Preparation and characterization of modified caramel with binary carboxylic acids

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ABSTRACT The caramel color is one of the most widely used natural food colorants. At present, there are few reports about its modification. The modification of caramel is expected to improve its stability, color indexes and compatibility with food. In this paper, succinic and maleic acid modified caramels were prepared by esterification reaction of the raw caramel with succinic anhydride and maleic anhydride, and characterized by ultraviolet-visible (UV-vis) spectra, Fourier transform infrared (FTIR) analysis, and scanning electron microscope (SEM), etc. The significant changes of color, yellow and red indexes of the modified caramels indicated that the modification technique can be used to adjust the color properties of caramel to meet the various needs of different fields and expand the application of caramel. Compared with raw caramel, though the antioxidant ability of caramel was decreased, the thermal stability was improved, which made it more suitable for cooking and high temperature sterilization environment compared to the raw caramel. This study provided an alternative method for improving the performance of the caramel as food colors.

1. Introduction
Caramel color is one of the oldest and common water-soluble colorings in food. All along, caramel coloring is widely used in the pharmaceutical and food industries due to its good water solubility and good coloring ability. It is one of the most widely used coloring agents so far[1]. Caramel is the most famous dietary material which gained from carbohydrates by heating. The caramel pigment contains furan ring skeletons, sugar, as well as abundant phenolic hydroxyl, aldehyde and carboxyl groups, making it have good hydrophilicity and other physicochemical properties[2]. Due to the presence of aldehyde groups in its furan series, it has the antioxidant capacity and free radical scavenging activity.

Caramel have various properties, such as color index, solubility and stability, for its different applications. For a long time, the performance adjustment of caramel is done simply by changing the raw material composition or preparation conditions[3], which confines the performance improvement. In this study, the performance improvement of caramel as food pigment by the modification of it with succinic and maleic anhydrides was investigated.

2. Materials and methods

2.1. Materials.
Caramel was purchased from energy-chemical Technology Co., Ltd. (Shanghai, China). Succinic
anhydride and maleic anhydride were obtained from Innochem Technology Co., Ltd. (Beijing, China). Anhydrous pyridine, and anhydrous N,N-dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). All other chemicals were purchased from Sigma-Aldrich (Beijing, China).

2.2. The synthesis of binary fatty acid modified caramels
The synthesis of binary fatty acid modified caramel was carried out in one step. Caramel (0.5 g) and catalyst anhydrous pyridine (1 mL) were mixed in 5 mL anhydrous N,N-dimethylformamide (DMF) in a 50mL flask. Then, succinic anhydride (0.5 g, 5.0 mmol) or maleic anhydride (0.5 g, 5.1 mmol) was added into the mixture and stirred, the reaction was maintained at 80°C. After 4 h reaction, the mixture was sealed in dialysis tubes (MWCO 3,500 Da, Solarbio Biotechnology Co., LTD), and dialyzed with distilled water at 25 °C for 48 h and the water was changed at 4h intervals. Subsequently, the samples inside the tubes were dried by freeze-drying (FD-1B-50 Freezer Dryer, Beijing Boyikang Instrument Co. LTD) to obtain brown powder (succinate and maleate caramel monoesters).

2.3. Ultraviolet-visible (UV-vis) absorption spectrum analysis.
The UV-vis spectra of the raw caramel and the succinate and maleate caramel monoesters were determined with (TU-1808 UV-vis spectrophotometer, SHIMADZU Co. Ltd. Japan) and 0.01% (w/w) of aqueous solution of raw material caramel, succinate and maleate caramel monoesters at the wavelength from 190 nm-800 nm.

2.4. Fourier Transform Infrared (FTIR) Analysis.
The FTIR spectra of the samples were conducted with TENSOR 27 Fourier Transform Infrared Spectrometer (Bruker Co., Ltd. Germany) by using potassium bromide (KBr) tablet method. The scanning wavelength range was 500-4000 cm⁻¹ with scan times of 16 and resolution of 4 cm⁻¹.

2.5. Determination of the color, red, and yellow indexes.
Color index (CI, European Brewery Convention unit) is an important indicator of caramel color depth. The red index (RI) and yellow index (YI) were indicators of the red or yellow hue in the caramel color. In our experiments, the color properties of samples (caramel, succinate and maleate caramel monoesters) were measured by the UV-vis spectrophotometer at the wavelength of 610 nm. In brief, 1.00 g of the samples were accurately weighted respectively, dissolved in distilled water to obtain 100mL solutions (1%, w/w). The solutions were diluted 10 times further with distilled water to form the 0.1% (w/w) of sample solutions respectively. The absorbances of the three sample solutions were determined by the UV-vis spectrophotometer at the wavelengths of 610 nm, 510 nm and 460 nm. The calculations of CI (1), RI (2), and YI (3) were based on the formulas[4].

2.6 Scanning Electron Microscopy (SEM) Analysis.
The morphology analysis of the raw caramel and binary fatty acid modified caramels was conducted using a Hitachi SU1510 scanning electron microscope (SEM, Japan). The dry samples were put onto a copper holder with conductive adhesive and then a layer of conductive metal film (i.e. gold) was applied on the surface of the samples. The prepared samples were observed by SEM at the acceleration voltage of 10 kV and magnification of 500 folds.

