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Multivariate Modeling of Aging in Bottled Lager Beer by Principal Component Analysis and Multiple Regression Methods

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Data collected from the sensory test score evaluation of bottled lager beer, together with the chemical components related to aging, including carbonyl compounds, higher alcohols, unsaturated fatty acid, organic acids, α-amino acids, dissolved oxygen, and staling evaluation indices, including lag time of electron spin resonance (ESR) curve, 1,1′-diphenyl-2-picrylhydrazyl (DPPH) scavenged amounts, and thiobarbituric acid (TBA) values, were used to predict the extent of aging in bottled lager beer, using both multiple linear regression and principal component analysis methods. Carbonyl compounds, higher alcohols, and TBA value were significantly and positively correlated with sensory evaluation of staling flavor. While lag time and DPPH scavenging amount were negatively correlated with taste test score. Multiple regression analysis was used to fit the sensory test data using the above chemical compound aging related parameters and evaluation indices as predictors. A variable selection method based on high loadings of varimax rotated principal components was used to obtain subsets of the predominant predictor variables to be included in the regression model of beer aging, so as to eliminate the multicollinearity of the original nine variables. It was found that staling extent was influenced significantly by higher alcohols, TBA value, and DPPH scavenging amount, and the multicollinearity of the regression model was found to be weak by examining the variance inflation factors of the new predictor variables. A mathematic model of the organoleptic test score for beer aging using these three predictors was obtained by multiple linear regression, showing that the major contributors to the sensory taste of beer aging were higher alcohols, TBA index, and DPPH scavenging amount, with the adjusted R^2 of the model being 0.62.

KEYWORDS: Statistical analysis; principal component analysis; regression analysis; beer aging

INTRODUCTION

It is generally recognized that the staling of bottled beer is very complex because of the thousands of flavor chemicals involved (*1*). Oxidatively produced unsaturated carbonyl compounds play a major role in the development of stale flavor in beer. There are several hypotheses, such as Strecker degradation of α-amino acids (*2*), oxidation of fatty acids (especially unsaturated fatty acids) (*3, 4*), condensation of higher alcohols (*5*), and so on. All of these reactions contribute to the course of aging of bottled beer.

Although the results of taste panel sensory analysis vary in the response between individuals and different circumstances and time, such sensory tests are the most widely and commonly used method to examine the aging extent of beer (*4, 6*). To date, no single instrument has been devised that satisfactorily evaluates flavor in a similar way in which the consumer does.

Multiple regression analysis is one of the most widely used methodologies for expressing the dependence of a response variable on several independent (predictor) variables. In spite of its evident success in many applications, the regression approach can face serious difficulties when the independent variables are correlated with each other (*7*). Multicollinearity, or high correlation between the independent variables in a regression equation, can make it difficult to correctly identify the most important contributors to a complicated process. One method for removing multicollinearity and redundant independent variables is to use multivariate data analysis techniques (*8*).

One such method is principal component analysis (PCA), which is used to separate interrelationships into statistically independent, basic components. It is useful in regression analysis to mitigate the problem of multicollinearity and to explore the

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relations among the independent variables, particularly if it is not obvious which of the variables should be the primary predictors. The new variables from the PCA are more valid to use as predictors in a regression equation, since they optimize spatial patterns and remove possible complications caused by multicollinearity (*9*). Subsequently, they allow the identification of the primary predictors with minimal multicollinearity.

The objective of this study was to use statistical analysis, including multiple regression and the principal component analysis, to develop models that predict beer aging as determined by chemical compounds related to staling and parameters of evaluating indices of staling as predictor variables. This approach provided a deeper knowledge of the important factors that influence the aging, and provide a simple and effective model of prediction for brewers to monitor the aging of their beer.

MATERIALS AND METHODS

Beer. Four brands of bottled lager beer commonly purchased by Chinese customers, produced by different Chinese brewing groups, were used for this study. The beers included the following: (a) Suntory, with original gravity 10 °P, from Suntory Brewing Co. Ltd. (Shanghai); (b) Tsingtao, with original gravity 10 °P, from Tsingtao Brewery Co. Ltd. (Qingdao); (c) Budweiser, with original gravity 10 °P, from Budweiser Wuhan International Brewing Co. Ltd. (Wuhan); and (d) Taihushui, with original gravity 9.5 °P, from China Resources Snow Breweries Co. Ltd. (Wuxi). Of the investigated beer samples, their shelf time ranged from shorter than 1 week to 12 months, according to the production date. All the samples were naturally aged by storing at room temperature.

