New Model for Flavour Quality Evaluation of Soy Sauce

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Abstract

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The soy sauce samples established a model for its flavour quality evaluation. Initially, 39 types of flavour compounds, organic acids and free amino acids in six different types of soy sauce were identified and determined by HS-SPME GC/MS and HPLC. The model was developed based on the principal component analysis method for assessing and ranking of flavour quality of soy sauce. Using the principal component analysis which simplifies complex information, our correlative evaluation model was established, tested by comparing the traditional sensory evaluation method, providing a new methodology for objective evaluation of the flavour quality of soy sauce.

Keywords: headspace solid phase micro-extraction (HS-SPME); gas chromatography mass spectrum (GC/MS); high performance liquid chromatography (HPLC); principal component analysis; sensory evaluation; modelling

Soy sauce is a traditional condiment widely used in China with the annual production of soy sauce around 5 million tons, with the sales value of around 20 billion Yuan Renminbi (Chinese currency). It is made from a mixture of soybeans and wheat using a well-established two-step fermentation process which not only imparts a delicious flavour but also facilitates digestion (Kataoka 2005).

Chinese soy sauce is an essential traditional condiment with more than 3000 years of history in China (Zhang & Tau 2009). The process of soy sauce fermentation production has been based on the enzymatic activities of relevant microorganisms. Various agricultural product-based substrates were hydrolysed and fermented by enzymatic catalysis with suitable microorganisms during the process of soy sauce production. It involves complex multi-step enzymatic conversions with various microbial species to produce traditional soy sauce. Various metabolites are released after microbial autolysis constituting a rich sauce paste.

Soy sauce is a fermented product which includes volatile organic flavour compounds consisting of alcohols, esters, phenols, acids, and heterocyclics. Among them, the flavour compounds, amino acids and organic acids are important indicators of the quality evaluation of soy sauce. The flavour compounds make a critical contribution to the typical flavour of the soy sauce and classification of the type of soy sauce. Many types of flavour compounds were identified which add to the flavour of the soy sauce that can be detected using qualitative and quantitative methodologies. The reasons for the formation of soy sauce flavour is rather complicated, the processing of raw materials contributes to the flavour precursors. Flavour is produced during bacterial fermentation added to the soy sauce production process. The flavour of

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soy sauce has been studied since the 1940s. With the continuing improvement of analytical instruments and technology, the composition of the soy sauce flavour compounds can be identified and quantified. However, there are large numbers of various soy sauce flavour compounds at low concentrations affecting the quality of the soy sauce flavour requiring further investigations. Currently, the sensory evaluation methodology is mainly used to examine the flavour of soy sauce. The flavour components are the main factors determining the quality of the soy sauce. Consequently, further research and analyses of the objective evaluation methods and system of soy sauce flavour quality are extremely important. It also enriches and improves the quality of the evaluation system of soy sauce which has important significance.

Principal component analysis (PCA) is one of the most useful methodologies in applied linear algebra (Yang *et al.* 2009). PCA is frequently used in all forms of analysis from neuroscience to computer graphics because it is a simple, non-parametric method of extracting relevant information from complicated data sets. With minimal additional effort, PCA provides a roadmap to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structure (He *et al.* 2004).

With the development of chemometrics, pattern recognition is used to deal with experimental data in order to solve the problem of classification and qualification. For example, Lee and co-workers assayed fatty acids and three phthalic glycerols of vegetable oil by gas chromatography. The authenticity of sesame oil was identified using the principal component analysis of experimental data of Lee *et al.* (1998). Setuer *et al.* assayed the olefin composition of fruit oil by gas chromatography and infrared spectroscopy. Different varieties of fruit oil were classified by using the principal component analysis of data (Setuer *et al.* 2001). Keiko and Tetsuo (1999) tested various types of soy sauce using infrared spectral properties and pattern recognition.

PCA is an orthogonal transformation that allows the building of more compact linear combinations of data, which is optimal with respect to the mean square error criterion. The new orthogonal basis is composed of vectors known as principal components (Aires *et al.* 2000).

Principal component analysis is a common method of statistical pattern recognition. Principal component analysis uses dimensionality reduction to exclude overlapping chemical data. It makes a few new variables which are linear combinations of original variables as required by the representative data structure of original variables without loss of information. These new variables were arranged by the decreasing order of variance (Aceves-Lara *et al.* 2008). Principal components of large variance contain the maximum amount of information. Generally, variance contribution of the first few principal components was large enough, basically reflecting the original variable information (Rhee *et al.* 2006; Park *et al.* 2010). Thus, most of the information of the original multidimensional space was expressed by low-dimensional space from the first several principal components (Fonville *et al.* 2010; Worden *et al.* 2011).

We propose using principal component analysis to create a soy sauce flavour quality evaluation model. Flavour components of soy sauce samples were analysed by an objective statistical analysis and to find an objective evaluation method better than the traditional sensory evaluation.

MATERIAL AND METHODS

Material. Six soy sauce samples (S1–S6) purchased from the Chinese grocery stores at Wuxi. The soy sauces were prepared from soybeans, wheat, water, and salt without other additives. All were produced by the high salt liquid state fermentation process. The samples, which were representative soy sauce brands from various regions in China, were light brown in colour.

Determination of conventional physical and chemical indicators. Amino nitrogen: formol titration method (Boutry *et al.* 2008); total nitrogen: Kjeldahl method (FOSS-TECATOR Instrument Co., Ltd, Höganäs, Sweden; Model 2300) (Barros *et al.* 2002); total acids: alkali titration method (Erinc *et al.* 2009); soluble saltless solid: drying constant weighing method (Li *et al.* 2010a); reducing sugars: DNS colorimetry method (Baer *et al.* 2010).

Determination of organic acids. For determination of organic acids, 5 ml samples were precipitated by 5 ml of absolute ethanol and settled for 30 min at ambient temperature to remove large peptides and then centrifuged at 10 000 rpm for 10 minutes. The supernatant was filtered through 0.22 µm filter. The 200 µl sample solution was analysed. Organic acids were determined by the Agilent 1200 HPLC under the following conditions: Agilent ZORBAX SB-Aq column (150 × 4.6 mm, 5 μm), column temperature at 30°C, the injection volume 10 μl and the detection wavelength 210 nm, flow rate 0.5 ml/min for the mobile phase containing 0.5% (w/v) acetonitrile, 99.5% (v/v) 0.02M KH_2PO_4 (pH adjusted to 2.0 with phosphoric acid).

