

Article

Morphological Changes and Component Characterization of Coffee Silverskin

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Abstract: Nuclear magnetic resonance (NMR) spectroscopy was used for the qualitative and quantitative analysis of aqueous extracts of unroasted and roasted coffee silverskin (CS). Twenty compounds were identified from 1D and 2D NMR spectra, including caffeine, chlorogenic acid (CGA), trigonelline, fructose, glucose, sucrose, etc. For the first time, the presence of trigonelline was detected in CS. Results of the quantitative analysis showed that the total amount of the main components after roasting was reduced by 45.6% compared with values before roasting. Sugars in the water extracts were the main components in CS, and fructose was the most abundant sugar, its relative content accounting for 38.7% and 38.4% in unroasted and roasted CS, respectively. Moreover, 1D NMR combined with 2D NMR technology shows application prospects in the rapid, non-destructive detection of CS. In addition, it was observed by optical microscopy and scanning electron microscopy (SEM) that the morphology of CS changed obviously before and after roasting.

Keywords: NMR spectroscopy; coffee silverskin; qualitative analysis; quantitative analysis



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1. Introduction

According to the International Coffee Organization (ICO), the global production of green coffee beans reached 1.014 million tons in 2019 [1]. The processing of coffee cherries results in the generation of a large number of coffee by-products, including pulp, parchment, coffee silverskin (CS), and coffee grounds. The effective utilization of these coffee by-products is not only environmentally beneficial but also conducive to the sustainable development of the coffee industry. Therefore, in recent years, many studies have begun to pay attention to the application of the wastes from coffee bean processing, and these studies have been summarized [2,3]. Among them, CS has been proven to be rich in dietary fiber [4] and antioxidant substances [5,6], and it can be used as an additive for functional foods [7,8] and cosmetics [9], as well as in adsorbent materials [10,11], showing good product development potential.

CS is a thin layer of skin that adheres tightly to an oval coffee bean; it accounts for 4% of the total dry weight of a green coffee bean, accounting for 0.6% of the dry weight of coffee berries [12]. If mixed into coffee powder, it will increase the bitterness. CS is mainly produced in two stages during the production process: (a) during the shelling and peeling stage of green coffee beans before export, the CS is removed from the surfaces of coffee beans together with the seed shell, but it is not completely removed; (b) after roasting, the whole CS of coffee is peeled from the surfaces of the coffee beans. Approximately 40 substances including cellulose, hemicellulose, fructose, sucrose, glucose, caffeine, chlorogenic acid, vitamin E, 5-hydroxymethylfural, and fatty acid have been reported in CS. Among

the total ingredients, sugars, caffeine, and chlorogenic acid have received special attention as functional substances in CS. Borrelli et al. [12] used enzyme gravity and chemical methods to determine the composition of CS and found that the main components were polysaccharides and proteins; total dietary fiber reached 60%, and soluble dietary fiber reached 8%. Ballesteros et al. [4] analyzed the cellulose, hemicellulose, lignin, and other sugar components' content in CS by HPLC, and their results showed that water-insoluble cellulose and hemicellulose accounted for 40.45% of the total mass. Martinez-Saez et al. [13] used capillary electrophoresis to measure the caffeine and chlorogenic acid content in CS and found that their quantities were 3.02 g/100 g and 751.2 mg/100 g, respectively, while another study based on LC–MS found that their quantities were 1.0 g/100 g and 198.7 mg/100 g, respectively [14]. The difference between these measurement data may be due to the inherent differences in the CS itself, such as its species, source, and processing method, or the use of different analysis methods. It should be noted that the above analysis and identification methods for CS require complicated sample preparation processes, which may affect the results regarding the composition of CS.

NMR characterization is the use of a variety of NMR detection methods to directly obtain the complete composition information of complex samples. It is non-destructive, simple, and fast, and it can be used to perform qualitative and quantitative analysis on a variety of substances at the same time. It is now widely used in the food industry [15–17]. At the same time, NMR spectroscopy has been successfully used in the analysis of the composition of green coffee [18], roasted coffee [19], the effect of roasting on coffee composition [20], and coffee flavor [21]. In addition, our research team is proficient in the use of NMR to characterize the chemical composition of coffee [22–24]. However, there is no research using this method to characterize the composition of CS.

Thus far, the relative content of caffeine, chlorogenic acid, sugars, and other major bioactive components in green coffee or roasted coffee beans has been fully studied. However, the main chemical compositions of CS, especially unroasted CS, have not been clarified. Therefore, in order to obtain comprehensive information on the compositions of unroasted and roasted CS, we chose to use NMR spectroscopy to characterize a water extract of CS.

2. Results and Discussion

2.1. Changes in Microstructure of CS

It was found by light microscopy that the melanin produced by the reaction of the sugars and the amine compounds via the Maillard reaction in lightly roasted CS was unevenly distributed in the center of the cells (Figure 1B), indicating that the compounds involved in the reaction during roasting are unevenly distributed in CS cells. Comparing the scanning electron microscopy (SEM) photographs of deep roasted and unroasted CS, it was found that the CS before roasting exhibited a complete cell outline (Figure 1C), and the cell structure of the CS after deep roasting was destroyed, making it difficult to distinguish the contours of the cells (Figure 1D).

