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Use of Chemical Indicators of Beer Aging for Ex-post Checking of Storage Conditions and Prediction of the Sensory Stability of Beer

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ABSTRACT: The rate of beer aging is affected by storage conditions including largely time and temperature. Although bottled beer is commonly stored for up to 1 year, sensorial damage of it is quite frequent. Therefore, a method for retrospective determination of temperature of stored beer was developed. The method is based on the determination of selected carbonyl compounds called as "aging indicators", which are formed during beer aging. The aging indicators were determined using GC-MS after precolumn derivatization with *O*-(2,3,4,5,6-pentaflourobenzyl)hydroxylamine hydrochloride, and their profile was correlated with the development of old flavor evolving under defined conditions (temperature, time) using both a mathematical and statistical apparatus. Three approaches, including calculation from regression graph, multiple linear regression, and neural networks, were employed. The ultimate uncertainty of the method ranged from 3.0 to 11.0 °C depending on the approach used. Furthermore, the assay was extended to include prediction of beer tendency to sensory aging from freshly bottled beer.

KEYWORDS: *beer, stale flavor, sensory aging of beer, aging indicators, carbonyl compound, GC-MS, multiple linear regression, neural networks, storage conditions*

■ INTRODUCTION

Sensory aging of beer is currently considered to be one of the most serious problems of the brewing industry and, thus, of brewing science. In contrast to the problem of colloidal stability of beer, which was successfully resolved 50 years ago, beer sensory aging is a complex phenomenon dependent on many different factors. In a narrower sense, the sensory aging concerns beer stored in small containers (bottles, cans) with a guaranteed period commonly ranging from a few months to about 1 year. Cask (draft) beer is stored in refrigerated areas (up to 5 °C), and its shelf life is limited.

The beer aging rate is affected by both chemical composition and storage conditions, such as time and temperature of storage. Many sensory changes can occur during beer aging. Primarily, the bitterness is reduced and its character is changed, and also fruity aroma decreases. Moreover, aromas such as catty, black currant, or wet paper/cardboard together with other aromas and flavors such as sweet, caramel, honey, bread, earthy, straw, after hay, wood, and sherry can arise in some beers.[1] Meanwhile, beers aging at room temperature (20−25 °C) usually acquire mainly a caramel aroma, and the cardboard character prevails in beers aging at 30 °C and higher temperatures.[2]

Food aging in general can be described by the following formula:[2,3]

$$\mathrm{RQD} = k(C_i, E_j)$$

RQD is the rate of quality deterioration, C_i represents compositional factors (e.g., content of reactive species, catalysts, inhibitors, pH, etc.), E_j represents environmental factors (e.g., temperature, light, mechanical stress), and k is a proportionality constant.

The equation describes also the aging of beer; identifying the relationships among these factors is a challenge for future research.

It has been shown that the main compounds arising during beer aging are carbonyl and heterocyclic compounds.[4] The most important factors and processes that affect beer aging include oxygen, reactive oxygen species, transition metal ions, Fenton reaction, sources of hydrogen peroxide, sulfur compounds, radicals producing other radicals, enzymatic oxidation of unsaturated fatty acids, nonenzymatic oxidation of unsaturated fatty acids, oxidation of iso-α-acids, oxidation of higher alcohols, Strecker degradation of amino acids, aldol condensations, binding of carbonyls by sulfur dioxide, release of flavor active compounds by enzymes from yeast, changes in ester levels, and so on.[2,5−8]

Because these factors contribute to the aging process to various degrees and can affect each other, it is very difficult to understand or describe the aging process of the beer in a simple way. However, it was observed that the concentration of multiple substances significantly increases during the aging process. Some of these compounds are sensorially active, and the increase of their concentration therefore causes (mostly adverse) beer taste changes.[9] The most important of these compounds are carbonyl compounds (aldehydes and ketones), the precursors of which are formed in the course of the technological process.[10,11]

Previously, it was observed that the concentrations of some substances, which are found in fresh beer at a low level, significantly increase in aged beer. At concentrations typical for fresh beer these compounds are mostly sensorially inactive[9,12] and were referred to as "aging indicators" as they could be advantageously used for the monitoring of beer aging.[12] In other words, if a correlation between the rate of formation of

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Table 1. Experimental Design of Treatment of Beer Samples[a]

beer	freshly bottled	stored for 2 months at 0, 8, 20, and 30 °C	stored for 4 months at 0, 8, 20, and 30 °C	stored for 6 months at 0, 8, 20, and 30 °C	shocked for 6 days at 45 °C
A1	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
A2	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
A3	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
A4	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
B	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
C	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
D	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
E	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
F	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
G	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y
H	x, y	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y (0, 8, 20, 30 °C)	x, y

[a]x, carbonyl compound estimation (2-furfural, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and 2-phenylacetaldehyde); y, sensory evaluation.

these compounds and beer aging is known, the storage conditions could be retrospectively determined.

