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Colorimetric parameters correlated with the variation in the marker constituent contents during the stir-fry processing of Schizonepetae Spica

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Abstract

Background: Color is an important method of authentication to evaluate the degree of Chinese medicinal material (CMM) processing. This traditional color description used for identification needs to be confirmed by modern scientific analysis. Schizonepetae Spica (SS), the dried spike of *Schizonepeta tenuifolia* Briq., is a traditional Chinese medicinal herb. Raw SS has the actions of dispelling the common cold and fever. Schizonepetae Spica Carbonisata (SSC; raw SS processed by stir-frying until the surface becomes blackish-brown and the interior turns dark yellow) has particularly strong efficacy in arresting bleeding from bloody stool and metrorrhagia.

Methods: In this paper, a high-performance liquid chromatography-diode array detector method and colorimetry were employed to determine 6 nonvolatile marker constituents and the color parameters (L^*, a^* and b^*) of SS during stir-fry processing, respectively.

Results: According to Pearson correlation analysis, L^*, a^* and b^* showed significant correlations with the levels of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid, while a^* was significantly correlated with the amounts of luteolin and apigenin. The corresponding regression models were good fits for the color parameters as respective functions of the 6 marker constituents according to enter multiple linear regression and third-order polynomial curve fitting methods (adjusted $R^2 > 0.800$). **Conclusion:** Surrogate prediction of marker compounds using color parameters provides a valuable and cost-saving tool for rapid or online monitoring of the processing degree of SS. Raw SS and SSC could be determined based on the color parameters and 6 marker constituent contents for a comprehensive quality evaluation for the first time. This paper provides scientific data to validate the relationships between color features and quality during the processing of SS.

Background

Chinese medicinal materials (CMMs) often have to be processed by using physical or chemical treatment before prescription or clinical usage. The aims of processing are to alter the clinical efficacy and/or reduce the toxicity of CMMs to fulfill the different requirements of therapy [1]. There is a close relationship between the efficacy, safety and processing of CMMs. Improper processing methods may produce poor clinical effects or even result in poisoning [2]. Characteristics of CMMs such as color are usually used to assess the degree of processing. For example, when dry Zingiberis Rhizoma is roasted with sand until it becomes brown externally, it is called Zingiberis Rhizoma Praeparatum. Cirsii Japonici Herba is called Cirsii Japonici Herba Carbonisata when it is stir-fried until the surface becomes black. However, this approach often depends to a certain extent on the practitioner's experience, which is strongly subjective and lacks objective criteria, so modern scientific analytical techniques are needed to confirm the validity of associations between the color and processing degree of CMMs.

Schizonepetae Spica (SS) is the dried spike of *Schizonepeta tenuifolia* Briq. (Chinese Pharmacopoeia, 2020 edition) [3]. It was first recorded in *Shen Nong Ben Cao Jing* (Shen Nong's herbal classic), a book written 2000 years ago [4]. Chemical studies revealed that SS contains volatile oils, flavonoids, organic acids and so on [5–7]. Pharmacological analyses have shown that SS has three main biological properties, namely, anti-inflammatory [8], antiviral [9, 10], and hemostatic activity [11–13]. The essential oils of SS are recognized as the major constituents responsible for its anti-inflammatory and antiviral effects [8, 14]. In addition, the nonvolatile

compounds such as flavonoids and organic acids from SS exhibit various biological activities. For example, luteolin-7-O-β-D-glucoside has been demonstrated to have good biological activity in terms of anti-inflammatory and antiviral effects [15, 16]. Apigenin-7-O-β-D-glucoside, hesperidin and luteolin have also been documented to possess significant antiviral activity [5, 17, 18]. Moreover, luteolin has been shown to be a potent hemostatic drug candidate [19]. Rosmarinic acid exhibits many biological properties, including antioxidant, antibacterial, anti-inflammatory and antiallergic effects [20]. Apigenin has notable anticancer activity in vitro and in vivo [21]. Thus, the contents of flavonoids and organic acids could be used as evaluation markers for SS. The herb is commonly used in traditional Chinese medicine (TCM) prescriptions to treat the common cold, fever, bloody stool and metrorrhagia [22]. To treat the common cold and fever, TCM practitioners often prescribe raw SS, while to treat bloody stool and metrorrhagia, they usually use Schizonepetae Spica Carbonisata (SSC; raw SS processed by stir-frying until the surface becomes blackish-brown and the interior turns dark yellow) [3]. However, the quality control standards of processing practices are inconsistent in terms of the national standard and various local standards. For example, the processing method “stir-frying until the surface becomes blackish-brown and the interior turns dark yellow” is recorded in the Chinese Pharmacopoeia (2020 edition), while stir-frying until the surface becomes dark brown and the interior turns brown is recorded in “The Practices of Processing Chinese Crude Drugs in Jiangsu Province” [23]. In addition, color discrimination of SSC is mainly based on the experience of individual operators. To guarantee the quality of SSC, a standardized processing method is critical. Therefore, the above problems of “SS with several processing methods and differences in different areas” should be harmonized, and consistent practices based on modern scientific studies need to be established.