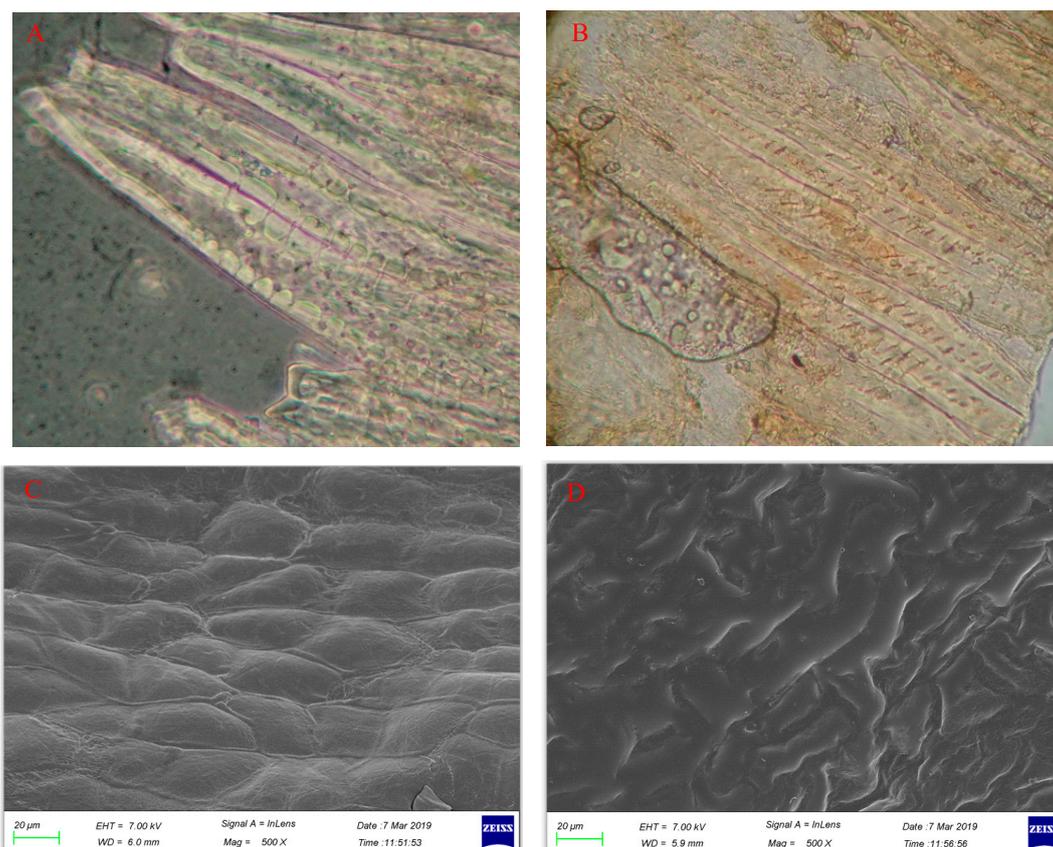


Figure 1. Microstructure of CS. (A): optical micrograph of unroasted CS ($\times 1000$); (B): optical micrograph of lightly roasted CS ($\times 1000$); (C): SEM graph of unroasted CS ($\times 500$); (D): SEM graph of deep-roasted CS ($\times 500$).

2.2. Qualitative Analysis of CS

In this study, ^1H NMR was used to compare the composition of the heavy water extract of CS before and after roasting (Figures S1 and S2). The spectra showed that there was no significant difference in the composition of the CS before and after roasting. Twenty compounds were identified with the aid of 1D and 2D NMR spectra (Figure 2). In addition, the 1D NMR spectrum of lightly roasted A1 was used as an example to identify 20 compounds (Figures S3 and S4). The main compounds in the aqueous extract of CS were sugars, organic acids, alkaloids, and amino acids, including caffeine, trigonelline, 5-chlorogenic acid (5-CGA), fructose, sucrose, glucose, malic acid, and glycine.

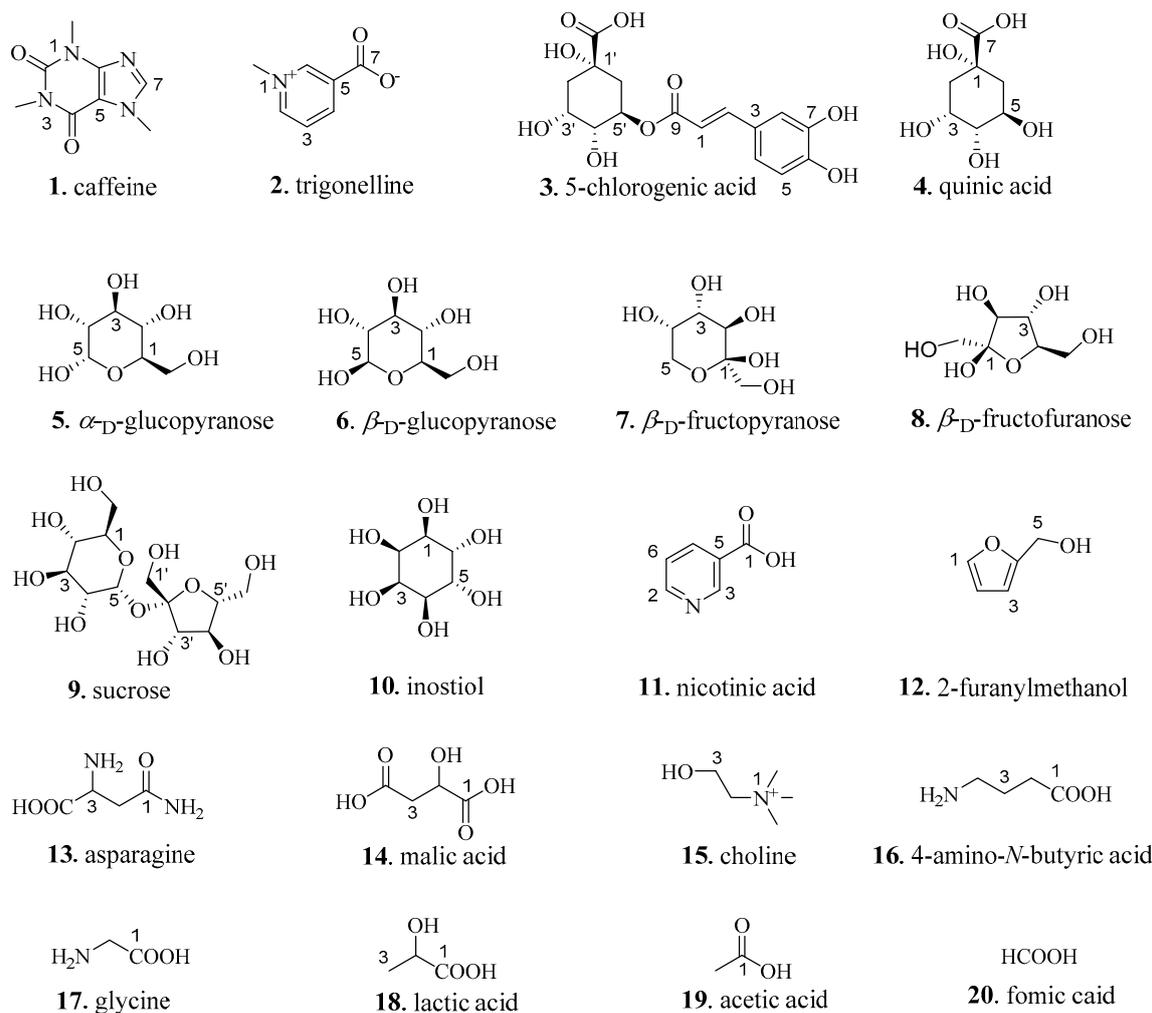


Figure 2. The compounds (1-20) of CS aqueous extract were identified by 2D NMR spectra.