Determination of amino acids. For determination of individual free amino acids, 10 ml samples were precipitated by 50 ml of 10% trichloroacetic acid (TCA) for 2 h at ambient temperature to remove large peptides and then centrifuged at 10 000 rpm for 10 minutes. The supernatant was filtered through 0.22 μm filter. The 200 μl sample solution was analysed. Amino acids were determined with the amino acid analyser (Agilent Technologies, Santa Clara, USA)under the following conditions: column at 40°C, injection volume 20 μl, detection wavelength 338 nm, flow rate of the mobile phase 1 ml/minute. Composition of the mobile phase was as follows: A phase: 8.0 g sodium acetate crystals were added into the 1000 ml beaker, then 1000 ml water was added and stirred until all the crystals were dissolved in water. Thereafter, 225 μl triethylamine was added, stirred with pH adjusted to 7.20 ± 0.05 with 5% (w/v) acetic acid and 5 ml tetrahydrofuran was subsequently added. The resulting system was ready for use. B phase: 8.0 g sodium acetate crystals were added into the 800 ml beaker with 400 ml water and stirred until all the crystals were dissolved in water. The pH was adjusted to 7.20 ± 0.05 with 5% (w/v) acetic acid. This solution was added to 800 ml methanol and 800 ml acetonitrile and used after mixing. The gradient elution methodology was used. The linear elution gradient was A:B (by volume) from 100:0 to 50:50 for 0–17 min, 50:50 to 0:100 for 17–20 min, and 100:0 for 20–24 minutes. Each amino acid was identified by comparing the samples with a standard (Sigma-Aldrich Co., St. Louis, USA) analysed under the same conditions and quantified by the calibration curve of the authentic compound.

Volatile flavour compounds collection by SPME. 10 ml of the soy sauce sample was placed into a 15 ml headspace vial and pre-equilibrated for 15 min at 50°C in a thermostatic bath with a vial capped using a silicon septum. Afterwards, a stainless steel needle, in which 85 μm polyacrylate (PA) (Swagelok, Solon, USA) fibre was housed, was pushed through the vial septum. The fibre was pushed out of the housing and exposed to the headspace (3 cm in depth) at 50°C for 40 minutes. After extraction, the fibre was pulled into the housing, and the SPME device was removed from the vial and inserted into the injection port of GC for thermal desorption of the analysis.

Determination of volatile flavour compounds. Gas chromatography tandem mass spectrometry 1200 L GC/MS-MS (Varian Co., Palo Alto, USA) was used. Chromatographic conditions: the column was DB-WAX, 30 m × 0.25 mm×0.25 μm capillary column, carrier gas helium gas, 0.8 ml/min for flow rate. Temperature program: initial temperature at 40°C, maintained for 4 min, heating rate at 6°C/min to 160°C, then heating rate at 10°C/min rate to 220°C and maintained for 6 minutes. Mass spectrometry conditions: interface temperature 250°C, ion source temperature 200°C, ionization mode EI, electron energy 70 eV, detection voltage 350 V, emission current 200 μA. The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliographic data on known compounds from the WILEY 6 library and NIST 98 library (both Hewlett-Packard Co., Palo Alto, USA) mass spectral database on the basis of the criterion similarity (SI) > 800 (the highest value is 1000). According to the method (Wanakhachornkrai & Lertsiri 2003) approximate quantification of volatile compounds was estimated by the external standard of peaks on the total ion chromatogram using Xcalibur software (Vienna, USA). Qualitative analyses were based on retention index and mass spectral data of authentic substances, unless otherwise indicated. Each flavour component was identified by comparing with the analytical grade standards purchased from Sigma-Aldrich Company (St. Louis, USA) under the same conditions and quantified by the calibration curve of the authentic compound. All analyses were performed in duplicate.

Development of index evaluation model. Correlation matrix of principal component analysis was used to analyse the data in different soy sauce samples. Data was evaluated according to the differential linear combination and the contribution rate was obtained by principal component analysis. Specific steps are as follows:

(1) P indexes of m samples were regarded as p random variables, denoted by $X_1, X_2, ..., X_p$, principal component analysis is to change the p indicators for discussion a linear combination of p indexes. These new indicators $F_1, F_2, ..., F_k$ ($k \le p$) according to retain the principles of main

information to entirely reflect the information of original index. These indicators are independent of each other as the first principal component, the second principal component, ... and so on of the original variable, expressed as:

$$F_1 = u_{11}X_1 + u_{21}X_2 + \ldots + u_{p1}X_p$$
$$F_2 = u_{12}X_1 + u_{22}X_2 + \ldots + u_{p2}X_p$$
$$F_p = u_{1p}X_1 + u_{2p}X_2 + \ldots + u_{pp}X_p$$

(2) Each principal component corresponding to the eigen value λ_i (i = 1, 2, ..., k) divides the total characteristic value as weight and linear weighted sum required by the general evaluation index (GEI):

$$GEI = (\lambda_1 F_1 + \lambda_2 F_2 + \ldots + \lambda_i F_i)/(\lambda_1 + \lambda_2 + \ldots + \lambda_i)$$

Finally, according to the GEI value sort the index of related indicators.

Sensory evaluation. Quantitative descriptive analysis was applied for evaluation of the samples, using a 20 cm line scale, by a well-trained panel consisting of 10 trained evaluators. Soy sauce sample (50 ml) was prepared in a disposable plastic cup, covered with a plastic petri dish and served to the panellists at 25°C. The program started with the observation of colour, detection of the flavour and taste of the relish. Quantitative descriptive analysis was performed on all the samples, divided randomly into four sessions involving six samples each. Each sample was coded with a three-digit random number. In each session, samples were presented randomly to each panellist. All samples were evaluated once.

Statistical analysis. To verify the statistical significance of all results, the values of means and standard deviations (SD) were calculated. The results were processed using a one-way analysis of variance (ANOVA). The $P < 0.05$ was adopted as statistically significant. All data are means and standard deviations of three determinations. The software used for analysis was SPSS 17.0 for Windows (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Conventional physical and chemical indicators of different soy sauce samples

The pH values of the six different soy sauce samples were between 4.47 and 4.53. In the soluble saltless solid index, S6 sample concentration was the highest and reached 16.07% (w/v). Total nitrogen contents of S2, S3, S4, and S6 samples were similar and S3 sample was the highest at 1.45% (w/v). Amino nitrogen contents of S4 and S6 were similar, 1.11% (w/v) and 1.12% (w/v), respectively. As shown in Table 1, the total acid content of S6 was the highest at 0.18% (w/v) and reducing sugars were 1.096% (w/v). The total acid content of S1 was the lowest at 0.11% (w/v) and reducing sugars were the highest at 1.57% (w/v). It was rather difficult to determine from the above index analysis which type of soy sauce samples was better. Consequently it was necessary to explore a more suitable evaluation method.

