

Aluminium and Aroma Compound Concentration in Beer During Storage at Different Temperatures

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Summary

Problem of aluminium in beer has been elaborated in several papers over the last decade. However, the effect of aluminium on organoleptic properties of beer has been observed in few papers where it has been stated that aluminium gives beer a »metallic« and bitter flavour without any observations on particular aroma compound changes. Also, the number of reports on precise changes of aroma components throughout different storage conditions is surprisingly scarce. In order to investigate the changes of aluminium concentration along with aroma compound changes, graphite furnace-atomic absorption spectrophotometry (GF-AAS) with Zeeman background correction and gas chromatography with static headspace sampler (GC-HSS) were used in this work. Analyses were conducted periodically throughout seven months of storage on three different brands of beer from name breweries. Samples were taken before and after filling in aluminium cans. One part of samples was stored in a refrigerator (4 °C) and the other in a thermostatic chamber (22 °C). The effects of beer brand and storage conditions on aluminium concentration and level of aroma compounds were measured. To prove the effect of aluminium concentration on the changes of aroma compounds, the adequate level of aluminium sulphate was added to bottled beer samples stored at 28 °C. Although different beer types showed significantly different aluminium concentration, it could be the result of other factors (different batches of identical beer type showed significantly different aluminium concentration as well). Samples that were stored in the refrigerator were protected from aluminium migration from the can to the beer and showed increased aroma stability. Level of aroma constituents of analyzed beer brands was significantly different. Elevated aluminium concentration did not have any noticeable effect on the level of aroma compounds in beer samples stored at 28 °C.

Key words: aluminium, beer, aroma compounds (esters and higher alcohols), storage

Introduction

Aluminium in beer originates from brewing raw materials (water, hops, malt, yeast and adjuncts). As the manufacturing process progresses from wort to beer, aluminium appears at higher concentration (1). This in-

dicates aluminium pick up from processing equipment and/or filter aids. This is especially prominent if fermentors are made of 99.5 % aluminium, while for fermentors made of 99.99 % aluminium the pick up is negligible (2).

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Furthermore, after filling the matured beer into bottles, kegs or cans this process goes on, especially in aluminium cans and kegs.

The process of aluminium corrosion and migration from can to beer has been elaborated over the last decade in several papers. The factors of the main influence are: the type and quality of cans, the type and thickness of protective can coating, pH of the beer, the length of contact between the can and the beer, thermal treatment, storage temperature, and presence of any corrosive substances (3–6). Also, it has been concluded that the amount of aluminium in canned beer could not be considered as health hazard (3,4). On the other hand, primary packaging material could influence beer quality, aroma and flavour stability, which has been the subject of previous researches (7,8).

Although it is well known that dissolved oxygen concentration has decisive influence on beer stability, primary packaging material can facilitate various processes with negative influence on colloidal and/or flavour stability of beer. Beer filled in glass bottles is sensitive to light-struck flavour formation (9). Canned beer is protected from the influence of light, but aluminium migration from can to beer could aggravate colloidal and flavour stability (7).

Esters and higher alcohols are volatile constituents that form the major part of beer flavour. Mainly the wort composition, fermentation conditions, and yeast strain (10,11) influence their production during the brewing process. However, the presence of different esters can have a synergistic effect on the individual flavours, which means that esters can also affect beer flavour well below their individual threshold concentration (12). The process of ester formation during beer production is extremely complex and difficult to predict. The decrease in ester concentration is often monitored during beer storage (13). However, there are scarce literature data available on flavour changes that take place during beer storage (8,14–16). That is particularly true when it comes to flavour changes in relationship to different beer packages. Bellido-Milla *et al.* (17) found differences between bottled and canned beer that relate to taste and stability. A trend with storage time for the flavour of all the canned lager samples is to increase in cabbage, watery and metallic characteristics and decrease in fruity, buttery and aromatic ones. Bottled lagers do not show this trend (8).

Results of our previous research showed that at the end of storage time in nonpasteurized canned beer samples, presumably *via* the activity of present microorganisms, more expressive can corrosion and aluminium migration were observed compared to pasteurized samples. Pasteurization also contributed to aroma stability during storage, although it changed the original taste of beer (6).

