Discrimination of Camellia seed oils processed by different extraction methods based on electronic tongue technology

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September 11, 2020

Abstract

Analytical methods involving electronic tongue technique combined with chemometrics analysis was proposed to discriminate oil variety and predict oil quality parameters. All the studied Camellia oil samples from pressing, n-hexane extraction and scCO2 extraction, were successfully discriminated by principal component analysis (PCA) and hierarchical cluster analysis (HCA). Furthermore, Multi Factor Linear Regression Model (MLRM) was established allowing predictive capacity of oil quality indicators, such as acid value (AV) and peroxide value (POV). The practical potential of e-tongue for the discrimination and assessment of Camellia oils has shown promising application in the characterization of Camellia oils in the oil quality evaluation.

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Abstract

Analytical methods involving electronic tongue technique combined with chemometrics analysis was proposed to discriminate oil variety and predict oil quality parameters. All the studied *Camellia* oil samples from pressing, n-hexane extraction and $scCO_2$ extraction, were successfully discriminated by principal component analysis (PCA) and hierarchical cluster analysis (HCA). Furthermore, Multi Factor Linear Regression Model (MLRM) was established allowing predictive capacity of oil quality indicators, such as acid value (AV) and peroxide value (POV). The practical potential of e-tongue for the discrimination and assessment of *Camellia* oils has shown promising application in the characterization of *Camellia* oils in the oil quality evaluation.

Keywords: Electronic tongue; *Camellia* oil; Physicochemical property; Chemometrics; Discrimination

1. Introduction

The evergreen shrub *Camellia oleifera* Abel., belonging to *Camellia* genus of Theaceae family, is a unique woody edible oil species widely distributed in southern China (Gao et al., 2020). *Camellia*seed oil, known as the "oriental olive oil" (Cheng et al., 2018; Ma et al., 2011), contains abundant unsaturated fatty acids, among which the total amount of oleic acid and linoleic acid is over 85% (Wei et al., 2015; Yang et al., 2016; Zhu et al., 2019). The edible value of *Camellia* seed oil is high, for it also contains various nutritional ingredients such as polyphenols, sterols and tocopherols (Wu et al., 2018; Ye et al., 2014; Zhou et al., 2019). Meanwhile, its bioactive properties, e.g., anti-inflammatory, anti-oxidant activity and anti-obesity (Fattahi-far et al., 2006; Guan et al., 2011; Wang et al., 2019), rendering *Camellia* seed oil a great application potential in cosmetic and medicinal field (Chaikul et al., 2017). Recommended by FAO as high quality edible oil (Zhou et al., 2019), *Camellia* seed oil is now in great demand for people have higher requirements for health, and thus the corresponding industry has a promising prospect in the future.

Cold pressing and solvent extraction are the most commonly used industrial methods in oil extraction process (Yang et al., 2019). Over refinement could be avoided by cold pressing via cutting down the high temperature pretreatment of *Camellia* seed, the decolorization and deodorization of crude oil (Moslavac et al., 2014). Thus the quality of cold pressing oil is guaranteed by reducing the loss of bioactive compound which is sensitive to heat and also by reducing the formation of benzopyrene and glycidyl ester at high temperature (Qi et al., 2015; Wei et al., 2015; Wu et al., 2012). Organic solvent extraction method has obvious defects such as solvent residue, and environmental pollution. This method is still in use especially in the treatment of pressed seed cake for the economic reason (Shao et al., 2015). In comparison, cold pressed oil showed stronger antioxidant and antimicrobial activities than oil extracted by organic solvent (Zhou et al., 2014). Supercritical CO_2 extraction (SCCE) has been widely applied as a green technology. It is superior to traditional technology as it is non-toxic, fast and high efficient. Besides that, its critical temperature and critical pressure (T_c = 304.2 K, P_c = 7.38 MPa) are moderate to reach (Duan et al., 2013). The oil quality extracted by SCCE is superior both in higher bioactive constituents content such as squalene and sterols, and also better physicochemical characteristics like lighter color, stronger aroma, lower acid value (AV) and peroxide value (POV) (Shao et al., 2015). The oil quality differs obviously when extracted by different processes, and this implies that developing a proper methodology to distinguish oil from different sources appears to be meaningful.