Determination of the results of flavour components in different soy sauce samples

Based on our previous study, volatile flavour compounds were produced and analysed by solid phase micro-extraction mass spectrometry and with the spectra of six different soy sauce samples and 39 types of major volatile flavour compounds in fixed concentrations (Table 2). Different soy sauce samples contain alcohols, phenols, esters, aldehydes, heterocyclics which are volatile flavour components.

Table 1. Conventional physical and chemical indicators in different soy sauce samples (S1–S6)

	S1	S2	S3	S4	S5	S6
pH	4.49 ± 0.15	4.50 ± 0.15	4.53 ± 0.18	4.500 ± 0.14	4.51 ± 0.12	4.47 ± 0.13
Soluble saltless solid (%)	12.212 ± 0.250	14.807 ± 0.260	13.826 ± 0.230	12.604 ± 0.240	14.503 ± 0.250	16.074 ± 0.220
Total nitrogen (%)	1.350 ± 0.05	1.560 ± 0.03	1.573 ± 0.04	1.560 ± 0.07	1.464 ± 0.06	1.550 ± 0.07
Amino nitrogen (%)	0.750 ± 0.002	0.940 ± 0.003	0.910 ± 0.002	1.150 ± 0.004	1.040 ± 0.005	1.160 ± 0.003
Total acid (%)	0.114 ± 0.004	0.160 ± 0.007	0.141 ± 0.005	0.170 ± 0.006	0.177 ± 0.005	0.180 ± 0.006
Reducing sugars (%)	1.572 ± 0.045	1.117 ± 0.040	1.005 ± 0.035	0.914 ± 0.035	0.914 ± 0.030	1.096 ± 0.040

Each value is presented as mean ± standard deviation (n = 3)

Table 2. Comparison of volatile flavour components (mg/l) in soy sauce samples (S1–S6)

	Name	S1	S2	S3	S4	S5	S6
Alcohols	alcohol	101.5 ± 0.2	261.8 ± 0.6	259.3 ± 0.4	288.0 ± 0.5	141.2 ± 0.3	269.6 ± 0.5
	isopropyl alcohol	0.087 ± 0.003	0.025 ± 0.001	0.031 ± 0.002	0.019 ± 0.001	0.069 ± 0.002	0.028 ± 0.001
	propanol	0.049 ± 0.002	0.031 ± 0.001	0.031 ± 0.001	0.022 ± 0.001	0.025 ± 0.003	0.026 ± 0.002
	isobutyl alcohol	0.227 ± 0.008	3.025 ± 0.012	5.211 ± 0.023	0.018 ± 0.002	0.005 ± 0.001	1.914 ± 0.006
	butyl alcohol	0.090 ± 0.003	0.026 ± 0.001	0.278 ± 0.002	0.007 ± 0.001	0.011 ± 0.001	0.010 ± 0.001
	isoamyl alcohol	0.049 ± 0.002	0.043 ± 0.002	0.005 ± 0.001	0.005 ± 0.001	0.281 ± 0.003	0.008 ± 0.001
	hexanol	1.205 ± 0.010	1.492 ± 0.010	1.334 ± 0.013	0.012 ± 0.008	0.004 ± 0.001	1.364 ± 0.011
	β-mercaptoethanol	0.088 ± 0.001	0.145 ± 0.003	0.000 ± 0.000	0.112 ± 0.002	0.000 ± 0.000	0.044 ± 0.001
	1-octanol	0.856 ± 0.011	1.089 ± 0.013	3.501 ± 0.108	10.186 ± 0.300	2.118 ± 0.030	2.491 ± 0.040
	furfuryl alcohol	34.062 ± 0.900	30.487 ± 0.800	31.536 ± 0.600	29.523 ± 0.300	28.933 ± 0.400	29.815 ± 0.500
	3-methylthiopropanol	11.074 ± 0.200	14.028 ± 0.200	13.962 ± 0.400	13.059 ± 0.400	14.730 ± 0.300	15.498 ± 0.300
	benzyl alcohol	6.381 ± 0.060	6.470 ± 0.050	6.773 ± 0.050	5.868 ± 0.060	5.617 ± 0.070	6.053 ± 0.040
	2-phenylethanol	37.615 ± 0.800	62.323 ± 1.200	63.808 ± 1.500	63.869 ± 1.300	63.360 ± 1.200	67.283 ± 1.100
Phenols	2-methoxyphenol	11.252 ± 0.100	9.485 ± 0.200	10.466 ± 0.200	9.883 ± 0.100	8.993 ± 0.100	8.829 ± 0.100
	phenol	9.398 ± 0.200	9.952 ± 0.300	9.964 ± 0.300	10.761 ± 0.600	10.188 ± 0.600	10.683 ± 0.500
	4-ethylphenol	3.299 ± 0.030	3.063 ± 0.030	3.164 ± 0.020	3.105 ± 0.050	2.704 ± 0.040	2.106 ± 0.020
	4-ethyl-2-methoxyphenol	120.2 ± 1.0	123.8 ± 1.2	104.1 ± 1.0	85.96 ± 0.8	90.44 ± 0.8	103.2 ± 0.9
Esters	ethyl acetate	0.012 ± 0.001	0.000 ± 0.001	0.007 ± 0.001	0.000 ± 0.000	0.014 ± 0.002	0.005 ± 0.001
	isopropenyl acetate	16.227 ± 0.300	0.000 ± 0.000	56.632 ± 1.100	31.407 ± 1.000	30.630 ± 0.900	10.391 ± 0.700
	butyl formate	0.035 ± 0.005	0.025 ± 0.001	0.023 ± 0.002	9.288 ± 0.006	0.000 ± 0.000	0.006 ± 0.001
	ethyl lactate	0.035 ± 0.004	0.048 ± 0.003	0.000 ± 0.000	0.009 ± 0.001	0.354 ± 0.005	0.000 ± 0.000
	methyl glycolate	0.048 ± 0.006	0.000 ± 0.000	0.000 ± 0.000	2.923 ± 0.500	0.018 ± 0.006	2.536 ± 0.200
	phenethyl acetate	1.557 ± 0.030	1.030 ± 0.020	1.553 ± 0.040	2.747 ± 0.060	2.036 ± 0.060	1.099 ± 0.030
	ethyl myristate	0.000 ± 0.000	0.000 ± 0.000	0.588 ± 0.006	4.175 ± 0.100	0.597 ± 0.008	0.494 ± 0.006
	palmitic acid ethyl ester	0.053 ± 0.006	0.455 ± 0.010	0.830 ± 0.030	3.872 ± 0.090	0.810 ± 0.040	0.485 ± 0.020
	diisobutyl phthalate	1.516 ± 0.040	1.833 ± 0.060	1.539 ± 0.080	2.002 ± 0.090	1.043 ± 0.060	0.700 ± 0.002
Aldehydes	2-methylbutyraldehyde	0.058 ± 0.010	0.005 ± 0.001	0.006 ± 0.001	0.000 ± 0.000	0.031 ± 0.002	0.014 ± 0.003
	2-methyl-2-butenal	0.022 ± 0.005	0.016 ± 0.002	0.007 ± 0.003	6.213 ± 0.200	0.011 ± 0.003	0.017 ± 0.004
	octanal	0.010 ± 0.001	0.028 ± 0.002	4.540 ± 0.080	34.715 ± 1.000	0.005 ± 0.001	2.226 ± 0.400
	n-nonaldehyde	3.134 ± 0.090	0.000 ± 0.000	7.434 ± 0.100	7.028 ± 0.100	2.124 ± 0.090	3.159 ± 0.080
	furfural	11.916 ± 0.400	9.565 ± 0.200	8.274 ± 0.200	9.263 ± 0.300	0.014 ± 0.003	9.125 ± 0.400
	decanal	1.067 ± 0.200	0.168 ± 0.006	5.330 ± 0.500	17.723 ± 0.900	1.653 ± 0.400	0.370 ± 0.008
	benzaldehyde	7.583 ± 0.100	7.172 ± 0.100	8.301 ± 0.200	19.133 ± 0.700	12.062 ± 0.700	6.444 ± 0.100
Heterocycles	2-methylpyrazine	10.168 ± 0.300	7.658 ± 0.100	7.996 ± 0.100	7.322 ± 0.100	4.066 ± 0.080	6.270 ± 0.100
	hydroxyacetone	0.000 ± 0.000	0.018 ± 0.002	0.007 ± 0.001	0.022 ± 0.001	0.004 ± 0.001	0.000 ± 0.000
	2-ethylpiperazine	0.911 ± 0.020	0.926 ± 0.080	1.165 ± 0.070	1.295 ± 0.060	0.000 ± 0.000	0.869 ± 0.080
	2-acetyl furan	2.157 ± 0.090	1.948 ± 0.090	2.223 ± 0.080	2.841 ± 0.080	1.602 ± 0.070	1.564 ± 0.070
	HDMF[a]	0.340 ± 0.010	0.310 ± 0.010	0.003 ± 0.001	0.002 ± 0.001	0.271 ± 0.010	0.258 ± 0.020
	HEMF[b]	0.675 ± 0.030	0.443 ± 0.020	0.284 ± 0.010	0.173 ± 0.008	0.198 ± 0.010	0.415 ± 0.020

