Research article

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A study on kinetics of beer ageing and development of methods for predicting the time to detection of flavour changes in beer

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The kinetics of beer ageing were studied based on the development of beer stale flavour with storage time. Results showed that the beer ageing rates at 50 and 60°C were 30.0 and 56 times as fast as those at room temperature, respectively. Based on these findings, two methods (method A and B) for predicting the 'time to detection of flavour change' (TDFC) of beer were developed. TDFC is the beer shelf-life in terms of flavour stability. In method A, beers were stored in a 50°C water bath and the intensity of beer ageing was scored daily. Thus, the range of TDFC of the beer was acquired according to the maximum number of days within which the intensity of beer ageing was ≤2 and the minimum number of days within which the intensity of beer ageing was >2. In method B, the 2-thiobarbituric acid (TBA) values of a beer were determined before and after 1 day of storage in a 50°C water bath, and the TDFC of the beer was calculated using the equation:

$$\text{TDFC (days)} = \frac{5.19}{\Delta \text{TBA}}$$

where ΔTBA is the increment of TBA value during 1 day of storage at 50°C. Both methods were simple, rapid and accurate.

Keywords: ageing; beer; flavour stability; kinetics; prediction; staling; thiobarbituric acid (TBS); time to detection of flavour changes (TDFC)

Introduction

Besides food safety, flavour is one of the most important quality properties of beer. Beer flavour involves many aspects such as profile, consistency and stability. Flavour is a sensory phenomenon. The brewer hopes to offer consumers a flavour experience that consumers regard as pleasurable. Beer flavour had been reported to be unstable and to change during storage *(1,2)*. Beer flavour change with time of storage is called ageing or flavour instability. In contrast to other alcoholic beverages such as wine, Chinese rice wine, whiskey, brandy and Chinese liquor (a traditional Chinese distilled spirit), beer ageing is usually considered unfavourable for flavour quality. There are two important reasons for the negative effects. One is that in most cases stale flavour occurring during storage results in off-flavours and seriously impairs beer quality and the other is that stale flavours make beer lose the characteristics that consumers expect *(3)*. Some may argue that ageing flavours are not always regarded as off-flavours and that consumers do not necessarily dislike the flavour of an aged beer. Nevertheless, brewers always still hope that their beer flavours are controllable and are stable to some extent.

In order to understand the mechanism of beer ageing and to control beer flavour changes, many studies have focused on the chemical aspects of beer ageing and the main changes in components and the corresponding pathways have now been elucidated *(4–6)*. Apart from these, there have been many studies on indices assessing beer flavour stability *(7–10)*. Several indices have been developed, for example, the 2-thiobarbituric acid (TBA) value, the resistance-to-staling value, 2,2-diphenyl-1-picrylhydrazyl, TRAP (total reactive antioxidant potential) and lag time of electron spin resonance *(11,12)*. Of these, the TBA value has wide application among Chinese breweries as this assay is cost-effective and can be conducted without the use of complicated instruments *(13–15)*. Additionally, the performance of this method is also acceptable *(16)*. TBA forms sensitive reactions with aldehydes, which are the important compounds resulting from ageing. The reaction products show a maximum absorption at a wavelength of 530 nm and thus absorbance at 530 nm can reflect the levels of aldehydes in beer.

All of these parameters that evaluate beer flavour stability only reflect the anti-oxidative activity of a beer; they do not reveal the 'time to detection of flavour changes' (TDFC) in a beer. TDFC is the maximum time within which a beer is still considered fresh for most consumers.

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In evaluating beer flavour stability, brewers prefer to know the rate at which their beer is ageing under normal conditions and the TDFC of their beer *(17)*. There are currently no reports on methods that can predict the TDFC of a beer. Thus it is meaningful to develop a prediction method that could estimate the TDFC of a certain beer in advance. In this study, first the kinetics of beer flavour ageing during storage were established, as studies on beer ageing rate are important *(18,19)*. Based on the kinetics, two methods for predicting the TDFC of a beer were developed.

