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Investigating the Variation of Volatile Compound Composition in Maotai-Flavoured Liquor During Its Multiple Fermentation Steps Using Statistical Methods

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Summary

The use of multiple fermentations is one of the most specific characteristics of Maotai-flavoured liquor production. In this research, the variation of volatile composition of Maotai-flavoured liquor during its multiple fermentations is investigated using statistical approaches. Cluster analysis shows that the obtained samples are grouped mainly according to the fermentation steps rather than the distillery they originate from, and the samples from the first two fermentation steps show the greatest difference, suggesting that multiple fermentation and distillation steps result in the end in similar volatile composition of the liquor. Back-propagation neural network (BNN) models were developed that satisfactorily predict the number of fermentation steps and the organoleptic evaluation scores of liquor samples from their volatile compositions. Mean impact value (MIV) analysis shows that ethyl lactate, furfural and some high-boiling-point acids play important roles, while pyrazine contributes much less to the improvement of the flavour and taste of Maotai-flavoured liquor during its production. This study contributes to further understanding of the mechanisms of Maotai-flavoured liquor production.

Key words: Maotai-flavoured liquor, multiple fermentations, volatile compounds, statistical analysis, back-propagation neural network

Introduction

Maotai-flavoured liquor, generally described as a highly complex-flavoured, sweet and refreshing soy sauce aroma style alcoholic drink, is one of the most popular and representative liquors in China. The formation of the special flavour of Maotai-flavoured liquor can be largely attributed to its unique and complicated production techniques. The process of Maotai-flavoured liquor production differs from those of other liquors in many aspects including starter preparation, grain (mainly sorghum and wheat) piling and liquor distillation. Briefly, the production of Maotai-flavoured liquor consists in nine fermentation steps and the whole process lasts almost a year. Each fermentation step includes starter addition, piling (putting the mixture of cooked grains and starter powder on the ground, making it into a small hill, and then undergoing fermentation for 4–5 days), fermentation in a pit and distillation. After each fermentation step, the fermented mixture is distilled, the liquor is collected, and the fermented grains are used as the material for the next step. The liquor from the first two fermentations, due to its coarse taste, is poured back on the piled mixture, while the liquor from the other seven fermentations is stored separately for further blending to form the final product.

Much effort has been made in recent years to find the complicated flavour composition of Maotai-flavoured liquor, how it is formed and changes during the brewing process. The research includes microorganism composition analysis (*1,2*), the isolation and characterization of

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functional strains (*3*), flavour component determination (*4,5*), and the analysis of the relationships between the microorganisms and flavour compounds (*6,7*). However, the utilization of multiple fermentation and distillation steps, a very special technique for Maotai-flavoured liquor manufacturing, has not received enough academic attention so far.

The flavour of this Chinese liquor is rather complex and it is generally presented in the form of numerous gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) data, for which statistical approach is a necessity to process and analyze the data. Cluster analysis (CA), discriminant analysis (DA), principal component analysis (PCA) and partial least square (PLS) regression have been widely adopted statistical methods in recent years. All these approaches have shown good performance in many cases of liquor flavour research, such as spectral analysis (*8*), artificial nose (*9*), liquor discrimination and identification (*10–12*). However, most of these methods are linear in nature, thus may not be capable of describing non-linear systems satisfactorily. As a promising alternative, artificial neural network (ANN) has a lot of advantages in parallel processing, classification, learning and pattern recognition. ANN has also been successfully used in researching the productions of wine and beer, such as prediction of process problems (*13*), sensory evaluation (*14*) and process optimization (*15*). As far as we know, ANN has seldom been applied in flavour research of traditional Chinese liquor, especially Maotai-flavoured liquor, whose flavour composition is regarded to be the most complicated among Chinese liquors.

The aim of this work, therefore, is to analyze the variations of the flavour composition of Maotai-flavoured liquor during its multiple fermentation process with statistical approaches, and to provide useful information for better understanding of the formation of its flavour style.

Materials and Methods

Liquor samples

The raw liquor samples were collected from nine distilleries of Langjiu Group Co., Ltd., Sichuan, PR China. Raw liquor was sampled after each of the seven fermentation and distillation steps. A total of 63 liquor samples were used for flavour compound analysis.

Analytical methods

Gas chromatography (GC) analyses of liquor samples were performed on an Agilent 7890A gas chromatograph (Agilent Technologies Co. Ltd., Santa Clara, CA, USA) equipped with automatic sampler and flame ionization detector. Samples were analyzed on a CP-Wax 57 CB column (50 m×0.25 mm×0.2 μm). The injector, detector and column temperatures were set at 125, 120 and 90 °C, respectively. The carrier gas was N_2. The flow rates of N_2, H_2 and air were set at 20, 20 and 230 mL/min, respectively. A total of 68 flavour compounds were determined by comparing their peak areas to those of the standards. All chemicals used in the analyses were of chromatographic grade.

