

## Kinetics and Influencing Factors of Nonenzymatic Browning in Apple Juice Concentrate

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**Abstract:** The effects of various processing operation units on browning and the rate of nonenzymatic browning in commercial apple juice concentrates (70.5 °Brix) stored at 5, 20 or 37 °C were investigated. The changes of color value ( $T_{440}$ ), browning index ( $A_{420nm}$ ), amino nitrogen content and 5-hydroxymethylfurfural content were evaluated. The rates of browning at all three temperatures obeyed the first-order kinetic model. Browning reaction occurred more obviously at high temperature. There was a general decrease in amino nitrogen content and an increase in 5-hydroxymethylfurfural content with prolonged storage; this effect was most pronounced for samples stored at 37 °C. The relation between 5-hydroxymethylfurfural content and fruit browning could be described as an exponential explanation for the reason why browning occurs in apple juice concentrate during storage is Maillard reaction, which could also be validated by the linear relation between amino nitrogen and browning. During the early processing stages, including crushing, pressing, enzymolysis, ultrafiltration and first pasteurization, no obvious Maillard reaction was found. The follow-up operation unit, adsorption, resulted in the removal of amino nitrogen and other dark colored compounds. However, Maillard reaction was undesirably promoted, leading to an obvious reduction of amino nitrogen in apple juice.

**Key words:** nonenzymatic browning; apple juice concentrate; process; storage; Maillard reaction

## 苹果浓缩汁非酶褐变动力学及影响因素

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**摘 要:** 通过检测苹果汁的色值( $T_{440nm}$ )、褐变指数( $A_{420nm}$ )、氨基态氮、5-羟甲基糠醛含量等指标研究加工过程对苹果汁褐变的影响以及在不同贮藏温度(5、20℃和37℃)下苹果浓缩汁(70.5°Brix)的非酶褐变速率。3个贮藏温度下的苹果浓缩汁褐变速率均符合一级反应动力学模式,而且高温情况下褐变更明显。随着贮藏时间的延长,氨基态氮含量明显下降而5-羟甲基糠醛含量上升,尤其是贮藏在37℃的苹果汁这一变化更明显。5-羟甲基糠醛与果汁褐变效应呈指数关系,可以说明苹果汁在贮存中的褐变主因是发生美拉德反应,而且氨基态氮含量与果汁褐变效应的线性关系也验证了这一结论。在果汁的初始加工过程中,破碎、压榨、酶解、超滤、前巴氏杀菌等单元操作中美拉德反应并不明显;吸附操作可以去除果汁中的氨基态氮以及其他色素物质;而其他操作过程如浓缩和后巴氏杀菌则促进了美拉德反应,导致果汁氨基态氮含量明显下降。

**关键词:** 非酶褐变; 苹果浓缩汁; 加工过程; 贮藏; 美拉德反应

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Darkening and browning has long been a worldwide problem associated with fruit juices during thermal processing and storage. Brown colour appearing in juices is often perceived as deterioration of quality. In apple juice concentrates (AJC), accumulation of brown colour during process-

ing is due to enzymatic browning and nonenzymatic browning. However, one of the main causes of deterioration is nonenzymatic browning, since enzymatic browning can be eliminated by heat treatment in processing during first pasteurization<sup>[1]</sup>, so in the following process and storage, accumula-

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tion of brown color is mainly due to nonenzymatic reactions<sup>[2]</sup>. These reactions involve caramelization, ascorbic acid degradation and the Maillard reaction<sup>[3]</sup>. Since nonenzymatic browning influences the flavor, aroma and colour characteristics of cooked foods, it may be desirable in baked, fried or roasted foods such as coffee, tea, beer, bread and cake<sup>[4]</sup>. However, nonenzymatic browning reactions are the most common quality problem of many concentrated fruit juices, causing loss of nutrients and the formation of intermediate undesirable compounds, like furfural and 5-hydroxymethylfurfural (5-HMF)<sup>[5]</sup> which are an intermediate product in the formation of brown pigments during the Maillard reaction<sup>[6]</sup> indicating severity of heating applied to fruit juices during processing<sup>[7]</sup> and the level of browning in juice<sup>[8]</sup>. Among nonenzymatic browning reactions, the Maillard reaction, taking place between  $\alpha$ -amino groups and reducing sugars, is the most important cause of browning in apple juice<sup>[9]</sup>. Further more, the presence of amino acids in juice systems containing ascorbate is also considered a major contributor to the development of browning<sup>[8]</sup>.

