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Aspects of internal recycling of recoverable yeast beer

The influence of blending fresh and aged yeast beers to pitching wort and to green beer on various parameters of the final beer is demonstrated within this study. Most of these parameters are not or only slightly influenced by the addition of yeast beers, but especially sensory attributes like foam and flavour were negatively affected by higher blending ratios of aged yeast beer. Also a discrepancy between measurable and sensory perceived flavour stability was observed. Within this aspect, 2-furfural plays a key role as the formation of this compound is repressed in yeast beers. Further studies are necessary to clarify this aspect. In order to recover high quality yeast beer it is recommended to avoid long storage periods of surplus yeast suspensions, especially at high temperatures, before processing and to flash pasteurize the recovered yeast beers to inactivate yeast enzymes. Blending ratios of 5 % v/v to pitching wort or to green beer should not be exceeded.

Descriptors: surplus yeast, yeast beer, recovery, separation, flavour stability, foam stability

1 Introduction

In the course of main fermentation, yeast cell mass increases three- to sixfold. Typically, the total amount of surplus yeast of a lager fermentation is about 170–230 g/hl of the final product [1]. This by-product has a dry matter content of 10 % w/w and is responsible for beer losses of between 1.5–3.0 % of total annual beer production volume [2]. In literature, two possibilities of recycling surplus yeast are described in principle [3, 4]. In case of an external recycling, surplus yeast is given directly to the processing industry for further treatment [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16] or to agriculture as feeding material [3, 17, 18, 19]. In case of an internal recycling, the dosage of surplus yeast to the mashing process [20, 21] and to spent grain [22] was investigated. Various methods for recycling surplus yeast were analyzed by Zürcher [23], regarding their ability to reduce extract loss. According to this investigation, the best compensation of extract loss can be achieved by a combination of recovering yeast beer and selling the remaining yeast sludge. In practice, mainly centrifuge and membrane separation techniques are used for yeast beer recovery. An overview of existing recovery systems is given by Hansen [24] and Schneeberger et al. [25]. The quality of recovered yeast beer is not only influenced by these separation techniques but also by means of surplus yeast storage conditions before processing [1, 4, 26]. The internal recycling of yeast beer is widely discussed in the literature. Schwill-Miedaner [4] investigated the dosage of yeast beer to different stages of wort production. Results showed that foam stability and taste of the final beer were not influenced with blending yeast beer to wort boiling, while blending to later stages of wort production these two parameters were clearly affected negatively. Müller et al. [27] analyzed the effects of blending yeast beers into the whirlpool showing no negative influences on

processing parameters. These findings were confirmed by Hansen [28], who also found no negative effects by blending yeast beer into hot wort. In contrast, Narziss et al. [29] found that already small amounts of 1–3 % of yeast beer led to darker wort and beer colours, higher amounts of polyphenols and gums as well as very high amounts of isobutyric acid and isovaleric acid. Therefore, Narziss [30] recommended to filtrate and sterilize (sterile filtration or flash pasteurisation) the yeast beer before blending it to pitching wort. Donhauser et al. [31] also investigated the effects of blending yeast beer to pitching wort. A blending ratio of 2–5 % did not affect tasting results, while from a blending ratio of above 10 % clearly a decrease in beer quality was detected. Hoggan [32] showed that a dosage of yeast beer directly before main filtration did not influence quality parameters of the blended beers. In this context, Oechlse [33] showed that over 90 % of high molecular β -glucans were separated while using membrane filtration. Therefore, the author did not expect to have any filtration problems within the main beer filtration process. These results were confirmed by Girr et al. [34], who also found high decreases of β -glucans. Bilge [35] described a possibility to recycle valuable ingredients from yeast beer by means of ultra filtration. Particularly, certain fractions of proteins were thought to improve foam stability after addition to the production process.

Due to recent developments in separation technology it was necessary to re-investigate systematically the influences of the addition of yeast beers to different steps of the brewing process.

