent /100g FW. According to the Tukey's test, there was no significant difference in terms of the content of triterpenes among the cultivars BJ, BZ, JB, LB, NP, YZ, PB, PZ and YL (p<0.05). In addition, as shown in **Table 3.3**, the content of triterpenes presented a negative correlation with the antioxidant capacities except the ABTS scavenging activity (r=0.5446, p<0.01).

3.4 Total Phenolic Contents and Total Flavonoid Content Analysis

Total phenolic contents (TPC) were measured by the Folin-Ciocalteu method, of which the principle relies on the phenolic compounds to donate the electrons to phosphotungstic acid complexes in base condition to form a blue color complexes (*32*). Meanwhile, flavonoids as a subgroup of phenolic compounds were determined by the aluminum chloride method.

The results of the TPC and TFC are listed in **Table 3.2**. Among the cultivars, the results of TPC in different cultivars were significantly different (p<0.05). LZ contained the lowest TPC as 330.74 mg GAE/ 100g FW, and YZ had the highest TPC as 571.44 mg GAE/ 100g FW. The range of TFC were in a range from 154.09 mg RE/ 100g FW (NP) to 43.14 mg RE/ 100g FW (JB). The TFC value in five cultivars, including JB, BZ, HP,

JD and LB, were less than 100 mg RE/ 100g FW, and more than 100 mg RE/ 100g FW in other cultivars. These results were agreed with a previous report from Gao (*11*), who reported that the TPC was from 275.6 mg GAE/ 100g FW to 541.8 mg GAE/ 100g FW, and TFC was from 62.0 mg RE/100g FW to 284.9 mg RE/100g FW in fresh jujube fruits. Compared to the Kou's study which reported that the TPC was from 0.558 mg GAE/100g FW to 2.520 mg GAE/100g FW, and the value of TFC varied from 0.47 mg RE/ 100g FW to 2.00 mg RE/ 100g FW (*25*), our study showed close values of the TFC but higher value of TPC. Li *et al*. detected the TPCs in five cultivars (e.g., *Ziziphus jujuba* Mill. *cv.* Jinsixiaozao, Yazao, Jianzao, Junzao and Sanbianhong) of jujube, and obtained higher content of TPC (5.18 mg GAE/ g to 8.53 mg GAE/ g) (*15*) compared to the results in this study (330.74 mg GAE/100g FW to 571.44 mg GAE/100g FW). In addition, the TPC of 12 Indian cultivars of *Ziziphus mauritiana* Lamk. were reported in a range from 172.08 mg GAE/100g to 328.65 mg GAE/ 100g, which were lower than the contents in this study (*22*).

Moreover, further studies on the correlation of TPC and TFC with the antioxidant capacity revealed that the TPC had positive correlations with the values of DPPH, FRAP, HRSA, of which the correlation coefficients were 0.5118, 0.4523, 0.7149 respectively; but a low correlation with ABTS (r=0.2113). In comparison, the TFC presented positive correlations with DPPH (r=0.5971, $p<0.01$), FRAP (r=0.4523, $p<0.01$), and a low correlation with HRSA (r=0.1749), as well as almost no correlation with ABTS (r=-0.0509). (**Table 3.3**)

3.5 Determination of Antioxidant Capacity

Antioxidant capacity were determined by four methods, including the DPPH, ABTS, HRSA and FRAP assays. The former three involved in measurements of the free radical scavenging capacity, while the last one measured the reducing power. Trolox was used as an equivalent standard chemical in all the methods. Except the ABTS analysis, the other results have shown significant differences among the cultivars (p<0.05). In the DPPH analysis, YZ (1.84 mmol TE/100g FW) possessed the strongest scavenging capacity, while JB (0.603 mmol TE/100g FW) had the weakest capacity. Besides, 6 cultivars of jujube, including BZ, JB, LB, PB, XZ, and YL, showed less than 1 mmol TE/100g FW in the DPPH capacity, while other cultivars had the values in agreement with that in the Kou's report (*25*). Twelve cultivars of Indian jujube (*Ziziphus mauritiana* Lamk.) were also reported with similar DPPH scavenging capacity in a range from 14.18 to 39.64 μmol TE/g, but some cultivars such as Gola, Seb, ZG-3 and Elaichi had higher values (*22*). The DPPH scavenging capacity of Jujube which were picked up from Yulin in China were almost twice (1.35 to 3.81 mmol Trolox / 100g FW) than the results in this study (*11*).

Data of ABTS capacity are shown in **Table 3.2**. Among most cultivars such as BJ, BZ, DB, HP, JB, JD, JS, LB, NP, PZ and YZ, there was no significant difference in their ABTS capacity (p<0.05). In regards of the ABTS capacity of all the cultivars in **Table 3.2**, its value was between 2.276 mmol Trolox/ 100g FW to 2.786 mmol Trolox /100g FW. Particularly, the cultivar XZ showed the highest ABTS capacity, while the cultivar YL had the lowest value in this study. Compared with the previous study, the results obtained from this study were higher than that in other cultivars of jujube fruits (0.959

mmol Trolox/ 100g FW to 1.951 mmol Trolox /100g FW) (*25*) which are grown in the same province, but lower than that in other jujubes (1.74 mmol Trolox /100g FW to 7.75 mmol Trolox /100g FW) (*11*) in Shaanxi province. On the other hand, according to another previous study, antioxidant capacity can be affected by different ripening stages (*33*). For example, pear jujube, which was investigated by Wu et al., which showed that its ABTS capacity approached to the highest value in its green stage, and gradually decreased along with the fruit ripening until half of the fruit was red, when its ABTS capacity became stable (*33*).

FRAP is another common method that is often used to measure the antioxidant power, which can be measured at 593 nm in light of the color changes involving in the formation of a blue color complex in low pH condition (*23*). As shown in **Table 3.2**, the capacity of FRAP was in a range from 1.228 mmol Trolox/100g FW to 3.823 mmol Trolox/ 100g FW. In comparison, Wojdylo et al. measured four cultivars of Spanish jujube, which showed their FRAP values were from 17.66 mmol Trolox /100g DW to 34.31 mmol Trolox /100g DW (*34*). The pulp of three cultivars of jujube including Dongzao, Muzao and Hamidazao were used as samples to measure the FRAP capacity, resulting in 982.31, 382.15, and 252.94 mg ascorbic acid/ 100g DW respectively (*35*). In the FRAP assay, ferrous phosphate can be used as another standard equivalent besides the trolox and ascorbic acid, by which the FRAP value of *Ziziphus mauritiana* which was grown in Bangladesh was determined to be 6336.71 μmol Fe (II)/g (*33*). On the other hand, this antioxidant capacity was also found to be affected by drying methods such as convective drying, vacuum microwave drying and freeze drying. Particularly, the latter

was found to be able to keep the highest antioxidant capacity, while the former method led jujube having the lowest FRAP value among these methods (*17*).

Hydroxyl radical scavenging activity (HRSA) is related to metal ions transition. In this study, this antioxidant capacity was observed to have a significant difference among the aforementioned cultivars ($p<0.05$). The cultivar XZ exhibited the smallest value of HRSA with 1.353 mmol Trolox/ 100g FW, while the cultivar YZ had the highest value of HRSA at 3.560 mmol Trolox /100g FW. Zhao et al. compared the HRSA of seven cultivars including Ziziphus jujuba Mill. cv. Pozao, Jinsizao, Junzao, Xiaozao, Yuzao, Goutou and Banzao, and found that the extract from the cultivar Goutou could inhibit 45.9% of hydroxyl radicals, but the cultivar Ban could only inhibit 10.7% of hydroxyl radical (*18*).

3.6 Hierarchical Cluster Analysis (HCA) and Principle Component Analysis (PCA)

Hierarchical cluster analysis was conducted by the Ward method, by which the result is shown in a dendrogram to indicate the distance between clusters (**Figure 3.1**), which is often used to indicate the similarity of objects in biological studies. The cultivars in the same cluster are more similar to each other, or have similar physio-chemical properties than the other cultivars outside this cluster. The scree plot on the left side of the dendrogram in **Figure 3.1** presents the points for the joint of each cluster. According to the natural break where the distance jumps up suddenly (or it is called the cutting point), the jujube cultivars are classified into five clusters. Cluster 1 includes the cultivars BZ, JB, LB and XZ; cluster 2 includes the cultivars DB, PZ and BJ; cluster 3 includes the cultivars HP, JD and YL; cluster 4 includes the cultivars LZ and PB; and cluster 5

includes the cultivars NP, JS and YZ. Based on the color map, cultivars in cluster 1 had relatively lower amount of cAMP, ascorbic acid, TPC, TFC, and the weaker antioxidant capacity including DPPH, FRAP and HRSA, except triterpenes and ABTS, which made these cultivars clustered together. In comparison, most cultivars in the cluster 2 contained middle levels of contents of antioxidant components and antioxidant capacity. The cultivars in the cluster 3 contained very low content of cAMP, and other values in middle levels in terms of their contents (gray color). The cultivars in the cluster 4 had relatively higher amount of cAMP but relatively lower amount of other measurements. Finally, the cultivars in the cluster 5 had relatively stronger antioxidant capacity in DPPH, FRAP and HRSA.

Principal component analysis (PCA) was also conducted to reveal the correlations between the cultivars. **Figure 3.2 A** shows the eigenvalues of the principal components. When the eigenvalues of PCs are larger than 1, they are considered as the main principal components, in this context, the first three principal components (PC1, PC2, and PC3) are considered the main PCs. However, because the cumulative percentage of these three PCs was still less than 80% of total data variance, in this case, two dimensional plot (PC1 and PC2) was chosen, where the PC1 and PC2 explained 64.0% of total data variance. In addition, according to the loading matrix (**Table 3.4**), PC1 was determined to include ascorbic acid, DPPH and FRAP, and PC2 included triterpenes and ABTS. In **Figure 3.2 B**, cultivars of cluster 1 are located in the II quadrant because of the positive effect of contents of triterpenes and ABTS capacity, while the cultivars of cluster 2, 3 and 4 cannot be separated very well based on the scores of PC1 and PC2 because all of them are

around the central of the coordinate system. The cultivars in cluster 5 are located in I and IV quadrant, because their values in terms of TPC, HRSA, DPPH, FRAP and ascorbic acid content in the loading plot had positive effects. **Figure 3.2 C** shows the loading plot of the variance. Longer arrow of the variance means this PCA can explain more information of that variance. In this study, much information of TFC and cAMP has been lost because the PC1 and PC2 only explained 64.0% of total data variance.

Figure 3.3 represents the bivariate fit of score of PC1 by score of PC2, which is performed under p=0.95. The density ellipse indicates how the score plot of PC1 and PC2 is distributed; in this study, it was grouped by hierarchical cluster analysis which is shown in **Figure 3.1**. The ellipses of cluster1, cluster 3, and cluster 5 have no intersections that means these clusters are discriminated from each other by the PCA method. In contrast, the cluster 2, cluster 3, and cluster 4, as same as the score plot shown in **Figure 3.2 B** of the PCA method, they could not be distinguished from each other, because of their overlapping ellipses. As shown in the **Figure 3.2 B**, there were no evidence to demonstrate that cluster 1 and cluster 4, cluster 2 and cluster 5 had the intersection, but based on the ellipses shown in **Figure 3.3**, these clusters are crossed together, indicating that the cluster 1 (BZ, JB, LB, XZ) and cluster 4 (LZ and PB) as the same as the cluster 2 (DB, PZ and BJ) and cluster 5 (NP, JS and YZ) cannot be distinguished from each other. As aforementioned, cluster 1, cluster 3 (HP, JD and YL) and cluster 5 were discriminated from each other; moreover, the cluster 2 (DB, PZ and BJ), cluster 4 (LZ and PB), and cluster 3 did not have significant differences. In addition,

cluster 1 and cluster 2, as well as the cluster 4 and cluster 5, can be discriminated from each other.

4. Conclusion

Overall, in this study, the cultivar NP contained the highest content of cAMP (2716.88 μg/100g FW), the cultivar XZ had the highest amount of triterpenes (18.19 μg UE/100g FW), and the cultivar PB possessed the highest content of ascorbic acid (679.6 mg/100g FW). In addition, the cultivar YZ contained the highest TPC (571.44 mg GAE/100g FW) and DPPH capacity (1.842 mmol Trolox/100g FW), while the cultivar NP contained the highest content of flavonoids (154.09 mg RE/100g FW) and HRSA capacity (3.523 mmol Trolox /100g FW); the strongest ABTS and FRAP capacities were shown in the cultivar XZ (2.786 mmol Trolox/100g FW) and JS (3.823 mmol Trolox/100g FW), respectively.

According to the hierarchical cluster analysis and principal component analysis, these 15 cultivars of jujube could be classified into five clusters. The cluster 1 includes the cultivars BZ, JB, LB and XZ, cluster 3 includes the cultivars HP, JD and YL, and the cluster 5 includes the cultivars NP, JS and YZ. These three clusters were discriminated from each other. However, the cluster 2 (DB, PZ, BJ), cluster 3 and cluster 4 (LZ and PB) could not be discriminated from each other based on the density ellipse.

In summary, these 15 cultivars of jujube possessed high antioxidant capacities, which indicates that they can be a natural source of antioxidants for potential applications in food and pharmaceutical products. Especially for the cultivar NP, JS, and YZ, these

three cultivars contained much higher contents of antioxidant components (cAMP, ascorbic acid and triterpenes) and stronger antioxidant capacity than the other cultivars.

Table 3. 1 Contents of Antioxidant Components in 15 Cultivars of Jujube Fruits

	cAMP μg/100g FW	triterpenes mg UE[1]/100g FW	ascorbic acid mg/100g FW
BJ	66.33±1.90 k	11.94±0.22 bcd	575.8±25.4 ab
BZ	593.75±49.25 f	15.46±0.67 ab	317.9±6.9 e
DB	645.13±0.63 ef	10.16±0.06 cde	544.9±13.1 abc
HP	79.25±1.57 jk	6.66±0.33 e	455.8±89.8 bcde
JB	402.50±0.82 g	14.46±0.18 ab	321.2±24.7 e
JD	226.71±5.85 hi	9.97±0.41 cde	568.2±1.9 ab
JS	1479.81±1.27 b	8.54±0.70 de	587.9±4.4 ab
LB	163.75±7.50 ij	15.18±0.24 ab	409.4±4.6 cde
LZ	1115.00±16.55 c	9.49±0.18 cde	384.3±24.8 de
NP	2716.88±14.37 a	13.15±0.40 bc	541.1±25.4 abc
PB	1116.50±6.48 c	12.37±0.82 bc	341.6±18.3 e
PZ	998.63±8.05 d	12.32±0.21 bc	679.6±10.5 a
XZ	249.00±15.76 h	18.19±0.34 a	356.4±5.4 e
YL	73.63±6.19 k	14.71±2.31 ab	517.5±3.4 bcd
YZ	721.75±23.80 e	12.57±0.30 bc	573.5±6.7 ab

Data were expressed as mean value ± standard error; UE[1] represented ursolic acid equivalent; Different letters in the same column followed the value means significant difference (p<0.05).

