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Effects of pasteurization on aluminium content and aroma compound changes in beer

Although beer pasteurization has been the object of a number of studies, there is no published data dedicated to investigation of the effects of pasteurization on aluminium content of beer and aluminium migration from aluminium package to beer. Thus, in order to investigate the changes of aluminium content along with aroma compounds changes, GF-AAS method with Zeeman background correction and GC-HSS method were used in this work. Also, for other analytical determinations, classic physical and chemical methods were applied. Pitting corrosion of aluminium cans was determined by microscopic analysis. Analyses were conducted periodically throughout seven months of storage on two different brands of beer filled in aluminium cans (pasteurized and non-pasteurized samples). One part of samples was stored in a refrigerator (4 °C) and the other in a thermostatic chamber (22 °C). The effects of pasteurization on aluminium content and aroma compounds changes were observed. Pasteurized samples, at the beginning of storage (22 °C), showed a higher content of aluminium and can corrosion. At the end of storage time in non-pasteurized samples, presumably via the activity of present microorganisms, more expressive can corrosion and aluminium migration was observed. Levels of aroma compounds in both pasteurized and non-pasteurized beer samples decreased during storage negligibly.

Descriptors: Aluminium, beer, pasteurization, aroma compounds (esters and higher alcohols), storage.

1 Introduction

Beer pasteurization is the most common method used for prolongation of beer shelf life. Pasteurization is necessary to ensure the microbiological stability of the final product. Currently, heat treatment is mainly performed by flash pasteurization or tunnel-pasteurization. Cold filtration appears interesting to replace pasteurization by heat treatment and allows the elimination of the organoleptic problems induced by thermal processing (1, 2).

Longer heating, residence and cooling times during pasteurization (especially in tunnel pasteurizators applied primarily for pasteurization of beer cans) lead to the beer flavour changes (undesirable flavour "after pasteurization" or bread-like flavour) and can sometimes change the colour of beer. Likewise, high aluminium content of beer can trigger the onset of "metallic" beer flavour.

Esters and higher alcohols are volatile constituents that form the major part of beer flavour. Their production during the brewing process is influenced mainly by the wort composition, fermentation parameters, and yeast strain.

Esters and higher alcohols are measured routinely in brewing, however, it is not clear if these components change in a predictable direction during the ageing process, although, the decrease in ester character of beer has been observed during ageing (3). An extracellular yeast esterase found in non-pasteurized beer hydrolyzes long chain esters. Lower levels of total esters and higher levels of total fatty acids were found in non-pasteurized (cold-filtered) beer stored at 30 °C (4).

Bellido-Milla et al. found differences between bottled and caned beer that relate to taste and stability (5). A trend with storage time for the flavour of all the canned lager samples is to increase in cabbage and decrease in fruity, buttery and aromatic characteristics. The bottled lagers do not show this trend (6).

The factors that effect the corrosion of aluminium cans, and process of aluminium migration from can to beer are: the type and quality of cans; the type and thickness of protective can coating; pH of beer; the length of contact between the can and the beer; storage temperature; presence of any corrosive substances (8 – 10).

Because beers are slightly acidic media and they do not contain strongly corrosive substances only a slight dissolution of aluminium in contact with beer occurs. The increase of the aluminium concentration in canned beer during the storage is relatively small compared to increases of the aluminium concentration in some canned soft drinks for the same storage period, which can be up to 2600 % (7, 9).

The aim of this work was to determine effects of thermal treatment (pasteurization) of beer cans on aluminium content, aluminium migration, and aroma compounds (esters and higher alcohols) content in beer during storage. For that purpose highly sensitive GF-AAS method with Zeeman background correction and GC-HSS method were used in this work.

2 Materials and methods

2.1 Samples

Analyses were conducted periodically throughout seven months of storage on two different brands of beer (A and C brand) filled in aluminium cans (pasteurized and non-pasteurized samples from same batch). One part of samples was stored in a refrigerator (4 °C) and the other in a thermostatic chamber (22 °C). Brand A was lager beer and brand C was premium lager beer. Numbers after letters A or C designate heat treatment and storage conditions of samples:

1 = pasteurized, 22 °C;
2 = pasteurized, 4 °C;
3 = non-pasteurized, 22 °C;
4 = non-pasteurized, 4 °C.