2 Material and Methods

Brewing process

Yeast beer was recovered by means of disc centrifugation. In sterilized and counter-pressed kegs, yeast beer and aerated wort were mixed according to Table 1 and fermentations were conducted at 12 °C with freshly propagated yeast and in duplicate.

From day five of fermentation on, extract and diacetyl level were measured in regular intervals. As soon as diacetyl level was below 0,1 ppm, green beer was transferred into a new keg in order to separate it from the sedimented yeast. Maturation was conducted over a period of two weeks at 0 °C. By means of bunging,

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the CO₂-content was fixed to 6 g/l. After the maturation, beer was kieselguhr-filtered and filled in 0,33 l bottles. For the green beer trials, yeast beer and a standardized green beer were mixed according to Table 1. The further treatment was conducted as described above.

While fresh yeast beer was recovered with only a very short storage time of the primary yeast (10 hours at 4 °C) before processing, for aged yeast beer the primary yeast was stored for six days without cooling ($T_{\max} = 10\text{ °C}$). Table 2 shows the main chemical-technical results of these two beers.

pH and bitter units

pH and bitter units were measured as described in MEBAK, Vol. 2 [36].

Beer flavour compounds

Beer flavour compounds were analyzed by means of GC-FID according to the method of Lustig [37]. Table 3 shows the flavour compounds belonging to the sum parameters used within this study.

Stale flavour compounds

Stale flavour compounds were analyzed by means of GC-FID as described by Kaltner [38]. For aging, beer samples were pretreated with shaking for 24 hr and storage for four days at 40 °C according to the method of Eichhorn [39].

Foam stability

Foam stability was measured using a NIBEM foam stability tester, type NIBEM-TPH (Haffmans, Venlo, The Netherlands).

Stability index (SI)

For investigating the antioxidative activity of beer, all beers were judged using the stability index according to Franz [40]. For the calculation of the SI, lag-time, anti-radical behaviour and anti-radical potential using an ESR spectrometer (Bruker BioSpin, Rheinstetten, Germany) and reducing power using a tannometer (Pfeuffer, Kitzingen, Germany) were determined.

Activity of extracellular esterase

The activity of extracellular esterase was detected according to the method of Horstedt [41]. 200 µl of sample were mixed with 600 µl substrate solution (1.2 mM *p*-Nitrophenyl-dodecanoate, 10 % DMSO and 60 mM MES buffer (all chemicals were purchased from Sigma-Aldrich Chemie, Taufkirchen Germany). The assay was carried out for 30 min at 40 °C and stopped with 600 µl of 1 M NaHCO₃, pH 9.0. The adsorption of the liberated *p*-Nitrophenol was measured spectrophotometrically at 400 nm using a CADAS 200 spectrophotometer (Dr. Lange, Berlin, Germany). One mU of esterase activity was defined as the amount of esterase that liberates one nmol of *p*-Nitrophenol per minute under the assay conditions.

Sensory evaluation

Sensory evaluation was done by judging the attributes according to the method of DLG [36] and to the method of Eichhorn [39]. Within the DLG test, the following attributes of a beer were analysed: "aroma", "taste", "mouthfeel", "liveliness" and "bitter taste". Each attribute was ranked with scores between "1" (worst) and "5" (best). Within the Eichhorn test (aging test), the following attributes of a beer were analysed: "aroma", "taste" and "bitter taste". Each attribute was ranked with scores between "1" (not aged) and "4" (aged). All samples were judged in fresh and forced aged status.

3 Results and Discussion

Blending of yeast beer to pitching wort

Due to higher amounts of alcohol and corresponding original gravity in the yeast beer, higher levels of these two parameters were determined in the final beers with increasing blending ratios. Similar observations were made for the pH values and for the bitter units, whereas beers containing the aged yeast beer showed a higher increase of pH and bitter units (cf. Fig. 1 and 2).