Table 3. 2 Antioxidant Capacity in 15 Cultivars of Jujube Fruits

	mg equivalent[1] / 100 g FW		mmol Trolox / 100g FW			
	TPC	TFC	DPPH	ABTS	FRAP	HRSA
BJ	392.03±7.73 def	152.98±0.37 a	1.541±0.009 c	2.529±0.068 abcd	2.651±0.112 bc	2.394±0.109 cd
BZ	357.86±9.64 ef	72.78±0.42 g	0.700±0.011 gh	2.714±0.034 ab	1.383±0.261 de	1.934±0.108 def
DB	392.26±13.45 def	151.60±0.73 a	1.092±0.024 d	2.555±0.048 abcd	1.975±0.303 cde	1.489±0.175 fg
HP	393.93±6.70 de	87.32±0.14 f	1.054±0.011 de	2.455±0.011 bcd	1.564±0.029 de	2.584±0.031 bc
JB	350.61±20.62 ef	43.14±0.74 h	0.603±0.012 h	2.604±0.036 abc	1.494±0.142 de	1.673±0.039 efg
JD	451.53±13.59 bcd	88.67±0.71 f	1.114±0.022 d	2.465±0.043 bcd	2.137±0.116 bcde	2.446±0.018 c
JS	452.64±2.20 bcd	121.06±0.14 c	1.687±0.037 b	2.464±0.047 bcd	3.823±0.307 a	2.493±0.174 c
LB	437.06±17.32 cd	71.77±1.21 g	0.660±0.010 h	2.725±0.026 ab	1.228±0.156 e	1.667±0.013 efg
LZ	330.74±1.10 f	119.34±0.74 c	1.145±0.019 d	2.312±0.007 cd	1.992±0.178 cde	1.952±0.057 de
NP	503.00±18.43 b	154.09±0.24 a	1.742±0.019 ab	2.689±0.041 ab	2.728±0.047 bc	3.523±0.009 a
PB	402.18±17.02 ef	104.91±0.24 de	0.798±0.019 fg	2.363±0.152 cd	1.357±0.099 de	1.607±0.056 efg
PZ	370.56±10.70 ef	124.20±0.49 c	1.550±0.018 c	2.496±0.018 abcd	2.298±0.115 bcd	2.217±0.030 cd

	mg equivalent[1] / 100 g FW		mmol Trolox / 100g FW			
	TPC	TFC	DPPH	ABTS	FRAP	HRSA
XZ	411.91±8.70 de	136.86±2.80 b	0.835±0.008 f	2.786±0.052 a	1.573±0.291 de	1.353±0.127 g
YL	489.64±7.18 bc	106.99±0.75 d	0.981±0.002 e	2.276±0.051 d	1.789±0.047 cde	3.029±0.079 b
YZ	571.44±5.08 a	101.07±0.74 e	1.842±0.041 a	2.695±0.089 ab	3.068±0.229 ab	3.560±0.024 a

Data were expressed as mean value± standard error;
Equivalent [1]: for TPC, the equivalent represented gallic acid; for TFC, the equivalent represented rutin;
Different letters followed the value in the same column means significant difference ($p<0.05$).

Table 3. 3 Pearson Correlation of cAMP, Triterpenes, Ascorbic acid, TPC, TFC, DPPH, ABTS, FRAP and HRSA

	cAMP	triterpenes	ascorbic acid	TPC	TFC	DPPH	ABTS	FRAP	HRSA
cAMP	1.0000								
triterpenes	-0.1267	1.0000							
ascorbic acid	0.1758	-0.3516*	1.0000						
TPC	0.2010	0.0853	0.4101**	1.0000					
TFC	0.4041**	-0.1233	0.4806**	0.1521	1.0000				
DPPH	0.5150**	-0.3729*	0.7648**	0.5118**	0.5971**	1.0000			
ABTS	0.0527	0.5446**	-0.1324	0.2113	-0.0509	-0.0260	1.0000		
FRAP	0.4470**	-0.3338*	0.6420**	0.4523**	0.4548**	0.8504**	0.0113	1.0000	
HRSA	0.3494*	-0.1966	0.5266**	0.7149**	0.1749	0.7079**	-0.0582	0.5710**	1.0000

*, $P<0.05$; **, $P<0.01$

Table 3. 4 Loading Matrix for Different Variables of Principal Component Analysis

	Prin[a] 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6	Prin 7	Prin 8	Prin 9
cAMP	0.5477	0.0099	0.5065	-0.6242	0.0423	0.0761	0.1409	-0.1271	0.0167
Tripterpenes	-0.3906	0.7788	0.1413	0.1362	0.3074	0.3135	-0.0936	-0.0008	-0.0268
Ascorbic acid	0.7984	-0.1356	-0.1060	0.4283	-0.0487	0.2079	0.2834	-0.1459	0.0464
TPC	0.6160	0.5148	-0.4366	-0.0346	0.2095	-0.2501	-0.0499	-0.2329	-0.0210
TFC	0.5963	-0.0749	0.6080	0.3277	0.3256	-0.2077	-0.0416	0.0902	0.0524
DPPH	0.9632	-0.0035	0.0715	0.0461	-0.0872	0.0325	-0.0078	0.1087	-0.2108
ABTS	-0.0804	0.8432	0.2087	0.1170	-0.4238	-0.1448	0.1334	0.0786	0.0272
FRAP	0.8670	0.0139	0.0618	0.0238	-0.2903	0.1240	-0.3672	-0.0598	0.0756
HRSA	0.7758	0.1865	-0.4342	-0.2276	0.1473	0.0543	0.0672	0.2948	0.0832

Prin[a] represented principal component

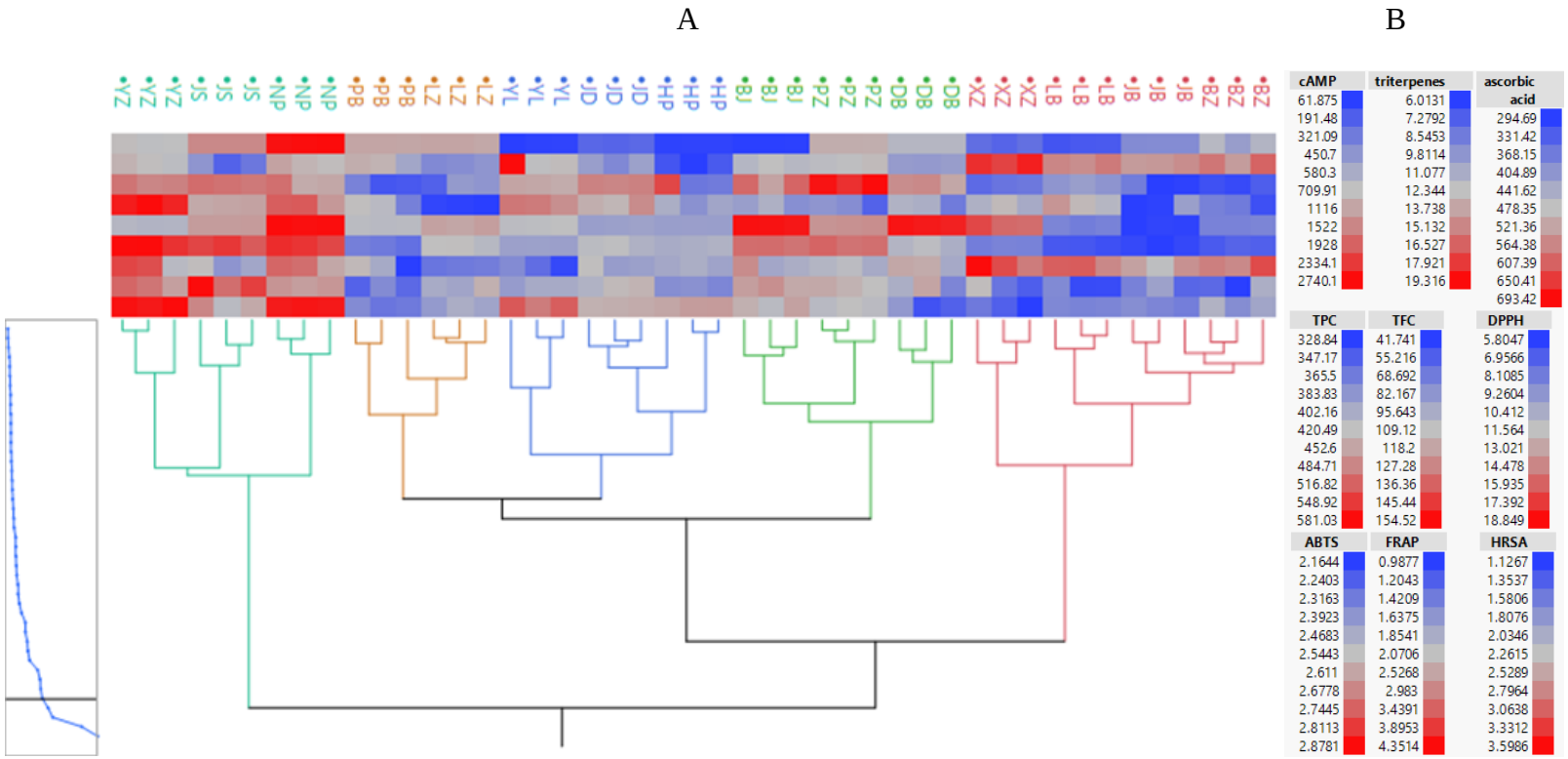

Figure 3. 1 Hierarchical Cluster of Antioxidant Components and Antioxidant Capacity of 15 Cultivars of Jujube Fruits (A, dengraogram with color map, B, legend of color map, color from blue to gray to red, that means the value was from lowest to medium to the larges, the units of the value were the same as Table 3.1 and Table 3.2), color map from top to the bottom were represent the value of cAMP, triterpenes, ascorbic acid, TPC, TFC, DPPH, ABTS, FRAP, and HRSA in sequence.

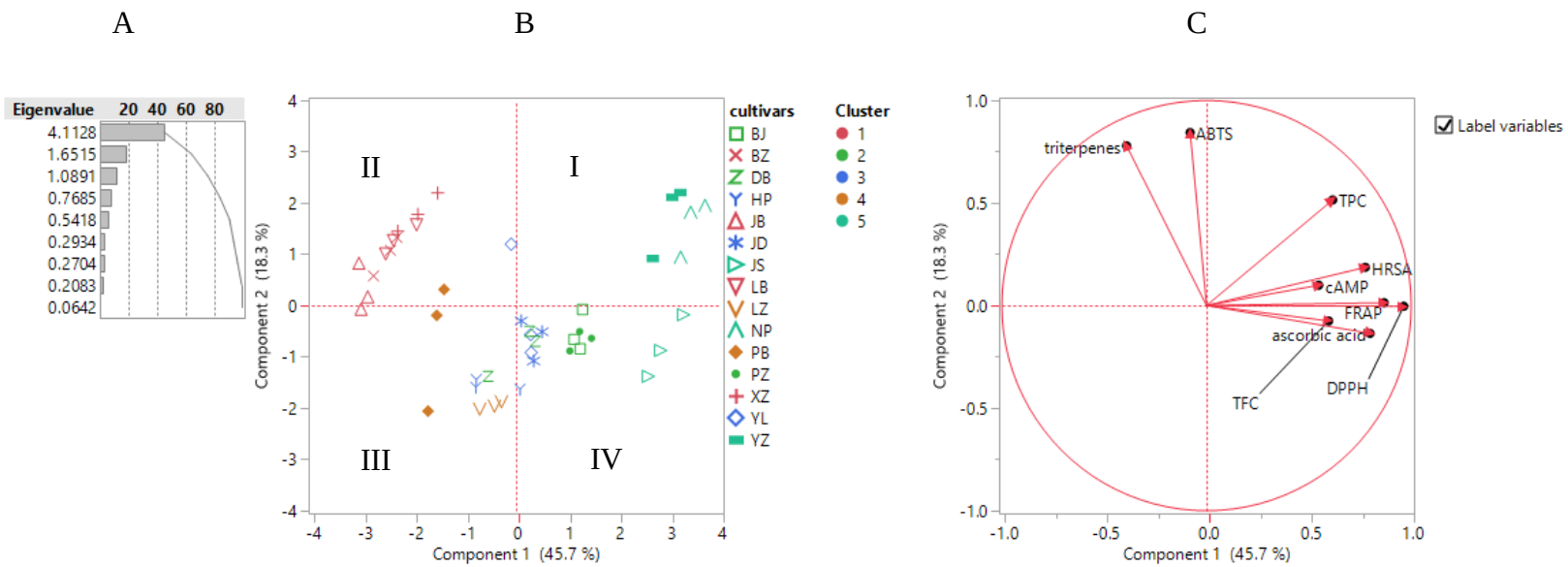

Figure 3. 2 Principal Component Analysis of 15 Cultivars of Jujube [A, eigenvalues of principal components; B, score plot of first two principal components (the same color in the score plot means they were in the same cluster, which were cataloged by HCA); C, loading plot of different variances], legend of cultivars in B: different marks represented different cultivars; legend of cluster in B: different color means different cluster.

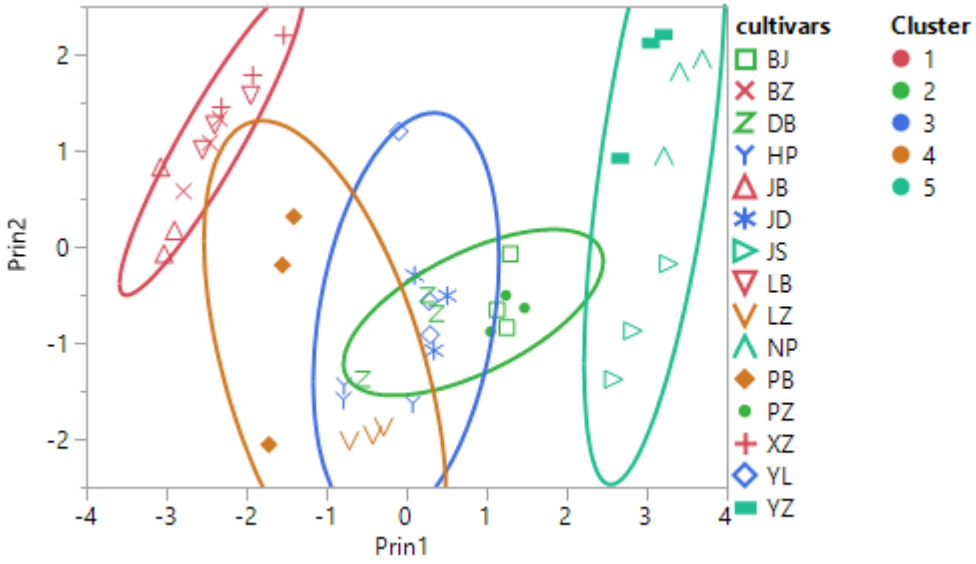

Figure 3. 3 BivariateFit of Score of Principal Component 2 (Prin 2) by Score of Principal Component 1 (Prin 1), legend of cultivars: different marks represented different cultivars; legend of cluster: different color means different cluster. Density ellipses were shown in cluster grouped, p=0.95.

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CHAPTER FOUR

CHARACTERIZATION OF VOLATILE COMPOUNDS IN JUJUBE FRUIT AND CLASSIFICATION OF 15 CHINESE JUJUBE CULTIVARS

Abstract

Ziziphus jujuba Mill. is also called jujube or Chinese date, which is commonly used for making flavoring ingredients due to its unique flavor. In this study, solid phase micro extraction (SPME) method was used to extract volatile compounds from fresh jujube, with the aid of GC-MS for further chemical separation and identification. According to the result, 33 volatile compounds, including aldehydes, alcohols, acids, ketones and esters, were identified. Among them, hexanal (276.5 to 1314 μg/100g FW), (*E*)-2-hexanal, (145.1 to 1876 μg/100g FW), nonanal (188.2 to 1047 μg/100g FW), and n-decanoic acid (58.42 to 1268 μg/100g FW) were found to be the major volatile compounds in fresh jujube fruit. In comparison, volatile compounds extracted from dried jujube fruits by both SDE and SPME methods were also investigated. It was found that (*E*)-2-hexenal and hexanal were the major aldehyde in the SDE extract, while nonanal and benzaldehyde were the major aldehyde compounds extracted by SPME. Classification of 15 Chinese jujube cultivars, based on the type and amounts of their volatile compounds, were conducted by hierarchical cluster analysis and principal component analysis, which helped categorizing the jujubes into five clusters, including cluster 1 (LB, HP, LZ, NP, JS, PZ, and YL), cluster 2 (BJ, DB), cluster 3 (PB, BZ, JD and XZ), cluster 4 (JB) and cluster 5 (YZ). Cluster 1, cluster 2 and cluster 3 that crossed

over together could not be discriminated from each other, but cluster 4 and cluster 5 could be separated very well from each other.