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2.2 Procedures

Prior to aluminium content determination 40 ml of beer were withdrawn from can. Containers and laboratory materials were washed with warm, diluted nitric acid and subsequently rinsed with double-distilled water (l = 0.055 μS/cm). Beer sample was placed in ultrasonic water bath to eliminate carbonation and than 5 ml of de-gassed beer were diluted to 50 ml with 0.2 % nitric acid (in double distilled water). After that sample was placed in the autosampler cup and shortly before Al determination 20 μl of sample were mixed with 15 μl/l of matrix modifier ($Mg(NO_3)_2$, 1 g/l dissolved in double distilled water). Aluminium content was determined by direct aspiration of the beer sample into a Perkin-Elmer model 4110 ZL atomic-absorption spectrophotometer with following equipment:

- Zeeman's graphite furnace (PE THGA- System);
- graphite tubes (PE THGA);
- Autosampler AS-72;
- Al hollow cathode lamp (Perkin-Elmer);
- Software (Perkin-Elmer AAWinLab ver. 2.50.).

The concentration of Al present in the beer samples was determined by comparing the absorbance of each sample with that of standards of known concentration. A stock solution of 1000 mg Al/l was used as the Al reference standard. Al standards in the concentration range 0-100 μg Al/l were prepared from the stock (reference) standard by dilution with double distilled water (λ = 0.055 μS/cm).

The graphite furnace program and other instrumentation conditions were as follows:

- Resonance wavelength: 309.3 nm;
- slit width: 0.7 nm;
- lamp current: 25 mA;
- signal processing parameter: peak-area mode;
- number of consecutive measurements of the sample: 3;
- injection temperature: 20 °C.

The temperature and gas programs were:

- step 1
  – temperature 110 °C,
  – 1-s ramp time,
  – 40-s hold time,
  – argon flow 250 ml/min;
- step 2
  – temperature 130 °C,
  – 15-s ramp time,
  – 40-s hold time,
  – argon flow 250 ml/min;
- step 3
  – temperature 1200 °C,
  –10-s ramp time,
  –20-s hold time, argon flow 250 ml/min;
- step 4
  – temperature 2350 °C,
  – 0-s ramp time,
  –3-s hold time,
  –argon flow stop;
- step 5
  – temperature 2450 °C,
  – 1-s ramp time,
  – 3-s hold time,
  – argon flow 250 ml/min^{-1}.

Measurements with standard deviation greater than 10 % between three consecutive measurements of the sample were redone. All samples were done in duplicate.

The pH values of the beer samples were determined by pH-meter Methrom, model 744.

Pitting corrosion (localized corrosion on a small surface area) of aluminium cans was determined by microscopic analysis using Olympus BH2-UMA microscope, TV camera Sony CCD XC-77, IMAGE monitor and software (LECO 2001 ver. 1.09.). Samples of aluminium cans (20 X 50 mm) were placed under the microscope (with internal surface up) and focused under 100 X enlargement. Picture of the sample surface was taken and digitized by camera and presented on monitor of the apparatus. With the software (LECO 2001 ver. 1.09.) digitized picture was colored in 256 different shades of gray (white-1, black-256). Dark areas that represent pitting corrosion are then colored in different color (red) and quantified (% of the surface area). Measurements were performed at the beginning (day 0) and at the end of storage (day 202) of storage. All samples were done in triplicate.

For the determination of beer aroma components, well-established static headspace method (SHS) was used. Standards of analyzed compounds (isoamyl alcohol, isoamyl acetate, 2-phenyl ethanol, acetaldehyde, ethyl acetate, isobutanol, ethyl decanoate, ethyl hexanoate, 1-propanol, 1-butanol, n-amyl alcohol, ethyl octanoate) were of >98% purity. Analyses were done on a Hewlett-Packard HP 5890 series 2 chromatograph with a split-splitless injector and a FID detector. For the SHS analysis Hewlett-Packard headspace sampler HP 7694 was used. Preliminary qualitative analysis was done by comparison of retention times of standards and corresponding peaks in beer samples. Compounds of interest were resolved on a Stabilwax capillary column (30 m x 0.25 mm; 0.25 μm) in following parameters: initial oven temperature was 35 °C kept for 4 min, than raised at 10 °C/min to 80 °C followed by 25 °C/min to 180 °C and by 10 °C/min to 210 °C and than kept for 2.5 min at 210 °C. Samples were injected by means of the headspace sampler in splitless mode (2 min). Injection port temperature was kept at 180 °C, pressure was 68947 Pa (10 psi) and carrier gas (nitrogen) flow was 3 ml/min. Detector temperature was 250 °C. A headspace sampler was equipped with a standard 1 ml loop. Carrier gas pressure was 117210 Pa (17 psi), vial pressure was 48263 Pa (7 psi) and injection time vas 0.2 min. Samples were heated for 20 min at 50 °C. Peak areas of beer samples have been measured and expressed in the integrator units (counts). Measurements were performed at the beginning (day 1) and after 150 days of storage. All samples were done in triplicate.