2.2.1. Alkaloids

As active alkaloids in coffee bean, caffeine and trigonelline are widely distributed in various parts of coffee, including the seeds, flowers, honey, and leaves [25–27]. However, previous analyses of the composition of CS have not detected the presence of trigonelline. The possible reasons for this are: (a) the roasting degree of the CS sample itself has a greater influence on the trigonelline content, and roasting causes the degradation of trigonelline; (b) the measurement method is for a certain kind of compound. As a result, researchers have not considered the existence of trigonelline during HPLC and LC–MS analysis, so the existence of trigonelline in CS has not been reported. To the best of our knowledge, this is the first time that the presence of trigonelline has been detected in CS. Meanwhile, trigonelline has been shown to exert multiple biological activities, including anti-diabetic [28], anti-obesity [29], anti-inflammatory [30], and neuroprotective effects [31]. Therefore, the discovery of trigonelline increases the potential of CS application.

2.2.2. Sugars

During the signal assignment process, there was serious signal overlap between the 1D NMR data of the carbohydrate components, but six sugars, including α -D-glucopyranose, β -D-glucopyranose, β -D-pyranose, β -D-fructofuranose, sucrose, and inositol, were determined by the correlation signals of the C/H core in the same coupling system in the HSQC-TOCSY spectrum or other 2D NMR spectrum (^1H - ^1H COSY, HSQC, and HMBC spectrum). Taking the 2D NMR spectrum of sample A1 as an example to analyze sucrose

(Figure 3), starting from the correlation peaks of the terminal hydrogen δ_H 5.41 (H-1, $J = 3.9$ Hz) and the terminal carbon δ_C 92.1 (C-1) of the glucose in sucrose, the process of confirming signal attribution by 2D NMR is as follows: straight line from the low field to the high field along the ^{13}C spectrum and the ^1H spectrum. The carbon spectrum has δ_C 69.2, 72.5, 71.1, and the hydrogen spectrum has δ_H 3.84 and 3.54. Further, four groups of signals, δ 69.2 \rightarrow 3.46 (C-4), δ 72.5 \rightarrow 3.76 (C-3), δ 71.1 \rightarrow 3.54 (C-2), δ 72.4 \rightarrow 3.84 (C-5), were determined by the HSQC spectrum. In the 1H-1H COSY spectrum, δ 5.41 (H-1) \rightarrow 3.54 (H-2), 3.54 \rightarrow 3.76 (H-3), 3.76 \rightarrow 3.46 (H-4), 3.46 \rightarrow 3.84 (H-5). C-6 and C-1 had no correlation signals, but C-5 (δ 72.4, 3.84) had a TOCSY correlation with δ 3.82, 60.5, respectively, and δ 60.5 \rightarrow 3.82 in the HSQC spectrum, thus indicating that δ 3.82, 60.5 (C-6) belongs to the same coupling system.

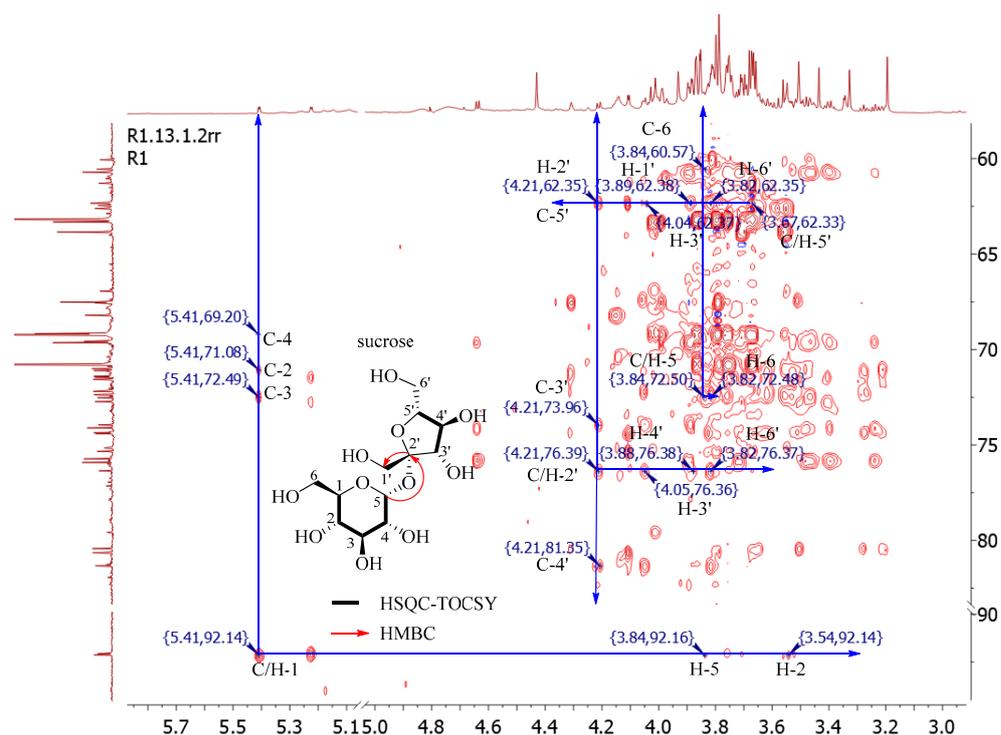


Figure 3. The HSQC-TOCSY correlations of sucrose from CS aqueous extract.

