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Influence of Different Storage Times and Temperatures on the Jujube (*Ziziphus jujuba* Mill.) Cloudy Juice Stability

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Jujube (*Ziziphus jujuba* Mill.) is one of China's unique agricultural and sideline products. However, the haze in cloudy jujube juice during storage affects the value of food and commodity seriously, which restricts the large-scale juice processed. In this study, the influence of storage time and temperature has been researched on soluble protein content, polyphenol content, pH and so on. The soluble protein content, turbidity and browning index went up when storage time and storage temperature were increased, but total phenolics, soluble pectin content and clarity and stability of jujube juice declined. However, pH in the entire storage period had changed little; temperature had a greater impact on stability during storage of fruit juice. That is to sayÿthe higher the storage temperature, the worse the stability of fruit juice. So the paper, improved the stability of jujube juice, which revealed the mechanism to provide data for reference.

Key words: Jujube, Storage, Cloudy juice, Stability.

Chinese jujube (*Ziziphus jujuba* Mill.) is the fruit tree pertaining to the Rhamnaceae family and has been cultivated throughout the reforested areas within the Yellow River valley since 4000 years ago, and the Chinese share o[1]f the world jujube production is about 90% (Zheng, *et al*., 2010). It is widely grown in loess plateau of China with arid climate. In 2009, the total grown area in Yulin, a main jujube producing region in loess plateau of China with a total area of 43,578 km2, was virtually 133,000 ha and the annual output was 433,800 tons on a fresh weight basis according to the statistics from local forestry bureau (Gao, *et al*., 2011).

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Jujube fruit is a popular and lucrative fruit, and is much admired for its high nutritional value (Gao, *et al*., 2011). Thus, Chinese jujube has been customarily utilized in traditional Chinese medicine and also commonly used as food for thousands of years. The Chinese occupying the world jujube output has risen in the last 10 years because of the demands for food and pharmaceutical applications (Li, *et al*., 2004). Juice and fruit juice products stand for a very crucial portion of the total processed fruit industry and their consumption significantly added during the last years (Beatriz *et al*., 2009). There are various components like pectine, protein, polyphenols in jujube juice, especially in cloudy juice. Thus, there is a growing market for natural cloudy jujube juice. The main problem with cloudy jujube juice production is the assurance of color and cloud stability, which are related to enzyme activities (Lu, *et al*., 2009).

The aim of this work was to evaluate changes of the quality of cloudy jujube juice during storage at 4, 25 and 37 °C, and after 0, 1, 2···68 days of storage. This study would provide technical support for commercial application of jujube juice processed, reveal the mechanism of cloudy jujube juice, and provide efficient information to improve the stability of the cloudy jujube juice.

MATERIALS AND METHODS

Jujube at commercial maturity was purchased from Yulin in Shaanxi Province. All reagents used were of analytical grade or purer.

Preparation of cloudy jujube juice

After being washed, fruits were immersed in hot water to remove nuclear easily. Then, jujubes were placed into a pulper (ACA Co., America) and pulped for 1 min. fruit pulp was packed by a 4 layer cheese cloth and juiced manually, then cloudy jujube juice was centrifuged in 5000 × g for 5 min (LXJ-II B, Shanghai Anting Scientific Equipment Factory, Shanghai, China). After the resulting cloudy jujube juice was stored at 4±1 °C, 25±1 °C and 37±1 °C, respectively, the indices of qualities were determined immediately (Zhao, *et al.*, 2005).

Turbidity determination

The turbidity of cloudy jujube juice was measured with a digital photoelectrical turbidimeter (WZS-185, Shanghai Precision Scientific Instruments Co.Ltd., Shanghai, China) using 5 mm cuvette at color correction mode. Cloudy apple juice was diluted in 1/20 with distilled water. Turbidity was expressed in nephelometric turbidity units (NTU).

Soluble protein determination

The soluble protein of cloudy jujube juice was analyzed using a spectrophotometric method described by (Beveridge, *et al.*, 1997). Cloudy jujube juice was decolorized with activated charcoal. The soluble protein was determined by measuring the A (absorbance at 595 nm) value using a 751-GD spectrophotometer (Shanghai Analytical Instrument Factory, Shanghai, China. The results were compared to a bovine serum albumin calibration curve.

Determination of total phenolic compounds (TPC)

The TPC was determined by Folin-Ciocalteu colorimetricmethod. Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid (Hao., 2005). TPC were expressed as gallic acid equivalents (mg GAE/g DW).