The high amounts of bitter units could be explained by resorption effects of bitter substances from yeast cell surface, where a prolonged storage time of surplus yeast leads to a distinct higher amount. The antioxidative capacity of final beers was improved by the addition of yeast beers. This was mainly influenced by the two parameters "reducing power" and "antioxidative potential" showing better levels for the blended beers (cf. Fig. 3).

For foam stability, no consistent tendencies could be observed. In previous studies a clear negative effect of poor quality yeast beer on foam stability was demonstrated [26]. However, for trials A–C and especially H–J no drawback was measured. Within the gas chromatographic investigations the behaviour of higher aliphatic alcohols, esters and short and medium chain fatty acids were studied. By the addition of fresh yeast beer to pitching wort, no negative influences were measured, whereas by the addition of aged yeast beer clear higher levels of fatty acids could be observed (cf. Fig. 4 and 5).

While moderate blending ratios of yeast beer led to slight increases of acetate and fatty acid esters, a further increase in blending ratio led to lower levels of these parameters (cf. Fig. 6 and 7).

With rising blending ratios also lower levels of the thermal indicator 2-furfural were measured. This comes along with also lower levels in the total sum of staling components, where apparently the quality of yeast beer is of minor importance (cf. Fig. 8).

Within the sensory tests, blended beers were judged inferior, whereas for trials A–C the differences were not significant. For trials H–J significant poorer tasting results were found for the blended beers, especially for bitter taste (cf. Fig. 9 and 10). Surprisingly also the tasting results for the forced aged beers were judged inferior for blended beers despite better analytical data for especially the stale flavour compounds were measured.

Blending of yeast beer to green beer

Higher amounts of alcohol and corresponding original gravity in the yeast beer led to higher levels of these two parameters only after blending aged yeast beer. Fresh yeast beer did not affect these parameters. Analogue to previous trials higher values for pH and bitter units were determined in the final beers with increasing blending ratios. (cf. Fig. 11 and 12).

The antioxidative capacity of final beers was not influenced by the addition of yeast beers (cf. Fig. 13).

Contrary to trials A–C and H–J a clear tendency was observed for foam stability. While foam stability was not affected by fresh yeast beer, NIBEM value was about 50 seconds lower for beers L and M compared to beer K (cf. Fig. 14) caused by a high proteolytic activity.

By the addition of fresh yeast beer to green beer, only minor influences on higher aliphatic alcohols and short and medium chain fatty acids were observed. By blending aged yeast beer, these parameters were highly affected negatively (cf. Fig. 15 and 16).

The addition of fresh yeast beer had no influence on esters. Blending

of aged yeast beer led to distinct lower levels of esters compared to the reference beer. (cf. Fig. 17 and 18).

The results for the thermal indicator 2-furfural were almost the same as within the previous trials. Contrary to previous trials, the decrease of the 2-furfural-level is dominated by yeast beer quality within trials D–F and K–M (cf. Fig. 19).

Within the sensory tests, blended beers were also judged inferior, whereas the differences were not significant for trials D–F. For trials K–M significant poorer tasting results were observed for the blended beers (cf. Fig. 20 and 21). The tasting results of trials D–F and K–M reflect the results from trials A–C and H–J as blended beers were also judged inferior despite better analytical data for stale flavour compounds.

4 Discussion

An increase of alcohol content and a therefore associated increase of original gravity could be observed for yeast beers, especially for aged yeast beers. McCaig et al. [42] described a process called “autofermentation” which starts during the storage of yeast suspensions mainly at higher temperatures. Within this process, intracellular storage carbohydrates like glycogen and trehalose are transformed to ethanol and carbon dioxide. By treating yeast cells with Lugol’s iodine, intracellular carbohydrates could be stained. In Figure 22 microscopic pictures of yeast cells are presented after dyeing with Lugol’s iodine, showing freshly cropped yeast cells (left) and yeast cells after a storage period of 10d at 10 °C (right).

In this context, Imai et al. [43] demonstrated a clear negative effect of ethanol on yeast physiology. This could be confirmed by our trials, measuring intracellular pH in yeast cells and ethanol in the corresponding yeast beer (cf. Fig. 23).

