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Effect of Maillard reaction browning factors on color of membrane clarification non-centrifugal cane sugar during storage

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Abstract

The effect of Maillard reaction browning factors on the color enhancement of membrane clarification non-centrifugal cane sugar (M-NCS) in storage was explored. During storage, the color of M-NCS gradually browned and blackened. pH, the contents of glucose, fructose, protein, total amino acids and 5-hydroxymethylfurfural (5-HMF) decreased significantly. Meanwhile, the moisture content, relative percentages of volatile Maillard reaction products (MRPs) and the content of 3-deoxyglucosone (3-DG) increased during storage. The sucrose content remained stable. Correlation analysis shows all browning factors were significantly correlated with color. Multivariate data analysis results demonstrated that the compound effects of volatile MRPs, 3-DG, pH, glucose and total amino acids led to the color change of stored M-NCS. The order of effect on the color change of stored M-NCS was volatile MRPs > 3-DG > pH > glucose > total amino acids.

Keywords: M-NCS; Maillard reaction; browning indicators; storage.

Practical Application: The mechanism behind color changes were revealed by determining Maillard reaction browning indicators. Correlation analysis and multivariate data analysis were used reveal the effect of Maillard reaction browning indicators on color changes of stored M-NCS and provide a theoretical basis for the quality maintenance.

1 Introduction

Non-centrifugal cane sugar (NCS) is a traditional and functional sugar with molasses presented in lump or powder form, which is traditionally produced using light limed clarification and manual removal of floating foam while boiling in open pan (Meerod et al., 2019). Apart from sugar, NCS also contains various amino acids, proteins, organic acids, phenols, flavonoids, policosanols, and minerals (Chand et al., 2011; Jaffé, 2015; Weerawatanakorn et al., 2016; García et al., 2017). NCS is also known for its various biological functions including anti-cariogenic, anti-toxicity, anti-carcinogenic effects, anti-oxidation, cyto-protection, skin damage protection and immunity improvement (Payet et al., 2005; Jaffé, 2012; Asikin et al., 2013, 2014, 2016; Lee et al., 2018). Thus, NCS is used not only in food and beverage as a sweetener but also in the pharmaceutical industry and health and skin care industry. Many countries or regions across the world produce or consume NCS, which is known in different names, such as kokuto (Japan), raqadura (Brazil), jaggery (Southeast Asia and Africa), panela (Latin America), brown sugar (China, Europe, and North America), and raw sugar (USA).

The properties of NCS, especially sensory characteristics, nutrition, active components, and antioxidant activity, are influenced by sugarcane variety (Singh et al., 2019), clarification procedure (Meerod et al., 2019), and drying-solidification methods (Weerawatanakorn et al., 2016) and storage conditions (Chand et al., 2011). Keeping the quality stability for NCS, as a typical seasonal production and year-round sales product, is important because of its instability in physicochemical characteristics and flavor during storage (Asikin et al., 2014). A total of 5%-10% NCS was reported to be lost during storage due to undesirable deteriorations in aroma, flavor, and quality (Kumar et al., 2017). Asikin examined the changes in physiochemical characteristics and flavor components and Maillard reaction products (MRPs) of NCS during 12 months, and the results showed that the browning rate in NCS was positively correlated with the contents of volatile products from Maillard reaction (Asikin et al., 2014). However, no further research on the relationships between the browning and Maillard reaction indicators in NCS during storage is available. Such study is essential to predict and control the browning reaction of NCS during storage.

Nowadays, nano ceramic-membrane is applied commonly to produce NCS (M-NCS) in China, the study has examined the nutritional and antioxidant properties of a sugar product obtained from sugarcane juice clarified with 50 nm ceramic membrane (M-NCS) and compares these properties with commercially available NCS products. M-NCS contains the potent antioxidants caffeic acid and gallic acids, and the overall antioxidant properties based on α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power are comparable to the commercial products. However, it contains 22-34% less essential amino acids, although comparable

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proportions of arginine, glutamine and leucine. As M-NCS has a lower turbidity, starch and amino acid contents than the NCS products, it would be a better choice for the beverage industry as it would sig- nificantly reduce the unpleasant haze formed in drink formulations (Zhu et al., 2020). However, the storage properties, especially browning properties of M-NCS remain unexplored. Such investigation is crucial to maintain the quality stability of M-NCS in storage given that it is stored and sold for the next whole year.

Therefore, the present work intended to reveal the rule and mechanism involved of the color enhancement in stored M-NCS. Meanwhile, the mechanism behind color changes were revealed by determining pH, moisture content, sugars, protein, total amino acids, volatile MRPs, 3-deoxyglucosone (3-DG) and 5-hydroxymethylfurfural (5-HMF).In addition, multivariate data analysis were used to analyze the complicated results. The observations made in this study reveal the effect of Maillard reaction browning indicators on color changes of stored M-NCS and provide a theoretical basis for the quality maintenance.

2 Materials and methods

2.1 Standards and reagents

Chemicals used as standards for analyzing sugars (fructose, glucose, and sucrose), 5-HMF, 3-DG, and 2,3-diaminonaphthalene were obtained from Yuanye Reagent Co., Inc. (Shanghai, China). Acetonitrile, formic acid, and methanol were all chromatographic pure and purchased from Xingke High-Purity Solvents Co., Ltd. (Shanghai, China). Zinc sulfate, potassium ferrocyanide, trichloroacetic acid, Coomassie brilliant blue G-250, ethanol, and phosphoric acid were from Guoyao Group Chemical Reagent Co., Inc. (Shanghai, China) and were of analytical grade.

2.2 Samples

Heart-shaped M-NCS (mass 20 ± 2 g/piece, size 3 cm × 3 cm × 2 cm) was obtained from Guangxi Baigui Sugar Food Technology Co., Ltd., China and was newly produced in the 2019/2020 production season. The samples and desiccants were placed together into the PV plastic tanks and then sealed and stored in dark at room temperature for 0, 2, 4, 6, 8, and 10 months without humidity control. The monthly average temperature and humidity changes for 10 months can be seen in Figure S1 (supplementary data). After storage, the M-NCS was milled into powder to pass a screen of 1.70 mm. The powdered M-NCS samples were kept at –30 °C prior to analysis.

2.3 Color, moisture content and pH analysis

The color of M-NCS was determined using the procedure of Asikin et al. (2014), in terms of the international unit of color for sugary products as per the standard ICUMSA (International Commission for Uniform Methods of Sugar Analysis) GS1/3-7 protocol. 1 g of sample was dissolved in 100 mL distilled water to obtain M-NCS solution. Adjust the pH of the solution to 7.0 with NaOH solution, and then the solution was filtered by 0.45 μM microporous filter membrane. The absorbance of the solution was measured by ultraviolet spectrophotometer at the wavelength of 420 nm.