Organoleptic evaluation

Organoleptic evaluation of liquor samples was conducted according to a literature method (*16*). All liquor samples were evaluated by ten tasters, and the average score of the flavour and taste of each sample was calculated.

Data analysis

Student-Newman-Keuls test, correlation analysis and principal component analysis (PCA) were performed using SAS v. 8.1 software (*17*). Cluster analysis, neural network model development and the calculation of each input neuron mean impact value (MIV) were carried out using MATLAB v. 7.1 software (*18*).

Cluster analysis was used to group the liquor samples according to Euclidean distances between the samples based on their volatile compound compositions. PCA was applied to reduce the dimensionality of the original data matrix and allow the visualization of liquor samples with different origins in a lower dimensional space.

Back-propagation neural network (BNN) models were established to predict fermentation steps and organoleptic evaluation scores of liquor samples based on their volatile compound compositions. The architecture of the neural network consisted of an input layer, a hidden layer and an output layer. The input nodes were the concentrations of the 68 volatile compounds of a liquor sample or corresponding principal components (PCs). The output is the fermentation step or the organoleptic evaluation score of the liquor sample. The number of the nodes in the hidden layer was selected according to the following equation:

$$n = \sqrt{n_1 + n_2} + a \qquad /1/$$

where n is the number of the nodes in the hidden layer, n_1 is the number of the input nodes, n_2 is the number of the output nodes, and a is a constant between 0–10.

The total dataset of 63 liquor samples was randomly split into two subdatasets, 48 samples for training and 15 samples for testing. The input variables for training and testing were standardized by using 'prestd' and 'trastd' functions, respectively, while the output variables were postprocessed by using a 'poststd' function in the neural network toolbox of MATLAB v. 7.1. For fermentation step prediction, the output variable was rounded to the nearest integral number. Bayesian regularization was adopted in training the neural network to avoid overtraining, and this was realized by using a 'trainbr' function in the neural network toolbox of MATLAB v. 7.1. After BNN models with satisfactory predictive ability were established, MIVs were calculated to screen the most influential volatile compounds (*19*).

Results and Discussion

Variations of volatile compound composition of liquor samples from different fermentation steps

The average concentrations of the volatile compounds in liquor samples from different fermentation steps in the nine distilleries are listed in Table 1. It can be seen that

Table 1. Volatile compounds in the samples from different fermentation steps of Maotai-flavoured liquor production in nine distilleries