The purpose of this work was to determine: (i) aluminium concentration in beer (before filling); (ii) aluminium migration from can to beer and aroma compound (esters and higher alcohols) changes in canned beer during storage, and (iii) possible effects of elevated aluminium ion concentration on aroma compound changes.

Materials and Methods

Samples

Analyses were conducted periodically throughout seven months of storage on three different brands of beer (A, B and C brand). Brand A was standard lager beer and brands B and C were premium lager beers. One part of samples was withdrawn from Bright Beer Tank (BBT) prior to filling and the other were samples of beer filled in aluminium cans, pasteurized and stored in a refrigerator (4 °C) or in a thermostatic chamber (22 °C). Numbers after letters A, B and C designate package and storage conditions of samples (Table 1).

Table 1. Plan of experiments

Beer sample designation	A	B	C
Beer type	A	B	C
BBT tanks	1	1	1
BBT – different batch	–	b	–
Canned beer stored at 4 °C	2	–	2
Canned beer stored at 22 °C	3	–	3
Canned beer stored upside down at 22 °C	d	–	–
Bottled beer with addition of Al or K sulphate stored at 28 °C	as or ks	–	–
Mash composition – malt/corn grits / %	70/30	90/10	100/0

To prove the effect of aluminium concentration on aroma compound changes aluminium sulphate was added to brand A beer filled in bottles under anoxic conditions (nitrogen atmosphere). Concentration of the added aluminium was 10 mg/L and that is approximately 50 times higher than the usually found concentration in beer. Samples were designated Aas. For control, potassium sulphate was added to beer from the same batch in the exact manner and samples were designated Aks (Table 1). To eliminate possible sulphate effect, both Aas and Aks samples had equal molar concentration of sulphate. Potassium sulphate was chosen because it is the most ubiquitous metal in beer ranging from 100–265 mg/L (17) and could not have significant influence on beer flavour after the addition. Samples were stored in a thermostatic chamber at 28 °C for two weeks.

Procedures

Prior to aluminium concentration determination, 40 mL of beer were withdrawn from BBT, cans or bottles. Containers and laboratory materials were washed with warm, diluted nitric acid and subsequently rinsed with double distilled water (conductivity=0.055 µS/cm). Beer sample was placed in ultrasonic water bath to eliminate carbonation and then 5 mL of degassed beer were diluted to 50 mL with 0.2 % nitric acid (in double distilled water). Afterwards, the sample was placed in the auto-sampler cup and shortly before Al determination 20 µL of the sample were mixed with 15 µL of matrix modifier [Mg(NO₃)₂, 1 g/L dissolved in double distilled water]. Aluminium concentration was determined by direct injection of 35 µL of the sample into a Perkin-Elmer (PE)

model 4110 ZL atomic absorption spectrophotometer with the following equipment (produced by Perkin-Elmer, United States): Zeeman's graphite furnace (PE THGA – System); graphite tubes (PE THGA); autosampler (PE model AS-72); Al hollow cathode lamp (Perkin-Elmer); and 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/L aluminium was used as the Al reference standard. Al standards in the concentration range 0–100 µg/L were prepared from the stock solution standard by dilution with double distilled water (conductivity=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; and injection temperature, 20 °C.

The temperature and gas programs are given in Table 2. Measurements with relative standard deviation (RSD) values greater than 10 % among three consecutive measurements of the sample were redone. Some typical values of the RSDs found were between 1.5–5 %. All samples were done in duplicate.

Table 2. The temperature and gas programs of Perkin-Elmer 4110 ZL atomic-absorption spectrophotometer

Step	Temperature °C	Ramp time s	Hold time s	Argon flow mL/min
1	110	1	40	250
2	130	15	40	250
3	1200	10	20	250
4	2350	0	3	stop
5	2450	1	3	250

For the determination of beer aroma components, a 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 × 0.25 mm; 0.25 µm) with the following parameters: initial oven temperature was 35 °C for 4 min, then 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 then 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 68 947 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 117 210 Pa (17 psi), vial pressure was 48 263 Pa (7 psi) and injection time was 0.2 min. Samples were heated for 20 min at 50 °C. Peak areas of beer samples were measured and expressed in the integrator units (counts). Results of the measurements performed at the beginning (day 1) and after 150 days of storage are given in Results and Discussion.