Moisture content was evaluated on the wet basis of the weight loss of a 2 g sample during oven drying at 105 °C for 2-4 h under atmospheric pressure until the mass difference between two results was less than 2 mg. The result was expressed as percentage (%) of the total weight.

The pH of M-NCS was measured with a digital pH meter FE22 (Mettler Toledo instrument Co. Ltd, Shanghai, China), 1 g of sample was dissolved in 100 mL distilled water to obtain M-NCS solution.

2.4 Sugar composition analysis

The sugar composition of M-NCS was determined by HPLC (Asikin et al., 2014). A 1160 Infinity HPLC system (Agilent Technologies, Inc., Santa Clara, USA) equipped with a refractive index detector model G1362A was used. The mobile phase consists of acetonitrile: water (70 : 30, v/v). Fructose, glucose, and sucrose were separated with an Agilent NH_2, 250 x 4.6 mm, 5 μm column (Santa Clara, CA, USA) with a flow rate of 1 mL/min. The column oven temperature was 35 °C. The sugar composition and concentration were calibrated by plotting peak areas against the concentrations for the respective sugar standards, and they were expressed as grams per 100 g M-NCS.

2.5 Protein and amino acid analysis

The protein content of M-NCS was determined using Coomassie brilliant blue G-250 method adapted from Grintzalis et al. (2015). 5 g of sample was dissolved in 50 mL distilled water to obtain M-NCS solution, and put 5 mL of it into a stoppered tube. 5 mL of Coomassie brilliant blue G-250 reagent was added into stoppered tube, standing for 10 min. The samples were measured with a UV spectrophotometer SP752 (Spectrum instrument Co., Ltd, Shanghai, China). Bovine serum albumin was used as the preparation material of protein standard solution, and the standard curve of protein concentration absorbance was made.

The amino acid contents of the M-NCS were determined using an automatic amino acid analyzer L-8900 (Hitachi, Tokyo, Japan) following the method of Shim et al. (2013).

2.6 Volatile MRPs analysis

Headspace solid phase microextraction (HS-SPME) method was applied to isolate the flavor components and analyzed by gas chromatography-mass spectrophotometry (GC-MS) (Asikin et al., 2014). 6 g of sample was placed in a closed vial and heated in a water bath at 60 °C for 20 min. The volatile flavour components were then absorbed onto an SPME fiber containing divinylbenzene/carboxen/polydimethylsiloxane (Supelco Inc., Bellefonte, PA, USA) whilst heating for 50 min. The GC-FID analysis was performed using an Agilent 7890B GC system equipped with a fused silica capillary column (DB-Wax, 60 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent J&W, Santa Clara, CA, USA).

2.7 5-HMF and 3-DG content analysis

The content of 5-HMF in M-NCS was evaluated using HPLC method adapted from Polovková & Šimko (2017). 4 g of sample into a 10 mL volumetric flask, dissolved in 8 mL of ultrapure water, add 0.3 mL of 15% potassium ferrocyanide solution and 0.3 mL of 20% zinc sulfate solution to precipitate the substances interfering with the measurement in the brown sugar sample, and fix the volume to 10 mL. The mobile phase consists of methanol: water (80 : 20, v/v), with an Agilent HC C18, 250 x 4.6 mm, 5 μm column (Santa Clara, CA, USA) with a flow rate of 0.7 mL/min. The column oven temperature was 40 °C and the wavelength was 284 nm. The standard curve of 5-HMF concentration peak area was made with 5-HMF standard.

The content of 3-DG in M-NCS was evaluated using HPLC method (Asikin et al., 2014). 1 g of sample was dissolved in 10 mL distilled water to obtain M-NCS solution. 5 mL of 1.6 mmol/L 2.3-diaminonaphthalene was added into M-NCS solution and then in a water bath at 50 °C for 24 h. The mobile phase consists of methanol (0.1% formic acid): water (40 : 60, v/v), with an Agilent HC C18, 250 x 4.6 mm, 5 μm column (Santa Clara, CA, USA) with a flow rate of 0.9 mL/min. The column oven temperature was 40 °C and the wavelength was 268 nm. The standard curve of 3-DG concentration peak area was made with 3-DG standard.

A 1160 Infinity HPLC system (Agilent Technologies, Inc., Santa Clara, USA) equipped with a UV detector model G1314F was used. The concentrations of 5-HMF and 3-DG were expressed as milligrams per 100 g M-NCS.

2.8 Sensory evaluation

Sensory evaluation was conducted in isolated booths at the laboratory. Twenty sugar engineering students with good professional ability conducted the analysis. Before the analysis, the entire panelists were given oral instruction on the attributes (color, flavor, texture, and general acceptability) and the direction of testing but randomly. They were also provided with a palate cleanser (water), and also, scorecards were provided to each panelist. Panellists assessed the samples and were asked to indicate his or her opinion and the overall acceptance using a nine-point hedonic scale (from 1: dislike extremely to 9: like extremely), and the acceptability index (Lafarga et al., 2019; Nduko et al., 2018).

2.9 Statistical analysis

The mean value and standard deviation of the results were obtained by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 23.0 (IBM Inc., USA). All experiments were repeated at least 3 times, and $p < 0.05$ was considered significant. Principal component analysis (PCA) was conducted by IBM SPSS Statistics 23.0 (IBM Inc., USA). Orthogonal partial least squares analysis (OPLS) was conducted by SIMCA 13.0 (Umetrics, Sweden). Data chart was made with Origin 9.65 software (OriginLab Inc., Northampton, USA).

3 Results and discussion

3.1 Color, pH, and moisture content in stored M-NCS

The increase of color comes from the production of melanoidins, this results in the formation of a large group ofhigh molecular weight, brown-colored and nitrogen-containing polymerized products, known collectively as melanoidins (Henle et al., 1996; Capuano & Fogliano, 2011). The M-NCS IU increased significantly by 70.13% ($p < 0.05$) from 15400 ± 57.74 IU to 26200 ± 25.16 IU over 10 months of storage (Figure 1A) with an average rate 14.07% every 2 months, while the actual rate of every storage stage varied significantly ($p < 0.05$). The Maillard reaction rate is different at each stage, affecting the rate of color formation. There are many browning factors that determine the rate of Maillard reaction, and the combined effects of different browning factors lead to the development of Maillard reaction in different directions. The browning development of M-NCS during storage was consistent with Asikin's findings (Asikin et al., 2014). However, the average browning rate of M-NCS was higher, and the fluctuation in browning rate was more obvious due to differences in sugarcane variety, clarification procedure, and NCS morphology (Zhu et al., 2020). Obviously, the change of color is directly proportional to the storage time.