3 Results and discussion

Results of aluminium concentrations and pH-value changes during storage of beer samples are shown in Figs. 1 – 3 and Table 1.

During first 70 days of storage in refrigerator (Fig. 1) pasteurized samples of beer A had higher aluminium concentration when compared to non-pasteurized samples of same brand. At the same time observed pH-changes were similar for both pasteurized and non-pasteurized samples. After 70 days of storage in refrigerator, along with faster pH decrease in non-pasteurized samples more expressive aluminium migration from can to beer was observed. Therefore, at the end of storage (days 150 and 202) non-pasteurized samples had higher aluminium content. It is reasonable to assume that in non-pasteurized samples significant microbial growth took place which decreased pH-value bellow 4.1. Most probably, these microbes (contaminants) were species of lactic

acid bacteria because they find beer a suitable media for growth. Therefore, pasteurized samples exhibited faster aluminium migration due to the heat damage of internal can surface, until the moment when organic acids formed as a result of bacterial metabolism decreased pH-value bellow 4.2. It is already known that low pH-value of beer fastens process of protective coating corrosion which leads to enhanced migration of aluminium from can to beer and consequently to accumulation of aluminium in beer (8, 9). Further, one can see that on a last day of storage both pasteurized and non-pasteurized samples had substantially lower aluminium content when compared to previous analysis (day 150). That "lack" of aluminium is only apparent since the results could be influenced by colloid haze formed during long-time storage under conditions of low pH-value and elevated aluminium ion concentration (11, 12). Therefore, colloidal haze particles could contain more aluminium than rest of the sample. Colloids can precipitate and consequently influence on results of analysis. Obtained results could therefore appear lower due to poor homogeneity of the sample. The average aluminium concentration during storage of pasteurized samples was 231.17 µg/l whereas that of non-pasteurized samples was 217.34 µg/l. Aluminium migration from can to beer was more expressive in non-pasteurized beer (in first 150 days 333.56 µg/l).

Figure 2 shows that during storage in thermostatic chamber, pasteurized samples of brand A beer had higher aluminium concentration if compared to non-pasteurized samples only at the beginning of storage. After that, along with faster pH decrease in non-pasteurized samples more expressive aluminium migration from can to beer was observed. It can be concluded that at higher storage temperature in non-pasteurized samples microbes grow at faster pace decreasing the pH-value more rapidly and causing the more expressive increase of aluminium content in beer. Exceptions are samples withdrawn at 110 and 202 days of storage when in pasteurized and non-pasteurized samples similar pH-value and consequently similar aluminium content were observed. It could be due to various initial microorganism count in samples after filling of beer. Also, it is possible that higher storage temperature had more influence on aluminium migration then pH-value. In fact, on Figure 2, it can be seen that differences between pasteurized and non-pasteurized samples diminish over time of storage. The average aluminium concentration during storage of pasteurized samples was 289.04 µg/l whereas that of non-pasteurized samples was higher (348.5 µg/l). Aluminium migration from can to beer during storage was more expressive in non-pasteurized beer (418.6 µg/l) in comparison to pasteurized samples (335.96 µg/l).

Non-pasteurized samples stored at 22 °C had higher aluminium content during storage time than non-pasteurized samples stored at 4 °C (figures 1 and 2). Aluminium migration from can to beer was more expressive in non-pasteurized beer stored at 22 °C (418.6 µg/l).

Figure 3 shows that during storage in thermostatic chamber, pasteurized samples of brand C beer had higher aluminium concentration if compared to non-pasteurized samples only at the beginning of storage. After that, along with faster pH decrease in non-pasteurized samples more expressive aluminium migration from can to beer was observed. The most expressive increase of aluminium content was observed after 70 days of storage in non-pasteurized samples (from 150 to 532 µg/l). Except for aluminium migration this could be the result of actual differences among parallel samples (although parallel samples of cans and beer are withdrawn from same batches and were filled and stored identically), probably due to the differences in the thickness of the protective can layer. Besides, AAS-GF method is extremely sensitive on even minor contamination from particles of dust in air (13). The average aluminium concentration during storage of pasteurized samples was 208.24 µg/l whereas that of non-pasteurized samples was 388.65 µg/l. Aluminium migration from can to beer during storage was more expressive in non-pasteurized beer (435.95 µg/l) than in pasteurized samples (238.17 µg/l).