The structural determination of the fructose fraction of sucrose began with the HMBC association between δ_H 5.41 (H-1) and δ_C 103.6 (C-1'). δ_H 3.67 had HMBC-related signals with δ_C 103.6, which was determined as C-5' in combination with the HSQC signal (δ_C 62.3). In the HSQC-TOCSY spectrum, starting from C-5', along the direction of the hydrogen spectrum, there were δ_H 3.82, 3.88, 4.05, 4.21 from the high field to the low field. Four groups of signals, δ 3.82 \rightarrow 60.1 (C-6'), δ 3.88 \rightarrow 81.3 (C-4'), δ 4.05 \rightarrow 74.0 (C-3'), δ 4.21 \rightarrow 76.4 (C-2'), were obtained by combining the HSQC spectra and the connection sequence δ_H 4.21 (H-2') \rightarrow 4.05 (H-3') \rightarrow 3.88 (H-4') was determined by ^1H - ^1H COSY. Finally, the above signals were confirmed to be in the same coupling system by the relevant signals in the HSQC-TOCSY spectrum of C/H-2'. Based on the above analysis, it was determined that sucrose was contained in the CS. The main HSQC-TOCSY-related signals of other saccharide components are shown in Figure S11. The signal assignment processes for other compounds by the 1D and 2D NMR spectra were the same as those for sucrose.

2.2.3. Phenolic Acids

The phenolic acids in CS are mainly composed of CGA, cinnamoylquinic acid, feruloylquinic acid, and their isomers, among which CGA is the most abundant. CGA in the coffee beans mainly includes 3-CGA, 4-CGA, and 5-CGA. Wei et al. [18] used the ^1H

NMR spectrum to determine the presence of these three isomers by the difference between the esterification positions of quinic acid and the coupling constant J between adjacent H. Using this method, the phenolic acid with the highest content in CS was determined to be 5-CGA. Gokhan Zengin et al. [32] used HPLC–MS/MS to analyze the active compounds of silver peel extracted by water, and they found that the content of 5-CGA was the highest, followed by 4-CGA. After careful analysis of 2D NMR, although some structural fragments of other acids can be found (Figures S3 and S11), their structures cannot be determined, which may be due to the small amount of CS samples extracted, resulting in no corresponding NMR signals. This reflects the comprehensive and non-destructive characteristics of NMR analysis information.

The 1D NMR data of the 20 compounds are listed in Table 1, and the signal assignments are detailed in Figures S3 and S4. The 1D NMR data of all compounds are consistent with the literature data [18–21]. Additionally, we collected the NMR data under the same conditions after mixing the standard materials, and the results were in agreement with the experimental data (Figure S12).

Table 1. The 1D NMR data of CS components.

Compound	¹ H	¹³ C	Assignment	Compound	¹ H	¹³ C	Assignment
Caffeine	3.33	27.9 (q)	N1-CH ₃	5-CQA	6.37	114.6 (d)	C1-H
-	-	152.7 (s)	C2-O	-	7.61	145.9 (d)	C2-H
-	3.51	29.8 (q)	N3-CH ₃	-	-	126.8 (s)	C3
-	-	156.4 (s)	C4-O	-	7.08	122.5 (d)	C4-H
-	-	107.9 (s)	C5	-	6.91	116.1 (d)	C5-H
-	3.93	33.4 (q)	N6-CH ₃	-	-	147 (s)	C6
-	7.87	143.5 (d)	C7-H	-	-	144.2 (s)	C7
-	-	148.4 (s)	C8	-	7.14	114.9 (d)	C8-H
Trigonelline	4.43	48.2 (q)	N1-CH ₃	-	-	169 (s)	C9
-	8.84	145.8 (d)	C2-H	-	5.32	71.1 (d)	C5'-H
-	8.07	127.5 (d)	C3-H	-	3.88	72.8 (d)	C4'-H
-	8.83	144.7 (d)	C4-H	-	4.26	70.6 (d)	C3'-H
-	-	136.9 (s)	C5	-	2.15, 2.05	37.3 (t)	C2'-H ₂
-	9.12	145.9 (d)	C6-H	-	-	77.3 (s)	C1'
-	-	167.8 (s)	C7	-	2.69	39.8 (t)	C6'-H ₂
Quinic acid	-	75.8 (s)	C1	-	2.51	173.6 (s)	C7'
-	2.05, 1.98	37.3 (t)	C2-H ₂	α -D-glucopyranose	3.76	72.5 (d)	C1-H
-	4.15	70.4 (d)	C3-H	-	3.46	69.2 (d)	C2-H
-	3.55	75.2 (d)	C4-H	-	3.71	72.7 (d)	C3-H
-	4.02	66.9 (d)	C5-H	-	3.53	71.5 (d)	C4-H
-	2.07, 1.88	40.6 (t)	C6-H ₂	-	5.23	92.07 (d)	C5-H
-	-	173.3 (s)	C7	-	3.67, 3.81	62.3 (t)	C6-H ₂
β -D-glucopyranose	3.83	71.4 (d)	C1-H	β -D-fructopyranose	-	98 (s)	C1
-	3.4	69.6 (d)	C2-H	-	3.79	67.5 (d)	C2-H
-	3.47	75.9 (d)	C3-H	-	3.89	69.6 (d)	C3-H
-	3.24	74.1 (d)	C4-H	-	3.99	69.2 (d)	C4-H
-	4.64	95.9 (d)	C5-H	-	4.02, 3.67	63.3 (t)	C5-H ₂
-	3.89, 3.71	60.7 (t)	C6-H ₂	-	3.55, 3.70	63.8 (t)	C6-H ₂
β -D-fructofuranose	-	101.5 (s)	C1	Sucrose	3.84	72.4 (d)	C5-H
-	4.11	74.4 (s)	C2-H	-	3.46	69.2 (d)	C4-H
-	4.11	75.4 (s)	C3-H	-	3.76	72.5 (d)	C3-H
-	3.82	80.6 (s)	C4-H	-	3.54	71.1 (d)	C2-H
-	3.67, 3.81	62.3 (t)	C5-H ₂	-	5.41	92.12 (d)	C1-H
-	3.55, 3.58	62.6 (t)	C6-H ₂	-	3.82	60.5 (t)	C6-H ₂
Inositol	3.62	72.2 (d)	C1-H	-	3.67	62.3 (t)	C5'-H ₂
-	3.51	71.1 (d)	C2-H	-	-	103.6 (s)	C1'
-	4.31	71.3 (d)	C3-H	-	4.21	76.4 (d)	C2'-H
-	3.21	80.4 (d)	C4-H	-	4.05	74 (d)	C3'-H
-	3.65	71.6 (d)	C5-H	-	3.88	81.3 (d)	C4'-H
-	3.28	74.4 (d)	C6-H	-	3.82	60.1 (t)	C6'-H ₂

Table 1. Cont.