The initial pH of M-NCS was 5.93 (Figure 1B), and it significantly decreased to 5.72 during the whole storage ($p < 0.05$). At this time, the initial stage of the Maillard reaction will occur carbonyl ammonia condensation reaction, the carbonyl ammonia condensation is reversible, and the product of carbonyl ammonia condensation is easily hydrolyzed under acidic conditions, and the free amino acid is blocked during the carbonyl ammonia condensation process, causing the pH of the system to decrease (Van Boekel, 2006). The pH of M-NCS is less than 7 during storage, so the Maillard reaction during storage is mainly 1,2-enolation, which first generates key pigment intermediates such as 3-DG and 5-HMF, and finally generates melanoidins (Henle et al., 1996). The pH not only affects the Maillard reaction pathway, but also determines the browning intensity of MRPs. Lotfy et al. (2021) found that an increase in pH was positively correlated with the browning intensity of MRPs, that is, the intensity of Maillard reaction increased with the increase of pH. Comparing the decline rate of pH from June to October with the increase rate of color from June to October, it can be found that this conclusion is similar to Lotfy's finding.

The initial moisture content of M-NCS was 4.98% (Figure 1C). It increased significantly in the first 6 months ($p < 0.05$) to 5.12%, but it decreased to 5.02% in the fourth storage stage, which may be caused by wrong experimental operation. Because of the hygroscopic behavior of M-NCS during storage (Verma & Narain, 1990), it is unlikely to result in a drop in moisture content. Then, it increased again to 5.13% at the final storage stage. One accepted explanation for the Maillard browning bell curve is that as the moisture content increases, the reaction rate decreases due to dilution of the reactants (Maltini et al., 2003). Observing the changes of color and moisture content from June to August, it is found that the increase rate of color decreased after the decrease of moisture content, indicating that the Maillard reaction process can not be determined by the change of a browning factor alone. The change of moisture

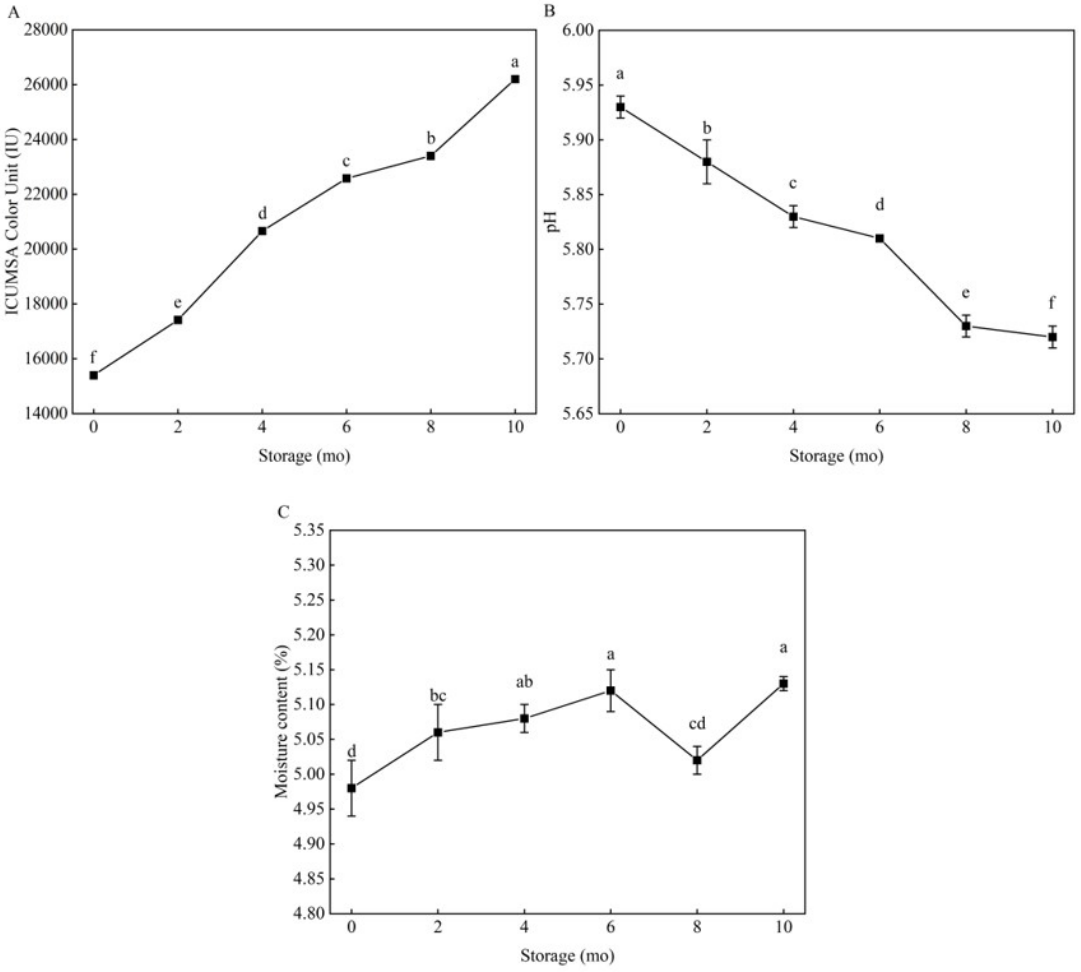

Figure 1. Changes in (A) color, (B) pH, and (C) moisture content of stored M-NCS. Different lowercase letters indicate significant difference ($p < 0.05$).

content is similar to that of ambient humidity (Figure S1). The best packaging material in terms of moisture barrier and quality conservation are the drying cum storage followed by polyethylene bags (Chand et al., 2011).

3.2 Sugar in stored M-NCS

Over 10 months of storage, sucrose content remained stable (81.36 ± 0.11-81.04 ± 0.03 g/100 g) (Figure 2A), whereas fructose content decreased by 17.50% (from 2.43 ± 0.05 g/100 g to 2.02 ± 0.03 g/100 g) (Figure 2B). Glucose content also changed from 2.61 ± 0.03 g/100 g to 1.21 ± 0.03 g/100 g with a decrease of 53.64%. Significant decreases in fructose and glucose were observed every 2 months and throughout the storage stages ($p < 0.05$). The higher consumption rate of glucose ($p < 0.05$) indicated that glucose is more prone to participate in Maillard reaction in M-NCS during storage at room temperature due to the steric hindrance of the carbonyl group in fructose (Yaylayan & Forage, 1991). In the final stage, fructose content in M-NCS increased significantly. The hygroscopic behavior of the product promotes microbial activity, triggering the biochemical degradation that is reflected in the production of inverted sugars (Verma & Narain, 1990), adding to the inherent content of inverted sugars in the product. Glucose and fructose participate in the Maillard reaction as reducing sugars, and determine the final M-NCS color through different reaction pathways. The fructose content did not decrease but increased in the final stage, which indicated that glucose was mainly involved in Maillard reaction as reducing sugar. The consumption rate of glucose is higher than fructose in both the final stage and the whole stage. It can be shown from the side that the decrease of glucose is more closely related to the increase of color, and the contribution of glucose to color enhancement is greater than that of fructose.

