

## **Physicochemical Properties and Nutritional Value of Jujube (*Ziziphus jujuba* Mill.) Fruit at Different Maturity and Ripening Stages**

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### **Abstract**

Jujube (*Ziziphus jujuba* Mill.) fruit is an important medicinal plant in Iran. The harvesting time is a crucial for jujube fruit quality. Several characteristics of jujube fruit when harvested at four development stages; white mature (light green), crisp mature (white-red), fully mature (red) and fully ripe (dehydrated brown) were evaluated. Fruit weight, diameter, volume, pulp to stone ratio, firmness, titratable acidity (TA), total soluble solids (TSS), ascorbic acid, total phenolics content, protein, ash and chlorophyll fluorescence parameters were determined. The results showed that fruit dry weight, TSS, TSS/TA were increased significantly, while firmness, protein content and ash weight were decreased as jujube fruit harvested at progressed development stages. Ascorbic acid in fruit was decreased from white to crisp mature stage and thereafter increased significantly to fully mature stage. However, the content of total phenol increased significantly from white to crisp mature stage and thereafter decreased at more maturity and ripening stages. Moreover, chlorophyll fluorescence parameters were influenced by harvesting time. The F0 and Fm showed a positive significant correlation with total phenolic, ascorbic acid and protein content and a negative significant correlation with DW. Therefore, chlorophyll fluorescence maybe a helpful, nondestructive technique to evaluate the nutritional quality changes in jujube fruit. Overall, jujube fruit harvested at crisp (white-red) and fully mature (red) stages, seem best in respect of postharvest handling and nutritional criteria.

**Keywords:** Ascorbic acid, chlorophyll fluorescence, harvesting time, jujube, phenolic compounds.

**Abbreviations:** AA, Ascorbic acid; DW, dry weight; F0, minimum fluorescence; Fm, maximum fluorescence; FW, fresh weight; LD, longitudinal diameter; TA, titratable acidity; TD, transverse diameter; TP, total phenolic; TSS, total soluble solids.

### **Introduction**

Chinese jujube (*Ziziphus jujuba* Mill.) is belonging to the Rhamnaceae family and is indigenous to China and has been cultivated since 4000 years ago (Li et al., 2007). In traditional Chinese medicine, jujube fruits are mainly used for the treatment of some

diseases such as tumors and cardiovascular diseases related to the production of radical species resulting from oxidative stress, commonly consumed in fresh and dried forms (Zhang et al., 2010). Jujube is one of the most valuable medicinal plants, which grows in South-Khorasan province, Iran, as the major producer of jujube in Iran (Golmohammadi, 2013).

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Jujube fruits can be eaten as fresh, dried, or candied. Compared with other fruits, fresh jujube is lower in water content, but higher in soluble solids, phenolics and ascorbic acid (Kader, 2000). In addition, jujube fruit contains amino acids, organic acids, polysaccharides, and microelements especially high potassium and iron content (Li et al., 2007). Many factors impact fruits quality, including appearance, color, texture and flavor, an equally important quality which is invisible is the nutritional quality. An increasingly important aspect of nutritional quality is the content of phytochemicals, which are responsible for health protection and disease prevention. The phytochemical content of fruit tissues is influenced by numerous preharvest factors, including genotype, rootstock, climatic conditions, agronomic practices and harvesting time, and also by postharvest factors, including storage conditions and processing procedures (Cevallos- Casals et al., 2006; Gil et al., 2002; Lee and Kader, 2000).

Fruit maturity at harvest time is one of the main factors that determine compositional quality of fruits and vegetables, as well as storage life and final quality (Lee and Kader, 2000). Vitamin C, as an antioxidant, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body (Lee and Kader, 2000). Also, they been reported that more than 90% of the vitamin C in human diets is supplied by fruits and vegetables. Previous studies showed that total vitamin C of red pepper was about 30% higher than that of green pepper (Howard et al., 1994). Also, ascorbic acid content increased with ripening on the plant in apricots, peaches, and papayas, but decreased in apples and mangoes (Lee and Kader, 2000).