For the purpose of quantitative analysis (effect of aluminium concentration on the concentration of aroma compounds), calibration of gas chromatograph was performed. Calibration samples were prepared using ethanol-free beer with the addition of internal standard (*n*-amyl alcohol, final concentration was 28.7 mg/L after the addition). Eighteen calibration samples were divided into six groups. Each group was further prepared by the addition of different amounts of compounds of interest (isoamyl alcohol, isoamyl acetate, 2-phenyl ethanol, acetaldehyde, ethyl acetate, isobutanol, ethyl decanoate, ethyl hexanoate, 1-propanol, 1-butanol, ethyl octanoate), with the exemption of the first group (without the addition). Concentration range of the compounds of interest was calculated using literature data (18). Finally, calibration was performed using software ChemStation 1990–2000. Experimental samples were done in triplicate according to the above-mentioned procedure.

Thickness of the internal lacquer layer of the can was determined by metallographic analysis. Can samples were covered with two-component mixture (liquid and powder) Durofix 2-Kit. Samples were further prepared by polishing (polishing device Pedenin 2, Struers). Pictures of lacquer layer were obtained using Microscope Olympus BH2-UMA. Pictures were digitized using CCD camera. Thickness of the internal lacquer layer was determined by direct measurement on IMAGE monitor using software LECO 2001 ver. 1.09.

Results and Discussion

Effect of beer brand on the concentration of aluminium

Initial aluminium concentrations in three beer brands were determined in nonpasteurized samples from BBT before filling and are presented in Fig. 1. It is obvious

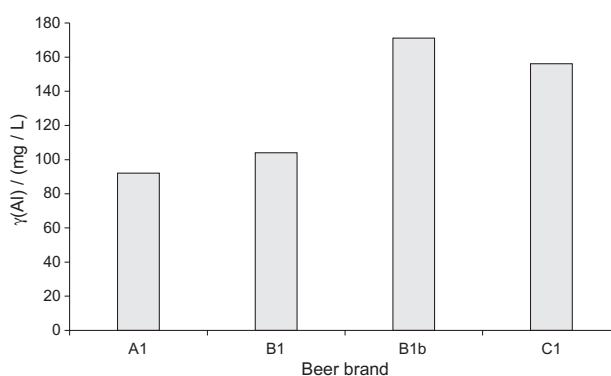


Fig. 1. Comparison of aluminium concentration in three beer brands (A, B and C) determined in nonpasteurized samples from BBT before filling (B1b=different batch of the beer brand B)

that the analyzed beers differ significantly regarding aluminium concentration, which is mainly influenced by various raw materials and technological processes employed for beer production.

The highest aluminium concentration (171 µg/L) was found in samples of premium beer brand (B1b) and the lowest (92 µg/L) in samples of standard lager beer (A1). It is worthwhile to notice that the same beer brands (B1 and B1b) from different batches could differ considerably regarding aluminium concentration. Thus, the range of determined aluminium concentrations was between 92–171 µg/L, which is in agreement with the published data. These data, a result of many different beer brand analyses, show that aluminium concentration ranges from 50–220 µg/L (19) and 20–505 µg/L (3). Our results just confirm that aluminium concentration in finished (clarified) beer differs among samples of different brands and also among samples of the same brand (different batch) because aluminium in beer is derived from various raw materials, equipment and brewing processes (4). It is obvious that premium beer produced exclusively from malt (C) or with small addition of adjunct such as corn grists (B) contains more aluminium when compared to standard lager beer (A) produced from 30 % or even more adjuncts in a grist. Therefore, malt is probably the main source of aluminium in clarified beer before filling, which is in agreement with results of Viñas *et al.* (1). However, the ingredient with potentially highest Al concentration is hop, but due to its broad range of aluminium concentration of 0.05–150 mg/kg according to Sharpe and Williams (3) and small mass contribution in beer making, it cannot be regarded as the main aluminium source in beer. Mashing liquor, on the other hand, contains less than 50 µg/L aluminium but is certainly a significant source of aluminium in beer before filling. Differences between two samples of the same beer brand (B1 and B1b) may be related to different batches of grist, the origin of used malt and hop and dose rate of diatomaceous earth during beer filtration.