In the PRC National Standard (GBT11765-2018), to classify *Camellia* seed oil by quality, the physicochemical parameters and the corresponding analytical procedures, are defined listed in Table 1. Conventional analytical techniques such as HPCL and GC-MS have certain drawbacks, e.g., expensive instruments, complex sample pretreatment and consuming more time (Aparicio et al., 2013; Semenov et al., 2019). In recent years, electronic tongue (e-tongue) technique, as an analytical technique mimicking the human senses of taste, owing to its simple, rapid and precise characters, has been widely explored in food, cosmetic and pharmaceutical industries (Sliwinska et al., 2014). Many qualitative and/or quantitative applications of e-tongue for olive oils have been reported successfully. Quality parameters such as peroxide, anisidine values and total tocopherols (Semenov et al., 2019), carotenoid (Semenov et al., 2019), oxidative stability and polyphenols (Rodrigues et al., 2019) were correlated and assessed using e-tongue system combined with chemometrics. Studies of classification of the olive oil base on quality (Tahri et al., 2018; Veloso et al., 2018), discrimination of adulterate olive oil (Harzalli et al., 2018; Oliveri et al., 2009), olive oil shelf-life estimation (Buratti et al., 2018; Rodrigues et al., 2017) and olive oil sensory assessment (Rodrigues et al., 2019; Rodrigues et al., 2018) were also well achieved indicating broad application of e-tongue in oil characterization. For *Camellia* oil, chemometrics combined with electronic nose was found effective to discriminate the geographical origins (Peng et al., 2020). Methods of FTIR (Han et al., 2020) or NIR (Chu et al., 2017) spectroscopy combined with chemometrics was developed to identify *Camellia* oil adulteration with rapeseed oil or vegetable oil. To the best of our knowledge, discrimination of *Camellia* seed oils produced by different processes has not been reported.

The aim of the present work was to evaluate the versatility of e-tongue for the characterization of *Camellia* seed oils (pressed, extracted with organic solvent and $scCO_2$) and simulation of peroxide value (POV) and acid value (AV) associated with sensory assessment. For this, chemometric quantitative approach (multiple linear regression models) is applied to establish predictive multivariate models.

2. Materials and methods

2.1 Chemicals and reagents

Commercial CO_2 (purity, 99.99%) was supplied by Air liquide Foshan Co., Ltd. (Foshan, China). Tartaric acid, silver chloride were supplied by Aladdin-Reagent Co, Ltd. (Shanghai, China). Isopropyl alcohol, ether, n-hexane, phenolphthalein, sodium hydroxide, acetic acid, chloroform, potassium iodide, sodium thiosulfate, anhydrous sodium sulfate, soluble starch, potassium chloride, potassium hydroxide, hydrochloric acid and ethanol were purchased from Guangzhou Chemical Regent Co, Ltd. (Guangzhou, China). Ultrapure water was produced by Milli-Q Reference (MERCK Millipore, Germany).

2.2 *Camellia* seed oil sample and physicochemical characteristic analysis

Five commercial pressed *Camellia* seed oil samples were obtained and denoted from S1 to S5 (Table 2). *Camellia* seeds were provided by Guangdong Fanlong Agricultural Technology Development Co., Ltd (Jieyang, China) and were grinded to a proper size by an experimental mill. Oil sample (No. S6) extracted by n-hexane was carried out by soxhlet extraction method (GBT14488.1-2008). Supercritical CO_2 extractions of *Camellia* seed were conducted in a HB120-50-05 device (Jiangsu Hongbo Machinery Manufacturing Co., Ltd). 1 kg of *Camellia* seeds were loaded in the extraction cell, and then CO_2 were pumped in to remove air. After reaching the set temperature, pressure was gradually increased by a CO_2 high-pressure pump. Extraction started in a cyclic manner for a period of time after reaching set pressure. Oil samples were collected in the pressure reduction container I per 0.5 h, and then merged and classified in the time period of 0-2h, 3h and 4-6h for the subsequent testing. Oil yield was classified as:

$$\text{Yield}\% = m_{\text{oil}} / M_{\text{seed}} * 100\%$$

Single factor analysis with temperature ranging from 313.15 K to 333.15 K and pressure ranging from 20 MPa to 30 MPa were investigated in this study. Samples achieved by SCCE were listed as S7 to S21 according to the condition and sampling time (Table 3). All the oils were sealed and refrigerated before further tests.

Physicochemical characteristic of the oil samples were analyzed according to the PRC National Standard. The moisture and volatile matter (GB 5009.236-2016), peroxide value (GB 5009.227-2016) and acid value (GB 5009.229-2016) were analyzed following the standard methods. Peroxide value and acid value were shown as mean ± standard deviation (SD) in Table 3.