Determination of total soluble solids (TSS)

The TSS of cloudy jujube juice was determined as oBrix using WYT-4 Hand-held Refractometer (Zhongyou Optical Instrument Co.Ltd., Wuzhou, China).

Clarity and variable index determination

The TSS of cloudy jujube juice was determined as T using a 751-GD UV-Vis spectrophotometer (Shanghai Analytical Instrument Factory, Shanghai, China). The absorbance of the sample and the blank which contained distilled water were both read at 660 nm. The variable index determination of cloudy jujube juice was determined using a 751-GD UV-Vis spectrophotometer (Shanghai Analytical Instrument Factory, Shanghai, China). The absorbance of the sample and the blank which contained distilled water were both read at 420 nm.

pH determination

The pH measurement was carried out using digital Thermo Orion 555 A pH meter (Thermo Fisher Scientific Inc, MA, USA). 10 ml cloudy jujube juice was inserted with a pH electrode (Thermo Orion Ross 9103BN, MA, USA) and pH was recorded after stabilization.

Pectin content

About 15 ml of jujube juice was poured in 50 ml of (95%) 25 ml hot ethanol and heated in a water bath of 85 °C for 10 min. The alcohol-insoluble solids (AIS) were then poured in a sintered glass filter (G3) and washed with cold 63% ethanol until the filtrate showed a negative reaction in the phenol-sulphuric acid test. To a AIS sample, distilled water was added to make 100 ml, mixed with 5 ml of NaOH (1 mol/L). The absorbance of the sample and the blank which contained distilled water were both read at 525 nm. The results were compared to a galacturonic acid calibration curve, and the analysis was operated with a UV-Vis spectrophotometer (UV751GD).

Statistical analysis

Analysis of variance (ANOVA) and paired t tests were accomplished with the software Microcal Origin 8.0 (Microcal Software, Inc., Northampton, USA). They were performed for all experimental run, to determine the significance at 95% confidence. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The cloudy jujube juice was observed during 68 days storage at 4, 25 and 37 °C. This study focused on the changes of quality of the juices during storage, and the analyses concerning the changes were presented as the following.

Changes of turbidity in cloudy jujube juice during storage

Changes of turbidity in cloudy jujube juice during 68-day storage were shown in Fig. 1. The turbidity in cloudy jujube juice decreased gradually with the increases of the storage times at 4 and 25 °C. The turbidity decreased in cloudy juice stored at 37 °C for first 8 days but increased as time went by. At early storage time, turbidity of jujube juice rapidly declined, and viscosity decreased gradually. As large particles in the upper decreased, the sinking rate of these became slowly. The generation of a large number of small particles leaded turbidity rising. In 37 °C, polymerization of phenolic oxidative was of the most intensity and browning was of more strength in jujube juice, probably resulting more dark pigment particles than that in other temperatures, which increased the turbidity of jujube juice. The maximum increase of turbidity in Fig.1 was that of 37 °C, which may lead to more extended protein molecules and more exposed hydrophobic groups, facilitating interactions of protein and polyphenols by hydrophobic bonding, which increased turbidity. For tannic acid, more protein binding sites were exposed and the turbidity increased due to heating. Therefore, with the extension of storage time, turbidity increased with higher temperature in jujube juice (Siebert, *et al.*, 1996).

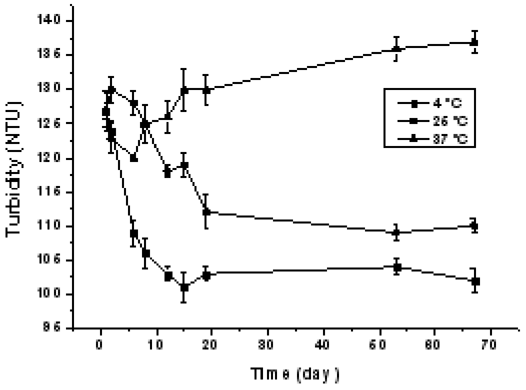

Fig.1. Effect of storage time and temperature on turbidity of jujube juice

Changes of soluble protein in cloudy jujube juice during storage

Changes of soluble protein in cloudy jujube juice during 68-day storage were shown in Fig. 2. With the extension of storage time, soluble protein decreased in jujube juice in three different storage temperatures, which probably cause the adsorption and precipitation of proteins in the process of suspension matter settling in jujube juice. Protein changed slowly at early storage time, but protein reduced significantly as time went by. In addition, the figure shows that storage temperature is not significant in soluble protein content.

