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Study of Maillard Reaction Inhibitors for the Sugar Cane Processing

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ABSTRACT

Maillard Reaction (MR) is a complicated non-enzymatic browning which can happen in many kinds of food, processed products such as breweries, dairy products and sugar products. Studies have done in order to remove the Maillard reaction products, to decolorize colored compounds or to treat wastewater from sugar cane factory. For this reason, this research was performed to study and identify MR inhibitors during sugar cane processing using model systems with different concentrations of amino acids and glucose, 0.09 mol L^{-1} for asparagine (Asn); 0.08 mol L^{-1} for glutamine (Gln) and 0.2 mol L^{-1} for glucose (Glc), which were heated at 85-90°C for two hours, based on physicochemical analyses and assays. The influence of the physical factors such as temperature, pH or water activity (a_w) were investigated as well as chemical agents such as Ascorbic Acid (AA), Cysteine (Cys), Nicotinic Acid (NA), NaCl and vitamin B6 at different concentrations (0.05, 0.1 and 0.15%), respectively. According to the results, AA 0.05%, NA 0.05% and Cys 0.1% have significantly reduced the browning effect, soluble matter (°Bx) and improved the pH compare with the untreated samples. These findings open new possibilities for strategies in the study and control of MR.

Key words: Maillard reaction, glucose, asparagine, glutamine, inhibitors

INTRODUCTION

During sugar cane processing many reactions and other factors affect quantity and quality of the final product (sucrose). These factors are related to the formation of non-sugars, mainly colored compounds and reducing sugars. The formation of these components during sugar cane processing is the result of physical variation such as pH, temperature, reaction time and autocatalytic effects (Bento and Sa, 1998). The thermal treatment of reducing sugars with amino acids or other proteins in an aqueous system with low water activity, colored compounds appeared in few minutes, browning increased with longer reaction times (Knerr *et al.*, 2001). In the past, many studies have been done on MR. More work has been concentrated on low molecular weight, fine particulates and colored compounds which could be the main constituents of Maillard reaction products. The principal components of the sugar cane are sugar and fiber (Honig, 1953). The average composition of sugar cane depends on the state of maturity of the plant (fiber 10-18%; water 70-77%; saccharose 12-16% and non-sugar compounds 2-5%). According to Roberts and Martin (1959), 100 mL of sugar cane juice contain about 15.5% of asparagine and glutamine which was the highest composition of amino acid.

Some developing countries where people are consuming non-refining sugar, the performance of sugar factories remains to be improved. The MR not only causes the loss of saccharose but also produces melanoidins (Coca *et al*., 2004); acrylamides (Zyzak *et al*., 2003; Knol *et al*., 2005) and the persistence of the unaccepted dark brown color (Pant and Adholeya, 2007). Engineers should take action to slow down the MR.

So far, few studies have concentrated on preventing the MR during sugar cane processing and its effect on non-refining sugar quality. Previous knowledge supports that Maillard reaction can be controlled by the physical factors such as temperature, reaction time, pH and water activity. The present paper concerns on the study and identification of the chemical inhibitors which can reduce or stop the Maillard reaction and its intermediary to be formed during the sugar cane processing before the crystallization (sugar boiling) phase.

MATERIALS AND METHODS

Materials: D-glucose-monohydrate, L-asparagine-monohydrate, L-glutamine, L-ascorbic acid, L-Cysteine, nicotinic acid, pyridoxine-hydrochloride, sodium hydroxide, all chemicals were the best available analytical grade reagents and supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium chloride was bought from the local market.