The most important indicators of aging include the following compounds: 2-furancarboxaldehyde (2-furfural), which arises in Maillard reaction of cyclization of pentoses or oxidation of hexoses; 2-methylpropanal, which is a product of thermal degradation of valine according to the Strecker reaction mechanism[13] or oxidation of 2-methylpropanol; 2-methylbutanal, which is a Strecker reaction product of leucine or an oxidation product of 2-methylbutanol; 3-methylbutanal, which arises by Strecker reaction from leucine under the action of heat or oxidation of 3-methylbutanol or catalytic activity of melanoidins; and 2-phenylacetaldehyde, which is a product of the Strecker thermal degradation of phenylalanine.[9]

It is common knowledge that maintaining optimal beer storage conditions below 10 °C (15 °C is maximal) is necessary to minimize sensory aging. Unfortunately, in cases of sensorial damage of the beer, the producer has only limited possibilities to assess the conditions under which the beer is distributed and stored. Development of effective assays for reliable determination of storage conditions of beer would help in the control of proper handling of beer during the passage from brewery to consumer.

The aim of this work was the development of a method for retrospective determination of temperature conditions to which the beer had been exposed. The method is based on the determination of carbonyl compounds using GS-MS analysis after precolumn derivatization and its correlation with the sensorial profile of beer under defined storage conditions (temperature, time) using both mathematical and statistical apparatus. Moreover, prediction of beer tendency to sensory aging from freshly bottled beer was also performed.

MATERIALS AND METHODS

Experimental Design. Samples of pale all-malt lager containing original extract ranging from 11.5 to 11.8% were taken immediately after bottling in dark green bottles in the eight major Czech breweries. Four samples differing in the time of bottling were taken in brewery A. The total number of samples studied was 11, and they were designated A_1−A_4 and B−H; for more details of experimental design see Table 1. All samples were stored for 6 months at 0, 8, 20, and 30 °C. Furthermore, fresh beer was exposed to heat shock of 45 °C for 6 days. This modification allowed acceleration of the aging process and comparison of the observed analytical and sensory changes with the natural aging conditions.

Carbonyl compounds were determined in fresh beer, in shocked beers, and in stored beers at 2 month intervals. At the same time, samples were periodically analyzed using sensorial evaluation. The obtained results were used as data input for a statistical model.

Chemicals and Materials. The standards of aldehydes, internal standard 3-fluorobenzaldehyde (IS). and the derivatization reagent *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA) were purchased from Sigma-Aldrich. A 4 mg/mL aqueous solution of derivatization reagent was prepared daily. Pure water was obtained from a Milli-Q purification system (Millipore). All other chemicals used were of reagent grade.

Derivatization Procedure. The standard mixture, as well the beer, were adjusted to pH 4.4 using 0.1% H_3PO_4. Five milliliters of beer or standard mixture was placed into the test tube, and 20 μL of IS (3-fluorobenzaldehyde, concentration approximately 120 mg/L), 50 μL of $Na_2S_2O_3{\cdot}5H_2O$ (0.1 M), and 500 μL PFBOA were added. The mixture was then thoroughly shaken and allowed to stand at room temperature for 1 h. The derivatization reaction was then stopped by adding 50 μL of 9 M H_2SO_4. The PFBOA derivatives were subsequently extracted with 1 mL of *n*-hexane. The hexane layer was removed, washed three times with 5 mL of 0.05 M H_2SO_4, and dried over anhydrous sodium sulfate. The dried hexane phase was then placed into screw-cap autosampler vials for GC-MS analysis.

Gas Chromatography with Mass Spectrometry Detection (GC-MS). The previously published methods of GC-MS determination of carbonyl compounds in beer[14,15] were slightly modified to obtain high repeatability of the results.