Change in fruit color is the main maturity index for Chinese jujube. If picked green they do not ripen satisfactorily off the tree. Color changes from green to whitish-green, then to reddish-brown and finally to dark-

brown, as the fruit ripen (Kader, 2000). Depending on the purpose, jujube fruit can be picked from the white mature stage, to the crisp mature stage, or the fully mature stage (Yao, 2013). Late harvesting of jujube fruit usually results in a dramatic decline in quality. It also has been reported that the time of harvest influences on total antioxidant capacity and phenolic compounds in jujube fruits (Gündüz and Saraçoğlu, 2014). Meanwhile, to ensure maximum resistance to mechanical damage and good shelf life, fruits are usually harvested well before physiological ripening, and at a stage characterized by high flesh firmness. Therefore, information on the degree of fruit ripeness is vitally important to growers to determine the best harvest date associated with best fruit quality. For example, the standard method to monitor grapes ripening is refractometrical determination of sugar concentrations in juice samples (Kader, 1999). In addition, attempts have been made to establish a maturity standard for mango based on firmness (Samson, 1980). However, the capacity to evaluate fruit maturity by fruit firmness, sugars, and other chemical parameters is limited and destructive. Therefore, nondestructive technologies to assess the degree of fruit ripeness accurately are desired.

Despite the potential of chlorophyll fluorescence measurements to nondestructively analyze the ripening of fruits (Smillie et al., 1987), we are unaware of studies relating chlorophyll fluorescence parameters with the ripening of jujube fruit, and very little is known about the changes in various growth parameters and fruit nutritional quality during maturity and ripening of jujube fruit, particularly produced in South Khorasan province, Iran. Therefore, we examined the levels of chlorophyll fluorescence in jujube fruit at various degrees of ripeness and compared these data with growth and quality parameters. The main objectives of this study therefore were to assess the effect of

harvesting time on jujube fruit attributes and to find possible correlations with quality evaluation of jujube nondestructively.

## Materials and Methods

### *Fruit material*

Twenty trees of jujube (*Ziziphus jujuba* Mill.), similar in vigor, age, and size, were selected from a jujube private orchard (Birjand, South Khorasan province, Iran). Fruits were hand harvested (about 200 fruits in each harvesting time) at approximately 12-day intervals to get fruit in four different stages from late in July to early in September, 2014. The maturation and ripening stages were classified by skin color (Liu, 2010) as, 1. white mature (light green), 2. crisp mature (white-red), 3. fully mature (red) and 4. fully ripe (dehydrated brown). Fruit were transferred from the field to the postharvest laboratory, University of Birjand, Birjand, Iran, within the same day for physical assessments and then samples were stored at -20°C for further experiments.

### *Chlorophyll fluorescence measurement*

Fruits were dark adapted for 30 min at first, and then chlorophyll fluorescence parameters, including the minimum fluorescence (F<sub>0</sub>) and the maximum fluorescence (F<sub>m</sub>), were measured using a MINIPAM (pulse-amplitude modulation) fluorometer (WALZ, Effeltrich, Germany) as reported by Dai et al. (2009). Photochemical efficiency of PSII (F<sub>v</sub>/F<sub>m</sub>) was also calculated. The chlorophyll fluorescence of each fruit was randomly and evenly measured at three locations on the peel.

### *Fruit weight, diameter, volume, pulp to stone ratio and firmness measurement*

After chlorophyll fluorescence measurements, their longitudinal diameter (LD), transverse diameter (TD), volume, fresh weight (FW), and dry weight (DW) were measured in each experiment. The LD and TD of fruit were measured with a digital caliper, the fruit volume was determined using water displacement

technique, the fruit weight and pulp/stone ratio were measured using a digital balance with an accuracy of 0.01 g. Fruit firmness was measured using a digital penetrometer (Extech Co., Fruit Hardness Tester, Model FHT 200, USA), fitted with a 3 mm diameter tip and data showed as Newton.

### *Titrateable acidity (TA), total soluble solids (TSS) and TSS/TA determination*

In order to measure acidity, 10 fruits were peeled in each experiment, cut into small pieces. One gram of the fruit samples with 10 mL of distilled water was filtered and centrifuged for 10 min at 5,000g; the supernatant was brought to 50 mL with distilled water. The samples were heated for 5 min at 100°C to eliminate CO<sub>2</sub>, and subsequently 2 mL of prepared solution titrated with 0.1 N sodium hydroxide. Titrateable acidity was calculated as percentage of malic acid by the following formula (Nielsen, 2010).

$$\% \text{ acid (wt/vol)} = N \times V1 \times \text{Eq wt} / V2 \times 10$$

where N = normality of titrant, usually NaOH (mEq mL<sup>-1</sup>), V1 = volume of titrant (mL), Eq. wt. = Equivalent weight of predominant acid (mg mEq<sup>-1</sup>), V2 = volume of sample (mL).

