Effects of high pressure homogenization on the stability of cloudy apple juice

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Abstract. The high pressure homogenization (HPH) process is a non-thermal technology that can be used to change the structure of fluid foods. Appropriate HPH treatment is benefit for uniform particle sizes distribution in juice and reducing pulp sedimentation. Cloudy apple juice was used as the experimental material in this study to evaluate the homogeneous pressure and homogeneous times (10^-1, 10^-2, 20^-1, 20^-2, 30^-1, 30^-2, 40^-1, 40^-2, 50^-1, 50^-2) on stabilities of juice. The indicators, such as, cloud value, cloudy stability, particle size distribution, ζ-potential and dynamic instability were investigated for juice stability. The results showed that, when homogenization at 20 Mpa for one time, the apple juice showed lower cloud value, higher cloudy stability, more uniform particle sizes, higher ζ-potential and lower dynamic instability. Therefore, the better HPH condition on stability of cloudy apple juice was 20 Mpa homogenizing for one time.

1. Introduction.
Apples are one of the most frequently consumed fruits. World apple production mounted up to 84.6 million tons in year 2014 and exhibited an ever-growing trend [1]. Approximately 35% of the total apple annual production is used for processed food, of which apple juice and cider represent up to 16% [2]. freshly pressed cloudy apple juice, one of the popular NFC juices, experiences an increasing market demand and value due to its mouthfeel sensation and health benefits [3]. Many researches showed that cloudy apple juice contains higher amounts of natural antioxidants (monomeric polyphenols, complex tannins, and non-polyphenolic compounds) than clear apple juice [4]. These compounds seem to have a positive effect on inhibiting cardiovascular diseases and cancer [5]. However, fast sedimentation, enzymatic browning, and flavor loss are the main challenges for cloudy apple juice manufacturing due to the presence of irregularly shaped particles, polymerized phenolic compounds, and the occurrence of enzymatic reactions [6].

Large and irregular particles in cloudy apple juice are the main reasons for juice sedimentation. High pressure homogenization (HPH) is a physical preprocessing step that can be considered to minimize the cloud loss in cloudy fruit juice. It was found that HPH was an effective approach to improve particle uniformity, reduce particle diameter, and modulate rheological characteristics of juice [7]. However, when the HPH was too intensive, the particles showed a tendency of aggregation for Brownian motion and intermolecular interaction.

The purpose of this work was to evaluate the effects of high pressure homogenization on particle stabilities in cloudy apple juice. This could provide some theoretical reference on stability improvement of cloudy apple juice.
2. Materials and Methods

2.1. Sample preparation
The apple cultivar of Ralls (Malus pumila) was chosen as the raw material for its most widely planting in western Liaoning Province of China and the better taste processed into juice. Apple fruits were purchased at a local market in Jinzhou, China, and then transported to the lab in Bohai University. Cored apples were pulped using DS-1 tissue blender (Wanhua experimental instrument factory, Jintan, China) with ascorbic acid (AA) added (1g/kg) as antioxidant. Then, juice was filtered through a 300-mesh filter cloth to remove impurities. The juice was homogenized under the pressure of 10 MPa, 20 MPa, 30 MPa, 40 MPa, and 50 MPa once and twice after filtered, and the obtained cloudy apple juice was subjected to various indexes. The results were expressed as 10-1, 10-2, 20-1, 20-2, 30-1, 30-2, 40-1, 40-2, 50-1 and 50-2.

2.2. Cloud Value and Cloudy Stability.
The juice was centrifuged at 760 × g for 10 min and the absorbance of the supernatant was measured at 660 nm using a 722N Visible Spectrophotometer (Precision Scientific Instruments, Shanghai, China) to determine the cloud values [8]. The samples were centrifuged at 4200 × g for 15 min, and the absorbance of the supernatant was measured at 625 nm. Cloud stability (CS) was calculated according to the following equation:

\[ CS\% = \frac{C_A}{C_0} \times 100 \]

where \( C_A \) is the absorbance after centrifuging and \( C_0 \) is the absorbance before centrifuging.

2.3. Particle Size Distribution (PSD).
The PSD was determined by the BT-9300ST Mastersizer (Better Instrument Co Ltd, Jinzhou, Liaoning, China). Laser light diffraction was used to measure particles from 0.1 to 1000 μm. The values of \( D_{10} \) (μm), \( D_{50} \) (μm), \( D_{90} \) (μm), \( D_{3,2} \) and \( D_{4,3} \) were calculated by the software provided by the equipment.

