

Physicochemical And Antioxidant Activity of Fruit Harvested From Eight Jujube (*Ziziphus Jujuba Mill.*) Cultivars At Different Development Stages

Min Yan

Tarim University

Yan Wang

Tarim University

Ritesh Balaso Watharkar

Karunya Institute of Technology and Sciences

Yunfeng Pu

Tarim University

Cuiyun Wu (✉ wcyby@163.com)

Tarim University

Minjuan Lin

Tarim University

Dengyang Lu

Tarim University

Mingzhe Liu

Tarim University

Jingkai Bao

Tarim University

Yilei Xia

Tarim University

Research Article

Keywords: jujube fruit, cultivar, development stage, physicochemical property, antioxidant activity

Posted Date: June 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-498991/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Scientific Reports on February 10th, 2022. See the published version at <https://doi.org/10.1038/s41598-022-06313-5>.

Abstract

Jujube is a crop highly resistant to drought and salinity, making it the main fruit tree in Xinjiang. The present study was to investigate the effect of development stage on physicochemical and antioxidant activity of fruit harvested from eight jujube cultivars situated in Xinjiang, China. The content of water, ascorbic acid, total polyphenols, catechin, epicatechin, proanthocyanidin and rutin decreased during development of jujube fruit harvested from the eight cultivars, while the contents of fructose, glucose, sucrose, cAMP, and cGMP greatly increased. The antioxidant activity determined by DPPH and ABTS radical scavenging decreased through the whole growth phase. After the early stages of development, a regular trend in changes of physicochemical and antioxidant characteristic was identified, while the differences among jujube cultivars gradually reduced with complete fruit development. This work contributes to understanding the changes that take place during the development of jujube fruit, indicating that development stage influenced the change in physicochemical and antioxidant activity more than cultivar, and the initial development stage was found to be rich in AA, phenolic compounds, and antioxidant activity, whereas the red maturity stage was rich in sugars, cAMP, and cGMP.

Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to the *Rhamnaceae* family, and it is generally recognized as the most important *Ziziphus* species for fruit production. The plant is indigenous to China (especially the north) and is widely distributed in the temperate and sub-tropical areas of the northern hemisphere¹. Jujube fruit production in China was recorded as about 7.21 million tons in 2017² which comprises 90% of total worldwide production. Jujube fruit has an exquisite taste, a unique flavour, and high nutritional value which make it popular and profitable. This fruit has commonly been consumed as a food additive, flavored fruit and medicinal purpose in Asian countries for thousands years³. Jujube fruit has been reported to be high in carbohydrates, organic acids, amino acids⁴, vitamin C and minerals⁵. Recent studies have reported that jujube fruit provides health benefits such as antioxidants and anti-cancer, hepatoprotective, gastrointestinal protective, anti-inflammatory, anti-insomnia, immunostimulating, and neuroprotective effects⁶. These medicinal properties have been shown to be closely related to the composition of jujube's bioactive components. Some bioactive components of jujube, like cyclic adenosine monophosphate⁷, γ -aminobutyric acid⁸, phenolic acids⁹, flavonoids and saponins¹⁰, have been investigated in previous studies.

The quality of marketed fruits depends on various factors, such as growing environment, cultivar, processing conditions, and storage, and consumers consider nutritional properties and health benefits to represent the quality of jujube fruit. A few studies have reported on changes in nutritional compounds, antioxidant activity, and sensory characteristics during the ripening stages of jujube fruit¹¹⁻¹³. Liu¹⁴ reported that 944 cultivars of jujube fruits occur in China and each one presents different physicochemical, physiological, and functional characteristics.

Most studies have been carried out on jujube cultivars grown in eastern regions with a warm continental climate. However, northwest China is also well known for jujube cultivation because of its arid continental climate, low rainfall, abundant sunshine, and fluctuating temperatures¹⁵. In the northwest region, Xinjiang is the major province for the cultivation of jujube fruit, with a recorded 476,250 ha of cultivated land and an annual production of about 3.47 megatons¹⁶. No reliable scientific reports were found regarding the nutritional and functional aspects of jujube fruit cultivated in north-western China. Therefore, the objective of the present study was framed to analyze the changes in physicochemical and antioxidant activity occurring in eight jujube cultivars grown in Xinjiang. The focus of the study was to facilitate and to identify suitable ripening stage and optimal harvest time to obtain high nutritional and functional characteristics in the fruit.

Materials And Methods

Chemicals

All the reagents, namely DPPH, ABTS, and Trolox were purchased from Sigma–Aldrich(St. Louis, MO, USA). HPLC grade phenolic standards (protocatechuic acid, (+)-catechin, p-hydroxybenzoic acid, chlorogenic acid, (-)-epicatechin, caffeic acid, p-cumaric acid, ferulic acid, rutin, quercetin, quercetin-3-rhamnoside, cAMP, and cGMP were also purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade sugar standards (glucose, fructose, and sucrose), ascorbic acid (AA), phloridzin and kaempferol were purchased from Yuanye Biotechnology Ltd (Shanghai, China). Methanol, acetonitrile and formic acid were purchased from Tedia Company, Inc (Fairfield, CT, USA). The Folin-Ciocalteu reagent and all other chemicals were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China).

Sample collection

Fruits were collected from the jujube germplasm resources nursery of Tarim University (40° 32' 23.50" N, 81° 17' 41.61" E), Alar, Xinjiang, China. In this experiment, permission for sample collection has been granted by Tarim University. Jujube plants with the same age, structure, and consistent management level were selected, and five trees were sampled for each cultivar. All methods involving plants were carried out in accordance with relevant national, international, or institutional guidelines and regulations.

A total of eight cultivars *Z. jujuba* were investigated, comprising cvs. Dabailing(DBL), Junzao(JZ), Junyou(JY), Spinosa(SZ), Huizao(HZ), Zanhuan(ZH), Fushuai(FS), and Fucui(FCM). The development stages were selected as per the stages of jujube fruit reported by Zhang¹. Jujube fruits were collected from July to October in 2019 at five stages (Fig. 1): young fruit stage (S1); fruit core-hardening stage (S2); green ripe stage, where peel is green (S3); half-red maturity stage, where 40–60% of the surface area is red (S4); red maturity stage where 100% of the surface area is red (S5). Twenty jujube fruits were picked randomly for each sample from different parts of five trees of the same species, which were free from any visible blemishes and diseases. The moisture and AA content of the fresh fruit were measured immediately after they had been transported to the laboratory. The sliced fruit were then frozen

using a freeze dryer (The FreeZone 6, Labconco, USA). Lyophilized samples were homogenized in a domestic blender (JYL-C022E, Jiuyang, China) and the powder was packed in airtight polyethylene bags. Finally, the powder was stored at -80 °C before further analysis.

Determination of water content and titratable acid (TA)

Water content was determined according to the method described by Watharkar¹⁷.

Titratable acid (TA) was measured according to the method described by Pu¹⁸, and the results were expressed in terms of % malic acid.