Materials and methods

Reagents and chemicals

The following substances, with corresponding purities were supplied by Sigma Aldrich (St Louis, MO, USA): 3-methyl-butanal (99.9%), 2-methyl-butanal (99.7%), 2-furfural (≥98.0%), 2-acetyl-furan (≥99%), 5-methyl-furfural (≥97.0%), benzaldehyde (90%), 2-phenylacetaldehyde (99.5%), diethyl succinate (98%), ethyl nicotinate (99%), ethyl phenylacetate (99.5%) and γ-nonalactone (99%). The compound 2-thiobarbituric acid (≥97.0%) was supplied by Fluka (Madrid, Spain). All other reagents used in this study were analytical reagent grade and were purchased from local suppliers. Water used throughout the study was ultrapure water (18.2 MΩ cm).

Beers

Two kinds of fresh beers were selected and both were commercially available from a Chinese brewery. One is identified as DB and the other was as PB in this paper. Both beers were light-coloured lagers. Except for using the same yeast, DB and PB were made according to different recipes and different processes. DB was a non-pasteurized beer that was sterilized by filtration through a 0.45 μm membrane at a cold temperature and bottled in green glass bottles. PB was a pasteurized beer and was bottled in clear glass bottles. The original extract of both beers was 10°P.

Beer ageing conditions

Two types of fresh beers, namely DB and PB, stored under five different conditions in the dark, were used to explore the kinetics of beer ageing. The five conditions are as follows: condition 1, room temperature; condition 2, 25°C water bath; condition 3, 40°C water bath; condition 4, 50°C water bath; and condition 5, 60°C water bath. The room temperature (i.e. the measured temperature in the storage room) ranged from 20 to 30°C.

Beer analysis

Analysis of TBA value. The TBA value of beer was measured according to the method described by Grigsby and Palamand *(7)*. A degassed beer aliquot of 5 mL was added to 2 mL of TBA 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 at 530 nm, with 2 mL of water as the blank, represented the TBA value.

Analysis of ageing compounds in beer. Ageing compounds in beer were determined using a GC/MS method similar to the one described by Herrmann *(20)*. The ageing compounds studied were 3-methyl-butanal, 2-methyl-butanal, 2-furfural, 5-methyl-furfural, benzaldehyde, 2-phenylacetaldehyde, diethyl succinate, ethyl phenylacetate, ethyl nicotinate, 2-acetyl-furan and γ-nonalactone.

The target compounds were identified by retention time and the proportion of qualitative ions. They were quantified based on their peak areas using the internal method.

Sensory evaluation of the degree of beer ageing. A system of intensity scoring was used, with 10 trained tasters asked to assess the beers on a scale of staleness from 0 to 5 with 0 = absent, 1 = slight, 2 = noticeable, 3 = obvious, 4 = strong, and 5 = extreme. Sensory evaluations were performed in a tasting room and beer samples were kept at room temperature for 30 min before the test. A 50 mL portion of each sample was poured into a clean glass. Evaluators drank mineral water to avoid interaction between samples. The arithmetical mean values of the tasting panel scores represented the degree of beer ageing.

Statistical analysis

Statistical techniques employed were regression analysis and Pearson correlation analysis using the statistical software package SPSS. The value $p < 0.05$ indicates statistical significance.

Results and discussion

Development of the intensity of stale flavour in beer during storage

Dalgliesh has described the actual sensory changes during beer storage in the most detail *(21)*. However, the Dalgliesh plot is just a generalization of the sensory evolution during beer storage and one cannot obtain specific data about the beer ageing rate from it. In order to study the actual evolution of stale flavour in a specific beer and the effect of storage temperature on the rate of beer ageing, two types of fresh bottled lagers were stored at room temperature and at 25, 40, 50 and 60°C temperatures. During storage, the degrees of beer ageing were analyzed at specific intervals until the stale flavour was obvious. The evolution of the intensity of stale flavour in both beers is shown in Fig. 1. It is apparent that the intensity of the stale flavour in the beer gradually increased with time and the higher the storage temperature was, the higher the rate of beer staling. The rate of beer ageing under room conditions was similar to that at 25°C.