Volatile compound	Fermentation step						
	1	2	3	4	5	6	7
	γ/(mg/100 mL)						
Acetaldehyde	(16.55±2.37)^{e}	(33.33±6.15)^{b}	(53.00±8.18)^{a}	(22.17±2.45)^{d}	(25.32±2.22)^{d}	(27.44±3.89)^{c,d}	(31.74±4.43)^{b,c}
n-Propanal	(2.11±0.75)^{a}	(0.65±0.28)^{b}	(0.08±0.12)^{c}	(0.02±0.07)^{c}	(0.06±0.09)^{c}	(0.16±0.13)^{c}	(0.28±0.19)^{c}
Isobutyraldehyde	(1.16±0.18)^{d}	(1.35±0.26)^{d}	(1.07±0.20)^{d}	(1.55±0.15)^{d}	(2.10±0.13)^{c}	(3.17±0.50)^{b}	(4.26±1.03)^{a}
Acetone	(2.50±0.86)^{b}	(1.59±0.27)^{c}	(1.47±0.23)^{c}	(1.35±0.145)^{c}	(1.78±0.13)^{c}	(2.55±0.30)^{b}	(5.11±0.98)^{a}
Ethyl formate	(0.38±0.23)^{d}	(1.37±0.13)^{a}	(0.80±0.19)^{c}	(0.71±0.11)^{c}	(0.80±0.14)^{c}	(0.85±0.21)^{c}	(1.09±0.38)^{b}
Ethyl acetate	(714.81±162.35)^{a}	(354.60±100.28)^{b}	(151.71±30.16)^{c}	(89.67±12.78)^{c}	(67.32±7.96)^{c}	(58.87±7.89)^{c}	(95.86±19.82)^{c}
Acetal	(15.30±4.27)^{d}	(41.36±10.84)^{b}	(62.85±9.72)^{a}	(23.82±2.64)^{c}	(24.92±2.00)^{c}	(26.47±4.22)^{c}	(28.05±4.59)^{c}
2-Butanone	(3.16±0.64)^{a}	(0.59±0.31)^{c}	(0.36±0.16)^{c}	(0.29±0.10)^{c}	(0.29±0.08)^{c}	(0.50±0.13)^{c}	(1.19±0.38)^{b}
Methanol	(27.81±4.48)^{a}	(18.08±3.19)^{b}	(12.75±2.32)^{c,d}	(11.39±1.32)^{d}	(10.42±0.62)^{d}	(11.38±0.73)^{d}	(14.81±2.14)^{c}
2-Methyl butyraldehyde	(5.53±1.18)^{b}	(5.55±0.94)^{b}	(3.38±0.58)^{c}	(3.29±0.33)^{c}	(3.30±0.37)^{c}	(5.02±0.73)^{b}	(8.54±1.13)^{a}
Isovaleraldehyde	(3.06±0.39)^{e}	(4.30±0.78)^{d,e}	(3.97±0.38)^{d,e}	(4.83±0.50)^{d}	(6.85±0.49)^{c}	(10.17±1.34)^{b}	(16.45±3.29)^{a}
Ethyl isobutyrate	(0.33±0.13)^{b}	(0.58±0.29)^{a}	(0.00±0.00)^{c}	(0.00±0.00)^{c}	(0.00±0.00)^{c}	(0.00±0.00)^{c}	(0.02±0.07)^{c}
2,3-Butanedione	(1290.2±815.49)^{a}	(92.32±86.48)^{b}	(5.29±2.35)^{b}	(3.77±1.10)^{b}	(5.02±1.66)^{b}	(12.76±4.73)^{b}	(13.75±5.51)^{b}
2-Pentanone	(0.19±0.11)^{c}	(0.34±0.23)^{c}	(0.53±0.18)^{c}	(0.42±0.22)^{c}	(0.85±0.37)^{c}	(2.81±0.82)^{b}	(4.47±1.55)^{a}
Ethyl butyrate	(4.06±1.33)^{a,b}	(2.96±0.98)^{b,c}	(1.74±0.57)^{c}	(1.90±0.42)^{c}	(2.36±0.60)^{c}	(3.81±1.47)^{a,b}	(4.34±1.53)^{a}
2-Butanol	(316.22±94.81)^{a}	(12.66±7.59)^{b}	(2.32±0.88)^{b}	(1.63±0.49)^{b}	(1.61±0.65)^{b}	(2.30±0.94)^{b}	(7.40±3.99)^{b}
n-Propanol	(11719±5010)^{a}	(1995.6±1505)^{b}	(48.55±16.58)^{b}	(23.27±3.42)^{b}	(20.01±2.33)^{b}	(26.59±5.44)^{b}	(58.44±25.98)^{b}
Ethyl isovalerate	(0.47±0.11)^{c}	(1.09±0.16)^{b}	(2.88±0.78)^{a}	(1.00±0.22)^{b}	(1.14±0.21)^{b}	(1.25±0.19)^{b}	(0.88±0.20)^{b}
Diethoxy-2-methyl butane	(0.13±0.02)^{e}	(0.22±0.03)^{b,c}	(0.16±0.03)^{d,e}	(0.18±0.026)^{c,d,e}	(0.21±0.04)^{b,c,d}	(0.26±0.05)^{b}	(0.37±0.11)^{a}
Diethoxy-3-methyl butane	(0.30±0.03)^{d}	(0.41±0.05)^{d}	(0.36±0.04)^{d}	(0.43±0.05)^{d}	(0.55±0.06)^{c}	(0.78±0.11)^{b}	(1.11±0.27)^{a}
Isobutanol	(42.82±5.41)^{a}	(23.49±3.48)^{c}	(19.51±3.22)^{d,e}	(18.41±2.56)^{d,e}	(16.22±2.81)^{e}	(21.48±2.31)^{c,d}	(32.86±3.63)^{b}
Isoamyl acetate	(5.15±1.38)^{a}	(1.30±0.43)^{b}	(0.57±0.10)^{c}	(0.43±0.07)^{c}	(0.36±0.07)^{c}	(0.40±0.05)^{c}	(0.75±0.15)^{c}
Ethyl valerate	(1.02±0.44)^{b}	(0.90±0.33)^{b}	(0.63±0.15)^{b}	(0.96±0.22)^{b}	(1.26±0.35)^{b}	(1.94±0.82)^{a}	(2.19±0.83)^{a}
2-Pentanol	(2.89±0.69)^{a}	(0.95±0.28)^{c,d}	(0.48±0.14)^{d}	(0.44±0.14)^{d}	(0.62±0.28)^{d}	(1.25±0.39)^{c}	(2.39±0.71)^{b}