2.4. ζ-potential.
ζ-potential was determined by using a Zetasizer Nano ZS (Malvern Instruments, Southborough UK). The concentrations of the samples in distilled water (pH 7.0) were 3 mg/mL. Measurements were conducted at a temperature of 25 °C with a balance time of 2 min. The values were measured by 3 repetitions.

2.5. Dynamic instability.
The dynamic instability was determined by the Turbiscan Lab Stability analyzer (Liquid dispersion, Beijing, China). 20 mL of apple juice was poured into a sample cell. The near-infrared light was used as the light source along with transmitted light detector and backscattered light detector to form a measuring probe which automatically measured at indoor temperature every 40 mm cell height. Each measurement cost 20 s per hour.

2.6. Statistical analysis.
One-way ANOVA test by SPSS Statistics 20.0 was used to tested significant differences (\( p < 0.05 \)) among different groups by HPH treatment of cloudy apple juice. Experimental dates were expressed as mean ± standard error (\( p < 0.05 \)).

3. Results and discussions

3.1. Cloud value and cloudy stability.
The cloud value and cloudy stability were major stability parameters of cloudy apple juice. The cloud stability indicates how much apple juice is stable after centrifugation at 4200 × g for 15 min. This
level of centrifugation is considered equivalent to one year of storage [9]. The results were showed in Figure 1. In this study, the HPH played a very important role on the cloud level and cloud stability. Samples treated by 10-1, 10-2, 20-1 and 20-2 had higher cloud stability values, that indicated these samples had higher suspension stability. The cloudy stability of samples after homogenization were significantly higher than that of untreated one (p < 0.05). It was probably because that the pectin, protein, polyphenols and other substances in the solution system of apple juice were balanced with other component under appropriate homogenization pressures, and the system was relative stable, and little sedimentation occurred [10].

![Figure 1](image)

Figure 1. Effects of homogenization on cloud value (A) and cloud stability (B) of cloudy apple juice. Different letter (a-e) meant significant difference (p < 0.05). Data are the mean of triple replicates and vertical bars indicate ±SD.

3.2. Particle size distribution (PSD).

The results of PSD of juice were showed in Table 1 and Figure 2. From Table 1, with homogenization pressure increased, D_{10}, D_{50}, D_{90}, D_{3,2} and D_{4,3} decreased significantly, and this indicated the particle size became smaller. From Figure 2, the PSD changing trends of the eleven groups were similar. D_{50} was an important indicator for particle size, and it showed that the times of homogenizations at same homogeneous pressure had little effect on the particle sizes.

|       | D_{10}(μm) | D_{50}(μm) | D_{90}(μm) | D_{3,2}(μm) | D_{4,3}(μm) |
|-------|------------|------------|------------|-------------|-------------|
| 0     | 12.5±0.1\(^a\) | 238.1±3.6\(^a\) | 537.3±5.7\(^a\) | 40.3±0.2\(^a\) | 205.0±2.4\(^a\) |
| 10-1  | 8.7±0.2\(^b\)  | 224.1±4.0\(^b\)  | 480.5±8.6\(^b\)  | 22.6±0.4\(^b\)  | 184.2±2.8\(^b\)  |
| 10-2  | 5.5±0.1\(^c\)   | 217.3±4.6\(^c\)   | 462.2±9.8\(^c\)   | 6.5±0.1\(^c\)   | 176.9±1.6\(^c\)   |
| 20-1  | 1.9±0.0\(^d\)   | 188.7±0.8\(^d\)   | 433.0±2.9\(^d\)   | 5.4±0.1\(^d\)   | 169.8±4.4\(^d\)   |
| 20-2  | 1.5±0.0\(^e\)   | 173.2±2.5\(^e\)   | 394.6±3.6\(^e\)   | 4.3±0.0\(^ef\)  | 153.7±1.4\(^e\)   |
| 30-1  | 1.3±0.0\(^f\)   | 166.4±1.5\(^f\)   | 356.4±3.0\(^f\)   | 4.6±0.1\(^f\)   | 136.7±1.5\(^f\)   |
| 30-2  | 1.3±0.0\(^f\)   | 144.9±3.2\(^f\)   | 300.4±4.5\(^f\)   | 3.9±0.1\(^f\)   | 138.4±2.6\(^f\)   |
| 40-1  | 1.3±0.0\(^f\)   | 118.7±1.0\(^f\)   | 219.3±2.0\(^f\)   | 4.4±0.0\(^f\)   | 117.9±1.0\(^g\)   |
| 40-2  | 1.1±0.0\(^g\)   | 115.8±2.3\(^g\)   | 210.7±2.7\(^g\)   | 4.3±0.1\(^g\)   | 114.2±2.3\(^g\)   |
| 50-1  | 1.0±0.0\(^h\)   | 108.3±0.7\(^h\)   | 215.7±1.3\(^h\)   | 4.1±0.1\(^fg\)  | 107.4±1.0\(^h\)   |
| 50-2  | 0.9±0.0\(^h\)   | 98.7±0.7\(^j\)    | 192.9±1.5\(^i\)   | 3.6±0.0\(^h\)   | 97.3±1.2\(^l\)    |

Different letters indicated highly significant difference at the same column (p < 0.05).
3.3. ζ-potential.