Effect of storage temperature on the concentration of aluminium

Regarding the fact that beer filled in cans always undergoes pasteurization to prolong biological stability, Figs. 2–4 show the results of storage temperature effect (4 and 22 °C) on the changes of aluminium concentration in beer. Pasteurized samples of beer A and beer C were monitored. Samples stored in refrigerator showed aluminium »pick up« during the first 70 days of storage (Fig. 2). After that, aluminium concentration is lower probably due to sedimentation of cold beer haze, which is formed as a result of can corrosion progress (7). Therefore, it was not possible to conclude that aluminium migration from the can actually took place in cold stored samples (low correlation coefficient: $R^2=0.0011$). On the other hand, samples stored in thermostatic chamber at 22 °C showed constant aluminium pick up from day 34 to the end of the storage (significant correlation coefficient: $R^2=0.9031$). Total aluminium migration from can to beer (A3) (difference between fresh filled and stored beer) was 336 µg/L (0–202 day). Medium aluminium concentration for A3 samples was 289 and for A2 samples 231 µg/L.

Results shown in Fig. 3 are similar to the results in Fig. 2. In cold stored samples aluminium migration was not established (low correlation coefficient: $R^2=0.1677$). Samples stored at 22 °C showed higher aluminium concentration after 70 days of storage, and the determined values were 2–3 times higher than in the refrigerator. Total aluminium migration from can to beer (A3d) was 319 µg/L (0–150 days). Medium aluminium concentration for A3d samples was 288 and for A2d samples 187 µg/L. Obviously, upside down storage had minor influence on

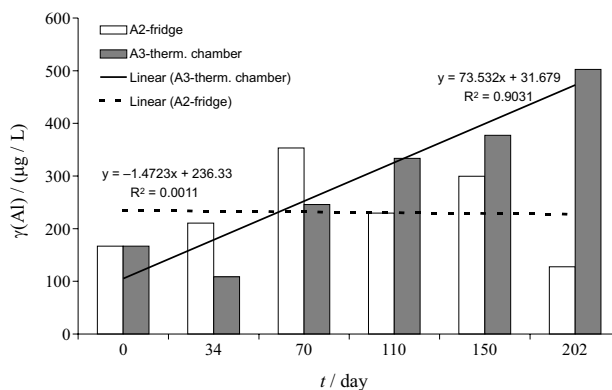


Fig. 2. Comparison of aluminium concentration changes in standard lager beer samples during storage at 4 (A2) and 22 °C (A3)

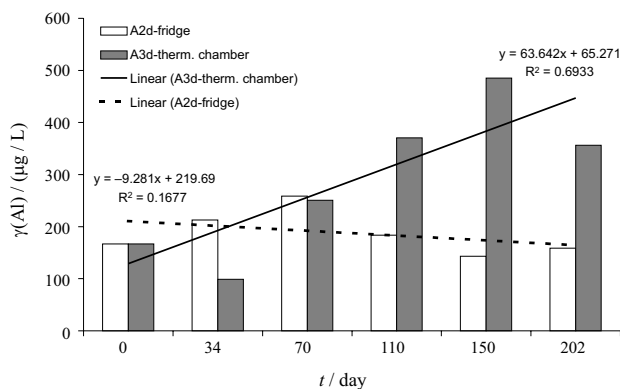


Fig. 3. Comparison of aluminium concentration changes in upside down stored cans of standard lager beer at 4 (A2d) and 22 °C (A3d)