In recent years, advances in sensor technologies such as colorimeters, electronic noses and electronic tongues have provided signals related to sensory attributes, making it easier to objectively characterize the color, aroma and taste of various products. Some studies have reported the use of a colorimeter to substitute the traditional color discrimination of CMMs [24, 25]. The colorimeter technique can be used to measure processed CMM products, with the additional advantage of controlling the technological parameters. Both colorimetry techniques and modern analytical techniques should be combined to map marker components in order to evaluate the scientific elucidation of color discrimination behind processing procedures and the quality of processing.

Therefore, the main purposes of this study were to investigate the relationship between chromatic aberrations and content variations in the marker nonvolatile substances of SS during processing in order to clarify the core scientific elucidation of color as an important indicator of the degree of processing. For this purpose, first, HPLC coupled with a diode array detector (DAD) was used to evaluate five flavonoids and one organic acid of SS during stir-fry processing, and the powder color was simultaneously determined using a colorimeter. Second, the correlations between marker constituents and chromatism were investigated by Pearson correlation analysis. Finally, polynomial regression models were established with the color parameters to rapidly predict the contents of the marker constituents. This was the first study to develop a method for the quantitative analysis of nonvolatile ingredients of SS using color parameters during stir-fry processing.

Materials And Methods

Materials and reagents

Raw SS, originating from Henan Province (China), was provided by Hexiang Pharmaceutical Co. Ltd., and authenticated in accordance with the SS monograph in the Chinese Pharmacopoeia (2020 edition) by Dr. Ying Zhang, Jinan University, P. R. China. The voucher specimen (No. HX18C01) was deposited at the Research Center for Traditional Chinese Medicine of Lingnan (Southern China), Jinan University.

Acetonitrile (HPLC grade), methanol (HPLC grade) and formic acid (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The chemical standards luteolin-7-O-β-D-glucoside (No. M-025-190115), hesperidin (No. C-006-180216), rosmarinic acid (No. 190921), luteolin (No. M-007-181216) and apigenin (No. Q-002-180131) were supplied by Chengdu Ruifensi Biotechnology (Sichuan, China). Apigenin-7-O-β-D-glucoside (No. T4S0295) was acquired from Tianjun Biotechnology (Guangzhou, China). The purity of the compounds was greater than 95%, as detected by HPLC-DAD. Deionized water was purified by passing distilled water through a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemical reagents were of analytical grade.

Sample preparation

Ten grams of dried SS slices (10–15 mm in length) were placed in a pan and stir-fried at different temperatures (240°C ± 10°C, 300°C ± 10°C, and 360°C ± 10°C) for 5, 5.5, 6, 6.5, 7 and 7.5 min. Thus, we prepared 18 batches of SSC samples, and then, we determined the contents of the marker constituents and the color parameters in each batch of the prepared SSC samples as well as the raw SS.

Quantitative analysis of the main chemical ingredient content of SS and SSC samples

The SS and SSC samples were ground and filtered through a 24-mesh sieve. The powder (2 g) was weighed accurately and macerated in 20 mL of 75% ethanol. Then, the sample was extracted for 60 min by hot reflux extraction in a water bath. A 10 μL supernatant of the extracts was injected for HPLC analysis after filtration with a 0.45-μm membrane.