In the world production of fruit juice, the apple juice output accounts for the proportion to be highest. Browning is an intractable problem and no reliable solution in commercial apple juice concentrate processing and storage. The objective of this study was to determine kinetics of non-enzymatic browning and factors which influence the browning of industrial apple juice concentrates in order to cope with it.

## 1 Materials and Methods

### 1.1 Materials, reagents and instruments

Commercial apple juice concentrate(70.5° Brix) produced by equipment and process of Tetra Pak Engineering Plant obtained from Shaanxi Hengxing Fruit Juice Co. Ltd.. All apple juice concentrates in sealed glass jars were stored in darkness at 5, 20 °C and 37 °C for 90 days.

UNICO 2000 (VIS) spectrophotometer Unico (Shanghai) Instruments Co.Ltd.; High-performance liquid chromatography(using a model SpectraSystem P4000 liquid chromatograph) Thermo Separation Products(USA); ATAGO PR301Brix spindle Atago Co.(Japan).

5- HMF was commercially purchased from the national institute for the control of pharmaceutical and biological products. Other reagents were all analytical reagent.

### 1.2 Methods

#### 1.2.1 Browning determination

Samples were diluted to 11.5 °Brix with distilled water. The transparency and the absorbance were determined in triplicate on a UNICO 2000 (VIS) spectrophotometer in 10 mm cells against water at 440nm (color value  $T_{440nm}$ )<sup>[10]</sup> and 420 nm (browning index  $A_{420nm}$ )<sup>[11]</sup>, respectively.

#### 1.2.2 Total amino acid (TAA) determination

Total amino acids determination was made in triplicate by the formol titration method, as recommended by AOAC<sup>[12]</sup>. The results are expressed as mean mmol/L at 11.5 ° Brix.

#### 1.2.3 Determination of 5-HMF

In this paper, 5-HMF was determined in triplicate by high-performance liquid chromatography using a model Spectra System P4000 liquid chromatograph according to Vural et al.<sup>[13]</sup> report.

#### 1.2.4 Colour development test during storage

Color value ( $T_{440nm}$ ), browning index ( $A_{420nm}$ ) and total amino acid of AJC stored at different temperatures were measured every 10 days, but 5-HMF was determined each month.

#### 1.2.5 Colour development analysis in process

In the complete apple juice concentrate product line of Shaanxi Hengxing Fruit Juice Co. Ltd. adopting equipment and process of Tetra Pak Engineering Plant, technological process time was calculated for the sampling can be comparable. Following the unit operations sequence, ground and pressed, first pasteurization, enzymolysis, ultrafiltration, adsorption, concentration and second pasteurization, apple juice was sampled in term of process time, in which Color value ( $T_{440nm}$ ), browning index ( $A_{420nm}$ ), total amino acid and 5-HMF were determined in triplicate. In the apple juice processing season time (from September to December), that on line measurements were repeated 5 times periodically every 15 days for reducing the difference of materials with different ripeness and the results are expressed as average mol/L at 11.5 °Brix.

#### 1.2.6 Statistical analyses

All determinations (except for being mentioned) were performed in triplicate and the mean values were calculated. Values were considered significantly different at  $P < 0.05$  level. Data were analyzed for degree of variation and significance of difference using an analysis of variance (ANOVA). All statistical analyses and charts were performed using Microsoft Office Excel 2003 or Origin Pro 7.5 (Origin Lab Corporation, MA, USA).