3.3 Protein and total amino acids in stored M-NCS

The initial content of protein in M-NCS was 23.45 ± 0.23 mg/100 g, which decreased to 16.67 ± 0.16 mg/100 g during storage ($p < 0.05$) (Figure 3A). The decrease rate in protein content was up to 11.56% during first storage stage, and then, it ranged from 4.77% to 6.40% during each subsequent storage stage.

A total of 17 kinds of free amino acids were identified in M-NCS: 3 alkaline amino acids, 2 acidic amino acids, and 12 neutral amino acids. The initial total free amino acid content was 303.84 ± 3.75 mg/100 g, and decreased by 38.71% during

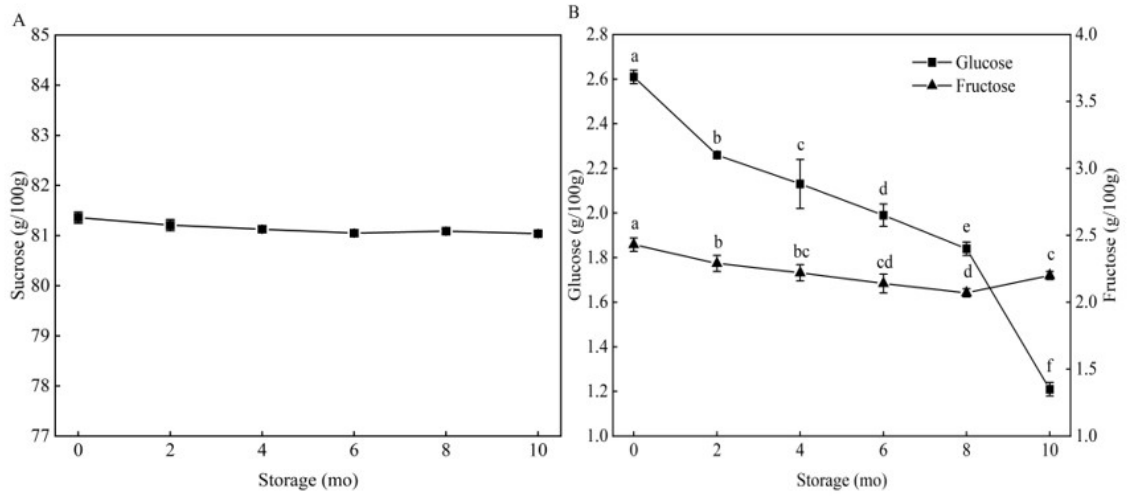

Figure 2. Changes in (A) sucrose and (B) glucose and fructose content of stored M-NCS. Different lowercase letters indicate significant difference ($p < 0.05$).

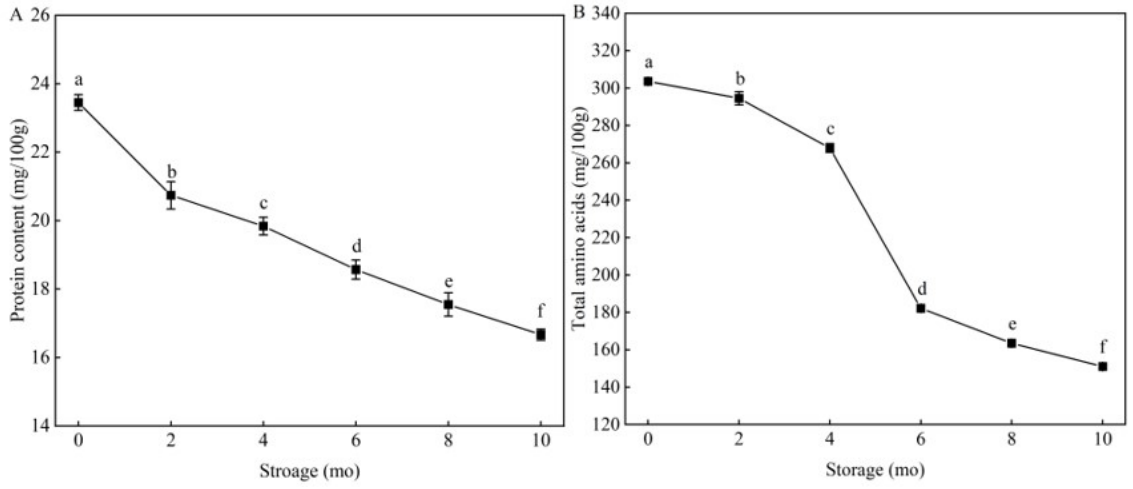

Figure 3. Changes in (A) protein and (B) total amino acids content of stored M-NCS. Different lowercase letters indicate significant difference ($p < 0.05$).

10 months of storage ($p < 0.05$) (Figure 3B). However, the consumption rate varied throughout the storage, and it was up to 32.02% in the third storage stage. It accounted for 82.72% of the total loss.

The contents of protein and amino acids, as the reactants that provide amino groups (Hodge, 1953), decreased significantly during storage ($p < 0.05$), but the changes were not the same. Except for the first stage, the protein has a faster decline rate, and the other stages have a relatively stable decline rate. However, the decreasing rate of total amino acid content accelerated at first and then slowed down, and the fluctuation was large. This suggests that proteins are more stable in the Maillard reaction, while amino acids may be more susceptible to other factors. The reason for the decrease of amino acid content is not only because it is the main reactant of Maillard reaction to participate in the formation of melanoidin to increase the color of M-NCS, but also because free amino acids participate in Strecker degradation and its own degradation reaction (Huyghues-Despointes & Yaylayan, 1996).