An UltiMate 3000 liquid chromatography system (Thermo Scientific, Bremen, Germany) equipped with a diode array detector and an ACE Excel 5 C18 column (4.6 mm ×250 mm, 5 μm; ACE, Scotland, UK) was used throughout this study. The mobile phase consisted of A (0.1% formic acid aqueous solution) and B (acetonitrile) using a gradient elution of 8%-20% B from 0–20 min, 20% B holding from 20–30 min, 20%-30% B from 30–50 min, and 30%-8% B from 50–60 min. The column temperature, flow rate, detection wavelength and injection volume were 30°C, 1 mL/min, 283 nm and 10 μL, respectively.

The stock solution of the mixture of the 6 marker components was prepared by dissolving the standards in methanol in a 25 mL volumetric flask. A series of appropriate concentrations were analyzed in triplicate to establish the calibration curve. The LODs and LOQs were determined as 3 and 10 times the signal-to-noise (S/N) ratios, respectively. The interday and intraday precisions were determined by analyzing the stock standard solution containing the 6 components, with six repetitions daily over three consecutive days. Six different working solutions prepared from the same sample were analyzed to confirm the repeatability. The same sample was tested at 0, 2, 4, 8, 12, 16, 20, and 24 h to confirm the stability. The recovery was determined by adding three concentration levels (80%, 100% and 120%) of the mixed standard solutions to known amounts of sample. Then, the fortified samples were extracted and analyzed by the proposed method. The mean recoveries were estimated using the following formula: recovery (%) = (amount found – original amount) / amount spiked × 100%.

Color measurement

The SS and SSC samples were ground and filtered through an 80-mesh sieve to ensure the uniformity of the particles. The color measurement was performed by a CR-410 colorimeter (Konica Minolta, Japan). A Fluke-63 portable infrared thermometer (Fluke, USA) was used to determine the temperature. The SS and SSC samples were filled into a powder box and observed under a D65 light source. The measuring diameter was set at 50 mm with a standard viewing angle of 2 degrees. The International Commission on Illumination (CIE) system was adopted for color quantization, with the chroma space represented by L^*, a^* and b^*. L^* represents the brightness from black to white, a^* represents the red-green axis, and b^* represents the yellow-blue axis.

Statistical analysis

The correlation between the contents of 6 main marker constituents and the color parameters was analyzed by Pearson correlation analysis. Multiple linear regression models and third-order polynomial functions for the marker components and the color parameters were established by using the enter linear regression method and curve fitting with a polynomial model. The data analysis of variables using Pearson correlation analysis, multiple linear regression analysis (enter) and third-order polynomial curve fitting were performed using SPSS 20.0 software (IBM Inc., USA).

Results And Discussion

HPLC method validation

Validation of the HPLC method was conducted by following the recommendation in the literature [26, 27]. The 6 marker compounds of SS and SSC were identified using reference standards of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin, rosmarinic acid, luteolin and apigenin, as well as comparisons with the literature [26, 28, 29]. The proposed HPLC method was validated by determining the linearity, LOD, LOQ, inter- and intraday precisions, repeatability, stability, and recoveries. The equations, linear ranges, LODs and LOQs for the 6 marker compounds are summarized in Table 1. The intra- and interday RSDs were 0.03%-0.50% and 0.22%-0.73%, respectively (Table 2). To confirm the repeatability, six parallel ethanol extracts of SS were analyzed. The RSDs of the 6 marker components detected were 0.56%-2.34%. The RSD values of the HPLC stability test were found to be less than 3.0% (Table 2). The measured recoveries of 6 marker compounds ranged from 95.47–104.54%, with an RSD between 0.13% and 2.91%. All these results demonstrated that the developed HPLC method was suitable for the analysis of the constituents in SS and SSC samples.