Simulation of MR: The present study was conducted in the years 2011-2012 at School of Food Science and Technology, Jiangnan University, People's Republic of China. To investigate the influence of different inhibitors on the MR, a simulating solution was prepared with glucose and amino acids. 0.025 mol of glucose was mixed with 0.09 mol of Asn, 0.08 mol of Gln. To obtain a homogeneous mixture both reactants were carefully heated (IKA Werke GmbH and Co. KG, Branch Laboratory Instrument Co., Ltd. Guangzhou, China) at 85-90°C. During the heating process, the pH of the two mixtures was adjusted to 8.5 with NaOH (0.5%) to initiate MR in the systems. The pH was measured using the METTLER TOLEDO pH-meter FE 20 Five Easy pH (Shanghai Toledo Instrument, Co., Ltd., China). The color was measured by the UV-VIS spectrophotometer UV1600 (Shanghai MAPADA Instruments, Co., Ltd., China). The dry matter content was measured by portable refractometer FG-108 and expressed on Brix.

Inhibition of MR: Three different concentrations (0.05, 0.1 and 0.15%) of each inhibitor (AA, Cys and NA) previously prepared were used to investigate their effect on the Maillard reaction of the systems. Each concentration of the inhibitors prepared was separately added to both mixtures during the heating and the process allowed to proceed for a little over two h. After two hours, the mixture was cooled to room temperature. A control experiment was carried out without addition of inhibitors. The resultant products obtained after the heating process were then considered as Maillard reaction products. The resultant products were subjected to the further analysis in order to determine the effects of the inhibitors on the reaction. All experiments were prepared in triplicate.

Analysis of samples: The digital pH-meter (METTLER TOLEDO) was used to measure the pH of the systems. The systems (Asn+Glc and Gln+Glc) had the same water activities (0.95) measured at room temperature using the formula:

$$a_w = \frac{p}{p^0} = \frac{n_2}{n_1 + n_2}$$

where, n_1 is the number of moles of solute and n_2 is the number of moles of water. Titration assay was used to add the different inhibitors in the samples in the order to control or to stop the Maillard reaction. Samples color was measured (brown pigment formation) at 560 nm (Clement *et al*., 2010). When necessary, samples were diluted with distilled water (1/5 v/v).

RESULTS AND DISCUSSION

Two model systems (Asn+Glc and Gln+Glc) were investigated for studying the effect of inhibitors on the Maillard reaction in the sugar cane process. Glucose is the typical reducing sugar in sugar cane and asparagine and glutamine have the highest percentage as amino acids in the sugar cane (Roberts and Martin, 1959). Chemical reagents were used to slow down the MR.

Effect of the inhibitors on the pH: The pH plays an important role in the reaction. Maillard reaction needs a particular pH (≤8.5) to initiate the reaction. At the beginning of the reaction, the pH of the systems was acidic (≤4.6) and at this pH the Maillard reaction cannot occur. To allow the reaction to run, sodium hydroxide 0.5% was added to raise the pH to the favorable value for Maillard reaction. The pH was recorded every ten minutes to see its variation and the results are shown in the Fig. 1a for the system Glc-Asn and Fig. 1b for the system Glc-Gln, which explained