3.4 Maillard reaction products in stored M-NCS

A total of 12 volatile MRPs were identified in M-NCS samples: 8 Pyrazines, 2 Furanols, 1 Furanone, and 1 Pyranone. the relative percentage of volatile MRPs increased from 26.43% to 35.78% (Figure 4A) with storage time ($p < 0.05$). This is not consistent with Asikin's findings (Asikin et al., 2014). The contents of glucose, fructose and total amino acids in this study decreased faster per unit time, which accelerated the occurrence of Maillard reaction. The change trend of the relative content of volatile MRPs is similar to that of color, indicating that its internal relationship may be closer. The only difference is that the increase rate of the relative content of volatile MRPs decreases and the increase rate of color increases from June to August.

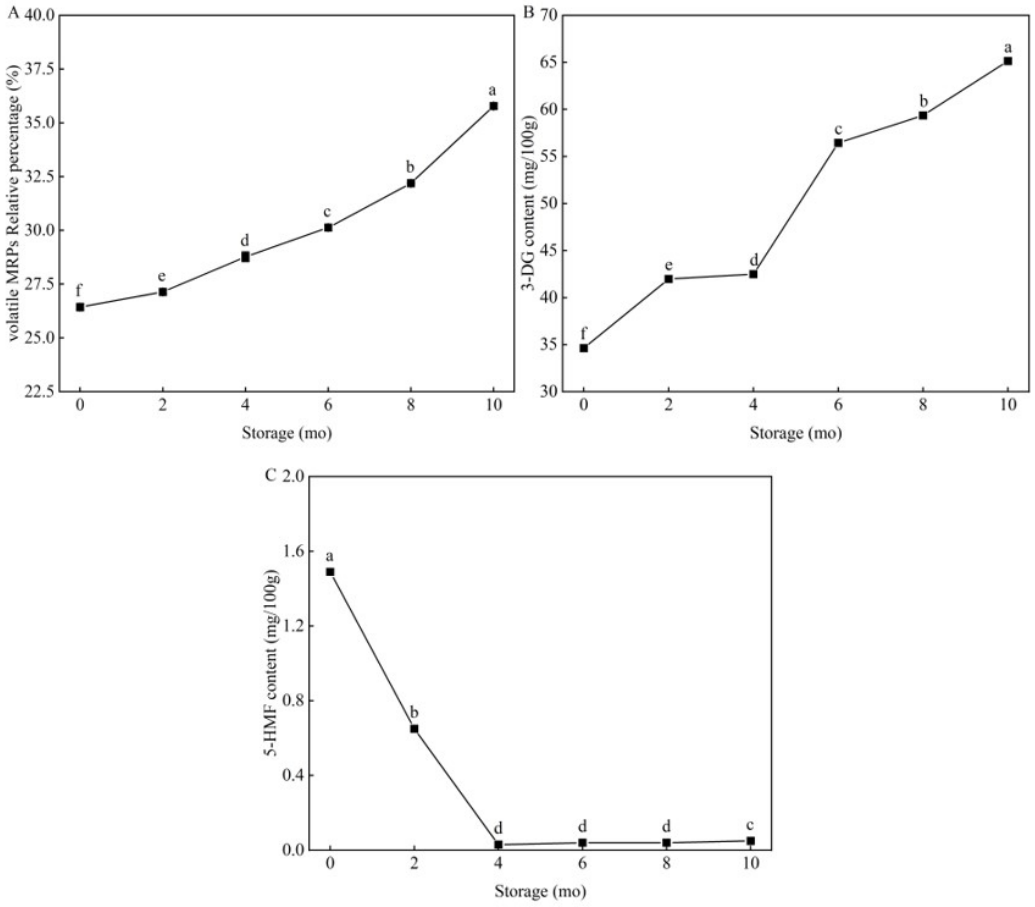

Figure 4. Changes in (A) volatile MRPs, (B) 3-DG and (C) 5-HMF contents of stored M-NCS. Different lowercase letters indicate significant difference ($p < 0.05$).

3-DG is believed to be a core intermediate product of melanoid (Yaylayan, 1990; Martins & Van Boekel, 2003), and previous research showed that the melanoid formation was related significantly to the increase in 3-DG content (Paravisini & Peterson, 2018). The first content of 3-DG in M-NCS was 34.63 ± 0.12 mg/100 g (Figure 4B), which was due to the Maillard reactions during the evaporation and solidification process of clear juice. Throughout storage, the 3-DG content increased significantly to 65.14 ± 0.07 mg/100 g ($p < 0.05$) at an increase of 88.10%, but the actual increase rate of each storage stage differed with the highest increase rate of the third stage (32.83%). 3-DG accumulation in M-NCS during storage was in agreement with Oliveira's finding that the increase in 3-DG was positively correlated with storage time (Oliveira et al., 2016). Similar findings were also found in the study of methylglyoxal (MGO) as α-dicarbonyl compounds (Uğur et al., 2022). The NCS used in this experiment is produced by clarifying sugarcane juice by membrane technology. M-NCS contains more reducing sugar and amino acids, which can produce more 3-DG. On the other hand, under normal temperature storage conditions, 3-DG decomposes slowly, and only a small part decomposes into 5-HMF. Since the increase of 3-DG is much greater than the consumption of 3-DG, the content of 3-DG increases significantly during storage. In this study, the M-NCS color also increased significantly during storage, which verified the statement that 3-DG was significantly correlated with the production of melanoidins.

During the first 4 months of storage, the 5-HMF content in M-NCS decreased significantly from 1.49 mg/100 g to 0.05 mg/100 g throughout storage ($p < 0.05$) with an overall reduction rate of 96.64% (Figure 4C). 5-HMF decreased rapidly to 0.03 mg/100 g with a decrease rate of 97.98%, and then, it remained relatively stable for the following 6 months. The reason for the decrease of 5-HMF content is that under the condition of acidic medium, the furan ring is cracked to produce levulinic acid, formic acid and various polymeric substances or 5-HMF continues to react with amino acids (Zhang & Weitz, 2012). 5-HMF is considered to be a core intermediate product of the Maillard reaction, which is due to the dehydration of fructosamine, and is chemically active and closely related with browning (Van Boekel, 2006). The fructofuranosyl cation due to acid-catalyzed degradation of fructose can be converted into 5-HMF, especially in dry systems (Locas & Yaylayan, 2008). Glucose and fructose can also form 3-DG by 1,2-enolization and dehydration, which then produced 5-HMF by cyclization (Capuano & Fogliano, 2011). It can be seen that the content of 3-DG is much more than that of 5-HMF, but the content of 5-HMF is very low. It can be judged that the generation of 5-HMF in this study is not mainly from the decomposition of 3-DG. Under the acidic conditions in this

study, the Maillard reaction generates hydroxymethylfurfural through the 1,2-enolation pathway, which may be the main pathway for the formation of 5-HMF. The research showed that activated carbon can inhibit the formation of 5-HMF by inhibiting Maillard reaction (Coklar & Akbulut, 2020).