Reagents. *N-tert*-butyl-α-phenylnitrone (PBN) was used as a spin-trapping reagent and was purchased from Aldrich (Steinheim, Germany). The free radical 1,1-diphenyl-2-picrylhydracyl (DPPH) was purchased from Tokyo Kasei TOSHIMA. KIT. (Tokyo, Japan). All other chemical standards were purchased from Sigma (St. Louis, MO) and were of the highest purity available. The other chemicals and solvents were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Aging Evaluation Indices. *Lag Time.* Lag time of beer samples was obtained from electron spin resonance spectrometry (ESR) (*6*). The ESR spectrum was measured with an electron spin resonance spectrometer WIN-EPR (Bruker, Rheinstetten, Germany), and the parameters for measuring the PBN spin adduct were the following: power, 20 mW; frequency, 9.7 GHz; center field, 3475.000 G; sweep width, 100.000 G; modulation frequency, 100.00 kHz; modulation amplitude, 1.00 G; conversion, 163.840 ms; time constant, 80.920 ms; and sweep time, 167.772 s.

DPPH Radical Scavenging Amount. DPPH radical scavenging activity of beer was determined by the absolute scavenging amount of radicals, according to the method of Brand-Williams et al. (*10*). A lower absorbance of the reaction mixture indicated a higher free radical scavenging activity. DPPH radical scavenging amount was calculated using the following formula:

$$\text{DPPH}^{\bullet}\ \text{scavenging amount (mol/L)} = \text{initial DPPH}^{\bullet}\ \text{concentration of reaction system (mol/L)} \times \text{DPPH}^{\bullet}\ \text{scavenging activity (\%)} \quad (1)$$

TBA. The TBA (thiobarbituric acid) method was used to evaluate the degree of beer staling and was based on the improved measurement of Li (*11*). Degassed beer of 5 mL was added to 2 mL of thiobarbutiric reactive reagent containing 0.33% (w/v) TBA in 50% (v/v) acetic acid. The mixture was incubated in a 60 °C water bath for 60 min. The absorbance of the solution was measured at 530nm, with 5 mL of 50% (v/v) acetic acid as the blank.

Sensory Test of Stale Flavor. A panel of 10 experienced panelists (6 males and 4 females) were selected on their ability to discriminate staling flavor of bottled beer. All the panelists had participated in a training program to improve their sensitivity and veracity before the final sensory evaluation. The training components were carried out from basic to advanced levels, including ranking, matching, staling flavor perception, and detection. These components focused on the main changes of flavor during beer aging, including cardboard flavor, the bitterness and sweet taste, and the toffeelike, or bready/winey flavor produced as a result of Maillard reactions. The last parameter was staling flavor discrimination of bottled beer that had been aged to different extents, which were produced by three different breweries in China, for adaption training. Adaption training involved the samples from each brewery being offered in an order of fresh to stale, that was from relatively fresh beer (shelf-time was not longer than 7 days) to extremely aged ones (force-aged beer at 60 °C for 12 days or close to expiration date).

Table 1. Judging of Stale Flavor by Organoleptic Flavor Panel Scores (Five-Point Scale)

	organoleptic sense description				
	fresh (not aged)[a]	slightly aged	moderately aged	relative strongly aged	strongly aged[b]
point	1	2	3	4	5

[a] Fresh beer, not longer than 1 week. Used in adapting training as the example of fresh flavor. [b] Forced-aged (37 °C, 30 days or more) beer and strongly aged bottled beer (close to the expired date but not out of date). Used in adapting training as the example of strongly staling flavor.

In the final sensory test, the beer samples were served randomly, and the panelists were asked to smell, taste, and finally mark the intensity of extent of staling. The judgment was done on a five-point scale as outlined in **Table 1**. The subjective evaluation of the organoleptic sense was indicated by the mean value of the total panel data. The task was carried out three times on three separate days, to reach the consensus and repeatability in the sensory test. Panelists' sensory evaluation results of a sample would not be adopted if they were not normal distribution. Overall, the sensory flavor evaluation protocol described used in this investigation meets the stringent requirements for valid flavor assessment proscribed by Meilgaard (*12*).

Compound Groups Related to Aging. *Carbonyl Compounds.* Carbonyl compounds were measured by headspace solid-phase microextraction and gas chromatography with mass detection (SPME-GC/MS) (*13, 14*) with some modification. Headspace SPME using a 75 μm Carboxen-polydimethylsiloxane (CAR-PDMS) fiber provided sample enrichment and enabled extraction of most forms of carbonyl compounds involved in beer aging.

Beer (5 g) was added to 2 g of NaCl that was put into a 15 mL vial for each sample. The sample vials were kept at 20 °C for at least 30 min to establish an equilibrium between the headspace and sample. The samples was then held at 20 °C in an ultrasonic bath for 40 min to further expose the fiber to the headspace for absorption of the target compounds.