GC-MS was carried out on the TRACE GC Ultra gas chromatograph coupled with the quadrupole mass spectrometer DSQ II (Thermo Electron Corp.). The polar column TR-WAX MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The carrier gas was set at 1.2 mL/min, 1 μL of extract was injected in a splitless mode, and the splitless time was 1 min. The injection temperature was 250 °C. The chromatographic oven was held at 40 °C for 1 min and then was increased to 250 °C at 10 °C/min and finally to 280 °C at 20 °C/min. The identification and quantitation of PFBOA derivatives of carbonyl compounds was accomplished using a mass spectrometer (EI-SIM/TIC, 70 eV).

Sensory Analysis. Sensory analysis was carried out by a 12-member sensory panel of the Research Institute for Brewing and Malting, Prague. During the sensorial evaluation, the main emphasis was placed on the development of the beer stale flavor, and the tasters evaluated this character using marks ranging from 0 to 5 (0, none; 1, very poor; 2, poor; 3, medium; 4, strong; 5, very strong). The mean from the obtained values was calculated; the lowest and highest values were omitted.

RESULTS AND DISCUSSION

Ex-post Determination of Storage Conditions. Determination of selected carbonyl compounds was performed in all beers according to the experimental design given in Table 1. Many relationships were obtained between increasing concentrations of individual aging indicators and ambient conditions. For simplicity, only a few selected dependences have been chosen for the discussion of results.

The dependence of the formation of aging indicators on storage time and temperature was the first to be studied. The buildup of the aging indicators depends linearly on storage time and exponentially on the temperature. An example is the 3-methylbutanal formation over 6 months at all storage temperatures in samples of beers A1−A4 (see Figure 1a). Figure 1b demonstrates the dependence of increasing concentration of 3-methylbutanal on storage time at 30 °C in eight beers (A1 and B−H). Similar results were obtained for all monitored indicators (data not shown). The experimentally determined range of the final concentrations of aging indicators in one brand of beer (in this case A1−A4) is seen to be narrower than the range of the results from eight different beers (A−H).

Simultaneously, the sensory evaluation of stored beers was performed under the conditions given in Table 1. Very similar results were obtained for tested beers; Figure 1c shows an example of the rate of evolution of stale flavor in beer A1.

The curves in Figure 1c clearly have a very similar trend as the curves in Figure 1a. This implies that sensory changes of aging beer approximately correspond to the rate and intensity of the formation of aging indicators.

On the basis of these results, we designed an approach that makes it possible to retrospectively determine the average storage temperature of an unknown beer sample. The necessary basic input information is the bottling time of the beer sample. The situation is simpler when one analyzes a beer brand for which the aging profile was studied before and relevant data are available (in our case beers A1−A4 from brewery A). In this case, the results of this method are very precise, and it can be reliably determined at which temperature the analyzed beer was stored. A somewhat more difficult situation arises for the analysis of beer for which the aging profile is not known. Then one has to use for the calculation a database of profile of a larger beer group (in our case beers A−H), which should, however, include only similar beer types, for example, lagers.

There are three possibilities for calculating the storage temperature of beer. The first approach is a deduction of the storage temperature from the dependence of indicator concentration in beer on storage time. Two other approaches use calculations using mathematical and statistical methods, namely, multiple linear regression and neural networks.

Deduction from the Obtained Dependence. The reading of the storage temperature from the graph is the simplest (see Figure 1a) but rather approximate. From a practical point of view and on the basis of our experience, 2-furfural is the most suitable representative of monitored indicators owing to its very intensive formation. Furthermore, if the GC-MS technique is not available, then a less precise method of 2-furfural determination by UV spectrophotometry can be alternatively used.[16]

Multiple Linear Regression. Calculation of storage temperature using multiple linear regression uses only one representative of the aging indicator. This calculation is explained on an example of production of 2-furfural in unknown beer sample. The data obtained according to the experimental scheme given in Table 1 document the dependence of 2-furfural concentration in four beer samples (brewery A) on time and temperature. The increasing concentration of 2-furfural, c(furfural), is exponentially dependent on storage conditions; the concentration was therefore converted to a linear dependence using natural logarithm ln c(furfural). Consequently, the regression equation