The ζ-potential is a measurement of the charged nature of the particle surface and can be used to assess the electrostatic stability of suspended particles in dispersion. When the absolute value of the ζ-potential is higher, the particles will repel each other to achieve a stable state of dispersion; when it is lower, there is not enough electrostatic repulsive force between the particles to prevent the particles from collecting, and the system is usually in an unstable state [11]. The suspended particles in the apple juice moved toward the positive electrode under the action of an applied electric field, indicating that the surface of the suspended particles was negatively charged. The ζ-potential results were presented in Figure 3. The ζ-potential of cloudy apple juice increased with the increasing of pressure firstly, and then decreased. Samples under 20 MPa homogeneous pressures for one time had the highest absolute value, which indicated that the apple juice at this condition had better stability. However, there was no significant (p < 0.05) difference between the samples of 20-2 and 30-1.

3.4. Dynamic instability.

The TSI results of juice were presented in Figure 4. The higher index of TSI was, the more unstable the juice would be. It was shown that the stability of the homogenized samples were better than that of the non-homogeneous one. With the storage time prolong, the TSI of the cloudy apple juice increased gradually. After storage for 10 h, the TSI of most group tended to change slower, except samples of 40-1, 40-2, 50-1 and 50-2, which showed a particularly unstable state. This may be because the homogeneous pressure was too strong to cause particle aggregation and apple juice instability. The stability of the 20-1 sample was the most stable one.
Figure 4. Effects of homogenization on dynamic instability of cloudy apple juice.

4. Conclusions
The homogenization pressure has a significant effect on the stability of apple juice. With the HPH of 20 MPa treated for once, particles in apple juice were more uniform, and juice showed more cloudy stability, higher Zeta potential and lower dynamic instability.

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References
[1] Huang Z., Hu H., Shen F. (2018) Relatively high acidity is an important breeding objective for fresh juice-specific apple cultivars. J. Scientia Horticulturae. 233 29-37.
[2] Nicklas TA., O’Neil CE., Iii VLF. (2015) Consumption of various forms of apples is associated with a better nutrient intake and improved nutrient adequacy in diets of children: National Health and Nutrition Examination Survey 2003-2010. J. Food & Nutrition Research. 5925948.
[3] Krapfenbauer, G., Kinner M., Gossinger M. (2006) Effect of thermal treatment on the quality of cloudy apple juice. J. Journal of Agricultural and Food Chemistry, 54(15), 5453–5460.
[4] Oszmianski, J., Wolniak, M., Wojdylo, A. (2007) Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. J. Journal of the Science of Food and Agriculture, 87(4), 573–579.
[5] Barth, S. W., Faechndrich, C., Bub, A., Watzl, B., Will, F., Dietrich, H., Briviba, K. (2007) Cloudy apple juice is more effective than apple polyphenols and an apple juice derived cloud fraction in a rat model of colon carcinogenesis. J. Journal of Agricultural and Food Chemistry, 55(4), 1181–1187.
[6] Beveridge, T. (2002) Opalescent and cloudy fruit juices: Formation and particle stability. J. Critical Reviews in Food Science and Nutrition, 42(4), 317–337.
[7] Betoret E, Sentandreu E, Betoret N, Fito P. (2012) Homogenization pressures applied to citrus juice manufacturing. Functional properties and application. J. Journal of Food Engineering, 111(1), 28–33.
[8] Erteguy M F, Baslar M. (2014) The effect of ultrasonic treatments on cloudy quality-related quality parameters in apple juice. J. Innovative Food Science & Emerging Technologies. 26 226-231.
[9] Beveridge T. (2002) Opalescent and cloudy fruit juices: formation and particle stability, J. C R C Critical Reviews in Food Technology. 42 317-337.

[10] Kroistiani K, Yi J, Kebede B. (2018) Minimizing quality changes of cloudy apple juice: the use of kiwifruit puree and high pressure homogenization. J. Food Chemistry. 249-202.

[11] Genovese DB, Lozano JE. (2001) The effect of hydrocolloids on the stability and viscosity of cloudy apple juices. J. Food Hydrocolloids. 15(1):1-7.