3.5 Correlations among color and browning indicators in stored M-NCS

Table 1 shows all browning factors were significantly correlated with color. Among them, 3-DG and Relative percentages of volatile MRPs were significantly positively correlated with color; other browning factors were significantly negatively correlated with color. The correlation between protein and color value was the highest, and the correlation between moisture content and color was the lowest. In order to further analyze the relationship between each browning factor and color, multivariate data analysis was used to study.

3.6 Multivariate data analysis of physicochemical characteristics and browning indicators in stored M-NCS

The correlation between Maillard reaction indicators of M-NCS and color during different time could be illustrated via PCA. First, PCA was run using the data matrix of M-NCS components and Maillard reaction indicators to visualize any differences between them. As shown in Figure 5A, 88.8% of the variance was explained by the first two components (PC1 = 80.8%

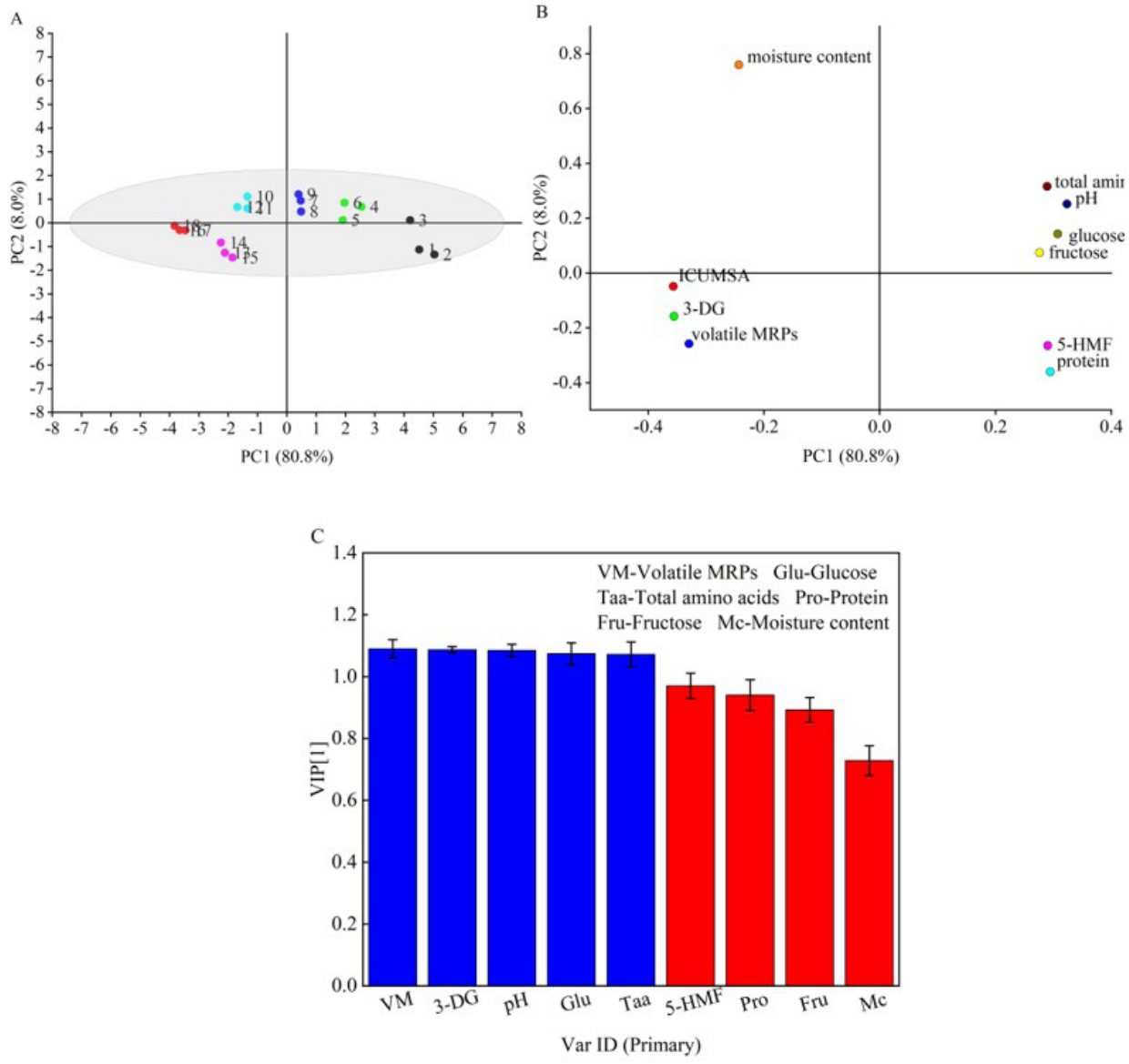

Figure 5. (A) Score plot of PCA. Samples of 1-3: M-NCS storeded at room temperature for 0 month; 4-6: M-NCS storeded at room temperature for 2 months; 7-9: M-NCS storeded at room temperature for 4 months; 10-12: M-NCS storeded at room temperature for 6 months; 13-15: M-NCS storeded at room temperature for 8 months; 16-18: M-NCS storeded at temperature for 10 months. (B) Loading plot of PCA. (C) VIP plot of browning indicators (defined as X matrix) vs. color (defined as Y matrix). The larger VIP values (> 1) were labeled in blue, which indicate the important X variables for explaining Y variables.

Table 1. Correlation coefficients between browning indicators and color.

	P	R^2
pH	0.000	-0.956
Moisture content	0.002	0.642
Glucose	0.000	-0.943
Fructose	0.000	-0.769
Protein	0.000	-0.975
Total amino acid	0.000	-0.945
Relative percentages of volatile MRPs	0.000	0.952
3-DG	0.000	0.959
5-HMF	0.000	-0.854

P: significance level. R2: correlation coefficient.

and PC2 = 8.0%). In the score plot, the sample stored for 0 month was located in the bottom right region of the score plot, samples stored for 2 and 4 months were located in the upper right of the diagram, the sample stored for 6 months was located in the upper left of the diagram, and samples stored for 8 and 10 months were located in the lower left of the diagram, which indicated that the last six months of storage resulted in a significant change in color. Combined with loading scatter plot of PCA (Figure 5B), pH and the contents of total amino acids, glucose, fructose, 5-HMF, and protein decreased significantly, while color, moisture content and the contents of 3-DG and volatile MRPs increased significantly throughout the period. This finding was completely consistent with the experiment results.The OPLS mathematical model ($R^2X = 0.868$, $R^2Y = 0.986$, $Q^2 = 0.982$) clearly showed that 3-DG, volatile MRPs, pH, glucose, and total amino acids were mainly responsible for the browning of M-NCS during storage [variable importance imputation (VIP) > 1], and the order of effect on the browning of M-NCS was volatile MRPs > 3-DG > pH > glucose > total amino acids (Figure 5C). Compared with the correlation analysis, it is not found that the browning factor with higher correlation with the color has a greater influence on the color.