Determination of ascorbic acid (AA)

Ascorbic acid content was determined according to the method described by Wojdyło¹⁹ with some modification. In this analysis 2 to 3 g of sliced fresh fruit was used. The sample was added to 50 mL of 2% oxalic acid solution and homogenized for 10 min using a magnetic stirrer. The homogenized suspension was centrifuged at 3,000 rpm for 15 min and the supernatant collected was filtered with a 0.22 µm syringe filter for chromatographic analysis. High Performance Liquid Chromatography was performed using an LC-20A system (Shimadzu, Kyoto, Japan) equipped with two LC-20AB pumps, a SIL-20A injector, and an SPD-M20A detector. A C18 column (I.D. 250 × 4.6 mm; Dikma Ltd, Tianjin, China) was used to separate the compounds. A 10 µL aliquot of the sample was injected into the system where column oven temperature was adjusted to 30 °C. Elution was performed using 0.5% formic acid at a flow rate of 0.6 mL/min. Absorbance was monitored at 243 nm where L-ascorbic acid was used as the standard. The AA concentration was expressed as g/Kg FW.

Determination of fructose, glucose and sucrose

Sugar profiles of jujube fruit were produced using HPLC, as per the method described in Pu¹⁸, and results were expressed as g/kg DW.

Determination of cyclic adenosine monophosphate(cAMP) and cyclic guanosine monophosphate(cGMP)

The method described by Chen²⁰ was slightly modified to measure cAMP and cGMP. Briefly, 5 g of sample was mixed with 100 mL of deionized water in a volumetric flask. The flask was placed in an ultrasonic bath for 30 min. Then, the extract was centrifuged (3000 rpm for 15 min) and the collected supernatant was filtered using a 0.22 µm syringe filter. The chromatographic system (Shimadzu, Kyoto, Japan) consisted of a LC-20A system equipped with two pumps (LC-20AB), an injector (SIL-20A), and a detector (SPD-M20A). Compounds were separated using a C18 column (I.D. 250 × 4.6 mm, Dikma Ltd, Tianjin, China) at 30 °C. The filtered extract (aliquot) was used for injection while methanol/water/formic acid (89.5:10:0.5, v/v/v) was used as a mobile phase which was pumped at a flow rate of 0.7 mL min⁻¹. The absorbance was monitored at 256 nm.

Determination of total polyphenol content (TPC) and total flavonoids content (TFC)

Total polyphenol content (TPC) was determined according to the Folin–Ciocalteu colorimetric method ¹⁸, and the results were expressed as gallic acid equivalent per gram of fruit (g GAE/Kg DW).

Total flavonoid content (TFC) was evaluated as per the process described in Pu ¹⁸, and the results were expressed as milligrams of rutin equivalent per gram of fruit (g RE/Kg DW).

Determination of phenolic profiling

The phenolic profile was produced according to the method described previously by Pu ¹⁸ with some modification. HPLC analysis was performed on a Shimadzu (Kyoto, Japan) LC-20A system equipped with two pumps (LC-20AB), an injector (SIL-20A), and a detector (SPD-M20A). Separation was performed on a ZORBAX SB-C18 column (I.D. 250 × 4.6 mm, Agilent, USA). The column oven temperature was set at 30 °C. Absorbance was monitored at 253, 279, 284, 310, 324, 327, 355, and 370 nm. The solvent system had a constant flow rate of 0.7 mL/min and 10 µL injection volume. Solvents such as 0.5% formic acid (eluant B) and methanol (eluant A) were used for the mobile phase. The gradient programs for the samples were as follows: 0–6 min, 90% B; 6–10 min, 90%–80% B; 10–11 min, 80%–75% B; 11–15 min, 75%–70% B; 15–25 min, 70%–60% B; 25–32 min, 60%–45% B; 32–40 min, 45%–0% B; 40–50 min, 0%B; 50–51 min, 0%–90% B; 51–60 min, 90% B. The injection volume was maintained at 10 µL and constant temperature remained at about 30 °C. Detection wavelengths were chosen considering the absorption maximums of UV spectra of the selected phenolic compounds.

Antioxidant activity assay

A DPPH radical scavenging assay was performed according to the method adopted by Tian ²¹ with some modifications. A 20 µL aliquot of the extract was mixed with 160 µL DPPH solution in a 96-well micro plate. The mixture was vortexed at 300 rpm in a dark environment for 30 min, and the absorbance was measured using a plate reader (1510, Thermo Fisher, USA) at 517 nm. Trolox was used as a standard, and the results were expressed as mmol TE/Kg DW.

The ABTS radical assay was performed according to the method adopted by Oney-Montalvo ²² with some modifications. A 20 µL aliquot of the extract was mixed with 170 µL ABTS solution in a 96-well microplate. The mixture was vortexed at 300 rpm in the dark for 10 min, and the absorbance was measured using a plate reader (1510, Thermo Fisher, USA) at 734 nm. Trolox was used as a standard, and the results were expressed as mmol TE/Kg DW.

Statistical analysis

SPSS version 20 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis of the collected data. The data were analyzed using one-way analysis of variance (ANOVA) and Duncan's test was performed to determine correlation at $p < 0.05$ significance level. Furthermore, principal composition analysis (PCA) was performed using Unscrambler 10.1 (CAMO AS, Trondheim, Norway) software.

Results And Discussion

Change in water content

The water content of jujube fruits at different development stages is presented in Fig. 2. The water content of all jujube fruit cultivars at different development stages differed significantly. In general, water content was found to decrease with respect to stage of development; the highest moisture levels, in the range of 86.52% (SZ) to 89.31% (DBL), were found at the young fruit stage (S1), and the lowest water content, in the range of 65.09% (HZ) to 76.60% (JY), at the red maturity stage (S5). These results are similar to the trend reported by Wu and Song^{23,24}, but the values for water content obtained in this study were significantly lower than the data reported in these two studies. The reason behind this discrepancy is probably that the climate (long periods of sunshine and a large temperature difference between day and night) during the growth of jujube fruit in Xinjiang results in the accumulation of solids in the fruit.

Change in titratable acid (TA)

TA was evaluated at different development stages of the jujube fruit and the results are shown in Fig. 3. TA at different development stages of all jujube fruit cultivars showed significant differences. The TA content of DBL was found to increase as development progressed, and the TA content of FCM was found to be higher in the early developmental stages (up to S3) and stabilized from S3 to S5, while the TA of other cultivars was found to increase during the early stage and then decrease as development progressed. These results are consistent with studies reported previously by Wu²⁴. Among all cultivars, SZ showed the highest concentration of TA, which was observed to almost double at the final stage of development. Increasing TA concentrations during development may be due to increasing ethylene production, which is at its peak at the early stages of development but declines progressively thereafter¹⁷.

Change in ascorbic acid

AA is a well-known compound in fruits that acts as an antioxidant and helps to scavenge free radicals by inhibiting radical chain reactions¹³. AA, present in abundance in jujube fruits, acts as a reducing and chelating agent²⁵. Jujube fruit provides a rich source of AA, meeting the adult daily requirement with the consumption of about 20 g of the fruit²⁶. The AA concentration at different development stages (Fig. 4) showed significant differences ($P < 0.05$) in all cultivars. During development the highest concentrations of AA were found in the HZ and FS cultivars, at about 8.51 g/Kg FW and 11.20 g/Kg FW, respectively, at the S1 stage, which gradually decreased thereafter. The highest rate of decrease of AA was found up to the S3 stage, whereas the rate of AA decline slowed thereafter. Wu²⁴ reported similar findings in the case of the ripening stages of 'pear-jujube' (*Zizyphus jujuba* Mill.). Up to the S5 stage, the AA content of all cultivars decreased, ranging between 0.79 g/Kg FW (JZ) and 4.86 g/Kg FW (FS). Generally, FS, FCM, and DBL cultivars are considered to be table fruit, whereas JZ, HZ, ZH, and JY are used for commercial processing. This discrimination has been made on the basis of AA concentrations, where fruit containing higher concentrations are consumed as table fruit and those with lower concentrations used

commercially. JY might be considered to be a potential commercial cultivar due to its having the highest amount of AA (4.48 g/Kg FW) compared to other commercial cultivars at the fully-matured stage (S5).