Furthermore, aged yeast beer did affect bitterness, flavour profile and flavour stability of blended beers. In this context, the apparent discrepancy between measurable and sensory perceived flavour stability was interesting. Within these trials a clear effect on flavour active ester contents by the addition of aged yeast beer was demonstrated. In literature, the existence of an extracellular esterase was described [41, 44, 45]. This esterase may have the ability to degrade flavour active esters and therefore reduce their influence on sensory properties of the beer. The reduction of esters is critical, since they are able to mask stale flavours for a long period of time. The extracellular esterase was described as very heat stable whereby after flash pasteurisation rest activities could be detected [41]. This could be confirmed by our trials, where beer had to be exposed to at least 220 PU to inactivate this enzyme (cf. Fig. 24).

The activity of this enzyme was also measured within the trials H–J and K–M. With increasing amounts of yeast beer, higher activities of esterase were measured (cf. Fig. 25).

Therefore it should be obligatory to flash pasteurize yeast beers in order to inactivate harmful influence of various yeast enzymes (esterase, proteinase A). On the other hand, with increasing blending ratios of yeast beer lower levels of 2-furfural were detected. No or only low concentrations of this compound even after excess heat treatment are a characteristic for yeast beers. Usually, 2-furfural is formed from 2-furfuryl alcohol during beer staling. As obviously this formation pathway did not proceed in yeast beers, another pathway must be existent for this phenomenon. Vanderhaegen et al. described the formation of 2-furfuryl-ethyl-ether (2-FEE) from 2-furfuryl alcohol and furfuryl acetate [46, 47]. The flavour

threshold for 2-FEE in beer was determined to 6 µg/l, thereby a solvent-like flavour could be perceived [48]. This flavour was only detected also in yeast beers but up to now this flavour was only correlated to the content of ethyl acetate. The formation of 2-FEE is favoured by higher levels of alcohol and darker beer colours. Both parameters are enhanced in yeast beers. Also Vanderhaegen et al. [48] described an enhanced formation of 2-FEE by yeast autolysate in bottle fermented beers, so probably the same mechanisms proceed in yeast beers. Finally, a statistical evaluation of all trials was conducted to extract the principal components. Figure 26 and 27 show the principal components and the rotated component matrix, respectively.

A clear differentiation of samples G1, G2, N1 and N2 (pure yeast beers) was noticeable. Factors, responsible for this differentiation are ethyl acetate, isovaleric acid, higher aliphatic alcohols and 2-furfural. For overall considerations, all samples showed a very homogenous behaviour leading to very small deviations. Except samples H–J, containing aged yeast beer, differ significantly. A characteristic parameter for these beers was a high level of acetate esters.

Trials showed that an internal recycling of yeast beers is possible in terms of a high beer quality if the following recommendations are followed:

- storage time of surplus yeast suspension must be as short as possible before processing;
- flash pasteurization of yeast beers in order to inactivate yeast enzymes;
- blending ratio should not be more than 5 % v/v.

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6 Literature

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Appendix

sample	Blending volume / % v/v	yeast beer quality	point of dosage
A	0	fresh	pitching wort
B	5	fresh	pitching wort
C	10	fresh	pitching wort
D	0	fresh	green beer
E	5	fresh	green beer
F	10	fresh	green beer
G	100	fresh	–
H	0	aged	pitching wort
I	5	aged	pitching wort
J	10	aged	pitching wort
K	0	aged	green beer
L	5	aged	green beer
M	10	aged	green beer
N	100	aged	–

parameter	fresh yeast beer	aged yeast beer	reference beer
original gravity / % w/w	12.59	12.97	11.80
alcohol / % v/v	5.58	5.74	5.00
pH / -	5.79	5.90	4.30
FAN / mg/l	127	200	100
total polyphenols / mg/l	146	260	n.d.
bitter units / EBC	80	85	40
colour / EBC	5.6	12.9	4.5

n.d. = not determined

sum of higher aliphatic alcohol	sum of short and medium chain fatty acids	sum of acetate esters	sum of fatty acid esters
1-hexanol	isovaleric acid	isobutyl acetate	ethyl butyrate
1-heptanol	hexanoic acid	hexyl acetate	ethyl hexanoate
1-octanol	octanoic acid	heptyl acetate	ethyl octanoate
1-decanol	nonanoic acid	octyl acetate	ethyl decanoate
	decanoic acid	furfuryl acetate	
	dodecanoic acid	2-phenyl-ethyl acetate	