[a]HDMF: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; [b]HEMF: 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone; each value is presented as mean ± standard deviation (n = 3)

Table 3. Concentrations of the principal volatile flavour compounds in different soy sauce samples by Z-Score normalisation

	Name	ZS1	ZS2	ZS3	ZS4	ZS5	ZS6
Alcohols	alcohol	–1.517	0.531	0.499	0.866	–1.009	0.631
	isopropyl alcohol	1.565	–0.649	–0.441	–0.879	0.936	–0.533
	propanol	1.903	0.036	–0.002	–0.922	–0.589	–0.426
	isobutyl alcohol	–0.718	0.616	1.658	–0.818	–0.824	0.086
	butyl alcohol	0.186	–0.417	1.950	–0.601	–0.555	–0.564
	isoamyl alcohol	–0.151	–0.204	–0.563	–0.556	2.007	–0.531
	hexanol	0.434	0.845	0.619	–1.274	–1.286	0.661
	β-mercaptoethanol	0.386	1.339	–1.079	0.781	–1.079	–0.349
	1-octanol	–0.725	–0.658	0.037	1.961	–0.361	–0.254
	furfuryl alcohol	1.792	–0.128	0.435	–0.646	–0.963	–0.489
	3-methylthiopropanol	–1.728	0.197	0.155	–0.434	0.655	1.155
	benzyl alcohol	0.440	0.648	1.362	–0.766	–1.355	–0.330
	2-phenylethanol	–2.017	0.239	0.374	0.380	0.333	0.691
Phenols	2-methoxyphenol	1.555	–0.362	0.703	0.070	–0.894	–1.073
	phenol	–1.492	–0.404	–0.379	1.185	0.059	1.030
	4-ethylphenol	0.892	0.356	0.585	0.451	–0.462	–1.822
	4-ethyl-2-methoxyphenol	1.019	1.261	–0.034	–1.224	–0.930	–0.092
Esters	ethyl acetate	0.925	–1.074	0.074	–1.074	1.340	–0.191
	isopropenyl acetate	–0.457	–0.592	–0.122	2.014	–0.338	–0.506
	butyl formate	–0.404	–0.406	–0.407	2.041	–0.413	–0.411
	ethyl lactate	–0.285	–0.193	–0.536	–0.471	2.021	–0.536
	methyl glycolate	–0.621	–0.655	–0.655	1.423	–0.642	1.149
	phenethyl acetate	–0.177	–0.998	–0.182	1.679	0.570	–0.891
	ethyl myristate	–0.613	–0.613	–0.244	2.010	–0.238	–0.303
	palmitic acid ethyl ester	–0.739	–0.451	–0.182	1.999	–0.197	–0.429
	diisobutyl phthalate	0.157	0.808	0.205	1.154	–0.811	–1.513
Aldehydes	2-methylbutyraldehyde	1.782	–0.642	–0.584	–0.852	0.536	–0.240
	2-methyl-2-butenal	–0.405	–0.408	–0.411	2.041	–0.410	–0.407
	octanal	–0.503	–0.502	–0.173	2.024	–0.503	–0.342
	n-nonaldehyde	–0.235	–1.320	1.253	1.113	–0.585	–0.226
	furfural	0.946	0.374	0.060	0.301	–1.949	0.267
	decanal	–0.488	–0.620	0.139	1.962	–0.402	–0.591
	benzaldehyde	–0.524	–0.609	–0.375	1.864	0.402	–0.759
Heterocycles	2-methylpyrazine	1.449	0.204	0.372	0.037	–1.577	–0.484
	hydroxyacetone	–0.917	0.989	–0.135	1.449	–0.469	–0.917
	2-ethylpiperazine	0.110	0.143	0.671	0.956	–1.898	0.018
	2-acetyl furan	0.215	–0.229	0.353	1.664	–0.961	–1.042
	HDMF[a]	0.931	0.730	–1.265	–1.271	0.482	0.393
	HEMF[b]	1.654	0.418	–0.431	–1.021	–0.889	0.269

[a]HDMF: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; [b]HEMF: 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone

All contain 13 types of alcohols, 4 types of phenols, 9 types of esters, 7 types of aldehydes and 6 types of heterocyclics, a combined total of 39 types.

