Influencing factors of hydrogen bonding intensity in beer

Chunfeng Liu · Jianjun Dong · Xiangsheng Yin · Qi Li · Guoxian Gu

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Abstract The hydrogen bonding was prone to be formed by many components in beer. Different sorts of flavor substances can affect the Chemical Shift due to their different concentrations in beer. Several key factors including 4 alcohols, 2 esters, 6 ions, 9 acids, 7 polyphenols, and 2 gravity indexes (OG and RG) were determined in this research. They could be used to investigate the relationship between hydrogen bonding intensity and the flavor components in bottled larger beers through the Correlation Analysis, Principal Component Analysis and Multiple Regression Analysis.

C. Liu · J. Dong
National Key Laboratory of Beer Biological Fermentation Engineering, Tsingtao Brewery Co. LTD,
Qingdao 266101 Shandong Province, People's Republic of China

C. Liu
e-mail: lcfidhr@126.com

J. Dong
e-mail: Dongjianjun@tsingtao.com.cn

C. Liu · Q. Li · G. Gu
Key Laboratory of Industrial Biotechnology,
Ministry of Education, Jiangnan University,
Wuxi 214122 Jiangsu Province, People's Republic of China

G. Gu
e-mail: gxgu@jiangnan.edu.cn

C. Liu · Q. Li · G. Gu
Laboratory of Brewing Science and Engineering,
Jiangnan University,
Wuxi 214122 Jiangsu Province, People's Republic of China

X. Yin
Cargill Malt,
McGinty Road West, MS 135,
Wayzata, MN 55391, USA
e-mail: Xiang_Yin@cargill.com

Q. Li (✉)
NO.1800, Lihu Road,
Wuxi, Jiangsu, People's Republic of China
e-mail: liqi@jiangnan.edu.cn

Results showed that ethanol content was the primary influencing factor, and its correlation coefficient was 0.629 for Correlation Analysis. Some factors had a positive correlation with hydrogen bonding intensity, including the content of original gravity, ethanol, isobutanol, Cl^- , K^+ , pyruvic acid, lactic acid, gallic acid, vanillic acid, and Catechin in beer. A mathematic model of hydrogen bonding Chemical Shift and the content of ethanol, pyruvic acid, K^+ , and gallic acid was obtained through the Principal Component Analysis and Multiple Regression Analysis, with the adjusted R^2 being 0.779 ($P=0.001$). Ethanol content was proved to be the most important factor which could impact on hydrogen bonding association in beer by Principal Component Analysis. And then, a multiple non-linearity model could be obtained as follows: *hydrogen bonding association intensity* (CS) = $4.917 + 0.002[E] - 0.006[P] + 5.141E - 8[K^2] + 5.378E - 5[G \times P] + 2.428E - 5[K \times P] + 2.369E - 5[E^2P] - 1.63E - 5[G^3] - 2.207E - 5[P^3] - 5.247E - 7[K^2P] - 3.037E - 5[E^2K]$. The average error was 1.23 % in the validated experiment.

Keywords Beer · Hydrogen bonding · NMR · Chemical shift

Abbreviations

NMR	Nuclear magnetic resonance
PCA	Principal component analysis
MRA	Multiple regression analysis
HPLC	High performance liquid chromatography
AAS	Atomic absorption spectrometry
HS-GC	Headspace gas chromatography
FID	Flame ionization detector
RP-	Reverse phase-high performance liquid
HPLC	chromatography
OA	Oxalic acid
TA	Tartaric acid
CS	Chemical shift
IA	Isoamyl alcohol
OG	Original gravity

AD	Alcohol degree
RG	Real gravity
PA	Pyruvic acid
EA	Ethyl acetate
MA	Malic acid
LA	Lactic acid
SA	Succinic acid
AA	Acetic acid
KA	Ketoglutaric acid
TA	Tartaric acid
CA	Citric acid
GA	Gallic acid
VA	Vanillic acid
P-CA	P-coumarilic acid
FA	Ferulic acid

Introduction

Hydrogen bond is one of the most important non-covalent interactions which attracts a lot of attentions in beer quality control research (Pejov et al. 2006). Various types of chemicals including alcohols, acids, saccharides, phenols, esters, and salts which presented in beer affect the flavor of beer, and they force the hydrogen bond formation. Studying the chemical composition and hydrogen bond of beer were valuable for beer quality assessment and new beer products development (Iola et al. 2002; Verhagen 2010).

High Performance Liquid chromatography (HPLC), Gas Chromatography (GC) and Atomic Absorption Spectrometry (AAS) were often used in the analysis of beer flavor compounds. However, these methods requires a crucial sample preparation before analysis. The Nuclear Magnetic Resonance (NMR) spectroscopy could rapidly detect the hydrogen bonds of different flavor substances in beverages with simple sample preparation (Iola et al. 2002). NMR is based on the magnetic moments of some atomic nuclei (e.g. ^1H). The exact value of the radiofrequency absorbed exquisitely depends on the chemical environment of the nucleus, so that each compound gives a well-defined set of ^1H absorption peaks (Chemical Shift) (Harris 1983; Derome 1987). The ^1H -NMR-based data can be used in the quality control or flavor metabolites analysis of beer (Lachenmeier et al. 2005; Rodrigues et al. 2010; Duarte et al. 2004; Rodrigues et al. 2011;), wine (Skogerson et al. 2009; Ramos and Santos 1999; Kosir and Kidric 2001), soy sauce (Ko et al. 2009), vinegar (Boffo et al. 2009), Coffee and tea (Charlton et al. 2002; Lee et al. 2010; Bosco et al. 1999), Olive oils (Sacchi et al. 1996), Fruit juices (Spraul et al. 2009; Le Gall et al. 2001; Gil et al. 2000), and so on.

It has been reported that hydrogen bonding association degree in pure water, beer, whiskey, japanese sake, and shochu (white liquor) was studied on the basis of ^1H -NMR

chemical shift (Nose and Hojo 2006; Hindman 1966; Muller 1965; Lachenmeier et al. 2005). Chemical Shift (CS) in low field corresponds to the strengthening of hydrogen bonding structure in the entire solution (Nose and Hojo 2006). It is well known that, water-ethanol hydrogen bonding structure is strengthened as the presence of some chemical components (Nose and Hojo 2006). The strength of hydrogen bond mainly depends on the nature of the proton donor and acceptor atoms. Additionally, it is also related to the substitution of these atoms (Ramasami and Ford 2010; Alkorta et al. 2002; Kawahara et al. 2003). The organic acids or (poly) phenols (proton donors), and conjugate base anions of weak acids (proton acceptors) are the main components to reduce organoleptical stimulation of alcoholic beverages (Nose and Hojo 2006). A few metal salts (MgCl_2 and KF) have a strengthening effect on the hydrogen-bonding structure. The OH proton chemical shifts from strong acids are related to the sizes and charges of the ions. Acids (H^+ and HA , undissociated acids) and bases (OH^- and A^- , conjugate-base anions from weak acids) can strengthen the structure of hydrogen-bonding via increasing concentration of solutes. Hydrogen bond donors as well as acceptors seem to cause the intimate (or tight) interaction between H_2O and ethanol molecules in alcoholic beverages (Nose et al. 2004; Fileti et al. 2004).

Some proposed NMR-based procedures used to control the quality or flavor of beers by correlating NMR data (hydrogen bond intensity) have been studied (Iola et al. 2002; Lachenmeier et al. 2005; Rodrigues et al. 2010; Duarte et al. 2004; Rodrigues et al. 2011; Almeida et al. 2006). ^1H -NMR-PCA data of beers with different types and origin has shown a good separation of samples (Duarte et al. 2004). The chemical changes occurring in a forced aging beer can be well monitored by the hydrogen bond intensity (Rodrigues et al. 2011).

Therefore, it is important to scientifically demonstrate the concrete relationship between hydrogen-bonding association and beer components using available statistical methods.