n-Butyl alcohol	(11.48±2.17)^{a}	(3.46±0.93)^{c}	(3.26±0.51)^{c}	(3.79±0.49)^{c}	(4.21±1.13)^{c}	(4.74±1.76)^{c}	(8.83±2.57)^{b}
Methyl-1-butanol	(17.85±1.96)^{a}	(10.73±1.95)^{c}	(9.63±2.00)^{c}	(10.53±1.61)^{c}	(9.95±1.31)^{c}	(10.70±1.28)^{c}	(16.14±1.61)^{b}
Isoamyl alcohol	(69.76±7.99)^{a}	(49.81±7.01)^{b}	(47.11±8.40)^{b}	(51.04±5.80)^{b}	(47.03±4.32)^{b}	(49.66±4.35)^{b}	(74.43±6.72)^{a}
Ethyl caproate	(4.66±1.29)^{d}	(4.48±0.74)^{d}	(4.43±2.03)^{d}	(7.79±1.49)^{c}	(12.65±2.34)^{b}	(16.17±3.79)^{a}	(17.49±4.29)^{a}
Pentanol	(0.77±0.23)^{c}	(0.53±0.09)^{c}	(0.45±0.05)^{c}	(0.49±0.06)^{c}	(0.66±0.13)^{c}	(1.06±0.31)^{b}	(1.76±0.55)^{a}
Hexyl acetate	(0.11±0.07)^{a}	(0.02±0.04)^{b}	(0.01±0.03)^{b}	(0.00±0.00)^{b}	(0.01±0.03)^{b}	(0.00±0.00)^{b}	(0.02±0.06)^{b}
3-Hydroxybutanone	(0.61±0.15)^{d}	(1.21±0.35)^{d}	(8.41±5.78)^{b,c}	(5.76±2.60)^{c,d}	(8.58±3.88)^{b,c}	(20.49±9.74)^{a}	(13.64±8.10)^{b}
2-Heptanol	(0.29±0.16)^{a}	(0.02±0.04)^{c}	(0.00±0.00)^{c}	(0.01±0.02)^{c}	(0.03±0.04)^{c}	(0.11±0.04)^{b}	(0.24±0.04)^{a}
Ethyl heptanoate	(0.32±0.15)^{d}	(0.39±0.09)^{c,d}	(0.34±0.07)^{d}	(0.51±0.11)^{c}	(0.83±0.18)^{b}	(0.88±0.13)^{a,b}	(1.01±0.20)^{a}
Ethyl lactate	(215.73±115.12)^{d}	(534.94±86.74)^{c}	(614.28±67.92)^{b}	(1021.0±105.7)^{a}	(662.96±71.88)^{b}	(509.43±35.25)^{c}	(449.39±58.96)^{c}
n-Hexanol	(3.05±0.46)^{c}	(1.84±0.21)^{d}	(1.67±0.25)^{d}	(1.97±0.17)^{c,d}	(2.73±0.41)^{c,d}	(4.72±0.97)^{b}	(8.19±2.22)^{a}
Butyl hexanoate	(0.08±0.06)^{a}	(0.05±0.05)^{a}	(0.04±0.05)^{a}	(0.06±0.06)^{a}	(0.05±0.05)^{a}	(0.07±0.04)^{a}	(0.26±0.52)^{a}
Trimethylpyrazine	(0.46±0.08)^{a}	(0.03±0.04)^{d}	(0.05±0.04)^{d}	(0.09±0.04)^{d}	(0.08±0.04)^{d}	(0.15±0.02)^{c}	(0.26±0.07)^{b}
Ethyl caprylate	(30.29±7.66)^{a}	(9.19±7.23)^{b}	(0.58±0.19)^{c}	(0.86±0.18)^{c}	(1.51±0.34)^{c}	(1.79±0.46)^{c}	(2.27±0.51)^{c}
Furfural	(0.29±0.75)^{g}	(4.98±2.15)^{f}	(17.61±1.26)^{e}	(27.43±2.61)^{d}	(39.30±3.19)^{c}	(56.76±6.10)^{a}	(48.29±7.44)^{b}
Acetic acid	(354.79±163.45)^{a}	(161.29±57.88)^{b}	(48.15±16.19)^{c}	(38.79±8.64)^{c}	(34.20±7.62)^{c}	(45.38±7.04)^{c}	(108.01±33.42)^{b,c}
Tetramethylpyrazine	(1.47±1.59)^{a}	(0.65±0.53)^{a,b}	(0.73±0.66)^{a,b}	(0.73±0.70)^{a,b}	(0.24±0.18)^{b}	(0.10±0.26)^{b}	(1.17±0.85)^{a,b}
Benzaldehyde	(0.34±0.15)^{b,c}	(0.50±0.10)^{a}	(0.22±0.07)^{c,d}	(0.37±0.19)^{b}	(0.17±0.05)^{d}	(0.19±0.07)^{d}	(0.29±0.10)^{b,c,d}
Ethyl pelargonate	(0.02±0.04)^{c}	(0.02±0.05)^{c}	(0.07±0.08)^{c}	(0.05±0.05)^{c}	(0.06±0.07)^{c}	(0.19±0.05)^{b}	(0.29±0.06)^{a}
L-2,3-butanediol	(0.36±0.49)^{g}	(1.54±0.20)^{f}	(3.16±0.30)^{e}	(4.88±0.43)^{d}	(6.01±0.28)^{c}	(7.28±0.47)^{b}	(10.21±1.25)^{a}
n-Caprylic alcohol	(0.15±0.19)^{b}	(0.36±0.32)^{a}	(0.31±0.22)^{a,b}	(0.09±0.02)^{b}	(0.10±0.01)^{b}	(0.13±0.01)^{b}	(0.17±0.03)^{b}
Propionic acid	(111.35±53.90)^{a}	(25.49±13.10)^{b}	(3.77±1.95)^{b}	(2.77±0.47)^{b}	(0.70±0.23)^{b}	(1.25±0.87)^{b}	(1.15±0.50)^{b}
D,L-2,3-butanediol	(1.72±1.91)^{f}	(3.10±1.45)^{d,e}	(2.18±0.89)^{e,f}	(3.89±0.99)^{c,d}	(4.49±0.74)^{b,c}	(5.35±0.37)^{b}	(8.18±1.02)^{a}
1,2-Propanediol	(2.28±0.78)^{b}	(4.30±1.35)^{b}	(3.76±2.08)^{b}	(3.78±1.28)^{b}	(3.01±1.03)^{b}	(3.85±1.23)^{b}	(10.41±3.33)^{a}