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) is a very popular fruit which is mainly grown in the north part of China. It is also used as a traditional medicine because of its rich amount of antioxidants such as vitamin C, polyphenols, terpenes (*1-4*) etc. Besides its nutritional values, jujube is also widely used as a unique food additive in food industry due to its desirable aromas.

Volatile compounds provide the major contributions to a food flavor, which often include low molecular esters, organic acids, fatty acids, alcohols, aldehydes, lactones and terpenes, etc. Many factors can affect the type and amount of volatile compounds in fruits, such as temperature of storage condition in the postharvest stage (*5*). For instance, the concentration of hexanal was decreased at 5 °C compared with that at 20 °C (*6*). Galindo et al. analyzed the effect of crop load on the volatile compounds of jujube fruits, and found that, after the reduction of crop load, the concentration of trans-2-hexenal or benzaldehyde increased, while the concentration of hexanal, heptanal, and nonanal decreased (*7*).

Extraction is an essential step for volatile compound analysis. Liquid-liquid extraction (LLE), solid phase micro-extraction (SPME), and simultaneous distillation and extraction (SDE) are three common methods for volatile extraction (*8-10*). LLE is usually used for aqueous samples, which often requires a large volume of organic solvent, resulting in some inevitable disadvantages such as high cost, hazardous risk, etc. (*11*). In 1990, solid phase micro-extraction was developed by Pawliszyn (*12*), who used it for analysis of chemicals in contaminated water. Later, application of SPME was rapidly

spread, and it has been widely used in food analysis. This method is simple and rapid, and is a solvent free method (*13*), which is suitable to analyze samples in gasous, solid or aqueous status. The principle of SPME relies on the diffusion of analytes from the sample matrix into the extraction phase (solid phase) to reach an equilibrium between the two phases (*14*). Technically, fused silica fiber or stainless wires with different types of polymer coatings are inserted into the liquid/gas sample or exposed to the head space to extract volatile compounds. After the phase equilibrium, the volatile compounds are desorbed by thermal process (*15*). Simultaneous distillation-extraction (SDE) was introduced in 1964. Compared to the LLE, SDE does not require a large volume of organic solvent due to its recycling extraction step. The steams (i.e., water steam in sample flask and solvent steam) are continually recycled. When they meet together, the volatile compounds are extracted from the water steam by the solvent steam. Due to high temperature used in this method, oxidation, degradation and loss of some volatile compounds might occur, which could affect the results. Regardless of the above disadvantages, SDE is still widely used for quantification analysis of volatile compounds because it has very high recovery of the analytes, particularly to those with less volatility (*16*).

Identification of volatile compounds is usually performed by gas chromatography (GC), which is also widely used in different areas such as environmental science (*17*), food analysis (*18, 19*), and clinic study (*20*), etc.. In many cases, mass spectrometry (MS) is often connected to GC to identify the chemicals because of its high sensitivity and high resolution (*21*). In this study, Trace 1300 gas chromatography from Thermo Scientific

with Tri plus RSH auto sampler was used. This instrument can automatically complete sample incubation, extraction, and fiber cleaning under the controlled temperature and time for incubation and extraction. This programmed procedure can improve the precision of the instrument and increase the repeatability (*22*).

Jujube has a unique desirable flavor, which make it often being used as a flavoring ingredient in food industry. Wang et al. studied the effects of different extraction methods on jujube aromas, including LLE, SDE, ultrasound-assisted solvent extraction (UAE) and head space solid-phase micro-extraction (HS-SPME). They found that LLE and UAE could extract similar compounds, but the volatile compounds extracted by SDE, HS-SPME, and LLE these three methods were very different (*23*).

The volatile compounds of the Spain jujube were reported to include aldehydes, terpenes, esters, ketones and hydrocarbons (*24*). Regarding the volatile compounds in jujube brandy wine and jujube leaves, the former were mainly composed of esters and acids (*25*), while the latter contained some the major components, such as z-ocimene and 1,1-dimethyl-3-methylene-2-ethenyl-cyclohexane acetate (*26*).

In general, the SPME method was adopted to extract the volatile compounds from the jujube fruits. Subsequently, the PCA was used to classify the 15 cultivars of jujube. In more detail, the specific aims of this study were to: 1) extract and identify the main volatile chemicals in the jujube fruit; 2) investigate the similarity between the aroma profiles of different jujube cultivars; 3) compare the effect of two extraction methods, i.e., SPME and SDE, on volatile chemicals.

2. Materials and Methods

2.1 Sample Collection

All the jujube fruits samples were collected from a farm in Shanxi province, China, in October 2015. The fruits were carefully picked up to avoid any broken part, and be kept in the same shape. After picking up the fresh jujube fruits from the trees, the samples were transported to the lab and directly frozen at -80 °C. The investigated jujube cultivars include *Ziziphus jujuba* Mill. *cv.* Banzao (BZ), *Ziziphus jujuba* Mill. *cv.* Dabailing (DB), *Ziziphus jujuba* Mill. *cv.* Cang county Jinsixiaozao (JS), *Ziziphus jujuba* Mill. *cv.* Huping (HP), *Ziziphus jujuba* Mill. *cv.* Lingbao (LB), *Ziziphus jujuba* Mill. *cv.* Yuanling (YL), *Ziziphus jujuba* Mill. *cv.* Jidan (JD), *Ziziphus jujuba* Mill. *cv.* Lizao (LZ), *Ziziphus jujuba* Mill. *cv.* Baode Youzao (YZ), *Ziziphus jujuba* Mill. *cv.* Bin county Jinzao (BJ), *Ziziphus jujuba* Mill. *cv.* Junzao (JB), *Ziziphus jujuba* Mill. *cv.* Pingshun Junzao (PB), *Ziziphus jujuba* Mill. *cv.* Xiangzao (XZ), *Ziziphus jujuba* Mill. *cv.* Pozao (PZ), *Ziziphus jujuba* Mill. *cv.* Neihuangbianhesuan (NP).

2.2 Chemicals

A mixture of alkane standard (C8-C20) and the internal standard, 6-methyl-5-hepten-2-ol, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Sample Preparation

All the fresh jujube samples were carefully peeled to remove the seed. The pulp was cut into small pieces in the same size for subsequent volatile compound extraction by the HS-SPME method. Moreover, the dried jujube fruit of the cultivar LZ was used to

compare the effect of SDE and SPME on extraction of volatile compounds from jujube fruits.

2.4 Optimization of the SPME method

Jujube sample NP was used to optimize the SPME method. In this study, the mixed coating fiber DVB/CAR/PDMS (50/30 μm) which was purchased from Supleco (Aldrich, Bellefonte, PA, USA) was used for the volatile extraction. The extraction condition included the following parameters: incubation temperature (40, 60, 80, 100 °C), incubation time (15, 30, 45 and 60 minutes), and extraction time (5, 15, 25, 35 minutes), which were tested to optimize the extraction condition. Based on the optimization result, the extraction condition was incubated at 80 °C for 30 minutes and extracted by 25 minutes for all the samples.

2.5 SDE method

Likens and Nickerson apparatus was applied in the SDE method. An amount of 150 grams of the LZ jujube musts added with 40 μL of 230 ppm internal standard, were mixed with 500 mL pure water in a 1000 mL flask which was placed above a heater at 100 °C. An amount of 50 mL of dichloromethane was placed in another flask which was heated at 62 °C. The extraction process lasted for 3 h. The extract was dehydrated by anhydrous sodium sulfate, then condensed to 0.5 mL by nitrogen purge.

2.6 GC-MS Method to Identify Volatile Compounds

Trace 1300 Gas Chromatograph with Tri plus RSH auto-sampler was connected to an ISQ single quadrupole mass spectrometry (Thermo Scientific, USA) for chemical

separation and identification. Thermo TR-5 capillary column (30 m × 0.25 mm × 0.25 μm) was used to separate the volatile compounds. Helium was used as the carrier gas, of which the flow rate was 1 mL/min. Injection port was in a splitless mode at 250 °C. Temperature program began from the initial temperature at 40 °C, hold for 2 min, then increased to 180 °C at the rate of 5 °C/min, hold for 5 min, then ramped to 240 °C at the rate of 10 °C/min hold for 10 min. The MS detector adopted an electronic ionization (EI) mode, of which the electron impact energy was 70 eV, ion source temperature was 280 °C, MS transfer line temperature was 280 °C. The scan mode was at the range of 40 – 700 *m/z*. In order to identify each chemical, two methods were used. First, the mass spectra of temporarily identified volatile compounds were compared with those in the NIST library (version 2.0); second, the retention index (RI) of those temporarily identified volatile compounds were compared with those of the references. RI were determined with aid of a series of alkanes (C8-C20) (Sigma-Aldrich, St. Louis, MO). RI was calculated by the following equation:

$$\mathrm{RI} = 100 \times [\mathrm{n} + (\mathrm{N} - \mathrm{n}) \times \frac{\log t - \log t_n}{\log t_N - \log t_n}]$$

t is the retention time of a detected compound;

t_n is the retention time of an alkane standard which was eluted before the sample, n is the number of carbons of that standard;

t_N is the retention time of an alkane standard which was eluted after the sample, N is the number of carbons of the standard.

Quantification of the compounds were calculated based on the peak area of the sample and internal standard (6-methyl-5-hepten-2-ol). All the samples were run in triplicate.

2.7 Statistics

The data was expressed as mean value ± standard error, which were conducted by one-way variance analysis (ANOVA). Significant level was obtained by the Tukey test ($p<0.05$) by JMP software. Principle component analysis (PCA) and hierarchical cluster analysis (HCA) were also operated by the JMP software.

3. Results and Discussion

3.1 Optimization of SMPE Method

In this study, the volatile compounds were divided into the following chemical classes including acid, aldehyde, ketone, ester, and alcohol according to their chemical structures. In order to find out the optimized conditions for volatile chemical extraction, the total peak areas of different compounds groups and the summation total peak areas of the chemical groups were compared so as to determine the highest level of chemical absorption by the SPME.

3.1.1 Effect of Temperature on Extraction Efficiency

Temperature is an important factor which can affect the efficiency of sampling from matrix to the SPME fiber. The diffusion of volatile compounds from the matrix to the head space could be dynamically achieved by heating because the volatile compounds need energy to overcome some barriers in the matrix (*27*). Generally speaking, the less

volatility the more energy they needed. **Figure 4.1** shows that the highest values of the total peak areas of all the volatile compounds, the chemical groups of acids and esters were achieved at 100 °C, while the highest values of peak areas of the aldehydes and ketones were achieved at 80 °C; the highest value of peak areas of the alcohols was at 60 °C, when 2,3-butanediol contributed about 92% of all the alcohol peak area. On the other hand, since the aldehydes are the major volatile compounds in the jujube fruit, 80 °C were chosen as the optimized temperature.

3.1.2 Effect of Incubation Time on Extraction Efficiency

In order to have sufficient volatile compounds vaporized from the matrix into the head space of the vial, the incubation time is another important factor which can affect the extraction efficiency. As shown in **Figure 4.2**, the total peak area of detected chemicals approaches to its highest value at 30 minutes of incubation, which means the most analytes were evaporated from solid sample into the gas phase, and adsorbed by the fiber. In regards of the total peak areas of different groups of the volatile compounds, such as aldehydes, acids, esters and ketones, the total peak areas of the aldehydes, acids, esters and the summation of these chemical groups were all at their highest values at 30 minutes, though the total peak areas of ketones and alcohols were obtained at different time. Since ketones and alcohols only accounted for less than 5% of volatile compounds in jujube, the incubation time for 30 minutes was selected as the optimized parameter.

3.1.3 Effect of Extraction Time on Extraction Efficiency

Under the fixed conditions of other parameters (80 °C for incubation temperature and 30 minutes for incubation time), when the SPME was exposed in the head space after 25 minutes, it was found that SPME could absorb the most amount of the volatile chemicals, particularly for the aldehydes, acids and ketones. **Figure 4.3** shows the comparison results of the SPME absorption at different extraction times. As a result, 25 minutes was chosen as the optimized condition for the SPME extraction.

3.2 Identification and Quantification of Volatile Compounds by GC-MS Method

Volatile compounds that were identified by GC-MS are listed in **Table 4.1**. Total 33 chemicals, including 18 aldehydes, 2 alcohols, 3 ketones, 5 acids and 5 esters, were detected, but not all of them were detected in all the cultivars. Except the cultivar HP that contained all the 6 detected esters, other cultivars only contained a few of them. Aldehyde and acids were the major volatile compounds in jujube fruits, which together accounted for more than 95% (see **Table 4.2**) of the volatile compounds in most cultivars. Alcohol and ketones were identified in most cultivars, but their combined contents only accounted for a small percentage.

Aldehydes consisted of the largest group of the volatile compounds in jujube fruits. According the comparison of their mass spectra and retention index with those in the NIST library and standards, 18 aldehydes were identified, including (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, heptanal, (*Z*)-2-heptenal, benzaldehyde, 2-phentyl furan, octanal, benzeneacetaldehyde, (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, decanal, (*E*)-2-decenal, 10-undecenal, undecanal, 2-undecenal and dodecanal (**Table 4.1**). According to **Table 4.2**, hexanal, (*E*)-2-hexenal, nonanal, and decanal were the major aldehydes in the jujube

fruits. This result is in agreement with the report by Hernandez et al. (*24*). The concentrations of the aldehydes were in significant difference ($p<0.05$). Concentration of hexanal was in a range from 276.5 to 1314 μg/100g FW; concentration of (*E*)-2-hexenal varied from 145.1 to 1876 μg/100g FW; nonanal was another major aldehyde, its amount was from 188.2 to 1047 μg/100g FW; the content of decanal was not as much as the other three aldehydes mentioned above, but its content was still higher than the other remaining aldehydes detected in jujube, of which the amount was from 73.77 to 246.1 μg/100g FW. Benzeneacetaldehyde was not detected in the cultivars of BJ, DB, HP, JD, LZ and NP. 10-Undecenal was not detected in HP, JB, JS, LB, PZ and YL. Similarly, 2-undecenal was found in the HP, JB, LB and YL, dodecanal was not detected in JB, LB and YL, and 2-pentenal was detected in all the cultivars except JB. Except major aldehydes including hexanal, (*E*)-2-hexenal, nonanal and decanal, the content of the other aldehydes in most cultivars was less than 100 μg/100g FW (see **Table 4.3**).

Only two alcohols in fresh jujube fruit were identified, they are 1-octen-3-ol and benzyl alcohol (**Table 4.1**). The former was identified in all cultivars except LB, and the content of this alcohol was in a range from 2.60 to 16.33 μg/100g FW. The latter was not identified in the cultivars of BZ, XZ, YL and YZ. Its content in other cultivars varied from 2.47 to 95.38 μg/100g FW (**Table 4.3**).