Multiple Regression Analysis (MRA) is one of the most widely use methodologies for expressing the dependence of a response variable on several independent (predictor) variables, and it need remove the multicollinearity and redundant independent variables (Siebert 2001). One such method is principal component analysis (PCA). The new variables from the PCA are more valid to use as predictors in a regression equation (Pages and Tenenhaus 2001). Subsequently, they allow the identification of the primary predictors with minimal multicollinearity.

The objectives of this study was to find out the relationship between beer flavor substances and ^1H -NMR CS through Correlation Analysis, MRA and PCA, and to develop a mathematical model which could predict the decisive factors of hydrogen bonding intensity in beer.

Materials and methods

Samples

The 35 bottled lager beer samples used for this study were purchased in Chinese market and produced by different Chinese brewery companies (Table 1). All of the beer samples were lager beers, and tasted like Miller beers which were much lighter than Pilsner beers. The samples included the following: (a) Tsingtao, from Tsingtao Brewery Co. Ltd. (Shanghai); (b) Budweiser, from Budweiser Wuhan International Brewing Co. Ltd. (Wuhan); (c) Suntory, from Suntory Brewing Co. Ltd. (Shanghai); (d) Snowbeer, from Huarun Snow Brewing Co. Ltd. (Suzhou); (e) Carlsberg, from Carlsberg Brewing Co. Ltd. (Huizhou); (f) Blue Ribbon, from Blue Ribbon Brewing Co. Ltd. (Gushangdong); (g) Yanjing, from Yanjing Brewing Co. Ltd. (Beijing); (h) Reep, Shanghai Asia Pacific Brewery Co. Ltd. (Shanghai); (i) Redrock, Inbos Redrock Brewing Co. Ltd. (Zhejiang). The raw materials used for the analysed beers were listed in Table 1. All the samples were stored at 4 °C before analysis.

Standards and reagents

Standards were purchased from Sigma (St. Louis, MO) and were of the highest purity available, including ethyl acetate, propanol, isobutanol, isoamyl acetate, isoamyl alcohol, oxalic acid, tartaric acid, pyruvic acid, malic acid, ketoglutaric acid, lactic acid, acetic acid, citric acid, succinic acid, gallic acid, vanillic acid, catechin, caffeic acid, P-coumarilic acid, ferulic acid, and syringic acid. The other chemicals and solvents were of the highest commercial grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

GC analysis of higher alcohols and volatile esters

The higher alcohols and volatile esters concentrations in beer were determined by Headspace Gas Chromatography (HS-GC) (Liu et al. 2008). Briefly, the reaction system contained NaCl (1.8 g), beer sample (4 ml), and 30 mg/l 3-heptanone (1 ml) as an internal standard solution. The analysis was performed using a Shimadzu GC-2010 gas chromatograph (Shimadzu, Japan) coupled with a FID detector. Separation was carried on a CP-WAX52CB capillary column (30 m×0.32 mm×0.52 µm, Shanghai Bioengineering Co. Ltd., Shanghai, China). Nitrogen was used as carrier gas with a flow rate of 3.0 ml/min. The column oven temperature was started at 40 °C for 2 min, and ramped at 10 °C/min to 180 °C with a 4 min hold. The temperature of gasification oven, detector and

Table 1 Characteristics of 35 kinds of beer samples

No.	Raw materials	Type ^a
1	Water, barely malt, rice, hop, yeast	Draft beer
2	Water, barely malt, hop, yeast	Pasteurimd beer
3	Water, barely malt, hop, yeast	Pasteurimd beer
4	Water, barely malt, rice, hop, yeast	Pasteurimd beer
5	Water, barely malt, hop, yeast	Pasteurimd beer
6	Water, barely malt, hop, yeast	Draft beer
7	Water, barely malt, hop, yeast	Draft beer
8	Water, barely malt, rice, hop, yeast	Pasteurimd beer
9	Water, wheat malt, hop, yeast	Pasteurimd beer
10	Water, barely malt, rice, hop, yeast	Pasteurimd beer
11	Water, barely malt, rice, hop, yeast	Draft beer
12	Water, barely malt, rice, hop, yeast	Draft beer
13	Water, barely malt, rice, hop, yeast	Pasteurimd beer
14	Water, barely malt, rice, hop, yeast	Draft beer
15	Water, Barely malt, hop, yeast	Pasteurimd beer
16	Water, barely, barely malt, hop, yeast	Pasteurimd beer
17	Water, barely malt, hop, yeast	Draft beer
18	Water, barely malt, rice, hop, yeast	Pasteurimd beer
19	Water, barely malt, rice, hop, yeast	Draft beer
20	Water, barely malt, hop, yeast	Pasteurimd beer
21	Water, barely malt, rice, hop, yeast	Pasteurimd beer
22	Water, barely malt, rice, hop, yeast	Pasteurimd beer
23	Water, wheat malt, rice, hop, yeast	Draft beer
24	Water, barely malt, hop, yeast	Pasteurimd beer
25	Water, barely malt, hop, yeast	Draft beer
26	Water, barely malt, rice, hop, yeast	Draft beer
27	Water, barely malt, rice, hop, yeast	Pasteurimd beer
28	Water, barely malt, hop, yeast	Pasteurimd beer
29	Water, barely malt, hop, yeast	Pasteurimd beer
30	Water, barely malt, hop, yeast	Draft beer
31	Water, barely malt, rice, hop, yeast	Pasteurimd beer
32	Water, barely malt, rice, hop, yeast	Pasteurimd beer
33	Water, barely malt, rice, hop, yeast	Pasteurimd beer
34	Water, barely malt, rice, hop, yeast	Pasteurimd beer
35	Water, barely malt, hop, yeast	Pasteurimd beer

^a All of the beer samples are lager beers, and they are more lighter than Pilsner beers, just like Miller beers

transmission line was 200 °C, 250 °C, and 130 °C, respectively.

HPLC analysis of organic acid

The organic acid concentration in beer was determined by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) at OD₂₁₀ as described by Liu et al. (2008).

Adding 5 ml degassed beer sample, 1 ml zinc sulfate (30 %, w/v) and 1 ml potassium ferrocyanide solution

(15 %, w/v) into a volumetric flask (25 ml), and make up to the scale using ultrapure water, mix well finally. And then, the solution was filtered by filter paper and Sep-Pak C18 Cartridge (Waters) successively. Purified sample (10 μ l) was prepared using a Waters Atlantis dC₁₈ column (5 μ m, 4.6 mm $\times$ 150 mm) at 30 °C. Elution was carried out in a mobile phase containing 20 mM NaH₂PO₄ (pH adjusted to 2.7 with H₃PO₄), with a flow rate of 0.5 ml/min.

AAS analysis of inorganic ions

The inorganic ion concentrations in beer were determined by AAS (Dolores et al. 2000; Hergenreder 1991; Cabrera et al. 1994). The analysis was performed using Spectra AA 220 (Varian, U.S.A), and the absorption spectrum for Na, K, Ca and Mg was at 589 nm, 766.5 nm, 422.7 nm and 285.2 nm, respectively.

Analysis of alcohol content, original gravity and real gravity

Fifty grams beer sample and 25 ml distilled water were added into a 250 ml distillation flask. 5 ml distilled water was added into a 50 ml volumetric flask, and was linked to the other ends of distillatory. Heating up the distillation flask till the distillate reached about 45 ml, and adjusting it to 50 $\pm$ 0.1 g. Finally, mensurating its specific gravity by pycnometer at 20 °C.

HPLC analysis of phenolic compounds

Seven phenolic compounds in 35 kinds of beers were analysed with the Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) (Waters 600, Waters Platform ZMD4000, Breeze workstation) and the Waters Atlantis dC₁₈ column (5 μ m, 4.6 mm $\times$ 150 mm) with a gradient elution system. Phenolic compounds were measured at 254 nm and 280 nm with a dual λ absorbance detector (Waters 2487), and the injection volume was 20 μ l. Mobile phases were 0.1 % glacial acetic acid in distilled water (solvent A) and 0.1 % glacial acetic acid in methanol (solvent B). All solvents for HPLC analysis were HPLC grade (Merck, German). Total HPLC run time was 60 min and the flow rate was 1 ml/min (García et al. 2004)

Analysis of ¹H-NMR chemical shift

One-dimensional NMR spectra of the degassed beer was recorded on Avance AV-500 spectrometer equipped with a 5 mm QNP (13C-1H-31P-19F) probe, operating at 500.130 MHz for proton analysis. The DSS ((CH₃)₃Si-(CH₃)₃-SO₃Na) was used as internal standard and the analysis was carried out at 18 °C.