For TSS measurement, about 100 g of fruit were frozen at -20°C. At the time of analysis, fruits were thawed and homogenized in a standard food blender. Slurries were used to determine TSS contents by a hand-held refractometer (0-32 Brix, RF 10, Extech, USA).

### *Ascorbic acid determination*

Each 5 g jujube flesh was ground with 2% oxalic acid. The slurry was filtered, and the final volume of filtrate was brought up to 100 mL with 2% oxalic acid. Ascorbic acid (AA) content was determined using 2, 6-dichlorophenol indophenols by visual titration (AOAC, 2005). Results of AA content were expressed as milligrams ascorbic acid per 100 g of fresh weight. Measurements were done in triplicate.

### **Total phenolics content determination**

The total phenolic (TP) content was determined colorimetrically using Folin–Ciocalteu reagent, as described by Emmons et al. (1999), with modifications. TP assay was conducted by mixing 8.25 mL of deionized water, 0.5 mL of extract (dry weight), 0.75 mL of 20% Na<sub>2</sub>CO<sub>3</sub>, and 0.5 mL of Folin–Ciocalteu reagent. After 40 min of reaction in a water bath at 40°C, the absorbance at 755 nm was measured using a spectrophotometer. Final results were expressed as milligrams gallic acid equivalents per gram of fresh jujube. The TP content was measured in triplicate.

### **Protein content and total ash**

Protein content was calculated from the nitrogen content (%N × 6.25) analyzed by Kjeldahl method. Ash was determined according to the AOAC methods (2005) at 550°C.

### **Statistical analysis**

The experiments were conducted using a completely randomized block design, with five replicates. Data from the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed using LSD test ( $P < 0.05$ ). All analyses were performed with GenStat program (version 12, 2010, VSN International, Ltd., UK). Correlations were done using SPSS program (version 22). Correlations between parameters were examined using the Pearson correlation.

## **Results**

### **Chlorophyll fluorescence**

Chlorophyll fluorescence parameters were

strongly influenced by harvesting time (Table 1). Harvested fruits in the first stage of maturity (white mature) had a higher value and were significantly different in F0 and Fm than other stages of harvest. The F0 and Fm declined continuously as fruit picked in more mature stages. The highest Fv/Fm ratio was observed in fruits picked at last time (fully ripe) compared to other harvest times.

### **Fruit weight, diameter, volume, pulp/stone ratio and firmness**

As shows in Table 2, fruit fresh weight was increased as jujube fruits harvested at more mature stages. The lowest fresh weight was obtained in fully ripe stage. Fruit dry weight was increased in fruits with more maturity at harvest, however, there was no significant difference between harvested fruit in fully mature (red) and fully ripe (dehydrated brown) stages. In present study, no significant changes was observed in longitudinal diameter (LD) and transverse diameter (TD) of jujube fruits during four stages of maturity and ripening. The lowest fruit volume showed in white mature stage compared to other harvest times. The lowest pulp/stone ratio was observed in fully ripe fruits because of less pulp weight in this harvest. Flesh firmness was decreased significantly with time of harvest, reaching optimal values (in the range 18-20 N) by fully mature stage (mean 19 N; Table 2). These firmness values also represent the optimal for a long storage of jujube fruit. There was no significant different between fruit firmness of white mature and crisp mature stages.

**Table 1. Changes in chlorophyll fluorescence parameters of jujube fruits harvested at different stages of maturity and ripening.**

Chlorophyll Fluorescence parameters	Harvesting time			
	White mature	Crisp mature	Fully mature	Fully ripe
F0	380.6a†	183.4b	161b	38.2b
Fm	436.8a	220.2b	176.4b	59.6b
Fv/Fm	0.117b	0.148b	0.086b	0.384a

† Means in each row not followed by the same letter are significantly different at  $P < 0.05$  according to the least significance difference (LSD).