Ketones were not the major compounds in the jujube fruits. Only three ketones were identified, they were 2-nonanone, 2-undecanone and 6,10-dimethyl, 5,9-undecadien-2-one. As shown in the **Table 4.3**, 2-nonanone and 6,10-dimethyl-5,9-undecadien-2-one were found in all cultivars, which ranged from 3.93 to 98.71 μg/100g

FW and 12.12 to 277.5 μg/100g FW, respectively. 2-Undecanone was identified in all the cultivars except YZ, with a range from 3.94 to 80.16 μg/100g FW.

Five short chain organic acids were identified in jujube fruits (**Table 4.1)**, including hexanoic acid, octanoic acid, nonanoic acid, n-decanoic acid, and dodecanoic acid. Among them, octanoic acid was detected in all cultivars except BJ, DB and XZ, nonanoic acid was not detected in BJ, DB, HP, JB, LB and YZ (**Table 4.3)**, and their contents were lower than that of the other acids. n-Decanoic acid and dodecanoic acid were identified as the major acids in all the cultivars. The content of n-decanoic acid ranged from 58.42 (YZ) to 1268 (YL) μg/100g FW. In comparison, the content of dodecanoic acid in the two cultivars, i.e., YL and JD, had a relatively higher amount in 1319 and 693 μg/100g FW, respectively, but its content in the other cultivars was lower than that of n-decanoic acid in the jujube fruits.

Esters were thought to be the most important aroma compounds in jujube brandy wine (*28*), which contributed a major portion in 81.7 % of all the volatile compounds (*29*). However, esters only accounted for a small portion in the fresh jujube fruit. As shown in **Table 4.1**, hexanoic acid methyl ester, hexanoic acid ethyl ester, benzoic acid ethyl ester, octanoic acid ethyl ester, and dodecanoic acid methyl ester were identified, but they were only found in a few cultivars (**Table 4.3)**, and their contents were very low too, except the cultivar HP and JB, in which the percentage of total esters in all volatile compounds were 15.64 % and 17.50%, respectively (**Table 4.2**)

3.3 Hierarchical Cluster Analysis and Principal Component Analysis

In multivariate statistics, principal component analysis (PCA) and hierarchical cluster analysis (HCA) are unsupervised methods, which do not request the prior information (*30*). These two methods were used in this study to determine the similarity of the cultivars based on the contents of the identified volatile chemicals, so as to classify the jujube cultivars based on their geographic origins.

The HCA method was calculated based on ward's method. As profiled in **Figure 4.4** and **Figure 4.5**, 15 cultivars were classified into five groups. Cluster 1 that includes the cultivars LB, HP, LZ, NP, PZ, JS, and YL contains relatively lower contents of volatile compounds. Cluster 2 includes BJ and DB, which had a higher concentration of aldehydes than cluster 1. Cluster 3 includes PB, BZ, JD and XZ, which contain similar contents of aldehydes, ketones, alcohols and esters as cluster 2, but higher content of acids than cluster 2. Cluster 4 only has the cultivar JB because it had very low contents of some aldehydes but higher contents of esters; similarly, cluster 5 only has the cultivar YZ because of its low contents of aldehydes, acids and esters.

PCA was used to decrease the dimension of data variance, which was calculated based on the correlation of contents of volatile compounds in this study. According to the eigenvalue shown in the **Figure 4.6 A**, there were eight eigenvalues large than 1, which suggested to use these eight principal components for further data analysis after the reduced dimension. However, two dimensions (or top two principal components, i.e., PC1 and PC2) were used in order to simplify the statistical analysis and obtain a planner score plot of PCs. According to **Figure 4.6 B**, PC1 and PC2 together can explain 42.7 % of total data variance that means some information of the volatile compounds have been

missed during the statistical re-modeling. As shown in the loading plot (**Figure 4.6 C**), the shorter the arrow, the more information has been lost in PCA. In this context, alcohols (benzyl alcohol and 1-octen-3-ol) and esters were the major groups of the volatile compounds that have lost the most information. In **Figure 4.6 B**, clusters 1, 2, 3 were close to each other, which cannot be distinguished from each other according to score plot in this PCA. However, based on the same PCA, the cluster 4 and cluster 5 were separated very well. In more details shown in the biplot (**Figure 4.7**), benzoic acid ethyl ester, octanoic acid ethyl ester and 2-undecanone are positively related to the cluster 4 (JB); (*E*)-2-decenal and (*E*)-2-nonenal are positively related to the cluster 5 (YZ); 10-undecenal, hexanal and 2-pentenal are positively related to the cluster 3; 2-pentenal is positively related to the cluster 2; octanal, octanoic acid and benzeneacetaldehyde are positively related to the cluster 1. These correlations are the base for the classification of different cultivars into different clusters.

Figure 4.8 explains how the clusters are close to each other. The density ellipse of each cluster was calculated under the 95% confidence interval. Obviously, the clusters 1, 2 and 3 are crossed over together so that they cannot be discriminated from each other. However, the cluster 4 and cluster 5 are separated far way, demonstrating they are significantly different from other three clusters. Therefore, only cultivar JB in the cluster 4 and YZ in the cluster 5 can be distinguished from other 13 cultivars based on the volatile compounds analysis.

3.4 Comparison of SDE and SPME on Chemical Extraction

Table 4.4 lists the major compounds which were extracted by SDE and SPME from the dried jujube fruits. The SDE method has extracted more efficiently the large molecular compounds, or less volatile chemicals, such as pyrazines (no pyryzine in SPME method), and a less percentage of aldehyde chemicals than SPME method; By the SDE method, the major aldehydes in the extract were (*E*)-2-hexenal and hexanal, while nonanal and benzaldehyde were found to be the major aldehydes in SPME method. Besides, even these two methods have extracted similar volatile compounds, the amount of the extracted volatile chemicals were quite different. SDE method could not extract octanoic acid, nonanoic acid, n-decanoic acid and dodecanoic acid, except hexanoic acid.

4. Conclusion

Overall, based on the SPME method, a total of 33 compounds were identified by the GC-MS, including aldehydes, alcohols, esters, acids and ketones. The contents of alcohols and esters were very low in jujube fruit, as well as ketones. The major volatile compounds of fresh jujube fruits were found to be aldehydes and acids, including hexanal, (*E*)-2-hexenal, nonanal, and n-decanoic acid that were the major volatile compounds based on their contents. In addition, based on the HCA and PCA, the 15 jujube cultivars could be classified into five clusters, cluster 1, cluster 2 and cluster 3 were not discriminated, while cluster 4 and cluster 5 could be discriminated from each other, also from the other three clusters. The comparison of the extraction efficiency of SDE and SPME showed that aldehydes were the most major compounds in both methods, except that the SDE method extract higher amount of alcohols, ketones and pyrazines than

SPME method, while the SPME method extracted higher amount of acids than SDE method.

Moreover, based on the HCA and PCA, the different cultivars of jujube fruits planted in the same place could not be differentiated well based on amount and class of the identified volatile compounds, this might be ascribed to the elimination of the effect of environmental factors. Therefore, classification of the samples from different locations based on other environmental factors such as soil, climate, etc., should be considered for further study.

Table 4. 1 Volatile Compounds in 15 Cultivars of Jujube Fruit Extracted by SPME, and the Identification by GC-MS

Retention time (min)	Chemical name	RI				Sensory descriptors
		EX	Reference			
			a	b	c	
4.48	(*E*)-2-Pentenal	<800			754	
5.45	Hexanal	803	802	803	800	fatty, green
6.88	(*E*)-2-Hexenal	860	855	855	860	apple, green, sweet
8.23	Heptanal	905	902	906	904	oily, woody, nutty
8.95	Hexanoic acid, methyl ester	931	927		932	
9.91	(*Z*)-2-Heptenal	962			964	
10.05	Benzaldehyde	967	960	981	966	almond, cherry, sweet
10.69	1-Octen-3-ol	986	979		986	
10.99	Furan, 2-pentyl-	994			993	
11.26	Hexanoic acid, ethyl ester	1002	998		1002	Floral, fruity, apple peel, pear
11.34	Octanal	1005	999	1009	1004	honey, fruity, citrus
11.62	Hexanoic acid	1015			1014	
12.53	Benzyl Alcohol	1046	1032		1042	
12.66	Benzeneacetaldehyde	1050	1042		1049	Hawthorne, honey, sweet
13.06	(*E*)-2-Octenal	1063		1067	1064	spicy, berbaceous
14.10	2-Nonanone	1095	1090		1096	
14.47	Nonanal	1107	1101	1113	1102	lemon, oily
16.13	(*E*)-2-Nonenal	1164	1162		1162	
16.54	Benzoic acid, ethyl ester	1177			1171	Ripen fruit
16.93	Octanoic acid	1190			1192	oily
17.23	Octanoic acid, ethyl ester	1199			1197	sweet, floral, fruity, pear
17.46	Decanal	1208	1202	1218	1208	wax, floral, citrus
19.07	(*E*)-2-Decenal	1266	1264	1277	1252	oil, floral, citrus
19.65	Nonanoic acid	1285	1271		1280	

Retention time (min)	Chemical name	RI				Sensory descriptors
		EX	Reference			
			a	b	c	
19.95	2-Undecanone	1295	1294		1294	
20.01	10-Undecenal	1297	1300		1297	
20.31	Undecanal	1309	1307		1308	orange, fatty, rose, waxy
21.86	2-Undecenal	1368			1368	
22.25	n-Decanoic acid	1382			1387	Fatty, citrus
23.01	Dodecanal	1411	1409		1412	
24.16	5,9-Undecadien-2-one, 6,10-dimethyl	1457			1452	
25.90	Dodecanoic acid, methyl ester	1527			1527	
27.02	Dodecanoic acid	1574	1567		1576	

RI, represented the retention index; EX, represented the experiment, by which the RI were calculated in this study; Reference a, the RI were calculated based on DB-5 column; Reference b was cited from Hernandez *et al.*, 2015; Reference c was cited from NIST (National Institute of Standards and Technology Standard Reference Database, Gaithersburg, USA); sensory descriptors were cited from Hernandez *et al.*, 2015, Shu *et al.*, 2014 and Andreu-Sevilla *et al.*, 2013 (*31*)

Table 4. 2 Percentage of Major Volatile Compounds and Different Chemical Groups in Total Volatile Compounds, %

Cultivars	Major volatile compounds					Different groups of volatile compounds				
	Hexanal	(*E*)-2-Hexenal	Nonanal	n-Decanoic acid	Dodecanoic acid	Aldehydes	Alcohols	Ketones	Acids	Esters
BJ	24.35±0.47	28.22±0.31	11.76±0.42	6.17±0.13	2.59±0.04	86.77±0.17	1.78±0.09	0.48±0.03	10.93±0.17	0.04±0.00
BZ	14.03±0.10	28.67±1.29	8.73±0.22	20.05±1.91	4.21±0.06	68.37±1.74	0.13±0.01	1.64±0.08	29.59±1.81	0.27±0.01
DB	15.48±0.51	28.23±0.26	8.94±0.30	7.18±0.31	3.96±0.19	77.82±0.47	2.14±0.07	1.10±0.03	14.98±0.36	3.95±0.19
HP	12.21±0.23	24.29±0.34	6.16±0.13	7.99±0.40	0.96±0.05	63.85±0.42	1.22±0.04	3.86±0.04	15.42±0.53	15.64±0.76
JB	1.23±0.04	2.91±0.04	11.14±0.22	13.54±0.48	21.08±0.23	39.23±0.51	1.05±0.05	5.41±0.23	36.55±0.55	17.50±0.04
JD	16.35±0.13	15.55±0.34	13.03±0.34	17.13±0.45	13.92±0.17	64.39±0.40	0.23±0.01	1.69±0.03	33.61±0.41	0.07±0.00
JS	15.86±0.23	16.10±0.34	8.01±0.21	31.16±0.63	4.28±0.23	59.37±0.38	1.05±0.01	1.42±0.06	38.09±0.43	0.07±0.00
LB	14.67±0.43	38.50±0.08	10.48±0.27	6.99±0.09	2.86±0.03	79.61±0.17	0.51±0.04	3.23±0.20	15.34±0.21	1.31±0.03
LZ	17.92±0.59	23.40±0.46	8.77±0.75	13.85±0.30	4.22±0.31	72.60±0.47	0.97±0.05	1.96±0.06	24.29±0.41	0.17±0.00
NP	13.16±0.26	19.07±0.27	10.20±0.41	25.30±0.58	2.84±0.07	62.45±0.45	2.01±0.04	2.38±0.05	32.91±0.51	0.24±0.01
PB	14.53±0.26	33.53±0.35	4.92±0.08	14.32±0.06	2.50±0.22	74.48±0.37	0.69±0.03	2.25±0.07	22.00±0.31	0.58±0.03
PZ	13.74±0.21	26.39±0.23	12.51±0.17	22.34±0.21	3.98±0.13	68.97±0.27	0.23±0.00	0.79±0.01	29.88±0.26	0.12±0.01
XZ	12.49±0.44	41.92±0.45	7.39±0.06	12.49±0.35	0.38±0.02	79.91±0.28	0.20±0.01	1.77±0.02	17.86±0.29	0.26±0.01
YL	6.32±0.20	11.09±0.31	4.76±0.18	26.43±0.86	27.44±1.30	35.82±0.88	0.12±0.00	2.99±0.09	60.56±0.99	0.49±0.02
YZ	10.16±0.79	9.67±0.67	39.29±1.13	2.14±0.08	0.31±0.01	82.63±0.09	0.58±0.02	10.76±0.12	5.34±0.05	0.68±0.05

Data were represented as mean value ± standard error

Table 4. 3 Concentration of Volatile Compounds in Jujube Fruits, μg/100g FW

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Aldehydes															
(*E*)-2-Pentenal	22.66±1.90 b	27.71 ± 0.42 a	14.12 ± 0.91 de	11.77 ± 0.48 defg	n.d.	15.38 ± 1.39 cd	13.43 ±0.92 def	14.19 ±0.80 de	15.79 ± 0.71 cd	9.27 ± 0.40 fg	27.79 ±0.66 a	10.67 ± 0.69 efg	18.74 ± 0.41 bc	8.47 ± 0.09 g	4.03 ± 0.25 h
Hexanal	1314±28 a	830.7 ± 21.3 b	763.1 ± 20.5 b	372.7 ± 11.3 ef	61.25 ± 3.02 h	814.6 ± 12.0 b	562.0 ±10.2 d	575.5 ±21.8 d	541.6 ± 15.0 d	431.1 ± 12.2 e	675.5 ±20.1 c	438.7 ± 10.9 e	558.8 ± 18.1 d	303.3 ±5.3 fg	276.5 ± 15.8 g
(*E*)-2-Hexenal	1524±12 c	1694 ±53 b	1392 ±27 d	741.8 ± 15.6 efg	145.1 ± 2.37 i	774.7 ± 20.8 ef	570.4 ±9.7 h	1510 ±33 cd	707.5 ± 16.8 fg	624.6 ± 14.2 gh	1557 ±19 c	842.6 ± 11.4 e	1876 ±30 a	531.9 ± 13.1 h	263.5 ± 14.9 i
Heptanal	77.57± 1.91 b	67.74 ± 1.63 bc	64.07 ± 2.94 bcd	39.13 ± 2.12 defg	67.39 ± 2.20 bc	64.45 ± 3.74 bcd	44.09 ±1.48 cdefg	57.82 ±2.33 bcdef	30.60 ± 3.13 g	31.68 ± 0.63 fg	60.36 ± 16.78 bcde	37.55 ± 0.95 efg	131.9 ±5.6 a	36.11 ± 1.40 efg	27.00 ± 2.51 g
(*Z*)-2-Heptenal	53.34± 4.83 cde	87.21 ± 2.81 b	63.82 ± 2.78 c	45.43 ± 1.88 def	25.15 ± 1.35 g	114.3 ±6.7 a	62.15 ±3.61 cd	37.33 ±0.46 efg	50.18 ± 2.35 cde	29.47 ± 1.51 fg	94.92 ±6.47 b	30.04 ± 1.25 fg	58.11 ± 1.16 cd	27.85 ± 0.50 g	26.59 ± 3.34 g
Benzaldehyde	342.5± 12.4 b	106.1 ±5.2 cde	455.7 ±8.4 a	190.1 ±8.6 c	512.0 ± 13.5 a	48.58 ± 0.44 def	84.11 ±3.04 def	57.88 ±2.96 def	177.6 ± 61.1 c	136.4 ±4.7 cd	109.5 ±4.7 cde	10.24 ± 0.36 f	68.51 ± 3.01 def	44.87 ± 0.96 ef	13.47 ± 0.60 f
Furan, 2-pentyl-	56.52± 5.05 de	116.8 ±1.5 b	65.74 ± 1.06 d	40.04 ± 3.96 f	20.25 ± 1.00 g	84.56 ± 2.07 c	53.65 ±2.10 e	59.28 ±1.80 de	56.95 ± 1.26 de	38.42 ± 1.81 f	164.0 ±3.2 a	28.78 ± 1.41 fg	67.09 ± 0.70 d	25.18 ± 0.35 g	59.66 ± 1.07 de
Octanal	356.9± 10.3 a	104.1 ±1.8 d	108.2 ±4.1 d	37.49 ± 1.19 f	134.5 ±4.1 c	173.1 ±3.6 b	110.1 ±4.8 d	122.5 ±2.4 cd	70.36 ± 2.40 e	136.6 ±6.1 c	n.d.	182.7 ±3.4 b	63.92 ± 2.31 e	172.4 ±3.0 b	22.25 ± 1.63 f
Benzeneacetaldehyde	n.d.	10.89 ±	n.d.	n.d.	32.13 ±	3.96 ±	n.d.	7.39± 0.12 d	n.d.	n.d.	15.72 ±0.31	4.48 ±	8.79 ±	5.30 ±	4.67 ±