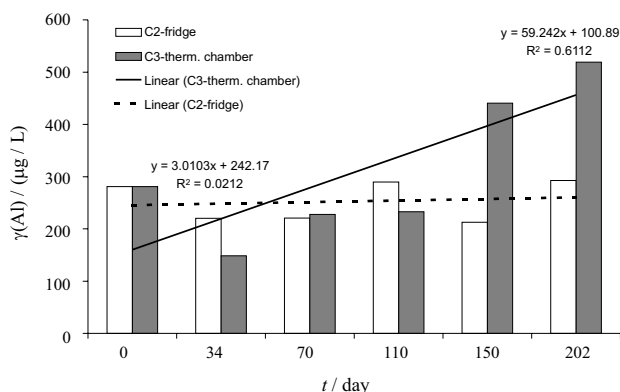


Fig. 4. Comparison of aluminium concentration changes in premium beer samples during storage at 4 (C2) and 22 °C (C3)

aluminium migration dynamics compared to »normal« storage. It was assumed that during upside down storage more prominent migration could occur due to the fact that beer is in contact with the edges of the can body and its lid on which more corrosion points could arise (20). Nevertheless, further metallographic analysis showed that internal lacquer layer is thicker on the top lid of the can (3–4 times), so it could be assumed that the edges of the can were more protected than the rest of the can.

Aluminium concentrations of refrigerated samples were not significantly affected by the time of storage, so aluminium migration was not established either (low correlation coefficient: $R^2=0.0212$; see Fig. 4). Warm stored samples showed an increase of aluminium concentration only after 110 days of storage. After that point migration is more prominent and on the last day of storage it was $238 \mu\text{g/L}$ (0–202 days). Medium aluminium concentration for C3 samples was 308 and for C2 samples $253 \mu\text{g/L}$.

Generally, temperature of storage influenced aluminium migration from can to beer, which confirms the published results by Šeruga *et al.* (4) and Vela *et al.* (5).

Effect of beer brand on the concentration of aroma compounds

Aroma constituents of beer samples (A1 and C1) before pasteurization, filling and storage are shown in Fig. 5. Concentration of aroma constituents of these two brands was significantly different. Compounds whose concentration was almost the same in both beers were

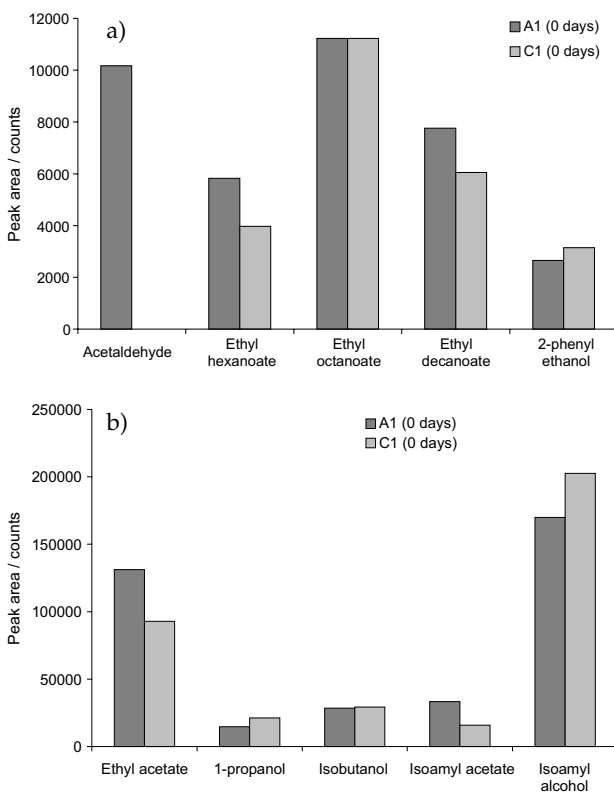


Fig. 5. Comparison of qualitative aroma compound content in standard lager (A) and premium beer (C) samples before filling (day 0)

- a) Compounds with lower peak area
b) Compounds with higher peak area

isobutanol and ethyl octanoate. In beer C higher amounts of 1-propanol, isoamyl alcohol and 2-phenyl ethanol were measured, and acetaldehyde concentration was below the limit of detection. In standard lager beer (A) considerably higher values of ethyl acetate, isoamyl acetate, acetaldehyde, ethyl hexanoate and ethyl decanoate were observed. Higher amounts of these compounds can contribute to the unacceptable beer aroma and taste (21,22). Although in this experiment aroma compounds were not quantified, it was assumed that these fresh beer samples did not contain any undesirable components and »off-flavours«. Standard lager beer (A) contained more constituents which are regarded extremely undesirable if present at higher levels (*i.e.* ethyl acetate and acetaldehyde). Isoamyl acetate (banana-like aroma) was higher in standard lager beer, and 2-phenyl ethanol (rose-like aroma) in premium beer (C). Differences in aroma constituents can be influenced by different grist composition (malt, corn grits), fermentation conditions (temperature profile, yeast strain) and maturation time. These results are to some extent comparable with the data published by Derdelinckx *et al.* (23). They found that beers of the same type (special bottle-refermented beers) could differ both organoleptically and analytically.