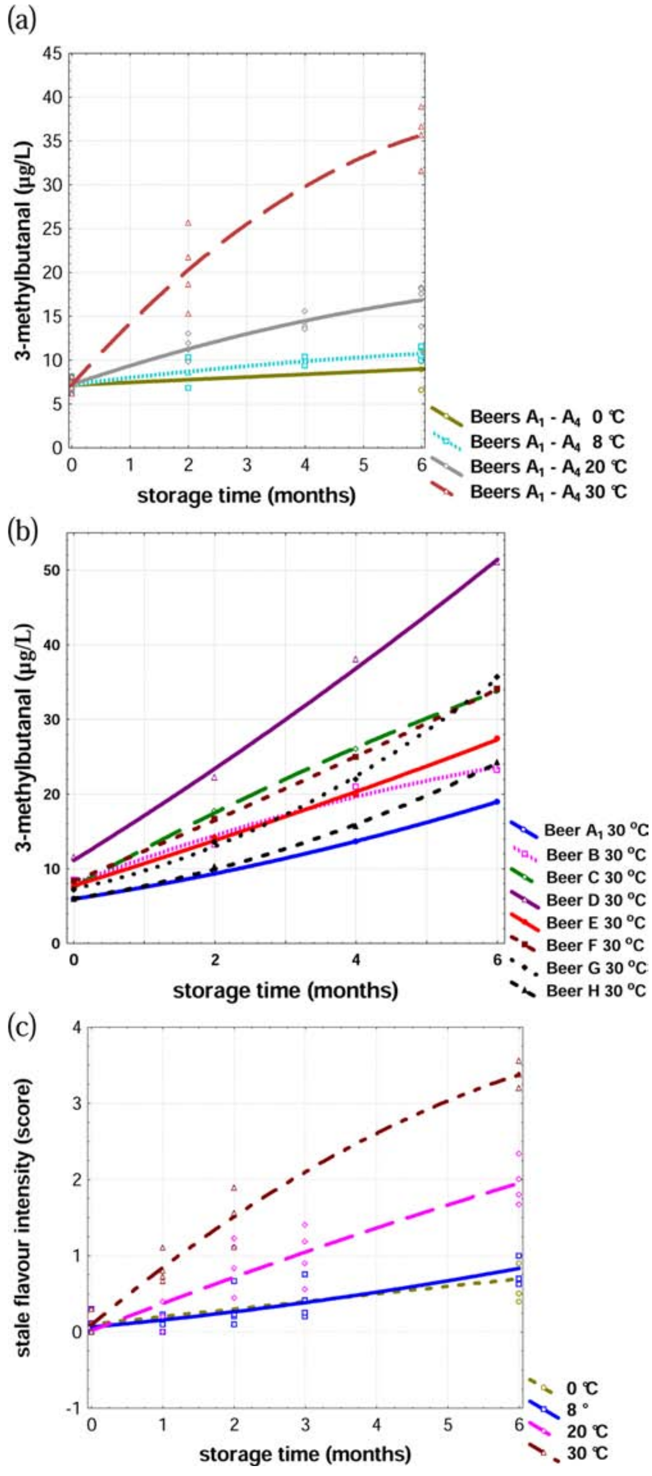

Figure 1. Effect of storage time and temperature (a) on the origin of 3-methylbutanal in samples A1−A4 at storage temperatures of 0, 8, 20, and 30 °C; (b) on the origin of 3-methylbutanal in eight samples A1 and B−H at 30 °C; and (c) on the development of the stale flavor intensity during storage of beers A1−A4.

$$\text{temperature } (^\circ\text{C}) = z + x \times \text{month} + y \times \ln c(\text{furfural})$$

Table 2. Storage Temperature Calculation Using Neural Networks for Beers A1−A4 (Partial)

storage time (months)	2-methylbutanal (μg/L)	3-methylbutanal (μg/L)	phenylacetaldehyde (μg/L)	3-methylpropanal (μg/L)	2-furfural (μg/L)	real storage temp (°C)	calcd storage temp (°C)
0	2.34	6.16	46.0	4.54	11.1	0	1
2	2.50	8.54	4.28	5.36	13.4	8	6
2	3.65	11.2	21.2	9.24	35.3	20	17
2	7.54	21.7	36.5	18.8	205	30	31
4	2.89	9.51	7.38	6.48	13.7	8	7
4	4.79	13.8	11.0	12.1	55.6	20	20
6	3.36	11.4	22.9	8.00	13.5	8	7
6	6.13	18.3	38.7	16.5	99.3	20	20
2	2.57	6.81	19.7	5.55	10.7	8	3
2	3.96	9.82	20.5	9.64	31.2	20	16
2	6.69	15.3	21.2	16.7	121	30	31
4	3.67	9.34	17.7	8.11	12.6	8	9
4	6.36	14.3	27.8	14.7	49.5	20	22
6	3.56	9.93	12.2	8.60	14.1	8	7
6	5.73	13.8	15.9	14.8	51.9	20	15
6	15.8	31.6	38.0	28.2	452	30	28
6	2.62	6.61	7.61	4.75	5.20	0	2