3.7 Visual and sensorial analysis

As is shown in Table S1, the analysis of variance revealed that the visual appearance, flavour, texture and overall acceptance of the stored M-NCS were significantly affected by storage time ($p < 0.05$). Finally, the acceptability index of the stored M-NCS ranged between 31.13 and 95.78%. Obviously, the longer the storage time, the lower people's acceptance of M-NCS.

4 Conclusions

The Maillard reaction browning indicators of M-NCS changed over 10 months of storage at room temperature. The color of M-NCS significantly increased, and its pH and contents of fructose, glucose, protein, total amino acids tended to decrease. Its moisture content slightly increased, and sucrose content remained stable. The relative percentage of volatile MRPs and 3-DG content increased significantly, while 5-HMF content decreased. Correlation analysis shows all browning factors were significantly correlated with color. Among them, 3-DG and Relative percentages of volatile MRPs were significantly positively correlated with color; other browning factors were significantly negatively correlated with color. Multivariate data analysis results demonstrated that the compound effects of volatile MRPs, 3-DG, pH, glucose, and total amino acids were mainly responsible for the color enhancement of M-NCS during storage. The order of effect on the color enhancement of stored M-NCS was volatile MRPs > 3-DG > pH > glucose > total amino acids. These results would provide a theoretical basis for understanding Maillard browning of stored M-NCS.

Ethical approval

This article does not contain any studies with human or animal subjects.

Conflict of interest

The authors have no conflict of interest to declare.

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References

Asikin, Y., Hirose, N., Tamaki, H., Ito, S., Oku, H., & Wada, K. (2016). Effects of different drying-solidification processes on physical properties, volatile fraction, and antioxidant activity of non-centrifugal cane brown sugar. *Lebensmittel-Wissenschaft + Technologie*, 66, 340-347. http://dx.doi.org/10.1016/j.lwt.2015.10.039.

Asikin, Y., Kamiya, A., Mizu, M., Takara, K., Tamaki, H., & Wada, K. (2014). Changes in the physicochemical characteristics, including flavour components and Maillard reaction products, of non-centrifugal cane brown sugar during storage. *Food Chemistry*, 149, 170-177. http://dx.doi.org/10.1016/j.foodchem.2013.10.089. PMid:24295691.

Asikin, Y., Takahashi, M., Mishima, T., Mizu, M., Takara, K., & Wada, K. (2013). Antioxidant activity of sugarcane molasses against 2,2′-azobis(2- amidinopropane) dihydrochloride-induced peroxyl radicals. *Food Chemistry*, 141(1), 466-472. http://dx.doi.org/10.1016/j.foodchem.2013.03.045. PMid:23768381.

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): a review on metabolism, toxicity, occurrence in food and mitigation strategies. *Lebensmittel-Wissenschaft + Technologie*, 44(4), 793-810. http://dx.doi.org/10.1016/j.lwt.2010.11.002.

Chand, K., Shahi, N. C., Lohani, U. C., & Garg, S. K. (2011). Effect of storage conditions on keeping qualities of jaggery. *Sugar Tech*, 13(1), 81-85. http://dx.doi.org/10.1007/s12355-010-0059-8.

Coklar, H., & Akbulut, M. (2020). The control of maillard reaction in white grape molasses by the method of reducireactant concentration. *Food Science and Technology*, 40(Suppl. 1), 179-189. http://dx.doi.org/10.1590/fst.07119.

García, J. M., Narváez, P. C., Heredia, F. J., Orjuela, Á., & Osorio, C. (2017). Physicochemical and sensory (aroma and colour) characterisation of a non-centrifugal cane sugar ("panela") beverage. *Food Chemistry*, 228, 7-13. http://dx.doi.org/10.1016/j.foodchem.2017.01.134. PMid:28317778.

Grintzalis, K., Georgiou, C. D., & Schneider, Y. J. (2015). An accurate and sensitive Coomassie Brilliant Blue G-250-based assay for protein

determination. *Analytical Biochemistry*, 480, 28-30. http://dx.doi.org/10.1016/j.ab.2015.03.024. PMid:25837770.

Henle, T., Deppisch, R., & Ritz, E. (1996). The Maillard reaction - from food chemistry to uraemia research. *Nephrology, Dialysis, Transplantation*, 11(9), 1718-1722. PMid:8918609.

Hodge, J. E. (1953). Dehydrated foods, chemistry of browning reactions in model systems. *Agricultural and Food Chemistry*, 1(15), 928-943. http://dx.doi.org/10.1021/jf60015a004.

Huyghues-Despointes, A., & Yaylayan, V. A. (1996). Retro-aldol and redox reactions of Amadori compounds: mechanistic studies with variously labeled D-[13C]glucose. *Journal of Agricultural and Food Chemistry*, 44(3), 672-681. http://dx.doi.org/10.1021/jf9502921.

Jaffé, W. R. (2012). Health effects of Non-Centrifugal Sugar (NCS): a review. *Sugar Tech*, 14(2), 87-94. http://dx.doi.org/10.1007/s12355-012-0145-1.

Jaffé, W. R. (2015). Nutritional and functional components of non centrifugal cane sugar: a compilation of the data from the analytical literature. *Journal of Food Composition and Analysis*, 43, 194-202. http://dx.doi.org/10.1016/j.jfca.2015.06.007.

Kumar, A., Chand, K., Shahi, N. C., Kumar, A., & Verma, A. K. (2017). Optimization of coating materials on jaggery for augmentation of storage quality. *Indian Journal of Agricultural Sciences*, 87(10), 1391-1397.

Lafarga, T., Acién-Fernández, F. G., Castellari, M., Villaró, S., Bobo, G., & Aguiló-Aguayo, I. (2019). Effect of microalgae incorporation on the physicochemical, nutritional, and sensorial properties of an innovative broccoli soup. *LWT*, 111, 167-174. http://dx.doi.org/10.1016/j.lwt.2019.05.037.

Lee, J. S., Ramalingam, S., Jo, I. G., Kwon, Y. S., Bahuguna, A., Oh, Y. S., Kwon, O. J., & Kim, M. (2018). Comparative study of the physicochemical, nutritional, and antioxidant properties of some commercial refined and non-centrifugal sugars. *Food Research International*, 109, 614-625. http://dx.doi.org/10.1016/j.foodres.2018.04.047. PMid:29803491.