Generally, it is obvious that in non-pasteurized samples pH-value drops more rapidly than in pasteurized samples. Along with faster pH decrease in non-pasteurized samples more expressive alumin-

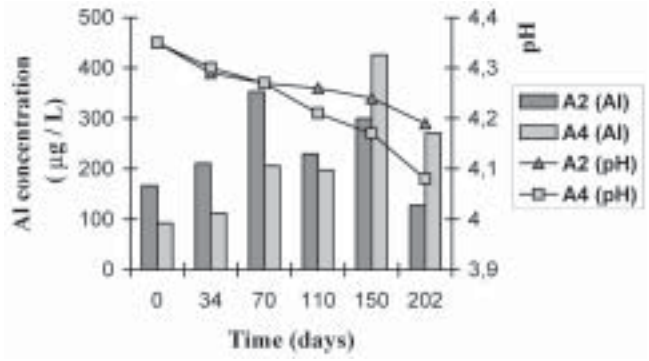

Fig. 1 Comparison of aluminium concentration and pH value changes in pasteurized (A2) and non-pasteurized (A4) lager beer samples during storage at 4 °C

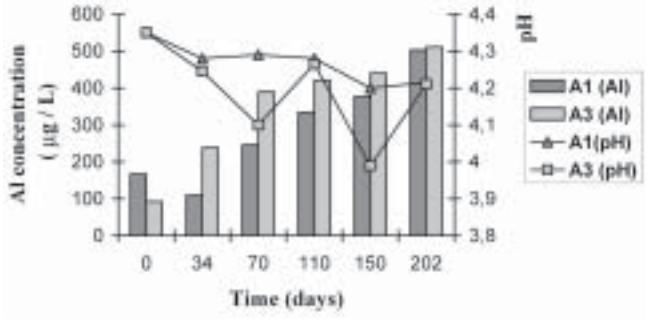

Fig. 2 Comparison of aluminium concentration and pH value changes in pasteurized (A1) and non-pasteurized (A3) lager beer samples during storage at 22 °C

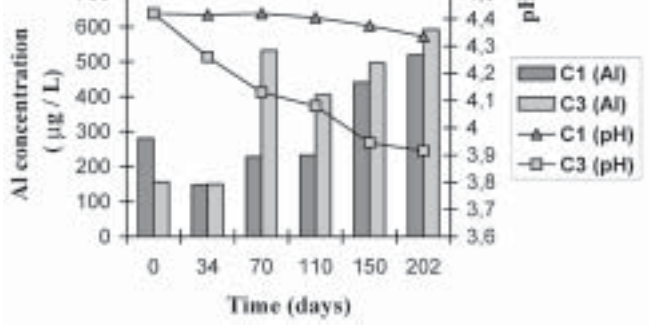

Fig. 3 Comparison of aluminium concentration and pH value changes in pasteurized (C1) and non-pasteurized (C3) premium lager beer samples during storage at 22 °C

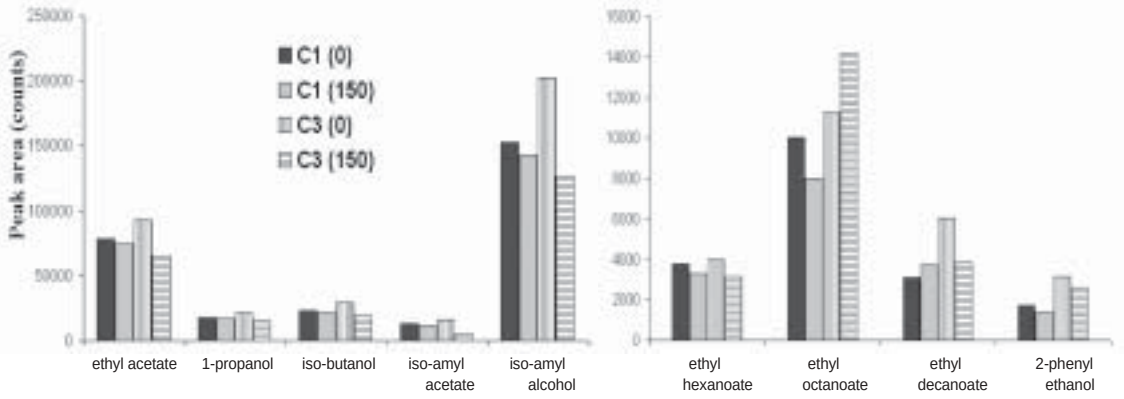

Fig. 4 Comparison of aroma compounds changes in pasteurized (C1) and non-pasteurized (C3) premium lager beer samples at the beginning (0) and after 150 days (150) of storage at 22 °C