**Table 2. Physical properties of jujube fruits harvested at different stages of maturity and ripening**

Constituents	Harvesting time			
	White mature	Crisp mature	Fully mature	Fully ripe
FW (g)	2.428a <sup>†</sup>	2.735a	2.756a	1.480b
DW (g)	0.628c	0.924bc	1.393ab	1.428a
LD (mm)	19.02a	18.56a	18.0a	18.62a
TD (mm)	16.71a	16.20a	16.77a	16.74a
Volume (cm <sup>3</sup> )	2.56b	3.7a	3.2ab	3.83a
Pulp/stone ratio	11.21a	13.68a	12.17a	6.73b
Firmness (N mm <sup>-2</sup> )	21.52a	18.99a	12.93b	-

<sup>†</sup> Means in each row not followed by the same letter are significantly different at  $P < 0.05$  according to the least significance difference (LSD).

### ***Titrateable acidity (TA), total soluble solids (TSS) and TSS/TA***

The highest titrateable acidity (TA) was shown at final harvest time (Table 3). Although total soluble solids (TSS) was increased significantly during maturity stages with the higher values at fully mature fruit, it reached the highest value during ripening in harvested fruit at fully ripe stage (Table 3).

Maturity index (TSS/TA) was increased as fruit harvested in more mature stages with the highest value at fully mature stage (Table 3). However, it was not significant different in crisp (38.7) and fully matures stages (41.1). Significant reduction in maturity index was recorded in harvested fruit at fully ripe stage (32.5).

### ***Ascorbic acid (AA)***

Harvesting time significantly influenced ascorbic acid of jujube fruits (Table 3). The highest value was recorded in harvested fruit at fully mature stage (637 mg per 100 g FW), while ascorbic acid was decreased

significantly in late harvested fruit at fully ripe stage (223 mg per 100 g FW).

### ***Total phenolics content***

Total phenolics content reached to the highest values in harvested fruit at crisp mature stage (Table 3). Flesh phenols decreased significantly during fully mature and fully ripe stages on the tree, however, no significant differences in phenols content were observed between these two stages.

### ***Protein content and total ash***

Protein content was decreased significantly as fruit picked at stages with more maturity (Table 3). The highest protein was recorded in harvested fruit at white mature (6.35%) followed by crisp mature (5.67%) stage. Similarly, total ash diminished as fruit harvested in more mature stages (Table 3), with the lowest values at fully ripe (1.6%) and the highest values in white mature stage (2.82%).

**Table 3. Chemical properties of jujube fruits harvested at different stages of maturity and ripening**

Constituents	Harvesting time			
	White mature	Crisp mature	Fully mature	Fully ripe
TA (%)	0.41b <sup>†</sup>	0.32c	0.36c	0.51a
TSS (°Brix)	10.6d	12.4c	14.8b	16.6a
TSS/TA (Maturity index)	25.8c	38.7a	41.1a	32.5b
Ascorbic acid (mg 100 g-1 fresh weight)	259.21b	138.69c	637.56a	223.85b
Phenolic content (mg g-1 dry weight)	0.621b	1.025a	0.400b	0.389b
Protein content (%)	6.35a	5.67ab	3.72b	3.34b
Total ash (%)	2.82a	1.94b	1.88b	1.6b

<sup>†</sup> Means in each row not followed by the same letter are significantly different at  $P < 0.05$  according to the least significance difference (LSD).

### ***Correlations of some physicochemical contents with chlorophyll fluorescence***

The Pearson correlations of physicochemical contents with chlorophyll fluorescence parameters (F0, Fm and Fv/Fm) during four maturity and ripening stages of jujube fruit are shown in Table 4. The F0 was the parameter that had the highest correlations with physicochemical contents followed by Fm, while Fv/Fm had no relationships with physicochemical

contents except a positive correlation with TA. The F0 had the best positive correlations with AA, followed by protein and phenol. The Fm had the best positive correlations with ash, followed by protein, AA and phenol. The Fv/Fm ratio had positive correlations with TA. The F0 showed negative correlations with DW, ash and TSS, and Fm had negative correlation with DW (Table 4).

