Kinetics Modelling on Malliard Reaction of Sugars with Amino Acids in Apple Juice

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Abstract: Maillard reaction is important for its effect on apple juice quality during heat processing. In order to control a complex reaction such as Maillard reaction, it is necessary to take a quantitative study. In this paper, a kinetic model was developed for the reaction among major sugars and amino acids in apple juice. A major guidance for understanding the mechanism of Maillard reaction in apple juice was given. To describe the quantitative changes of reaction and to predict the changes from certain time and temperature effects, the nonenzymatic browning of apple juice was discussed with the knowledge of kinetics.

Key words: kinetics modelling; Maillrd reaction; apple juice; nonenzymatic browning

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苹果汁中糖和氨基酸的美拉德反应动力学模拟研究

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摘要：在苹果汁的热加工过程中，美拉德反应对其质量的影响是极其重要的。为了能控制象美拉德反应这样复杂的反应，必须对其进行定量研究。本文通过对苹果汁中主要糖和氨基酸的美拉德反应动力学模拟。阐述了苹果汁中非酶褐变的美拉德反应机理，应用反应动力学原理，定量地描述反应的变化和预测反应随时间-温度的变化规律。

关键词：动力学模拟；美拉德反应；苹果汁；非酶褐变

Apples are the most widely grown and consumed temperate fruit crops. The annual yield of apple in the world was estimated to be more than 40 million tons 15 years ago, of which more than 5 million tons were processed to obtain apple juice[1,2]. Europe with the European part of the CIS and Turkey might make app. 40%~45% of the world apple juice concentrated product[3]. The P.R. of China is the biggest apple producing country in the world with an accounting for a half yield of the world. It was estimated roughly that by the end of 1998 China had 61 plants for apple jiuce concentration with 72 production lines and a production capacity of 180,000 t. The apple concentration capacity in China is already in significant scale, but in terms of apple production, the processing only uses a very small part of it. At present, many major fruit juice importing countries such as US, UK, Germany, France and Netherlands are in business relations to the fruit juice industry in China. Particularly, as China had taken part in the WTO, the apple juice production of China is increasing steadily with more export channels opening [4]. Thus, the apple juice industry is emphasized both in Europe and China.

The most commercial processing of apple juice includes several general steps. The process starts with the harvesting, transport and washing of the fresh fruit, and then they are milled to a pulp and pressed. During the pressing step pectolytic enzymes are added to the pulp in order to break down the fruit cell structure, so that pulp pressability and process yield can be improved. The raw apple juice obtained after pressing must be clarified prior to its commercial use. Apple juice clarification begins with depectinisation by pectinase. This process may result in a lower viscosity and larger particles cause by

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the flocculation of pectin-protein complexes so that the juice is much easier to filter. It is usually carried out at 50 ℃ for 2 h or 20 ℃ for 8~12 h. Those settled solids and other suspended matter are removed by conventional filtration[5].

Evaporation is a conventional method for concentrating clarified apple juice. It is performed in multiple effect evaporators at temperatures between 45 ℃ and 90 ℃[6]. Sometimes natural apple juice has to be produced under inhibiting enzymatic oxidation with ascorbic acid[7].

It has long been known that browning is the most common quality problem with apple juice during processing and storage. Browning is the result of a complex series of chemical reactions that cause the quality loss of apple juice, such as increase in colour intensity, decrease in brightness, formation of off-flavour, and nutrient losses[8-10]. As a matter of fact, color change and off-flavor formation are the major causes of apple juice rejection. It was reported that millions bottles of soviet apple juice became unsaleable because nobody liked the juice with dark and cloudy[3].

The major kinds of reactions leading to browning are enzymatic[11] and nonenzymatic[12,13]. The enzymatic browning is a result of the oxidation catalyzed by polyphenol oxidase (PPO) of phenolic compounds to quinones and their subsequent condensation to colored pigments. Enzymatic browning may be prevented by temperature and/or pressure or chemical agents via inactivation of polyphenoloxidases[14]. The nonenzymatic browning is favoured by heat treatments and includes a wide number of reactions such as Maillard reaction, caramellisation, ascorbic acid decomposition and chemical oxidation of polyphenol. Nonenzymatic browning is considered as the major cause of browning in the absence of active polyphenol oxidase after apple juice processing [15,16].

Chemical Composition of Apple Juice

The composition of apple juice depends on the variety, origin, growing conditions of apple, the quality of the fruit, processing procedures and storage. Table 1 and Table 2 showed the approximate composition of raw apple juice obtained from pressing apple [23-25].