Change in fructose, glucose and sucrose

The sugar compounds in fruit increase the sweetness of its taste and aroma, which helps to maximize consumer acceptance^{17,20}. In the present investigation fructose, glucose, and sucrose were the main sugars found in jujube fruit, which coincides with the result reported by Wu²⁵ and Song²⁴. As shown in Fig. 5, fructose and glucose were the main sugars at the early fruit stage (S1) and their levels increased during the early development stages then decreased as development progressed (Fig. 5A and 5B) because they can turn into polysaccharides and participate in various physical and chemical reactions²⁵. In addition, fruit weight increased by between 54% and 112% from stages S3 to S5 (Table 1), indicating that the concentration of fructose and glucose may be diluted as development progresses.

Meanwhile, sucrose was initially undetected in early development stages (S1 and S2) but started to appear thereafter and then levels substantially increased and sucrose became the dominant sugar in most of the jujube cultivars (Fig. 5C). Generally, the red maturity stage presented the highest amount of total sugar content, ranging from 615.33 g/Kg DW (SZ) to 813.26 g/Kg DW (HZ). These results corresponded with the fact that the sweetness of jujube fruits rapidly increases after the green ripe stage (S3), confirming that the accumulation of sugars in jujube fruits occurs mainly in the later stages of development¹⁷.

Change in cAMP and cGMP

As a second messenger in living organisms, cAMP metabolism is related to cell growth and differentiation and it exhibits anti-inflammatory effects⁷. In the present study a significant difference in cAMP and cGMP content was observed in jujube fruit (Fig. 6). cAMP and cGMP were initially undetected at the initial development stages (S1 and S2) but they started to occur at increasing concentrations at stages S3 to S5. Among all the cultivars evaluated, JY was found to contain the highest amount of cAMP, i.e. 480.92 mg/Kg DW at the fully ripened stage (S5), followed by JZ, FCM, HZ, DBL, ZH, FS, and SZ. SZ had the lowest level cAMP (15.51 mg/Kg DW at the S5 stage). These findings suggested that cultivar is the major factor affecting cAMP accumulation in jujube fruit. Also, cAMP levels were almost twice those of cGMP in all cultivars at development stage S5, which means that cAMP might be more important than cGMP in the case of jujube fruit. The cAMP content of jujube fruit has been reported to be 30–160 mg/Kg, which is the highest value observed in more than 180 plants⁷. Previous studies have reported that cGMP is also found at high concentrations in jujube fruit^{5,27}. Variation in compound concentrations may depend on the environment where the plant is cultivated, soil, and plant species.

Change in total phenolic content (TPC)

The jujube fruit is a promising source of antioxidants due to the considerable amount of phenolic compounds it contains. As shown in Fig. 7A, significant differences ($P < 0.05$) were found in the TPC of

jujube fruit at various stages of development. Generally, jujube fruit from the eight cultivars exhibited similar total phenolic patterns and a decreasing trend in TPC was observed with respect to development progression. The highest values were recorded at the young fruit stage (S1), ranging from 26.19 g GAE/Kg DW (HZ) to 35.06 g GAE/Kg DW (SZ), and the lowest at the red maturity stage (S5), ranging from 5.92 g GAE/Kg DW (DBL) to 9.68 g GAE/Kg DW (SZ). Remarkably, the decreasing trend was most pronounced from S1 to S3, but less pronounced thereafter until S5. The TPC of jujube fruit decreased with development stage, which was in agreement with those reported previously for jujube fruit^{28,29}. However, the values of TPC in jujube fruit (at S5) obtained from this study were higher than previously published results^{28,29}. These higher levels of TPC may partially be attributed to the effect of cultivar, tissue, harvesting time, and/or climatic conditions, as well as the high altitude of the study area³⁰.

Change in total flavonoid content (TFC)

Flavonoids are currently considered to be essential components for various applications, such as nutraceuticals, pharmaceuticals, medicines and cosmetics. It has also been reported that flavonoids determine anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties³¹. Jujube fruit contains considerable amounts of flavonoids, including rutin, and TFC is mainly determined using ethanol or methanol extracts of jujube fruit. As shown in Fig. 7B, jujube fruit TFC was found to differ significantly, at $P < 0.05$, at all development stages. TFC was found to exhibit a downward trend with respect to development progression, similar to that of TPC. The highest values were recorded at the young fruit stage (S1), ranging from 11.66 g RE/Kg DW (FS) to 19.30 g RE/Kg DW (DBL), and the lowest at the red maturity stage (S5), ranging from 0.29 g RE/Kg DW (ZH) to 5.99 g RE/Kg DW (DBL). The DBL cultivar of jujube exhibited a very much higher amount of TPC than that of other cultivars at all development stages. Wang²⁹ reported a similar trend and observed that rutin is the dominant flavonoid present in jujube fruit. Wang²⁸ also noted similar changes in TFC concentration during jujube fruit ripening. These results clearly indicate that the TPC of jujube fruit is dependent on developmental stage and cultivar.

Phenolic compounds profiling

Phenolics are important compounds present in fruit, which are beneficial to consumers because of their antioxidant activity. In the present study fifteen phenolic compounds, comprising hydroxybenzoic acid (p-hydroxybenzoic acid), hydroxycinnamic acids (caffeic acid, p-coumaric acid, and ferulic acid), other phenolic acids (chlorogenic acid), proanthocyanidins (catechin, epicatechin, and proanthocyanidins), and flavonoids (rutin, quercetin, phloridzin, quercetin-3-glucoside, quercetin-3-rhamnoside, quercetin 3-xylosyl-glucoside, and quercetin 3-rutinoside-7-pentos), were well separated and quantified by HPLC-DAD. As shown in Fig. 8, proanthocyanidins (Fig. 8D), catechin (Fig. 8A), epicatechin (Fig. 8B), and rutin (Fig. 8C) were found to be the predominant phenolic compounds of jujube fruit, and significant differences were noted between total and individual phenolic compounds among all cultivars at various development stages. The concentration of individual phenolic compounds was found to exhibit a downward trend with respect to development progression, which was similar to that of TPC and TFC. The highest phenolic

compound content varied from 20.41 g/Kg DW (JY) to 41.00 g/Kg DW (SZ) at the start of development (S1) while the lowest content was found to range from 0.28 g/Kg DW (ZH) to 6.49 g/Kg DW (FS) at the fully mature stage (S5). This change is consistent with the results reported by Wang³². Phenolic compounds, such as procyanidins, catechin, and epicatechin, were found to be predominant at the young fruit stage (S1), which is directly linked to sensory attributes such as bitterness and astringency³³, these unpleasant flavour characteristics prevent their consumption. However, these unpleasant taste characteristics were reduced in association with decreasing phenolic concentration correlated with respect to ripening, helping to develop a pleasant and sweet flavour in jujube fruit. Therefore, ripe and fully ripe stages are considered better for consumption and processing.