**Table 4. The Pearson correlation coefficients of chlorophyll fluorescence parameters with FW, DW, TA, TSS, AA, phenol, protein and ash during four maturity and ripening stages of jujube fruit**

	FW	DW	TA	TSS	AA	Phenol	Protein	Ash
F0	0.275†	-0.675**	-0.366	-0.487*	0.654*	0.448*	0.630**	-0.662**
Fm	0.255	-0.669**	0.372	-0.422	0.586*	0.453*	0.612**	0.636**
Fv/Fm	-0.435	0.348	0.462*	0.313	-0.077	-0.304	-0.442	0.415

† Significant correlations are denoted with asterisks at significance level \* P<0.05 and \*\* P<0.01

### **Discussion**

Postharvest strategies can be manipulated to increase the concentration of phytochemicals and nutrients in fruits and vegetables and this is certainly achievable to improve human health and well-being. It has long been recognized that naturally occurring substances in fruits and vegetables have antioxidant activity. Among those substances, the phenolic compounds widely distributed in fruits and vegetables. There is a consensus that the antioxidant capacity is directly correlated with phenolic compounds (Robards et al., 1999; Zozio et al., 2014). The high antioxidant activity of the extracts from different parts of jujube fruit such as peel and pulp has been reported (Xue et al., 2009). This antioxidant activity has been attributed to the high level of phenolic compounds including chlorogenic acid, gallic acid, protocatechuic acid and caffeic acid (Zhang et al., 2010). Similarly, the present study indicated that the jujube is rich source of phenolics and ascorbic acid as two main groups of antioxidants. However, the harvesting time is important as the higher amount of phenols was recorded at crisp mature stage, while fruit in fully mature stage had greater ascorbic acid.

Therefore, to recover the highest antioxidant capacity, fruit should be harvested at these two stages. CoSeteng and Lee (1987) reported that the changes in total phenolics in apple showed a decrease from the early stages of fruit development until harvest. In addition, the content of total phenolics was high in young loquat 'Mogi' and decreased steadily during growth between 4 and 2 weeks before harvest (Ding et al., 2001). Lu et al. (2012) stated that the pattern of changes in total phenolics content of Changhong jujube differs from many other fruits because of its different phenolic compounds content levels. The high ascorbic acid content recorded at fully mature stage (637.5 mg 100 g<sup>-1</sup> FW) is in agreement with previous reports in jujube (Kader, 2000; Li et al., 2007).

It is well known that high consumption of fruits and vegetables with high phytochemical content can inhibit, prevent or retard risk of major diseases including chronic disease and promoting good health (Birt et al., 2001). In order to increase the consumption of healthy foods, they must have an attractive taste. The maturity index (TSS/TA) is responsible for the taste and flavor of jujube fruit. The relationship between TSS and TA plays an important

role in consumer acceptance as it is reported for other commodities such as citrus, table grapes, kiwifruit and mango (Crisosto et al., 2003). In the present study, maturity index was found the highest in harvested fruit at fully mature and crisp mature stages. Celik et al. (2008) showed that TSS content in cranberry increased from 6 to 9.3% from the green to dark red stage. In agreement with Lu et al. (2012) that stated TSS continued to increase throughout the period of fruit growth and development in jujube, our results showed significant increase in TSS during maturity and ripening stages.

The results of our current study showed that picked fruit at crisp mature stage with high firmness had greater potential for long-term cold storage than other maturity stages at both 4 and 10°C (data not shown). Therefore, it can be suggested that from postharvest technology viewpoint harvested fruit at crisp mature stage seems best.

Correlations of phytochemical with chlorophyll fluorescence parameters (F0 and Fm) found at different maturity and ripening stages of jujube fruit in this study support a possible nondestructive method to evaluate the chemical changes and the best harvest date of jujube using chlorophyll fluorescence (Table 4). It was previously suggested by Lu et al. (2012) as they found correlations between phytochemical with chlorophyll fluorescence parameters at the last three

ripening stages than the correlations during growth of Changhong jujube fruit. Interestingly, F0 was the parameter that had the highest positive correlations with physicochemical contents in agreement with findings of Lu et al. (2012).

## Conclusions

In present study, we observed that the maturity at harvest is an important factor in determining jujube fruit nutritional quality, therefore the choice of the harvesting time is important. Harvesting time must take into account these parameters, along with the quality characteristics essential for postharvest technology but also for consumer acceptance. Overall, jujube fruit harvested at crisp (white-red) and fully mature (red) stages, seem best in respect of postharvest handling and storage, organoleptic properties and nutritional criteria. However, further research is needed regarding nondestructive method in jujube quality evaluation with attention to the fact that chlorophyll fluorescence is not only influenced by fruit maturity at harvest but also by other factors such as light, temperature, nutrients and cultivar.

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