Browning and Flavor Changing of Cloudy Apple Juice during Accelerated Storage

Danshi Zhu, Yusi Shen, Lingxia Xu, Xuehui Cao, Changxin Lv and Jianrong Li*

College of Food Science and Technology, Bohai University; Food Safety Key Lab of Liaoning Province; National & Local Joint Engineering Research Center of Storage, Processing and Safety Control Technology for Fresh Agricultural and Aquatic Products; Jinzhou, Liaoning, 121013, China

*Corresponding author

Abstract. In this paper, the browning and flavor changing of cloudy apple juice during accelerated storage were investigated with storage temperature at 37 °C. These indicators, such as, soluble solids content, titratable acid content, Vc, color difference, and turbidity of cloudy apple juice were measured every 1 or 2 hours during storage. Meanwhile, the volatile flavor of juice were also analyzed using electronic nose. The results showed that \( L^* \) and \( \Delta E^* \) values decreased during storage, while \( a^* \) and \( b^* \) values increased. The electronic nose could distinguish the apple juice stored for 0 hour from other storage time, and the sensor with the highest contribution rate was W1S, followed by W1C and W2S, which were sensitive to hydrocarbons, aromatics, and alcohols, respectively. The values of Vc content, sugar-acid ratio, and turbidity fluctuated slightly during storage. In general, the content of Vc decreased significantly, and the ratio of sugar to acid also decreased, while, turbidity increased during storage.

1. Introduction

Apple is one of the four most widely grown fruits and one of the most commonly consumed fruits [1]. Apple juice is the second most consumed juice among U.S. consumers due to its valuable nutrional properties and highly appreciated flavor[2]. However, long-term storage of apple juice at room temperature conditions lead to the accelerated development of brown color and off-flavors, which results in decreases of the commercial value of products[3]. Browning can be divided into enzymatic browning and non-enzymatic browning. The enzymatic browning reaction in fruits are mainly catalyzed by polyphenol oxidase (PPO) in the presence of oxygen and reducing substances[4]. However, the browning of juice after pasteurization is mainly due to non-enzymatic browning, which is the result of the Maillard reaction, including condensation between reducing sugars and amino acids, caramelization, ascorbic acid degradation and pigment damage[5]. This work was to explore browning and flavor changes during accelerated storage of cloudy apple juice. This could provide some theoretical references for understanding the relationship between browning and flavor changing in cloudy apple juice, and then better for preservation of the flavor components in the future.

2. Methods

2.1. Sample Preparation

Rall's ripe apple fruit (Malus pumila) were purchased from the fruit market in Jinzhou, Liaoning
Province, China. All apples had reached commercial maturity. Cored apples were pulped using DS-1 tissue blender (Wanhua experimental instrument factory, Jintan, China), with ascorbic acid (1g/kg) was added as antioxidant. Then, juice was filtered through a 300-mesh filter cloth to remove impurities. After that, the cloudy apple juice was placed in an open glass bottle and stored in a constant temperature incubator at 37 °C for 9 hours, and every 1 or 2 hours for sampling analysis.

2.2. Color Measurement
The color of cloudy apple juice was measured using a CR-400 chroma meter (Konica Minolta Sensing, Ink., Tokyo, Japan). The investigated parameters were as follows[6]: \( L^* \) (brightness), \( a^* \) (red-green component), \( b^* \) (yellow-blue component), \( \Delta E^* \) (the total color difference) (Eq. (1)), \( H^0 \) (hue angle) (Eq. (2)), and \( C^* \) (chroma, quantitative attribute for colorfulness) (Eq. (3)). The values were measured by 3 repetitions.

\[
\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \tag{1}
\]

\[
H^0 = \arctan(b^* / a^*). \tag{2}
\]

\[
C^* = \left[\left(a^*\right)^2 + \left(b^*\right)^2\right]^{1/2}. \tag{3}
\]

2.3. Electronic Nose
The smell characteristics of apple juice were analyzed using a portable PEN3 E-nose (Insent Company, Japan). The equipment of E-nose mainly consists of a pattern-recognition software, a sampling apparatus and a sensor array unit. The sensor array were composed often metal oxide semiconductor (MOS) sensors[7].

2.4. Sugars and Acidity
The soluble solid content (SSC) of apple juice was determined by PAL-3 hand-held sugar meter (ATAGO, Japan). The content of titratable acid was titrated with NaOH, and phenolphthalein solution was used as indicator[8].

2.5. Turbidity and Vitamin C
Turbidity was directly measured in a transparent glass tube of 30 mL using the WGZ-2000 nephelometer (INESA physico Opticaal Instrument Co, Ltd, Shanghai, China) at room temperature. Measurements were repeated for 3 times, and the results were expressed as nephelometric turbidity units (NTU). Vitamin C content was measured by the method of 2, 6-dichlorophenol indophenol titration.