Compound	¹ H	¹³ C	Assignment	Compound	¹ H	¹³ C	Assignment
Nicotinic acid	-	166.2 (s)	C1	2-Furanylmethanol	7.52	146.2 (d)	C1-H
-	8.83	153.2 (d)	C2-H	-	6.67	111 (d)	C2-H
-	9.13	150.2 (d)	C3-H	-	6.65	110.8 (d)	C3-H
-	8.32	136.9 (d)	C4-H	-	-	161.4 (s)	C4
-	-	126.7 (s)	C5	-	4.68	56.0 (t)	C5-H ₂
-	7.50	123.71 (d)	C6-h	Malic acid	-	179.4 (s)	C1
Asparagine	-	174.4 (s)	C1	-	4.01	66.9 (d)	C2-H
-	2.86, 2.95	34.4 (t)	C2-H ₂	-	2.69, 2.51	38.3 (t)	C3-H ₂
-	4.0	51.2 (d)	C3-H	-	-	179.2 (s)	C4
-	-	173 (s)	C4	4-Amino-N-butyric acid	-	180.5 (s)	C1
Choline	3.19	53.8 (q)	N1-CH ₃	-	2.35	33.4 (t)	C2-H ₂
-	3.19	53.8 (q)	N1-CH ₃	-	1.91	23.1 (t)	C3-H ₂
-	3.19	53.8 (q)	N1-CH ₃	-	3.02	39.1 (t)	C4-H ₂
-	3.51	67.4 (t)	C2-H ₂	Lactic acid	-	182 (s)	C1
-	4.06	55.5 (t)	C3-H ₂	-	4.15	68.2 (d)	C2-H
Glycine	-	170.7 (s)	C1	-	1.34	19.9 (q)	C3-H ₃
-	3.81	52.8 (t)	C2-H ₂	Acetic acid	-	178.8 (s)	C1
Formic acid	8.43	171.4 (d)	C1-H	-	2.0	21.6 (d)	C2-H

2.3. Quantitative Analysis of CS

TSP was used as an internal standard substance and the characteristic peak ¹H-NMR signal of the CS components was compared with the ¹H-NMR signal of TSP to obtain the relative content of CS components and the characteristic peak chemical shift, and a number of protons are shown in Table S2. The relative integral areas of different compounds before (B) and after roasting (A) are shown in Figure 4A. Garcia De Serna et al. [33] used CS as a sucrose substitute and stevia to improve the formulation of biscuits. Meanwhile, the results showed that the sugars in the water extract of CS before or after roasting were the main component of the active components, accounting for 87.2% and 77.1%, respectively, and fructose was the most abundant among the saccharides, accounting for 49.6% and 44.5%, respectively. Therefore, the analysis results further confirm that CS is rich in carbohydrates and can be used for food development.

In contrast to coffee beans, the relative content of caffeine was significantly higher than that of 5-CGA in CS (Figure S14), which is consistent with the results obtained by Iriondo-DeHond et al. [34], who used UPLC-MS/MS to determine the content of caffeine and 5-CQA. At the same time, the lowest reported caffeine content in CS was 4.4 mg/g [35]. Therefore, it is presumed that CS contains a certain amount of trigonelline. It is worth noting that the content of trigonelline in CS, which has not been detected before, was higher than that of caffeine (Figure S14). The total amounts of the main ingredients after roasting were reduced by 45.6% compared with values before roasting, which was mainly due to the Maillard reaction between sugar components (Figure 4B). Caffeine did not change significantly as it remained thermally stable during the roasting process. Trigonelline and 5-CGA showed only a small amount of degradation due to the shallow roasting (Figure 4B).

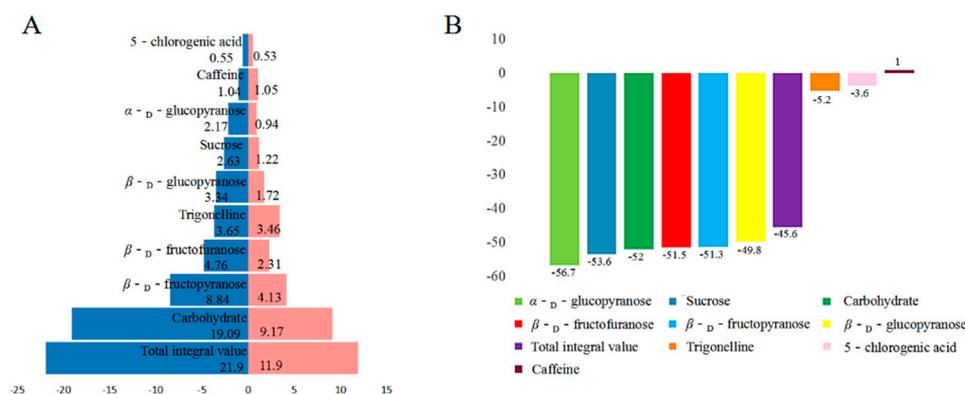


Figure 4. (A) the relative integral area of different compounds before (blue) and after roasting (pink); (B) the change rate of different compounds after roasting.

3. Materials and Methods

3.1. Instruments and Materials

The CS samples selected in this study were obtained from *Arabica* coffee planted in Yunnan, China. A Paic JB-3 coffee roaster was used for sample roasting. The samples were ground with a Jiuyang JYL-B060; a Kz-2758 drying oven was used for sample moisture control. SEM images were acquired with a Sigma300 (CARL ZEISS) field emission scanning electron microscope. Optical micrograph images were acquired with a dark-field microscope (Zhejiang Sunny Optical Co., Ltd., Ningbo, China). D₂O (99.9%) required for extraction was purchased from Beijing Yinuokai Technology Co. Ltd. An SZCL-4B intelligent magnetic heating stirrer was used for the sample heating, stirring, and extraction; a Genevac miVac vacuum centrifugal concentrator was used for sample extract centrifugation. The sample NMR data were acquired on a Bruker Avance III 800 MHz NMR spectrometer. TSP was purchased from Shanghai Source Ye Biotechnology Co. Ltd. Other standards required for the experiment were purchased from Aladdin Reagent (Shanghai, China) Co., Ltd.