The analyses were performed using a GC system associated with a mass spectrometry (MS) detector (Trace MS, Finnigan, U.S.A.). Helium was used as the carrier gas with a flow rate of 0.8 mL/min. The components were separated on a PEG-20,000 capillary column (30 m × 0.25 mm × 0.25 μm, Shanghai Bioengineering Co Ltd., Shanghai, China). The oven temperature program was 3.5 min at 40 °C, 5 °C/min to 60 °C, 6 °C/min to 120 °C, then 8 °C/min to 230 °C for 12 min. The injector temperature was set at 250 °C for the CAR-PDMS fiber. The temperature of gasification was 200 °C. Detection was by mass spectrometry of the total ion current obtained by electron impact at 70 eV. The constituents were identified by comparing the experimental spectra with those of the U.S. National Institute of Standards and Technology (NIST) 1998 data bank 1.6, U.S.A. (http://webbook.nist.gov/chemistry/). On the basis of the peak resolution, their areas were calculated from the GC spectrum, and their values were presented by the amount of internal standard 2-octanal.

Higher Alcohols. The higher alcohol content of beer was determined by a headspace gas chromatography (HS-GC) with a flame ionization detector (FID). The analysis was performed using a Shimadzu GC-

2010 gas chromatograph coupled with a FID detector (Shimadzu, Japan). Separation was performed with a CP-WAX52CB capillary column (30 m × 0.32 mm × 0.52 μm, Shanghai Bioengineering Co Ltd., Shanghai, China). The carrier gas was nitrogen with a flow rate of 3.0 mL/min. The oven temperature program was started at 40 °C for 2 min, then 10 °C/min to 180 °C for 4 min. The temperature of the detector was at 250 °C, and the temperature of the gasification oven was 200 °C. The equilibrium of vials between headspace and samples was performed at 70 °C for 30 min.

Unsaturated Fatty Acid. The concentration of unsaturated fatty acid in beer was also determined by gas chromatography after a precolumn derivatization to fatty acid methyl esters (FAMEs) of the samples. The preparation included the following steps: 150 mL of beer was extracted 3 times (30 mL, 30 mL, 20 mL) by dichloromethane/methanol solution of 3:1 (v/v), which then was concentrated to approximately 1 mL using a rotary evaporator. The concentrated samples were derivatized by an acidic methanol method by Wang et al. (*15*) in a 60 °C bath. The FAMEs were extracted into a small amount of hexane and then injected into the GC system for analysis. The concentration of unsaturated fatty acids was expressed as the amount of oleic acid, relative to methyl heptadecanoate as internal standard. The measurement was performed using a Shimadzu GC-2010 gas chromatograph coupled with a FID detector. Separation was performed with a CP-WAX52CB capillary column 30 m × 0.32 mm × 0.52 μm. Nitrogen/air was used as the carrier gas with a fractional flow ratio of 5.0, and the flow rate was 3.0 mL/min. The oven temperature program was started at 80 °C for 3min, 10 °C/min to 190 °C for 0.1 min, then 3 °C/min to 220 °C for 10 min. The temperature of the detector was 250 °C with the injection of 1.0 μL.

Organic Acids. The organic acid of beer was determined using a RP-HPLC method, performed using a Waters 1525 binary HPLC pump (Waters, Milford, MA) equipped with a Waters 717 plus autosampler coupled with a Waters 2478 dual λ absorbance detector at a UV absorbance of 210 nm. Purified extracts of 10 μL were separated by a Waters Atlantis dC_{18} column (5 μm, 4.6 mm × 150 mm) at the temperature of 30 °C. Elution was carried out with a mobile phase containing 20 mmol/L NaH_2PO_4 (pH adjusted to 2.7 with H_3PO_4), with a flow rate of 0.5 mL/min.

α-*Amino Acids.* The content of α-amino acid was also determined by the RP-HPLC system described above. The column used was a Hypersil reversed-phase column (4 μm, 3.9 mm × 250 mm) (Agilent, Santa Clara, CA), held at 40 °C. A two-solvent gradient was used to run the samples: solution A comprised 20 mM sodium acetate, methanol, and acetonitrile, in the proportions 1:2:2 (v/v); solution B was 20 mM sodium acetate, followed as solvent B changing from 0 to 7% (0−11 min), 7 to 12% (11−13.9 min), 12 to 15% (13.9−14 min), 15 to 34% (14−20 min), 34 to 0% (20−22 min), and 0% (22−50 min). A filtered sample of 10 μL was separated under a flow rate of 1.0 mL/min and was detected at an absorbance of 338 nm (262 nm for proline).

Dissolved Oxygen. The dissolved oxygen concentration of beer was analyzed by a portable analysis apparatus Micro Logger 3650 (Orbisphere Laboratories Neuchatel/Geneva, Switzerland), equipped with an Inpace 2000 sampler (Haffmans, Germany).