Effect of storage temperature on the concentration of aroma compounds

Qualitative comparison of aroma compound changes in pasteurized samples of premium A beer stored at 4°C (A2) and 22°C (A3) is given in Fig. 6. After 150 days

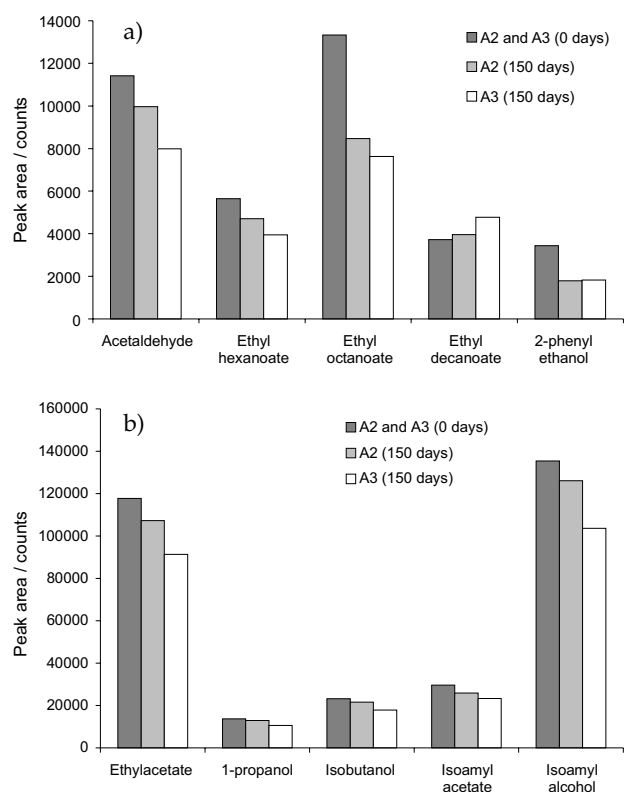


Fig. 6. Qualitative comparison of aroma compound changes in samples of standard lager A beer stored at 4°C (A2) and 22°C (A3) (day 0 and day 150)

- a) Compounds with lower peak area
b) Compounds with higher peak area

of storage it was clear that samples stored in the refrigerator contained higher levels of all analyzed aroma compounds, with the exception of ethyl decanoate, in comparison with samples stored at 22 °C. Concentration of 2-phenyl ethanol after 150 days of storage was similar. Difference in the concentration of aroma constituents was more expressive for the following compounds: ethyl hexanoate, ethyl octanoate, ethyl acetate and isoamyl alcohol, while for other compounds the difference was negligible. Analyzed samples showed certain aroma stability, which was more expressed for samples stored in the refrigerator. It is worthwhile noticing that pasteurization contributes to aroma stability during storage, but also changes the original taste of beer (6).

Effect of aluminium concentration in beer on the concentration of aroma compounds

To determine the effect of aluminium on beer aroma compounds, aluminium sulphate was added to samples of beer A (Aas). Beer A with the addition of potassium sulphate (Aks) served as control. Samples were stored in brown glass bottles closed by crown corks. Fig. 7 shows the comparison of quantified aroma compounds in these beers. Before analysis samples were stored in thermostatic chamber (28 °C) for two weeks. Differences among the determined aroma constituents in the samples were minor and hardly recognizable, with 1-butanol (10.5 %)