2.3 E-tongue analysis

2.3.1 Sample pretreatment

12 g of oil sample were added to 85 g ultrapure water (333.15 K), stirred by emulsifying mixer for 3 min. After cooling to room temperature, 50 g standard solution (2.24 g KCl and 0.045 g tartaric acid in 1000 mL ultrapure water) was added and then centrifuged for 10 min at 3000 rpm. Aqueous phase were taken for further determination.

2.3.2 Sensors

A commercial Taste-Sensing System SA 402B (Insent Company, Japan) was applied to test the simulated taste of *Camellia* oil samples. There are two reference sensors and five detecting sensors (Table 4) representing sourness (CA0), bitterness and its aftertaste (C00), astringency and its aftertaste (AE1), saltiness (CT0), umami and richness (AAE). All the sensors were activated for 24 h before of measurement.

2.3.3 Sample analysis

Sensors consists of artificial lipid membranes which are sensitive to different chemical substances and the potential difference between taste sensor and reference sensor is measured and recorded. Each experiment was repeated four times and the later three data profile were selected averaged for subsequent analysis, shown as mean ± standard deviation (SD) in Table 5.

2.4 Statistical analysis

To compare the taste characteristics of *Camellia* seed oil, correlation analysis (Pearson correlation), principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL) at test significant differences ($p<0.05$) (Lin et al., 2020). Estimation of the quality parameters, acid value and peroxide value were also carried out by SPSS 19.0 software using multivariate linear regression technique.

3. Results and Discussion

3.1 Single-Factor Experiments of *Camellia* seed scCO_2 extraction

The loading of crushed *Camellia* seeds and the flow rate of CO_2 were set fixed in the experiments. Varied extraction temperature and pressure were investigated and discussed in detail.

3.1.1 Effect of Extraction Temperature

Rising of the temperature could enhance both the saturated vapor pressure and the diffusivity coefficient of the solute, resulting in better solubility behavior in scCO_2, while at the same time, higher temperature also reduced the density of scCO_2 decreasing the dissolving capacity of scCO_2 (Bogdanovic et al., 2015; Pilavtepe et al., 2012). These two factors have opposing effects on the oil extraction yield, as a result, an optimum temperature could be predicted in theory. Temperature effects are shown in Fig. 1 (a). As the temperature increased, the oil yield decreased rapidly. This indicates that the optimal temperature might appear lower than 313.15 K. Dissolving power of scCO_2 plays an more important role in the extraction process. Higher yield was obtained with longer extraction time.

3.1.2 Effect of Extraction Pressure

As the pressure increased, the density and polarity of scCO_2 also increased which led to stronger dissolving capacity indicating higher extraction yield. Pressure effects are displayed in Fig. 1 (b). The oil yield increased gradually as the pressure increased, and the yield increased less at higher pressure. In a longer-time dynamic extraction process, more oils were extracted and the highest yield 22.60% was achieved at 30 MPa and 8 h. Within the first 4 hours, the oil yield increased fast, and after 4 hours, it enhanced slowly in a upward trend.

3.2 Oil physicochemical characteristics

Lower AV and POV represents better quality of oil. These two indicators of all the oil samples were determined and compared in Table 3. AVs of commercial pressed oils are all less than 1, while for samples extracted by n-hexane and scCO_2, the values are much higher, distributing between 3 and 1. The AV value contributes to distinguishing sourness tastes. SCCE samples were further investigated in Fig. 2. AVs declined obviously as extraction time increased, e.g., from 3.48 mg/g (0-2 h) to 0.64 mg/g (4-6 h) at the condition of 25 MPa and 333.15 K. While for POV, sample extracted by n-hexane was the highest up to 0.493 g/100 g, indicating that solvent reflux process is unfavorable for the oil quality. For SCCE samples, POV were ranging from 0.012 ~ 0.029 g/100g, significantly lower than other oil prepared samples, indicating scCO_2 extraction could be a better preparation method keeping oil quality. After further optimizing the supercritical extraction process, it seems promising to prepare*Camellia* oil of which AV and POV meet the first-grade standard (GBT11765-2018, AV [?] 0.50 mg/g, POV [?] 0.25 g/100g).

