A novel method for the discrimination of Hawthorn and its processed products using an intelligent sensory system and artificial neural networks

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Abstract Hawthorn (CFS) has commonly been applied as an important traditional Chinese medicine and food for thousands of years. The raw material of CFS is commonly processed by stir-frying to obtain yellow (CFY), dark brown (CFD), and carbon dark (CFC) colored products, which are used for different clinical uses. In this study, an intelligent sensory system (ISS) was used to obtain the color, gas, and flavor samples data, which were further employed to develop a novel and accurate method for the identification of CFS and its processed products using principal component analysis. Moreover, this research developed a model of an artificial neural network, which could be used to predict the total organic acid, total flavonoids, citric acid, hyperin, and 5-hydroxymethyl furfural via determination of the color, odor, and taste of a sample. In conclusion, the ISS and the artificial neural network are useful tools for rapid, accurate, and effective discrimination of CFS and its processed products.

Keywords: Hawthorn, intelligent sensory system, artificial neural networks, discrimination

Introduction

Hawthorn (CFS), the ripe fruit of the rose family plant *Crataegus pinnatifida* Bge. var. *major* N.E.Br. or *Crataegus pinnatifida* Bge, is a traditional food and herbal medicine for improving digestion and appetite that has been used for thousands of years in China (1). Numerous pharmacological investigations have demonstrated that CFS has hypocholesterolemic (2) and antioxidant effects (3).

For traditional use, CFS is commonly processed by stir-frying to obtain yellow (CFY), dark brown (CFD), and carbon dark (CFC) colored products, which are used for different clinical uses. After processing, the pharmacological effects of CFS and its processed products are different (4). Recently, the quality control of CFS and its processed products has been mainly based on two aspects: characteristics discrimination (commonly performed) and indicative component discrimination citric acid (AD), commonly conducted by high performance liquid chromatograph (HPLC) (5). However, both the abovementioned approaches have some limitations, such as the difficulty in reflecting the overall quality of CFS and its processed products. Moreover, the characteristics (including color, odor, taste) are closely related to the inherent quality of traditional Chinese medicine (TCM) (6–8). Characteristics identification mainly relies on

the experience of workers to distinguish the color, gas, and taste of CFS and its processed products. Moreover, empirical identification by people often has the disadvantages of poor operation and easy interference by extraneous factors (9). Therefore, finding a rapid and non-destructive way to comprehensively evaluate the quality of TCMs is extremely important.

Currently, an intelligent sensory system (ISS) has become a strong support for the quality assurance of foods and drugs. ISS mainly includes a computer vision (CV), an electronic nose (EN), and an electronic tongue (ET), and it is an objective evaluation way via simulation of the human sensory organs (10–13). The specific value of characteristics of samples (color, gas, and flavor) could reflect the overall characteristics of TCM. Previous researches have demonstrated that ISS combined with artificial neural networks (ANNs) could be successfully used to predict the related chemical constituents and bioactivities in foods and TCMs (14–16). However, there is no report regarding employing ISS combined with ANNs to predict the related active constituents in CFS and its processed products.

Thus, the objective of this study is aimed to identify CFS and its processed products based on ISS and to predict the related active constituents of CFS and its processed products based on ISS combined with ANNs (ISS–ANN) (Fig. 1).

Fig. 1. The scheme designed for prediction of the chemical attributes of CFS and its processed products using an intelligent sensory system and artificial neural networks.

Materials and Methods

Samples and chemicals CFS raw materials were purchased from Qingzhou, Shandong Province, processed by the Neautus Traditional Sichuan Chinese Medicine Co. (Chengdu, China), and identified by Prof. Wu C.J. (College of pharmacy, Chengdu University of TCM, Chengdu, China). Another 12 batches of CFS and its processed products were purchased from the Chinese herbal medicine market of *Hehuachi* (Chengdu, China). Phenolphthalein, ammonium dihydrogen phosphate, aluminum nitrate, methanol, ethanol, sodium hydroxide, and sodium hydroxide were obtained from the Chengdu Jinshan Chemical Reagent Co. (Chengdu, China); reference agents, including AD, rutin (RN), and 5-HMF, were obtained from the National Institutes for Food and Drug Control (Beijing, China); acetonitrile was purchased from the Thermo Fisher Scientific Ltd. (Shanghai, China). All other reagents used were of analytical grade.

Determination of chemical compositions in CFS and its processed products

Determination of total organic acid: The total organic acid (TOA) was determined according to a previously described method (5). Briefly, 100 mL of water was added to 1 g fine powder of the sample, and then soaked at room temperature for 4 h. After filtration, 50 mL of water and 2 drops phenolphthalein indicator solution were added to 25 mL of the filtrate; subsequently, titration was conducted using 0.1 M sodium hydroxide solution. The end point of the titration is controlled by the instantaneous color fading of phenolphthalein. The TOA was then expressed as AD equivalents in mg/g dry sample.