Table 1
Calibration data for the 6 marker compounds by HPLC

Compound	Regression equation	R^2	Linear range (μg/mL)	LOD[a] (μg/mL)	LOQ[b] (μg/mL)
Luteolin-7-O-β-D-glucoside	$y^c = 0.1497x^d + 0.2286$	0.9992	0.80-159.20	0.29	0.97
Apigenin-7-O-β-D-glucoside	$y = 0.2128x + 0.0483$	0.9999	0.48-48.00	0.37	1.24
Hesperidin	$y = 0.1912x - 0.4667$	0.9999	3.08–616.00	0.30	0.98
Rosmarinic acid	$y = 0.2063x - 0.2390$	0.9999	1.62–324.00	0.26	0.87
Luteolin	$y = 0.1966x + 0.1069$	0.9998	0.47–141.60	0.10	0.34
Apigenin	$y = 0.3038x - 0.0788$	0.9999	0.40–120.00	0.08	0.27

[a] Limit of detection

[b] Limit of quantification

[c] Peak area

[d] Amount of injected compound (μg/mL)

Table 2
Precision, repeatability, stability and recovery data for the 6 marker compounds by HPLC

Compound	Precision (RSD, %)		Repeatability (RSD, %, *n* = 6)	Stability (RSD, %, *n* = 6)	Recovery (*n* = 3)	
	Intraday (*n* = 6)	Interday (*n* = 3)			Mean (%)	RSD (%)
Luteolin-7-O-*β*-D-glucoside	0.03	0.28	1.77	2.76	98.52	0.72
					101.94	1.07
					100.24	1.80
Apigenin-7-O-*β*-D-glucoside	0.06	0.24	1.46	2.59	95.47	0.35
					97.35	0.50
					96.18	1.52
Hesperidin	0.09	0.44	0.56	0.28	104.54	0.36
					104.72	1.06
					101.17	1.81
Rosmarinic acid	0.04	0.22	0.93	1.29	102.92	0.13
					103.54	0.87
					100.54	1.71
Luteolin	0.09	0.65	0.57	2.79	100.97	0.90
					102.40	0.31
					99.35	2.91
Apigenin	0.50	0.73	2.34	0.79	99.18	1.25
					102.07	2.57
					99.47	1.55

Changes in marker compounds during the stir-fry processing of Schizonepetae Spica under different treatment conditions

The developed method was applied to simultaneously determine the amount of 6 marker compounds during the stir-fry processing of SS at different temperatures over time. Representative HPLC chromatograms of the standard analytes and the samples are shown in Fig. 1. Comparison of the chromatograms of samples at various stir-fry temperatures revealed that the contents of four major ingredients (luteolin-7-O-*β*-D-glucoside, apigenin-7-O-*β*-D-glucoside, hesperidin and rosmarinic acid) decreased with increasing temperature, whereas the levels of another two main components (luteolin and apigenin) first increased and then progressively decreased

with increasing processing temperature. The quantitative results showed remarkable variation among different samples in the contents of the 6 marker compounds (Table 3 and Fig. 2). The amounts of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid in the samples derived from the three different processing temperatures decreased with increasing processing time. In contrast, the amounts of luteolin and apigenin initially increased until they reached a plateau and decreased with time in all three cases. In the current study, the contents of these 6 marker compounds fluctuated slightly among different time points, which might be attributed to inhomogeneous stir-fry processing [24, 30]. Our recent study showed that the increased levels of luteolin and apigenin might result from the degradation of the glucosidic bond of luteolin-7-O-β-D-glucoside and apigenin-7-O-β-D-glucoside under high-temperature during stir-fry processing [31]. In general, the amounts of the 6 major constituents decreased gradually with the processing temperature over time. The occurrence of splitting decomposition or cracking of the chemical structures during the heating process may account for these declining phenomena.

Table 3
Contents of 6 marker constituents of Schizonepetae Spica samples during stir-fry processing at different temperatures over time