3.3 Taste characteristics

3.3.1 E-tongue profiles of*Camellia* oils

Fig. 3 shows the radar chart of taste variations determined by electronic tongue. Pressed and n-hexane extracted oils samples are displayed in Fig. 3 (a), and scCO_2 extracted samples are shown in Fig. 3 (b) (varied temperature) and Fig. 3 (c) (varied pressure). There were no obvious differences in bitterness, astringency, aftertaste-bitterness (aftertaste-B), aftertaste-astringency (aftertaste-A) and saltiness among these samples. However, for sourness, umami and richness, there were significant differences among these oil samples, especially in the SCCE samples. Negative correlations were found between umami and sourness ($R^2 = -0.997$, $p < 0.001$), richness and sourness ($R^2 = -0.912$, $p < 0.001$). E-tongue value of reference tartaric acid solution was set as zero, so oil sourness taste value were all negative. The larger the absolute value, the weaker sourness taste. Sourness taste of three major categories oil samples (in descending order) was: $scCO_2$ extracted oils, n-hexane extracted oil and pressed oils. The highest sourness was found in S13 (-0.99), and the lowest was in S2 (-10.32). For the SCCE samples at 25 MPa and different temperatures, shown in Fig. 3 (b), sourness gradually increased in ascending order: Samples (313.15 K) $<$ Samples (323.15 K) $<$ Samples (333.15 K). While for the SCCE samples at 313.15 K and different pressures (Fig. 3 (c)), sourness was highest in oils at 30 MPa. This may be due to that sourness substances are more easier to be extracted under higher temperature and pressure.

Differences of bitterness and astringency taste responses are relative small. For bitterness, the maximum value was in solvent extracted S6 (-1.05). Pressed oils have the smallest bitterness and astringency among all the samples. Similarly, high temperature (S13-S15) and high pressure (S19-S21) contribute to the astringency taste value.

3.3.2 Discrimination of *Camellia* oil

Principal component analysis (PCA) is mainly used to exhibit a clear visualization of multidimensional taste profiles in a reduced-dimension plot (Zhu et al., 2020). The relative variances of components suggest the relevant importance of the component expressed as percentages in Table 6. In the results from PCA with eigenvalue greater than 1 (Fig. S1 in supplementary material), the first two principal components (PC1 and PC2) accounted for 65.4% and 22.8% of total variance, respectively, with the total cumulative variance contribution for 88.2%. Although the pressed oils were successfully separated from extracted oils, the difference between SCCE samples and n-hexane extracted samples was not obvious. Fig. 4 illustrates three-dimensional score plot (a) and loading plot (b) in PCA for the difference *Camellia* oils by the first three principal components (PC1, PC2 and PC3), with the total cumulative variance contribution for 97.4%. The samples are well clustered and no overlap was observed among the three groups, implying the taste of three groups were different. Pressed oils showed relative narrow spatial distribution, while SCCE samples, exhibited a wide range of positive to negative scores, almost splitting into 3 subgroups along both PC1 and PC2 directions: G1 (S13~S15 and S19~S21), G2 (S10~S12), and G3 (S7~S9 and S16~S18). (Fig. S2 in supplementary material). These three groups well illustrated the $scCO_2$ extraction condition differences among the samples, as G1 represents better solubility of solute in $scCO_2$ due to high temperature (S13~S15), or stronger solvent power due to high pressure (S19~S21); G2 represents medium temperature and pressure (S10~S11); G3 represents relative low temperature and pressure (S7~S9 and S16~S18).

8 kinds of taste loading vectors were shown in Fig. 4 (b). Similar loading vectors suggest redundancy in the potential responses and high loading parameters contribute to discriminating among the samples (Saidi et al., 2018). The umami, richness, saltness, sourness, and astringency were mainly responsible for the oil discrimination in the direction of PC1 , whereas aftertaste-A and Aftertaste-B contributed a lot to the separation along with the direction of PC2, and for PC3, bitterness and astringency play an important role in the classification (Fig. S3 in supplementary material). The pressed, n-hexane extracted and $scCO_2$ extracted samples were mainly differentiated by umami, richness, saltness, aftertaste-A and Aftertaste-B with positive score values along PC1 and PC2. Combined the varied extraction processed, PC2 in general may be responsible for special flavor substances, as pressed and strong $scCO_2$ extraction method offered more abundant flavor chemicals in the oils. These clusters are clearly distinguished from each other (Fig. S2 (a) in supplementary material). To be noteworthy, most PC3 may represent free fatty acid, for the acid values of S6 and S13 were relative large in accordance with their high positive score along PC3. Also, peroxide value of the oil samples may involve in PC1, as their increasing trend is consistent. Not all the data fit these regulations, and the reason is hard to suggest because of the complicated multivariate nature of the score plots.