## 2 Results and Discussion

### 2.1 Browning in storage

Browning development in sample at 5, 20 °C and 37 °C for 90 days was described by  $T_{440nm}$  and  $A_{420nm}$  that changed with increasing storage time and with different temperature. When these values were plotted versus storage time, it was observed that the curve of nonenzymatic browning reaction followed a first-order reaction (Fig.1). The reaction model determined in this study was in agreement with previous studies<sup>[9,14]</sup>. In this study, nonenzymatic browning was considered as the Maillard reaction, since it is the most important cause of browning in apple juice<sup>[9]</sup>. However, some previous studies of the Maillard reaction mechanism have revealed that this reaction fits zero order and parabolic kinetic models. In model systems, Peterson et al.<sup>[15]</sup> and Stamp et al.<sup>[16]</sup> reported that Maillard browning follows a zero-order reaction. However, Cerrutti et al.<sup>[14]</sup> reported a first-order Maillard reaction in a glucose-lysine Maillard model system. A parabolic kinetic model of Maillard reaction of peach juice concentrate was also determined by Buedo et al.<sup>[5]</sup>. Fig.1 showed graph of the change colour of apple juice concentrate stored at 5, 20 °C and 37 °C over 90 days storage period. At all three storage temperatures, there was a progressive browning development with storage time. From the both figures, the browning reaction is prone to occur at high temperature, for the rate of this reaction at high temperature is faster than that at low temperature. Comparing with the initial sample, the final increased browning developments at the end of storage period were 12.45%, 46.86% and 415.28% for juice sample stored at 5, 20 °C and 37 °C, respectively.

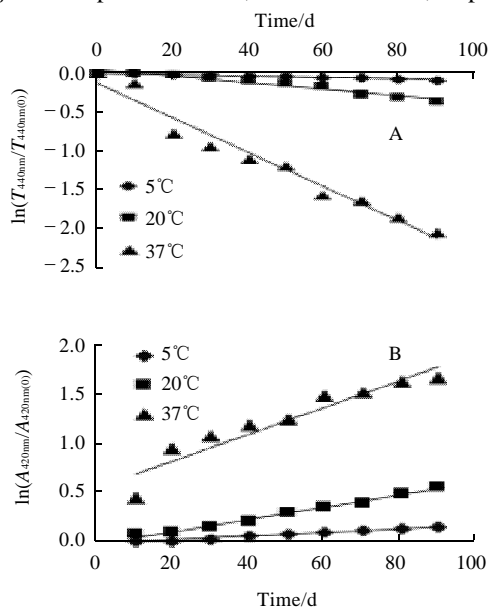


Fig.1 First-order reaction kinetics browning formation

The over all rates of Maillard reaction at all three temperatures, as measured by  $T_{440nm}$  and  $A_{420nm}$ , can be described by first-order reaction kinetics (Fig.1) using the following equations:

at 5 °C:

$$\ln(T_{440nm}/T_{440nm(0)}) = -0.0009t + 0.0036 (R^2 = 0.9672) \quad (1)$$

$$\ln(A_{420nm}/A_{420nm(0)}) = 0.0019t - 0.0253 (R^2 = 0.9833) \quad (2)$$

at 20 °C:

$$\ln(T_{440nm}/T_{440nm(0)}) = -0.0111t - 0.0514 (R^2 = 0.9397) \quad (3)$$

$$\ln(A_{420nm}/A_{420nm(0)}) = 0.0062t - 0.0186 (R^2 = 0.9870) \quad (4)$$

at 37 °C:

$$\ln(T_{440nm}/T_{440nm(0)}) = -0.0214t - 0.1799 (R^2 = 0.9515) \quad (5)$$

$$\ln(A_{420nm}/A_{420nm(0)}) = 0.0138t + 0.562 (R^2 = 0.9061) \quad (6)$$

Where  $T_{440nm}$ ,  $A_{420nm}$  were values at any time  $t$  (at 11.5 °Brix);  $T_{440nm(0)}$ ,  $A_{420nm(0)}$  were initial values (at 11.5 °Brix);  $t$  was storage time (0 — 90 days);  $R^2$  was correlation coefficient.