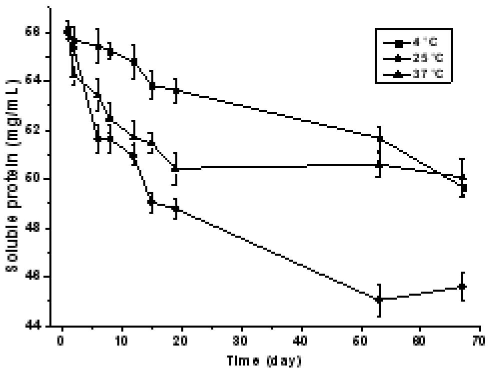

Fig.2. Effect of storage time and temperature on soluble protein of jujube juice

Changes of phenolic content in cloudy jujube juice during storage

Fig.3 showed the changes in phenolic content and degradation during storage for 68 days at three different temperatures. There was a significant decrease in total phenolics during 19 day under the experimental conditions applied. As it was shown in figure, all the treatments, time and temperature of storage significantly affected phenolic degradation. Since the polyphenol oxidase (PPO) in cloudy jujube juice was inactivated, the decrease of polyphenols was caused by non-enzymatic reaction (Ibrahima, *et al.*, 2011).

Changes of soluble solid in cloudy jujube juice during storage

Changes of soluble solid in cloudy jujube juice during 68-day storage were shown in Fig. 4. The soluble solid content of jujube juice during storage time generally firstly decreased then

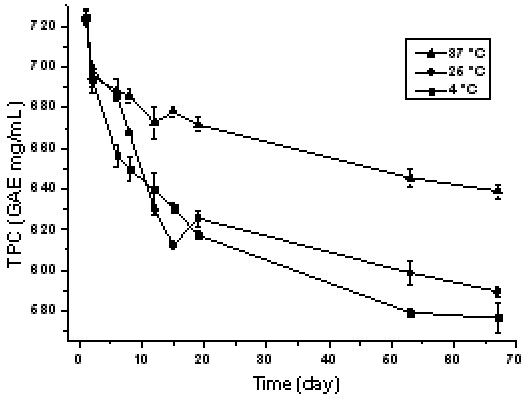

Fig. 3. Effect of storage time and temperature on TPC of jujube juice

increased, and then repeated. As 37 °C, data shows the change that soluble solid content on second day was about 9.23, the third day about 9.13, the fifteenth about 9.63, the nineteenth day about 9.53 and the fifty-third day about 9.76. Soluble solid content decreased at the later stage of storage may be due to the occurrence of polysaccharide hydrolysis, caramelization and Maillard reaction. During storage, the content of reducing sugar in cloudy jujube juice increased with the extension of storage time (Robert, *et al.*, 1997).

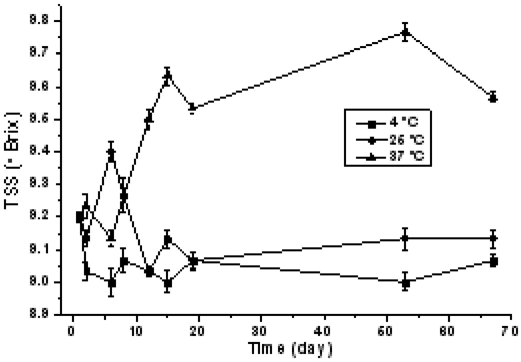

Fig.4. Effect of storage time and temperature on soluble solid of jujube juice

Changes of clarity in cloudy jujube juice during storage

Fig.5 showed the changes in clarity during storage for 68 days at three temperatures. Clarity increased at early storage time, which probable due to precipitation and polymerization of macromolecules suspended solids. It is obvious that the clarity of 37 °C gradually reduced at the later storage and then significantly lower than clarity of 25 °C and 37 °C. This indicates that the higher storage temperature leads to the lower clarity in cloudy juice, and with the extension of storage time, the clarity in jujube juice goes downward trend.

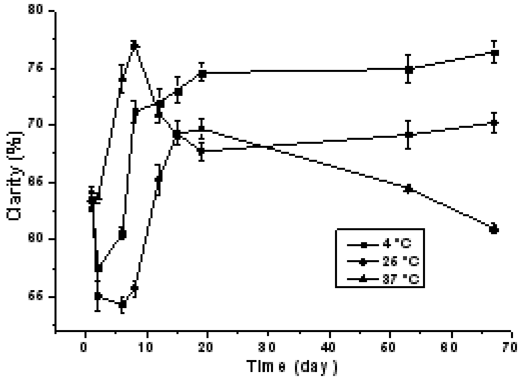

Fig. 5. Effect of storage time and temperature on clarity of jujube juice

Changes of browning in cloudy jujube juice during storage

Table.1 showed the changes in browning during storage for 67 days at three temperatures. Browning index in cloudy jujube juice during storage went upward trend. The browning index changed relatively slower when stored at 4 °C but changed rapidly when stored at 25 °C and 37 °C. As shown below, with the extension of storage time, browning index rising, and with the rising of storage temperature, browning became more and more seriously till the last day. The browning occurred during storage mainly the non-enzymatic browning, which related to the reaction of the