Determination of total flavonoids: The total flavonoids (TF) in CFS and its processed products were determined using ultraviolet–visible spectrophotometry according to the previously described method (5). Briefly, RN was used as the reference substance; the TF contents were then expressed as RN equivalents in mg/g dry sample. The

determining wavelength for the ultraviolet–visible spectrophotometry was set at 500 nm.

Determination of AD, hyperin (HN), and 5-hydroxymethyl furfural (5-HMF): AD and hyperin (HN) were determined by following previous reports (17–19). Briefly, HPLC analysis was used to determine AD using a Shimadzu LC-2010A system with a Phenomenex C₁₈ column (250 mm×4.60 mm, 5 µm). To determine the AD contents, the mobile phase used was 2% ammonium biphosphate solution (pH value was adjusted to 3.0 using phosphoric acid) with the flow rate of 1.0 mL/min. The column temperature and detecting wavelength were set at 30°C and 210 nm, respectively.

For the determination of HN, the mobile phase used was acetonitrile (A) and 0.3% phosphoric acid (B) with a flow rate of 1.0 mL/min. Gradient elution process was as follows: 0–60 min, 90%–73%; 60–75 min, 73% (B). The column temperature and wavelength were set at 30°C and 335 nm, respectively.

For the determination of 5-hydroxymethyl furfural (5-HMF), a mixture of acetonitrile (8%) and water (92%) was used as the mobile phase, and the flow rate was set to 1.0 mL/min. The column temperature and wavelength were maintained at 25°C and 283 nm. In previous researches, the five main chemical constituents were selected, possessing various pharmacological activities similar to that of CFS, including antioxidant activity, promoting digestive effect, and antihyperlipidemic effect (2,3).

ISS

CV assay: The CV system used in our present research comprises four major components: gray chamber (including lighting system), industrial camera, computer hardware and software. An 80×60×70 cm³ gray wooden box was prepared to act as the housing to isolate the lighting outside. To avoid undesirable reflections, the color of the internal stand walls of the CV was set to gray. We selected a D50 (Philips Master TL-D 90 De Luxe 36W/950; Philips, Amsterdam, Netherlands) light source, and the brightness control system of the lamp was arranged at the bottom of the box. A stable industrial camera (SXG80c; Baumer, Friedberg, Germany) connected to a laptop (EIZO CS230; Lenovo, Morrisville, NC, USA) with a remote Baumer Gapi viewer was used to view and to capture images (Baumer). Before capturing images, the system was calibrated for white balance with a white card (White Balance Card, 21.59×27.94 cm², X-rite). For the camera parameter settings, the aperture value was 16 and exposure time was 0.3 s.

To eliminate background interference, the threshold segmentation algorithm was used to obtain the images. The average RGB color values of samples were obtained and subsequently converted into HSV values for the following experiments. HSV is a color space where H represents hue and S and V represent the degree of saturation and brightness, respectively.

EN assay: In our present investigation, an FOX-4000 E-nose system (Alpha M.O.S., Toulouse, France) was employed, which comprises a sampling apparatus, a detector unit, an air generator equipment,

Table 1. The repeatability based on the detective method of EN and ET (n=6)

Sensor	RSD (%)	Sensor	RSD (%)	Sensor	RSD (%)
LY2/LG	1.027	T30/1	1.146	P30/1	1.135
LY2/G	0.7258	P10/1	1.100	P40/2	0.9758
LY2/AA	0.4484	P10/2	1.000	P30/2	1.830
LY2/GH	1.154	P40/1	1.156	T40/2	0.7477
LY2/gCTL	0.6167	T70/2	1.066	T40/1	0.7633
LY2/gCT	0.4631	PA/2	1.092	TA/2	0.8322
ZZ	0.22	GA	0.30	CA	0.56
AB	0.21	BB	1.22	DA	0.21
JE	0.25	-	-	-	-

and an HS-100 autosampler. In addition, the obtained data were analyzed by the pattern recognition software (Version 2012.45, Alpha M.O.S.). The sensor array comprised 18 metal oxide semi-conductors (MOS) chemical sensors. Furthermore, the chemical sensors were grouped into three types: 1) T-type, including, T30/1, T40/2, T40/1, TA/2, and T70/2; 2) P-type, including P10/1, P10/2, P40/1, PA/2, P30/1, P40/2, and P30/2; and 3) LY-type, including LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, and LY2/gCT. Good repeatability was investigated and shown in Table 1.

ET assay: We used the α Astree E-tongue (Alpha M.O.S.) for the ET assay on CFS. The E-tongue comprised seven cross-selective potentiometric sensors, an Ag/AgCl reference electrode, a 16-position sample changer, and an interface electronic module (Alpha M.O.S.). In addition, the Astree II software (Version 2012.45, Alpha M.O.S.) was used to record and analyze the data and good repeatability was investigated and shown in Table 1.