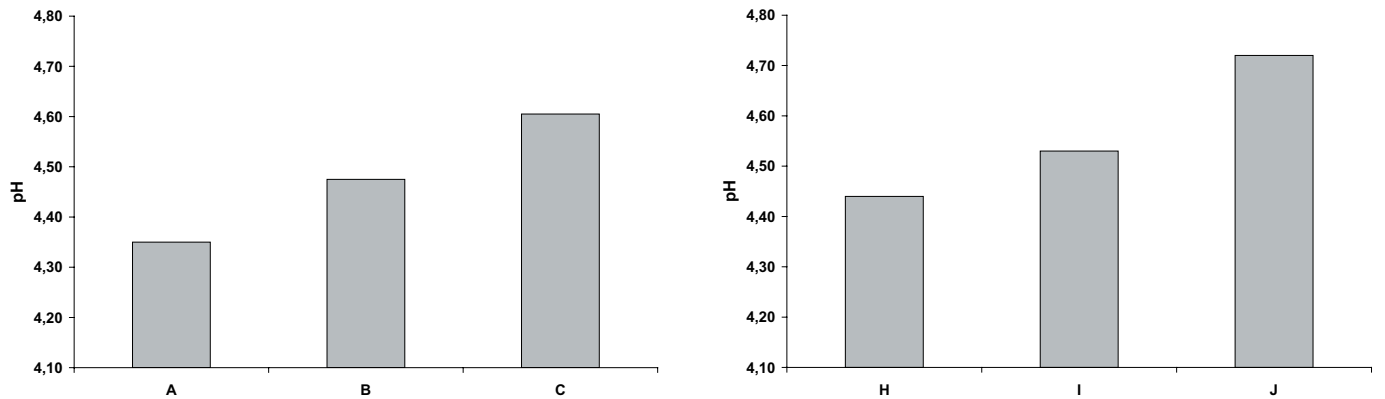


Fig. 1 pH-value of the final beer by means of addition of fresh (left) and aged yeast beer (right) to pitching wort