Multivariate Modeling Method. The analysis of the data was carried out using the statistical software, SPSS (Statistical Package for Social Science, version 13.0 for Windows, SPSS Inc., Chicago, IL).

Principal Component Analysis (PCA). Principal component analysis (PCA) maximizes the correlation between the original variables to form new variables that are mutually orthogonal, or uncorrelated (*16*). It is a special type of factor analysis that transforms the original set of intercorrelated variables into a new set of an equal number of independent uncorrelated variables or principal components (PCs) that are linear combinations of the original variables. The principal components are ordered in such a way that the first PC explains most of the variance in the data, and each subsequent PC accounts for the largest proportion of variability that has not been accounted for by its predecessors. Although the number of PCs equals the number of independent original variables, generally, most of the variation in the data set can be explained by the first few principal components used to represent the original observations.

Principal component methods are also used for selecting subsets of variables for regression equations. One such application is to obtain a varimax rotation of the principal components and to retain a subset of the original variables associated with each of the first few components, which are then used as predictors in the regression. Varimax rotation ensures that each variable is maximally correlated with only one principal component and a near zero association with the other components. More details on the application of these methods and others are further described in Statheropoulos et al. and Jolliffe (*17, 18*).

Multiple Regression Analysis. A bivariate correlation matrix of the data was produced to measure the association between the variables, displayed in Pearson's correlation coefficient. Before final modeling, a PCA was employed for two purposes. First, they were used for principal component regression analysis, applying the stepwise regression option in the choice of the principal components to enter the regression equation, with the sensory test as the dependent variable. Next, a varimax rotation of the principal components was used as a variable selection technique to choose the appropriate variables for inclusion in the ultimate regression model. The objective of this approach was to minimize the effect of multicollinearity on the estimation of the regression coefficients and achieve parsimony.

In this study, we combine the multiple regression method and the principal component analysis (PCA) to obtain prediction models for beer aging with the concentration of measured compounds and aging index parameters as predictor variables. Principal component analysis was used for fitting the data so that only the significant independent variables responsible for the staling extent observed could be determined. The relationships between beer aging and other measured parameters were modeled with three methods: multiple linear regression, principal component regression, and selected variables with high loadings on the rotated principal components that are used in a multiple regression analysis afterward.

RESULTS AND DISCUSSION

Analysis of Measurement and Evaluation Data. Beer samples of four commercial brands from different brewing groups were analyzed to determine their degree of staling by evaluation indices, including the sensory test score, lag time of ESR curve, DPPH scavenging amount, and TBA value. In addition, components that were indicative of staling were measured to collect the data for compounds related to beer aging; that is, staling-flavor chemicals (carbonyl compounds as typical staling compounds) and their precursors (higher alcohols, unsaturated fatty acids, organic acids, α-amino acids), as well as substances that are activated or accelerated by the staling of beer, such as oxygen.

For samples produced from different brewing groups and with different extents of aging, the staling is a result of numerous off-flavor components, which are usually measured by parameters or evaluation indices of staling. However, beer aging is partially evaluated by a simple staling parameter, representing just one or several typical flavor compounds as indicative chemicals (*19*). On the other hand, too many parameters in one model can lead to multicollinearity (*18*). Our clear goal was to find variables from these indices that were principal determinants of taste test score.

In this study, six measurement variables and four evaluation indices were analyzed by multivariate methods, in order to identify the principal variables for developing a mathematical model. The measurement and the evaluation indices data from different samples were carbonyl compounds (CC), higher alcohols (HA), unsaturated fatty acid (UFA), organic acid (OA), α-amino acid (AA), dissolved oxygen (DO), lag time of ESR curve (LT), DPPH scavenging amount (DPPH), and TBA value (TBA) as independent variables and organoleptic taste score (TS) as response variable.

Table 2. Summary of the Aging-Related Compounds and Indices of 12 Bottled Beers[a]

parameters[b]	range	mean	standard error
	components		
CC (μg/L)	322.0–2922.6	1342.6	804.2
HA (mg/L)	54.29–84.87	72.25	7.69
UFA (mg/L)	0.0496–0.291	0.1719	0.0838
OA (mg/L)	759.73–912.22	835.93	51.85
AA (mg/L)	307.91–460.40	398.92	50.16
DO (μg/L)	31–58	40.0	9.5
	indices		
LT (min)	0–120	44	44
DPPH ($\times 10^{-5}$ mol/L)	1.95–3.41	2.83	0.44
TBA	0.314–0.693	0.516	0.116
ST	2.10–3.30	2.73	0.34

[a] Samples are four brands of bottled lager beer with different aging extent, stored at room temperature. Their naturally aging time range from <1 week to 12 months. [b] The parameters are presented as follows: CC, carbonyl compounds; HA, higher alcohols; UFA, unsaturated fatty acid; OA, organic acid; AA, α-amino acid; DO, dissolved oxygen; LT, lag time of ESR; DPPH, DPPH scavenging amount; TBA, TBA value; ST, sensory test.