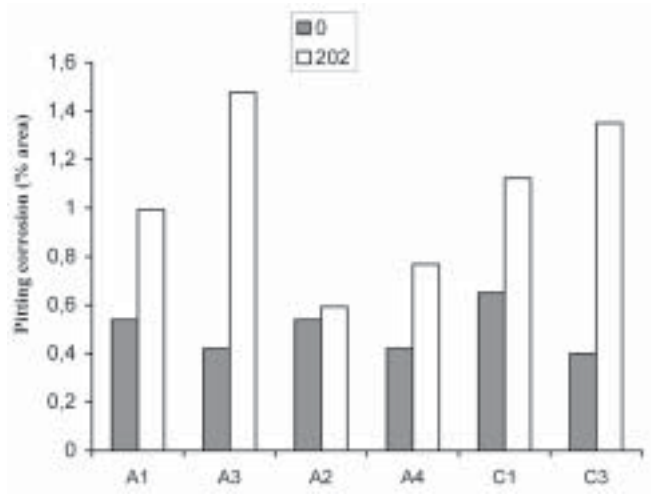

Fig. 5 Comparison of pitting corrosion of beer cans in all beer samples at the beginning (0) and after 202 days (202) of storage

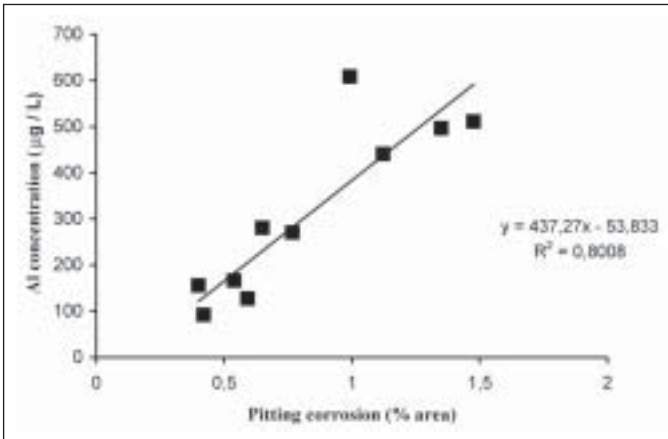

Fig. 6 Correlation between pitting corrosion of beer cans and aluminium concentration in beer samples (A1, A2, A3, A4, C1, C3)

ium migration from can to beer was observed. The most expressive aluminium migration was observed in non-pasteurized beer C samples stored at 22 °C (435.95 µg/l) with most expressive pH drop (3.905 on last day of storage).

Although effect of beer pasteurization on aluminium migration has not been subject of previously published papers experimental data from this work are in agreement with works of authors who emphasize positive influence of low pH-value on aluminium migration from aluminium container to certain beverage or food (7, 14 – 16), and positive influence of storage temperature on aluminium migration from can to beer (10).

Data presented in Table 1 show that pasteurized and non-pasteurized samples stored at same temperature did not differ significantly regarding aluminium concentration during storage (A1 vs. A3; A2 vs. A4; C1 vs. C3 — Fischer coefficient values were lower than F critical). As well, non-pasteurized and pasteurized samples stored at 22 and 4 °C respectively (A1 vs. A4) did not differ significantly. Non-pasteurized samples stored at 22 and 4 °C respectively (A3 vs. A4 — Fischer coefficient value (bold) were higher than F critical) differed significantly regarding aluminium concentration during storage.

Changes in aroma compounds are summarized in Figure 4.

Figure 4 shows comparison of aroma compounds changes during 150 days of storage at 22 °C between pasteurized (C1) and non-pasteurized (C3) samples. On the first day of storage it was observed that contents of all analyzed compounds (except acetaldehyde which was below limit of detection in both samples) was higher in non-pasteurized beer. After 150 days of storage faster decrease of all aroma compound content in non-pasteurized beer was detected with the exception of ethyl-octanoate. Content of this compound increases considerably in non-pasteurized beer. After 150 days of storage most of analyzed compounds contents were lower in non-pasteurized beer. Exceptions are ethyl-octanoate and 2-phenyl ethanol, whereas content of ethyl-decanoate didn't differ significantly. It is reasonable to assume that short exposure to elevated temperatures during pasteurization enhances chemical reactions that change quantities of aroma compounds in beer. Also, activity of microorganisms present in non-pasteurized beer stimulates more rapid aroma compound changes.