Antioxidant activity

Phenolic compounds act as antioxidants primarily by scavenging radicals via electron transfer mechanisms and by chelation with transition metals that are involved in generating free radicals⁴. The results obtained for the antioxidant activity of jujube fruit in terms of its free radical scavenging capacity (DPPH and ABTS) are shown in Fig. 9. The antioxidant activity in jujube fruit was found to differ significantly ($P < 0.05$) at different developmental stages. Radical scavenging activity, as determined by DPPH and ABTS assay, demonstrated a similar trend in the present investigation. The highest values were observed at the young fruit stage, and varied between 89.92 and 135.64 mmol TE/Kg DW (DPPH) and 119.03 and 345.87 mmol TE/Kg DW (ABTS), respectively, among all cultivars. The values of antioxidant activity were found to decrease with respect to developmental progression. Similar results, of decline in antioxidant activities along with increasing development stage, have also been reported by others^{29,34}. These results clearly suggest that antioxidant activity in jujube fruit is dependent on stage of development, and the highest levels of radical scavenging activity were found in the greener stages of ripening. Xie³⁵ reported that the antioxidant capacity of fruit could vary due to cultivar, soil conditions, postharvest practices, and the ripeness of the fruit. They also reported that the concentration of antioxidant compounds may vary according to cultivar.

Principal composition analysis (PCA)

Principal component analysis (PCA) is a well-known statistical technique that was used to understand the relationships among the quality of the different jujube cultivars from Xinjiang. All the chemical parameters detected in this study were used as variables in a PCA performed to visualize resemblances and differences by reducing the dimensionality of numerical datasets³⁶. Generally, the principal components (PCs) have more than 85% cumulated reliability of the original dataset, and then these PCs can be used to replace the original one³⁶.

In this study, 96% of the variability in the datasets could be explained by two PCs, where the first two PCs accounted for 92% and 4% of the total variance, respectively. As can be seen from the correlation loadings plot (Fig. 10), complete differentiation of these indices and jujube samples was not possible. However, the samples and indices can be appropriately divided into three groups. On the right plane,

moisture, AA, TPC, TFC, phenolic compounds, and antioxidant activities obviously gathered together, forming the first group. All samples in the second group centred in the middle, and TA, fructose, glucose, sucrose, cAMP, and cGMP were clearly scattered on the left plane and formed the third group. The correlation loadings plot demonstrated that group one had high levels of these indices during the early development stage, while group three had high levels of these indices in the final developmental stage of all jujube cultivars. Based on the size and distance between the samples and indices, AA, TPC, TFC, phenolic compounds, and antioxidant activities are characteristic components of unripe jujube fruit, while fructose, glucose, sucrose, cAMP, and cGMP are characteristic components of mature jujube fruit.

Conclusion

In the present study the changes in physicochemical and antioxidant activity during development were determined in eight jujube cultivars grown in Xinjiang, China. The results showed that development stage and cultivar had statistically significant effects, not only on the nutritional compound content but also on bioactive compound content and antioxidant activity. Of the five development stages studied, the young fruit stage (S1) showed the highest content of phenolic compounds, AA, and antioxidant activity, which suggests that this stage is suitable for use as a source of natural antioxidants in the diet or as a natural food additive, while the red maturity stage (S5) had the highest content of sugars, cAMP, and cGMP, making it suitable for consumption as table fruit or processing into jujube products, such as dried fruit, powder, juice, wine, etc. These results should be helpful in selecting superior jujube genotypes for commercial cultivation and choosing a suitable harvest time for jujube fruit. The results also provide important information regarding the nutritional and physicochemical properties of jujube cultivars, which can be useful for developing the fruit processing industry. In addition, although the trends in production of these compounds have been investigated during jujube fruit development, the mechanisms responsible for variation (such as gene expression and enzyme activity) are not clear and need to be investigated in further studies.

Declarations

Acknowledgements

This work was supported by the Key industry support plan project of XPCC2017DB006 and 2018DB002, Demonstration Base for Cultivating Innovative Talents2019CB001 and XPCC Young and Middle-aged Science and Technology Innovation Leading Talents Program (2018CB032) .

Author contributions

M.Y. and Y.W. contributed equally to the majority of the experimental work and its analysis; M.Z.L., D.Y.L., J.K.B., Y.L.X., R.B.W. and M.J.L. also contributed to both experimental work and analyses. C.Y.W. and Y.F.P.

were all closely involved in the supervision of the work. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

References

1. Zhang, Z. & Li, X. Genome-wide identification of AP2/ERF superfamily genes and their expression during fruit ripening of Chinese jujube. *Scientific reports* **8**, 15612, doi:10.1038/s41598-018-33744-w (2018).
2. Huang, B. The Chinese Rural Statistic Yearbook. *Beijing: China Statistic Press*, doi:10.7506/spkx1002-6630-201717004(2018).
3. Rostami, H. & Gharibzahedi, S. M. T. Mathematical Modeling of Mucilage Extraction Kinetic from the Waste Hydrolysates of Fruiting Bodies of Zizyphus jujuba Mill. *Journal of Food Processing and Preservation* **41**, e13064, doi:10.1111/jfpp.13064 (2017).
4. Choi, S. H. *et al.* Changes in free amino acid, protein, and flavonoid content in jujube (Ziziphus jujube) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. *Journal of agricultural and food chemistry* **60**, 10245–10255, doi:10.1021/jf302848u (2012).
5. Chen, J. *et al.* Fruit of Ziziphus jujuba (Jujube) at two stages of maturity: distinction by metabolic profiling and biological assessment. *Journal of agricultural and food chemistry* **63**, 739–744, doi:10.1021/jf5041564 (2015).
6. Memarpoor-Yazdi, M., Zare-Zardini, H., Mogharrab, N. & Navapour, L. Purification, Characterization and Mechanistic Evaluation of Angiotensin Converting Enzyme Inhibitory Peptides Derived from Zizyphus Jujuba Fruit. *Scientific reports* **10**, 3976, doi:10.1038/s41598-020-60972-w (2020).
7. Jiang, T. *et al.* Characterization of cAMP as an anti-allergic functional factor in Chinese jujube (Ziziphus jujuba Mill.). *Journal of Functional Foods* **60**, 103414, doi:10.1016/j.jff.2019.06.016 (2019).
8. Yunfeng Pu, A. J. S., Jianjun Zhong, Donghong Liu, Lijun Song. Determination of aminobutyric acid GABA in jujube fruit Ziziphus jujuba Mill. *CyTA - Journal of Food* **17**, 158–162, doi:10.1080/19476337.2019.1566277 (2019).
9. Zemouri-Alioui, S., Bachir bey, M., Kurt, B. Z., Sonmez, F. & Louaileche, H. Optimization of ultrasound-assisted extraction of total phenolic contents and antioxidant activity using response surface methodology from jujube leaves (Ziziphus jujuba) and evaluation of anticholinesterase inhibitory activity. *Journal of Food Measurement and Characterization* **13**, 321–329, doi:10.1007/s11694-018-9947-5 (2018).