A summary of the characteristics of those 10 parameters is displayed in **Table 2**, whose Pearson correlation matrix is shown in **Table 3**. Sensory test scores were positively correlated with carbonyl compounds, higher alcohols, and TBA value and were negatively correlated with lag time and DPPH scavenging amount. The Pearson coefficient for the sensory test and other parameters were generally weak. The sensory test score was positively correlated with carbonyl compounds in bottled lager beer, which was expected, since most of these compounds are known staling flavor components. This indicated that a rise in carbonyl compounds concentration was associated with the corresponding sensory identification of staling. The next set of highly positive correlation between variables and organoleptic taste score were with higher alcohols and TBA value. Previous studies have reported that the concentrations of the corresponding aldehydes increase during beer aging, with the participation of oxygen (*1*), which explained the positive and high coefficients. So, the correlation coefficient of carbonyl compounds and higher alcohols are significant with an interval of confidence of 95%, which indicates a close correlation between the two parameters. **Table 3** also showed the cross correlation between the different variables. The negative correlations were high between the sensory test scores and lag time and similarly for the evaluation of DPPH scavenging amount. The two indices both measure antioxidant potential of the bottled lager beer with respect to free radical reactions, which showed a good correlation with beer aging and the two radical-related indices, compared with that of beer staling and the parameters. It is widely regarded that a large number of precursors from nonenzymatic oxidation are involved in staling of beer, such as higher alcohols, unsaturated fatty acids, organic acids, and α-amino acids (*20*). Another important factor is dissolved oxygen, which in its activated form promotes oxidation reactions (*21, 22*). Most of the relationships between the variables was consistent with those identified in previous investigations, for instance, TBA value measured by the chromogenic reaction between thiobarbituric acid and unsaturated aldehydes (*23, 24*). Accordingly the correlation coefficient for the TBA value and carbonyl compounds was positive and high.

Multiple Regression Analysis. The score of organoleptic taste test assesses the combined impact on flavor of the complex reactions between precursors and production of off flavors. Staling in bottled beer is thus the result of the combined effects of numerous reactive products of aging flavor, including an increase of off-flavor compounds and a decrease, or even the disappearance, of desirable aroma and flavor compounds.

A stepwise multiple regression procedure was carried out with the following variables: CC, HA, UFA, OA, AA, DO, LT, DPPH, and TBA. The objective was to find the predictive variables that best fit the sensory test data, in their order of magnitude of influence. The result of multiple linear regression modeling of sensory test was listed in **Table 4**.

The coefficient of multiple determination, standard regression coefficient for the model parameters, indicates the proportion of the variation in the organoleptic evaluation explained by these variables in the model. Standard regression coefficient of an independent variable is an important parameter that indicates its predictive value for the dependent variable when the other factors are kept constant. The higher its absolute value for a variable, the more significant the influence it has on the dependent variable, thus the more important its role in the regression equation. The standard regression coefficient is obtained after the coefficient of multiple linear regression has been standardized. Thus, the standard regression coefficient (or parameter estimate) has no relationship with the unit of the corresponding factor. The absolute value of the independent variable indicates its influence on the dependent variable. **Table 4a** showed that, in the multiple linear model, the highest absolute value of standard regression coefficient in magnitude among independent variables was TBA, followed by DPPH, HA, LT, OA, UFA, CC, DO, and AA, in the order from high to low. This means that, in the linear model, most of the influence on the sensory evaluation of staling was explained by the TBA value, followed by DPPH scavenging amount, higher alcohols, and so forth.

The result of fitting the regression models on the sensory test data, using the nine chemicals parameters and evaluation indices as independent variables, gave the high adjusted R value (multiple correlation coefficient) for the model of 0.993.

Principal Component Analysis. The results of stepwise multiple regression were checked for multicollinearity by examining the variance inflation factors (VIFs) of the predictor variables, as shown in **Table 4a**. VIF is a parameter that measures the extent of linear dependence of the predictor and the other independent variables. There was a significant amount of multicollinearity among the predictive variables, judging by the discriminate threshold of VIF $\geq$ 10. The presence of such strong multicollinearity indicates that the model obtained in **Table 4a** is invalid.

