

Identifying and characterizing the components related to the brown color of Chinese sugar-smoked chicken during processing

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ABSTRACT The desired color is a key indicator for consumer acceptability of Chinese sugar-smoked chicken. To investigate the formation of color attributes of Chinese sugar-smoked chicken during processing, color values, structural characteristics, and components of brown pigment were evaluated in 2 groups, which were defined as brown skin (BS) and normal skin (NS) of Chinese sugar-smoked chicken based on their color values. Compared with the NS samples, the BS samples showed significantly lower values of lightness, redness, and yellowness and higher content of malondialdehyde and 5-hydroxymethylfurfural. UV–

visible and Fourier-transform infrared spectra suggested that the structure of brown pigment was similar to melanin. The brown pigment consisted of multiple chemical components including the polymer of fructose and glucose, and derivatives produced by lipid oxidation, which were identified by HPLC–tandem mass spectrometry. The polymer content of glucose and fructose, which was demonstrated as sucrose by HPLC analysis, was higher in the BS group than in the NS group. Our results indicated that the higher content of the polymer of glucose and fructose was mainly responsible for the brown color of Chinese sugar-smoked chicken.

Key words: Chinese sugar-smoked chicken, brown color, UV-VIS, FT-IR, HPLC–MS/MS

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INTRODUCTION

Chinese sugar-smoked chicken is a traditional meat product, with their unique sensory attributes including desired taste, aroma, and color (Chen et al., 2013). The entire production process of Chinese sugar-smoked chicken is composed of 5 steps, which mainly includes raw chicken preparation, salting, air-drying, baking, and smoking, and takes about 7–8 d. Among them, air-drying and baking are the key stages for the formation of pleasant sensory attributes during the processing of Chinese sugar-smoked chicken. Particularly, sugar-smoking technology, a popular smoking method involving preprocessing of meat and meat products, has been used as it imparts a characteristic flavor and

attractive color to meat products favored by consumers (Chen et al., 2013). Although the flavor of Chinese sugar-smoked chicken has been improved using sugar-smoking methods and fully discussed (Chang et al., 2020), it is still not expounded in the formation of color attributes of Chinese sugar-smoked chicken.

Color attributes of meat products are frequently examined owing to their strong impact on consumers' acceptability and marketability (Humaid et al., 2019). Therefore, the color of chicken skin is an important quality parameter in the manufacture. The Maillard reaction, caramelization, and lipid oxidation are the main factors that result in the brown color of chicken skin during the processing of Chinese sugar-smoked chicken. During the processing of Chinese sugar-smoked chicken, the temperature of baking stage is higher than 90°C, which resulted in caramelization as well as Maillard reactions (Kondjoyan et al., 2014). The latter is also known as nonenzymatic browning and is the reaction between the amino groups of proteins and reducing sugars, which is a way to develop the typical aroma, taste, and brown color of roasted and sugar-smoked meat products

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(Shahidi et al., 2014). The formation of colored compounds (low-molecular-weight) and melanoidins (high-molecular-weight) during the Maillard reaction is mainly responsible for browning (Namiki and Hayashi, 1981; Ledl and Schleicher, 1990; Ames et al., 1993). Furthermore, when the temperature exceeds 150°C, carbonization reaction occurs, which results easily in the formation of dark color and a burned appearance (Matsuda et al., 2013). For Chinese sugar-smoked chicken, the temperature of the sugar-smoking stage reached 155°C, which could result in the undesired color (brown color) of Chinese sugar-smoked chicken. Although the variation of color attributes during the processing of Chinese sugar-smoked chicken has been reported, the actual components responsible for the characteristics of desired color have not yet been well identified and discussed in the production of Chinese sugar-smoked chicken.

The aim of the present study was, therefore, to investigate the formation of color attributes of Chinese sugar-smoked chicken during processing, structural characteristics, and components of brown pigment by UV–visible (VIS) spectra, Fourier-transform infrared (FT-IR) spectra, and HPLC–tandem mass spectrometry (MS/MS), which aimed at providing theoretical guidance for the formation of brown pigment and further improving the color of chicken skin, consumers' acceptance, and marketability.

MATERIALS AND METHODS

Sample Preparation

Three yellow chickens were reared in a farm (Wenzhou, Zhejiang, China) and slaughtered in a local commercial slaughter house (conducted following the European Community, 1099/2099/EC 2009 guidelines), where the facilities of the slaughter house met the requirements of the Institute of Animal Care and Use Committee. Immediately after slaughter, carcasses were chilled at 4°C in a ventilated room for 24 h. One hundred chickens were randomly selected based on the carcass weight (500 ± 40 g) at 24 h postmortem. They were marinated for 24 h (0°C–4°C) and then air-dried for 3 d (10°C–12°C, 4 m/s). The air-dried samples were baked for 6–7 h (55°C) and then sugar smoked for 15 min (130°C–140°C). Finally, standing was performed to develop flavor of sugar-smoked chicken for 24 h at 10°C–12°C. The color values of these chicken samples were first evaluated. Chinese sugar-smoked chickens with brown skin (BS) and Chinese sugar-smoked chickens with normal skin (NS) were defined based on their color attributes and the study by Hashim et al. (1999), who demonstrated that lightness (L^*), redness (a^*), and yellowness (b^*) values of the desired chicken products were approximately 49.08, 8.50, and 22.97, respectively. Therefore, L^* and b^* values of chicken samples that ranged from 47.94 to 50.66 and from 14.88 to 19.12 were defined as NS, respectively; the L^* and b^* values of chicken samples in the

range of 42.51–44.53 and 6.82–8.52 were defined as BS. Thirty sugar-smoked chicken samples for each group were sampled, respectively. After sampling, all the samples were cut to small pieces (about 1 cm × 1 cm × 1 cm) and were wrapped in aluminum foil, frozen, and stored at –40°C before analysis.