is used, where month and ln c(furfural) are input variables and temperature storage is an output variable. The calculated coefficient of determination R^2, which indicates the tightness of the fit of the relevant regression points, was 93.8, and the resulting equation was temperature (°C) = −4.25 − 1.22 × month + 6.92 × ln c(furfural). Uncertainty of measurement, that is, an interval that contains the correct result with a probability of 95%, was determined as 5 °C.

Neural Networks. An accurate and reliable method to determine the storage temperature is to use all five monitored indicators of aging. They are formed by various biochemical mechanisms, and it can be assumed that they will characterize beer aging better than only a single indicator. From this perspective, a method of neural networks appears to be ideal. As an example, the calculation of the storage temperature from the data on five indicators for beers A1−A4 is given in Table 2 (only part of the table is shown). The input variables are the storage time and concentration of five selected indicators. The real and calculated values of storage temperature are in the two right-hand columns. As follows from Figure 2, the uncertainty of this measurement ranges from 2.5 to 4.5 °C.

If no data from the analyzed brand are available, one can use the data from a database of several similar beer types (A−H). The uncertainty of calculated temperature is then higher and ranges from 4 to 11 °C (see Figure 2). It is also evident that uncertainty of the determination decreases with the beer age.

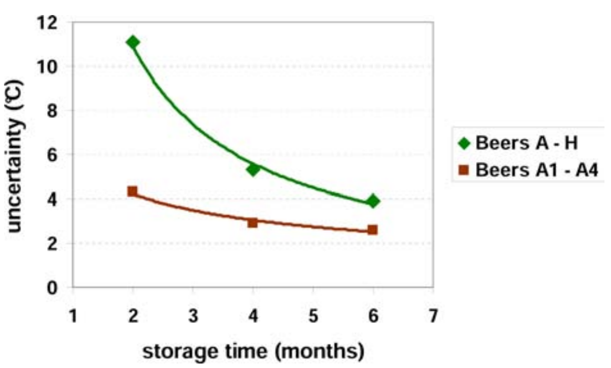

Figure 2. Comparison of estimation uncertainty of storage temperature from beers A1−A4 and beers A−H.

Prediction of Sensory Aging of Beer. The method of prediction of sensorial changes from the characteristics of freshly bottled beer was developed to obviate the impractical 6 month period needed for determining the actual profile of aging indicators in beer. For this purpose, the beer was exposed to a 6 day heat shock at 45 °C and the correlation between sensory indicator profiles of artificially aged beer (6D/45 °C) and naturally aged beer (6M/20 °C and 6M/30 °C) was studied. Numerous dependences were obtained between the formation of stale flavor and the concentration of monitored carbonyl compounds for both, beer storage at 20 and 30 °C and beer shocked at 45 °C. Only dependences with proven statistical significance and importance from a practical point of view are presented and explained below. When the data are analyzed and processed, it should be remembered that determination of aging indicators employing chemical analysis is more precise then that using sensory analysis.[17,18]

A statistically significant correlation (r = 0.82) was proved between the evolution of stale flavor in shocked beer (6D/45 °C) and stored beer (6M/20 °C) (data not shown). Hence, a sensory analysis of shocked beer can be used as a first-line means to assess the tendency of stored beer to aging.

Very approximately can be stated that the sensorial character (age) of beer artificially aged (shocked) over 6 days at 45 °C can be related to 6-week-old beer stored at 30 °C or ≥6-month-old beer stored at 20 °C.

The relationship was studied between the stale taste of beer and profiles of aging indicators in both stored and shocked beers. As demonstrated in Figure 3a, a clear correlation was found between the stale flavor of beer and 2-furfural content during the monitoring of eight beers stored at 8, 20, and 30 °C. When the 2-furfural content versus stale flavor is determined in a very wide interval of storage temperatures, the correlation coefficient is high (0.98).