Statistical analysis

The data was analyzed using the SPSS statistical software. (SPSS Inc., Chicago, IL, USA).

Correlation analysis

Correlation Analysis reflects the stochastic dependence of uncertainty changes by the dualistic phenomenal numerical values. A bivariate correlation matrix was conducted to measure the association between variables, which displayed in Pearson's correlation coefficient (*r*). The relation was entirely linear correlation if the *r* equaled to 1, positive correlation if *r*>0, and negative correlation if *r*<0, respectively (Statheropoulos et al. 1998).

Principal component analysis (PCA)

PCA maximizes the correlation between original variables to form new variables which were mutually orthogonal, or uncorrelated (Daems and Delvaux 1979). The main goal of this procedure was to find out the relationships among different parameters and to detect possible clusters within objects and variables. It was a special type of factor analysis that transformed original inter-correlated variables into a new set of independent uncorrelated variables or principal components (PCs) (Iola et al. 2002; Statheropoulos et al. 1998; Jolliffe 1986). The principal components are ordered in such a way: the first PC explains most of the data variance, and each subsequent PC accounts for the largest proportion of variability. Although the PCs number equal to independent original variables number, most of the variation in a data set could generally be explained by the first few principal components used to represent the original observations.

Multiple regression analysis (MRA)

A bivariate correlation matrix of data was produced to measure the association between variables, displayed in Pearson's correlation coefficient (Liu et al. 2008). Before final modeling, a PCA was employed for two purposes. First, 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. Varimax rotation ensured that each variable was maximally correlated with only one principal component. Next, the PCA were used for principal component regression analysis, and the stepwise regression option was applied to choose the dependent variable in the regression equation. The objective of this approach was to minimize the effect of multicollinearity on the regression coefficients estimation and achieve parsimony.

Table 2 The content of alcohols, esters, inorganic ions, organic acid, phenolic compounds and alcohol degree, real gravity, original gravity of beer samples (mg/l)

No.	EA	Propanol	Iso-butanol	Iso-amyl acetate	Isoamyl alcohol	AD (%vol)	OA	TA	PA	MA	KA
1	14.33±2.01	8.22±2.12	11.06±1.34	2.33±2.05	69.91±1.78	4.22±2.08	0.66±2.41	121.60±1.65	12.69±3.00	14.06±1.09	2.43±1.05
2	8.87±1.97	10.26±2.32	18.02±1.52	1.13±2.18	80.42±2.12	3.43±2.41	0.35±1.82	37.11±2.02	16.81±2.79	4.72±1.52	1.87±1.12
3	10.30±2.31	8.40±1.98	10.23±2.04	1.09±1.97	57.44±2.17	3.57±2.32	0.87±2.22	76.33±3.12	14.19±3.12	6.33±1.62	1.94±1.42
4	9.35±2.41	5.71±2.02	5.67±2.32	1.67±1.83	37.83±2.41	3.43±2.12	0.71±1.72	113.78±2.42	12.44±3.13	10.30±1.32	1.37±1.23
5	9.86±2.04	9.93±2.12	7.01±1.72	1.76±1.92	52.22±2.32	4.00±2.09	0.67±1.32	133.04±2.22	9.51±2.98	17.12±2.02	3.81±1.14
6	9.90±2.12	8.28±2.21	10.16±1.96	1.41±1.75	61.99±2.12	3.85±1.92	0.31±1.62	126.45±2.37	16.43±2.97	14.94±2.17	2.58±1.53
7	11.18±1.97	8.00±1.89	10.59±2.22	2.31±1.82	51.80±2.53	3.43±1.18	0.45±1.53	94.07±2.28	6.78±2.86	8.07±2.28	1.95±1.42
8	12.14±2.21	6.62±2.42	8.91±2.53	2.20±1.61	51.80±1.76	3.78±2.32	0.43±1.72	124.52±2.19	5.47±3.12	14.01±2.09	1.38±2.12
9	15.59±2.11	9.67±2.02	11.89±1.32	3.41±2.12	63.23±2.40	3.85±2.12	1.09±2.12	127.55±2.00	8.47±3.13	11.89±2.14	2.86±1.31
10	16.37±2.14	9.88±1.58	15.51±2.42	3.82±2.02	71.96±2.15	4.29±2.23	0.66±1.58	101.32±2.12	8.16±3.04	11.94±2.22	2.31±1.28
11	11.87±2.42	6.84±2.52	7.12±2.41	1.80±1.72	47.77±2.10	3.86±2.12	0.96±2.03	126.99±2.61	9.44±2.92	11.70±1.93	1.62±1.39
12	18.33±2.01	10.02±1.78	13.08±2.15	3.95±1.83	82.97±2.30	4.94±1.72	0.65±1.52	137.19±2.52	14.85±3.41	14.04±2.13	3.76±1.25
13	11.19±1.49	10.91±2.38	7.87±2.06	2.30±1.91	59.57±3.02	3.92±1.98	0.69±1.72	140.07±2.57	9.82±3.15	19.13±2.43	3.92±1.40
14	11.56±2.22	11.08±2.18	7.91±2.37	2.16±1.74	61.20±2.72	3.92±2.02	0.73±1.81	130.05±2.64	9.16±3.00	18.19±2.32	3.19±1.51
15	12.73±1.92	11.93±1.88	8.30±2.28	2.54±1.85	66.50±2.37	3.92±1.87	0.78±1.92	147.14±2.81	8.95±3.26	18.67±2.05	3.78±1.64
16	11.06±2.13	11.43±1.86	11.61±2.19	1.69±1.66	58.43±2.46	3.71±1.72	0.25±1.43	110.72±2.66	11.79±3.73	10.90±2.13	3.02±1.43
17	17.51±2.22	7.94±2.28	15.16±2.01	3.89±2.14	84.74±2.58	3.00±2.06	0.29±1.52	106.39±2.58	6.31±3.03	11.13±2.42	2.71±1.72
18	15.19±2.34	8.81±2.56	8.38±2.31	2.09±1.87	55.46±2.39	4.07±2.27	0.76±1.67	110.61±2.61	11.77±2.13	12.40±2.53	1.90±1.81
19	7.06±2.41	13.34±2.46	20.66±1.41	1.26±2.22	111.44±2.70	4.36±2.22	0.17±1.55	65.25±2.71	19.64±3.10	6.78±2.43	4.43±1.79
20	9.10±2.42	8.21±1.96	13.64±2.51	1.08±1.94	69.40±2.65	3.85±1.78	0.13±1.56	82.99±2.36	13.87±2.94	8.90±2.04	1.90±1.66
21	14.60±2.12	9.77±2.19	15.33±2.63	2.68±2.15	80.76±2.34	4.58±1.96	0.36±1.88	116.66±2.07	15.13±3.15	8.22±2.25	1.85±1.58
22	5.96±3.01	13.39±1.98	19.26±2.44	0.90±1.72	108.43±2.67	4.14±1.61	0.84±1.72	61.14±2.29	21.77±3.26	7.27±2.36	4.55±1.42
23	13.28±2.02	6.86±2.55	9.16±2.31	1.70±1.82	64.21±1.98	3.85±1.72	0.56±1.88	123.28±2.45	9.79±3.17	16.28±2.47	2.77±1.38
24	18.25±1.87	7.48±1.58	15.17±1.53	3.97±2.42	81.49±2.47	4.36±1.85	0.52±2.01	118.97±2.56	16.12±3.28	12.44±1.43	2.93±1.46
25	13.89±2.02	10.55±1.79	10.37±2.51	2.32±2.33	74.54±1.99	4.00±2.12	0.96±1.63	156.97±2.73	8.90±3.29	18.11±1.79	3.92±1.59
26	13.56±1.92	13.20±2.59	14.32±1.74	2.06±1.75	71.45±2.39	4.36±2.00	0.25±1.74	125.87±2.82	11.07±3.33	14.32±2.32	2.10±1.40
27	22.86±2.43	15.53±2.41	21.12±2.41	5.99±1.84	124.48±2.46	4.94±1.92	1.09±1.85	189.40±2.91	13.95±3.41	16.05±2.41	4.18±1.54
28	11.82±2.34	7.80±2.19	10.17±2.42	2.08±1.75	66.14±1.87	3.15±2.42	0.32±1.66	81.24±2.77	9.80±3.53	11.41±2.16	2.38±2.01
29	12.93±2.52	20.54±2.31	17.25±2.41	1.78±1.96	100.80±2.63	5.16±1.97	0.58±1.58	195.84±2.66	19.11±3.26	7.95±2.45	5.98±2.22
30	10.77±2.25	8.87±1.92	11.03±1.87	1.61±1.87	73.08±1.83	2.93±2.17	0.65±1.69	54.51±2.46	8.85±3.27	2.81±2.18	1.41±1.69
31	5.43±1.63	7.39±2.73	9.45±2.21	0.64±1.88	57.86±2.62	3.29±2.25	0.13±2.42	88.47±2.37	11.16±3.25	2.84±2.27	1.61±1.51
32	10.0±1.88	9.44±2.23	13.90±2.31	1.73±2.42	66.44±1.88	3.85±2.16	0.29±2.23	108.95±2.68	14.16±3.13	4.80±2.38	2.45±1.47
33	6.78±2.21	6.62±1.93	6.06±2.21	0.71±2.14	41.35±2.72	2.51±1.94	0.33±1.34	103.14±2.48	10.57±3.24	6.73±1.69	3.70±1.39
34	6.89±2.55	7.15±2.24	5.05±2.40	0.55±1.82	37.88±2.52	2.72±1.82	0.33±1.54	93.49±2.39	9.06±3.35	8.99±2.52	2.63±1.63
35	6.99±2.05	6.23±2.33	5.54±2.06	0.68±1.72	35.84±2.38	2.65±2.24	0.92±1.85	134.27±2.51	8.86±3.16	6.63±2.40	3.94±1.55