Color Measurement

A Chroma Meter with a measuring area of 8-mm diameter, D65, illuminant, and 0° viewing angle (CR-400; KONICA MINOLTA, Co., Ltd., Tokyo, Japan) was used to determine the color values of the NS and BS samples. The standard white tile ($Y = 93.5$, $x = 0.3114$, and $y = 0.3190$) was used for calibration of the colorimeter for measurement of skin color. L^* , a^* , and b^* values were obtained at different locations of the chicken before removing the skin. The values of L^* , a^* , and b^* were expressed as the mean of thirty samples.

Chemical Analysis

The skin samples were homogenized in distilled water using a Waring disintegrator (Karl Kolb, Dreieich, Germany). The homogenate was then filtered for collection of the supernatant. 5-Hydroxymethylfurfural (5-HMF) content was determined by gas chromatography–MS. Thiobarbituric acid was analyzed for the measurement of malondialdehyde (MDA) content in the skin samples (Yang et al., 2017).

Extraction of Brown Pigment

Extraction of brown pigment was carried out by the method of Lu et al. (2019). In brief, 2 g of thawed and minced chicken skin was used. Twenty milliliters of ethanol (85%) was added. The solution was subjected to ultrasonic treatment for 32 min (25°C, 400 W) and then centrifuged at 6,000 g for 15 min. The supernatant was filtered using a neutral filter paper, and the filtrate was defined as the brown pigment solution.

UV–VIS Spectrum Analysis of Brown Pigment

The UV–VIS spectrum of the brown pigment sample was obtained in ethanol using a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) in the spectral range of 200–800 nm.

Fourier-Transform Infrared Spectra Analysis of Brown Pigment

The brown pigment sample was mixed with spectroscopic-grade potassium bromide powder and then ground and pressed into pellets for FT-IR measurement. Fourier-transform infrared spectra were recorded using a Nexus 470 FT-IR spectrometer (Thermo Scientific, Waltham, MA) at the frequency range of 4,000–400 cm^{-1} .

HPLC–MS Analysis of Brown Pigment

The solution of brown pigment was filtered using a syringe filter (Anpel Laboratory Co., Ltd., Shanghai, China) for HPLC analysis. The separation and determination of the brown pigment was performed using an Agilent HPLC system (Agilent, California, CA) and a DAD detector (Agilent) as well as an Agilent ZORBAX SB-C18 (Agilent; 150 × 4.6 mm, i.d., 5 μm). The mobile phase was formic acid (0.1%) (A) and acetonitrile (B) by gradient elution. The procedure of gradient elution by HPLC for brown pigment was as follows: 0 min (A:B = 60:40), 4 min (A:B = 40:60), 6 min (A:B = 20:80), 8 min (A:B = 10:90), and 15 min (A:B = 5:95). The injection volume was 10 μL, and the flow rate was 1 mL/min. All samples were detected at 205 nm.

The identification of brown pigment was performed using a Waters UPLC–Synapt G2 LC–MS/MS system (Agilent) and a UV photodiode array detector. The mobile phases and gradient program were the same as those used for the HPLC analysis.

High-resolution mass spectra were recorded using a Bruker Daltonics microTOF instrument (Bruker Daltonics, Massachusetts, MA) using both negative and positive electrospray ionization (ESI) modes. The microTOF Focus mass spectrometer (Bruker Daltonics) was fitted with an ESI source, and internal calibration was achieved using 10 mL of 0.1 M sodium formate solution. Calibration was carried out using the enhanced quadratic calibration mode. All MS measurements were performed in both negative and positive ion modes. The capillary voltage was 3.0 kV, the sample cone voltage was 30 V, and the sample extraction voltage was 5.0 V. The ion source temperature was 120°C, and the desolvent gas temperature was 350°C. High-purity nitrogen (N₂) was used as the spray gas. The collision gas was high-purity argon (Ar), and the backflow gas flow rate was 80 L/h. The desolvent gas flow rate was 800 L/h. The mass spectrum scan range was 100–1200 Da, and the scan time was 0.3 s.

Statistical Analysis

The results were expressed as mean values and SD. ANOVA was performed using the t test via SAS 8.0 (SAS Institute Inc., Cary, NC). All graphs were drawn using Originpro8.5 software (SAS 8.0; SAS Institute Inc.).

RESULTS AND DISCUSSION

Changes in Color Parameters, 5-Hydroxymethylfurfural Content, and MDA Content of Two Kinds of Chicken Skin

Color is one of the most important attributes of meat products as it directly affects overall quality and further influences the acceptability of consumers (Passetti et al., 2019). The color values of NS and BS samples are shown in Table 1. Hue angle values between 40° and 75° represent brown color, with a lower angle indicating more

Table 1. Changes in color parameters, 5-hydroxymethyl furfural content, and malondialdehyde content of 2 kinds of Chinese sugar-smoked chicken.