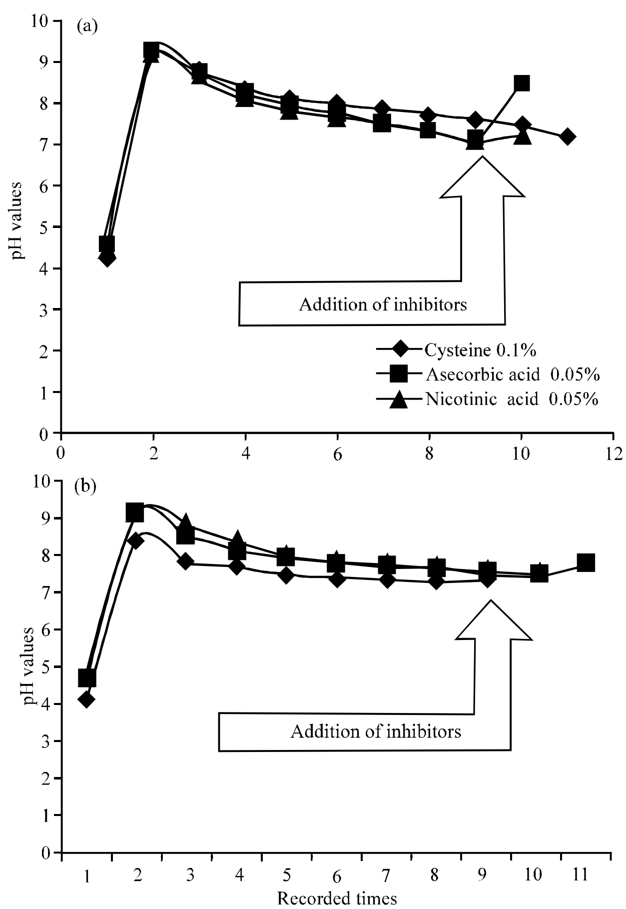

Fig. 1(a-b): Illustration of pH before, during and after the reaction according to time in the model systems (a) Gln+Glc and (b) Asn+Glc

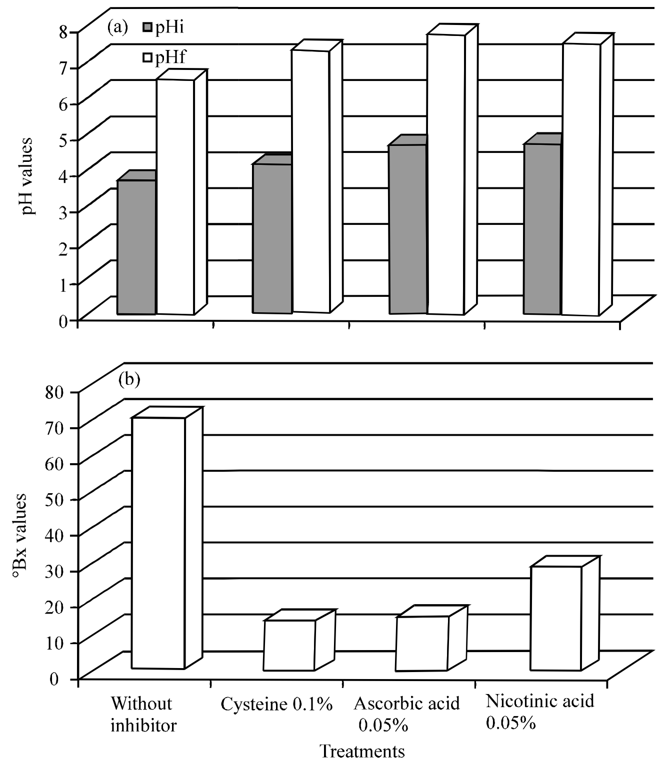

Fig. 2(a-b): Illustration of the difference between pHi and pHf in the model systems (a) Asn+Glc and (b) Gln/Glc to compare treated samples (with inhibitors) to untreated samples

that after the NaOH was added in the model systems, the pH started to decrease with the reaction time, temperature and water activity. This fall of pH was to promote the MR. The pH can be used as an indication for the degree to which the MR has occurred.

Contrary to temperature and water activity which favored the occurrence of MR, the effect of inhibitors on the pH increases or stabilizes this one. An increase in the final pH (pHf) was observed after the addition of chemical inhibitors. Figure 2a showed that in the Asn-Glc system AA (7.74) has the highest pHf, after NA (7.49) and the smallest is Cyst (7.3). Figure 2b showed in the Gln/Glc model that AA (8.47) has the highest pHf, after NA (7.24) and finally Cyst (7.19) has the smallest pHf. This augmentation of pH confirmed as well as the one of the important factors of Maillard reaction (Mao *et al*., 2007).