It is known that beer aging is a very complex process because there are many chemical groups that have been attributed involvement in staling. However, some of the indices to evaluate beer aging are set up to measure the concentration of one or several kinds of typical staling chemicals. Multicollinearity thus is caused by the affinitive linear correlation between variables. As discussed above, the unsaturated aldehydes in carbonyl compounds are the chromophore of the TBA method, resulting in a high correlation coefficient between these two related variables and subsequently the likely prospect of multicollinearity in the multiple regression analysis. The objective of principal component analysis before modeling was to obtain parsimonious prediction models (i.e., models that depend on as few variables as possible) for aging with compounds or evaluation indices data as predictor variables.

To counter the problem of multicollinearity and achieve model parsimony, a variable selection method based on principal component regression analysis was applied to yielded predomi-

Table 3. Pearson Correlation Matrix of Variables[a] Related to Beer Aging

	CC	HA	UFA	OA	AA	DO	LT	DPPH	TBA	ST
CC	1	0.648[b]	−0.013	0.544	−0.047	−0.418	−0.705[c]	−0.782[c]	0.760[c]	0.711[c]
HA		1	0.203	0.439	−0.097	−0.631[b]	−0.586[b]	−0.430	0.712[c]	0.645[b]
UFA			1	0.122	0.116	−0.459	0.160	0.014	0.021	−0.148
OA				1	0.089	−0.149	−0.286	−0.344	0.209	0.472
AA					1	0.065	0.174	0.058	−0.075	−0.306
DO						1	0.642[b]	0.580[b]	−0.734[c]	−0.425
LT							1	0.712[c]	−0.809[c]	−0.722[c]
DPPH								1	−0.864[c]	−0.638[b]
TBA									1	0.578[b]
ST										1

[a] The parameters are presented as follows: CC, carbonyl compounds; HA, higher alcohols; UFA, unsaturated fatty acid; OA, organic acid; AA, α-amino acid; DO, dissolved oxygen; LT, lag time of ESR; DPPH, DPPH scavenging amount; TBA, TBA value; ST, sensory test. [b] The correlation coefficient is significant with an interval of confidence of 95%. [c] The correlation coefficient is significant with an interval of confidence of 99%.

Table 4. Multiple Linear Regression Models for Prediction of Organolepic Taste Evaluations

(**a**) original[b]

predictors[a]	constant	AA	DO	CC	UFA	OA	LT	HA	DPPH	TBA
standard regression coefficient[d]		−0.063	0.072	−0.093	−0.175	−0.446	−0.689	1.540	−1.926	−2.523
estimated regression coefficient	8.719	0.000	0.003	-3.9×10^{-5}	−0.708	−0.003	−0.005	0.068	−1.476	−7.375
standard error	0.409	0.000	0.003	0.000	0.180	0.000	0.000	0.003	0.076	0.367
variance inflation factors (VIF)		1.164	7.622	6.738	3.016	3.147	5.874	8.697	15.040	24.020
adjusted R^2 of model	0.993									

(**b**) after PCA[c]

predictors	constant	PC7	PC8	PC3	PC5	PC6	PC4	PC2	PC1	PC9
standard regression coefficient[d]		−0.077	0.113	−0.157	−0.269	−0.295	0.344	0.406	−0.480	−0.531
estimated regression coefficient	2.733	−0.026	0.038	−0.053	−0.091	−0.100	0.117	0.138	−0.163	−0.180
standard error	0.008	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
variance inflation factors (VIF)		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
adjusted R^2 of model	0.993									

[a] The predictors are presented as follows: CC, carbonyl compounds; HA, higher alcohols; UFA, unsaturated fatty acid; OA, organic acid; AA, α-amino acid; DO, dissolved oxygen; LT, lag time of ESR; DPPH, DPPH scavenging amount;, TBA, TBA value; with sensory test score as dependent variable. [b] The analysis is performed on the data of original variables. [c] The analysis is performed on the principal components as variables, after PCA of original variables. [d] Otherwise known as equation parameter estimate.

Table 5. Rotated Principal Component Loadings of Variables of Measured Compounds Parameters and Evaluating Indices

predictors[a]	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
CC	−0.710	0.346	−0.015	0.359	−0.018	−0.178	0.079	0.456	−0.001
HA	−0.302	0.887	0.130	0.240	−0.060	0.137	−0.145	0.062	−0.006
UFA	0.038	0.078	0.986	0.058	0.061	0.058	−0.095	−0.001	2.07×10^{-5}
OA	−0.162	0.163	0.059	0.968	0.055	−0.048	−0.002	0.040	−0.002
AA	0.032	−0.043	0.054	0.047	0.995	0.040	0.015	−0.003	0.000
DO	0.463	−0.352	−0.411	0.018	0.036	0.259	0.650	0.035	−0.003
LT	0.593	−0.292	0.146	−0.125	0.109	0.675	0.231	−0.069	−0.004
DPPH	0.964	−0.070	0.000	−0.179	0.027	0.094	0.133	−0.003	0.075
TBA	−0.827	0.458	0.011	−0.008	−0.017	−0.205	−0.201	−0.008	0.151
eigenvalue	4.561	1.361	1.124	0.871	0.500	0.319	0.181	0.060	0.022
% of variance	50.683	15.127	12.485	9.677	5.554	3.546	2.010	0.671	0.246
cumulative %	50.683	65.810	78.296	87.973	93.527	97.073	99.083	99.754	100.000