Locas, C. P., & Yaylayan, V. A. (2008). Further insight into thermally and pH-induced generation of acrylamide from glucose/asparagine model systems. *Journal of Agricultural and Food Chemistry*, 56(15), 6069-6074. http://dx.doi.org/10.1021/jf073055u. PMid:18624447.

Lotfy, S. N., Saad, R., El-Massrey, K. F., & Fadel, H. H. M. (2021). Effects of pH on headspace volatiles and properties of Maillard reaction products derived from enzymatically hydrolyzed quinoa protein-xylose model system. *LWT*, 145, 111328. http://dx.doi.org/10.1016/j.lwt.2021.111328.

Maltini, E., Torreggiani, D., Venir, E., & Bertolo, G. (2003). Water activity and the preservation of plant foods. *Food Chemistry*, 82(1), 79-86. http://dx.doi.org/10.1016/S0308-8146(02)00581-2.

Martins, S. I. F. S., & Van Boekel, M. A. J. S. (2003). Kinetic modelling of Amadori N-(1-deoxy-d-fructos-1-yl)-glycine degradation pathways. Part II—kinetic analysis. *Carbohydrate Research*, 338(16), 1665-1678. http://dx.doi.org/10.1016/S0008-6215(03)00174-5. PMid:12873422.

Meerod, K., Weerawatanakorn, M., & Pansak, W. (2019). Impact of sugarcane juice clarification on physicochemical properties, some nutraceuticals and antioxidant activities of non-centrifugal sugar. *Sugar Tech*, 21(3), 471-480. http://dx.doi.org/10.1007/s12355-018-0646-7.

Nduko, J. M., Maina, R. W., Muchina, R. K., & Kibitok, S. K. (2018). Application of chia (Salvia hispanica) seeds as a functional component in the fortification of pineapple jam. *Food Science & Nutrition*, 6(8), 2344-2349. http://dx.doi.org/10.1002/fsn3.819. PMid:30510734.

Oliveira, C. M., Santos, S. A. O., Silvestre, A. J. D., Barros, A. S., Ferreira, A. C. S., & Silva, A. M. S. (2016). Quantification of 3-deoxyglucosone (3DG) as an aging marker in natural and forced aged wines. *Journal of Food Composition and Analysis*, 50, 70-76. http://dx.doi.org/10.1016/j.jfca.2016.05.009.

Paravisini, L., & Peterson, D. G. (2018). Role of reactive carbonyl species in non-enzymatic browning of apple juice during storage. *Food Chemistry*, 245, 1010-1017. http://dx.doi.org/10.1016/j.foodchem.2017.11.071. PMid:29287316.

Payet, B., Sing, A. S. C., & Smadja, J. (2005). Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. *Journal of Agricultural and Food Chemistry*, 53(26), 10074-10079. http://dx.doi.org/10.1021/jf0517703. PMid:16366697.

Polovková, M., & Šimko, P. (2017). Determination and occurrence of 5-hydroxymethyl-2-furaldehyde in white and brown sugar by high performance liquid chromatography. *Food Control*, 78, 183-186. http://dx.doi.org/10.1016/j.foodcont.2017.02.059.

Shim, Y.-S., Yoon, W.-J., Ha, J., Seo, D., Lee, K.-W., Lee, W.-Y., Kwon, K.-I., Kang, T.-S., Lee, J.-H., Kim, H.-J., Kwak, H.-J., Lee, S.-P., Kim, S.-J., Yun, W.-K., Lee, J., & Hwang, J.-B. (2013). Method validation of 16 types of structural amino acids using an automated amino acid analyzer. *Food Science and Biotechnology*, 22(6), 1567-1571. http://dx.doi.org/10.1007/s10068-013-0252-0.

Singh, P., Bhatnagar, A., Singh, M. M., & Singh, A. (2019). Validation of elite sugarcane varieties for quality jaggery production in subtropical India. *Sugar Tech*, 21(4), 682-685. http://dx.doi.org/10.1007/s12355-018-0647-6.

Uğur, H., Görünmek, M., Çatak, J., Efe, E., Özgür, B., Duman, S., & Yaman, M. (2022). Determination and assessment of the most potent precursors of advanced glycation end products in baklava and Turkish delight by HPLC. *Food Science and Technology*, 42, e08522. http://dx.doi.org/10.1590/fst.08522.

Van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, 24(2), 230-233. http://dx.doi.org/10.1016/j.biotechadv.2005.11.004. PMid:16386869.

Verma, V. K., & Narain, M. (1990). Moisture absorption isotherms of jaggery. *Journal of Stored Products Research*, 26(2), 61-66. http://dx.doi.org/10.1016/0022-474X(90)90001-9.

Weerawatanakorn, M., Asikin, Y., Takahashi, M., Tamaki, H., Wada, K., Ho, C. T., & Chuekittisak, R. (2016). Physico-chemical properties, wax composition, aroma profiles, and antioxidant activity of granulated non-centrifugal sugars from sugarcane cultivars of Thailand. *Journal of Food Science and Technology*, 53(11), 4084-4092. http://dx.doi.org/10.1007/s13197-016-2415-5. PMid:28035164.

Yaylayan, V. (1990). In search of alternative mechanisms for the Maillard reaction. *Trends in Food Science & Technology*, 1(C), 20-22. http://dx.doi.org/10.1016/0924-2244(90)90006-K.

Yaylayan, V., & Forage, N. G. (1991). Determination of the kinetics and mechanism of decomposition of tryptophan Amadori rearrangement product by RP-HPLC analysis. *Journal of Agricultural and Food Chemistry*, 39(2), 364-369. http://dx.doi.org/10.1021/jf00002a028.

Zhang, J., & Weitz, E. (2012). An in situ NMR study of the mechanism for the catalytic conversion of fructose to 5-Hydroxymethylfurfural and then to levulinic acid using 13c labeled d -fructose. *ACS Catalysis*, 2(6), 1211-1218. http://dx.doi.org/10.1021/cs300045r.

Zhu, Z., Xie, C., Li, W., Hang, F., Li, K., Shi, C., & Doherty, W. O. S. (2020). Nutritional and antioxidant properties of non-centrifugal cane sugar derived from membrane clarified juice. *LWT*, 131, 109717. http://dx.doi.org/10.1016/j.lwt.2020.109717.

Supplementary Material

Supplementary material accompanies this paper.

Figure S1. The monthly average temperature and humidity changes for 10 months.
Table S1. Visual and sensorial analysis of stored M-NCS ($p < 0.05$).

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