Effect of the inhibitors on the soluble matter (°Bx): The soluble matter was expressed in °Bx. It increases when the temperature increases and the water activity decreases (Hedegaard *et al*., 2007). Chou *et al*. (2002) have reported that the ultrafiltration reduced the concentration of macro-molecules and well dispersed fine particulates which are nothing other than Maillard reaction products. Figure 3a (Asn-Glc) showed that the untreated sample (70.5°Bx) has the highest concentration of soluble matter compare to the samples treated with inhibitors. Soluble matter has been affected more by Cyst (14°Bx) than AA (15°Bx) and NA (29°Bx). The Gln-Glc (Fig. 3b) model was treated in the same conditions and the result shown that AA (9°Bx) has more effect on the system more than NA (10°Bx) and Cys (11°Bx). However, this study has shown that the inhibitors

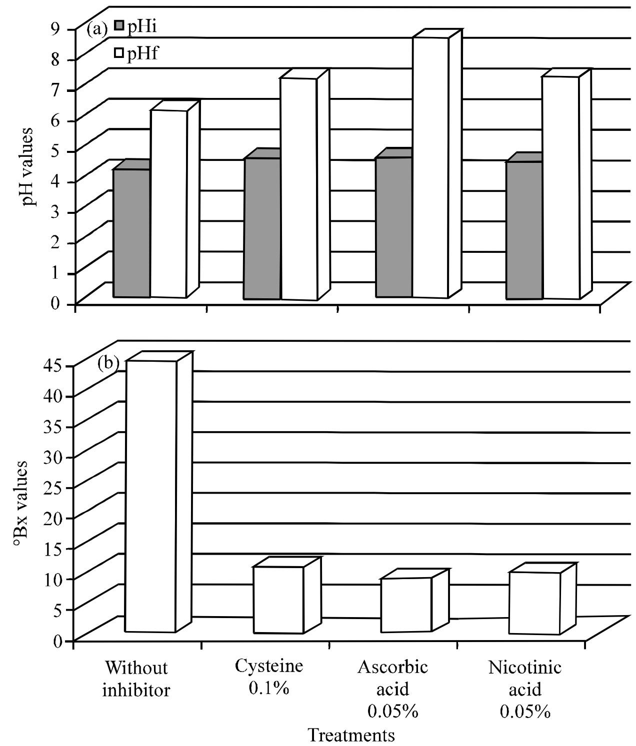

Fig. 3(a-b): Illustration of the inhibitors effects on the soluble solids (Brix) in the model systems (a) Asn/Glc and (b) Gln/Glc treated samples compared to untreated samples

strongly affected (reduced or stopped) the Maillard reaction products which happened during the experiments. Therefore, the percentage of soluble matter (°Bx) was reduced in both systems. This effect of the inhibitors on the soluble matter can facilitate the crystallization (sugar boiling) of sucrose and reduce the percentage of Maillard reaction products such as melanoidins, acrylamide, macro-molecules and other colored compounds.

The Maillard reaction produces many kinds of products such as organic acids (De Vleeschouwer *et al.*, 2010); acrylamides melanoidins (Ibarz *et al.*, 2009) and unknown ones which increased with the temperature and heighten the concentration of soluble matter in the samples which can affected the quality of the final product.

Effect of inhibitors on color: Browning is a classical feature of the extent of the Maillard reaction in its advanced and final stages and it has been directly related to the colored compounds formation in both model systems (Asn+Glc and Gln+Glc). Thus, the rates of Maillard reaction could be represented by the color formation and expressed directly by the optical density. The variation of color between the samples (Fig. 4a for Asn-Glc and Fig. 4b for Gln-Glc) was the effect of inhibitors on the reaction. Table 1 (Asn-Glc) showed that the untreated sample (without inhibitors)has the highest value (0.156 nm) compare to the samples which were treated with inhibitors. The addition of inhibitors reduced significantly the browning with NA (0.065 nm) afterward Cyst (0.077 nm) and then AA (0.078 nm). Table 2 (Gln-Glc) showed the same phenomena but the difference was with Cys (0.056 nm) which has more effect than NA (0.063 nm) and AA (0.062 nm).