When only the results for beer stored at 30 °C (see Figure 3b, which represents the upper right part of Figure 3a) are shown, a statistically significant association between the stale flavor of beer and content of 2-furfural is found also at the same

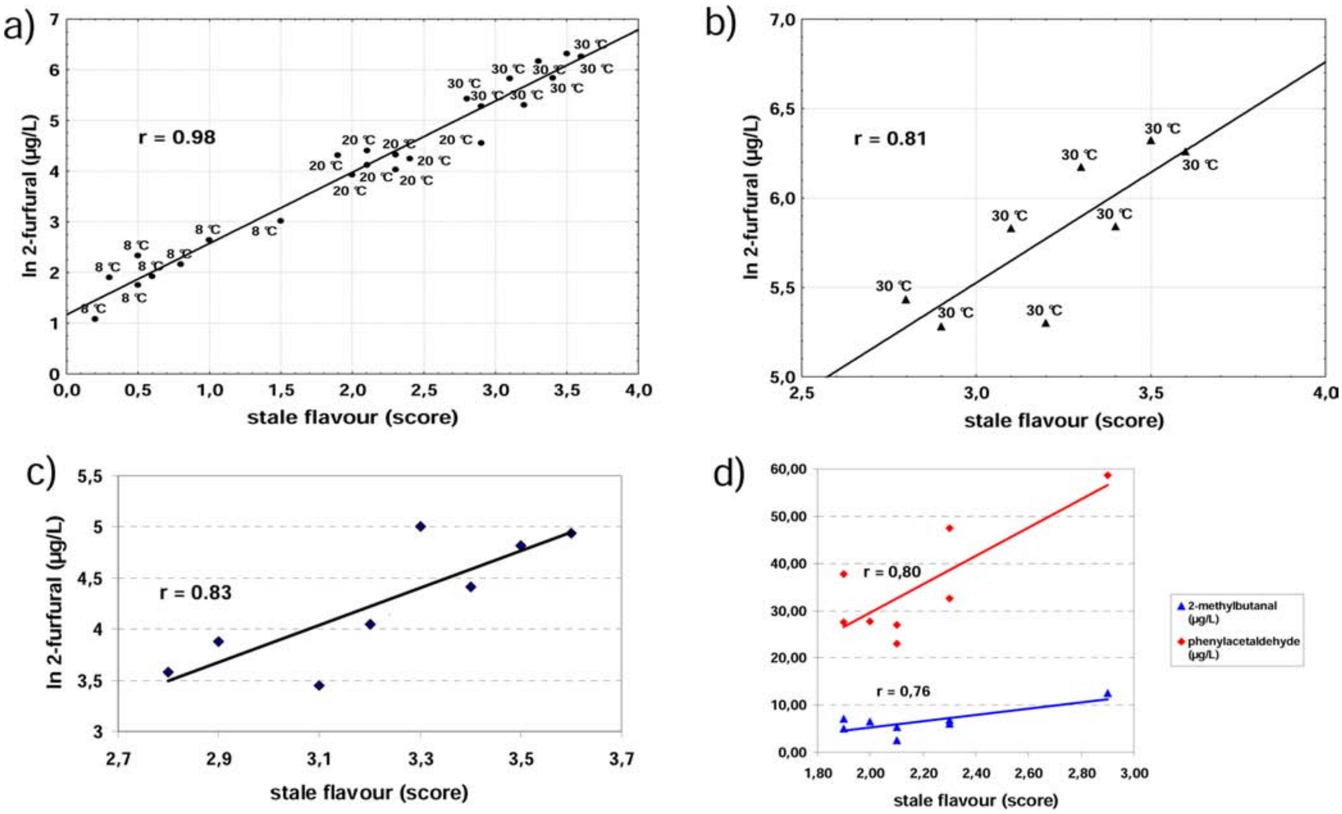

Figure 3. Carbonyl compounds content versus stale flavor: (a) 2-furfural in stored beer at 6M/8, 20, 30 °C; (b) 2-furfural in stored beer at 6M/30 °C; (c) 2-furfural in shocked beer stored at 6M/30 °C; (d) 2-methylbutanal and phenylacetaldehyde in shocked beer stored at 6M/20 °C.

temperature. As already mentioned in the Introduction,[9,12] this concentration of 2-furfural does not participate in stale flavor, but correlates with other sensorially active ingredients of old beer (r = 0.81).