Table 1. – continued

Volatile compound	Fermentation step						
	1	2	3	4	5	6	7
	γ/(mg/100 mL)						
Isobutyric acid	(0.00±0.00)^{b}	(0.00±0.00)^{b}	(0.06±0.17)^{b}	(0.28±0.51)^{a,b}	(0.48±0.25)^{a,b}	(0.59±0.79)^{a}	(0.29±0.17)^{a,b}
Ethyl caprate	(0.29±0.16)^{a}	(0.12±0.07)^{b}	(0.16±0.17)^{a,b}	(0.20±0.13)^{a,b}	(0.15±0.10)^{a,b}	(0.19±0.03)^{a,b}	(0.25±0.03)^{a,b}
Diethyl succinate	(0.17±0.14)^{b}	(0.41±0.10)^{b}	(1.39±0.51)^{b}	(0.49±0.06)^{b}	(0.94±1.02)^{b}	(1.46±1.41)^{b}	(16.04±4.25)^{a}
Butyric acid	(4.08±1.59)^{b}	(0.29±0.55)^{d}	(0.34±0.15)^{d}	(1.45±0.75)^{c,d}	(2.90±1.21)^{b,c}	(6.61±3.54)^{a}	(3.64±1.06)^{b}
Isovaleric acid	(0.41±0.26)^{b}	(0.34±0.32)^{b}	(0.19±0.29)^{b}	(0.35±0.39)^{b}	(0.34±0.16)^{b}	(0.81±0.45)^{a,b}	(1.13±1.04)^{a}
Ethyl phenylacetate	(0.11±0.04)^{b}	(0.02±0.04)^{b}	(0.00±0.00)^{b}	(0.04±0.12)^{b}	(0.01±0.02)^{b}	(0.08±0.23)^{b}	(0.70±0.41)^{a}
1,3-Propanediol	(1.24±0.27)^{a}	(1.15±0.16)^{a,b}	(1.07±0.18)^{a,b}	(1.03±0.15)^{a,b}	(0.83±0.10)^{b}	(0.92±0.27)^{a,b}	(1.28±0.56)^{a}
Valeric acid	(0.12±0.20)^{d}	(0.33±0.05)^{c,d}	(0.45±0.05)^{b,c}	(0.57±0.17)^{b,c}	(0.76±0.07)^{b}	(1.07±0.30)^{a}	(0.57±0.57)^{b,c}
Ethyl laurate	(0.18±0.05)^{a}	(0.06±0.03)^{b,c}	(0.06±0.04)^{b,c}	(0.04±0.04)^{c}	(0.07±0.03)^{b,c}	(0.06±0.05)^{b,c}	(0.09±0.01)^{b}
Benzyl alcohol	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.01±0.04)^{a}	(0.02±0.05)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00) ^{a}
Ethyl phenylpropionate	(0.08±0.03)^{e}	(0.10±0.03)^{e}	(0.11±0.02)^{d,e}	(0.14±0.02)^{c,d}	(0.16±0.02)^{c}	(0.20±0.03)^{b}	(0.29±0.05)^{a}
β-Phenethyl alcohol	(0.46±0.07)^{e}	(0.56±0.09)^{e}	(0.95±0.11)^{d}	(1.30±0.07)^{c}	(1.61±0.08)^{b,c}	(1.77±0.51)^{b}	(2.79±0.69)^{a}
Hexanoic acid	(4.21±4.02)^{a}	(0.04±0.11)^{b}	(0.10±0.23)^{b}	(1.75±1.40)^{b}	(4.91±2.57)^{a}	(5.37±3.50)^{a}	(1.15±2.11)^{b}
Heptanoic acid	(0.02±0.04)^{b}	(0.04±0.07)^{a,b}	(0.08±0.07)^{a,b}	(0.10±0.06)^{a,b}	(0.10±0.05)^{a,b}	(0.04±0.06)^{a,b}	(0.12±0.07)^{a}
Caprylic acid	(0.02±0.05)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}
Ethyl palmitate	(2.47±0.71)^{e}	(3.12±0.52)^{d}	(3.49±0.42)^{d}	(4.05±0.59)^{c}	(4.46±0.35)^{b,c}	(4.71±0.43)^{b}	(6.58±0.44)^{a}
Nonanoic acid	(0.04±0.07)^{b,c}	(0.00±0.00)^{c}	(0.03±0.09)^{b,c}	(0.15±0.09)^{a}	(0.12±0.10)^{a,b}	(0.16±0.13)^{a}	(0.18±0.12)^{a}
Decanoic acid	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.02±0.05)^{a}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	(0.02±0.06)^{a}
Ethyl oleate	(0.92±0.29)^{e}	(1.20±0.25)^{d}	(1.27±0.19)^{d}	(1.43±0.23)^{c,d}	(1.56±0.19)^{c}	(1.82±0.27)^{b}	(2.91±0.35)^{a}
Ethyl linoleate	(2.06±0.81)^{c}	(2.73±0.64)^{b}	(2.79±0.43)^{b}	(3.04±0.60)^{b}	(3.24±0.37)^{b}	(3.49±0.42)^{b}	(5.17±0.64)^{a}