Quality attributes	NS	BS
L*	49.30 ± 1.36 ^a	43.52 ± 1.01 ^b
a*	5.90 ± 0.68 ^a	4.47 ± 0.61 ^b
b*	17.00 ± 2.12 ^a	7.67 ± 0.85 ^b
Caramel red index	2.57 ± 0.05 ^a	1.49 ± 0.01 ^b
Caramel yellow index	5.00 ± 0.07 ^a	3.35 ± 0.02 ^b
MDA (mg per 100 g of muscle)	5.80 ± 0.78 ^b	6.54 ± 0.85 ^a
5-HMF (ng/g)	244.3 ± 16.54 ^b	270.6 ± 10.25 ^a

^{a,b}Different letters indicate that there is significant difference ($P < 0.05$).

Data are expressed as mean ± SD.

Abbreviations: 5-HMF, 5-hydroxymethyl furfural; a*, redness; b*, yellowness; BS, brown skin; L*, lightness; MDA, malondialdehyde; NS, normal skin.

brown color than a higher angle (Hashim et al., 1999). The L*, a*, and b* values of the NS samples were 49.30, 5.90, and 17.00, respectively; compared with NS samples, the relatively lower L*, a*, and b* values (less than 0.88-fold, 0.76-fold, 0.45-fold, respectively) were assigned to the BS samples ($P < 0.05$), implying that the NS samples showed more attractive color (Hashim et al., 1999). The L* values of the NS samples were higher than those in the report of Hashim et al. (1999), who suggested that L* values of smoked chickens were 49.08, whereas a* and b* values of the NS and BS samples were lower than the results of Hashim et al. (1999). The reduction of L* values of the BS samples represents a shift toward darker coloration, which may be attributed to the faster speed of nonenzymatic browning reaction including the Maillard reaction and lipid oxidation (Liu et al., 2017). The reduction of b* values of the BS samples is due to the lower speed of sugar dehydration and ring splitting (Kamuf et al., 2003). Compared with the BS samples, the higher a* values of the NS samples could be due to polymerizing or condensing reactions forming colored high-molecular-weight components (Kamuf et al., 2003). As shown in Table 1, the relatively lower caramel red and yellow index (less than 0.58-fold/0.67-fold) was shown in the BS samples than in the NS samples, and the results of the caramel red and yellow index in both the NS and BS samples were consistent with the changes of a* and b* values. However, our results were lower than the range of the hue index for caramel color (approximately 3.5–7.5), which is reported by Kamuf et al. (2003). These results could be assumed that low degree of caramelization reactions took place in the Chinese sugar-smoked chickens.

5-Hydroxymethylfurfural, a classic product of the Maillard reaction involving hexose degradation such as glucose and sucrose, has previously been used as a browning marker and indicator of inadequate storage in various food products (Martins et al., 2010; Serracayuela et al., 2013). Malondialdehyde, a secondary decomposition product of polyunsaturated fatty acids with 3 or more double bonds, is widely used to evaluate lipid oxidation in meat products (Sun et al., 2001). As shown in Table 1, there was a significant difference in

the content of 5-HMF and MDA between the NS and BS samples ($P < 0.05$). The content of 5-HMF in the NS samples was 244.3 ng/g, whereas its content was 0.9-fold higher in the BS samples; the content of MDA in the BS samples was 6.54 mg per 100 g, which was 1.13-fold higher than that of the NS samples. These results revealed that the higher content of 5-HMF and MDA in the BS samples was related to serious Maillard reaction and oxidation during processing.

UV–VIS Spectrum Assay of Brown Pigment of Two Kinds of Chicken Skin

The UV–VIS absorption spectrum of brown pigment is shown in Figure 1. The results showed that the absorption range of brown pigment occurred at 200 to 400 nm, which had a maximum absorption at 205 nm, followed by 280 nm, and the values of absorption decreased toward the visible region (380–780 nm). These results were in line with the results reported by Tu et al. (2009), who found that both synthetic melanin and Taihe Black-bone silky fowl melanin showed a steadily increased absorption at wavelengths ranging from 200 to 400 nm and that Taihe Black-bone silky fowl melanin exhibited an additional absorption peak at wavelength 280 nm compared with synthetic melanin. The absorption characteristic of the brown pigment at 205 nm was also similar to the UV–VIS absorption spectrum of melanin pigmentation (Kimura et al., 2015). As shown in Figure 1, the structure of 2 kinds of chicken skin showed no obvious difference, which indicated that they could possess identical compounds. However, it also could be seen that the absorbance of BS was higher than that of NS at 205 nm, which could be attributed to

excessive accumulation of melanin pigmentation by the Maillard reaction and oxidation in the BS. The typical absorption peak at 270–280 nm could be explained by the fact that proteins were bound to melanin, which resulted in absorption at 280 nm of brown pigment of the NS and BS (Ruan et al., 2002; Kimura et al., 2015).

Infrared Spectrum Assay of Brown Pigment of Two Kinds of Chicken Skin

Infrared spectroscopy is an important nondestructive method, providing information on functional groups shown in samples. Infrared spectroscopy has been used to investigate structural characteristics of isolated neuromelanin (Double et al., 2000), hair melanin (Liu et al., 2005), iris melanin, and choroid melanin (Hong and Simon, 2006).