No.	LA	AA	CA	SA	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻
1	108.28±4.06	64.90±3.52	131.85±2.48	235.39±4.32	69.65±3.45	78.25±2.03	63.25±3.14	38.2±1.85	89.49±3.75	266.55±3.03
2	60.82±4.12	26.59±3.32	99.37±3.03	160.67±3.52	69.90±3.38	51.60±2.15	48.00±3.23	69.15±1.78	113.18±4.03	162.16±3.16
3	87.46±3.62	25.53±2.81	83.62±2.53	155.60±3.26	96.25±3.62	34.45±2.18	28.90±3.42	32.45±1.97	110.32±3.62	189.50±2.55
4	115.01±3.55	14.83±2.62	146.99±3.12	291.72±4.22	127.25±3.71	50.75±2.36	53.00±3.27	30.15±2.35	108.96±4.17	56.52±3.65
5	72.56±3.82	55.68±2.92	105.49±2.62	189.29±4.13	94.60±3.55	45.75±2.27	57.75±3.19	41.30±1.83	61.25±3.12	172.10±4.01
6	150.56±3.71	93.02±3.12	145.65±2.43	274.02±4.05	185.00±4.02	72.50±2.19	59.75±2.90	22.35±1.90	100.20±4.03	266.55±4.32

Table 2 (continued)

No.	LA	AA	CA	SA	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻
7	45.70±3.62	24.82±2.86	153.95±2.72	92.45±3.42	129.75±3.19	74.50±2.43	49.25±2.88	47.10±2.41	120.65±3.36	153.46±4.23
8	67.08±3.12	15.84±2.75	119.64±2.81	230.27±4.57	165.75±3.28	34.25±2.35	47.25±3.26	25.38±1.70	56.38±2.95	153.46±4.52
9	51.95±3.43	29.01±3.03	121.14±2.65	94.98±3.66	132.75±3.56	80.25±2.38	53.50±2.41	36.80±2.25	89.16±2.47	83.86±3.65
10	50.23±3.84	26.63±3.22	101.47±2.54	74.54±4.22	167.60±3.44	72.95±2.42	50.50±2.39	30.25±2.34	86.89±2.66	90.08±3.77
11	97.07±3.75	17.72±2.96	166.51±2.27	187.46±3.92	167.00±3.22	39.25±2.51	52.00±3.18	27.30±1.73	58.98±2.88	163.40±4.18
12	82.95±3.46	17.88±3.15	78.05±2.43	145.30±3.88	89.70±2.95	72.00±2.36	59.00±3.29	53.20±2.42	118.38±3.49	158.43±3.29
13	58.37±3.57	21.03±2.86	74.73±2.59	265.48±4.53	47.45±2.88	141.70±2.37	38.55±2.65	40.95±2.38	50.21±3.27	168.37±4.27
14	69.81±3.48	13.40±2.73	79.40±2.70	187.72±3.61	54.40±2.84	58.35±2.62	42.15±2.45	48.75±2.45	56.05±2.55	163.40±3.92
15	66.31±3.59	19.27±3.46	70.84±2.67	293.66±3.59	69.60±3.47	82.50±2.51	48.95±2.85	24.50±2.13	53.13±3.36	159.67±4.55
16	60.87±3.81	19.32±2.68	109.67±2.38	254.79±4.08	99.60±3.51	82.50±2.64	58.95±2.97	24.50±1.67	85.27±3.18	100.02±4.17
17	45.75±3.70	15.43±3.24	70.74±2.49	71.01±3.74	70.70±2.69	47.75±2.73	52.00±3.16	36.70±2.58	83.71±2.40	106.23±3.68
18	73.83±3.19	19.50±2.38	95.30±2.47	186.25±4.15	67.25±2.78	50.75±2.28	53.03±3.23	30.15±2.04	108.96±3.29	56.52±3.79
19	126.38±3.25	15.98±3.19	66.75±2.62	233.11±4.27	104.00±3.42	36.25±3.05	54.05±2.80	28.05±1.21	104.82±2.84	114.93±4.47
20	51.85±3.28	43.60±2.58	54.90±2.73	208.21±4.36	41.60±3.28	38.50±2.75	45.25±3.48	57.30±2.14	99.87±2.93	180.80±4.26
21	79.33±3.37	11.48±3.28	74.89±2.54	205.41±3.87	116.40±3.17	74.00±2.82	61.75±3.27	30.45±1.56	112.86±3.05	169.62±3.18
22	72.15±3.48	16.52±2.67	68.98±2.39	238.88±4.64	148.80±3.33	50.75±2.61	54.25±2.70	59.40±2.50	93.26±3.13	113.69±4.22
23	100.35±3.69	17.05±3.29	101.94±2.66	267.64±4.32	41.20±3.20	48.90±2.45	44.80±3.66	49.65±2.19	86.89±2.97	229.27±3.01
24	46.78±3.00	23.91±3.35	42.36±2.58	81.46±3.65	65.60±3.46	40.50±2.56	36.90±2.71	46.80±2.26	93.01±3.19	81.38±3.84
25	68.68±3.82	77.69±3.61	74.06±2.90	172.96±3.99	148.75±3.57	69.20±2.84	58.25±3.29	22.75±2.17	59.30±2.86	169.62±4.11
26	78.76±3.73	52.72±2.93	143.53±2.17	217.35±4.33	76.90±3.31	61.00±2.65	51.75±2.99	38.90±2.58	109.61±2.70	95.05±4.53
27	85.87±3.68	15.34±2.84	259.02±2.26	136.37±3.77	62.00±2.16	66.75±2.54	72.95±3.18	58.90±2.46	115.59±2.51	143.52±3.44
28	90.01±3.59	17.49±3.28	36.24±2.57	231.74±3.84	59.30±3.48	47.25±2.72	38.75±3.26	34.04±2.14	83.78±2.45	209.38±4.28
29	207.04±3.75	47.47±2.66	53.71±2.73	190.55±3.89	102.50±2.96	25.90±2.68	46.65±2.84	29.45±1.96	165.77±3.06	152.22±3.06
30	39.79±3.90	16.02±2.75	50.06±2.84	138.18±4.51	65.40±3.28	69.80±2.71	42.40±3.45	38.40±1.88	85.27±2.73	201.93±4.27
31	45.15±3.04	16.42±2.69	29.37±2.65	101.51±4.42	69.70±3.36	33.60±2.85	58.70±3.63	48.80±1.73	104.49±2.85	198.20±3.36
32	103.75±3.38	17.45±3.27	38.21±2.72	261.46±4.31	66.50±2.97	30.75±3.04	33.70±2.88	36.65±2.00	84.31±3.05	154.70±2.95
33	47.34±3.46	17.17±3.48	71.77±2.69	147.56±4.28	62.00±3.49	66.75±2.95	52.95±3.51	58.90±1.75	75.87±2.71	150.97±4.13
34	51.58±3.73	16.72±2.95	85.55±2.81	247.17±4.39	59.30±2.55	47.25±2.78	38.75±2.92	34.02±2.31	81.71±2.92	131.09±4.32
35	65.05±3.84	17.46±3.60	162.57±2.77	234.78±3.64	66.50±2.71	30.75±2.83	33.70±3.00	36.65±2.22	81.38±3.38	104.99±2.68