Table 1. Browning index changes during storage

Time (day)	4 °C	25 °C	37 °C
1	1.12±0.03	1.13±0.01	1.13±0.02
2	1.13±0.02	1.14±0.06	1.14±0.05
6	1.14±0.07	1.15±0.02	1.16±0.04
8	1.15±0.04	1.16±0.03	1.16±0.04
12	1.17±0.09	1.17±0.01	1.17±0.02
15	1.19±0.02	1.19±0.04	1.19±0.02
19	1.22±0.01	1.22±0.02	1.23±0.02
53	1.24±0.02	1.24±0.01	1.26±0.04
67	1.26±0.04	1.31±0.01	1.31±0.02

Changes of pH in cloudy jujube juice during storage

Maillard, caramelization, and change of amino acids, vitamin C degradation and oxidation and polymerization of the phenolic compounds.

Table 1. pH changes during storage

Time (day)	4 °C	25 °C	37 °C
1	4.47±0.02	4.26±0.01	4.29±0.03
2	4.54±0.01	4.26±0.02	4.28±0.03
6	4.5±0.01	4.23±0.03	4.27±0.01
8	4.49±0.02	4.22±0.01	4.27±0.02
12	4.48±0.04	4.21±0.03	4.28±0.02
15	4.46±0.02	4.19±0.04	4.26±0.01
19	4.42±0.03	4.22±0.01	4.25±0.04
53	4.38±0.02	4.24±0.02	4.19±0.02
67	4.48±0.02	4.33±0.02	4.18±0.02

Table. 2 showed the changes in pH during storage for 67 days at three temperatures. The pH of cloudy jujube juice at 25 °C first decreased and then increased, which may because that the PE, the intracellular enzyme of cell wall, when heated, the residues will cause the pectin taking of methoxy group and forming free carboxyl demethoxylation group. At early storage time, relatively active residual PE enzyme produced freer carboxyl groups, which led to the decline of pH. However, PE gradually hydrolyzed semi poly galacturonic acid methyl ester and reduced its esterification degree, once reached the critical degree of esterification, the divalent ions (mainly Ca^{2+}) could make free carboxyl groups on adjacent pectin chain crosslinked to each other, formed insoluble polymer and adsorbed other substances in the turbid substances. At late storage time, pH increased may be due to the loss of cloudy state and the increased precipitation, thus, the jujube juice content relatively decreased and the acidity increased. pH changed slightly or almost the same in jujube juice during storage.

Table. 3 showed the changes in phenolic content and degradation during storage for 68 days at three temperatures. Highest pectin content was easy to be seen in the early storage of cloudy jujube juice at 25 °C, while the lowest content of pectin in the later storage of 4 °C sample. Therefore, we can conclude that with the extension of storage time soluble pectin content in cloudy juice decreased, which mainly due to the decomposition of pectin by residual unpassivated pectinase during the heating process, leading soluble pectin content decreased. When stored to 68-day, as the temperature raised, the pectin content went upward trend, which may be due to the adsorption and precipitation of protein when suspended solids in the process of settlement in cloudy jujube juice.

Table 3. Pectin index changes during storage

Time (day)	4 °C	25 °C	37 °C
1	-	7.24±0.03	-
68	5.76±0.05	6.38±0.02	6.59±0.02

CONCLUSION

The aim of this work was to evaluate changes of the quality of cloudy jujube juice during storage at 4, 25 and 37°C, and to estimate these changes. This study would provide technical support for commercial application in jujube juice processing. The results showed that: soluble protein content increased with storage time and storage temperature raised; TPC and soluble pectin content with storage time declined; clarity and stability went downward trend with the storage temperature and storage time extended; turbidity and browning index with storage period and storage temperature rose; However, pH in the entire storage period changed little; temperature had a greater impact on stability during storage of fruit juice , the higher the storage temperature, the worse the stability of fruit juice. The results can improve the stability of jujube juice, cloudy juice, cloudy juice jujube reveal the mechanism to provide data for reference after the turbidity.

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