[a] The variables are presented as follows: CC, carbonyl compounds; HA, higher alcohols; UFA, unsaturated fatty acid; OA, organic acid; AA, α-amino acid; DO, dissolved oxygen; LT, lag time of ESR; DPPH, DPPH scavenging amount; TBA, TBA value.

nant variables for the model. These variables would be used as new predicting variables in multiple regression analyses to obtain the final models of beer aging.

First, the predictor variables were transformed by PCA into nine principal components of equal number, to select a smaller number of components that would explain most (typically 60−90%) of the total variation in the predictor variables.

The nine original variables were then evaluated for influence by principal component analysis. After the transformation, varimax rotation was used to maximize the loading of a predictor variable on one component. In general, application of PCA procedures followed by a varimax rotation produced a ranked series of factors. **Table 5** summarizes the results of the varimax rotation on the nine principal components together with the amount of variance explained by each component respectively. The higher the loading of a variable, the more that variable contributes to the variation accounted for by that particular principal component. In practice, only loadings with absolute values greater than 50% are selected for the principal component interpretation. A principal component with an eigenvalue ≥ 1, is usually considered as being statistically significant (*18*). The variables affected different contribution to each PC, with the

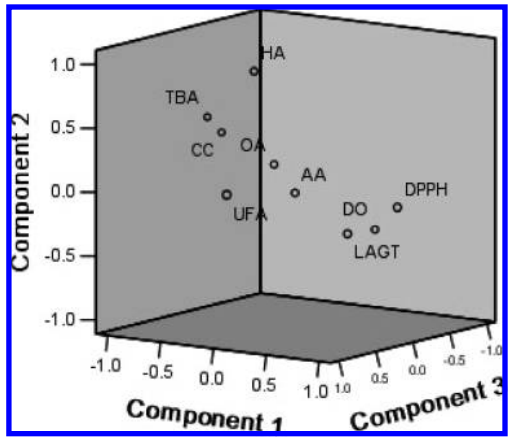

Figure 1. Rotated component plot of first three factors after principal components analysis (PCA) of nine predictors: CC, carbonyl compounds; HA, higher alcohols; UFA, unsaturated fatty acid; OA, organic acid; AA, α-amino acid; DO, dissolved oxygen; LT, lag time of ESR; DPPH, DPPH scavenging amount; TBA, TBA value.

Table 6. Coefficients of Multiple Linear Regression Models Fitting with Aging Parameters after PCA

predictors	constant	HA	TBA	DPPH
standard regression coefficient		0.932	−1.143	−1.224
estimated regression coefficient	4.141	0.041	−0.938	−3.342
standard error	1.332	0.014	0.332	1.630
variance inflation factors		2.790	5.408	8.943

loadings of the first three factors (eigenvalues ≥ 1) plotted in **Figure 1**. It was noted that all variables dispersed separately on the three-axis chart. The indices of higher alcohols (HA), TBA value (TBA), carbonyl compounds (CC), unsaturated fatty acid (UFA), and DPPH scavenging amount (DPPH) were observed to have higher loadings for their principal components than the other original variables.

Table 5 shows the rotated principal component loadings of variables. It was noted that the first three principal components accounted for 78.3% of the total variation. In the data for beer differing in quality and extent of aging, the first principal component PC1 accounted for 50.7% of the total variation. It is loaded heavily on DPPH, TBA, and CC with little contributions from other variables. The second PC, which accounted for about 15.1% of the total variation, indicated the importance in variation in the compounds measure by the index HA. The third PC loaded heavily on UFA, explaining 12.5% of the variation. The remaining variables were represented by the rest of the principal components accounting successively for less of the total variation. Principal components four, five, six, and seven loaded heavily on factors OA, AA, LT, and DO, respectively. The last two principal components PC8 and PC9 were contributed by original variables with lower loadings of original variables, compared to the first seven PCs. Of all variables, CC explained most on PC8, and TBA did most on PC9.