Kinetics of beer ageing during storage

Equation of the rate of development of stale flavour in beer. To find the relationship between the rate of development of stale flavour in beer and storage time, regression analyses of scores of stale flavour in beer vs storage time were conducted (see Table 1). The linear regression showed good fits for the data and was statistically significant in all cases, meaning that the development of stale flavour in the studied beers had the feature of a zero-order reaction.

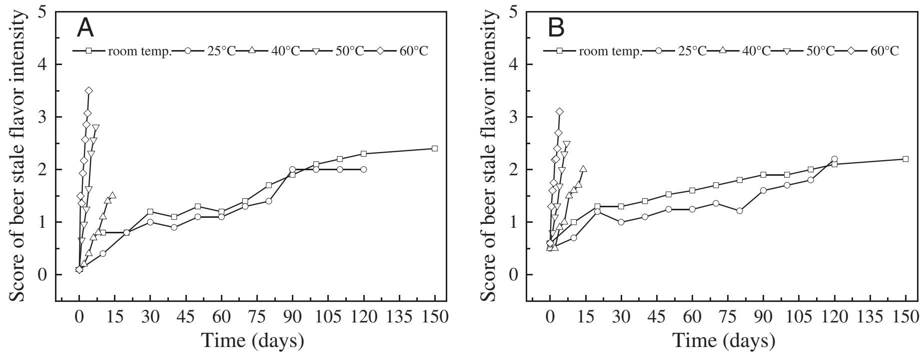

Figure 1. Development of the intensity of stale flavour in beer at different storage temperatures. (A) Beer DB and (B) beer PB.

Table 1. Linear fitting of results of beer stale flavour vs storage time

Beer	Storage temperature (°C)	Intercept	Slope[a]	R^2	k^b	Ratio of rate constant[c]
DB	Room	0.6324	0.0131	0.9445*		1.0
	25	0.3022	0.0156	0.9328*	0.0428	1.2
	40	0.025	0.1071	0.9865*	0.1565	8.2
	50	0.1656	0.3920	0.9878*	0.3474	29.9
	60	0.6536	0.7319	0.9297*	0.7351	55.9
PB	Room	0.3022	0.0096	0.9115*		1.0
	25	0.6425	0.0109	0.8769*	0.0350	1.1
	40	0.4167	0.1137	0.9656*	0.1225	11.8
	50	0.5427	0.2807	0.9953*	0.2647	29.2
	60	0.5437	0.5437	0.9559*	0.5461	56.6

[a]Slope is just the rate constant of beer ageing.
[b]Estimated rate constant according to the Arrhenius equation.
[c]Namely the ratios of slope.
* Regression is significant at the 0.05 level.

Effect of storage temperature on the rate of development of stale flavour in beer. Generally the effect of temperature on a chemical reaction rate may be represented by the Arrhenius equation:

$$k = A\ e^{-E_a/RT}$$

where k is the rate constant, A is the pre-exponential factor assumed to be independent of temperature; E_a is activation energy; R is the gas constant; and T is the temperature in Kelvin.

In Table 1, the slope is just the rate constant of beer ageing. According to these rate constants of beer ageing at different temperatures, together with the corresponding temperature, the A and E_a were calculated. Thus, the specific Arrhenius equations for both beers were also acquired (data not shown). In turn, the rate constants at different temperatures were estimated according to the acquired Arrhenius equation (see k in Table 1). It was found that the estimated rate constants had huge deviations from the actual rate constants. This indicated that the effect of storage temperature on the rate of beer ageing did not comply with the Arrhenius law. Therefore the rate of beer ageing at a certain temperature cannot be speculated based on the Arrhenius equation.

The ratios of the rate of development of stale flavour in these two beers during storage at different temperatures were calculated by dividing the rate constants at different temperatures by those at room temperature. The results are shown in the last column in Table 1. It can be seen that the ratios in both beers were similar, especially in the cases of 50 and 60°C. These results demostrate that the rate of beer ageing at 50°C is nearly 30 times that at room temperature; the rate of beer ageing at 60°C is nearly 56 times that at room temperature. In other words, 1 day at 50°C was equivalent in stale flavour development to 4 weeks at room temperature. Similarly, 1 day at 60°C was equivalent to 8 weeks at room temperature. Thus, the rate of beer ageing at room temperature could be predicted according to the development of beer stale flavour at 50 or 60°C.