Principal component analysis and evaluation results of volatile flavour compounds in different soy sauce samples

In the principal component analysis, in order to eliminate the influence of dimensional data types and size of the absolute value, raw data need to be standardised and weighted. Indicators of the original volatile flavour compounds were standardised by Z-Score and the standardised sample correlation matrix is shown in Table 3, which were coded as ZS1, ZS2, ZS3, ZS4, ZS5, and ZS6 to express the six different soy sauce samples which were standardised.

The indexes of the content of the volatile flavour compounds for principal component analysis by SPSS 17.0 software and correlation matrix characteristic value and feature vectors are shown in Tables 4 and 5, respectively.

Extraction principle of the number of principal components is the principal component corresponding to the characteristic value of the first *m* principal components which are higher than one. Eigen values can be considered in an index that affects the intensity of the principal components. If the characteristic value is lower than one, it explains that the explanatory power of principal components is lower than the average explanatory power of the original variables. Generally, if eigen value is higher than one, it can be used as an inclusion criterion. As shown in Table 4, the first five eigen values were higher than one. Therefore, the number of principal components can be identified as five. The accumulated variance contribution rate of the first five principal components reached 100% of the original information with no information loss. It met the requirement of statistical analysis.

As shown in Table 5, the first five principal components were in linear relationship with the original 39 indicators. Therefore, the five new variables replaced the original 39 variables. The flavour components of soy sauce were classified according to the principal component analysis. However, it cannot fix the superiority and inferiority of soy sauce samples through the numbers of flavour components. Therefore, it is essential to construct the general evaluation index of volatile flavour compounds to evaluate the quality of soy sauce samples. Each principal component corresponding to the eigen value divided the total characteristic value as weight and linear weighted sum required by the general evaluation index.

Principal component analysis was used to construct indices of volatile flavour compounds for six different soy sauce samples and the evaluation results are shown in Figure 1. It is apparent from Figure 1 that the value of the general evaluation index of volatile flavour compounds in various soy sauce samples was significantly different. According to the data from the general evaluation index analysis of volatile flavour compounds, the volatile flavour compounds index from the superior to the inferior was S4, S3, S2, S1, S6, S5, respectively.

Evaluation results of free amino acids and organic acids in different soy sauce samples

The HPLC method was used to produce and analyse free amino acids and organic acids of six different soy sauce samples with fixed concentration (Tables 6 and 7). As shown in Table 6, the soy sauce samples contain 17 types of free amino acids because different soy sauce samples contain different free amino acids. As shown in Table 7, different soy sauce samples contain 10 types of organic acids: oxalic acid, tartaric acid, formic acid, pyruvic acid, malic acid, lactic acid,

Table 4. Explanation of total variance of volatile flavour compounds in different soy sauce samples

Component	Extraction sums of squared loadings		
	total	variance (%)	cumulative (%)
1	16.608	42.583	42.583
2	10.112	25.928	68.511
3	6.547	16.787	85.299
4	3.437	8.812	94.111
5	2.297	5.889	100.000

Table 5. Component matrix of volatile flavour compounds in different soy sauce samples

Name	Component				
	1	2	3	4	5
Alcohol	0.632	0.086	–0.769	–0.025	0.035
Isopropyl alcohol	–0.640	–0.066	0.756	0.049	–0.111
Propanol	–0.713	0.595	0.347	–0.068	–0.115
Isobutyl alcohol	–0.186	0.271	–0.761	0.522	0.201
Butyl alcohol	–0.209	0.448	–0.189	0.848	0.007
Isoamyl alcohol	–0.236	–0.759	0.512	0.096	0.311
Hexanol	–0.610	0.461	–0.637	–0.054	–0.079
β-Mercaptoethanol	0.194	0.514	–0.037	–0.769	0.325
1-Octanol	0.978	0.117	0.115	0.059	–0.117
Furfuryl alcohol	–0.556	0.766	0.260	0.092	–0.170
3-Methylthiopropanol	0.108	–0.750	–0.648	0.076	–0.012
Benzyl alcohol	–0.369	0.728	–0.448	0.335	0.147
2-Phenylethanol	0.508	–0.552	–0.633	0.145	0.124
2-Methoxyphenol	–0.198	0.841	0.418	0.277	–0.046
Phenol	0.759	–0.452	–0.338	–0.161	–0.282
4-Ethylphenol	0.044	0.710	0.433	0.240	0.498
4-Ethyl-2-methoxyphenol	–0.727	0.522	–0.240	–0.298	0.227
Ethyl acetate	–0.585	–0.369	0.585	0.365	–0.216
Isopropenyl acetate	0.946	0.190	0.255	–0.002	–0.055
Butyl formate	0.935	0.196	0.243	–0.164	–0.045
Ethyl lactate	–0.177	–0.781	0.487	0.109	0.331
Methyl glycolate	0.717	–0.070	–0.180	–0.356	–0.568
Phenethyl acetate	0.761	–0.063	0.623	0.172	0.000
Ethyl myristate	0.968	0.081	0.211	–0.048	–0.095
Palmitic acid ethyl ester	0.981	0.072	0.179	–0.029	0.010
Diisobutyl phthalate	0.443	0.669	0.166	–0.046	0.571
2-Methylbutyraldehyde	–0.666	0.063	0.690	–0.086	–0.261
2-Methyl-2-butenal	0.935	0.193	0.243	–0.166	–0.046
Octanal	0.955	0.215	0.170	–0.068	–0.088
n-Nonaldehyde	0.597	0.324	0.049	0.634	–0.366
Furfural	–0.030	0.879	–0.237	–0.313	–0.268
Decanal	0.939	0.240	0.218	0.110	–0.036
Benzaldehyde	0.881	–0.082	0.456	0.010	0.094
2-Methylpyrazine	–0.216	0.965	0.080	–0.065	–0.108
Hydroxyacetone	0.734	0.240	–0.116	–0.198	0.593
2-Ethylpiperazine	0.416	0.821	–0.354	0.001	–0.170
2-Acetyl furan	0.690	0.664	0.257	0.099	0.091
HDMF[a]	–0.762	–0.211	0.151	–0.591	0.055
HEMF[b]	–0.734	0.518	0.061	–0.381	–0.210