3. Results and discussion

3.1. UV spectra analysis
The raw caramel has two distinct absorption peaks at 230 nm and 275 nm, which indicated that there are conjugated systems in the structure of the caramel[5]. Among them, the absorption peak at 275 nm may be generated by the absorption of R band with strong hydroxyl group the absorption peak at 230 nm may be the absorption peak of the superposition of the K, E and B bands caused by the benzene
ring and other conjugated structures in the molecule. All of these show that in the raw caramel molecule, besides the chromophore groups *i.e.* benzene ring conjugated system, there are also hydroxyl functional groups. The ultraviolet-visible (UV-vis) spectra of the two binary fatty acid modified caramels showed peak shape changes and a slight shift of the maximum peak to shorter wavelength compared to the raw caramel. The substitution of the hydroxyl group and the introduction of the ester bond caused the blue shift of the absorption peak. It can be deduced that the hydroxyl groups in the raw material were consumed and the esterification reaction was taken place to produce the binary fatty acid modified caramels.

3.2. *FT-IR* spectra analysis

The FTIR spectra of raw caramel and two binary fatty acid modified caramels are showed in Fig. 1. The peaks at 3430 cm\(^{-1}\), 1724 cm\(^{-1}\) and 1546 cm\(^{-1}\) are the main characteristic absorptions of the raw caramel (Fig. 1a)[6], among them, the blunt absorption peak at 3430 cm\(^{-1}\) is the absorption of hydroxyl group. After the formation of binary fatty acid modified caramel monoester, the absorption intensity at 3430 cm\(^{-1}\) became weaker, which indicated the consumption of the hydroxyl group in the raw caramel after the esterification reaction. Additionally, after the modification, the intensity of ester carbonyl absorption peak (1724 cm\(^{-1}\)) increased. At 1564 cm\(^{-1}\), a new absorption peak appeared due to the antisymmetric stretching vibration of the carboxy anion (COO\(^{-}\))[7]. These changes can be attributed to the formation of ester bond and carboxyl group, and the reduction of hydroxyl group after the esterification of caramel with binary aliphatic anhydride.

![Fig. 1. FTIR spectra of caramel (a), succinate caramel monoester (b) and maleate caramel monoester (c).](image)

3.3. *Determination of the color, red, and yellow indexes.*

CI is an important indicator of caramel color depth. The red index (RI) and yellow index (YI) are an indicator of the strength of the red hue or yellow hue in the caramel color[8]. The absorbance at 610 nm, 510 nm, and 460 nm of the samples were measured by UV-vis spectrophotometer and recorded as A1, A2, and A3, respectively. The results of CI, RI and YI of the three samples were calculated according to eq(1),(2) and (3) were shown in Fig. 2.

The CI of succinate caramel monoester was greatly improved (reach 397368 EBC units), which was 2.1 times higher than that of the raw caramel (187368 EBC units), but both the RI and YI declined (2.46 and 2.58 respectively) compared with that of raw caramel (3.80 and 5.45 respectively). On the contrary, the CI of maleate caramel monoester was 69473 EBC units, which was lower than that of the raw caramel, but the RI and YI of maleate caramel monoester were 4.51 and 6.95 respectively, which increased compared with that of the raw material.

CI, RI and YI are the important indexes for the application of caramel as food color. The experimental results above showed that the modification of caramel with binary fatty acids can significantly alter the values of these indexes so that the modified caramel can fulfill different
requirements of the food industry.

![Bar chart showing the relationship between the color index (CI), red index (RI) and yellow index (YI) of raw caramel(A), succinate caramel monoester(B) and maleate caramel monoester(C).]

**Fig. 2.** Relationship between the color index (CI), red index (RI) and yellow index (YI) of raw caramel(A), succinate caramel monoester(B) and maleate caramel monoester(C).

### 3.4. Ferric Reducing Antioxidant Power (FRAP)

The FRAP antioxidant capacity (Total antioxidant capacity, TAC) of raw caramel, succinate and maleate caramel monoesters are shown in Table 1.

| Sample                                                                 | A          | B          | C          |
|------------------------------------------------------------------------|------------|------------|------------|
| TAC (mM/0.1%)                                                          | 0.0755±0.012 | 0.0360±0.007 | 0.0197±0.004 |

The total antioxidant capacities of the three samples (raw caramel, succinate caramel monoester and maleate caramel monoester) with a concentration of 0.1%(w/w) were: 0.0755mM, 0.0360mM, and 0.0197mM, respectively. The result indicated that the total antioxidant capacity of binary fatty acid modified caramel samples were decreased compared to the raw caramel. This result may be due to the decrease of the phenolic hydroxyl groups which were considered to have the antioxidant properties[9] and the generated carboxyl group didn’t have corresponding antioxidant capacity. The decrease of active hydroxyl group and the increase of stable ester bond are also important reasons for the increase of thermal stability of caramel after modification.

### 3.5. SEM Analysis

The morphology characteristic of the raw and modified caramels were observed with Hitachi SU1510 scanning electron microscope (SEM, Japan). The SEM image of each sample at 500 magnification was shown in Fig. 3. The raw caramel had a smooth spherical structure before modification (Fig. 3a). However, the caramel morphology changed greatly after modification. Both the two modified caramels were powder with thin flakes.
4. Conclusion

To sum up, two modified caramels, succinate and maleate caramel monoesters were prepared by the esterification reaction between the caramel and two binary anhydrides: succinic anhydride (saturated fatty acid anhydride) and maleic anhydride (unsaturated fatty acid anhydride). UV-vis and FTIR spectra showed the obvious differences between the raw and two modified caramels. The appearance of ester bond absorption peaks indicated the formation of acylated products. The significant changes of CI, YI and RI of the modified caramels indicated that the modification technique can be used to adjust the color properties of caramel to meet the various needs of different fields and expand the application of caramel. After modification, the active hydroxyl group in caramel was esterified to produce more stable ester group. Though the antioxidant ability of caramel was decreased, but the thermal stability was improved, which made it more suitable for cooking and high temperature sterilization environment compared to the raw caramel.

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