Data are expressed as mean value±standard deviation (N=9). The same letter in superscript in the same row denotes values that are not significantly different

most of the volatile compounds underwent significant concentration change during the multiple fermentation steps. Some components, like acetic acid, ethyl acetate, 2,3-butanedione, 2-butanol and *n*-propanol, decreased dramatically after one or two fermentations, while other components, like ethyl caproate, ethyl lactate, ethyl palmitate, ethyl oleate and ethyl linoleate, increased gradually during the whole process. Organoleptic evaluation showed that the liquor after 3–6 fermentations had softer and sweeter flavour compared to those after fermentations 1 and 2. This implies that some of the components that decreased significantly during the multiple fermentations very possibly stimulate flavour and taste of the liquor.

Correlation analysis suggests close relationships among some compounds (Table 2). These relationships can be partly explained by the sharing of common metabolic pathways or enzymes used for the formation of different compounds (acetaldehyde and acetal, *n*-propanal and *n*-propanol, 2-butanone and 2-butanol, for example). However, the close relationships among some other components (ethyl acetate and methanol, for example) are not fully understood and thus need further research in the future.

Table 2. Close relationships among volatile compounds in Maotai-flavoured liquor samples revealed by correlation analysis

Group	Volatile compound
1	acetaldehyde, acetal
2	*n*-propanal, ethyl acetate, methanol, 2,3-butanedione, 2-butanol, *n*-propanol, isoamyl acetate
3	isobutyraldehyde, isovaleraldehyde, 2-pentanone, diethoxy-2-methyl butane, diethoxy-3-methyl butane
4	2-butanone, 2-butanol
5	pentanol, hexyl acetate, butyl hexanoate, *n*-caprylic alcohol
6	ethyl caprylate, propionic acid
7	acetic acid, propionic acid
8	ethyl phenylpropionate, ethyl palmitate, ethyl oleate

Pearson's correlation coefficients among the components in the same rows are higher than 0.9

Cluster and principal component analyses of liquor samples after different fermentation steps

Cluster analysis was performed to find similarities among liquor samples after different fermentation steps and distilleries based on their volatile compound compositions. The results show that except for several samples from the first two fermentations, most samples from a same fermentation are clustered together (details not shown), suggesting that the fermentation step plays a more important role in forming the liquor style than the distillery where it is produced.

Comparing the samples from the same distillery, those after 3 to 7 fermentations are similar to each other, while those after the first two rounds, especially the first one, show much difference (Fig. 1). This means that although there may be considerable differences in the be-

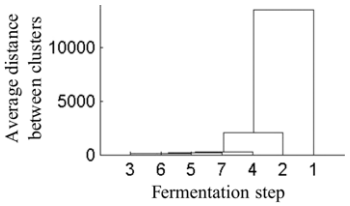

Fig. 1. A representative cluster analysis result of liquor samples from seven fermentation and distillation steps in a same distillery based on volatile compound composition

ginning, multiple fermentations and distillations lead to similar flavour compound composition of the liquor after several steps.