No.	GA	Catechin	VA	Caffeic acid	Syringic acid	P-CA	FA	OG(°P)	RG(°P)
1	5.59±1.35	0.39±1.26	0.46±2.07	0.22±2.75	0.38±2.81	0.65±2.42	2.23±3.05	10.00±2.35	3.43±2.12
2	3.96±1.46	0.36±1.57	0.34±2.14	0.16±2.88	0.42±3.05	0.54±2.83	1.94±3.17	8.18±2.56	2.80±2.61
3	4.12±1.44	0.38±2.00	0.39±2.45	0.20±3.07	0.45±2.96	0.62±2.78	2.05±3.29	8.12±2.64	2.50±2.82
4	4.21±1.57	0.32±1.63	0.35±2.62	0.21±2.76	0.43±3.14	0.59±2.51	2.13±3.11	8.06±2.58	2.67±2.45
5	6.25±1.91	0.76±1.49	0.51±2.53	0.08±3.12	0.19±3.05	0.65±2.43	1.79±2.99	9.49±2.73	3.25±2.27
6	6.10±1.87	0.59±2.02	0.46±2.38	0.23±2.81	0.22±3.26	0.46±2.61	1.58±2.84	9.17±2.66	3.14±2.38
7	3.48±1.49	0.40±1.51	0.39±2.23	0.20±4.05	0.28±3.55	0.42±2.27	1.45±2.90	8.05±2.35	2.66±2.42
8	4.21±1.63	0.46±1.43	0.31±2.62	0.12±2.76	0.21±3.14	0.46±2.43	1.23±2.82	8.54±2.80	2.61±2.64
9	3.96±1.52	0.45±1.47	0.38±2.45	0.15±2.88	0.32±3.49	0.41±2.42	1.25±1.73	9.19±2.64	3.17±2.42
10	3.87±1.84	0.43±1.66	0.42±2.66	0.17±3.26	0.29±3.36	0.38±2.13	1.34±2.45	9.48±2.38	2.78±2.88

Table 2 (continued)

No.	GA	Catechin	VA	Caffeic acid	Syringic acid	P-CA	FA	OG(°P)	RG(°P)
11	4.14±1.43	0.35±1.49	0.37±2.42	0.25±3.29	0.41±3.29	0.52±2.25	1.95±2.13	8.48±2.42	2.86±2.38
12	4.37±2.02	0.13±2.25	0.39±2.13	0.23±2.41	0.39±3.27	0.56±2.73	1.54±2.74	11.40±2.84	3.52±2.17
13	6.09±1.73	0.48±2.40	0.49±2.58	0.41±2.74	0.49±2.74	0.77±2.55	1.83±2.88	9.57±2.38	3.32±2.82
14	5.57±2.25	0.46±1.67	0.51±2.04	0.23±3.49	0.14±2.97	0.58±2.25	1.59±2.80	9.53±2.42	3.40±2.58
15	5.89±2.13	0.50±1.83	0.56±2.73	0.37±3.29	0.28±2.80	0.63±2.41	1.64±2.64	9.43±2.17	3.30±2.80
16	3.86±2.40	0.47±1.65	0.42±2.62	0.21±2.80	0.28±3.18	0.40±2.88	1.49±2.38	8.44±2.82	2.61±2.84
17	4.02±1.84	0.58±2.15	0.49±2.75	0.24±3.27	0.31±2.93	0.58±2.84	1.53±2.42	7.77±2.58	3.03±2.93
18	4.53±1.67	0.40±2.22	0.38±2.82	0.22±3.23	0.45±3.05	0.60±2.80	2.10±2.17	9.88±2.93	2.84±2.40
19	3.03±2.28	0.22±1.66	0.14±2.51	0.09±2.97	0.24±3.25	0.32±2.64	1.21±2.58	9.72±2.19	3.13±2.75
20	4.03±2.19	0.37±1.73	0.35±2.74	0.18±2.84	0.44±3.47	0.57±2.82	1.99±2.93	8.12±3.16	2.07±3.05
21	4.75±2.17	0.47±2.17	0.38±2.80	0.13±3.27	0.14±3.16	0.51±2.58	1.37±2.40	10.49±2.75	3.37±2.19
22	3.14±1.88	0.25±2.31	0.19±2.28	0.10±2.71	0.28±2.93	0.37±2.26	1.24±3.05	9.56±2.70	3.08±2.00
23	5.87±1.96	0.66±1.73	0.50±2.17	0.18±3.65	0.14±2.31	0.63±2.00	1.55±2.22	9.05±2.45	3.02±3.16
24	3.96±2.14	0.57±2.28	0.46±2.50	0.21±3.28	0.22±2.00	0.54±2.45	1.49±2.14	9.59±2.21	2.78±2.22
25	5.17±2.26	0.3±1.96	0.48±2.31	0.18±3.17	0.23±2.70	0.52±2.31	1.59±2.70	9.29±2.26	3.03±2.01
26	4.02±1.73	0.35±1.77	0.32±2.85	0.17±3.46	0.39±2.45	0.58±2.14	1.95±2.21	9.71±2.01	2.90±2.14
27	3.45±2.00	0.29±2.21	0.28±2.88	0.13±2.83	0.36±2.22	0.47±2.01	1.35±3.05	11.12±2.31	3.45±2.92
28	4.30±2.31	0.36±2.73	0.42±2.56	0.2±3.45	0.21±2.14	0.52±2.21	1.54±2.26	7.51±2.14	2.55±2.45
29	5.18±2.22	0.29±2.14	0.33±2.26	0.24±2.87	0.06±2.01	0.49±2.92	1.48±2.31	11.24±2.13	3.11±2.83
30	3.82±1.97	0.35±2.01	0.32±2.19	0.15±2.69	0.40±2.21	0.50±3.38	1.89±2.31	7.16±2.78	2.52±3.38
31	3.74±2.21	0.38±2.08	0.34±2.31	0.17±2.91	0.43±3.05	0.54±2.83	1.94±2.06	8.26±2.26	3.09±3.05
32	4.09±2.14	0.20±1.95	0.36±2.22	0.18±3.38	0.29±2.92	0.51±2.50	1.52±2.68	9.08±2.70	3.06±2.31
33	3.06±2.01	0.25±1.69	0.26±2.92	0.10±3.00	0.32±2.26	0.43±2.78	1.30±2.22	5.88±2.26	1.90±2.45
34	3.34±1.96	0.28±1.47	0.28±2.37	0.12±2.92	0.35±3.38	0.45±2.70	1.33±2.26	6.27±2.50	1.95±2.06
35	2.96±2.06	0.26±1.63	0.24±2.09	0.11±3.63	0.28±2.83	0.40±2.68	1.31±2.45	6.27±3.05	2.05±2.78