### 2.2 Total amino acid evolution

#### 2.2.1 Total amino acid decrease in storage

Decreases of TAA, in apple juice concentrate samples stored for 90 days at 5, 20 °C and 37 °C, are presented in Fig.2. The concentrations of TAA declined with storage time, and the rate of decrease was faster at higher temperature. The results were well consistent with kinetics of browning formation in Fig.1. Total amino acid loss is attributed to the Maillard reaction with glucose mainly, which is naturally present in the juice, but is also produced in larger quantities by the hydrolysis of the main sugar, sucrose, during storage<sup>[17]</sup>. The change of TAA (at the above mentioned temperatures) were shown as the following equations, which were in agreement with previous study<sup>[2]</sup>:

at 5 °C:

$$\ln(TAA/TAA_0) = -0.0005t - 0.0039 (R^2 = 0.9783) \quad (7)$$

at 20 °C:

$$\ln(TAA/TAA_0) = -0.0021t - 0.0035 (R^2 = 0.9826) \quad (8)$$

at 37 °C:

$$\ln(TAA/TAA_0) = -0.0043t - 0.0437 (R^2 = 0.9618) \quad (9)$$

Where TAA was concentration at any time  $t$  (mg/L at 11.5 °Brix);  $TAA_0$  was initial concentration (mg/L at 11.5 °Brix);  $t$  was storage time (0—90 days);  $R^2$  was correlation coefficient.

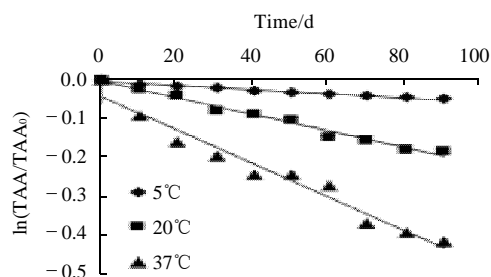


Fig.2 Total amino acid changes in AJC over 90 days storage

## 2.2.2 Total amino acid decline in industrial process

In order to obtain comparable samples, technological process time was calculated. Following the unit operations sequence, ground and pressing, first pasteurization, enzymolysis, ultrafiltration, adsorption, concentration and second pasteurization, apple juice should be sampled at the time 0, 17, 200, 230, 240, 250, 260 min, respectively. Because the apple juice production season lasts several months and the quality of apple was different with production season, sampling was repeated 5 times periodically every 15 days in a production season, and then, total amino acid was determined.

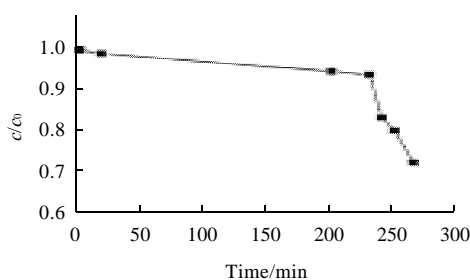


Fig.3 Total amino acid evolution in industrial process

During industrial process time, the concentrations of total amino acid described as Fig.3. At initial process time, ground and pressed, first pasteurization, enzymolysis and ultrafiltration have no significant effect on decrease of TAA ( $P > 0.05$ ). However, adsorption, concentration and second pasteurization can obviously reduce the content of TAA ( $P < 0.05$ ), especially in concentration. In the complete process, the concentration of TAA was descending, in agreement with 5-HMF ascending in process (Fig.6), which confirmed juice browning during process after first pasteurization is Maillard reaction.

## 2.3 5-HMF evolution

### 2.3.1 5-HMF formation in storage time

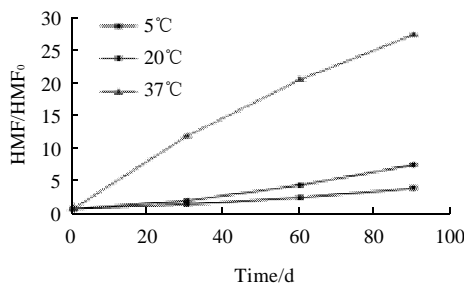


Fig.4 5-HMF formation over storage at 5, 20°C and 37 °C

The contents of 5-HMF increase significantly at all three temperatures (Fig.4) during storage time. After 90 days

storage and comparing with the initial sample, the contents of 5-HMF at 5, 20 °C and 37 °C samples have reached 4.09, 7.70 and 27.58 times, respectively, which according with the results of Fig.1. Lee et al.<sup>[7]</sup> have reported the same results and suggested that temperature is an important factor affecting browning, and that the rate of browning appears to increase sharply in the initial storage time at high temperature.