[a]HDMF: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; [b]HEMF: 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone

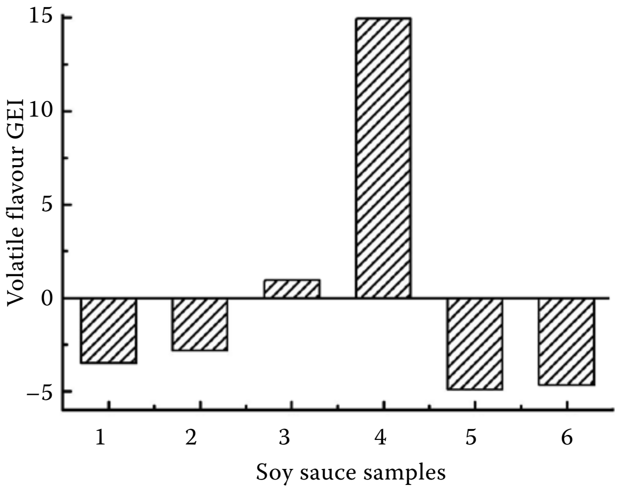

Figure 1. General evaluation index (GEI) of volatile flavour components in different soy sauce samples

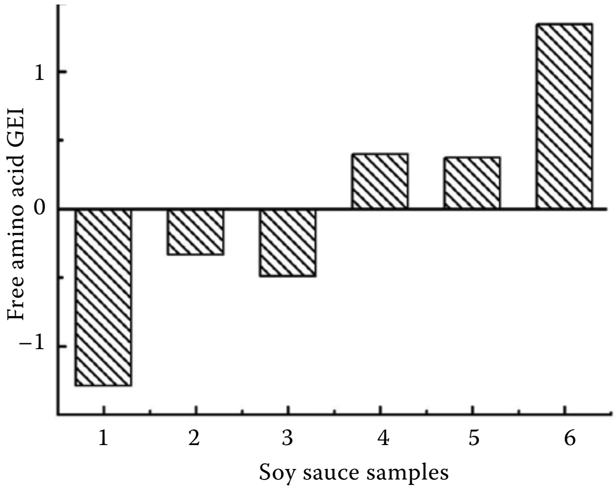

Figure 2. General evaluation index of free amino acids in different soy sauce samples

α-ketoglutarate, citric acid, succinic acid and propionic acid because different soy sauce samples contain different organic acids.

Indicators of the original free amino acids and organic acids were standardised using Z-Score. The indexes of the content of free amino acids and organic acids for principal component analysis were analysed by the SPSS 17.0 software. Finally, each principal component corresponding to eigen value divided the total characteristic value as weight and linear weighted sum required by the general evaluation index of free amino acids and organic acids (Figures 2 and 3). It is apparent from Figures 2 and 3 that the values of the general evaluation index of free amino acids and organic acids in different soy sauce samples were significantly different. The analysis of data from the general evaluation index analysis of free amino acids revealed that the free amino acids index from the superior to the inferior was S6, S4, S5, S2, S3, S1, respectively. The analysis of data from the general evaluation index analysis of organic acids indicated that the organic acids index from the superior to the inferior was S1, S6, S5, S2, S3, S4, respectively. The above analyses show that three types of indicators to determine the superiority of the general evaluation index of soy sauce are different. Therefore, in order to obtain the reliable result, it is necessary to consider the weight relationship of the three evaluation indexes.

Total evaluation results of volatile flavour compounds, free amino acids and organic acids in different soy sauce samples

Indicators of the original volatile flavour compounds, free amino acids and organic acids were

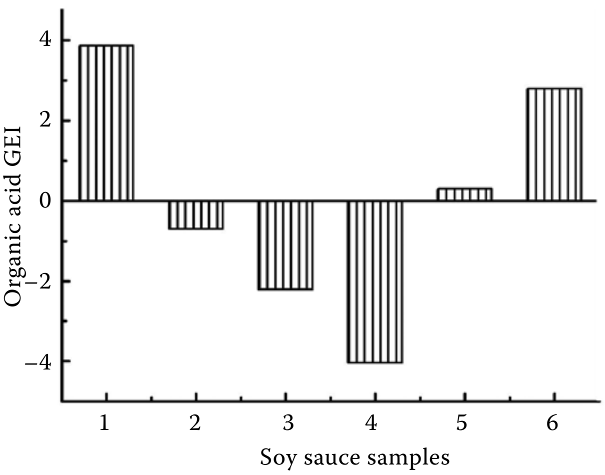

Figure 3. General evaluation index of organic acids in different soy sauce samples

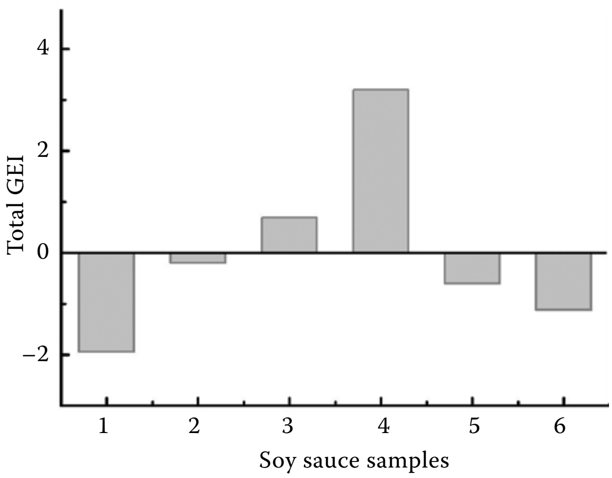

Figure 4. Total general evaluation index in different soy sauce samples

Table 6. Comparison of free amino acids (in g/l) in different soy sauce samples (S1–S6)