2.6. Statistical Analysis
The values were expressed as mean ± standard deviation. Data were analyzed by one-way analysis of variance test using SPSS Statistics 20.0 followed by drawing with Origin 9.0. Significance was determined at 95% confidence level.

3. Results
3.1. Color Difference
Table 1 showed the results of the change in color difference of cloudy apple juice. \( L^* \) and \( \Delta E^* \) values decreased while \( a^* \) and \( b^* \) values increased during storage. This indicated the loss of the typical apple juice’s color (yellowness) and formation of browning (enhanced dark and redness) during storage at 37 °C.
Table 1. Changes in color difference of apple juice during storage at 37 °C.

| Time/h | $L^*$     | $a^*$     | $b^*$     | $\Delta E^*$ |
|--------|-----------|-----------|-----------|--------------|
| 0      | 38.69 ± 0.08<sup>a</sup> | -7.49 ± 0.04<sup>c</sup> | 14.25 ± 0.13<sup>b</sup> | 36.21 ± 0.10<sup>g</sup> |
| 1      | 38.33 ± 0.07<sup>a</sup> | -7.10 ± 0.06<sup>c</sup> | 14.87 ± 0.40<sup>b</sup> | 35.98 ± 0.15<sup>h</sup> |
| 2      | 38.36 ± 0.14<sup>a</sup> | -6.93 ± 0.04<sup>c</sup> | 15.23 ± 0.45<sup>a</sup> | 36.09 ± 0.20<sup>g</sup> |
| 3      | 38.28 ± 0.13<sup>a</sup> | -6.52 ± 0.04<sup>d</sup> | 15.49 ± 0.16<sup>a</sup> | 36.03 ± 0.17<sup>h</sup> |
| 4      | 38.26 ± 0.39<sup>a</sup> | -6.36 ± 0.05<sup>d</sup> | 15.42 ± 0.68<sup>a</sup> | 35.98 ± 0.18<sup>h</sup> |
| 5      | 38.59 ± 0.13<sup>a</sup> | -6.07 ± 0.09<sup>c</sup> | 15.21 ± 0.24<sup>a</sup> | 36.19 ± 0.11<sup>g</sup> |
| 7      | 36.67 ± 0.41<sup>b</sup> | -4.38 ± 0.18<sup>b</sup> | 15.75 ± 0.68<sup>a</sup> | 34.37 ± 0.23<sup>b</sup> |
| 9      | 34.87 ± 0.17<sup>c</sup> | -2.73 ± 0.37<sup>c</sup> | 15.28 ± 0.59<sup>c</sup> | 32.42 ± 0.33<sup>c</sup> |

Values in the same column with different lower-case letters (a-f) are significantly different at $p < 0.05$.

The results of hue angle ($H^\circ$) and chroma ($C^*$) of cloudy apple juice were shown in Figure 1. $H^\circ$ and $C^*$ are vital parameters for appraisal of fruit juice color. From Figure 1 (A), the value of hue angle dropped significantly ($p < 0.05$). It showed that cloudy apple juice continuous browning visually during 9 hours accelerated storage. This indicated that, hue angle could be used to describe the changes of fruit juice browning. However, the chroma increased slowly and then decreased (Figure 1 (B)) during storage, and this might not be a good indicator for browning.

![Figure 1](image-url)

**Figure 1.** Changes of hue angle (A) and chroma (B) of cloudy apple juice during accelerated storage. Data were the means of three replicates ± SD. Different letters (a-e) indicate significant differences ($p < 0.05$).

3.2. Volatile Flavor Change

Electronic nose is a common method for detecting juice flavor. The detection limit of the electronic nose can reach $10^{-9}$. It has strong sensitivity to odor, and has the advantages of short time and fast detection speed[9]. Figure 2 showed the linear discriminant analysis (LDA) and sensor load analysis (LA) plots of cloudy apple juice during storage. From Figure 2(A), the first linear discriminant (LD1) and the second linear discriminant (LD2) explained 90.79% of the total variance (LD1 for 57% and LD2 for 33.79%). From the LD1, samples that stored 0 hour and other storage times could be well distinguished. And from the LD2, the flavor substances at different storage time were well differentiated except 5h and 7h. From Figure 2(B), the first sensor load accounted for 98.52%, and the second sensor load accounts for 0.72%. From the first main axis, the highest contribution sensor was W1S, followed by W1C and W2S. The sensors W1S, W1C, and W2S were sensitive to hydrocarbons, aromatics, and alcohols, respectively.
Figure 2. Linear discriminant analysis, LDA (A) and sensor load analysis, LA (B) of cloudy apple juice analyzed using electronic nose.