10. Zeyadi, M. & Almulaiky, Y. Q. A novel peroxidase from *Ziziphus jujuba* fruit: purification, thermodynamics and biochemical characterization properties. *Scientific reports* **10**, 8007, doi:10.1038/s41598-020-64599-9 (2020).
11. Reche, J. *et al.* Physicochemical and nutritional composition, volatile profile and antioxidant activity differences in Spanish jujube fruits. *Lwt* **98**, 1–8, doi:10.1016/j.lwt.2018.08.023 (2018).
12. Chiti, S., Basiri, S., Mortazavi, A., & Sharifi, A. Evaluation on Physicochemical Properties and Antioxidant Capacity of Two Iranian Jujube (*Ziziphus jujuba* Mill.) Cultivars. *Journal of Medicinal plants and By-product* **8**, 85–93 (2019).
13. Francisca Hernández *et al.* Physico-chemical, nutritional, and volatile composition and sensory profile of Spanish jujube (*Ziziphus jujuba* Mill.) fruits. *Journal of the science of food and agriculture* **96**, 2682–2691, doi:10.1002/jsfa.7386 (2016).
14. Mengjun Liu, M. W. Germplasm resources of Chinese jujube. *Beijing: China Forestry Publishing House* (2008).
15. Guang Yang, F. L., Lijun Tian, Xinlin He, Yongli Gao, Zelin Wang, Futian Ren. Soil physicochemical properties and cotton (*Gossypium hirsutum* L.) yield under brackish water mulched drip irrigation. *Soil and Tillage Research* **199**, 104592, doi:https://doi.org/10.1016/j.still.2020.104592 (2020).
16. Gao Weihong, Y. L. The Xinjiang Statistic Yearbook. *Beijing: China Statistic Press* (2018).
17. Watharkar, R. B. *et al.* Change in physicochemical characteristics and volatile compounds during different stage of banana (*Musa nana* Lour vs. Dwarf Cavendish) ripening. *Journal of Food Measurement and Characterization* **14**, 2040–2050, doi:10.1007/s11694-020-00450-z (2020).
18. Yunfeng Pu *et al.* Effect of harvest, drying and storage on the bitterness, moisture, sugars, free amino acids and phenolic compounds of jujube fruit (*Zizyphus jujuba* cv. Junzao). *Journal of the science of food and agriculture* **98**, 628–634, doi:10.1002/jsfa.8507 (2018).
19. Wojdylo, A. *et al.* Chemical composition, antioxidant capacity, and sensory quality of dried jujube fruits as affected by cultivar and drying method. *Food chemistry* **207**, 170–179, doi:10.1016/j.foodchem.2016.03.099 (2016).
20. Chen, K., Fan, D., Fu, B., Zhou, J. & Li, H. Comparison of physical and chemical composition of three chinese jujube (*Ziziphus jujuba* Mill.) cultivars cultivated in four districts of Xinjiang region in China. *Food Science and Technology* **39**, 912–921, doi:10.1590/fst.111118 (2019).
21. Jinhu Tian, J. C., Feiyan Lv, Shiguo Chen, Jianchu Chen, Donghong Liu, Xingqian Ye. Domestic cooking methods affect the phytochemical composition and antioxidant activity of purple-fleshed potatoes. *Food chemistry* **197**, 1264–1270, doi:https://doi.org/10.1016/j.foodchem.2015.11.049 (2016).
22. Julio Enrique Oney-Montalvo, Kevin Alejandro Avilés-Betanzos, Emmanuel de Jesús Ramírez-Rivera, Manuel Octavio Ramírez-Sucre & Rodríguez-Buenfil, I. M. Polyphenols Content in *Capsicum chinense* Fruits at Different Harvest Times and Their Correlation with the Antioxidant Activity. *Plants* **9** (2020).
23. Song, L. *et al.* Phytochemical Profiling and Fingerprint Analysis of Chinese Jujube (*Ziziphus jujuba* Mill.) Leaves of 66 Cultivars from Xinjiang Province. *Molecules* **24**, doi:10.3390/molecules24244528

- (2019).
24. Wu, C.-S., Gao, Q.-H., Guo, X.-D., Yu, J.-G. & Wang, M. Effect of ripening stage on physicochemical properties and antioxidant profiles of a promising table fruit 'pear-jujube' (*Zizyphus jujuba* Mill.). *Scientia Horticulturae* **148**, 177–184, doi:10.1016/j.scienta.2012.09.026 (2012).
 25. Song, J. *et al.* Assessment of sugar content, fatty acids, free amino acids, and volatile profiles in jujube fruits at different ripening stages. *Food chemistry* **270**, 344–352, doi:10.1016/j.foodchem.2018.07.102 (2019).
 26. Kou, X. *et al.* Quantitative assessment of bioactive compounds and the antioxidant activity of 15 jujube cultivars. *Food chemistry* **173**, 1037–1044, doi:10.1016/j.foodchem.2014.10.110 (2015).
 27. Jianping Chen *et al.* Chemical and biological assessment of *Zizyphus jujuba* fruits from China: different geographical sources and developmental stages. *Journal of agricultural and food chemistry* **61**, 7315–7324, doi:10.1021/jf402379u (2013).
 28. Wang, C., Cheng, D., Cao, J. & Jiang, W. Antioxidant capacity and chemical constituents of Chinese jujube (*Zizyphus jujuba* Mill.) at different ripening stages. *Food Science and Biotechnology* **22**, 639–644, doi:10.1007/s10068-013-0125-6 (2013).
 29. Wang, B. *et al.* Changes in phenolic compounds and their antioxidant capacities in jujube (*Zizyphus jujuba* Miller) during three edible maturity stages. *LWT - Food Science and Technology* **66**, 56–62, doi:10.1016/j.lwt.2015.10.005 (2016).
 30. Gao, Q.-H. *et al.* Physico-chemical properties and antioxidant capacity of different jujube (*Zizyphus jujuba* Mill.) cultivars grown in loess plateau of China. *Scientia Horticulturae* **130**, 67–72, doi:10.1016/j.scienta.2011.06.005 (2011).
 31. Zhao, J., Yang, J. & Xie, Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. *International journal of pharmaceutics* **570**, 118642, doi:10.1016/j.ijpharm.2019.118642 (2019).
 32. Wang, Y. *et al.* Different responses of photosystem II and antioxidants to drought stress in two contrasting populations of Sour jujube from the Loess Plateau, China. *Ecological Research* **31**, 761–775, doi:10.1007/s11284-016-1384-5 (2016).
 33. Aneta Wojdyło, J. O., Paweł Bielicki. Polyphenolic Composition, Antioxidant Activity, and Polyphenol Oxidase (PPO) Activity of Quince (*Cydonia oblonga* Miller) Varieties. *Journal of agricultural and food chemistry* **61**, 2762–2772, doi:https://doi.org/10.1021/jf304969b (2013).
 34. Cosmulescu, S. *et al.* Variation of Bioactive Compounds and Antioxidant Activity of Jujube (*Zizyphus jujuba*) Fruits at Different Stages of Ripening. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **46**, 134, doi:10.15835/nbha46110752 (2017).
 35. Pu-jun Xie, Feng Youa, Li-xin Huang & Zhang, C.-h. Comprehensive assessment of phenolic compounds and antioxidant performance in the developmental process of jujube (*Zizyphus jujuba* Mill.). *Journal of Functional Foods* **36**, 233–242, doi:10.1016/j.jff.2017.07.012 (2017).
 36. Cevoli, C., Cerretani, L., Gori, A., Caboni, M. F., Gallina Toschi, T., and Fabbri, A. Classification of Pecorino cheeses using electronic nose combined with artificial neural network and comparison with