Thus for the particular dataset, reliable discrimination of oil samples of different extraction method can be illustrated by the e-tongue technique.

3.3.3 Cluster analysis

Hierarchical cluster analysis (HCA) discovered and identified relationships between oil varieties by the distance of taste response of the samples (Zhu et al., 2020). HCA provides insight into the taste profiles by dividing similar samples into groups (clusters) (Liu et al., 2020). In this study, HCA was conducted by Ward's method for aggregation and the squared Euclidean distance as diversity test. Dendrogram of clustered oil samples base on similarities is shown in Fig. 5. The clustering result is almost the same with that of PCA analysis. All the samples were first divided into two main clusters. Cluster I is SCCE samples and Cluster II involved pressed oils and n-hexane extracted oils. $ScCO_2$ conditions related with solvent extraction power contributes a lot to Cluster I. Cluster II was further divided into two sub-clusters, one was consist of pressed oils and the other was n-hexane extracted oil S6, when the number of overall cluster is set to 5 or more, indicating the difference between the pressed and n-hexane extracted samples is less than the SCCE samples of varied conditions. Thus the HCA method was able to distinguish oil samples of different extraction processes base on the e-tongue profiles.

3.4 Correlation between E-tongue analysis and chemical properties

Acid value (AV) and peroxide value (POV) are key parameters to assess *Camellia* oil quality and gradation. The possibility of estimating the AV and POV level based on e-tongue profiles processed with Multi Factor Linear Regression Model (MLRM) was evaluated, applying the AV and POV data of *Camellia* oils produced from different extraction methods determined by titration according to National Standard. The model results were clearly shown in Table 7. Based on 8 kind of potential signals recorded by 5 sensors, which was further selected using stepwise regression method, MLRM models were established with certain ability predicting AV and PV of *Camellia* oils (AV: $R^2 = 0.702$, $p < 0.001$; POV: $R^2 = 0.632$, $p < 0.001$). Lack of the linearity of the models may be due to the inhomogeneity in 1 kg scale $scCO_2$ extraction process which resulted AV and POV fluctuating. Fig. 6 shows "determined value versus predicted value" plot of the MLRM derived for acid value (a) and peroxide value (b). For assessing these physiochemical characterizations using e-tongue technique coupled with chemometrics, satisfactory results were also reported (Rodrigues et al., 2019; Semenov et al., 2019). Although the precision of the models could be further improved, the method still seems attractive for obtaining two key quality values in one measurement. This indicates that e-tongue system would be a promising technique for quantification of *Camellia* oil quality parameters.

4. Conclusions

$ScCO_2$ extracted *Camellia* oils were prepared under various pressure, temperature and period conditions. The oil quality parameter acid value and peroxide value of pressed, n-hexane extracted and $scCO_2$ extracted oils were investigated according to National Standard. An analytical method involving electronic tongue technique combined with qualitative and quantitative analysis was proposed to discriminate oil variety and quantify oil acid value and peroxide value. The PCA and HCA results obtained in this work successfully discriminated oils by different processed methods, even further classification among $scCO_2$ samples were achieved satisfactorily. Furthermore, the use of MLRM has evidenced good predictive capacity of acid value and peroxide value, regardless of different extraction process. More work remains to be done to improve the prediction precision and to validate the suggested approach as only limited samples was determined in this preliminary study. Nevertheless, the practical potential of e-tongue as taste sensor for the successful classification and assessment of *Camellia* oils has been demonstrated, which is promising to be a complementary technique in the characterization of *Camellia* oils in the quality evaluation.

Acknowledgement

The financial supports from the Young Innovative Talents Program of Educational Commission of Guangdong Province (No.2019GKQNCX057), The Innovation Project of Guangdong Colleges and Universities (No.2018GKTSCX034 and No.2019GKTSCX011), Jieyang Science and Technology Project (No.2017xm017), Project of Zhujiang Distinguished Professor of Guangdong Province (No.KYRC2017-001), Talent Program of Guangdong Industry Technical College (No.KYRC2019-008), Innovation and entrepreneurship exquisite education project of Guangdong Industry Technical College (No.2020JZYR010) are gratefully acknowledged.

Conflict of Interest

The authors declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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