Table 1 Composition of apple juice

Compound	Concentration/(g/L)	Compound	Concentration/(g/L)
Water	860~900	Malic acid	3~7
Sugars	100~120	Ascorbic acid	0.05
Fructose	46~70	Pectin	1~5
Glucose	20	Polyphenols	1
Sucrose	27	Proteins	0.6
Starch	0.5~5	Ashes	2

Table 2 Composition of free amino acid in apple juice

Amino acid(10^{-2} mg/g)	72° Brix (concentrated)	11.5° Brix (fresh) Min	Max	Mean
Aspar agine	296.8	18.6	116.7	41.1
Glutamic acid	57.6	2.3	5.6	3.5
Aspartic acid	40.7	5.9	27.9	13.9
Serine	9.6	0.81	2.2	1.4
Glutamine	6.7	<0.16	0.97	0.55
Alanin	4.8	0.9	2.14	1.31
Amino Butyric acid	4.3	0.46	1.41	0.7
Proline	3.9	0.52	2.5	0.99
Isoleucine	3.8	0.23	0.89	0.50

Sugars

The most important sugars in apple juice are glucose and fructose and sucrose. The fructose content ranges from 46~70 g/L. Glucose concentration is 20 g/L. Sucrose, 27 g/L [23]. It was reported that glucose was more reactive than fructose[21]. If the content of reducing sugars (fructose+glucose) to total sugars was increased the browning would be faster.

Amino acids

Amino acids, commonly found in apple juice and highly reactive during the Maillard browning were asparagine (Asn), aspartic acid (Asp) and glutamic acid (Glu)[15]. Literature[26] reported that with reference to colourless intermediate formation (A_{280} and HMF), Asp was the most reactive amino acid. while in brown pigment formation, it was Asn. Glutamic acid was the least reactive amino acid for both processes. The results were consistent with the reports in which Asn was nearly 70% of the total amino compounds in clarified apple juice, and 90% of it disappeared after 100 days of storage at 37 ℃.

Ascorbic acid

The chemistry of the degradation of ascorbic acid is related to the Maillard reaction. Ascorbic acid undergoes a reaction chemically similar to that of sugars except that amino acids are not necessary for browning. since ascorbic acid is very reactive, it degrades by two pathways, both of which lead to the formation of dicarbonyl intermediates and subsequently to browning compounds[12]. Literature values[27] for ascorbic acid content in apple juices are 0.7~2.0 mg/100 g.

The formation of melanoidins in Maillard reaction can be enhanced by a reductone as it exerts an influence on the Maillard reaction[28].

Organic acids

The main organic acid in apple juice is malic acid. its content ranged from 3~7 g/L. Lewis et al[29] suggested that organic acids can react with reducing sugars to produce brown pigments. The color produced as a result of this reaction was shown to be of the same order as that produced in the reaction of the amino acids with glucose. other results[21] indicated when the malic acid content ranged from 6 to 43 g/L in a model solution of constant composition, an increase in malic acid actually accelerated the rate of browning during storage, although the participation of malic acid in the nonenzymatic browning, via Maillard reaction, was judged to be catalysed[30].

During the enzymatic clarification process, the natural pectic substances are broken by pectinases which are able to hydrolyse pectins to their basic units. The free galacturonic acid could be present in clarified apple juice after the enzyme treatments. The effect of 60 mg/L of galacturonic acid in a model solution was reported and the results showed that after only 25 days the A_{420} values exceeded the absorbance scale, indicating a very important contribution of galacturonic acid in the colour formation[21].

Total phenolic compounds

Nonenzymatic chemical oxidation may occur in the absence of active polyphenol oxidase, causing oxidation of phenol to brown polymeric compounds in fruit products[49]. The phenolic composition in apple juice consists of cinamic acids such as esters of caffeic and coumaric acid with quinic acid[31], flavonols such as quercetin glucoside[32], dihydrochalcones such as phloridzin[33], catechins such as epicatechin, and procyanidins such as dimer B2[30]. Spanos. et.al. reported[31] that the storage of concentrates for 9 months at 25℃ resulted in the formation of HMF, degradation of cinnamics (ca. 36%) and quercetin and phloretin glycosides (ca.60%), as well as a total loss of procyanidins.

Quality Changes of Apple Juice

The nonenzymatic browning of apple juice results in considerable changes in quality:

Nutritional value

Nutrientsubstance and nutritional value can be lost: degradation of vitamins and carbohydrate, amino acid loss, and amino acid unavailability for human metabolism[17].

Off-flavour compounds

Off-flavour compounds can be formed: formation of several off- flavor substances such as hydroxymethylfural (HMF), furfural, and 2,5-dimethyl-4-3-(2H)-furanon. HMF may be resulted from heating and concentration, in storage of apple juice and so can be often used as a quality indicator[18]. It is responsible for the cooked taste of apple juices[19].

Brown colour

Brown colour can be developed: the formation of melanoidins from advanced Maillard reaction[21] and the formation of brown polymeric compoundsby from phenolic compounds in apple juice[22].

Oxidative compounds

Oxidative compounds can be formed in Maillard reaction [20].