Table

Table 1 is not available with this version

Figures

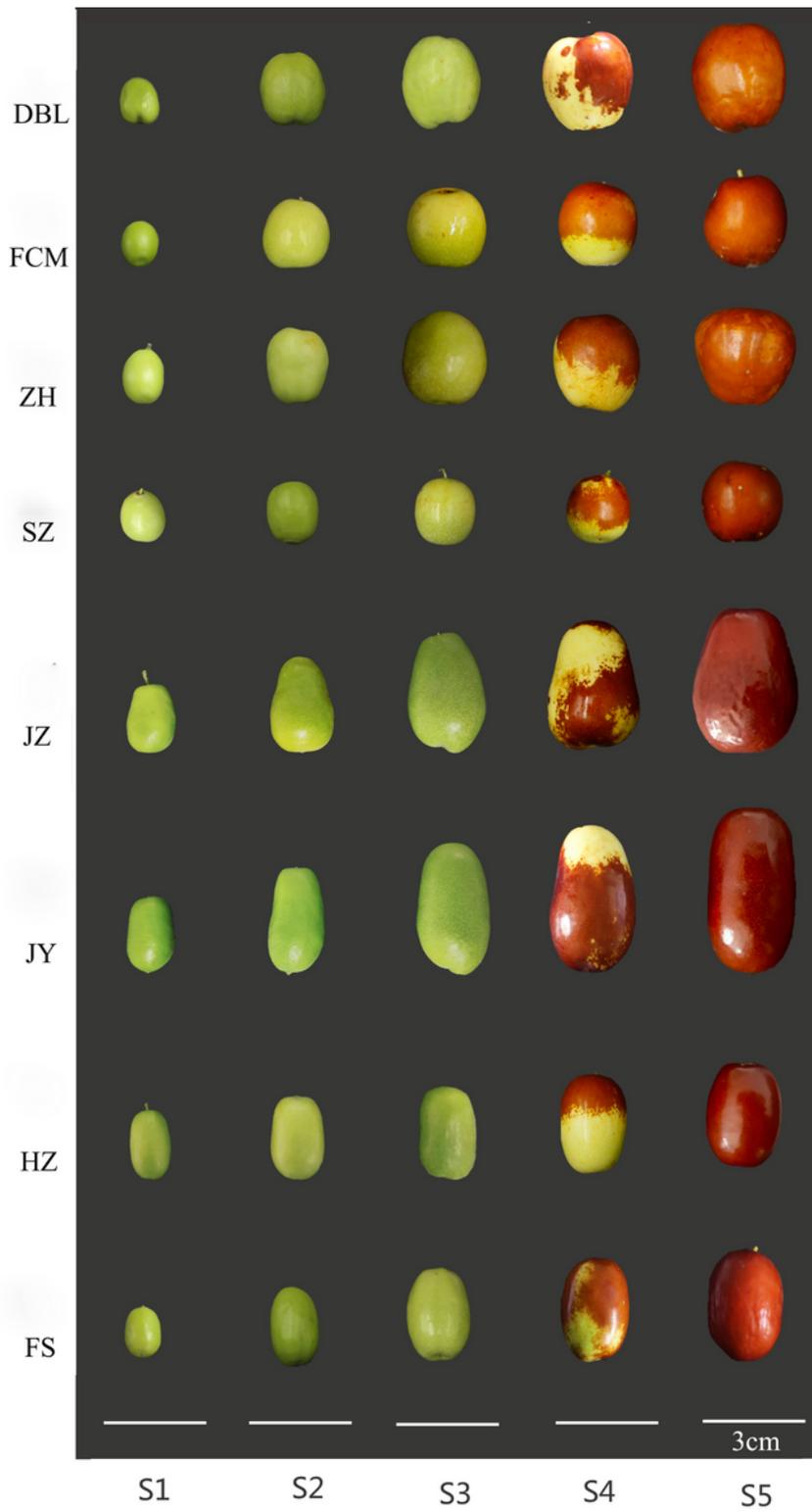


Figure 1

Photographs of jujube fruits used in this experiment

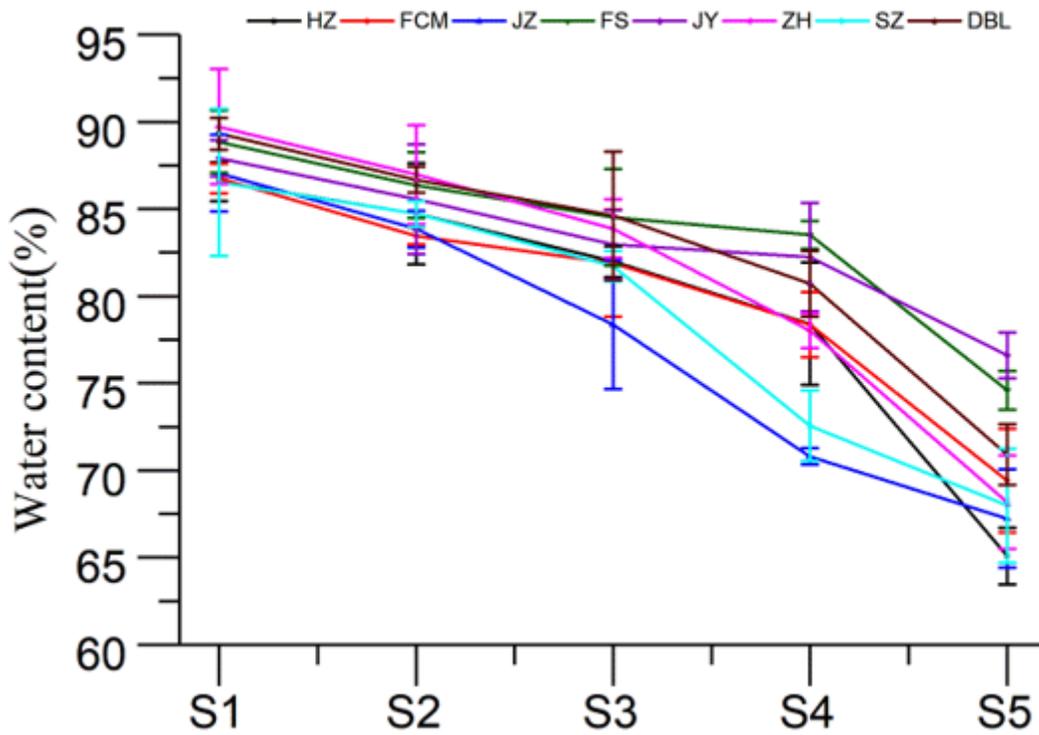


Figure 2

Change in moisture level of jujube fruit at various development stages.