No.	Heating temperature (°C)	Heating time (min)	Contents of flavonoids and phenolic acid (mg/g, *n* = 2)					
			Luteolin-7-O-*β*-D-glucoside	Apigenin-7-O-*β*-D-glucoside	Hesperidin	Rosmarinic acid	Luteolin	Apigenin
1	0	0	0.38	0.26	2.06	0.97	0.10	0.02
2	240 ± 10	5	0.16	0.14	1.67	0.71	0.25	0.21
3		5.5	0.14	0.12	1.37	0.62	0.30	0.20
4		6	0.12	0.11	1.27	0.60	0.34	0.20
5		6.5	0.12	0.10	1.28	0.65	0.35	0.21
6		7	0.10	0.09	1.11	0.60	0.36	0.19
7		7.5	0.10	0.09	1.21	0.63	0.35	0.20
8	300 ± 10	5	0.05	0.03	0.66	0.39	0.28	0.12
9		5.5	0.03	0.01	0.41	0.25	0.25	0.08
10		6	0.03	0.01	0.51	0.29	0.23	0.10
11		6.5	0.03	0.01	0.39	0.24	0.20	0.08
12		7	0.01	-[a]	0.20	0.12	0.15	0.05
13		7.5	0.02	-	0.32	0.18	0.17	0.07
14	360 ± 10	5	0.05	0.04	0.81	0.31	0.12	0.07
15		5.5	0.06	0.04	0.87	0.32	0.11	0.06
16		6	0.04	0.03	0.71	0.26	0.09	0.06
17		6.5	0.01	-	0.21	0.05	0.03	0.03
18		7	0.01	-	0.13	0.02	0.01	0.03
19		7.5	0.01	-	0.19	0.03	0.02	0.02

[a] Below the LOQ

Color analysis

The color parameters of SS during stir-fry processing in this experiment are shown in Table 4. It was apparent that the L^*, a^* and b^* values decreased gradually, and the corresponding sample powder changed from brown to black.

The color of CMMs is an important parameter to evaluate the processing degree. Modern analytical methods have validated that there is a close correlation between marker components and the color of CMMs [24, 32].

However, there have been limited scientific data and theoretical bases to validate the relationships between color and the quality of CMMs. Considering that the color indicates that a CMM reaches a certain processing state, could color parameters be used as surrogates of changes in chemical constituents during processing?

Table 4
The color measurement values of the Schizonepetae Spica samples subjected to the three processing temperatures over time (n = 3)

No.	L^*	a^*	b^*
1	40.45	2.73	5.61
2	35.14	2.49	1.49
3	34.62	2.51	1.12
4	34.08	2.33	0.60
5	34.41	2.36	0.75
6	34.30	2.39	0.62
7	34.40	2.40	0.62
8	33.33	1.96	-0.47
9	33.10	1.86	-0.82
10	33.09	1.90	-0.74
11	32.65	1.91	-0.80
12	32.62	1.77	-1.07
13	32.70	1.79	-1.16
14	33.06	1.80	-0.79
15	32.83	1.78	-1.14
16	33.09	1.68	-0.89
17	32.87	1.62	-1.29
18	32.54	1.66	-1.18
19	32.56	1.65	-1.24

Correlation analysis

The correlations between the color parameters and chemical marker contents during the stir-fry processing of SS were investigated. The Pearson correlation coefficients and corresponding p-values in two sets of variables are listed in Table 5. Significantly positive correlations were observed between the contents of four major ingredients

(luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid) and the L^*, a^* and b^* values. The levels of another two main components (luteolin and apigenin) were found to be significantly and positively correlated with a^*. However, the amounts of luteolin and apigenin had no significant correlation with L^* and b^*. Previous reports have shown that there is a certain correlation between the color parameters and chemical compositions in CMMs during the course of processing [33–36]. In this study, the three color parameters were associated significantly with the contents of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid, whereas a^* was most significantly correlated with the luteolin and apigenin contents.

Table 5
Pearson correlation analysis results between the color parameters and chemical marker contents during the stir-fry processing of Schizonepetae Spica

Compound	L^*		a^*		b^*	
	r	*P*	*r*	*P*	*r*	*P*
Luteolin-7-O-β-D-glucoside	$0.985^{\#\#\#a}$	0.000	$0.854^{\#\#\#}$	0.000	$0.991^{\#\#\#}$	0.000
Apigenin-7-O-β-D-glucoside	$0.945^{\#\#\#}$	0.000	$0.917^{\#\#\#}$	0.000	$0.972^{\#\#\#}$	0.000
Hesperidin	$0.840^{\#\#\#}$	0.000	$0.922^{\#\#\#}$	0.000	$0.883^{\#\#\#}$	0.000
Rosmarinic acid	$0.836^{\#\#\#}$	0.000	$0.965^{\#\#\#}$	0.000	$0.887^{\#\#\#}$	0.000
Luteolin	0.165	0.499	$0.659^{\#\#b}$	0.002	0.269	0.266
Apigenin	0.151	0.537	$0.687^{\#\#}$	0.001	0.268	0.268
[a] Correlation is significant at the 0.001 level						
[b] Correlation is significant at the 0.01 level						