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
		0.43 c			1.27 a	0.36 e					b	0.16 e	0.38 d	0.06 e	0.23 e
(*E*)-2-Octenal	87.08± 6.78 de	111.2 ±3.7 bc	124.3 ±2.3 ab	103.4 ±3.5 cd	81.27 ± 3.17 e	115.9 ±5.2 abc	80.21 ±2.85 e	57.14 ±1.45 f	80.49 ± 1.65 e	56.85 ± 2.05 f	131.4 ±5.38 a	41.35 ± 3.01 f	53.73 ± 1.24 f	57.55 ± 2.61 f	49.09 ± 0.79 f
Nonanal	635.0± 21.3 b	516.6 ± 16.5 cd	440.9 ± 17.0 de	188.2 ±6.5 i	555.7 ± 16.6 bc	649.2 ± 16.1 b	284.3 ±15.9 gh	411.1 ±13.7 ef	264.1 ± 16.4 ghi	333.8 ± 10.3 fg	228.3 ±3.7 hi	399.6 ±8.5 ef	330.8 ±2.7 fg	228.0 ±3.4 hi	1047 ±50 a
(*E*)-2-Nonenal	47.79± 3.71 bcd	50.16 ± 0.57 bcd	71.98 ± 2.24 bc	42.26 ± 2.50 cd	77.24 ± 2.00 bc	83.73 ± 1.18 b	44.50 ±1.22 cd	35.41 ±1.06 d	41.34 ± 2.38 cd	27.13 ± 0.78 d	61.72 ± 15.98 bcd	26.92 ± 0.68 d	50.74 ± 1.94 bcd	41.18 ± 0.99 cd	201.6 ± 20.5 a
Decanal	131.5±4.4 cde	210.3 ±3.8 b	215.6 ± 11.7 ab	100.6 ±3.2 ef	192.8 ±6.5 b	192.2 ±2.9 b	151.4 ±12.6 c	150.3 ±4.9 c	110.8 ±2.9 de	139.2 ±2.9 cd	246.1 ±9.6 a	121.2 ±5.1 cde	200.3 ±9.0 b	143.3 ±2.6 cd	73.77 ± 4.33 f
(*E*)-2-Decenal	6.57±0.71 b	11.14 ± 0.43 b	8.83 ± 0.19 b	11.17 ± 0.33 b	13.95 ± 1.58 b	12.76 ± 1.60 b	5.71± 0.38 b	7.31± 0.32 b	6.42 ± 0.16 b	6.65 ± 0.32 b	13.08 ±0.77 b	4.36 ± 0.28 b	6.06 ± 0.24 b	9.31 ± 0.24 b	64.58 ± 6.71 a
10-Undecenal	4.24±0.35 d	24.64 ± 2.09 a	4.33 ± 0.22	n.d.	n.d.	7.40 ± 0.22 bcd	n.d.	n.d.	5.16 ± 0.22 cd	4.87 ± 0.17 cd	8.57± 0.67 bc	n.d.	26.53 ± 1.62 a	n.d.	11.12 ± 0.43 b
Undecanal	15.55± 1.24 g	50.51 ± 4.28 b	27.93 ± 1.63 ef	16.53 ± 0.33 g	37.98 ± 0.72 cd	27.97 ± 3.12 ef	26.12 ±0.54 f	19.68 ±1.82 fg	21.62 ± 0.53 fg	28.92 ± 2.09 def	36.33 ±1.41 de	15.04 ± 1.00 g	46.82 ± 1.04 bc	82.86 ± 0.85 a	81.03 ± 1.34 a
2-Undecenal	4.67±0.51 d	7.47 ± 0.24 c	7.67 ± 0.56 c	n.d.	n.d.	11.38 ± 0.42 b	4.13± 0.10 d	n.d.	4.70 ± 0.03 d	3.87 ± 0.14 de	8.39± 0.59 c	1.85 ± 0.07 ef	3.44 ± 0.19 de	n.d.	27.45 ± 1.06 a
Dodecanal	5.21±0.85 g	15.93 ± 0.12 b	9.70 ± 0.59 de	8.49 ± 0.21 ef	n.d.	13.65 ± 0.88 bc	8.74± 0.69 de	n.d.	11.42 ± 0.97 cd	5.80 ± 0.18 fg	21.51 ±1.08 a	5.68 ± 0.31 fg	5.60 ± 0.21 fg	n.d.	4.07 ± 0.25 g

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Alcohols															
1-Octen-3-ol	10.34±0.15 c	7.59±0.48 de	10.02±0.63 c	16.33±0.59 b	14.05±0.70 b	3.78±0.22 gh	2.60±0.20 h	n.d.	4.05±0.02 gh	6.50±0.16 def	27.50±0.86 a	4.83±0.13 fgh	8.76±0.29 cd	5.95±0.12 efg	15.80±0.77 b
Benzyl alcohol	85.66±5.24 a	n.d.	95.38±3.80 a	21.09±1.29 e	38.22±4.03 c	7.52±0.22 f	34.66±1.18 cd	19.91±2.17 e	25.24±1.01 de	59.48±1.62 b	4.41±0.14 f	2.47±0.16 f	n.d.	n.d.	n.d.
Acids															
Hexanoic acid	116.8±5.42 ef	222.6±20.1 a	189.5±5.7 abc	155.4±5.1 cd	n.d.	94.09±1.77 fg	54.51±1.61 h	197.1±6.6 ab	151.2±3.2 de	74.84±2.95 gh	189.4±5.3 abc	56.41±2.15 h	205.5±7.1 ab	172.4±3.0 bcd	57.93±5.23 h
Octanoic Acid	n.d.	83.88±5.15 c	n.d.	42.03±3.54 de	99.38±1.84 b	19.34±2.51 f	35.80±2.40 e	18.25±1.32 f	29.39±2.17 ef	79.17±2.87 c	42.75±1.93 de	52.09±1.99 d	n.d.	136.4±5.1 a	21.12±1.36 f
Nonanoic acid	n.d.	9.46±1.16 c	n.d.	n.d.	n.d.	14.83±1.53 b	3.81±0.14 ef	n.d.	7.50±0.13 cd	1.89±0.04 fg	8.95±0.19 c	5.35±0.17 de	17.58±0.42 a	12.81±0.25 b	n.d.
n-Decanoic acid	333.5±8.3 e	1189±129 a	354.2±13.9 de	244.2±16.3 ef	674.6±12.9 bc	852.7±17.4 b	1106±56 a	274.4±9.2 e	419.6±20.4 de	828.1±17.2 b	665.3±8.7 bc	713.3±11.4 bc	558.9±14.4 cd	1268±50 a	58.42±1.50 f
Dodecanoic acid	139.9±1.8 defg	249.4±1.9 d	195.1±7.2 de	29.39±2.03 fgh	1048±24 b	693.3±7.4 c	151.4±4.0 def	112.2±2.6 efgh	127.3±7.7 defgh	93.20±1.58 efgh	115.7±9.2 efgh	126.9±5.2 defgh	17.00±0.57 gh	1319±89 a	8.52±0.04 h
Esters															
Hexanoic acid, methyl ester	n.d.	5.26±0.10 a	n.d.	2.67±0.04 c	n.d.	n.d.	n.d.	5.43±0.25 a	1.96±0.05 d	n.d.	3.66±0.17 b	n.d.	2.50±0.19 c	2.83±0.09 c	n.d.
Hexanoic acid, ethyl ester	n.d.	n.d.	n.d.	37.35±1.30 a	n.d.	n.d.	n.d.	16.49±1.14 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Benzoic acid, ethyl ester	2.02±0.08 c	n.d.	n.d.	424.2 ± 23.8 b	822.9 ± 21.2 a	n.d.	n.d.	8.74± 0.81 c	n.d.	n.d.	17.70 ±0.96 c	3.85 ± 0.20 c	n.d.	n.d.	18.67 ± 1.33 c
Octanoic acid, ethyl ester	n.d.	n.d.	n.d.	10.59 ± 0.66 c	31.02 ± 2.47 a	n.d.	n.d.	14.37 ±1.03 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dodecanoic acid, methyl ester	n.d.	10.60 ± 0.87 d	195.1 ±7.2 a	2.34 ± 0.17 de	31.26 ± 1.54 b	3.46 ± 0.19 de	2.57± 0.06 de	6.42± 0.34 de	3.24 ± 0.17 de	7.88 ± 0.57 de	5.75± 0.20 de	n.d.	9.33 ± 0.13 de	20.88 ± 0.46 c	n.d.
Ketones															
2-Nonanone	3.93±0.08 i	14.62 ± 0.32 fgh	8.61 ± 0.37 hi	57.11 ± 1.61 c	98.71 ± 3.91 a	10.66 ± 0.19 ghi	8.66± 0.12 hi	87.99 ±2.59 b	10.17 ± 0.34 ghi	11.22 ± 0.38 gh	32.99 ±0.88 d	7.51 ± 0.24 hi	21.89 ± 1.33 e	21.06 ± 0.21 ef	16.57 ± 0.54 efg
2-Undecanone	3.94±0.32 ij	15.65 ± 1.01 ef	6.38 ± 0.11 hi	24.37 ± 1.78 d	80.16 ± 2.65 a	13.86 ± 1.22 fg	13.16 ±0.56 fg	21.55 ±1.62 de	7.85 ± 0.25 ghi	9.99 ± 1.05 fgh	14.24 ±0.35 f	5.74 ± 0.27 hij	33.74 ± 0.37 c	62.03 ± 1.74 b	n.d.
5,9-Undecadien-2-one, 6,10-dimethyl-	18.09± 1.28 hi	66.92 ± 3.24 c	39.38 ± 1.57 efg	36.34 ± 2.27 fgh	91.47 ± 10.49 b	59.58 ± 1.15 cde	28.32 ±1.15 ghi	16.99 ±1.71 hi	41.30 ± 0.46 defg	56.80 ± 1.55 cdef	57.41 ±1.56 cde	12.12 ± 0.33 i	23.79 ± 1.32 ghi	60.64 ± 0.77 cd	277.5 ± 10.1 a

Data were represented by mean value ± standard error;

n.d. means not detected;

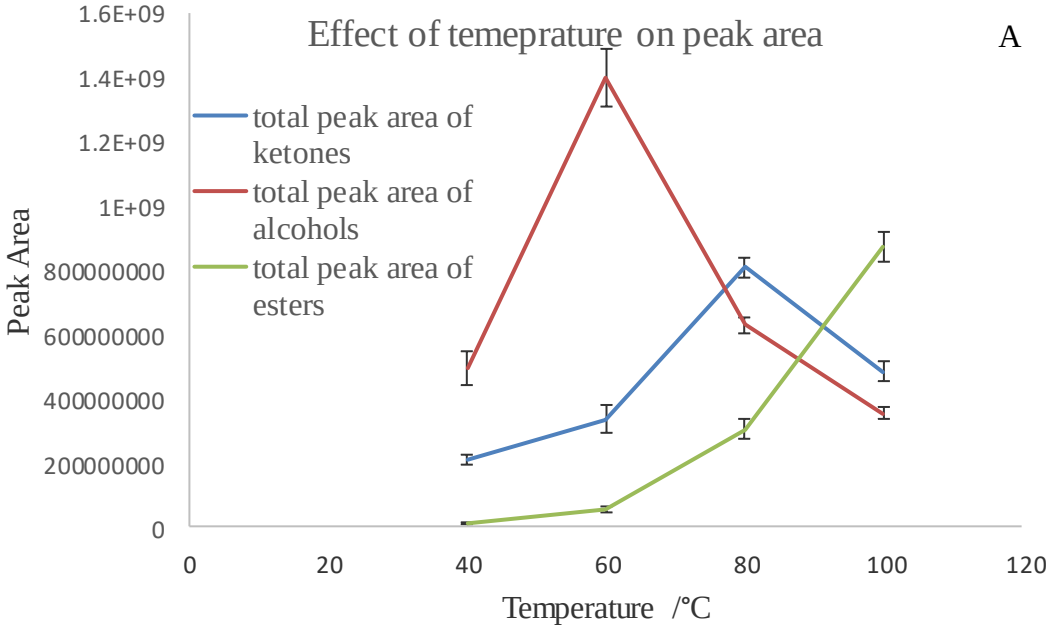
The same letters in the same row followed the data means no significant difference, (p<0.05)

Table 4. 4 Comparison of SDE and SPME, % of Major Volatile Compounds in Total Volatiles (percentage of volatile compounds < 1% were not included)

SDE			SPME		
Chemical name	%	SD	Chemical name	%	SD
Hexanal	9.50	0.17	n.d.		
(*E*)-2-Hexenal	13.38	0.23	(*E*)-2-Hexenal	1.16	0.04
(*E*)-2-Heptenal	1.09	0.02	(*Z*)-2-Heptenal	0.61	0.00
Octanal	1.70	0.03	Octanal	7.66	0.08
Nonanal	1.30	0.02	Nonanal	28.54	0.16
(*E*)-2-Octenal	4.23	0.08	(*E*)-2-Octenal,	2.13	0.05
Decanal	n.d.		Decanal	2.59	0.04
Benzaldehyde	1.28	0.02	Benzaldehyde	17.13	0.20
n.d.			Furfural	1.85	0.10
n.d.			Heptanal	4.09	0.14
1-Penten-3-ol	6.74	0.13	n.d.		
Hexanol	4.09	0.08	n.d.		
Benzyl alcohol	10.87	0.11	Benzyl alcohol	2.13	0.09
Phenylethyl alcohol	1.39	0.023	n.d.		
2,3-Butanedione	13.31	0.27	2-Butanone, 3-hydroxy-	2.24	0.07
n.d.			2-Undecanone	2.89	0.08
hexanoic acid	3.68	0.07	Hexanoic acid	4.89	0.12
n.d.			Octanoic acid	3.06	0.20
n.d.			Nonanoic acid	1.41	0.05
n.d.			n-Decanoic acid	3.99	0.13
n.d.			Dodecanoic acid	1.66	0.09

SDE			SPME		
Chemical name	%	SD	Chemical name	%	SD
ethyl hexanoate	2.48	0.04	n.d.		
2-methypyrazine	1.90	0.04	n.d.		
2,5-dimethylpyrazine	4.75	0.09	n.d.		
2,6-dimethylpyrazine	9.49	0.16	n.d.		
2-ethylpyrazine	2.76	0.06	n.d.		
trimethylpyrazine	1.80	0.03	n.d.		