Alcohol Degree (*AD*)-A measure of alcohol content of a solution as a percentage of the volume of alcohol per volume of beer, Real Gravity (*RG*)- The actual extract (dissolved solids) of a beer devoid of alcohol and carbon dioxide, Original Gravity (*OG*)-The specific gravity of wort as calculated from the alcohol content and real extract of the beer, Ethyl Acetate (*EA*), Oxalic Acid (*OA*), Tartaric Acid (*TA*), Pyruvic Acid (*PA*), Malic Acid (*MA*), Ketoglutaric Acid (*KA*), Lactic Acid (*LA*), Acetic Acid (*AA*), Citric Acid (*CA*), Succinic Acid (*SA*), Gallic acid (*GA*), Vanillic acid (*VA*), P-coumarilic acid (*P-CA*), Ferulic acid (*FA*), Chemical Shift (*CS*)

Degrees Plato (°P)-1 Plato is equal to 1 g of sugar per 100 g beer, All of the data were the mean of three parallel values

The results were shown as mean ± standard deviation

Table 3 Pearson correlation matrix of alcohols, esters, inorganic ions, organic acid, polyphenols content, alcohol degree, real gravity and original gravity to proton chemical shift (CS)

Predictors ^a	AD	RG	OG	EA	Propanol	Iso-butanol	Isoamyl acetate	Isoamyl alcohol	CS
AD	1	0.706 ^b	0.969 ^b	0.567 ^b	0.672 ^b	0.581 ^b	0.532 ^b	0.652 ^b	0.629 ^b
RG		1	0.841 ^b	0.474 ^b	0.470 ^b	0.367 ^b	0.541 ^b	0.522 ^b	0.316
OG			1	0.590 ^b	0.650 ^b	0.532 ^b	0.563 ^b	0.639 ^b	0.562 ^b
EA				1	0.229	0.340 ^b	0.941 ^b	0.411 ^a	0.202
Propanol					1	0.636 ^b	0.246	0.726 ^b	0.342 ^a
Iso-butanol						1	0.407 ^a	0.923 ^b	0.462 ^b
Isoamyl acetate							1	0.473 ^b	0.213
Isoamyl alcohol								1	0.386 ^c
CS									1
Predictors ^a	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻	CS		
K ⁺	1	-0.066	0.293	-0.443 ^b	0.246	-0.268	0.423 ^c		
Na ⁺		1	0.262	-0.021	-0.453 ^b	0.099	-0.177		
Mg ²⁺			1	0.042	0.067	0.049	0.272		
Ca ²⁺				1	0.004	0.070	0.000		
Cl ⁻					1	-0.159	0.551 ^b		
SO ₄ ²⁻						1	-0.187		
CS							1		
Predictors ^a	OA	TA	PA	MA	KA	LA	AA	CA	SA
OA	1	0.429 ^c	-0.130	0.364 ^c	0.257	0.011	-0.004	0.440 ^b	-0.038
TA		1	-0.136	0.630 ^c	0.489 ^c	0.384 ^c	0.269	0.381 ^c	0.228
PA			1	-0.301	0.374 ^c	0.532 ^c	0.126	-0.163	0.070
MA				1	0.245	0.056	0.325	0.295	0.399 ^c
KA					1	0.367 ^c	0.130	-0.047	0.047
LA						1	0.366 ^c	0.104	0.452 ^c
AA							1	0.127	0.148
CA								1	0.020
SA									1
CS									0.012
Predictors ^a	GA	Catech-in	VA	Caffeic acid	Syringic acid	P-CA	FA	CS	
GA	1	0.605 ^b	0.794 ^b	0.550 ^b	-0.273	0.691 ^b	0.338 ^c	0.628 ^b	
Catechin		1	0.694 ^b	0.199	-0.301	0.397 ^c	0.115	0.548 ^b	
VA			1	0.633 ^b	-0.163	0.654 ^b	0.308	0.533 ^b	
Caffeic acid				1	0.210	0.603 ^b	0.380 ^c	0.201	
Syringic acid					1	0.288	0.598 ^b	0.004	
P-CA						1	0.706 ^b	0.464 ^b	
FA							1	0.471 ^b	
CS								1	

^aThe predictors were presented as follows: Alcohol Degree (AD), Real Gravity (RG), Original Gravity (OG), Ethyl Acetate (EA), Oxalic Acid (OA), Tartaric Acid (TA), Pyruvic Acid (PA), Malic Acid (MA), Ketoglutaric Acid (KA), Lactic Acid (LA), Acetic Acid (AA), Citric Acid (CA), Succinic Acid (SA), Gallic acid (GA), Vanillic acid (VA), P-coumaric acid (P-CA), Ferulic acid (FA), Chemical Shift (CS)

^bThe correlation coefficient is significant with an confidence level of 99 %

^cThe correlation coefficient is significant with an confidence level of 95 %

Table 5 Multiple linear regression analysis for the principal components to proton CS

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard regression coefficient	0.462	0.082	0.405	−0.174	0.156	0.362	0.105	0.154
t	2.732	0.687	2.304	−0.219	0.639	2.189	1.306	2.390
p	0.021	0.516	0.162	0.749	0.994	0.036	0.295	0.023
VIF	1.181	1.093	1.282	1.150	1.421	1.052	1.085	1.182

In addition, seven major polyphenols (gallic acid, catechin, vanillic acid, caffeic acid, P-coumarilic acid, ferulic acid, syringic acid) were measured by HPLC (Table 2). The contents of polyphenols in the observed beers varied between 3 and 6 mg/l, 0.1 and 0.8 mg/l, 0.1 and 0.6 mg/l, 0.1 and 0.4 mg/l, 0.3 and 0.8 mg/l, 1 and 2.2 mg/l, 0.1 and 0.5 mg/l. The Pearson correlation matrix of polyphenols to CS was shown in Table 3. GA, VA, and Catechin were positively correlated with CS and Pearson correlation coefficient was 0.628, 0.533, 0.528 with an confidence level of 99 %, respectively. It may be assumed that the phenolic hydroxyl group is a key factor that have a positive effect on hydrogen bonding intensity.

Principal component analysis (PCA)

A significant amount of multicollinearity was observed among the origin predictive variables using Correlation Analysis. So, the objective of PCA before modeling was to obtain prediction convergence parameters for models. These variables would be used as new predicting variables in MRA to obtain a final model, which could be used to calculate the proton CS value in beer. The first step in the PCA was data exploration, which allowed the main variability aspects of a data set to be visualized, without constraint of an initial hypothesis concerning the relationship.

The results of eigenvalue and variance contribution after varimax rotation transformation were shown in Table 4. Eight principal components were revealed, which explained 89.64 % of the total variance in the predictor variables, and could well reflect the most information of predictor variables. The first three principal components of PC1, PC2, and PC3 accounted for 23.50 %, 10.00 %, and 7.60 % of the variance (eigenvalue >1), respectively.

Higher variable loading values means more contributions to the principal component. Results of the varimax rotation on the eight principal components showed that PC1 positively correlated with the content of isoamyl alcohol, isobutanol, original gravity, alcohol degree, real gravity, propanol, pyruvic acid, and gallic acid, indicating the importance of ethanol factor. PC2 had a higher loading value on ethyl acetate, isoamyl acetate, and P-coumarilic acid content, so it could be defined as ester factor. PC3 loaded mainly on the content of Cl^- , Na^+ , malic acid, lactic acid, and syringic acid. The remaining variables were represented

by the rest of the principal components (PC4, PC5, PC6, PC7, and PC8). The loading of the first three PCs were plotted in Fig. 1, which intuitionistically showed the correlation between the first three PCs and all former variables. It should be noted that all variables dispersed separately on the three-axis chart.