The correlation between browning, described by  $T_{440nm}$  and  $A_{420nm}$ , and 5-HMF values are evaluated in Fig.5, and the equations were shown:

$$T_{440nm}/T_{440nm(0)} = 0.226 + 0.879e^{-0.1075HMF/HMF_0} \quad (R^2=0.9780) \quad (10)$$

$$A_{420nm}/A_{420nm(0)} = 5.4948 - 4.4221e^{-0.0250HMF/HMF_0} \quad (R^2=0.9689) \quad (11)$$

Where  $T_{440nm}$ ,  $A_{420nm}$  were values at any time  $t$  (at 11.5 °Brix);  $T_{440nm(0)}$ ,  $A_{420nm(0)}$  were initial values (at 11.5 °Brix); HMF was values at any time  $t$  (μg/L at 11.5 °Brix);  $HMF_0$  was initial values (g/L at 11.5 °Brix);  $R^2$  was correlation coefficient.

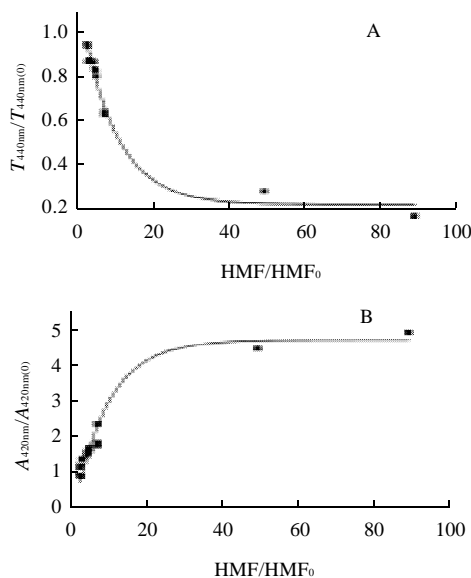


Fig.5 Correlations between  $T_{440nm}$  and 5-HMF(A),  $A_{420nm}$  and 5-HMF(B)

The relation between 5-HMF and browning of juice can be described as exponential and the regression coefficients ( $R^2$ ) were higher than 0.90. So, the reason why AJC becomes browning during storage is Maillard reaction; browning changes greatly by little change of HMF. At the beginning of storage, lower temperature can retard browning in juice.

### 2.3.2 5-HMF mounts up in industrial process

The method of sampling as 2.2.2 mentioned, sampling was also repeated 5 times periodically every 15 days in a production season, and then, 5-HMF was determined.

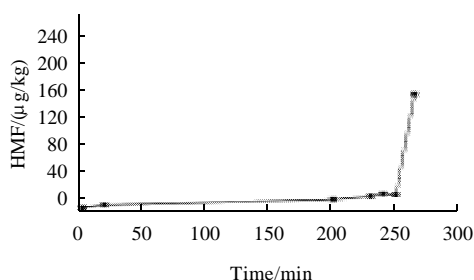


Fig.6 5-HMF formation in industrial process

At initial processing stage (Fig.6), low temperature and instant high temperature sterilization, browning reacts slowly, which has no obvious formation of 5-HMF ( $P > 0.05$ ). However, at the end of process concentration and second pasteurization, the rate of browning reaction increases significantly ( $P < 0.05$ ) for the sake of high temperature and long-playing, as well as Maillard reaction readily reacting at water activity  $0.6 - 0.7^{[18]}$ , which indicated that the cause of browning in these process is mainly Maillard reaction.

### 3 Conclusions

The rates of browning in commercial apple juice concentrates (70.5 °Brix) stored at 5, 20 °C and 37 °C fit first-order kinetic model. The browning reaction is susceptible to high temperature. There was a general decrease in amino nitrogen content and an increase in 5-hydroxymethylfurfural content with storage especially for samples stored at higher temperature (37 °C). The relation between 5-hydroxymethylfurfural and browning of juice can be described as exponential indicating apple juice concentrates becomes browning during storage is Maillard reaction. Amino nitrogen changes also supported the same reason. Ground, pressing, enzymolysis, ultrafiltration and first pasteurization have no obvious effect on the nonenzymatic browning of apple juice. Adsorption can absorb amino nitrogen and other dark colored compounds reducing the nonenzymatic browning. Concentration and second pasteurization can reduce the content of amino nitrogen for promoting Maillard reaction.

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