SD means standard deviation; n.d. means not detected



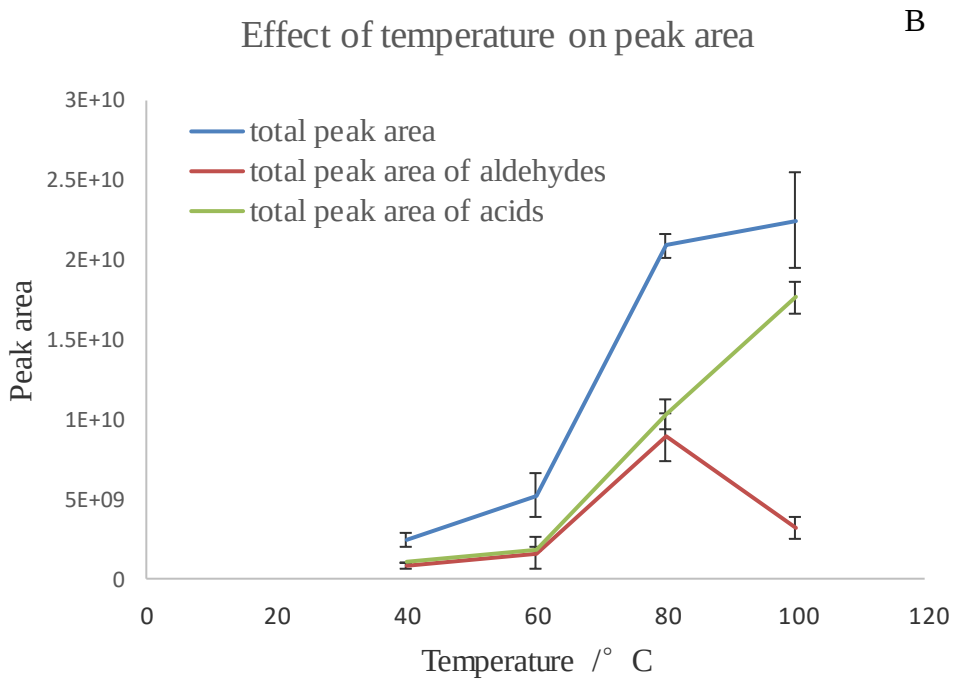


Figure 4. 1 Optimization of Incubation Temperature, Fixed Incubation Time for 30 min, Extraction Time for 25 min

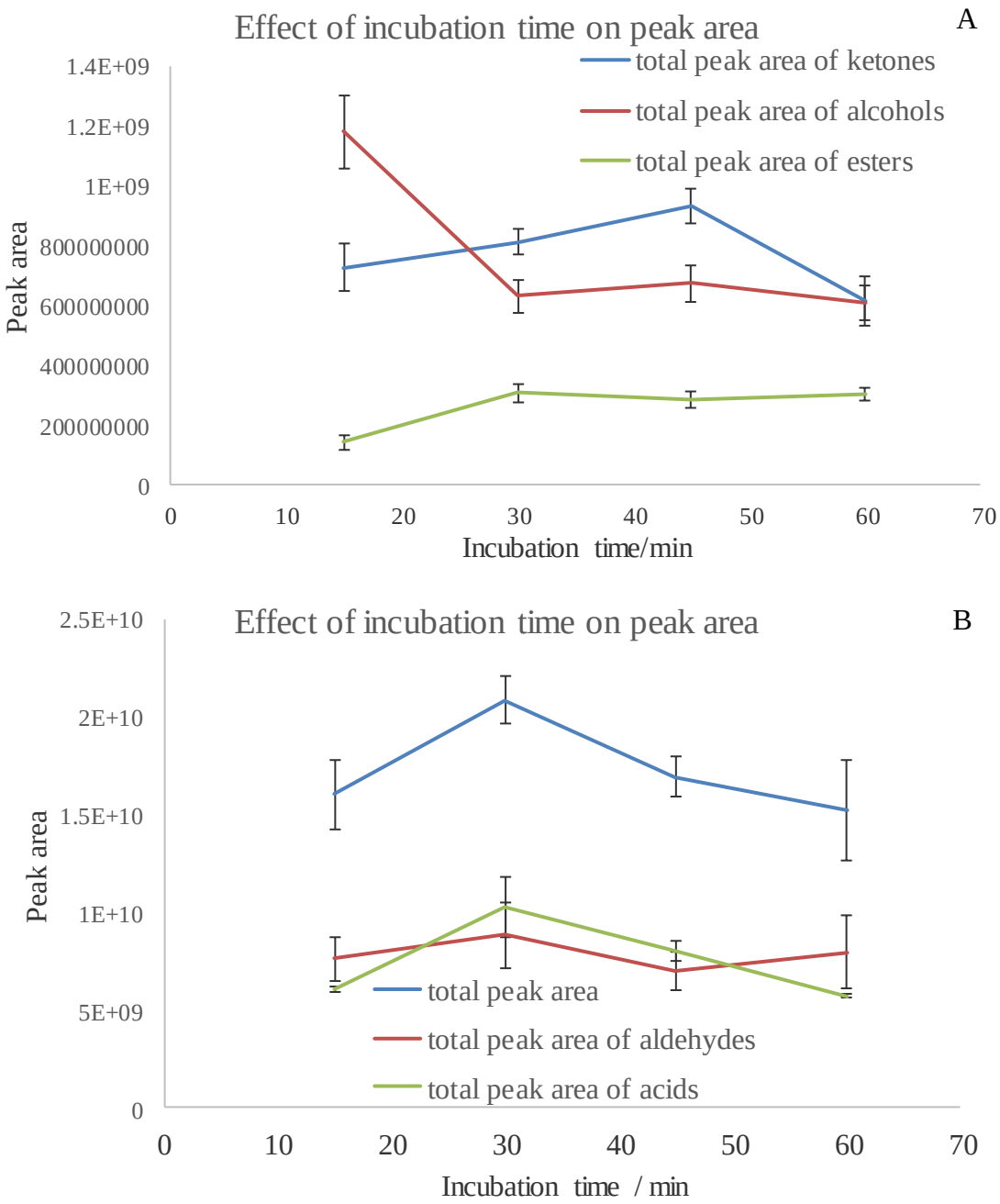


Figure 4. 2 Optimization of Incubation Time, Fixed Incubation Temperature at 80 °C, Extraction Time for 25 min

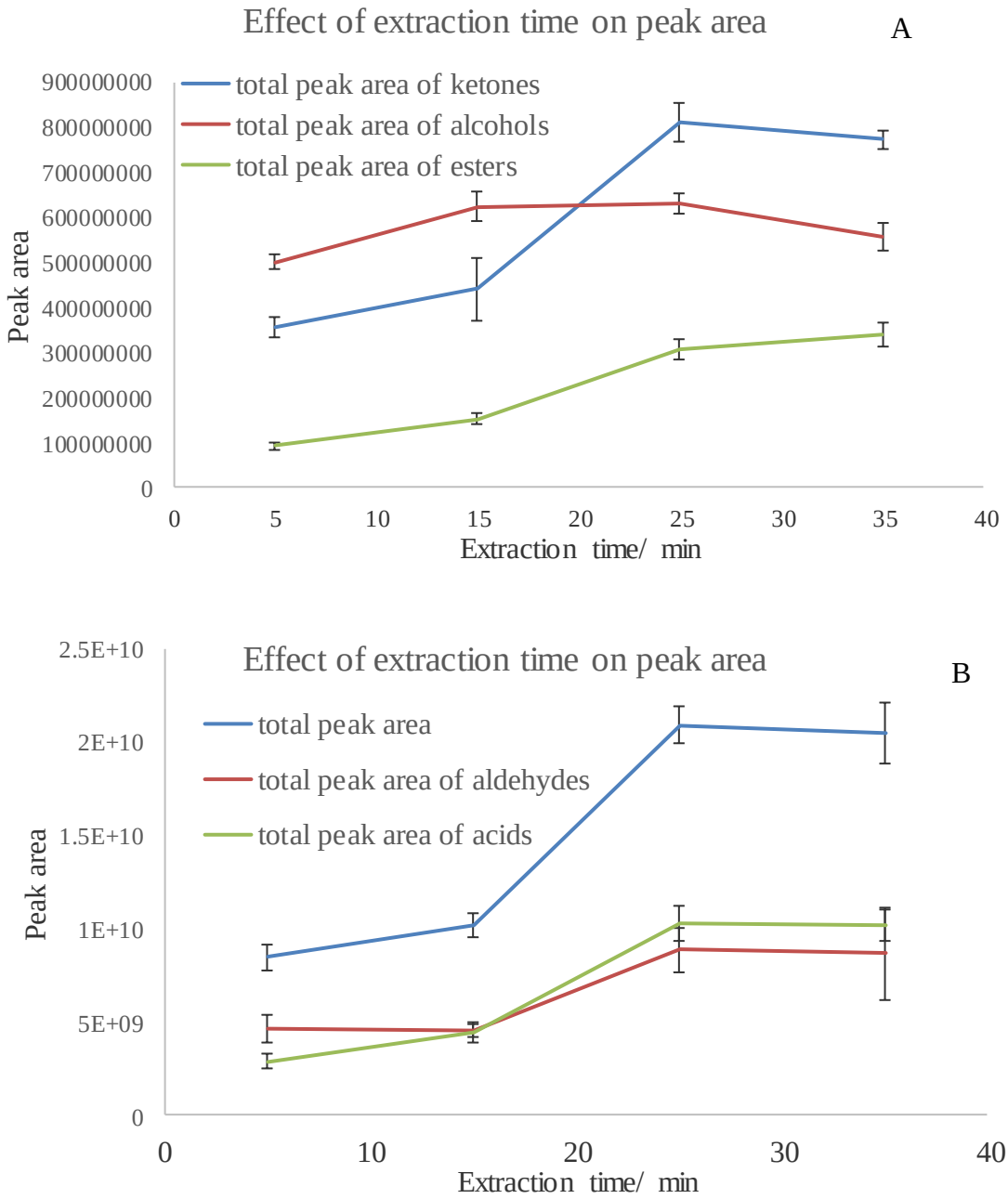


Figure 4. 3 Optimization of Extraction Time, Fixed Incubation Temperature at 80 °C, Incubation Time for 30 min

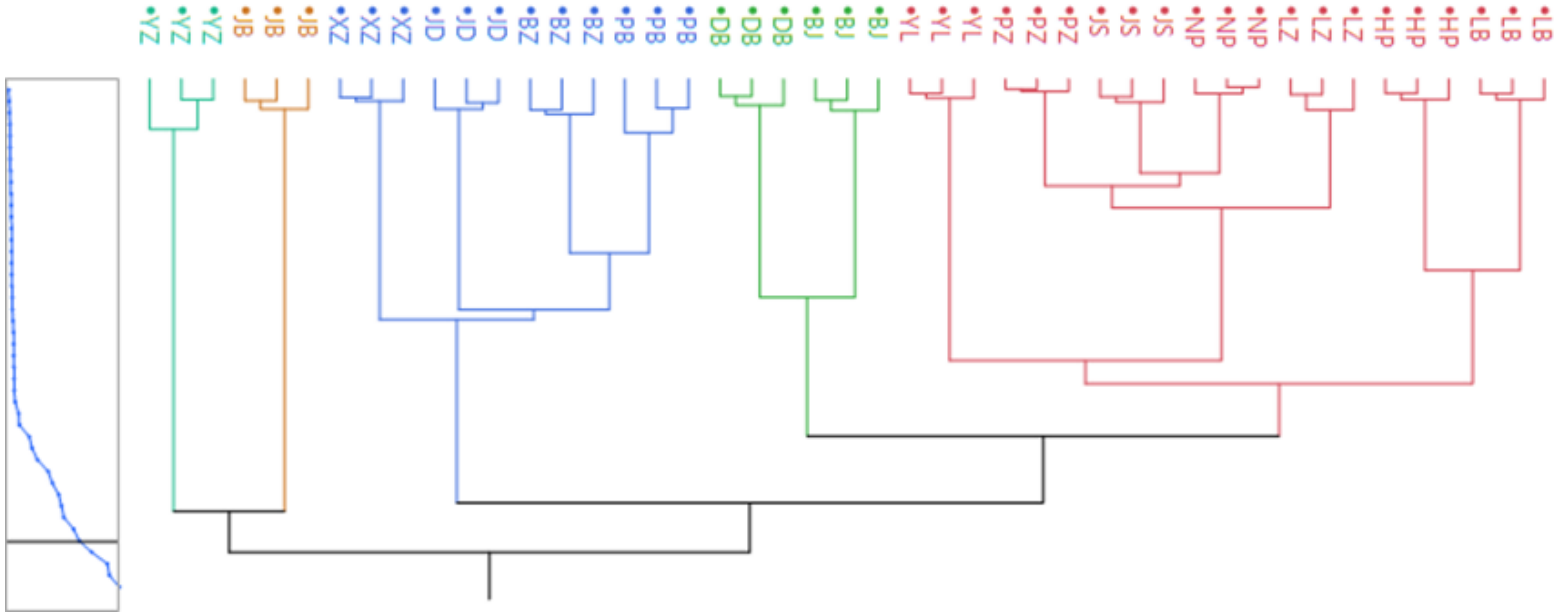


Figure 4. 4 Hierarchical Cluster of Volatile Compounds Analysis of 15 Cultivars of Jujube Fruits