Method A for predicting the TDFC of a beer

TDFC is the beer shelf-life in terms of flavour stability. In practical terms, it is assumed that most consumers can perceive beer stale flavour when the score of the intensity of stale flavour in beer is >2 on a six-point scale (0–5) in this study. Of course, different brewers may have different criteria, for example, it is also feasible for some brewers to set the score of the intensity of stale flavour in beer to 1.

For brewers, it is very useful to know the TDFC of their beers in advance. Brewers could take relevant precautions if they knew their beer's TDFC prior to it entering into the marketplace. Based on the rates of development of stale flavour in two different beers, a method has been proposed for predicting the TDFC of a beer based on sensory evaluation. This prediction method has been called 'method A' to differentiate it from the other prediction method that was developed next.

The main steps of the method A are as follows:

Step 1 - 12 bottles of fresh beer are selected.
Step 2 - all of these bottles are forced aged in a 50°C water bath.
Step 3 - one bottle of the forced aged beer is collected every day and the intensity of stale flavour is scored through a tasting panel.
Step 4 - the mean of the tasting panel scores is calculated and the TDFC of the beer is judged according to mean scores.

How is the TDFC of beer judged? For example, when the score of stale flavour of a beer is <2 after a 2 day storage at 50°C, but >2 after a 3 day storage at 50°C, one can judge that the TDFC of the beer is between 2 and 3 months.

Method B for predicting the TDFC of a beer

Method A depends on the scores of a tasting panel so its application will be restricted when there is no qualified tasting panel available. Therefore it was necessary to develop another method that could predict the TDFC of a beer based on a certain physicochemical index. This prediction method was called 'method B'.

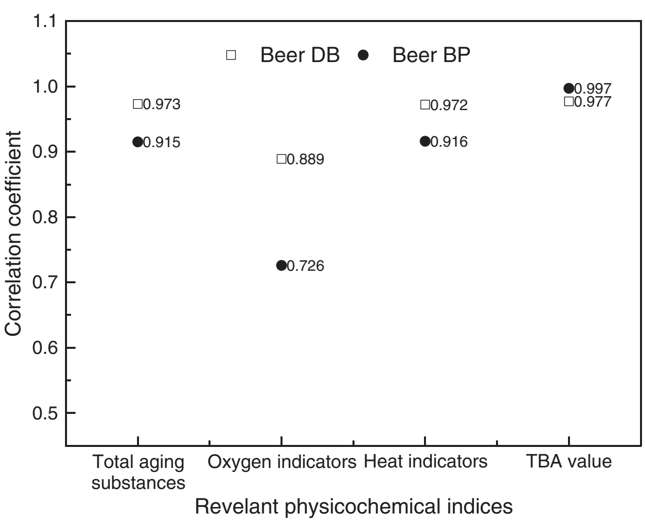

Figure 2. Correlations between physicochemical indices and the extent of beer ageing.

The best physicochemical indices indicating the extent of beer ageing. In order to establish method B, first it was key to find the best physicochemical index that correlated best with the intensity of beer stale flavour. Correlation analyses between the intensity of beer stale flavour and several relevant physicochemical indices such as TBA value, and ageing substances measured by GC/MS were conducted. The ageing substances determined in this study included 3-methyl-butanal, 2-methyl-butanal, 2-furfural, 5-methyl-furfural, benzaldehyde, 2-phenylacetaldehyde, diethyl succinate, ethyl phenylacetate, ethyl nicotinate, 2-acetylfuran and γ-nonalactone. Of these compounds, 3-methyl-butanal, 2-methyl-butanal, benzaldehyde and 2-phenylacetaldehyde are oxygen indicators, as the concentrations of these three compounds increase more at elevated oxygen concentrations *(5)*. The levels of these three compounds can reflect the extent of beer oxidation. The compounds 2-furfural and γ-nonalactone belong to heat indicators, as these two compounds can be largely induced by heat *(5)*. The levels of these two compounds can reflect the extent of heat-induced damage to beer. The correlation coefficients for both beers are shown in Fig. 2.