Name	S1	S2	S3	S4	S5	S6
Asp	3.398 ± 0.100	4.271 ± 0.150	4.144 ± 0.160	4.875 ± 0.130	4.567 ± 0.140	5.467 ± 0.130
Glu	4.618 ± 0.170	5.762 ± 0.190	5.580 ± 0.190	6.530 ± 0.210	6.203 ± 0.200	7.373 ± 0.250
Ser	1.081 ± 0.050	1.319 ± 0.070	1.308 ± 0.070	1.598 ± 0.060	1.439 ± 0.060	1.772 ± 0.080
His	0.472 ± 0.003	0.611 ± 0.004	0.583 ± 0.003	0.684 ± 0.006	0.611 ± 0.006	0.782 ± 0.008
Gly	2.901 ± 0.080	3.558 ± 0.110	3.443 ± 0.110	4.015 ± 0.120	3.811 ± 0.120	4.520 ± 0.140
Thr	1.726 ± 0.080	2.174 ± 0.090	2.096 ± 0.090	2.443 ± 0.090	2.333 ± 0.080	2.800 ± 0.080
Arg	1.149 ± 0.040	1.422 ± 0.060	1.367 ± 0.060	1.600 ± 0.080	1.538 ± 0.060	1.824 ± 0.090
Ala	2.760 ± 0.090	3.408 ± 0.090	3.298 ± 0.100	3.869 ± 0.110	3.685 ± 0.130	4.391 ± 0.160
Tyr	1.223 ± 0.030	1.525 ± 0.030	1.480 ± 0.050	1.724 ± 0.070	1.643 ± 0.070	1.924 ± 0.060
Cys	0.012 ± 0.001	0.017 ± 0.001	0.015 ± 0.001	0.016 ± 0.001	0.042 ± 0.001	0.023 ± 0.001
Val	2.743 ± 0.090	3.402 ± 0.110	3.281 ± 0.110	3.835 ± 0.120	3.668 ± 0.120	4.397 ± 0.140
Met	0.303 ± 0.002	0.400 ± 0.002	0.385 ± 0.002	0.460 ± 0.003	0.433 ± 0.003	0.536 ± 0.003
Phe	1.952 ± 0.060	2.427 ± 0.080	2.346 ± 0.080	2.660 ± 0.080	2.608 ± 0.080	3.130 ± 0.080
Ile	1.783 ± 0.060	2.206 ± 0.070	2.130 ± 0.070	2.488 ± 0.050	2.338 ± 0.090	2.835 ± 0.080
Leu	2.879 ± 0.100	3.514 ± 0.120	3.855 ± 0.140	3.947 ± 0.140	3.776 ± 0.130	5.123 ± 0.150
Lys	1.417 ± 0.070	1.716 ± 0.070	1.650 ± 0.050	1.932 ± 0.060	1.883 ± 0.060	2.197 ± 0.090
Pro	2.600 ± 0.110	1.961 ± 0.100	1.935 ± 0.110	2.252 ± 0.130	2.899 ± 0.140	2.526 ± 0.120
Total (g/l)	33.019 ± 1.136	39.694 ± 1.347	38.897 ± 1.396	44.927 ± 1.460	43.478 ± 1.490	51.621 ± 1.662

Each value is presented as mean ± standard deviation (n = 3)

standardised using the Z-Score again. The indexes of the content of volatile flavour compounds, free amino acids and organic acids for principal component analysis were analysed by the SPSS 17.0 software. Finally, each principal component corresponding to the eigen value divided the total characteristic value as weight and linear weighted sum required by the total general evaluation index (Figure 4). It is apparent from Figure 4 that the data from the general evaluation index in various soy sauce samples were significantly different. The analysis of data from the general evaluation index

Table 7. Comparison of organic acids (in g/l) in different soy sauce samples (S1–S6)

Name	S1	S2	S3	S4	S5	S6
Oxalic acid	0.133 ± 0.006	0.118 ± 0.004	0.121 ± 0.004	0.106 ± 0.002	0.119 ± 0.002	0.121 ± 0.005
Tartaric acid	0.188 ± 0.008	0.183 ± 0.009	0.174 ± 0.008	0.177 ± 0.008	0.196 ± 0.009	0.203 ± 0.011
Formic acid	0.613 ± 0.020	0.553 ± 0.020	0.533 ± 0.018	0.504 ± 0.018	0.552 ± 0.016	0.572 ± 0.014
Pyruvic acid	0.069 ± 0.002	0.068 ± 0.002	0.066 ± 0.002	0.065 ± 0.002	0.072 ± 0.003	0.071 ± 0.003
Malic acid	1.661 ± 0.010	0.860 ± 0.012	0.755 ± 0.012	1.426 ± 0.011	1.425 ± 0.013	1.187 ± 0.010
Lactic acid	0.000 ± 0.000	1.688 ± 0.015	1.183 ± 0.012	0.000 ± 0.000	0.000 ± 0.000	1.705 ± 0.020
α-Ketoglutarate	0.952 ± 0.009	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.397 ± 0.015	0.512 ± 0.018
Citric acid	0.000 ± 0.000	0.978 ± 0.014	0.987 ± 0.013	0.889 ± 0.010	1.133 ± 0.018	0.841 ± 0.011
Succinic acid	2.434 ± 0.100	1.964 ± 0.080	1.808 ± 0.080	1.624 ± 0.060	1.595 ± 0.060	1.580 ± 0.060
Propionic acid	3.028 ± 0.130	2.856 ± 0.110	2.705 ± 0.110	2.568 ± 0.120	2.848 ± 0.150	3.403 ± 0.200
Total	9.079 ± 0.285	9.269 ± 0.266	8.331 ± 0.259	7.358 ± 0.231	8.336 ± 0.286	10.196 ± 0.352

Each value is presented as mean ± standard deviation (n = 3)

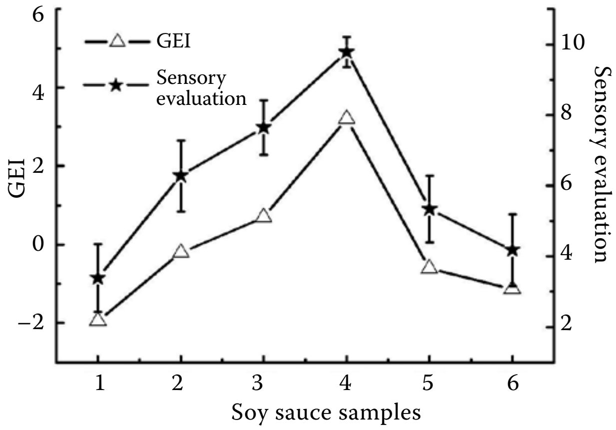

Figure 5. Comparison with total general evaluation index and sensory evaluation score in different soy sauce samples

revealed that the total index from the superior to the inferior was S4, S3, S2, S5, S6, S1, respectively.

Model validation

In order to test the flavour quality evaluation of soy sauce and the general evaluation index, the traditional sensory evaluation method comparing the flavour quality evaluation of soy sauce based on the results of general evaluation was adopted. Initially, sensory evaluation was conducted by 10 trained evaluators using coded samples. The sensory values were determined based on the sensory evaluation of the average value of the same solution by various evaluators. Observations of the colour, then the smell of the flavour and subsequently the taste of the relish were done according to the program. Weighted distribution of soy sauce sensory attributes (Liu 2004; Li *et al.* 2010a,b) and soy sauce sensory evaluation by quantitative descriptive analysis are shown in Tables 8 and 9, respectively.