Figure 2 shows the FT-IR spectra of the brown pigment extracted from 2 kinds of chicken skin. It can be seen that the structure showed no noticeable difference between the NS and BS samples, whereas the intensities of absorbance showed obvious difference (Figure 2). As shown in Figure 2, the infrared spectra of the brown pigment displayed a broad and intense peak at around $3,412\text{ cm}^{-1}$ for the characteristic absorption of hydroxyl groups ($-\text{OH}$) or amino groups ($-\text{NH}$) (Kanmani et al., 2011). This result was probably due to the formation of the hydrogen bond (Liu et al., 2017), which made the peak wide and strong or led to NH stretching (Tu et al., 2009). The absorption peaks at $2,922$ and $2,850\text{ cm}^{-1}$ were the resonance absorption peaks of $-\text{CH}$, which were formed by the asymmetrical and symmetrical stretching of CH_3 (Tu et al., 2009). The absorption peak at $1,711$ and $1,627\text{ cm}^{-1}$ specified

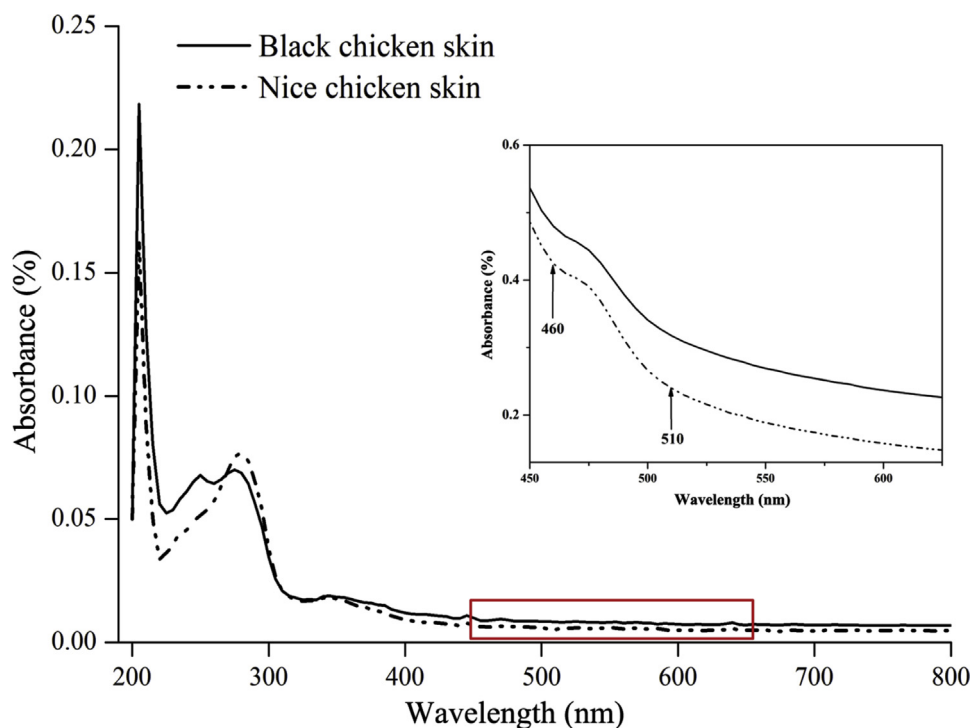


Figure 1. The UV–VIS spectrum of 2 kinds of Chinese sugar-smoked chicken skin. Abbreviation: VIS, visible.

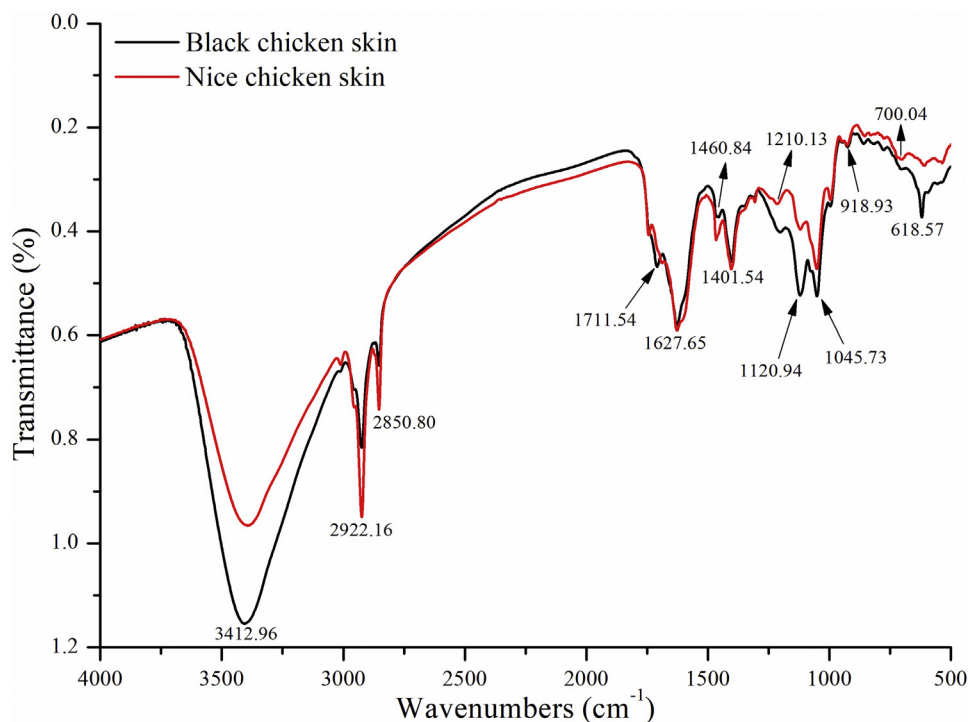


Figure 2. The FT-IR spectrum of 2 kinds of Chinese sugar-smoked chicken skin. Abbreviation: FT-IR, Fourier-transform infrared.

the presence of C=O in the brown pigment molecule (Akyuz et al., 2012). The absorption peak at $1,460\text{ cm}^{-1}$ was caused by the bending vibration of $\text{-CH}_2\text{CH}_3$ (Liu et al., 2017). The absorption peak at $1,401\text{ cm}^{-1}$ was the characteristic methyl group peak or CN stretching (Tu et al., 2009). The stretching vibration of C–O caused the formation of peak at $1,200\text{--}1,045\text{ cm}^{-1}$. C–O–C symmetrical stretching could result in the presence of peak at 918 cm^{-1} . The weak absorption peaks in the range of $600\text{--}700\text{ cm}^{-1}$ indicated that the hydrogen atoms on the phenyl groups had been substituted and a conjugated system was formed (Liu et al., 2017). It also could be the presence of long-chain molecules that contain more than 4 CH_2 groups (Liu et al., 2017).