Regression analysis

To date, a series of quality control methods focusing on the essential oil of SS have been reported, including steam distillation, gas chromatography-mass spectrometry (GC-MS) and fingerprinting [37, 38]. However, there are few reports regarding the determination of nonvolatile constituents from raw and processed SS. Additionally, laboratory instruments such as HPLC could provide accurate measurement results of marker compounds in SS during processing. However, these methods involve laborious, complex and time-consuming pretreatment of samples with extensive use of organic reagents that are unfriendly to the environment. Therefore, possible models of surrogate color parameters for marker constituents in SS during processing will be explored. According to the above results of correlation analysis, color analysis and the variation in the major constituents of SS during processing, statistically significant parameters (L^*, a^* and b^*) were analyzed by the enter linear regression method to establish the regression model of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid. The optimal models were determined according to the maximum adjusted R^2 values (Table 6). The best individual regression models were established as follows:

Luteolin-7-O-β-*D-glucoside* = -0.604 + 0.019·L^* + 0.014·a^* + 0.03·b^* (1)

Apigenin-7-O-β-D-glucoside = -0.361 + 0.009·L^* + 0.066·a^* + 0.018·b^* (2)

Hesperidin = -11.237 + 0.275·L^* + 1.35·a^* − 0.256·b^* (3)

Rosmarinic acid = -7.354 + 0.179·L^* + 0.833·a^* − 0.208·b^* (4)

For luteolin and apigenin, third-order polynomial curve fitting was performed for the data of each sample. The SPSS analysis process excluded a^{*2} as an unimportant factor, and the formulas and adjusted R^2 values (Table 6) were determined as follows:

Luteolin = -2.977 + 2.217·a^* − 0.146·a^{*3} (5)

Apigenin = -1.562 + 1.154·a^* − 0.074·a^{*3} (6)

The ANOVA test results of coefficient values, standard errors and *t*-values for the color parameters included in each proposed model are presented in Table 7. These results showed that the contents of 6 marker constituents could be represented well by polynomials of the color parameters, including L^*, a^* and b^*. The models were statistically significant (p = 0.000), with adjusted R^2 values greater than 0.800, which indicated that good correlations were established between the color parameters and concentrations of different marker compounds. Thus, the contents of marker constituents of SS could be rapidly predicted by color parameters during processing, which represented the scientific elucidation of color as an important indicator for the identification of SS processing degree. However, a unified quantitative relationship between the color parameters and chemical marker contents for various SS samples could not be established because of the differences in a number of factors—such as plant cultivars, harvesting time, degradation degrees and many other poorly controlled aspects during stir-fry processing. Nevertheless, it is possible to develop specific prediction models for marker compounds in specific SS samples to monitor the degree of processing using color parameters. Further studies are warranted to establish the relationship between the alteration of color parameters and the changes in medicinal properties during processing.

Table 6
Statistical evaluation for the prediction models

Compound	R	R^2	Adjusted R^2	F	P
Luteolin-7-O-β-D-glucoside	0.991	0.983	0.980	289.785	0.000
Apigenin-7-O-β-D-glucoside	0.982	0.965	0.958	137.046	0.000
Hesperidin	0.938	0.880	0.856	36.758	0.000
Rosmarinic acid	0.974	0.948	0.938	91.242	0.000
Luteolin	0.971	0.942	0.935	129.771	0.000
Apigenin	0.910	0.828	0.807	38.570	0.000