In order to reduce the data dimensionality and visualize different liquor samples in a lower dimensional space, PCA was conducted on the data matrix of 63 liquor samples×68 volatile compounds. The results reveal that the first ten principal components (PCs) extracted are needed to account for 86 % of the total variance in the data matrix. The first three PCs (PC1, PC2 and PC3), however, explain only 15.8, 14.1 and 9.4 % of the total variance, respectively. The three-dimensional plot of the PCA (Fig. 2) shows that liquor samples taken after fermentations 1 and 7 can be separated appropriately based on these three PCs, while other samples, especially those taken after fermentations 3–5, are very closely located. Samples from the same fermentation step from different distilleries also failed to be separated satisfactorily from each other (details not shown). On the whole, the PCA here does not provide much insight for understanding the differences among the liquor samples.

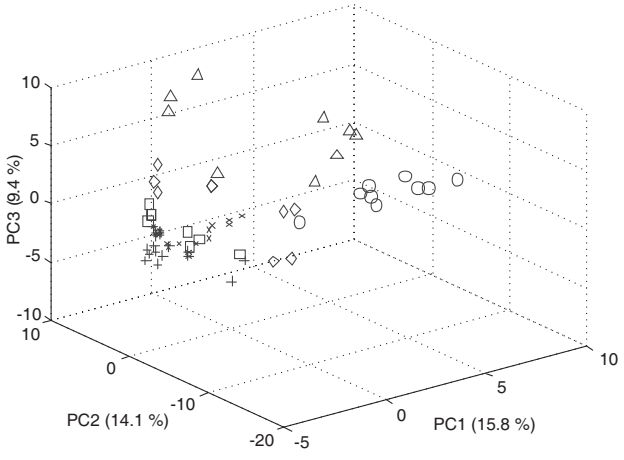

Fig. 2. Principal component analysis (PCA) plots for 63 Maotai-flavoured liquor samples. (○) fermentation 1, (x) fermentation 2, (+) fermentation 3, (*) fermentation 4, (□) fermentation 5, (◇) fermentation 6, (△) fermentation 7

Developing BNN models for predicting the number of fermentation steps and organoleptic evaluation score of liquor samples and variable screening

BNN models were developed to predict the number of fermentation steps and organoleptic evaluation scores of the liquor samples based on their volatile compositions and PCs, respectively. After a trial of the topological structure, it was found that BNN models with nine nodes in the hidden layer could provide satisfactory prediction when 68 volatile compound concentrations were used as inputs, while six nodes in the hidden layer were appropriate when ten PCs were used as inputs. Some representative predictions in the test are shown in Fig. 3. The accuracy of brewing round prediction in the test was between 80 and 100 % when 68 volatile compound concentrations were used as inputs, while 60–90 % of accuracy was obtained with ten PC inputs. For organoleptic evaluation score prediction, BNN model using 68 volatile compound concentrations as inputs also had better performance (R^2 value between 0.80 and 0.95) than those using the ten PCs as inputs (R^2 value between 0.70 and 0.90). This result was not entirely unexpected since the ten PCs account for only 86 % of the data variance and the linear PCA may lose some non-linear information of the investigated system.

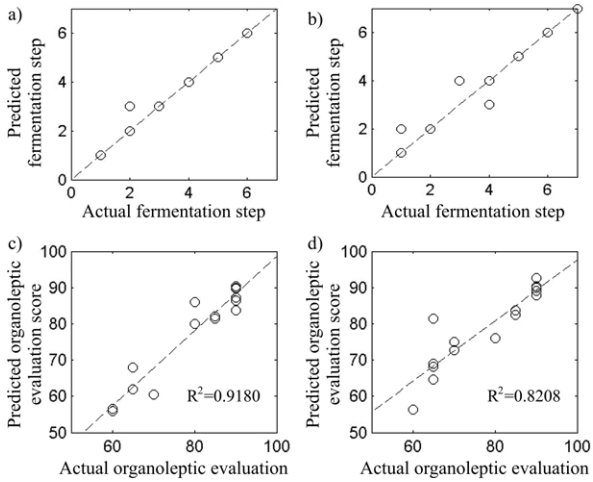

Fig. 3. Validation of BNN model for the predictions of fermentation steps and organoleptic evaluation score of liquor samples: a) fermentation step prediction by BNN with the concentrations of 68 volatile compounds as inputs, b) fermentation step prediction by BNN with ten PCs as inputs, c) organoleptic evaluation score prediction by BNN with the concentrations of 68 volatile compounds as inputs, and d) organoleptic evaluation score prediction by BNN with ten PCs as inputs. BNN= back-propagation neural network

As the BNN model with 68 volatile compound concentrations as inputs represents well the relationship between the volatile compositions and organoleptic evaluation results of the liquor samples, mean impact value (MIV) analysis was adopted to find which volatile compounds play more important roles in forming the liquor flavour style (Table 3). High MIVs of many alcohols and esters were observed. However, most of these MIVs are negative, suggesting that high concentration of these compounds may degrade the flavour and taste of the liquor. Noticeable volatile compounds that showed positive and relatively high MIVs are ethyl lactate, furfural and several acids including valeric acid, heptanoic acid, isobutyric acid and nonanoic acid. This implies that these compounds contribute greatly to the formation of the flavour of the liquor. The concentrations of almost all these