T30/1, T40/2, T40/1, TA/2, T70/2, P10/1, P10/2, P40/1, PA/2, P30/1, P40/2, P30/2, LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, and LY2/gCT are the different sensors of EN; ZZ, AB, GA, BB, CA, DA, and JE are the different type sensor of ET, and all these abbreviations are names of the commercial sensor produced by Alpha M.O.S.

ANN assay: The ANN model includes the input, hidden, and output layers (20), and each layer contains numerous nerve cells. In previous researches, it is reported that a single hidden layer is adequate for the ANN to predict results and nonlinear functions (21–23). In this investigation, one feed-forward networks with input, output, and hidden layers were used for predicting TOA, AD, TF, HN, and 5-HMF. In the input layer, the network for predictions possesses 28 neurons according to 28 character features extracted from each sample (color, odor, and taste). In the output layer, five neurons represented of five compositions about CFS and its processed products. Back-propagation algorithm was obtained to train the ANN model, and the input data were furthermore normalized and ranged from 1 to 1 before being fed to the ANN. In addition, log-sigmoid transfer functions were used for data transfer in the hidden and output layers. The error minimization was analyzed by the Levenberg–Marquardt algorithm. Moreover, a trial-and-error approach was used to set neurons' number in the hidden layer. In this aspect, the

nodes' number is optimized using repeated training to 1–10 in the hidden layer.

Statistical analysis Data were represented as mean±standard deviation and principal component analysis (PCA) was conducted to discriminate different samples. In addition, Pearson correlation coefficients were used to analyze the correlation of different samples. The differences were recognized as significant if the *p* values were less than 0.05.

Results and Discussion

Discrimination of different CFS and its processed products In our research, PCA was conducted to discriminate CFS, CFY, CFD, and CFC. According to the results acquired by CV, EN, and ET, the color data (H, S, and V) and sensor responses (18 and 7 variables for EN and ET, respectively) were analyzed via PCA, and the scores plots of PCA are shown in Fig. 2. The three principal components are shown in Fig. 2A (PC1=95.907%; PC2=3.872%; PC3=0.2208%), and the four sample groups can be obviously discriminated. Similarly and interestingly, the PCA results of EN (Fig. 2B, PC1=94.237%; PC2=4.533%; PC3=0.693%) and ET (Fig. 2C, PC1=87.648%; PC2=9.511%; PC3=1.669%) also demonstrated that the four sample groups can be significantly discriminated. Collectively, our results indicated that ISS can be used to discriminate CFS and its processed products.

PCA is one of the most useful multivariate statistical techniques, and it aims at reducing the dimension of the original data, i.e., the multi indicators into a small number of integrated indicators (24). Thus, in our present research, we analyzed all the obtained data using PCA, and the results significantly discriminate CFS and its processed products.

Correlations between instrumental values and components The color and chemical attributes of different samples were determined, as shown in Table 2. Colors of the different samples were noticeably different and varied from light to dark yellow. During processing, the odor changed from a faint scent to empyreumatic and finally to bitterish; from the point of view of the taste, the acidity of samples is gradually lower, and finally into a bitter taste. No reliable assay methods are available to detect the change in odor and taste; therefore, EN and ET will be used to reflect the variety. With the changes of firepower and the extension of processing time, the chemical changes in processing are clearly, the contents of TOA, AD, TF, and HN are gradually decreased. At the same time, the content of 5-HMF gradually increases.

In this experiment, the original data obtained by CV, EN, and ET were transformed to the mean values. Then, the information representing integrated indexes of color parameters and electronic sensory signals was acquired, and the correlations between these factors and components content were investigated. One factor was obtained for CV (FAC1, 87.805%) and EN (FAC1, 93.974%); two factors (FAC1, 69.809%; FAC2, 14.849%) were extracted for ET. The relations between samples character (color, odor, and taste) and TOA, AD, TF, HN, and 5-HMF are represented in the correlation matrix presented below (Pearson correlation coefficients) (Table 3).

The results of this research demonstrated that color, odor, and taste are important features of CFS. Thus, measuring these features using proper instrumentation to predict the different dependent parameters of CFS and its processed products such as TOA, AD, TF, HN, and 5-HMF is possible.

ISS and ANN combination (ISS-ANN) The aim of this research was to prove the applicability of ISS-ANN (EN, ET, and CV) as a simple and potential tool for prediction of TOA, AD, TF, HN, and 5-HMF. The

Fig. 2. PCA scores plots for discriminating Hawthorn (CFS) and processed samples according to CV (A), EN (B), and ET (C).