Pitting corrosion of beer cans are presented in Figure 5, and its influence on aluminium concentration in beer in Figure 6.

Pitting corrosion of aluminium cans (Fig. 5) was always higher after storage period of 202 days. Highest pitting corrosion intensity was detected in non-pasteurized samples stored at 22 °C for both beer A (A3) and beer C (C3). Lowest pitting corrosion intensity was detected in pasteurized samples stored at 4 °C (A2). Pasteurized samples exhibited higher corrosion intensity at the beginning of the storage in comparison to pasteurized samples (A1 vs. A3; A2 vs. A4; C1 vs. C3). Non-pasteurized samples exhibited higher corrosion intensity at the end of the storage.

Data shown on Figure 6 indicate good correlation between pitting corrosion of can and aluminium content of beer.

4 Conclusions

Pasteurized samples at the beginning of storage showed higher aluminium content and pitting corrosion of the can because the heat applied during pasteurization damages the protective coating on the can surface and stimulates the process of aluminium migration from can to beer.

During storage time in non-pasteurized beer samples, presumably via the activity of present microorganisms, more distinctive decrease of pH value was observed in comparison to pasteurized samples. Lower pH values stimulate can corrosion and therefore aluminium migration from can to beer.

At the end of storage time in non-pasteurized samples more expressive can corrosion and aluminium migration were observed.

Levels of aroma compounds in both pasteurized and non-pasteurized beer samples decreased during storage negligibly. Exceptions are significant decrease of iso-amyl alcohol and ethyl decanoate in non-pasteurized samples and significant increase of ethyl octanoate, also in non-pasteurized beer samples.

Table 1 ANOVA comparison of aluminium levels in beer based on heat treatment method (pasteurized vs. non- pasteurized samples)

Beer samples	F	P- value	F critical
A1 vs. A3	3.185	0.134	6.607
A2 vs. A4	0.079	0.79	6.607
C1 vs. C3	1.804	0.236	6.607
A1 vs. A4	3.026	0.142	6.607
A3 vs. A4	**14.846**	0.018	7.708

5 References

1. Gaub, R.: Brauwelt Int. **5**, 448-457, 1993.
2. Leeder, G.: Brauwelt Int. **4**, 372-373, 1993.
3. Meidaner, H., Narziss, L., and Eichorn, P.,: Proc. Congr. Eur. Brew. Conv. 23, 401, 1991.
4. Vesely, P., Volgyi, A., Lusk, L.T., Navarro, A., Seabrooks, J., and Ryder, D.: ASBC Annual Meeting, P-12, June 8 – 12, 2002.
5. Bellido-Milla, D., Moreno-Perez, J. M., and Hernández-Artiga, M.P.: Spectrochim. Acta B, **55**, 855-864, 2000.
6. Griffiths, N.M.: J. Inst. Brew. **97**, 173-179, (1991).
7. Šeruga, M., Grgi, J., and Mandi, M.: Z. Lebensm. Unters. Forsch. **198**, 313-316, 1994.
8. Sharpe, F.R., and Williams, D.R.: J. Am. Soc. Brew. Chem. **53,** 85-92, 1995.
9. Šeruga, M., Grgi, J., Grgi, Z., and Šeruga, M.: Z. Lebensm. Unters. Forsch. **204**, 221-226, 1997.
10. Vela, M.M., Toma, R.B., Reiboldt, W., and Pierri, A.: Food Chem. **63**, 235-239, 1998.
11. Mebak: "Brautechnische Analysenmethoden II", VLB, Berlin, 1996.
12. Kaneda, H., Takashio, M., and Tamaki, T.: J. Inst. Brew. **103**, 21-23, 1997.
13. Perkin-Elmer: "Analytische Methoden für Atomabsorptions-Spektroskopie", Bodenseewerk Perkin-Elmer & Co. GmbH, Überlingen, 1981.
14. Evenshtein, Z.M.: Pishch. Tekhnol. **2**, 56-59, 1971.
15. Ondreicka, R., Kortus, J., and Ginter, E.: "Intestinal Absorption of Metal Ions, Trace Elements and Radionucleotides", Pergamon Press, New York, 1971.
16. Samsahl, K., and Wester, P.O.: Sci. Total Environ. **8**, 165-177, 1977.

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