Table 3. Mean impact value (MIV) analysis of the volatile compounds for the organoleptic evaluation score

Volatile compound	MIV	Rank	Volatile compound	MIV	Rank
Ethyl isobutyrate	–0.070536	1	Nonanoic acid	0.024576	35
2-Methyl butyraldehyde	–0.068213	2	*n*-Propanol	–0.023981	36
Ethyl lactate	0.064073	3	Trimethylpyrazine	–0.023199	37
Isobutanol	–0.057357	4	Benzyl alcohol	0.022346	38
2-Pentanol	–0.053145	5	L-2,3-butanediol	0.020594	39
Ethyl formate	–0.052818	6	Ethyl laurate	–0.019764	40
Methanol	–0.050198	7	2-Butanol	–0.019722	41
Ethyl acetate	–0.045195	8	2-Pentanone	–0.019569	42
Ethyl butyrate	–0.044636	9	Hexyl acetate	–0.019485	43
Benzaldehyde	–0.042708	10	Hexanoic acid	0.018534	44
Acetic acid	–0.042306	11	3-Hydroxybutanone	0.017463	45
Methyl-1-butanol	–0.041465	12	Isovaleric acid	–0.015082	46
n-Propanal	–0.038482	13	Diethoxy-3-methyl butane	–0.014975	47
Isoamyl alcohol	–0.038121	14	Isovaleraldehyde	–0.014823	48
2-Heptanol	–0.037807	15	Ethyl pelargonate	–0.014777	49
n-Caprylic alcohol	–0.037405	16	Ethyl linoleate	–0.014753	50
2-Butanone	–0.036517	17	Ethyl oleate	–0.014225	51
Ethyl caprylate	–0.036170	18	2,3-Butanedione	–0.014009	52
Acetone	–0.034515	19	β-Phenethyl alcohol	0.013055	53
Furfural	0.033426	20	Tetramethylpyrazine	–0.012898	54
Isoamyl acetate	–0.031415	21	Isobutyraldehyde	–0.012627	55
Diethoxy-2-methyl butane	–0.029032	22	Butyl hexanoate	–0.012366	56
Ethyl isovalerate	0.028823	23	Ethyl valerate	–0.011957	57
Pentanol	–0.028756	24	Ethyl heptanoate	0.011339	58
1,2-Propanediol	–0.028752	25	Caprylic acid	–0.011201	59
Valeric acid	0.027971	26	Ethyl caproate	0.010988	60
Heptanoic acid	0.027790	27	Acetal	–0.009885	61
Diethyl succinate	–0.026526	28	Decanoic acid	0.005609	62
n-Butyl alcohol	–0.026290	29	Ethyl caprate	0.005334	63
Propionic acid	–0.026065	30	Acetaldehyde	–0.005082	64
n-Hexanol	–0.025835	31	D,L-2,3-butanediol	–0.004520	65
Ethyl phenylacetate	–0.025591	32	Ethyl phenylpropionate	0.004460	66
1,3-Propanediol	–0.025232	33	Butyric acid	–0.002125	67
Isobutyric acid	0.024669	34	Ethyl palmitate	0.002073	68

The MIV of each compound is the average value of calculation results based on five randomly selected trained BNN models

compounds increased with the number of fermenations (Table 1), suggesting multiple fermentations are vital in forming the liquor flavour.

Major flavour components in Maotai-flavoured liquor have been discussed extensively in recent years but no consistent opinion has been obtained so far (*20,21*). Furfural (*22*), acids with high boiling point (*23*), and pyrazines (*24*) have all been suggested to be the major flavour compounds in Maotai-flavoured liquor. Our results show that ethyl lactate, furfural and some acids with high boiling points do play important roles in forming the liquor style. Pyrazines, however, seem to contribute less or even negatively according to their MIVs in Table 3. This is supported by a previous report where the concentration of pyrazines varied significantly in different Maotai-flavoured liquor samples (*20*). However, as the analyses here are based exclusively on Maotai-flavoured liquor samples, we cannot assert that the components with moderate or low MIVs are unimportant or even unnecessary in forming the liquor flavour. Undoubtedly, further elucidation of major flavour components in Maotai-flavoured liquor requires more samples with different flavour characteristics.

Conclusion

The results of this research show that fermentation steps exert much more influence on the volatile composition of Maotai-flavoured liquor than the distillery. Although there may be considerable differences in the volatile com-

position among the liquor samples at the beginning, multiple fermentations and distillations ultimately lead to similar volatile composition of the liquor. Based on the statistical analyses, we suggest that ethyl lactate, furfural and some high-boiling-point acids make relatively high contribution in forming the special flavour of Maotai-flavoured liquor.

Acknowledgements

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