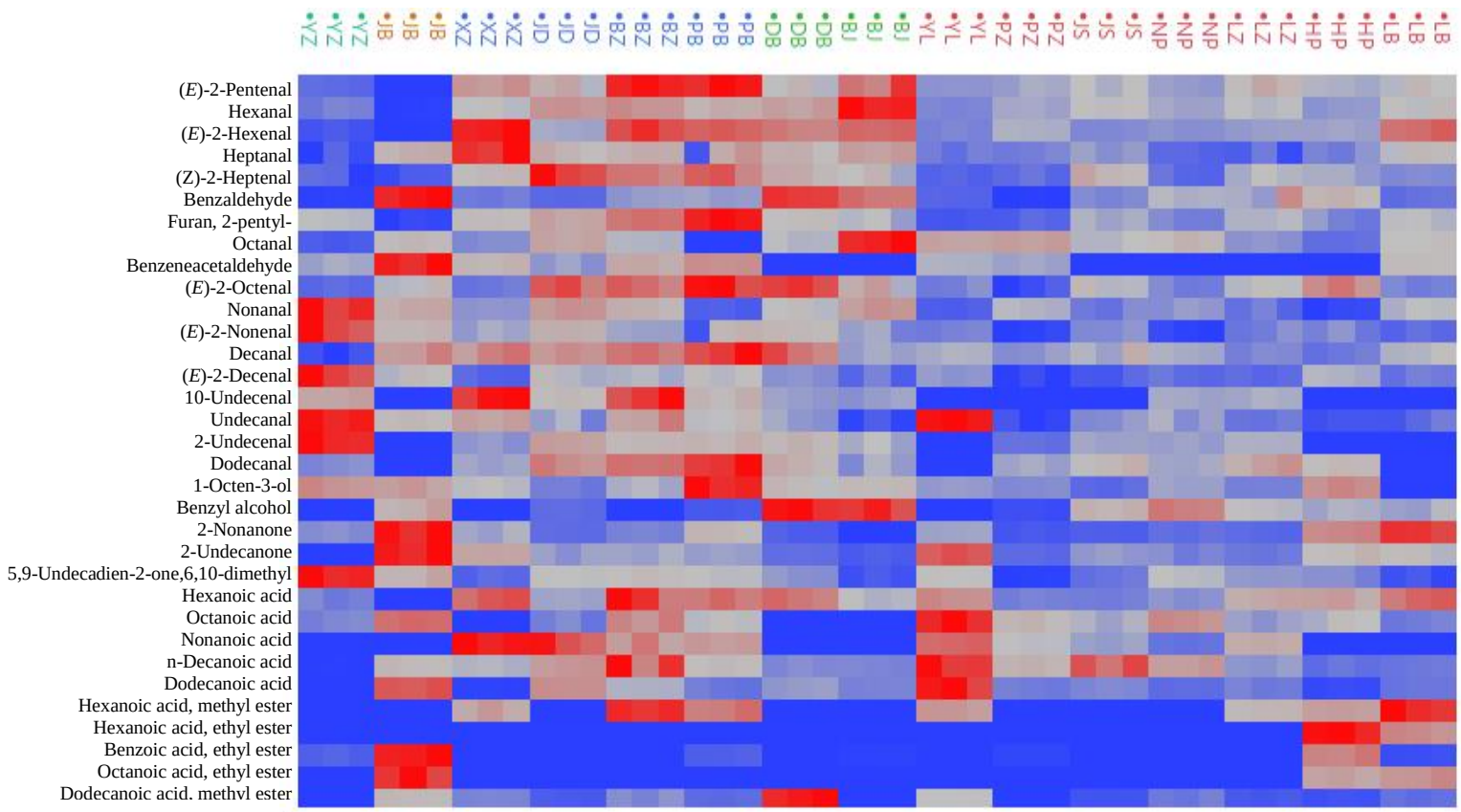


Figure 4. 5 Color Map of Concentrations of Different Volatile Compounds in Jujube Fruit (Color from blue to gray to red means value of concentration varied from lowest to medium to highest)

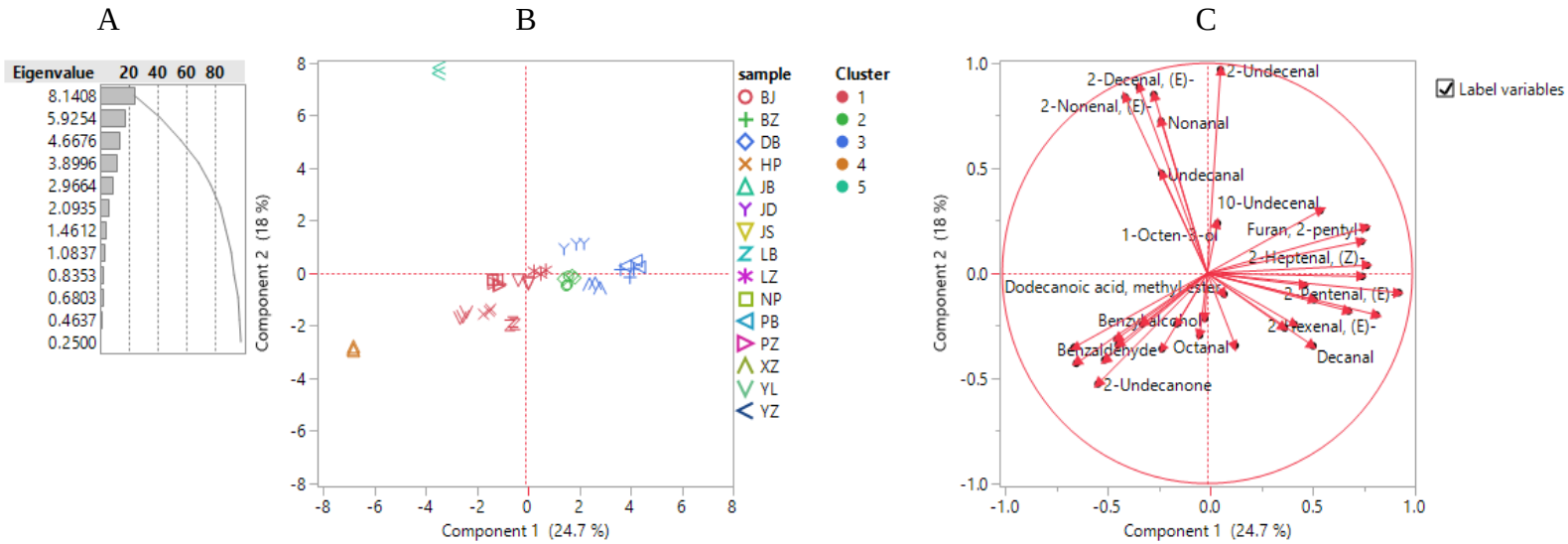


Figure 4. 6 Principal Component Analysis of 15 Cultivars of Jujube [A, eigenvalues of principal components; B, score plot of first two principal components (the same color in the score plot means they were in the same cluster, which were cataloged by HCA); C, loading plot of different variances], legend of cultivars in B: different marks represented different cultivars, the colors of the mark were meaningless; legend of cluster in B: different color means different cluster.

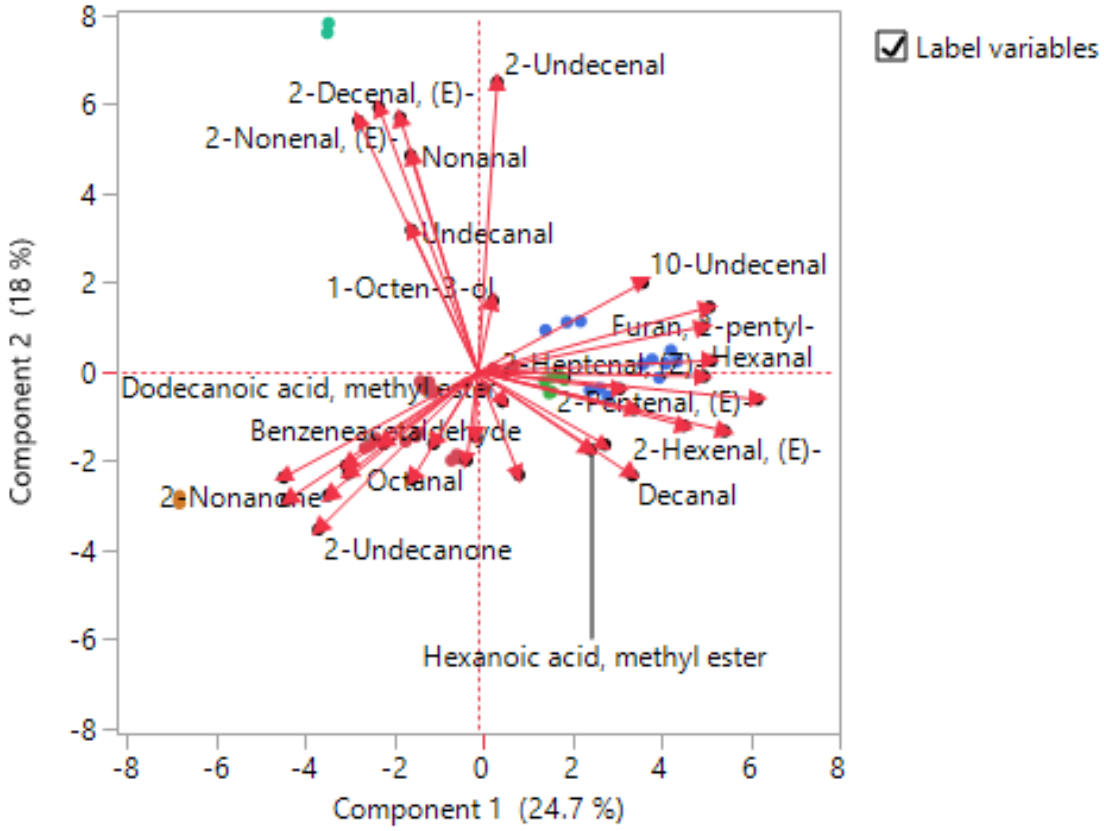


Figure 4. 7 Biplot of Principal Component Analysis

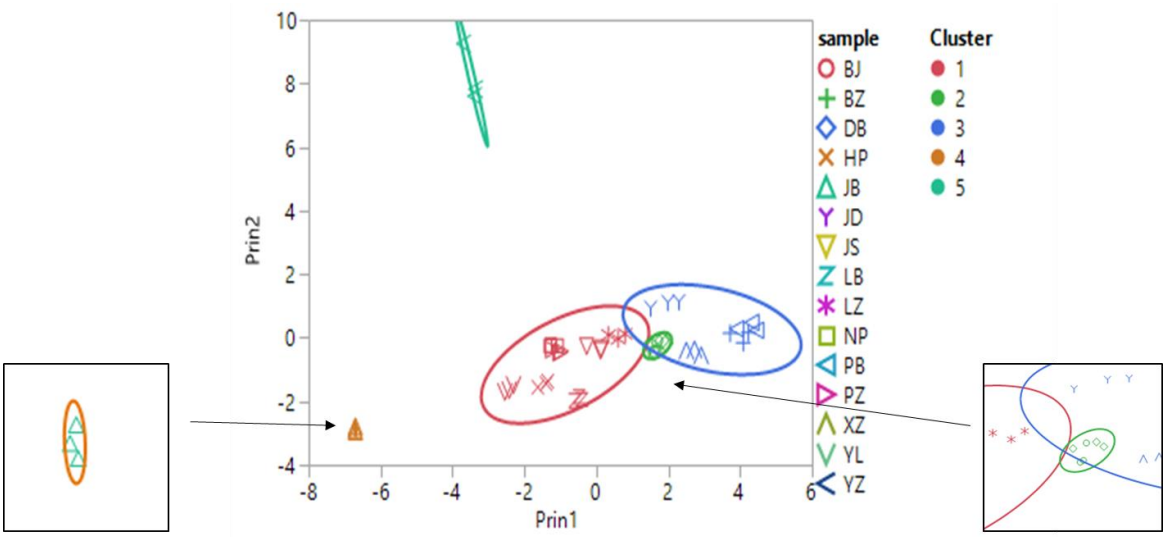


Figure 4. 8 Bivariate Fit of Principal Component 2 by Principal Component 1, legend of cultivars: different marks represented different cultivars, the colors of the mark were meaningless; legend of cluster: different color means different cluster. Density ellipses were shown in cluster grouping, p=0.95

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CHAPTER FIVE

SUMMARY

In this study, jujube was analyzed in regards of its basic composition in terms of the reducing sugars, organic acids, free amino acids, fatty acids and minerals. Its bioactive compounds such as ascorbic acid, triterpenes, cAMP and antioxidant capacity were also analyzed. The volatile compounds in jujube were detected by GC-MS. As a result, it was found that most of the analytes in different jujubes existed in significant difference ($p<0.05$). However, the fatty acids and minerals did not show the significant differences among some cultivars. The antioxidant capacity such as DPPH, FRAP and HRSA were highly correlated with the total phenolic content and the content of ascorbic acid; ABTS was highly correlated with total content of triterpenes. Volatile compounds extracted by the SPME method included six major chemical groups, including aldehydes, acids, alcohols, esters and ketones. Among them, the aldehydes and acids were the major group, which accounted for more than 95% of total amount of the identified volatile compounds.

In order to classify the cultivars, principal components analysis and hierarchical cluster analysis were used. It was found that principal component analysis based on the contents of reducing sugars were more reliable than PCA based on the other analytes because this PC can explain more than 80% of data variance if a two-dimensional plot is used. In particularly, the discrimination of different cultivars based on volatile compounds analysis was not well, most cultivars did not show the significant difference.

Overall, these comprehensive data including reducing sugars, organic acids, free

amino acids, free fatty acids, minerals, antioxidants, antioxidant capacity and the volatile compounds in jujube can provide more information about nutritional values of jujube fruits, to improve the quality of processing products which related to jujube. The classification based on different analytes can help us to choose proper cultivars of jujube for better utilization.

Because of jujube fruits were collected from the same place, the environmental factors such as the soil, sunlight, rainfalls were ignored, the classification only based on the cultivars. In future, the genotype of different cultivars can be analyzed to confirm the classification; and the samples from different location should be analyzed, try to find if the environmental factors can affect the contents of compounds and the classification effectively.

APPENDICES

Appendix A

Water Contents in Different Cultivars of Jujube Fruits

Table A. 1 Water Contents in 15 Cultivars of Jujube, %

Sample	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
Water content	76.7± 2.2	68.3± 1.2	77.6± 0.8	68.9± 0.9	74.1± 0.3	79.5± 2.5	76.8± 0.3	75.1± 0.4	79.3± 0.5	74.3± 0.5	75.0± 1.0	77.4± 0.6	73.3± 1.4	74.6± 0.6	74.0± 0.2

Data represent as mean value ± standard error

Appendix B

Contents of Minerals and Fatty Acids in Different Cultivars of Jujube per 100g FW

Table B. 1 Contents of Minerals in 15 Cultivars of Jujube, mg/100g FW

	Mn	Fe	Cu	Zn	Ca	Mg	Ni	Al	B	Pb	Ti	Cr
BJ	0.118± 0.007 gh	1.61± 0.10 defg	0.165± 0.008 bcd	0.361± 0.040 bcd	6.70± 0.44 ab	14.53± 0.48 def	0.057± 0.002 bcd	3.84± 0.19 cd	0.604± 0.032 bcd	n.a.	0.057± 0.001 bcdef	0.089± 0.002 d
BZ	0.264± 0.006 a	3.97± 0.17 a	0.192± 0.002 ab	0.510± 0.007 a	6.10± 0.21 bcd	18.94± 0.49 a	0.081± 0.007 a	4.59± 0.05 a	0.517± 0.009 cde	0.041± 0.003 a	0.066± 0.001 abcd	0.185± 0.017 ab
DB	0.239± 0.006 a	1.61± 0.02 defg	0.107± 0.008 fg	0.384± 0.001 bc	6.76± 0.09 ab	15.68± 0.21 cd	0.066± 0.008 abc	3.08± 0.02 f	0.524± 0.019 cde	0.012± 0.001 d	0.043± 0.004 f	0.104± 0.005 cd
HP	0.161± 0.014 def	2.08 ±0.03 bcde	0.151± 0.002 cde	0.418± 0.006 b	6.13± 0.08 bcd	15.92± 0.13 cd	0.072± 0.004 ab	4.38± 0.13 ab	0.650± 0.032 bc	0.028± 0.001 bc	0.071± 0.009 abc	0.180± 0.009 ab
JB	0.198± 0.006 b	2.17± 0.07 bc	0.139± 0.002 def	0.420± 0.029 ab	4.65± 0.15 fg	15.90± 0.37 cd	0.062± 0.001 bcd	3.75± 0.10 cd	0.539± 0.015 cde	n.a.	0.055± 0.003 cdef	0.168± 0.010 b
JD	0.148± 0.003 ef	1.28± 0.01 g	0.123± 0.001 efg	0.360± 0.009 bcd	5.11± 0.18 ef	13.27± 0.23 ef	0.045± 0.005 d	3.04± 0.05 f	0.517± 0.056 cde	n.a.	0.042± 0.001 f	0.079± 0.002 d
JS	0.111± 0.005 h	1.48± 0.08 g	0.102± 0.003 g	0.285± 0.005 d	6.44± 0.12 abc	14.25± 0.30 def	0.054± 0.003 bcd	3.89± 0.09 cd	0.526± 0.081 cde	n.a.	0.062± 0.006 abcde	0.091± 0.001 d
LB	0.171± 0.023 cde	1.58± 0.10 efg	0.095± 0.003 g	0.277± 0.004 d	5.46± 0.09 de	13.30± 0.28 ef	0.058± 0.001 bcd	3.62± 0.06 de	0.510± 0.007 cde	0.031± 0.001 b	0.054± 0.002 cdef	0.102± 0.011 cd