3.2. Sample Preparation

For dried fruits (1.5 kg), the pulp and parchment were removed to obtain 760 g of green beans covered with CS. Then, the beans were divided into three portions (A.350 g, B.350 g, C.60 g). Parts A and C were roasted with a coffee roaster to obtain lightly roasted beans (LRB) and dark-roasted beans (DRB), respectively. Part B was used for the collection of unroasted CS samples. The roasting degree was mirrored by the color value of the beans (refer to Specialty Coffee Association of America color cards): LRB, 80–95; DRB, 40–55.

The water content of CS samples A and B was determined by the weight loss method. The two samples were ground in a grinder, placed in a beaker, dried in a 50 °C oven at constant temperature, and weighed once every half hour until the difference between the two drying times was less than 0.3 g, so that the water content of the two samples was consistent, and they were sealed for storage. We accurately weighed the CS powder of 0.5 g \times 2 A sample and B sample and placed them in 4 round-bottomed flasks (5 mL), respectively. We then sealed each sample and stirred it with 3.5 mL D₂O at 60 °C (1500 r/m) for 2 h to obtain 4 sample water extracts. The resulting extracts were simply referred to as A1, A2, B1, and B2. After the sample was cooled at room temperature, the four extracts were transferred to PE tubes and centrifuged at 4000 rpm for 30 min; 400 μ L supernatant and 10 μ L TSP were separately added to the NMR tube; then, ¹H-NMR and 2D NMR data of the D₂O extracts of unroasted and lightly roasted CS samples were acquired using an 800 MHz NMR spectrometer.

3.3. NMR Data Acquisition Conditions

All 1D and 2D NMR data were collected on a Bruker Avance III 800 MHz NMR spectrometer at a temperature of 27 °C. All experimental parameters are listed in Table S1.

3.4. Morphological Characterization of CS with Different Roasting Degrees

For light microscope image collection, unroasted (sample B) and lightly roasted (sample A) CS with similar shapes were selected, respectively. The surface was evenly immersed in oil, and then the sample was placed under dark-field microscopy for observation. For SEM characterization, unroasted (sample B) and deep-roasted (sample C) CS with similar shapes were selected. The surface was evenly plated with silver, and then placed under SEM (CARL ZEISS) to collect images. SEM magnification was 40 to 600 times.

3.5. Data Processing

All 1D and 2D NMR spectral data were processed using the MestReNova software (Mestrelab Research, Santiago de Compostela, Spain). For qualitative analysis, the components of CS water extracts A (roasted) and B (unroasted) were analyzed and identified by using the 1D NMR and 2D NMR spectra of related components [19–22], and the accuracy of component identification was confirmed by the NMR spectra of the mixed standard samples under the same conditions. For quantitative analysis, the characteristic ^1H signals (Table S2) of the main components in the ^1H NMR spectrum were normalized and integrated with respect to the ^1H signal of the internal standard TSP, and the characteristic peak areas A_s and B_s before and after roasting were obtained, respectively. Then, the relative content changes in each substance before and after roasting were calculated.

Calculation formula:

$$\text{Relativecontent}(\%) = \frac{A_x}{T_s} \times 100\% \quad \text{Rateofchange}(\%) = \frac{(A_s - B_s)}{B_s} \times 100\%$$

Formula: A_x , the characteristic peak H signal area of CS component; T_s , the total area of the characteristic peak H signal of the CS components; A_s , the characteristic peak H signal area of roasted CS component; B_s , the characteristic peak H signal area of unroasted CS component.

4. Conclusions

The morphology of CS before and after roasting was observed by optical microscopy and SEM. The Maillard reaction occurred unevenly in the CS cells under light roasting, which may have resulted from the uneven distribution of sugars and amines in CS cells. Deeply roasted CS cells were severely damaged, making it difficult to distinguish their contours under SEM.

NMR spectroscopy is the most important tool to identify the structure of organic compounds, because it can provide a variety of 1D and 2D NMR spectral data, reflecting a large amount of structural information. Therefore, it is becoming increasingly popular for researchers to characterize mixed-component samples by NMR spectroscopy. The water extract of CS before and after roasting was characterized by NMR spectroscopy, and 20 compounds, including caffeine, 5-CGA, trigonelline, fructose, glucose, sucrose, etc., were identified. Meanwhile, trigonelline was first found in the water extract of CS, which increases the potential application prospects of CS. NMR spectroscopy was proven to be a convenient means to characterize CS compounds.

The relative content of the main components in CS was detected and their changes during the roasting process were discussed. Even though the Maillard reaction during the roasting process caused a large reduction in sugars, they were still the main ingredients in the aqueous extract of both unroasted and roasted CS. Moreover, 5-CGA and trigonelline underwent light degradation. In total, the main ingredients in roasted CS were reduced by 45.6% compared with unroasted CS. Considering that the utilization of CS is mainly

based on its rich sugars, caffeine, CGA, and trigonelline content, the unroasted CS is more valuable for recycling than roasted CS.