Determination of 2-furfural concentration after the heat shock (6D/45 °C) in beer and comparison with stale flavor of naturally stored beer (see Figure 3c) were performed to reduce the time required for monitoring the indicator profile under natural conditions (i.e., after 6 months).

The results are convincing because the coefficient of determination (square of the correlation coefficient), indicating how closely two variables are related, is in the range of approximately 50−70%. It is obvious that 2-furfural as an indicator of aging is more suitable for prediction of sensory stability of beer at a higher temperature.

Two other important indicators, 2-methylbutanal and phenylacetaldehyde, have a similar value for the prediction of flavor development as 2-furfural; however, higher correlations were found at 20 °C (Figure 3d). The relatively high correlation coefficient is in both cases affected by the points situated at the top right of the figure.

These results were confirmed by using factor analysis. Figure 4 shows that the indicators 2- and 3-methylbutanal are more convenient for predicting the sensorial stability of beer stored at 20 °C (they are located at higher values of factor 1), whereas the prediction for sensory stability of beer stored at 30 °C is more precise when the monitoring of 2-furfural is used. This conclusion corresponds to the observed correlations, which are higher at 20 °C for 2- and 3-methylbutanal and at 30 °C for 2-furfural.

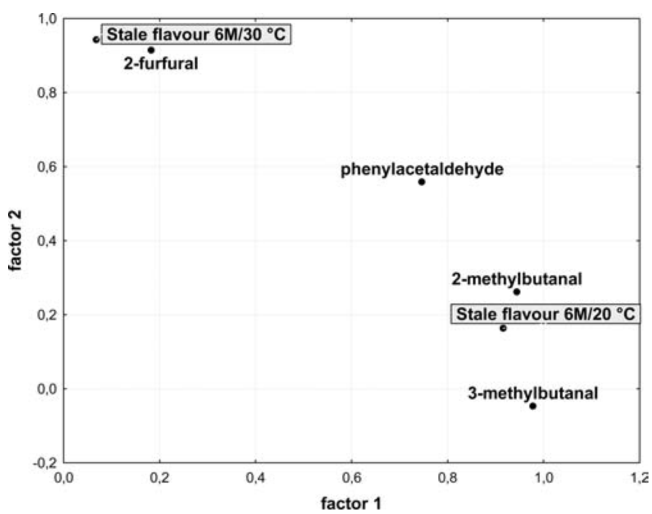

Figure 4. Factor analysis of the aging indicators as variables in relation to temperature of stored beer.

Overall, it can be concluded that the determination of aging indicators in beer after the 6D/45 °C shock may to some extent predict the tendency to aging of the sample beer.

Moreover, the probability of estimating sensorial stability increases with higher concentrations of aging indicators; that is, the beer with lower sensory stability can be identified more easily.

On the basis of the database of indicator profiles, the following procedure can be used for predicting sensorial stability. Fresh beer is shocked for 6 days at 45 °C, and the determination of aging indicators and the sensory evaluation focused on the development of stale flavor are performed. Finally, the parameters of the analyzed sample are compared to the parameters of a database. The probability of a correct estimation of sensory stability will be higher in the case of samples having parameters that are deposited in the database.

Aging indicators were found to characterize very well the changes taking place during the storage of beer; 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, phenylacetaldehyde, and 2-furfural in particular were shown as the best ones.

The determination of these substances in beer allows a very reliable estimation of the degree of damage caused by aging and also a retrospective control of the storage temperature. The sensorial damage after a thermal load can therefore be very competently estimated, and an ex-post control of transportation and storage conditions can be performed.

Furthermore, not an excessively strong but still statistically significant correlation was found between the formation of stale flavor in naturally aging beers and in beers that were shocked at higher temperature.

Similar correlations were found between the formation of stale taste of both naturally aged and shocked beers and the development of some aging indicators, specifically carbonyl compounds. On the basis of these findings, a convenient and useful method for prediction of the sensory stability of beer was proposed and verified. Fresh beer was artificially aged, and aging indicators were determined using a GC-MS method and sensorial analysis. The calculation serving as the basis for prediction of the storage conditions is based on the comparison of parameters and data obtained from a prebuilt database.

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Notes

The authors declare no competing financial interest.

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