The presence of C=O and long-chain compounds revealed that lipid oxidation might explain the formation of brown pigment (Liu et al., 2017). The peaks at $1,401\text{ cm}^{-1}$ strongly imply a pyrrole or indole NH group (Tu et al., 2009). These FT-IR features in the main absorbance bands showed obvious similarities to previous studies of melanin (Zhang et al., 2007). The most evident differences between the NS and BS samples were the peaks at $3,412\text{ cm}^{-1}$, $2,922\text{ cm}^{-1}$, and $2,850\text{ cm}^{-1}$, which also implied the presence of a substantial amount of aliphatic groups in the BS structure. It could be concluded that the brown pigment isolated from chicken skin is a type of melanin-like pigment.

The processing of Chinese sugar-smoked chicken predominantly generates furanoid species similar to sucrose, which indicates that the presence of sugar derivatives is linked by the glycosidic bond. Meanwhile, FT-IR data confirmed the presence of CH_3 , CH_2 , C–O–H, and

C–O–C moieties. As suggested in the research study reported by Yaylayan and Kaminsky (1998), the monomeric units could be generated from furanose forms of glucoses or 3-deoxyglucosone, which could form carboxylic acid derivatives after oxidation. On the other hand, 1-deoxyglucosone could exist in furanose. Polymerization of carboxylic acid derivatives and furanose could generate furan derivatives that are consistent with the FT-IR experimental data. From the aforementioned results, we could conclude that the pigment extracted from the pigment of Chinese sugar-smoked chicken is similar to melanin. In the present study, higher pigment content in BS could result in darker color.

Identification of Brown Pigment of Chicken Skin

The brown pigment of chicken skin was analyzed by HPLC (Supplementary Figure 1). As shown in Supplementary Figure 1, the retention time of the main component of brown pigment is about 1.2 min. To characterize the composition of brown pigment, the time-of-flight mass analyzer with ESI was used to identify the components of the brown pigment of chicken skin. The chromatogram of chicken skin was acquired in both positive and negative ion modes. The brown pigment was found to be a mixture of compounds containing a variety of chemical components. Table 2 displays the mass-to-charge ratio (m/z) of the product ions, elemental composition, and average mass error of chicken skin.

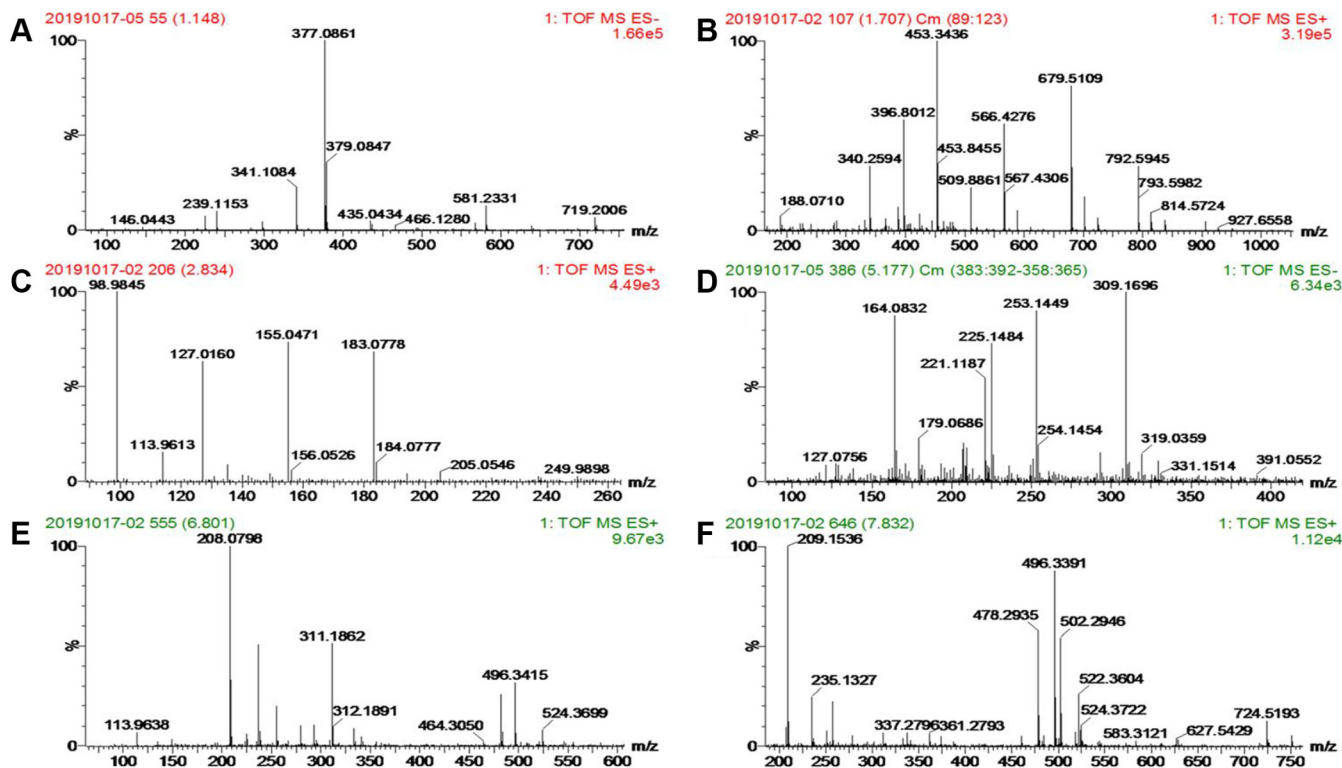
As shown in Figure 3 and Supplementary Figure 2, the highest intensity of the quasi-molecular ion peak was