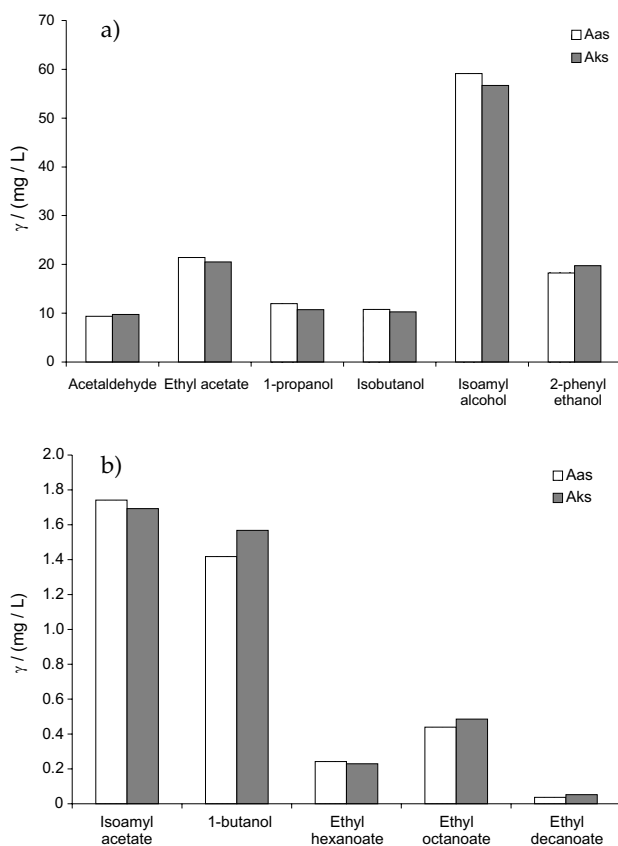


Fig. 7. Comparison of quantified aroma constituents in Aas beer samples (addition of aluminium sulphate) and in control samples Aks (addition of potassium sulphate)
 a) Compounds with higher concentration range (more than 2 mg/L)
 b) Compounds with lower concentration range (up to 2 mg/L)

and isoamyl alcohol (4.3 %) as the most expressive. Therefore, the added aluminium (10 mg/L) did not have a significant influence on the changes of the quantity of the analyzed compounds during 2 weeks of storage. Aluminium concentration in the beer with added aluminium sulphate exceeded approximately 20–100 times the usual aluminium concentration in bottled beer, which is around 100–540 µg/L (4). Böröcz-Szabó (24) found that the addition of as little as 0.25 mg/L iron in form of chloride or sulphate to the beer caused some assessors to find the samples worse in flavour and taste than the control. When 0.5 mg/L was added, a significant difference was found. The threshold value of sensorially detectable aluminium contamination in beer originating from the added aluminium salts is substantially higher than with Fe²⁺ or Fe³⁺ (24). In contrast, aluminium contamination of beer, as a product of can corrosion, is similar to iron contamination detectable at a much lower concentration. However, the same author found that both the added aluminium salts (KAl(SO₄)₂·12 H₂O, Al₂(SO₄)₃·18 H₂O and AlCl₃) and aluminium corroded in the beer changed the original taste of beer to give a product of totally different taste (7). Therefore, it could be assumed that in this work the added aluminium also had some effect on the sensory properties of beer. Maybe increased aluminium concentration influenced the change of other aroma compounds, not measured in this work. It is possible that carbonyl compounds, even in very small concentrations (in µg/L), deteriorate sensory perception of beer, and aluminium itself as cation has an effect on beer aroma perception. Influence of aluminium on sensory properties of beer has not yet been fully elucidated, but it is obvious that aluminium vessels in contact with beer have to be well coated to protect the quality of beer.

Conclusions

Aluminium concentration in beer prior to can filling depends on beer type, namely on raw materials used for brewing. Premium beers manufactured from barley malt (C) or premium beers from barley malt and small concentration of adjuncts (corn grits) (B) contain more aluminium in comparison with standard lager beer (A) manufactured from malt and more adjuncts in grist. Aluminium concentration changes in canned beer depend on storage temperature. Storage in the refrigerator protects against aluminium migration from the can, while storage at 22 °C facilitates aluminium migration from can to beer. Likewise, aroma stability is more expressed in cold stored beer. Concentration of aroma constituents of analyzed beer brands was significantly different. In premium beer higher levels of 1-propanol, isoamyl alcohol and 2-phenyl ethanol were observed. Standard lager beer contained higher concentration of constituents regarded as undesirable if present in extremely high amounts. Addition of aluminium sulphate in beer had no effect on concentrations of analyzed aroma compounds.

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