Compared with oxygen indicators, heat indicators and total ageing compounds, the correlation coefficients between the TBA value and the intensity of stale flavour were the highest. Thus the TBA value was chosen as the parameter indicating the extent of beer ageing.

The critical TBA value of beer ageing. In this study, the critical TBA value (TBA_c) of beer ageing was the TBA value at which the stale flavour could just be perceived by most consumers. As mentioned earlier, it is assumed that most consumers can perceive stale flavour when the score of the intensity of stale flavour in the beer is 2 or 1 on a six-point scale (0–5). Because the TBA value and the intensity of stale flavour are positively correlated, the TBA_c of a beer can be calculated through the regression equation between TBA value and ageing intensity. In order to obtain the TBA_c of the DB and PB beers, the beer TBA value and the corresponding score of stale flavour were analyzed and regression analyses were conducted. The results are shown in Fig. 3, and it can be seen that the TBA_c of DB and PB were 0.379 and 0.426, respectively. Although correlation analyses showed that the TBA value had statistically a significant positive correlation

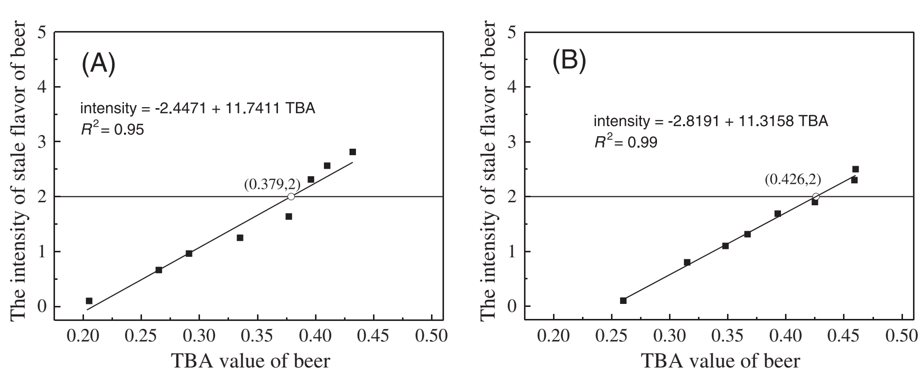

Figure 3. Relationship between the intensity of stale flavour in beer and 2-thiobarbituric acid (TBA) value. (A) Beer DB and (B) beer PB.

Table 2. Verification of the accuracy of time to detection of flavour change (TDFC) predicted by method A

Sample	Staleness after 1 day of storage at 50°C	Staleness after 2 days of storage at 50°C	Staleness after 3 days of storage at 50°C	Staleness after 4 days of storage at 50°C	Predicted TDFC[a] (months)	Actual TDFC[b] (months)
DB 1	0.7	0.8	1.2	1.6	>4	>4
DB 2	0.6	0.8	1.4	1.7	>4	>4
DB 3	0.8	0.9	1.3	1.6	>4	>4
DB 4	0.6	0.9	1.2	1.4	>4	>4
DB 5	0.7	0.9	1.2	1.5	>4	>4
PB 1	0.8	1.4	1.8	2.3	>3, <4	>3, <4
PB 2	0.6	0.9	1.7	1.9	>4	>4
PB 3	0.7	0.9	1.5	1.6	>4	>4
PP 4	0.9	1.2	1.8	2.1	>3, <4	>3, <4
PB 5	0.9	1.4	1.8	2.1	>3, <4	>3, <4

[a]Predicted by method A.
[b]Acquired through tracking the beer under actual storage condition.