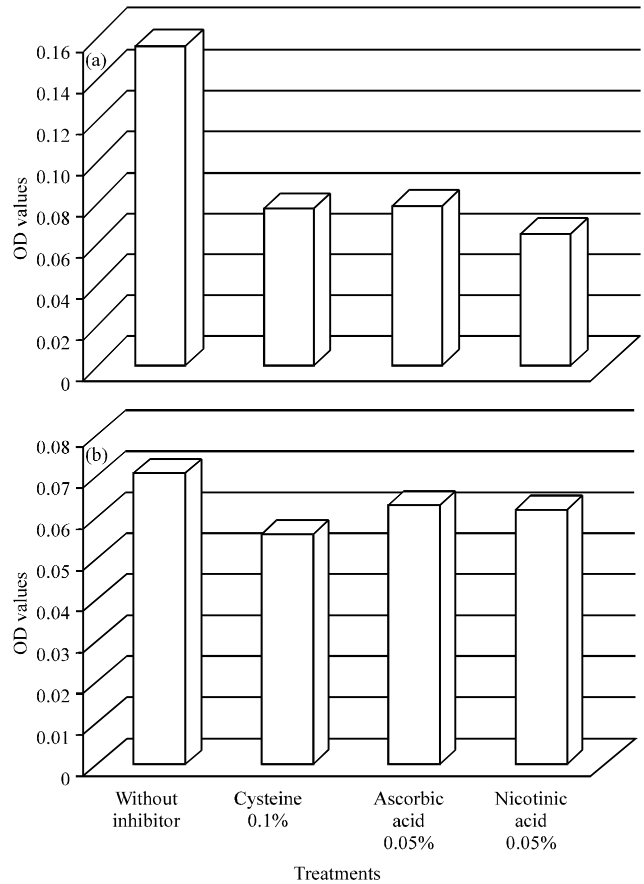

Fig. 4(a-b): Illustration of inhibitors effect on color (OD value) in the systems (a) Asn/Glc and (b) Gln/Glc to see the difference between treated samples (with inhibitors) and untreated samples

Table 1: Inhibitors effects on different parameters compare to the untreated samples in the model systems Asn/Glc

Asn/Glc*	Bx	pHi	pHf	OD
Without inhibitor	70.5±3.0	3.70±0.5	6.51±0.5	0.156±0.002
Cysteine 1%	14.0±3.0	4.15±0.5	7.30±0.5	0.077±0.002
Ascorbic acid 0.5%	15.0±3.0	4.67±0.5	7.74±0.5	0.078±0.002
Nicotinic acid 0.5%	29.0±3.0	4.71±0.5	7.49±0.5	0.065±0.002

*Parameter values (1, 0.5 and 0.5%) are transformed values

Table 2: Inhibitors effects on different parameters compare to the untreated samples in the model systems Gln/Glc

Glc/Glc*	Bx	pHi	pHf	OD
Without inhibitor	44.5±3.0	4.21±0.5	6.14±0.5	0.071±0.002
Cysteine 1%	11.0±3.0	4.58±0.5	7.19±0.5	0.056±0.002
Ascorbic acid 0.5%	9.0±3.0	4.60±0.5	8.47±0.5	0.063±0.002
Nicotinic acid 0.5%	10.0±3.0	4.45±0.5	7.24±0.5	0.062±0.002

*Parameter values (1, 0.5 and 0.5%) are transformed values

Sugar refining processes used the operations such as carbonation; filtration and ion exchange to remove colored substances before crystallization to produce white sugar. Any or combination of above operations selected should have performed the highest color removal with minimum environmental problems. In the order to produce good quality of sugar, many studies have concentrated on removing of color during the sugar cane processing. Donovan and Williams (1992) have concluded that the color occlusion in sugar crystal which gave the higher molecular weight colorants can be separated by membrane. Chou *et al.* (2002) concluded that the SAT process is a direct replacement of the sulfitation, carbonation and blanco directo processes for plantation white sugar productions.