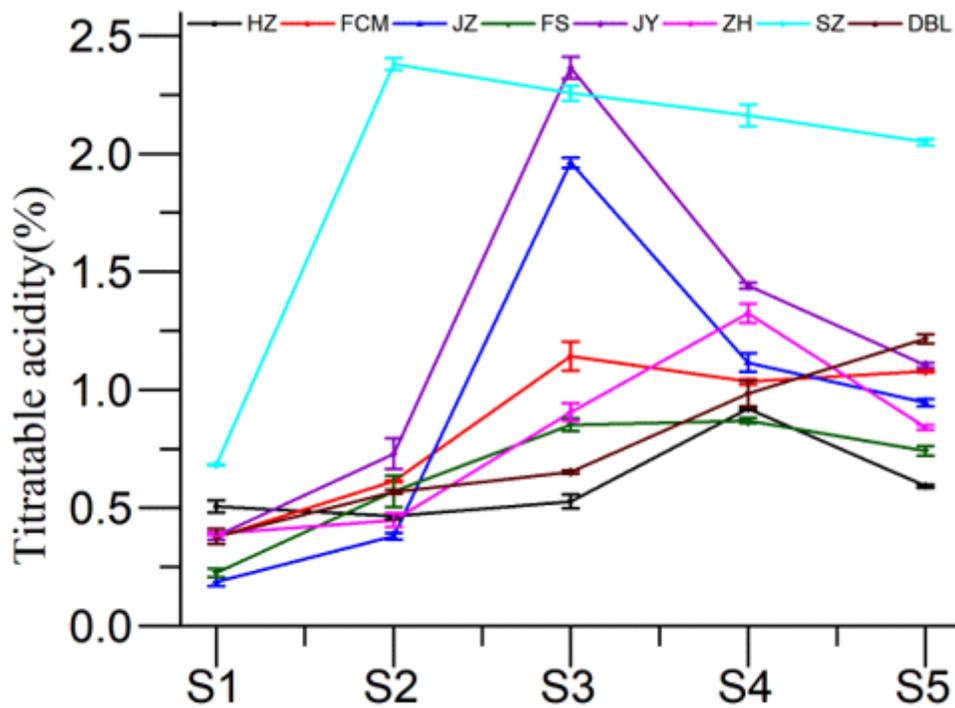


Figure 3

Change in titratable acid content of jujube fruit at various development stages.

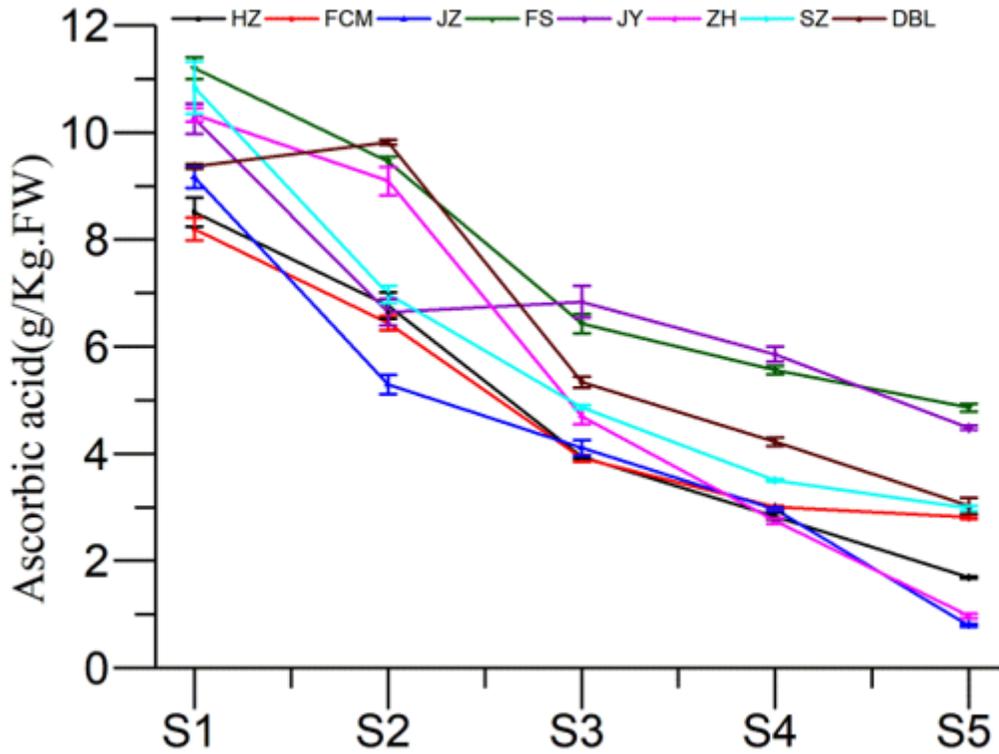


Figure 4

Change in ascorbic acid content of jujube fruit at various development stages.

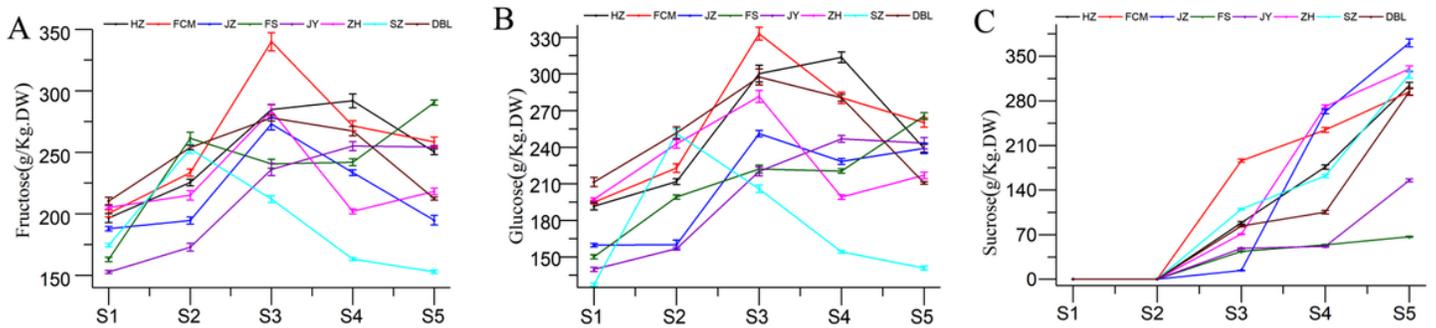


Figure 5

Changes in (A) fructose, (B) glucose, and (C) sucrose content of jujube fruit at various development stages.

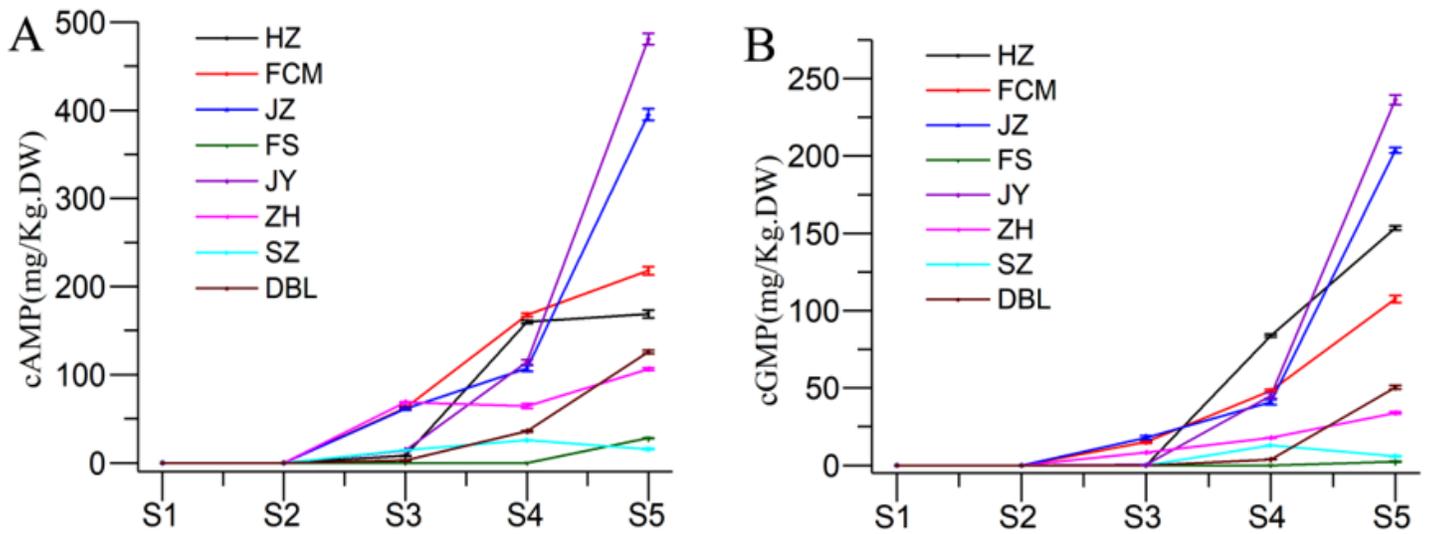


Figure 6

Changes in (A) cAMP and (B) cGMP of jujube fruit at various development stages.