Table 7
Regression analysis coefficients for the prediction models

Compound	Variable	Coefficient value	Standard error	*T*-value	Sig.
Luteolin-7-O-β-D-glucoside	Constant	-0.604		-0.799	0.437
	L^*	0.019	0.397	0.935	0.365
	a^*	0.014	0.055	0.426	0.676
	b^*	0.030	0.550	1.060	0.306
Apigenin-7-O-β-D-glucoside	Constant	-0.361		-0.432	0.672
	L^*	0.009	0.227	0.371	0.716
	a^*	0.066	0.344	1.860	0.083
	b^*	0.018	0.447	0.598	0.559
Hesperidin	Constant	-11.237		-0.887	0.389
	L^*	0.275	0.895	0.793	0.440
	a^*	1.350	0.863	2.528	0.023
	b^*	-0.256	-0.756	-0.548	0.592
Rosmarinic acid	Constant	-7.354		-1.831	0.087
	L^*	0.179	1.207	1.624	0.125
	a^*	0.833	1.107	4.921	0.000
	b^*	-0.208	-1.273	-1.400	0.182
Luteolin	Constant	-2.977		-12.710	0.000
	a^*	2.217	6.700	13.034	0.000
	a^{*3}	-0.146	-6.083	-11.834	0.000
Apigenin	Constant	-1.562		-6.386	0.000
	a^*	1.154	5.747	6.500	0.000
	a^{*3}	-0.074	-5.096	-5.763	0.000

Conclusions

Flavonoids and organic acids are the main nonvolatile constituents of SS, and they possess many pharmacological activities. The present study examined variations in the levels of six marker nonvolatile

constituents and the color parameters of SS during stir-fry processing by using HPLC-DAD and colorimetry, respectively. The amounts of the four major ingredients (luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid) and the three color parameters (L^*, a^* and b^*) decreased with increasing temperature over time, whereas the contents of another two main components (luteolin and apigenin) initially increased before reaching a plateau and then decreased during processing. L^*, a^* and b^* were recognized as the relevant parameters significantly associated with the luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin and rosmarinic acid contents in SS, while a^* was most significantly related to the luteolin and apigenin contents in SS. By regression analysis, four optimal multiple linear regression models and two third-order polynomial models were established for the corresponding marker compounds as a function of relevant color parameters, with an adjusted R^2 value significantly greater than 0.800. For the first time, surrogate prediction using color parameters was developed for the simultaneous quantification of 6 marker substances including flavonoids and an organic acid during the processing of SS. Although these models could not be applied to various SS samples, the chemical marker contents could be rapidly predicted by the color parameters through the specific models. In short, colorimeter sensor technology and phytochemical analysis combined with chemometric methods provided scientific data and a theoretical basis to validate the relationships between color features and the processing degree of SS. The developed strategy offers new clues for the quality control standards of nonvolatile constituents of SS processing practices and provides useful references for other processing methods.

Abbreviations

CIE, International Commission on Illumination; CMM, Chinese medicinal material; DAD, diode array detector; SS, Schizonepetae Spica; SSC, Schizonepetae Spica Carbonisata; TCM, traditional Chinese medicine

Declarations

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Not applicable.

Authors' contributions

HC and XL conceived and designed the experiments. XL performed the experiment and prepared the manuscript. HC, MW, YZ and ZM revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets used during this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