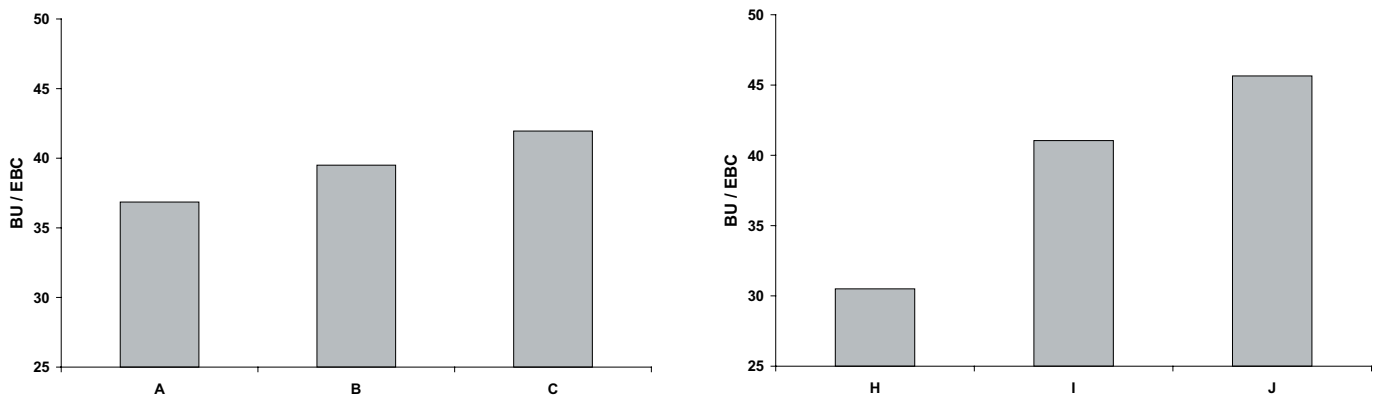


Fig. 2 Bitter units of the final beer by means of addition of fresh (left) and aged yeast beer (right) to pitching wort