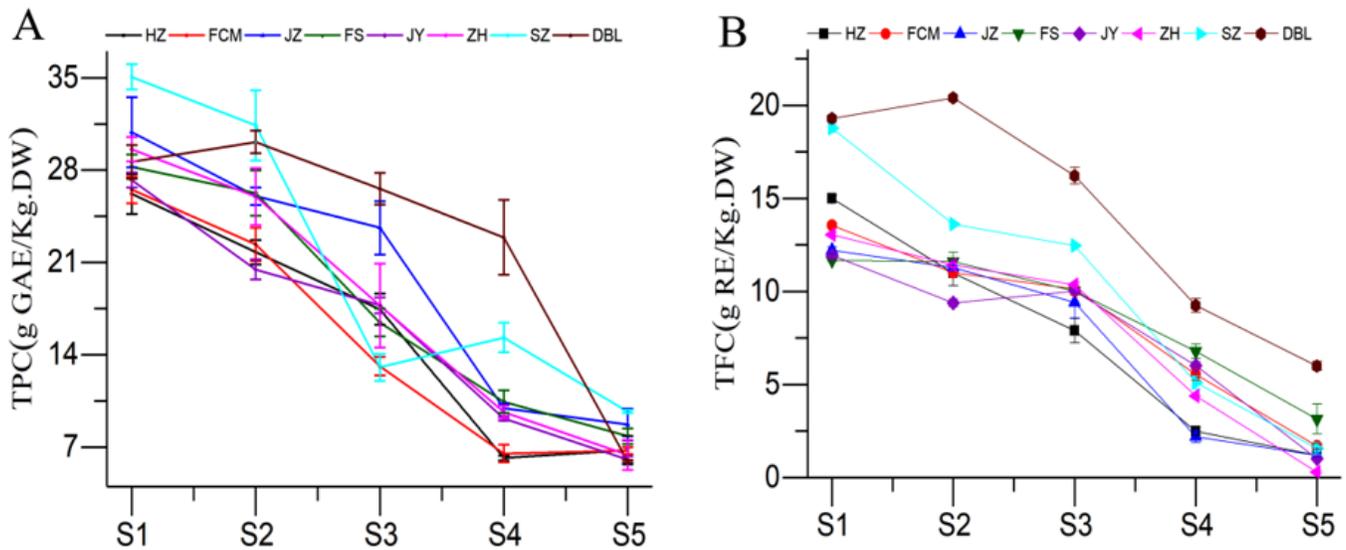


Figure 7

Changes in (A) total phenol content (TPC) and (B) total flavonoid content (TFC) of jujube fruit at various development stages.

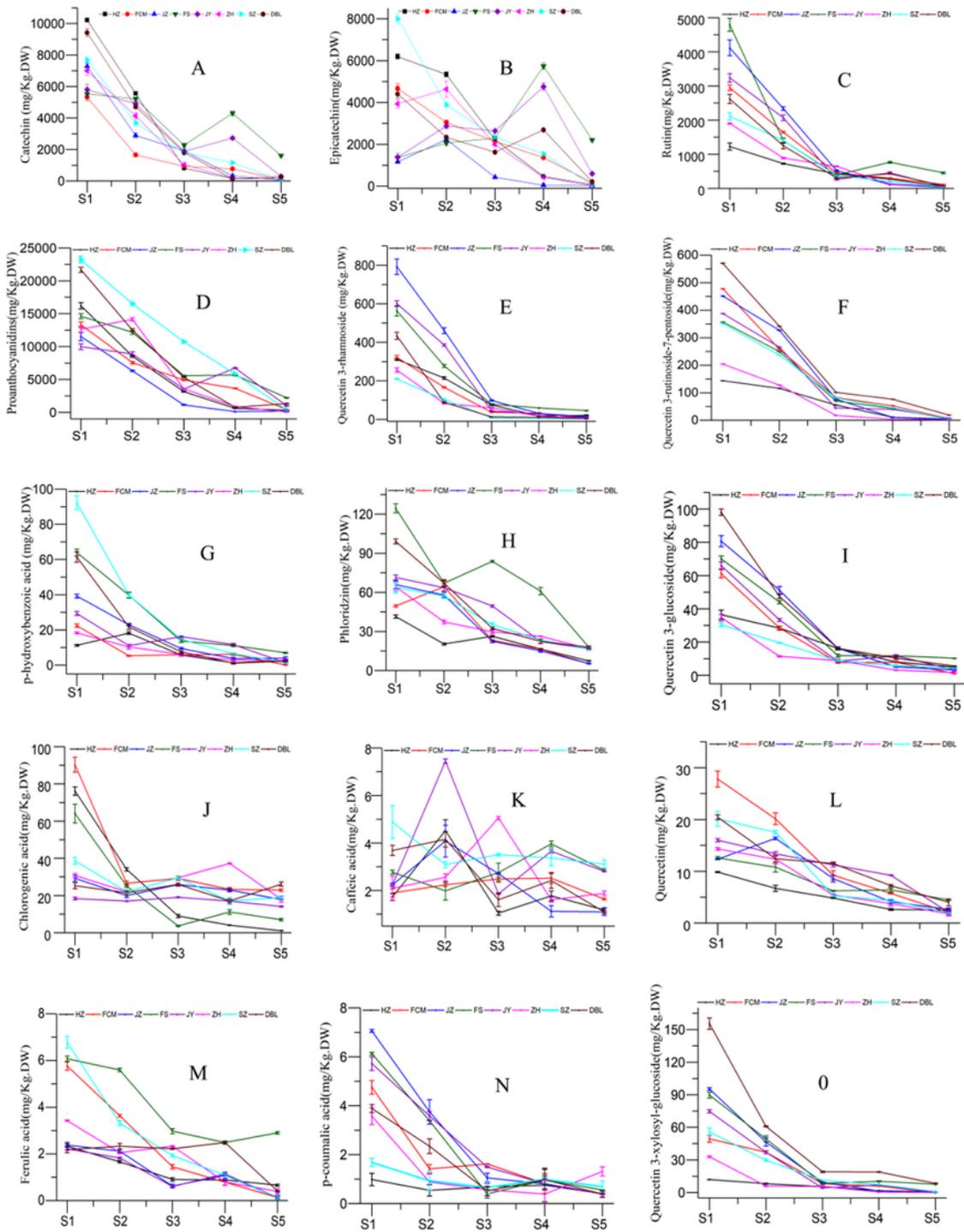


Figure 8

Change in phenolic profile of jujube fruit at various development stages.