	Mn	Fe	Cu	Zn	Ca	Mg	Ni	Al	B	Pb	Ti	Cr
LZ	0.171± 0.009 cde	1.53 ±0.13 fg	0.136± 0.001 def	0.333 ±0.021 bcd	4.48± 0.08 fg	12.93± 0.07 f	0.052± 0.001 cd	3.07± 0.02 f	0.634± 0.018 bcd	0.022± 0.002 c	0.052± 0.003 def	0.110± 0.008 cd
NP	0.194± 0.008 bc	1.63± 0.04 defg	0.173± 0.012 abc	0.399± 0.019 b	6.07± 0.16 bcd	17.92± 0.67 ab	0.062± 0.003 bcd	4.16± 0.04 bc	0.733± 0.046 b	0.021± 0.003 c	0.072± 0.002 ab	0.103± 0.003 cd
PB	0.178± 0.006 bcd	1.64± 0.07 cdefg	0.164± 0.012 bcd	0.402± 0.013 b	6.55± 0.03 ab	14.64± 0.21 edf	0.063± 0.002 bcd	3.95± 0.08 cd	0.592± 0.015 bcde	0.005± 0.001 de	0.073± 0.002 ab	0.095± 0.001 cd
PZ	0.140± 0.004 fg	2.13± 0.25 bcd	0.106± 0.001 fg	0.307± 0.006 cd	6.41± 0.11 abc	14.34± 0.31 def	0.053± 0.004 cd	3.22± 0.05 ef	0.476± 0.021 de	n.a.	0.046± 0.001 ef	0.224± 0.021 a
XZ	0.155± 0.007 def	2.05± 0.06 bcdef	0.148± 0.002 cde	0.331± 0.002 bcd	5.72± 0.14 cde	14.45± 0.38 def	0.064± 0.001 bc	3.88± 0.03 cd	0.425± 0.011 e	n.a.	0.051± 0.002 def	0.143± 0.003 bc
YL	0.176± 0.001 bcd	2.17± 0.05 b	0.206± 0.008 a	0.348± 0.021 bcd	4.12± 0.18 g	14.96± 0.14 cde	0.060± 0.001 bcd	3.97± 0.04 bcd	0.911± 0.010 a	n.a.	0.078± 0.001 a	0.120± 0.008 cd
YZ	0.167± 0.002 de	1.37± 0.07 g	0.156± 0.009 cde	0.420± 0.016 ab	6.93± 0.13 a	16.45± 0.11 bc	0.061± 0.005 bcd	3.78± 0.03 cd	0.586± 0.014 bcde	n.a.	0.053± 0.003 cdef	0.095± 0.001 d

Data represent as mean value ± standard error,
Different letters followed the data in the same column means significant difference (p<0.05)
n.d. means not detected

Table B. 2 Contents of Saturated Fatty Acids in 15 Cultivars of Jujube, μg/100g FW

	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C23:0	C24:0
	3.22±	28.19±	16.73±	2.66±	29.52±	4.09±	6.23±	5.59±	2.97±	14.11±	14.77±	16.23±
BJ	0.04 gh	2.43 e	0.83 gh	0.09 bc	0.57 de	0.39 b	0.26 ij	0.48 c	0.07 ab	0.15 a	0.04 abcd	0.41 cde
	4.27±	46.08±	24.48±	6.24±	26.91±	0.34±	30.61±	7.01±	3.05±	14.72±	15.25±	25.51±
BZ	0.22 fg	1.25 c	0.11 de	0.63 a	0.49 ef	0.01 g	0.15 a	0.43 abc	0.02 ab	0.12 a	0.05 ab	1.21 a
	5.69±	38.99±	16.96±	1.31±	15.36±	2.42±	4.96±	6.13±	2.74±	2.79±	14.28±	10.08±
DB	0.19 def	1.59 cde	0.45 gh	0.09 d	0.21 i	0.01 de	0.08 j	0.11 abc	0.21 b	0.28 b	0.51 abcd	0.02 hij
	4.61±	30.08±	14.99±	2.32±	32.59±	4.91±	8.47±	6.20±	3.05±	14.20±	13.43±	15.29±
HP	0.35 feg	0.69 de	0.17 hi	0.06 cd	0.93 cd	0.13 a	0.05 h	0.28 abc	0.03 ab	0.17 a	0.35 d	0.36 def
	18.89±	96.75±	31.95±	5.64±	25.49±	5.43±	16.03±	6.16±	3.06±	14.60±	13.51±	27.44±
JB	0.50 b	1.30 a	1.65 ab	0.10 a	0.67 f	0.05 a	0.22 de	0.11 abc	0.01 ab	0.08 a	0.28 cd	1.50 a
	8.19±	42.53±	12.97±	1.49±	39.71±	2.81±	16.29±	6.28±	3.01±	14.51±	14.75±	12.55±
JD	0.24 c	1.44 cd	0.56 i	0.09 d	0.65 b	0.19 cde	0.43 d	0.05 abc	0.03 ab	0.13 a	0.19 abcd	0.22 fgh
	22.11±	92.16±	27.09±	3.40±	25.00±	4.13±	6.28±	7.15±	2.96±	14.49±	15.16±	18.11±
JS	1.10 a	0.87 a	0.70 cd	0.08 b	1.08 fg	0.11 b	0.40 ij	0.17 ab	0.06 ab	0.03 a	0.34 ab	0.15 bcd
	6.16±	29.43±	17.84±	2.69±	21.31±	3.13±	5.39±	5.64±	3.02±	1.54±	15.08±	8.85±
LB	0.28 cdef	1.33 e	0.14 gh	0.21 bc	0.56 gh	0.17 cd	0.39 ij	0.34 c	0.01 ab	0.04 c	0.14 ab	0.18 ij
	2.17±	31.66±	18.33±	1.55±	25.19±	2.28±	11.78±	6.04±	2.98±	1.88±	13.87±	11.04±
LZ	0.08 h	0.64 de	0.35 fgh	0.05 d	0.43 f	0.16 e	0.32 g	0.24 abc	0.04 ab	0.07 c	0.60 bcd	0.24 ghi
	23.87±	71.66±	21.58±	1.72±	47.51±	3.28±	13.95±	6.83±	3.05±	14.56±	15.33±	12.03±
NP	0.38 a	8.09 b	0.74 ef	0.14 cd	1.06 a	0.06 c	0.49 f	0.52 abc	0.03 ab	0.12 a	0.05 a	0.48 fghi
	6.03±	100.89±	28.47±	1.94±	44.30±	0.31±	22.27±	6.59±	3.10±	14.51±	15.20±	21.00±
PB	0.10 def	0.68 a	0.76 bc	0.03 cd	0.54 a	0.02 g	0.79 b	0.27 abc	0.07 ab	0.15 a	0.22 ab	0.66 b
	19.02±	71.03±	33.34±	5.89±	31.71±	0.32±	19.51±	6.73±	3.03±	14.05±	14.07±	18.78±
PZ	0.38 b	2.13 b	0.85 a	0.18 a	0.48 d	0.01 g	0.46 c	0.13 abc	0.02 ab	0.16 a	0.24 abcd	0.51 bc
	7.30±	73.65±	17.84±	3.59±	36.07±	0.30±	14.21±	5.56±	3.11±	14.55±	15.01±	14.47±
XZ	0.49 cd	1.22 b	0.28 gh	0.24 b	0.42 bc	0.01 g	0.34 ef	0.26 c	0.08 a	0.15 a	0.05 ab	0.25 efg
	19.60±	71.08±	19.60±	1.67±	20.91±	1.37±	7.08±	5.87±	3.01±	14.40±	14.93±	7.46±
YL	0.26 b	0.58 b	0.36 fg	0.03 cd	0.89 h	0.04 f	0.13 hi	0.16 bc	0.02 ab	0.02 a	0.16 abc	0.55 j
	6.37±	42.36±	16.59±	2.28±	24.31±	0.32±	21.53±	7.36±	3.01±	14.75±	15.24±	17.17±
YZ	0.18 cde	1.30 cd	0.53 gh	0.11 cd	1.19 fgh	0.02 g	0.45 b	0.17 a	0.05 ab	0.21 a	0.23 ab	1.03 cde

Data represent as mean value ± standard error, Different letters followed the data in the same column means significant difference (p<0.05)

Table B. 3 Contents of Unsaturated Fatty Acids in 15 Cultivars of Jujube, μg/100g FW

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
C1 4:1	83.44±2.84 efg	94.43±1.48 de	88.13±1.30 def	79.99±0.67 fg	135.17±2.95 ab	88.88±3.89 def	136.01±2.49 a	99.97±2.13 d	74.05±2.48 g	121.76±2.59 c	140.51±2.12 a	123.16±1.54 bc	45.89±2.12 h	92.59±3.92 de	119.26±2.57 c
C1 5:1	2.49±0.19 de	2.98±0.07 bcde	3.13±0.13 bcde	3.59±0.31 bcd	3.75±0.18 bc	2.45±0.04 e	3.01±0.22 bcde	2.89±0.13 bcde	3.15±0.30 bcde	3.98±0.21 b	14.07±0.26 a	3.41±0.29 bcde	3.97±0.14 b	3.11±0.29 bcde	2.83±0.17 cde
C1 6:1	4.68±0.23 h	4.99±0.10 h	9.50±0.35 e	6.03±0.23 fgh	11.75±0.43 e	11.39±0.32 e	26.98±1.55 b	8.64±0.19 efg	5.64±0.07 gh	17.47±0.49 d	16.62±0.25 d	21.19±0.64 c	5.34±0.21 h	31.37±1.24 a	9.12±0.56 ef
C1 7:1	3.60±0.25 ef	5.66±0.12 ab	3.42±0.03 ef	4.47±0.26 cde	5.67±0.21 ab	3.67±0.19 ef	3.79±0.07 def	3.24±0.07 f	3.32±0.21 f	3.60±0.02 ef	5.14±0.43 abc	4.22±0.20 cdef	6.07±0.09 a	3.64±0.32 ef	4.75±0.07 bcd
C1 8:1	14.19±0.50 e	16.01±0.07 e	45.68±1.56 c	13.21±0.13 e	74.20±1.09 a	14.47±0.47 e	16.35±0.77 e	27.02±1.03 d	12.88±0.11 e	15.07±0.31 e	29.40±2.50 d	57.69±0.76 b	15.55±0.43 e	14.69±0.69 e	17.17±0.72 e
C2 0:1	5.24±0.17 ab	5.40±0.14 ab	5.19±0.09 ab	5.24±0.22 ab	4.60±0.24 b	5.32±0.05 ab	5.99±0.14 a	4.98±0.28 ab	5.16±0.30 ab	5.75±0.41 ab	5.56±0.22 ab	5.67±0.10 ab	4.78±0.25 b	4.85±0.25 ab	6.03±0.27 a
C2 2:1	1.63±0.07 def	1.67±0.03 def	2.03±0.08 bcd	1.59±0.06 ef	1.80±0.02 cdef	1.44±0.02 f	2.45±0.19 b	14.44±0.02 a	1.87±0.07 cdef	2.11±0.07 bc	1.72±0.13 cdef	2.05±0.05 bcd	1.63±0.09 def	1.96±0.10 cde	1.51±0.04 f
C2 4:1	1.19±0.07 d	4.34±0.23 b	2.62±0.14 c	1.60±0.06 d	2.82±0.21 c	1.09±0.05 d	3.12±0.17 c	2.98±0.15 c	1.45±0.07 d	1.36±0.06 d	1.75±0.09 d	2.92±0.16 c	1.46±0.04 d	1.16±0.12 d	5.85±0.20 a
C1 8:2	110.51±1.73 e	195.21±2.09 a	14.15±0.53 j	47.02±1.94 hi	15.47±0.97 j	82.28±2.03 f	170.07±1.52 b	13.28±0.54 j	44.17±1.58 i	119.76±1.40 d	53.44±1.24 h	16.27±0.34 j	14.62±0.24 j	62.78±2.39 g	137.08±1.25 c
C1 8:3	3.48±0.06 ab	3.49±0.04 ab	3.52±0.08 ab	3.54±0.09 ab	3.72±0.10 a	3.43±0.02 ab	3.39±0.07 ab	3.38±0.03 ab	3.48±0.06 ab	3.48±1.10 ab	3.65±0.21 ab	3.18±0.13 b	3.44±0.02 ab	3.47±0.02 ab	3.53±0.05 ab
C2 0:2	1.91±0.02 g	3.13±0.16 c	14.45±0.03 a	1.92±0.14 g	3.01±0.01 cd	2.33±0.08 efg	5.00±0.06 b	2.53±0.12 def	2.37±0.19 efg	2.23±0.07 fg	2.71±0.11 cdef	3.10±0.08 c	2.85±0.08 cde	2.96±0.02 cd	2.86±0.14 cde

	BJ	BZ	DB	HP	JB	JD	JS	LB	LZ	NP	PB	PZ	XZ	YL	YZ
C2 0:3	1.19±0.06 fg	5.25±0.14 a	1.24±0.04 fg	1.60±0.02 f	3.08±0.14 c	1.17±0.05 fg	1.21±0.03 fg	1.45±0.08 fg	1.10±0.01 g	1.27±0.06 fg	2.47±0.07 de	2.10±0.07 e	2.75±0.14 cd	1.25±0.10 fg	3.59±0.16 b
C2 0:4	4.91±0.05 a	4.81±0.05 a	4.97±0.06 a	4.91±0.04 a	4.86±0.01 a	4.81±0.05 a	5.00±0.14 a	5.02±0.11 a	4.92±0.02 a	4.91±0.04 a	4.96±0.06 a	4.98±0.06 a	4.99±0.05 a	4.87±0.04 a	4.97±0.10 a
C2 0:5	5.38±0.11 bc	7.68±0.45 a	3.33±0.27 def	4.08±0.10 cde	7.77±0.32 a	4.20±0.20 cde	5.85±0.06 b	5.21±0.03 bc	2.77±0.08 ef	3.64±0.32 def	5.31±0.44 bc	4.76±0.16 bcd	8.00±0.36 a	2.29±0.14 f	4.63±0.51 bcd
C2 2:2	13.32±0.21 bc	13.35±0.07 bc	14.61±0.21 abc	14.77±0.11 abc	14.78±0.10 abc	13.73±0.24 abc	15.11±0.37 ab	13.85±0.11 abc	14.28±0.48 abc	14.59±0.30 abc	15.20±0.92 a	15.11±0.20 ab	13.04±0.26 c	13.52±0.18 abc	14.07±0.42 abc
C2 2:6	5.20±0.08 cd	11.02±0.51 a	4.12±0.20 de	3.88±0.08 de	9.12±0.43 b	3.39±0.12 ef	6.76±0.15 c	5.57±0.19 cd	2.71±0.21 ef	4.01±0.27 de	6.75±0.18 c	5.94±0.27 c	9.88±0.36 ab	2.05±0.06 f	6.43±0.83 c

Data represent as mean value ± standard error,

Different letters followed the data in the same row means significant difference (p<0.05)