Table 2. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) data for brown pigment in the positive/negative ion mode.

Peak numbering	Retention time (min)	Assignment	Positive ion mode			Negative ion mode		
			Experimental m/z [M+H] ⁺	Molecular formula	Relative error (ppm)	Experimental m/z [M-H] ⁻	Molecular formula	Relative error (ppm)
1	1.18	Glu-Fru	365.1053	C ₁₂ H ₂₂ O ₁₁	-1.9	341.1084	C ₁₂ H ₂₁ O ₁₁	0
2	1.72		566.4276	C ₃₀ H ₅₆ N ₅ O ₅	-0.9			
3	2.82		183.0778	C ₈ H ₁₁ N ₂ O ₃	4.4			
4	5.18					164.0832	C ₈ H ₁₀ N ₃	4.9
5	6.79		311.1862	C ₁₇ H ₂₇ O ₃	1.3			
6	7.73		502.2976	C ₁₇ H ₃₆ N ₁₂ O ₅	-3.2			
7	8.69		235.1694	C ₁₅ H ₂₃ O ₂	-1.7			
8	9.20		301.1407	C ₁₆ H ₂₂ O ₄	-3.0			
9	10.69					277.2180	C ₁₈ H ₂₉ O ₂	4.3
10	11.54					303.2331	C ₂₀ H ₃₁ O ₂	2.3
11	12.19		353.2690	C ₂₁ H ₃₇ O ₄	-0.6	279.2327	C ₁₈ H ₃₁ O ₂	1.1

obtained at an m/z of 365.10 (Figure 3A), which indicated that there was a compound with the molecular weight of 365.10 (named compound 1) in the pigment. A neutral loss of 74 was found in Figure 3A, combined with the result of FT-IR, which suggested that it contained a long-chain hydrocarbon. This compound may be a polymer of glucose and fructose, which was consistent with the result reported by Golon (2012). Similarly, the quasi-molecular ion peak at the m/z of 311.18 (Figure 3E), 235.17 (Supplementary Figure 2A), 301.14 (Supplementary Figure 2B), 277.22 (Supplementary Figure 2C), 303.23 (Supplementary Figure 2D), and 353.27 (Supplementary Figure 2E) showed that the molecular weight of these compounds was 311.18, 235.17, 301.14, 277.22, 303.23, and 353.27, respectively. The fragment ion peak at an m/z of

277.22 (Supplementary Figure 2C) indicated the loss of -OH from MS². Supplementary Figures 2A, 2E, and 2F revealed the loss of -CHO groups, in which it would be easy to lose a molecule of water through the elimination reaction. As per the van Krevelen diagrams of sucrose, glucose, and fructose, there could be the presence of a series of minor compounds presumably formed in a redox reaction as “cross peaks” with respect to the dehydration diagonal or lipid-like heterocycles owing to the higher values of H/C and the lower values of O/C (Kim et al., 2003). Similar redox disproportionation products have been suggested by Limacher et al. (2008) when studying the formation of furans in Maillard reactions. It was concluded that these compounds could be derived from the reaction between lipid oxidation and caramelization reactions. The brown pigment

**Figure 3.** The secondary mass spectrogram (MS²) of compounds. (A) Compound 1 (m/z = 356.11), (B) compound 2 (m/z = 566.43), (C) compound 3 (m/z = 183.08), (D) compound 4 (m/z = 164.08), (E) compound 5 (m/z = 311.19), and (F) compound 6 (m/z = 502.30).

also contained compounds whose molecular weights were 566.43 (Figure 3B), 164.08 (Figure 3C), 183.08 (Figure 3D), and 502.30 (Figure 3F), respectively. As shown in Figure 3D, the neutral loss of 44 could be caused by the loss of $-\text{CONH}_2$, which would mean that there were amide groups. The aforementioned compounds could be attributed to the Maillard reaction.

Overall, the exploration of MS would suggest that the brown pigment consisted of multiple chemical components including long-chain fatty acid esters, oligomerization, some redox disproportionation reaction products, and derivatives produced by lipid oxidation. The presence of amides and aldehydes in the brown pigment could be a result of the Maillard reaction. However, some data were different from the result reported by Golon (2012). Further studies were needed to identify the molecular structure of brown pigment to clarify the browning mechanism of Chinese sugar-smoked chicken skin.

Change of Brown Pigment of Chicken Skin

The changes in the content of pigment compounds in the skin of Chinese sugar-smoked chicken are summarized in Figure 4. We found 11 compounds by MS, whereas only 7 compounds were separated by HPLC. Thus, we only analyzed the same compounds identified by HPLC and MS. Further analysis needed to be carried out, and this is the limitation of this manuscript.