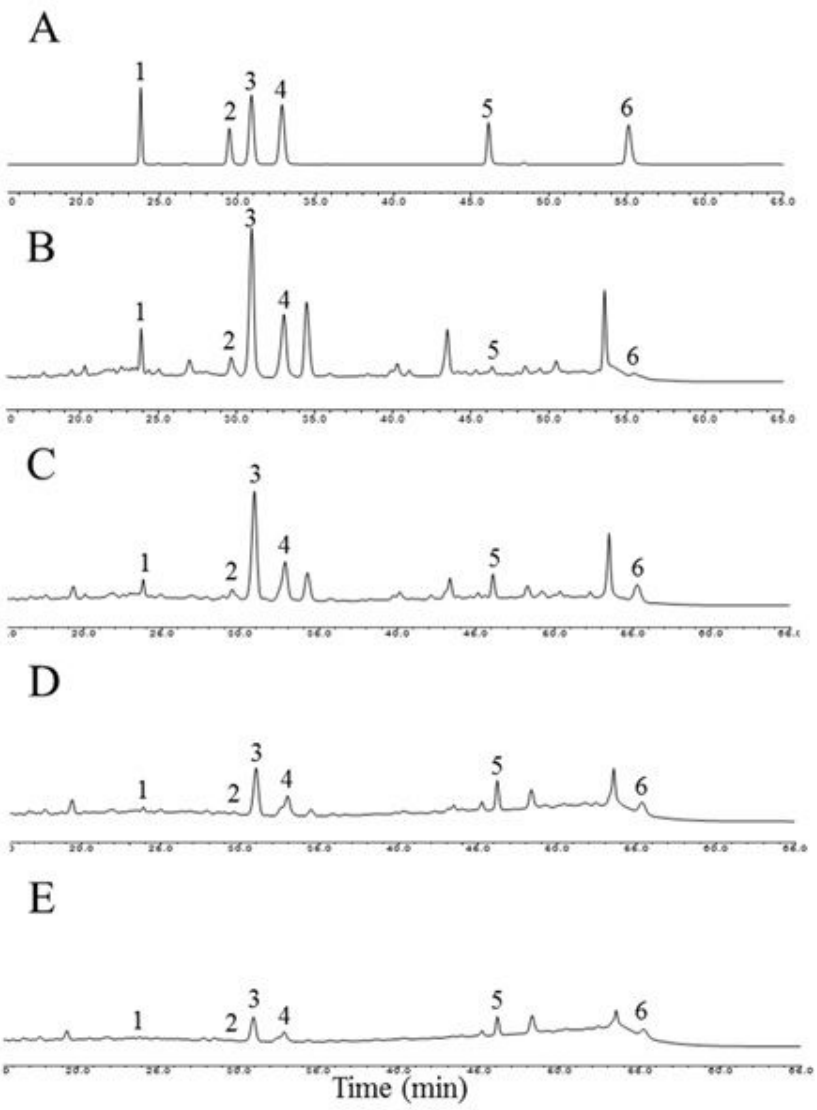

Figure 1

Representative chromatograms of reference substances (A) and samples (B-E). B, Raw Schizonepetae Spica; C-E, Representative chromatograms of Schizonepetae Spica during stir-fry processing under different treatment conditions (C, 240°C ± 10°C, 5 min; D, 300°C ± 10°C, 5 min; E, 360°C ± 10°C, 5 min); 1, luteolin-7-O-β-D-glucoside; 2, apigenin-7-O-β-D-glucoside; 3, hesperidin; 4, rosmarinic acid; 5, luteolin; 6, apigenin

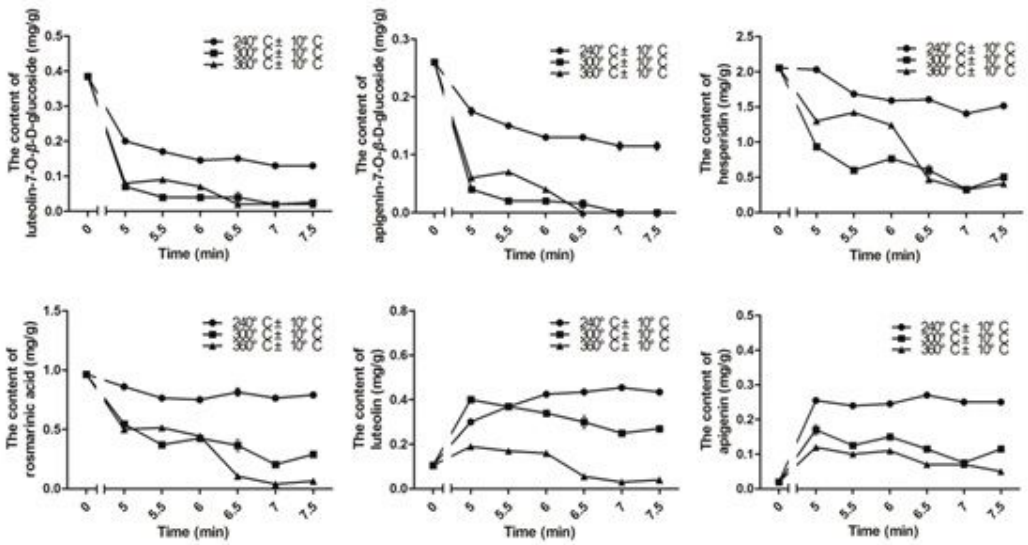

Figure 2

Changes in the amounts of luteolin-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, hesperidin, rosmarinic acid, luteolin and apigenin in the Schizonepetae Spica samples subjected to the three processing temperatures over time (n = 2)