Multiple regression analysis (MRA)

Beer contains a very complex nutrient mixture. Besides water and ethanol, higher alcohols, volatile esters, organic acids, carbohydrates, phenols, and salts are major flavor components of beer, which could affect the hydrogen-bonding structure of water-ethanol (Nose and Hojo 2006). In order to investigate their contributions and interrelationships with hydrogen-bonding, a mathematic model of CS and the major flavor components in beer was obtained by MRA.

A stepwise multiple regression procedure was carried out between the eight independent PCs and CS. As shown in Table 5, PC1, PC3, PC6 had the major impact on CS. The regression equation was drawn as Eq. 1, where “E” represents the concentration of ethanol. The model had an adjusted R^2 of 0.868 and well statistically significant ($P < 0.05$).

Fig. 2 Comparison between chemical shift by proposed model and those observed for 35 bottled lager beer

Fig. 3 Scatter plot of dependent variable with predicted value

$$CS(ppm) = 4.919 + 8.796E - 4 \times PC1 + 3.853E - 5 \times PC3 + 4.836E - 5 \times PC6 \quad (1)$$

PC1 had the most important impact on CS, indicating the ethanol factor was the main factor responsible for the hydrogen-bonding association. PC3 and PC6 showed more impact on CS among the rest of the PC variables. The PCs in regression were matched to new independent variables with high standard regression coefficients (PC1-0.462, PC1-0.405, PC1-0.362).

Different compounds are used to give a scientific explanation to sources of the flavor profile and oral sensation. Ethanol has an important impact on sweet and bitter tastes of beer (Perpète and Collin 2000). The high alcohols affect drinkability because beer flavor is considered heavier as amyl alcohol concentration increases (Tan and Siebert 2004). Beer flavor becomes fruity and undesirable when the concentrations of esters are high, among which ethyl acetate and isoamyl acetate are representative (Verstrepen et al. 2003). In this study, isoamyl alcohol, isobutanol, original gravity, alcohol degree, real gravity, propanol, and pyruvic acid content were selected from PC1.

Inorganic ions contribute to the saline taste of beer, and the low molecular weight organic acids are intermediates or final metabolites of beer fermentation which can provide a sour flavor character (Hufnagel and Hofmann 2008; Mato et al. 2005). Also, there are many polyphenolic compounds which can affect the taste, smell, and nonbiological stability of beer. Monophenols can jointly enrich beer flavour with

spicy, vanilla-like flavour aspects (Sterckx et al. 2011). In this research, Cl^- , Na^+ , Ca^{2+} , K^+ , Mg^{2+} , malic acid, syringic acid and lactic acid contents were selected from PC3 and PC6. Consequently, these variables were used as new predictor variables in subsequent regression analysis. A new model was constructed (Eq. 2), where “K” represents the concentration of K^+ , “P” represents the concentration of pyruvic acid, and “E” represents the concentration of ethanol, and “G” represents the concentration of gallic acid. The decision coefficient (R^2) was 0.779 ($P=0.001$), so the Eq. 2 had a better response for the relationship between the variables and hydrogen bonding intensity, and was very markable and trustful.

$$CS(ppm) = 4.917 + 0.002[E] - 0.006[P] + 5.141E - 8[K^2] + 5.378E - 5[G \times P] + 2.428E - 5[K \times P] + 2.369E - 5[E^2P] - 1.63E - 5[G^3] - 2.207E - 5[P^3] - 5.247E - 7[K^2P] - 3.037E - 5[E^2K] \quad (2)$$

The correlation between the predicted and practiced CS for 35 bottled lager beers was shown in Fig. 2. The normal probability plot of standardized residual showed that most data were located in the beeline of normal distribution, and most observed values appeared to fit the predicted values well. Some discrepancies between the predicted and observed values could be from the factors which were not considered in the study, such as aldehydes, saccharides, and so on. The scatter plot of dependent variable with regression standardized predicted value was presented in Fig. 3. The residual scatter situated in the range of -2 – 2 , and was randomly homogeneous distributed. Thus, there was a rather independence between residual and dependent factors according to the supposed theoretics.

Verification of models

Non-covalent interactions play an important role in the fields of chemistry, physics, and biology (Scheiner 1997). In chemistry, the hydrogen bond is a type of attractive intermolecular force that exists between two partial electric charges of opposite polarity, and its intensity is related to the CS (Pejov et al.

Table 6 Verification of multiple regression model

Sample	1	2	3	4	5	6	7	8	9
Predictive value	4.8557	4.8495	4.7793	4.8095	4.7892	4.8598	4.8659	4.9257	4.9453
Practical value	4.9118	4.912	4.9183	4.918	4.9039	4.9121	4.9137	4.9175	4.9163
Error (%)	−1.14	−1.27	−2.83	−2.21	−2.34	−1.06	−0.97	0.17	0.59
Average error (%)				−1.23					

2006). In this research, the alcohol degree, pyruvic acid, gallic acid, K^+ content, and CS were determined for another 9 bottled lager beers purchased in Chinese market, and the results were considered as predictive variables in the above regression model. As shown in Table 6 where the predictive value and practical test value were list, the error range was between -2.34% and 0.59% , and the average error value was -1.23% , indicating the multiple regression model was well fitted with proton CS in beer.

Conclusions

Different sorts of flavor substances can affect the Chemical Shift due to their different concentrations in beer. Several key factors including 4 alcohols, 2 esters, 6 ions, 9 acids, 7 polyphenols, and 2 gravity indexes (OG and RG) were determined in this study. They could be used to investigate the relationship between hydrogen bonding intensity and the flavor components in bottled larger beers through the Correlation Analysis, PCA and MRA. Alcohol was the primary factor with the highest correlation coefficient to CS, excepting its important effect on sweet and bitter tastes. The ethanol, gallic acid, pyruvic acid, original gravity, Cl^- , Vanillic acid, Catechin, lactic acid, and K^+ had positive correlations with the CS, and the Pearson correlation coefficient was 0.629, 0.628, 0.595, 0.562, 0.551, 0.533, 0.528, 0.515, 0.423 with an confidence level of 99 % respectively. Moreover, the contents of ethanol, pyruvic acid, gallic acid, and K^+ were suggested to be the decisive factors for beer hydrogen bonding CS via PCA and MRA. And finally, a mathematical model was developed which could predict the decisive factors of hydrogen bonding intensity in beer: *hydrogen bonding association intensity* (CS) = $4.917 + 0.002[E] - 0.006[P] + 5.141E - 8[K^2] + 5.378E - 5[G \times P] + 2.428E - 5[K \times P] + 2.369E - 5[E^2P] - 1.63E - 5[G^3] - 2.207E - 5[P^3] - 5.247E - 7[K^2P] - 3.037E - 5[E^2K]$. The average error was 1.23 % in the validated experiment.

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References

Alkorta I, Rozas I, Elguero J (2002) Interaction of anions with perfluoro aromatic compounds. *J Am Chem Soc* 124(29):8593–8598