We could know that compound 1 was mainly a polymer of glucose and fructose confirmed by MS and spectral data. To confirm this compound, the standard substance of sucrose was further analyzed by HPLC, and compound 1 was demonstrated as sucrose by comparing retention time and mass spectra of the sucrose standard (Supplementary Figure 3). As shown in Figure 4, compounds 1 and 2 had a significant difference ($P < 0.05$). The concentration of compound 1 was 0.609 mg/mL in the NS, which was significantly lower

than that in BS (0.624 mg/mL) ($P < 0.05$). This may itself relate to ions, such as iron, phosphate, and inorganic salt, and other differences (Wang and Zhang, 2006). As shown in Figure 4, it could be seen that the contents of compound 1 and compound 2 account for more than 90% of all compounds. These are important reasons for the difference in brown pigment between the 2 types of chicken skin. However, the type of compound 2 needs further research.

CONCLUSION

In this article, chemical characteristics and structural changes of pigment in 2 kinds of Chinese sugar-smoked chicken were compared. The accumulation of brown pigment was the key discrimination between the BS and NS samples. The brown pigment formed in Chinese sugar-smoked chicken is derived from the intermediates in the Maillard reaction, by thermal degradation of caramelization, and by the polymerization of compounds. The significantly lower content of the polymer of glucose and fructose, which was demonstrated as sucrose, was responsible for development of normal Chinese sugar-smoked chicken, compared with brown Chinese sugar-smoked chicken.

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DISCLOSURES

No conflict of interest exists in the submission of this manuscript.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.psj.2020.12.034>.

REFERENCES

- Akyuz, S., T. Akyuz, G. Emre, A. Gulec, and S. Basaran. 2012. Pigment analyses of a portrait and paint box of Turkish artist Feyhaman Duran (1886–1970): the EDXRF, FT-IR and micro Raman spectroscopic studies. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 89:74–81.
- Ames, J. M., A. Apriyantono, and A. Arnoldi. 1993. Low molecular weight coloured compounds formed in xylose–lysine model systems. *Food Chem.* 46:121–127.
- Chen, S., T. H. Kao, C. J. Chen, C. W. Huang, and B. H. Chen. 2013. Reduction of Carcinogenic Polycyclic Aromatic Hydrocarbons in meat by sugar-smoking and Dietary Exposure assessment in Taiwan. *J. Agr. Food Chem.* 61:7645–7653.
- Chang, H., Y. Wang, Q. Xia, D. Pan, J. He, H. Zhang, and J. Cao. 2021. Characterization of the physicochemical changes and volatile compound fingerprinting during the chicken sugar-smoking process. *Poult. Sci.* 100:377–387.

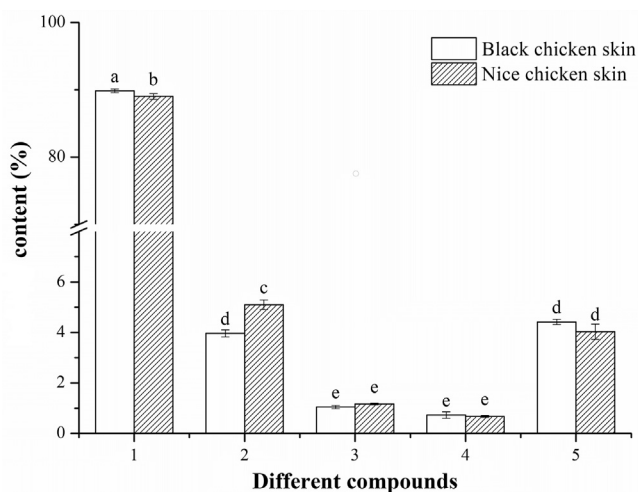


Figure 4. The relative content of brown pigment for 2 kinds of Chinese sugar-smoked chicken skin. 1 = $\text{C}_{12}\text{H}_{22}\text{O}_{11}$; 2 = $\text{C}_8\text{H}_{10}\text{N}_3$; 3 = $\text{C}_{18}\text{H}_{29}\text{O}_2$; 4 = $\text{C}_{20}\text{H}_{31}\text{O}_2$; 5 = $\text{C}_{18}\text{H}_{31}\text{O}_2$. ^{a–e}Different letters indicate that there is significant difference ($P < 0.05$).