Table 3. Verification of the accuracy of TDFC predicted by method B

Sample	TBA_i	TBA_f	Predicted TDFC[a] (days)	Actual TDFC[b] (months)
DB 1	0.136	0.169	157	>4
DB 2	0.129	0.166	140	>4
DB 3	0.111	0.145	153	>4
DB 4	0.126	0.150	216	>4
DB 5	0.136	0.162	200	>4
PB 1	0.297	0.348	102	>3, <4
PB 2	0.314	0.355	127	>4
PB 3	0.303	0.340	140	>4
PB 4	0.157	0.202	115	>3, <4
PB 5	0.339	0.383	118	>3, <4

TBA, 2-thiobarbituric acid; TBA_i, initial TBA; TBA_f, final TBA.
[a]Predicted by method B.
[b]Acquired through tracing the beer under actual storage condition.

with the intensity of stale flavour, the values of TBA_c of different beers were highly variable, as in the case of DB and PB.

However, from Fig. 3, one can see that the slopes of the regression equation, for both beers, were extraordinarily similar. Thus the increment of the TBA value will tend to be constant when the increment of the intensity of stale flavour of the beer is identical. According to the regression equations, the increment in the TBA value (namely the difference between TBA_c and the initial TBA value) of DB and PB was about 0.173 when the intensity of the beer stale flavour increased to 2 from 0.

The main steps of method B. Based on the kinetics of the development of stale flavour of beer, the linear correlation between the TBA value and the extent of beer ageing, and the difference between TBA_c and initial TBA value (TBA_i), method B for predicting TDFC of beer was developed.

The procedure is as follows:

Step 1 - six bottles of beer are selected.
Step 2 - three bottles are forced aged at a temperature of 50°C for 24 h and three bottles beer are stored at 4°C.
Step 3 - the TBA_i and the final TBA value (TBA_f) are determined after forced aging at 50°C for 24 h in triplicate. The averages of TBA_i and of TBA_f are calculated.
Step 4 - the TDFC according to equation 1 is calculated and 'day' is the unit of the TDFC.

$$\mathrm{TDFC\ (days)} = \frac{TBA_c - TBA_i}{TBA_f - TBA_i} \times 30 \quad (1)$$

Because the difference between TBA_c and TBA_i is 0.173, the formula can be simplified to:

$$\mathrm{TDFC\ (days)} = \frac{0.173}{TBA_f - TBA_i} \times 30 = \frac{5.19}{\Delta TBA} \quad (2)$$

where ΔTBA is the increment of the TBA value during 1 day of storage at 50°C.

When it is assumed that the intensity of beer ageing at which most consumers can perceive a beer beginning to exhibit a stale flavour is 1, the prediction equation can be simplified to:

$$\mathrm{TDFC\ (days)} = \frac{0.0865}{TBA_f - TBA_i} \times 30 = \frac{2.6}{\Delta TBA} \quad (3)$$

Validation of these two prediction methods

The accuracy of these two methods for predicting the TDFC of a beer required verification. Thus five batches of DB and PB beers were collected and stored at room temperature in the dark. The intensity of stale flavour of these two beers was scored every month to determine the actual TDFC (i.e. until the stale intensity approached or exceeded 2) . All results are shown in Table 2 and Table 3. These beer TDFCs predicted through methods A and B were consistent with the actual TDFCs obtained under the natural conditions. These results suggest that these two methods for predicting the TDFC of a beer are accurate for the two beers studied.

Conclusions

The development of stale flavour in a beer on the one hand changes the expected flavour of beer and on the other hand, in most cases, results in off-flavours in the beer. Therefore it is very important to be able to rapidly forecast the TDFC of a beer in advance. The methods developed in this study make it possible to predict the TDFC of a specific beer in advance.

The kinetics of beer ageing complied with the first-order linear equation. The rate of beer ageing rose with the increase in storage temperature. The development of stale flavour during 1 day at 50°C was equivalent to that during 4 weeks of storage at room temperature. Similarly, 1 day at 60°C was equivalent to 8 weeks of storage at room temperature.

Correlation analyses showed that the TBA value had statistically a significant correlation with the intensity of beer stale flavour, but that the TBA value showed large variation amongst different beers. An interesting phenomenon was observed in that the difference between TBA_c and TBA_i had a small variation and this tended to be 0.173.

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