Supplementary Materials: The following are available online. Table S1: NMR data acquisition conditions, Table S2: Comparison between the compounds from unroasted (B) and roasted (A) coffee silverskin aqueous extracts, Figure S1: The comparison of ^1H NMR spectra between unroasted B1 (up) and lightly roasted A1 (down) CS aqueous extracts. Roasted and unroasted CS are similar in chemical composition, but the total content of unroasted CS is higher than roasted CS, Figure S2: ^1H NMR spectrum signal of unroasted CS and lightly roasted aqueous extracts, Figure S3: ^1H NMR spectrum signal identification of lightly roasted A1 CS aqueous extracts (A-C). caffeine (C), trigonelline (T), quinic acid (Q), β -D-glucopyranose (1G), β -D-fructofuranose (1F), inositol (I), nicotinic acid (N), asparagine (A), choline (Ch), glycine (Gl), formic acid (F), 5-caffeoylquinic acid (5C), α -D-glucopyranose (2G), β -D-fructopyranose (2F), sucrose (S), 2-furanylmethanol (Fu), malic acid (M), 4-Amino-N-butyric acid (An), lactic acid (L), acetic acid (Ac), Figure S4: ^{13}C NMR spectrum signal identification of lightly roasted A1 CS aqueous extracts (A-C). caffeine (C), trigonelline (T), quinic acid (Q), β -D-glucopyranose (1G), β -D-fructofuranose (1F), inositol (I), nicotinic acid (N), asparagine (A), choline (Ch), glycine (Gl), formic acid (F), 5-caffeoylquinic acid (5C), α -D-glucopyranose (2G), β -D-fructopyranose (2F), sucrose (S), 2-furanylmethanol (Fu), malic acid (M), 4-Amino-N-butyric acid (An), lactic acid (L), acetic acid (Ac), Figure S5: ^1H NMR spectrum of lightly roasted A1 CS aqueous extracts, Figure S6: ^{13}C NMR spectrum of lightly roasted A1 CS aqueous extracts, Figure S7: HSQC spectrum of lightly roasted A1 CS aqueous extracts, Figure S8: HMBC spectrum of lightly roasted A1 CS aqueous extracts, Figure S9: ^1H - ^1H COSY spectrum of lightly roasted A1 CS aqueous extracts, Figure S10: HSQC-TOCSY spectrum of lightly roasted A1 CS aqueous extracts, Figure S11: Characteristic coupling constants J for 3-CQA, 4-CQA, 5-CQA, Figure S12: ^1H NMR spectrum of the mixed standards, Figure S13: The HSQC-TOCSY correlations of carbohydrates from silverskin aqueous extract, Figure S14: The relative content of different compounds before (A) and after roasting (B).

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Sample Availability: Samples of the compounds are not available from the authors.

References

1. International Coffee Organization (ICO). Statistics. Trade Statistics Tables. World Coffee Consumption. Available online: <http://www.ico.org/prices/new-consumption-table.pdf> (accessed on 29 April 2020).
2. Klingel, T.; Kremer, J.I.; Gottstein, V.; Rezende, T.; Schwarz, S.; Lachenmeier, D.W. A review of coffee by-products including leaf, flower, cherry, husk, silver skin, and spent grounds as novel foods within the European Union. *Foods* **2020**, *9*, 665. [CrossRef]
3. Hejna, A. Potential applications of by-products from the coffee industry in polymer technology—current state and perspectives. *Waste Manag.* **2021**, *121*, 296–330. [CrossRef]
4. Behrouzian, F.; Amini, A.M.; Alghooneh, A.; Razavi, S.A. Characterization of dietary fibre from coffee silverskin: An optimization study using response surface methodology. *Bioact. Carbohydr. Diet. Fibre* **2016**, *8*, 58–64. [CrossRef]

5. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Costa, E.; Costa, C.S.G.; Antonia Nunes, M.; Almeida, A.A.; Santos-Sliva, A.; Oliveira, M.M.P.P. Nutritional, chemical and antioxidant/pro-oxidant profiles of silverskin, a coffee roasting by-product. *Food Chem.* **2017**, *267*, 28–35. [[CrossRef](#)]
6. Nzekouea, F.K.; Angelonia, S.; Navarinic, L.; Angelonia, C.; Freschid, M.; Hreliad, S.; Vitalia, L.A.; Sagratina, G.; Vittoria, S.; Caprioli, G. Coffee silverskin extracts: Quantification of 30 bioactive compounds by a new HPLC-MS/MS method and evaluation of their antioxidant and antibacterial activities. *Food Res. Int.* **2020**, *133*, 109128. [[CrossRef](#)] [[PubMed](#)]
7. Bertolino, M.; Barbosa-Pereira, L.; Ghirardello, D.; Botta, C.; Rolle, L.; Guglielmetti, A.; Vecchia, S.B.D.; Zappa, D. Coffee silverskin as nutraceutical ingredient in yogurt: Its effect on functional properties and its bioaccessibility. *J. Sci. Food Agric.* **2019**, *99*, 4267–4275. [[CrossRef](#)] [[PubMed](#)]
8. Gocmen, D.; Sahan, Y.; Yildiz, E.; Coskun, M.; Aroufai, D.A. Use of coffee silverskin to improve the functional properties of cookies. *J. Food. Sci. Technol.* **2019**, *56*, 2979–2988. [[CrossRef](#)]
9. Rodrigues, F.; Gaspar, C.; Palmeira-De-Oliveira, A.; Sarmiento, B.; Amaral, M.H.; Oliveira, M.B.P.P. Application of coffee silverskin in cosmetic formulations: Physical/antioxidant stability studies and cytotoxicity effects. *Drug Dev. Ind. Pharm.* **2016**, *42*, 99–106. [[CrossRef](#)] [[PubMed](#)]
10. Sung, S.H.; Chang, Y.; Han, J. Development of polylactic acid nanocomposite films reinforced with cellulose nanocrystals derived from coffee silverskin. *Carbohydr. Polym.* **2017**, *169*, 495–503. [[CrossRef](#)] [[PubMed](#)]
11. Oliveira, G.; Goncalves, I.; Barra, A.; Nunes, C.; Coimbra, M.A. Coffee silverskin and starch-rich potato washing slurries as raw materials for elastic, antioxidant, and uv-protective biobased films. *Food Res. Int.* **2020**, *138*, 109733. [[CrossRef](#)]
12. Borrelli, R.C.; Esposito, F.; Napolitano, A.; Fogliano, A. Characterization of a new potential functional ingredient: coffee silverskin. *J. Agric. Food Chem.* **2004**, *52*, 1338–1343. [[CrossRef](#)] [[PubMed](#)]
13. Martinez-Saez, N.; Ullate, M.; Martin-Cabrejas, M.A.; Martorell, P.; Genovés, S.; Ramon, D.; Castillo, M.D.D. A novel antioxidant beverage for body weight control based on coffee silverskin. *Food Chem.* **2014**, *150*, 227–234. [[CrossRef](#)] [[PubMed](#)]
14. Bresciani, L.; Calani, L.; Bruni, R.; Brighenti, F.; Rio, D.D. Phenolic composition, caffeine content and antioxidant capacity of coffee silverskin. *Food Res. Int.* **2014**, *61*, 196–201. [[CrossRef](#)]
15. Navarro, Y.; Soengas, R.; Iglesias, M.J.; Ortiz, F.L. Use of NMR for the analysis and quantification of the sugar composition in fresh and store-bought fruit juices. *J. Chem. Educ.* **2020**, *97*, 831–837. [[CrossRef](#)]
16. Solovyev, P.A.; Fahl-Hassek, C.; Riedl, J.; Esslinger, S.; Bontempo, L.; Camin, F. NMR spectroscopy in wine authentication: An official control perspective. *Compr. Rev. Food. Sci. Food Saf.* **2021**, *20*, 2040–2062. [[CrossRef](#)]
17. Soyler, A.; Cikrikci, S.; Cavdaroglu, C.; Bouillaud, D.; Oztop, M.H. Multi-scale benchtop ¹H NMR spectroscopy for milk analysis. *LWT Food Sci. Technol.* **2020**, *139*, 110557. [[CrossRef](#)]
18. Wei, F.; Furihata, K.; Hu, F.; Miyakawa, T.; Tanokura, M. Complex mixture analysis of organic compounds in green coffee bean extract by two-dimensional NMR spectroscopy. *Magn. Reson. Chem.* **2010**, *48*, 857–865. [[CrossRef](#)]
19. Wei, F.; Furihata, K.; Hu, F.; Miyakawa, T.; Tanokura, M. Two-dimensional ¹H-¹³C nuclear magnetic resonance (NMR)-based comprehensive analysis of roasted coffee bean extract. *J. Agric. Food Chem.* **2011**, *59*, 9065–9073. [[CrossRef](#)]
20. Wei, F.; Furihata, K.; Koda, M.; Hu, F.; Miyakawa, T. Roasting process of coffee beans as studied by nuclear magnetic resonance: Time course of changes in composition. *J. Agric. Food Chem.* **2012**, *60*, 1005–1012. [[CrossRef](#)]
21. Wei, F.; Furihata, K.; Miyakawa, T.; Tanokura, M. A pilot study of NMR-based sensory prediction of roasted coffee bean extracts. *Food Chem.* **2014**, *152*, 363–369. [[CrossRef](#)]
22. Hu, G.L.; Peng, X.R.; Wang, X.; Li, X.; Qiu, M.H. Excavation of coffee maturity markers and further research on their changes in coffee cherries of different maturity. *Food Res. Int.* **2020**, *132*, 109121. [[CrossRef](#)] [[PubMed](#)]
23. Hu, G.L.; Peng, X.R.; Gao, Y.; Huang, Y.J.; Qiu, M.H. Effect of roasting degree of coffee beans on sensory evaluation: Research from the perspective of major chemical ingredients. *Food Chem.* **2020**, *331*, 127329. [[CrossRef](#)]
24. Wang, Y.B.; Wang, X.Y.; Hu, G.L.; Hong, D.F.; Qiu, M.H. Chemical ingredients characterization basing on ¹H NMR and SHS-GC/MS in twelve cultivars of *coffea arabica* roasted beans. *Food Res. Int.* **2021**, *147*, 110544. [[CrossRef](#)]
25. Schievano, E.; Finotello, C.; Mammì, S.; Illy Belci, A.; Colomban, S.; Navarini, L. Preliminary characterization of monofloral *coffea* spp. honey: Correlation between potential biomarkers and pollen content. *J. Agric. Food Chem.* **2015**, *63*, 5858–5863. [[CrossRef](#)] [[PubMed](#)]
26. Kadri, S.M.; Zaluski, R.; Lima, G.; Mazzafera, P.; Ricardo, D. Characterization of *Coffea arabica* monofloral honey from Espírito Santo, Brazil. *Food Chem.* **2016**, *203*, 252–257. [[CrossRef](#)]
27. Chen, X.M.; Ma, Z.; Kitts, D.D. Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves. *Food Chem.* **2017**, *249*, 143–153. [[CrossRef](#)]
28. Zhou, J.; Chan, L.; Zhou, S. Trigonelline: A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Curr. Med. Chem.* **2012**, *19*, 3523–3531. [[CrossRef](#)]
29. Choi, M.; Mukherjee, S.; Un, J.W. Trigonelline induces browning in 3T3-L1 white adipocytes. *Phytother. Res.* **2020**, *35*, 1113–1124. [[CrossRef](#)] [[PubMed](#)]
30. Mohsen, K.; Mitra, A.; Elham, E.J.; Tourandokht, B.; Mehrdad, R. Trigonelline mitigates lipopolysaccharide-induced learning and memory impairment in the rat due to its anti-oxidative and anti-inflammatory effect. *Int. Immunopharmacol.* **2018**, *61*, 355–362.
31. Qiu, Z.; Wang, K.; Jiang, C.; Su, Y.; Yao, L. Trigonelline protects hippocampal neurons from oxygen-glucose deprivation-induced injury through activating the PI3K/Akt pathway. *Chem. Biol. Interact.* **2020**, *317*, 108946. [[CrossRef](#)]

32. Zengin, G.; Sinan, K.I.; Mahomoodally, M.F.; Angeloni, S.; Mustafa, A.M.; Vittori, S.; Maggi, F.; Gaprioli, G. Chemical composition, antioxidant and enzyme inhibitory properties of different extracts obtained from spent coffee ground and coffee silverskin. *Foods* **2020**, *9*, 173. [[CrossRef](#)]
33. García-Serna, E.; Martínez-Saez, N.; Mesias, M.; Morales, F.J.; Del Castillo, M.D. Use of coffee silverskin and stevia to improve the formulation of biscuits. *Pol. J. Food Nutr. Sci.* **2014**, *64*, 243–251. [[CrossRef](#)]
34. Iriondo-DeHond, A.; Garcia, N.A.; Fernandez-Gomez, B.; Guisantes-Batan, E.; Escobar, F.V.; Blanch, G.P.; San-Andres, M.I.; Sanchez-Fortun, S.; Del-Castillo, M.D. Validation of coffee byproducts as novel food ingredients. *Inno. Food Sci. Emerg. Tech.* **2019**, *51*, 194–204. [[CrossRef](#)]
35. Narita, Y.; Inouye, K. High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. *Food Chem.* **2012**, *135*, 943–949. [[CrossRef](#)] [[PubMed](#)]