Maillard Reaction

Maillard browning reaction

In concentrated apple juice the main reaction contribution to nonenzymic browning is considered to be the Maillard browning reaction[24,36,37]. It causes an interesting research area for the implications in apple juice stability and technology, as well as in nutrition and health.

Stages of Maillard reaction

The Maillard reaction is the reaction between carbonyls and amines. It involves three stages[38]: the initial stage, intermediate stage and final stage.

The first stage involves the sugar-amine condensation and the Amadori rearrangement, to form the so-called Amadori product. The second stage involves sugar dehydration and fragmentation and amino acid degradation especially at high temperature. In the final stage of browning, the intermediates polymerize and unsaturated, fluorescent, colored polymers, known as melanoidins are formed. The chief reactions involved are thought to be aldol condensation, aldehyde-amine polymerization, and the formation of heterocyclic nitrogen compounds. The chemistry of these compounds is not well-known and their formation mechanism also remains obscure. Browning occurs at this stage.

Affecting factors

The course of Maillard reaction is strongly affected by various factors[39]. They can be considered as food processing and storage variables. These are temperature, duration of heating, pH, water content, type of reactant, ratio of amino acid to sugar, oxygen, metals, type of buffer and the presence of any reaction inhibitors, such as sulfur dioxide. Tmperature, pH, and water activity are believed to play the crucial role.

Kinetics Study

Undoubtedly, Maillard reaction is one of the most complex reactions given up to hundreds of reaction products. In order to be able to control these complex chemical reactions, they need to be studied in a quantitative way. With knowledge of kinetics, it becomes easier to describe the changes quantitatively and to predict the changes from certain time and temperature effects. Kinetics is also a tool for understanding the reaction mechanism.

Simple kinetics

Most literature results dealed with kinetics of Maillard reaction in apple juice using simple kinetics to describe changes[21,40,41]. The following part describes the methods used by various researchers.

(a) Measuring the absorbance due to coloured products at 420 nm was the most common method[21,40~43].

(b) The loss of amino acids and sugars had also been used to follow the Maillard reaction [42,43].

(c) The productions of Amadori rearrangement products (ARP) and the accumulation of HMF were followed. But the accumulation of ARP was found not to correlate with the rate of browning [44] and HMF was relatively low reactive in the Maillard reaction [45].

The Maillard reaction involves a complicated reaction mechanism. It is not easy for a complicated reaction to use of simple kinetics. The use of such simple kinetics is not providing mechanistic insight in the Maillard reaction. It can only be useful as a mathematical tool for engineering purposes. All of the kinetic work on apple juice was done in this way.

Multiresponse modelling

Recently, there was some development in the kinetics of Maillard browning. Van Boekel introduced the multriresponse approach in the area of Maillard kinetics [46]. The Basic idea was to take into account as many responses as possible at once, i.e. together with sugar, amino acids, Amadori product, degradation products, as opposed to only one response. There are two major advantages in this approach: models can be tested much more rigorously, and once a model seems acceptable, estimation of parameters can be done much more precise.

The basic procedure consists of following steps:

(a) Determination of main reactants and products;

(b) Proposition of reaction mechanisms;

(c) Building of a kinetic model based on the proposed reaction mechanism;

(d) Fitting of the kinetic model with data.

Kinetics Modelling

Kinetic modelling has been applied to describe the chemical changes in food. Moreover, it can also help in understanding the chemistry and mechanism of Maillard reaction.

Proposition of the reaction mechanism;

The reaction mechanism described in this proposal can be summarized as Fig.1.

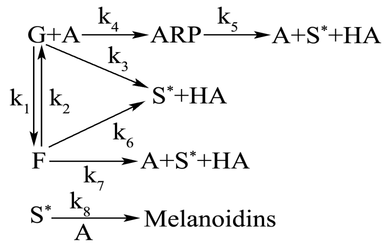

Fig.1 Kinetic model used to describe the parallel interaction of sugar isomerisation/degradation and Maillard reaction.

Note: G: open chain form of glucose; F: open chain form of fructose; A: amino acid; S^*: reactive sugar fragments (DH); HA: organic acids formed (acetic + formic acid); ARP: Amadori product.

Building of kinetic model based on the proposed mechanism

Fig.1 shows the kinetic model based on the mentioned above reaction.

Fitting the kinetic model with data.

Once the model is proposed, it should be fitted with experimental data.

Conclusion

Maillard reaction is a cascade of consecutive and parallel reaction steps during the heating and processing of apple juice for its contribution to juice quality. It is of utmost importance for the food technologist to be able to control the extent of Maillard reaction. In order to control the Maillard reaction, it is necessary to study quantitatively. Using the knowledge of kinetics, it is possible to describe the quantitative changes and to predict the changes from certain time and temperature effects. Kinetics is also a tool for understanding reaction mechanisms. The kinetic model for the reactions among the major sugars and amino acids in apple juice can also be developed .

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