- Almeida C, Duarte IF, Barros A, Rodrigues J, Spraul M, Gil AM (2006) Composition of beer by 1H NMR spectroscopy: effects of brewing site and date of production. *J Agric Food Chem* 54:700–706
- Boffo EF, Tavares LA, Ferreira MMC, Ferreira AG (2009) Classification of Brazilian vinegars according to their 1H NMR spectra by pattern recognition analysis. *LWT- Food Sci Technol* 42:1455–1460
- Bosco M, Toffanin R, Palo D, Zatti L, Segre A (1999) High resolution 1H -NMR investigation of coffee. *J Sci Food Agric* 79:869–878
- Cabrera C, Madrid Y, Camara C (1994) Determination of lead in wine, other beverages and fruit slurries by flow injection hydride generation atomic absorption spectrometry with on-line microwave digestion. *J Anal At Spectrom* 9:1423–1428
- Charlton AJ, Farrington WHH, Brereton P (2002) Application of H NMR and multivariate statistics for screening complex mixtures: quality control and authenticity of instant coffee. *J Agric Food Chem* 50:3098–3103
- Daems V, Delvaux F (1979) Multivariate analysis of descriptive sensory data on 40 commercial beers. *Food Quality Preference* 8:373–380
- Derome AE (1987) In: Baldwin JE (ed) *Modern NMR techniques for chemistry research: Organic chemistry series*, Vol. 6. Pergamon Press, Oxford
- Dolores BM, Juana MP, Maria PH (2000) Differentiation and classification of beers with flame atomic spectrometry and molecular absorption spectrometry and sample preparation assisted by microwaves. *Spectrochimica Acta Part B* 55:855–864
- Duarte IF, Barros A, Almeida C, Spraul M, Gil AM (2004) Multivariate analysis of NMR and FTIR data as a potential tool for the quality control of beer. *J Agric Food Chem* 52:1031–1038
- Fileti EE, Chaudhuri P, Canuto S (2004) Relative strength of hydrogen bond interaction in alcohol-water complexes. *J Chem Phys Lett* 400:494–499
- García AA, Grande BC, Gándara JS (2004) Development of a rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for the determination of polyphenols in alcohol-free beers. *J Chromatogr A* 1054:175–180
- Gil AM, Duarte IF, Delgadillo I, Colquhoun IJ, Casuscelli F, Humpfer E, Spraul M (2000) Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J Agric Food Chem* 48:1524–1536
- Harris RK (1983) *Nuclear magnetic resonance spectroscopy: A physicochemical view*. Longman Scientific & Technical, Essex
- Hergenreder RL (1991) Determination of iron in beer by flow injection-flame atomic absorption spectroscopy. *At Spectrosc* 12:74–76
- Hindman JC (1966) Proton resonance shift of water in the gas and liquid states. *J Chem Phys* 44:4582–4592
- Hufnagel JC, Hofmann T (2008) Quantitative reconstruction of the nonvolatile Sensometabolome of a red wine. *J Agric Food Chem* 56(19):9190–9199
- Iola D, Antonio B, Peter SB, Renton R (2002) High-resolution nuclear magnetic resonance spectroscopy and multivariate analysis for the characterization of beer. *J Agric Food Chem* 50:2475–2481
- Jolliffe IT (1986) *Principal component analysis*. Springer, New York
- Kawahara S, Uchimaru T, Taira K (2003) 2-Pyridone and 3-oxo-1,2,6-thiadiazine-1,1-dioxide derivatives: a new class of hydrogen bond equivalents of uracil. *J Comput Aided Mol Des* 17:329–334
- Ko BK, Ahn HJ, van den Berg F, Lee CH, Hong YS (2009) Metabolomic insight into soy sauce through 1H NMR spectroscopy. *J Agric Food Chem* 57:6862–6870
- Kosir IJ, Kidric J (2001) Identification of amino acids in wine by one- and two-dimensional nuclear magnetic resonance spectroscopy. *J Agric Food Chem* 49:50–56
- Lachenmeier DW, Frank W, Humpfer E, Schäfer H, Keller S, Mortter M, Spraul M (2005) Quality control of beer using high-resolution nuclear magnetic resonance spectroscopy and multivariate analysis. *Eur Food Res Technol* 220:215–221

- Le Gall G, Puaud M, Colquhoun IJ (2001) Discrimination between orange juice and pulp wash by ^1H nuclear magnetic resonance spectroscopy: identification of marker compounds. *J Agric Food Chem* 49:580–588
- Lee JE, Lee BJ, Chung JO, Hwang JA, Lee SJ, Lee CH, Hong YS (2010) Geographical and climatic dependencies of green tea (*Camellia sinensis*) metabolites: a ^1H NMR-based metabolomics study. *J Agric Food Chem* 58:10582–10589
- Liu J, Li Q, Dong JJ, Chen J, Gu GX (2008) Multivariate modeling of aging in bottled lager beer by principal component analysis and multiple regression methods. *J Agric Food Chem* 56:7106–7112
- Mannina L, Sobolev AP, Viel S (2012) Liquid state ^1H high field NMR in food analysis. *Prog Nucl Magn Reson Spectrosc*. doi:10.1016/j.pnmrs.2012.02.001
- Mato I, Suárez-Luque S, Huidobro JF (2005) A review of the analytical methods to determine organic acids in grape juices and wines. *Food Res Int* 38(10):1175–1188
- Muller N (1965) Concerning structural models for water and chemical-shift data. *J Chem Phys* 43:2555–2556
- Nose A, Hojo M (2006) Hydrogen bonding of water–ethanol in alcoholic beverages. *J Bios Bioe* 10:269–280
- Nose A, Hojo M, Ueda T (2004) Effects of salts, acids, and phenols on the hydrogen-bonding structure of water-mixtures. *J Phys Chem* 108:798–804
- Pages J, Tenenhaus M (2001) Multiple factor analysis combined with PLS path modelings Application to the analysis of relations between physicochemical variables, sensory profiles and hedonic judgments. *Chemom Intell Lab Sys* 58:261–273
- Pejov L, Solimannejad M, Stefov V (2006) The π -type hydrogen bond with triple C–C bond acting as a proton-acceptor. A gradient-corected hybrid HF-DFT and MP2 study of the phenol = acetylene dimer in the neutral S_0 ground. *Chem Phys* 32:259–260
- Perpète P, Collin S (2000) Influence of beer ethanol content on the wort flavour perception. *Food Chem* 71:379–385
- Ramasami P, Ford TA (2010) Ab initio studies of the vibrational spectra of some hydrogen-bonded complexes of fluoroacetylene. *Can J Chem* 88:716–724
- Ramos A, Santos H (1999) NMR studies of wine chemistry and wine bacteria. *Annu Rep NMR Spectrosc* 37:179–202
- Rodrigues JEA, Erny GL, Barros AS, Esteves VI, Brandão T, Ferreira AA, Cabrita E, Gil AM (2010) Quantification of organic acids in beer by nuclear magnetic resonance (NMR)-based methods. *Anal Chim Acta* 674:166–175
- Rodrigues JA, Barros AS, Carvalho B, Brandão T, Gil AM (2011) Probing beer aging chemistry by nuclear magnetic resonance and multivariate analysis. *Anal Chim Acta* 702(2):178–187
- Sacchi R, Patumi M, Fontanazza G, Barone P, Fiordiponti P, Mannina L, Rossi E, Segre AL (1996) A high field ^1H magnetic resonance study of the minor components in virgin olive oils. *J Am Oil Chem Soc* 73:747–758
- Scheiner S (1997) Hydrogen bonding. Oxford University Press, New York
- Siebert KJ (2001) Chemometrics in brewings-A review. *J Am Soc Brew Chem* 59:147–156
- Skogerson K, Runnebaum R, Wohlgemuth G, De Ropp J, Heymann H, Fiehn O (2009) Comparison of gas chromatography-coupled time-of-flight mass spectrometry and ^1H nuclear magnetic resonance spectroscopy metabolite identification in white wines from a sensory study investigating wine body. *J Agric Food Chem* 57:6899–6907
- Spraul M, Schutz B, Humpfer E, Mortter M, Schafer H, Koswig S, Rinke P (2009) Mixture analysis by NMR as applied to fruit juice quality control. *Magn Reson Chem* 47:S130–S137
- Statheropoulos M, Vassiliadis N, Pappa A (1998) Principal component and canonical correlation analysis for examining air pollution and meteorological data. *Atmos Environ* 32:1087–1095
- Sterckx FL, Missiaen J, Saison D, Delvaux FR (2011) Contribution of monophenols to beer flavour based on flavour thresholds, interactions and recombination experiments. *Food Chem* 126(4):1679–1685
- Tan Y, Siebert KJ (2004) Quantitative structure-activity relationship modeling of alcohol, ester, aldehyde, and ketone flavor thresholds in beer from molecular features. *J Agric Food Chem* 52:3057–3064
- Verhagen LC (2010) Beer flavor Comprehensive Natural Products II, 3: 967–997
- Verstrepen KJ, Derdelinckx G, Dufour JP, Winderickx J, Thevelein JM, Pretorius IS, Delvaux FR (2003) Flavor-active esters: adding fruitiness to beer. *J Biosci Bioeng* 96:110–118