Table 8. Weighted distribution of soy sauce sensory attributes

Item	Characteristic	Colour and lustre	Aroma	Taste
Weight (%)	20	20	30	30

Table 9. Soy sauce sensory evaluation by quantitative descriptive analysis

Score	Characteristic	Colour and lustre	Aroma	Taste
10	very strong, clear, non-suspended solids and sediment	reddish-brown or shallow reddish brown, the colour is very bright, very shiny	very rich sauce flavour and ester fragrant	very delicious, very mellow, salt and sweet is delicious flavour
9	relatively strong, clear, non-suspended solids and sediment	reddish-brown or shallow reddish-brown, the colour is very bright, good gloss	rich sauce flavour and ester fragrant	very delicious, mellow, salt and sweet is delicious flavour
8	strong, clear, non-suspended solids and sediment	reddish-brown or shallow reddish-brown, the colour is very bright, relative shiny	relatively rich sauce flavour and ester fragrant	delicious, mellow, salt and sweet is delicious flavour
7	light strong, clear, non-suspended solids and sediment	reddish-brown or shallow reddish-brown, the colour is very bright, shiny general	sauce flavour and ester fragrant	delicious, relative mellow, salt and sweet is delicious flavour
6	dilute, clear, non-suspended solids and sediment	reddish-brown or shallow reddish brown, the colour is very bright, dull	rich sauce flavour and no ester fragrant	relatively delicious, relatively mellow, salt and sweet is delicious flavour
5	relatively dilute, clear, non-suspended solids and sediment	reddish-brown or shallow reddish brown, the colour is bright	relatively rich sauce flavour and no ester fragrant	delicious, salt and sweet is delicious flavour
4	very dilute, clear, non-suspended solids and sediment	reddish-brown or shallow reddish brown, the colour is relatively bright	sauce flavour and no ester fragrant	delicious, salt and sweet is relative delicious flavour
3	very dilute, relatively clear, non-suspended solids and sediment	reddish-brown or shallow reddish-brown, the colour is not bright	light sauce flavour and no ester fragrant	umami and salt is delicious flavour
2	very dilute, relatively turbid	light reddish-brown or very light shallow reddish-brown	unscented	umami and salt is relatively delicious flavour
1	suspended solids or sediment	dark reddish-brown	bad smell	odour, discomfort

Table 10. Sensory score in different soy sauce samples (S1–S6)

Sample name	Group	Characteristic	Colour and lustre	Aroma	Taste	Total score
S1	1	3	3	4	4	14
	2	3	5	3	4	15
	3	4	3	5	3	15
	4	3	3	3	5	14
	5	3	2	2	5	12
	6	3	3	3	3	12
	7	5	5	4	2	16
	8	3	3	3	3	12
	9	2	4	5	2	13
	10	3	3	3	3	12
Average score		3.2	3.4	3.5	3.4	13.5
S2	1	7	6	5	6	24
	2	6	6	8	7	27
	3	7	5	8	7	27
	4	7	6	6	6	25
	5	7	8	6	6	27
	6	6	6	7	5	24
	7	6	7	5	6	24
	8	5	5	7	5	22
	9	6	7	5	8	26
	10	8	7	6	5	26
Average score		6.5	6.3	6.3	6.1	25.2
S3	1	8	8	7	8	31
	2	7	8	7	8	30
	3	9	7	8	9	33
	4	8	7	8	9	32
	5	7	8	6	7	28
	6	8	9	7	8	32
	7	6	6	7	8	27
	8	7	8	8	9	32
	9	8	7	8	8	31
	10	7	7	8	7	29
Average score		7.5	7.5	7.4	8.1	30.5
S4	1	10	9	10	10	39
	2	9	10	10	10	39
	3	10	10	9	10	39
	4	9	10	10	10	39
	5	10	10	9	9	38
	6	10	9	10	9	38
	7	10	10	10	10	40
	8	9	10	10	10	39
	9	10	10	10	10	40
	10	10	10	10	10	40
Average score		9.7	9.8	9.8	9.8	39.1
S5	1	5	5	4	5	19
	2	4	5	4	6	19
	3	5	5	5	5	20
	4	5	6	4	7	22

Table 10 to be continued

Sample name	Group	Characteristic	Colour and lustre	Aroma	Taste	Total score
	5	6	7	6	5	24
	6	6	4	5	6	21
S5	7	5	4	5	8	22
	8	4	6	7	6	23
	9	4	6	6	5	21
	10	4	6	5	6	21
Average score		4.8	5.4	5.1	5.9	21.2
	1	4	3	5	6	18
	2	4	3	5	5	17
	3	5	3	3	6	17
	4	3	4	3	3	13
S6	5	3	4	3	4	14
	6	4	6	4	3	17
	7	3	5	4	4	16
	8	3	3	5	5	16
	9	5	4	6	4	19
	10	4	4	4	6	18
Average score		3.8	3.9	4.2	4.6	16.5

According to the indicators of fermented soy sauce, the experiment utilised the point system to evaluate soy sauce. The results of sensory evaluation for soy sauce samples are shown in Table 10. It is apparent from Table 10 that sensory evaluation scores in various soy sauce samples were significantly different. The analysis of the sensory evaluation scores showed that the index from the superior to the inferior was S4, S3, S2, S5, S6, S1, respectively.

Comprehensive weighted score and comparison with the total evaluation results are shown in Figure 5. These results were consistent with the conventional sensory evaluation which indicates that the model is acceptable and very useful for assessing the flavour quality of the soy sauce.

CONCLUSIONS

On the basis of our studies of the Chinese traditional soy sauce, the model for flavour quality evaluation of soy sauce was established. Initially, 39 types of flavour compounds in six different types of soy sauce samples were identified and determined by headspace solid phase micro-extraction gas chromatography-mass spectrometry. Organic acids and free amino acids were analysed by the HPLC method. The model was based on the principal component analysis method for assessing and ranking of the flavour quality of the soy sauce. It has been proved to be very useful for the evaluation of the flavour quality model for six types of soy sauce. Our results show that S4 sample is the best, followed by S3, S2, S6, S5, S1, which are completely consistent with the established method.

The evaluation model of soy sauce flavour quality based on principal component analysis, by comparing the traditional sensory evaluation method to further test the model showed that both methods have good consistency, substantiating that the proposed method is feasible. Consequently, in the flavour quality evaluation of the soy sauce, using principal component analysis which simplifies the complex information as well as our correlative evaluation model has been formulated, thus providing a new methodology for the objective evaluation of the flavour quality of soy sauce.

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