Table 2. Values of TOA, AD, TF, HN and 5-HMF content of CFS and processed samples

	H	S	V	TOA (mg AD/g)	AD (mg/g)	TF (mg RN/g)	HN (mg/g)	5-HMF (mg/g)
CFS	24.40±0.24	135.57±0.86	89.83±0.96	90.20±1.24	68.91±2.01	72.87±1.72	0.23±0.02	1.4±0.03
CFY	23.54±0.87	113.91±2.19	59.58±1.37	84.54±1.88	61.67±3.00	63.57±3.81	0.20±0.02	1.52±0.20
CFC	33.41±1.29	73.68±2.16	45.40±0.61	74.74±1.87	55.26±2.38	51.29±2.49	0.13±0.02	7.30±0.61
CFD	46.40±0.58	55.59±0.59	42.90±1.00	40.44±2.29	25.89±4.21	26.02±1.72	0.04±0.01	10.09±0.99

Table 3. The correlation of contents and characterization of CFS samples

		CV	EN	ET	
		FAC1	FAC1	FAC1	FAC2
TOA	Coefficients	0.901	0.995	0.920	0.986
	<i>p</i>	<0.05	<0.01	<0.05	<0.01
AD	Coefficients	0.895	0.996	0.905	0.892
	<i>p</i>	<0.05	<0.01	<0.05	<0.05
TF	Coefficients	0.950	0.976	0.787	0.956
	<i>p</i>	<0.05	<0.05	0.107	<0.05
HN	Coefficients	0.964	0.952	0.762	0.939
	<i>p</i>	<0.05	<0.05	0.119	<0.05
5-HMF	Coefficients	0.990	0.87	0.993	0.990
	<i>p</i>	<0.01	0.065	<0.01	<0.01

consistency of an illumination system is an important factor to maintain color reproducibility. A preliminary experiment conducted for sensitivity analysis of the lighting system indicated the uniformity

of the illumination setup over the effective illuminated area in the center of the image. The value of color for images captured in 30-min intervals during 2 h was lower than 0.02. The test proves the good stability of the system. To ensure the stability of EN and ET, a correction fluid is used to revise the original state of sensors. The response value of EN and ET for sensors acquired in a 30-min interval during 2 h was lower than 0.03. After the systems calibration, ANN was used for TOA, AD, TF, HN, and 5-HMF prediction for the samples.

The topology notably influences the final accuracy of the network; therefore, the network topology is an important factor in the design of ANNs. In the hidden layer, neuron number is an important factor for improving the accuracy of network training. In theory, the appropriate number of hidden layer nodes in a specific design is not determined, and the best nodes were determined by the training of different neuron number. In this experiment, we selected 10 neurons, which were determined to be the ideal neuron number for the hidden layer through continuous training. The original data set of

Fig. 3. Correlations between the predicted and experimental values of TOA, AD, TF, HN, and 5-HMF.

40 samples were divided into four blocks, thus establishing the model of ANN, and the 12 sets of data obtained from the sample were used as the verification test set.

Correlations between the predicted and experimental TOA, AD, TF, HN, and 5-HMF for the test sets are shown in Fig. 3. It can be observed that the optimal ANN could predict TOA, AD, TF, HN, and 5-HMF values of samples based on the color, odor, and taste data measured by ISS-ANN.

Correlations between the predicted and experimental TOA, AD, TF, HN and 5-HMF values for the 12 test sets are shown in Fig. 3. These results demonstrate that character parameters (color, odor, and taste) are good predictors for TOA, AD, TF, HN, and 5-HMF of CFS and its processed products. Our study results also suggested that ISS-ANN can be effectively used for predicting the main compositions of CFS and its processed samples.

In our present research, the distinctions between CFS and its different processed products were successfully and rapidly realized using machine vision, an electronic nose, and a tongue. In addition, our results demonstrated that organic acid, total flavones, and other active ingredients could be effectively predicted via determining the color, odor, and taste of CFS using EN, ET, and CV. In recent years, ISS has been widely used in food, agricultural products, chemical industry, and other fields; however, in the field of TCM, ISS is still in the initial stage, and the present investigation is the first report regarding the application of EN, ET, and CV to predict the constituents of TCM, which is a promising strategy for rapid, damage free, and accurate evaluation of the TCM quality.

Nowadays, ANNs are widely used for prediction and classification in various branches of biology, physics, and chemistry. It is revealed that an ANN is an objective mathematical representation for human neural architecture and is considered to be an ideal model for reflecting the “learning” and “generalization” abilities of human beings. ANNs can maintain more data of samples with accuracy prediction via a series of training. In this study, we utilized the ANN to build a model that could be used to determine the values of color, odor, and taste to predict its correlated chemical attributes, and the results effectively predict the main compositions of CFS and processed samples.

In conclusion, the ISS (EN, ET, and CV) and ANNs are useful tools for rapid, accurate, and effective discrimination of CFS and its processed products.

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