3.3. Taste Change
The changes of soluble solids content, titratable acid content, and sugar to acid ratio in cloudy apple juice during accelerated storage were shown in Figure 3. From 0 to 9 hours of storage time, the SSC scarcely changed except for a slight decrease in storage for 4 hours. In addition, the titratable acid appeared rising trend, and the ratio of sugar to acid showed a downward trend. Usually, the taste dropped during the browning process of juice, and generally this taste change will not be favored by consumers [2,10].

Figure 3. Changes of soluble solids content, titratable acid content (A) and sugar-acid ratio (B) in apple juice during storage. Different letters (A-D) and (a-d) meant significant difference ($p<0.05$).

3.4. The Change of Vc Content and Turbidity
Turbidity is an index to evaluate the degree of light scattered by particles (suspended solids) in water or other liquids. To some extent, it can indicate the degree of browning under certain circumstances. The changes of Vc content and turbidity in cloudy apple juice during storage were shown in Figure 4. The Vc content decreased firstly, and then increased gradually after storage for 2 hours, and dropped again after storage for 4 hours. The change of turbidity was exactly contrary to the change of Vc. There are many ascorbic acid and dehydroascorbate reductase in apple fruit. The partially dehydroascorbic acid could be reduced to ascorbic acid, and it led to the transitory increase of Vc content. However, the oxidation of Vc was the main reaction during juice storage, and the total content of Vc decreased finally. For turbidity, particles in cloudy juice aggregation and degradation caused by various oxidation-reduction reaction, which resulted in the decline and increase of turbidity[11].
4. Conclusions
Cloudy apple juice stored at accelerated conditions resulted in the color changes from the yellowness to red-brown, which apparently browning. The electronic nose could distinguish the apple juice stored at 0 hour from other storage time. And the flavor substances at different storage time were well differentiated except 5h and 7h. The highest contribution sensor was W1S, which was sensitive to hydrocarbons. During the browning process, the taste of apple juice became sour and the turbidity was increased, and also, the Vc loss was severe. The quality of cloudy apple juice deteriorated significantly during accelerated storage. The browning lead to losses of desirable flavour and nutritional value of juice.

Acknowledgements
This work was financed by National Key Research and Development Program (2017YFD0400704) and National Natural Science Foundation of China (31701618) and Natural Science Foundation of Liaoning province, china (20180551103). We thank Dr. Li Jianrong (Bohai University, CN) for his critical review of the manuscript.

References
[1] Huang Z, Hu H, Shen F, et al 2018 Relatively high acidity is an important breeding objective for fresh juice-specific apple cultivars[J] Scientia Horticulturae vol 233, pp 29-37
[2] Paravisini L, Peterson DG 2018 Role of Reactive Carbonyl Species in non-enzymatic browning of apple juice during storage[J] Food Chemistry vol 245, pp 1010-7
[3] Lu S, Luo Y, Turner E, Feng H 2007 Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices[J] Food Chemistry vol 104, pp 824-9
[4] López-Nicolas JM, Pérez-López AJ, Carbonell-Barrachina A, García-Carmona F 2007 Use of natural and modified cyclodextrins as inhibiting agents of peach juice enzymatic browning[J] Journal of Agricultural & Food Chemistry vol 55, pp 5312
[5] Burdurlu HS, Karadeniz F 2003 Effect of storage on nonenzymatic browning of apple juice concentrates[J] Food Chemistry vol 80, pp 91-7
[6] Yi J, Kebede BT, Grauwet T, et al 2016 A multivariate approach into physicochemical, biochemical and aromatic quality changes of purée based on Hayward kiwifruit during the final phase of ripening[J] Postharvest Biology and Technology vol 117, pp 206-16
[7] Ortega JB, Garcia JC, Ortega JG, editors 2015 Precision of volatile compound analysis in extra virgin olive oil: The influence of MOS electronic nose acquisition factors. IEEE International Conference on Industrial Technology vol 36, pp 72-6
[8] Cao J, Jiang W, Zhao Y 2007 Experiment Guidance of Postharvest Physiology and Biochemistry of Fruits and Vegetables (Beijing, China Light Industry Press) vol 2, pp 28-30
[9] Zhou Z, Zhu Y, Cheng C, Chen G, Qiong MA, Pan S 2014 Analysis and Evaluation on Overall
Flavor of Rosa roxburghii Juice by Electronic Nose[J] Journal of Hubei University for Nationalities vol 87, pp 33-9

[10] Klimczak I, Gliszczynska-Swiglo A 2017 Green tea extract as an anti-browning agent for cloudy apple juice[J] Journal of the science of food and agriculture vol 97, pp 1420-6

[11] Ma, YuHua, Ma, FengWang, Wang, YongHong, et al 2011 The responses of the enzymes related with ascorbate–glutathione cycle during drought stress in apple leaves[J] Acta Physiologiae Plantarum vol 33, pp 173-80