- Double, K., L. Zecca, P. Costi, M. Mauer, C. Griesinger, S. Ito, and ...P. Riederer. 2000. Structural characteristics of human *substantia nigra* neuromelanin and synthetic dopamine melanins. *J. Neurochem.* 75:2583–2589.
- Golon, A. 2012. Characterization of Complex Mixtures of the Light Shredder Waste Fraction and Caramelization Processes by Mass Spectrometry. Jacobs University Bremen. IRC-Library, Information Resource Center der Jacobs University Bremen. Accessed Jan. 2021. <https://opus.jacobs-university.de/frontdoor/index/index/docId/31>.
- Hashim, I., K. McWatters, and Y. C. Hung. 1999. Consumer acceptance of honey marinated chicken. *J. Muscle Foods* 10:231–244.
- Hong, L., and J. D. Simon. 2006. Insight into the binding of divalent cations to Sepia eumelanin from IR absorption spectroscopy. *Photochem. Photobiol.* 82:1265–1269.
- Humaid, S., D. Nayyar, J. Bolton, and D. I. Skonberg. 2019. Physicochemical properties and consumer acceptance of high-Pressure processed, sous Vide-Cooked Lobster Tails. *J. Food Sci.* 84:3454–3462.
- Kamuf, W., A. Nixon, O. Parker, G. Barnum, and D. Willamson. 2003. Overview of caramel colors. *Cereal Food World* 48:64–69.
- Kanmani, P., N. Yuvaraj, K. Paari, V. Pattukumar, and V. Arul. 2011. Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* PI80 and its functional characteristics activity in vitro. *Bioresour. Technol.* 102:4827–4833.
- Kim, S., R. W. Kramer, and P. G. Hatcher. 2003. Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the van Krevelen diagram. *Anal. Chem.* 75:5336–5344.
- Kimura, T., W. Fukuda, T. Sanada, and T. Imanaka. 2015. Characterization of water-soluble dark-brown pigment from Antarctic bacterium, *Lysobacter oligotrophicus*. *J. Biosci. Bioeng.* 120:58–61.
- Kondjoyan, A., A. Kohler, C. E. Realini, S. Portanguen, R. Kowalski, S. Clerjon, and ...L. Debrauwer. 2014. Towards models for the prediction of beef meat quality during cooking. *Meat Sci.* 97:323–331.
- Ledl, F., and E. Schleicher. 1990. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem. Int. Edition English* 29:565–594.
- Limacher, A., J. Kerler, T. Davidek, F. Schmalzried, and I. Blank. 2008. Formation of furan and methylfuran by Maillard-type reactions in model systems and food. *J. Agr. Food Chem.* 56:3639–3647.
- Liu, Y., L. Hong, K. Wakamatsu, S. Ito, B. Adhyaru, C. Y. Cheng, C. R. Bowers, and J. D. Simon. 2005. Comparison of structural and chemical properties of Black and red human hair Melanosomes. *Photochem. Photobiol.* 81:135–144.
- Liu, Y., W. Zheng, S. A. Ibrahim, H. Yang, and W. Huang. 2017. Chemical properties of vacuum-fried *Pleurotus eryngii* during storage and characterization of brown pigment. *Int. J. Food Prop* 20:S2349–S2358.
- Lu, F., Y. Zou, D. Liu, and J. Zhang. 2019. Response Surface methodology Applied to Optimize the extraction process of pigment from sugar smoked chicken skin. *Meat Sci.* 33:20–25 (in Chinese).
- Martins, S., A. Leussink, E. Rosing, G. Desclaux, and C. Boucon. 2010. Meat flavor generation in complex Maillard model systems: Reactants synergetic and/or competition effect? Pages 71–83 in *Controlling Maillard Pathways to Generate Flavors*. D. S. Mottram and A. J. Taylor, eds. ACS Publications, Washington, DC.
- Matsuda, H., Y. Llave, M. Fukuoka, and N. Sakai. 2013. Color changes in fish during grilling—Influences of heat transfer and heating medium on browning color. *J. Food Eng.* 116:130–137.
- Namiki, M., and T. Hayashi. 1981. Formation of novel free radical products in an early stage of Maillard reaction. *Prog. Food Nutri. Sci.* 5:81–91.
- Passetti, R. A. C., V. C. Resconi, C. Çakmakçı, M. del Mar Campo, J. K. Kirinus, L. C. G. Passetti, and ...C. Sañudo. 2019. Number of consumers and days of display necessary for the assessment of meat colour acceptability. *Food Res. Int.* 121:387–393.
- Ruan, L., Y. Huang, G. Zhang, D. Yu, and S. Ping. 2002. Expression of the mel gene from *Pseudomonas maltophilia* in *Bacillus thuringiensis*. *Lett. Appl. Microbiol.* 34:244–248.
- Serra-Cayuela, A., M. Aguilera-Curiel, M. Riu-Aumatell, S. Buxaderas, and E. López-Tamames. 2013. Browning during biological aging and commercial storage of Cava sparkling wine and the use of 5-HMF as a quality marker. *Food Res. Int.* 53:226–231.
- Shahidi, F., A. Samaranayaka, and R. Pegg. 2014. Maillard reaction and browning. Page 1712 in *Encyclopedia of Meat Sciences*. C. Devine and M. E. Dikeman, eds. Elsevier, Amsterdam, the Netherlands.
- Sun, Q., C. Faustman, A. Senecal, A. Wilkinson, and H. Furr. 2001. Aldehyde reactivity with 2-thiobarbituric acid and TBARS in freeze-dried beef during accelerated storage. *Meat Sci.* 57:55–60.
- Tu, Y., Y. Sun, Y. Tian, M. Xie, and J. Chen. 2009. Physicochemical characterisation and antioxidant activity of melanin from the muscles of Taihe Black-bone silky fowl (*Gallus gallus domesticus* Brisson). *Food Chem.* 114:1345–1350.
- Wang, D., and L. Zhang. 2006. Research progress on Nonenzymatic browning reaction. *Acad. Period. Farm Prod. Process.* 10:9–19 (in Chinese).
- Yang, Y., X. Zhang, Y. Wang, D. Pan, Y. Sun, and J. Cao. 2017. Study on the volatile compounds generated from lipid oxidation of Chinese bacon (unsmoked) during processing. *Eur. J. Lipid Sci. Tech.* 119:1600512.
- Yaylayan, V., and E. Kaminsky. 1998. Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system. *Food Chem.* 63:25–31.
- Zhang, J., J. Cai, Y. Deng, Y. Chen, and G. Ren. 2007. Characterization of melanin produced by a wild-type strain of *Bacillus cereus*. *Front. Biol. China* 2:26–29.