In the same way of thinking, chemical factors (inhibitors) were used in this study to reduce the browning substances generated by MR (Fig. 4a, 4b) showed the extent of browning in samples without inhibitors and samples with inhibitors. On the one hand, Asn/Glc system, browning increased rapidly with the increase of temperature and water activity to reach 0.156 nm (Fig. 4a). On the other hand, Gln/Glc system, browning increased with the raise of temperature, afterward became stable (Fig. 4b). At 85-90°C, the model Asn/Glc without inhibitors the absorbance reached the highest point 0.071 nm (Fig. 4a). However, for the Asn/Glc system, the color had fallen to 0.077 nm in presence of Cys 0.1 and 0.078% nm in the presence of AA 0.05 and 0.065% nm in the presence of NA 0.05%. For the Gln/Glc system treated in the same conditions, the results showed (Fig. 4b) that the samples without inhibitors, the browning was higher than the samples which were treated with inhibitors. However, the variation in the color between untreated samples and the treated samples was less than 0.015 nm for Cys 0.05%, less than 0.01 nm for AA 0.05% and NA 0.05%. These results have shown that the three inhibitors (AA, Cys and NA) strongly reduced the advanced and the final stages of the Maillard reaction and its intermediate compounds in the systems especially for Asn/Glc model.

The effects of different inhibitors on Maillard reaction in the systems (Asn/Glc and Gln/Glc) were investigated. For this purpose, three kinds of inhibitors such as ascorbic acid (Cortez-Vega *et al.*, 2008), cysteine (Tochi *et al.*, 2009) and nicotinic acid at different concentration (0.05, 0.1. 0.15%) were added into the model systems when the browning started between 85-90°C during two hours. Results are shown in Table 3 for Gln-Glc and Table 4 for Asn-Glc the percentage necessary of the inhibitors to inhibit the Maillard reaction compared to the samples without inhibitors. Both systems (Asn/Glc, Gln/Glc) have shown inhibition activities to some extent for all concentrations. However, the model systems have respondedpositively with AA, Cys and NA

Table 3: Results with all inhibitors and different concentrations in the model systems Gln/Glc

	Gln/Glc			
Inhibitors (%)	Bx	pHi	pHf	OD
Cysteine				
0.05	11	4.28	6.57	0.081
0.10	11	4.58	7.19	0.056
0.15	9	4.7	7.16	0.058
Ascorbic acid				
0.05	9	4.6	8.47	0.063
0.10	8	4.67	7.36	0.06
0.15	9	4.56	7.46	0.063
Nicotinic acid				
0.05	10	4.45	7.24	0.062
0.10	9	4.28	7.12	0.055
0.15	10.2	4.38	7.12	0.054

Table 4: Results with all inhibitors and different concentrations in the model systems Asn/Glc

	Asn/Glc			
Inhibitors (%)	Bx	pHi	pHf	OD
Cysteine				
0.05	20	4.1	7.28	0.124
0.10	14	4.15	7.3	0.077
0.15	19	4.17	6.9	0.083
Ascorbic acid				
0.05	15	4.67	7.74	0.078
0.10	18	4.35	7.45	0.075
0.15	21	4.71	7.7	0.077
Nicotinic acid				
0.05	29	4.71	7.49	0.065
0.10	25	4.43	7.43	0.069
0.15	20.5	4.5	7.23	0.058

(Table 1 for Asn-Glc and Table 2 for Gln-Glc) because inhibitors affected the different steps of Maillard reaction and stopped its intermediaries tobe formed (browning compounds). Among these inhibitors Cys provided the highest inhibition effects followed by AA and NA. This explains that Maillard reaction has been affected by these chemical factors (AA, Cys and NA) which greatly influenced the formation of browning compounds especially AA which is known to have antioxidant effect (this point will be more developed in the second part of our work).

CONCLUSION

The physico-chemical analyses andassays were used to identify the chemical inhibitors on Maillard reaction occurred during the sugar cane processing which may affected the final product. This study revealed that ascorbic acid, cysteine and nicotinic acid have inhibitory effects on the Maillard reaction. Despite all these, more investigations are required regarding the effect of the Maillard reaction in sugar cane processing before the crystallization (boiling sugar) of the sucrose. Future studies of MR inhibitors for the sugar cane processing should be targeted on the antioxidant effect of these three chemical inhibitors (AA, Cys and NA) and should be focused on their action in the different steps of MR.

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