Model Fitting. A stepwise regression analysis was then carried out, with principal components as independent variables, to determine which of the original independent variables are most predictive of variation of the sensory test score (**Table 4b**). The main objective of this data processing step was to select a subset of the variables that provides the best prediction equation for modeling of beer staling using the multiple regression method. The selected original independent variables were those with high loadings, associated with each of the

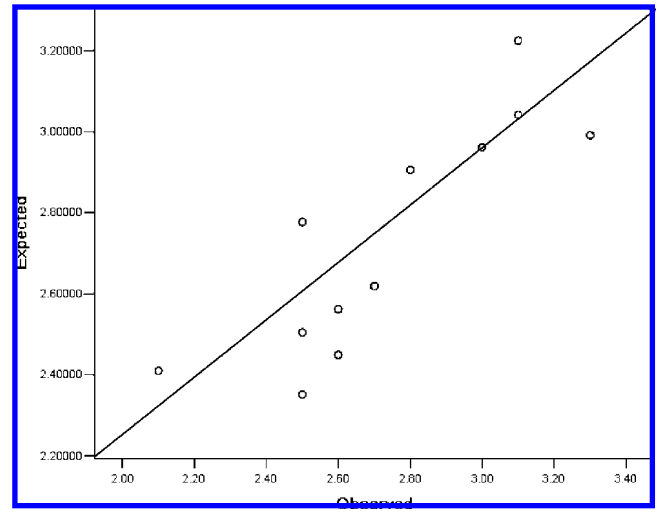

Figure 2. Comparison between sensory test scores expected by the proposed model and those observed for bottled lager beer. The beer samples differ by manufacturing brewery and aging time. Sample number $n = 12$.

principal components that were included in the regression equation and that had high coefficients of determination. From **Tables 4b** and **5**, the PCs in regression were matched to independent variables with both high standard regression coefficients of PCs and heavy loadings in these PCs. The first three PCs with the largest standard regression coefficients were chosen, that being PC9, PC1, and PC2 in the order from largest to smallest. Then, TBA was selected from PC1 and PC9, DPPH was selected from PC1, and HA from PC2, according to the loadings of the original variables in the PCs. These three variables were then used as the primary predictor variables in a subsequent regression analysis, and the following model was derived:

$$ST = 4.141 + 0.041[HA] - 0.938[TBA] - 3.342[DPPH] \quad (2)$$

The multiple regression model using the three variables, HA, TBA, and DPPH, had an adjusted R^2 of 0.617. The coefficients of the regression are listed in **Table 6** and were all statistically significant ($P < 0.05$). The distributions of the residuals were approximately normal, with zero means and no obvious serial correlation, which represented an indication of a valid model fit. The variance inflation factors of the coefficients were also shown, with 2.790 for higher alcohols, 5.408 for TBA, and 8.943 for DPPH, which indicated a weak level of multicollinearity in the new model. Fitting model with reduced multicollinearity resulted in an adjusted R^2 of 0.62, lower but closer in practice compared with **Table 4a** of the regression with the original variables.

The correlation between the predicted and calculated sensory test scores of bottled lager beer is shown in **Figure 2**, with eq 2 plotted against the standard expected values for sensory test score and the corresponding observed values. The scatterplot diagram shows that most observed values appear to fit the predicted values closely. The discrepancies between the predicted and the observed values could be due to factors that were not considered in the study, such as bitterness from hop, the Maillard products with thermal steps, the inevitable error of subjective judgment of organoleptic test, and so forth. On the other hand, the more sets of samples there are in the regression, the closer the model will be to the actual beer aging, as prospected in the future investigation.

Among the three predictor variables of the fitted model, higher alcohols were the first predominant factor, followed by TBA value, and DPPH scavenging amount. It has been reported that free radicals are one of the most important substance groups for oxidation during beer aging, with hydroxyl radicals activated for beer staling by reacting with ethanol (*25*). It has been suggested that these reactive oxygen species (ROS) may react by a similar mechanism with the main higher alcohols, producing the majority of carbonyl compounds in aged beer. TBA is a measurement index of typical unsaturated aldehydes that are the alkedienals, in carbonyl compounds, which account for the undesirable sensory profile of aged beer. As for the DPPH scavenging method, the capability to scavenge DPPH free radicals is counted by the antioxidative potential of beer, which is another influence in beer aging, as opposed to oxidation. Therefore, the complicated course of aging can be discussed in terms of oxidation and antioxidation. In the model in eq 2, higher alcohols and TBA value were parameters of oxidation aspect, while DPPH scavenging amount was the index reflecting the antioxidative potential of the beer.

In summary, the study showed that, in natural aging of bottled lager beer from Chinese breweries, the indices of higher alcohols and TBA value together with DPPH scavenging ability can be adequately used to estimate the extent of aging. The study also showed that PCA, combined with varimax rotation, provides a good method for variable selection to identify the most appropriate subset of variables to use for modeling beer aging. This statistical process mitigates the problem of multicollinearity and achieves model parsimony.

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