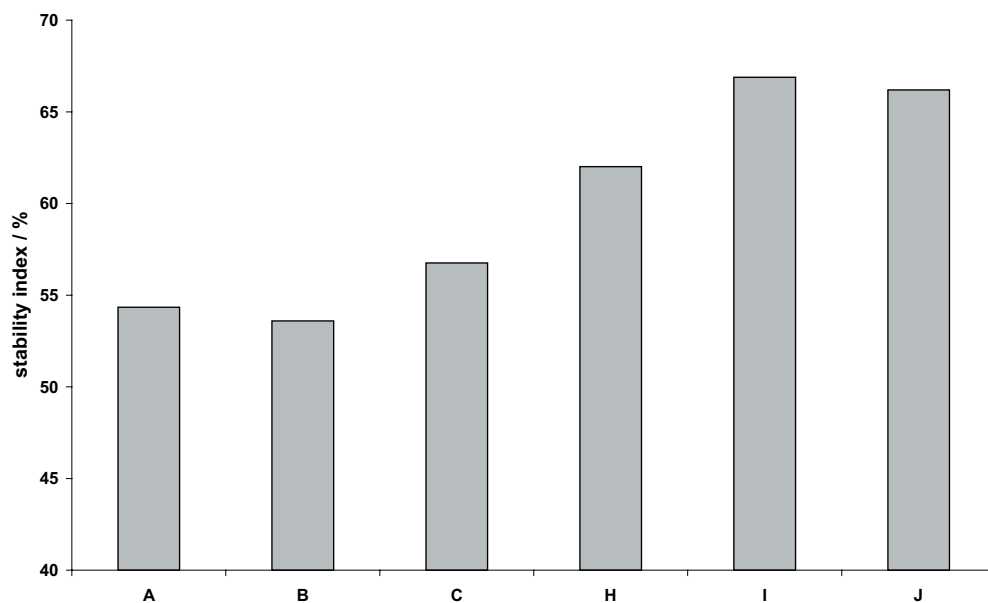
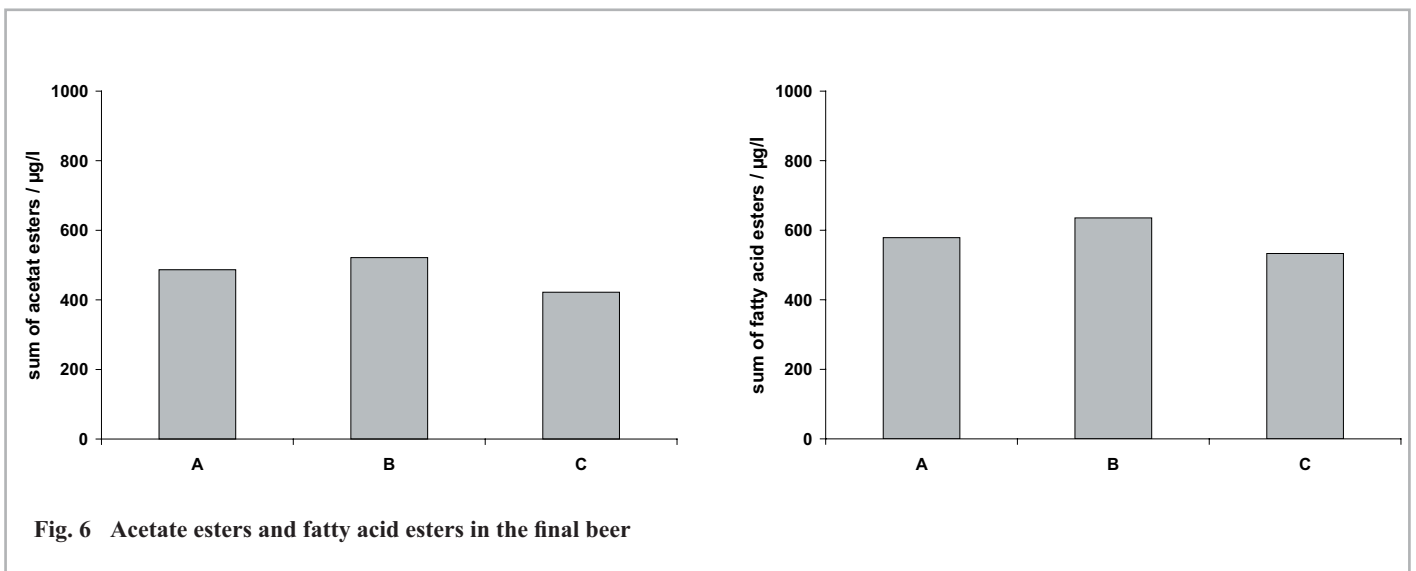
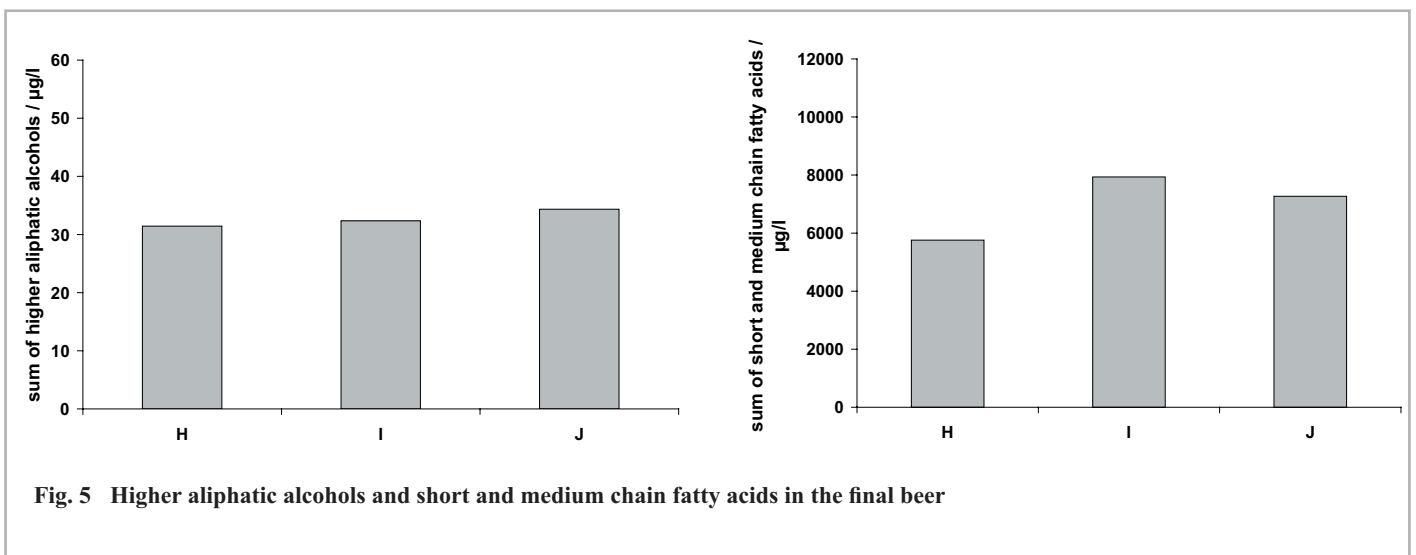
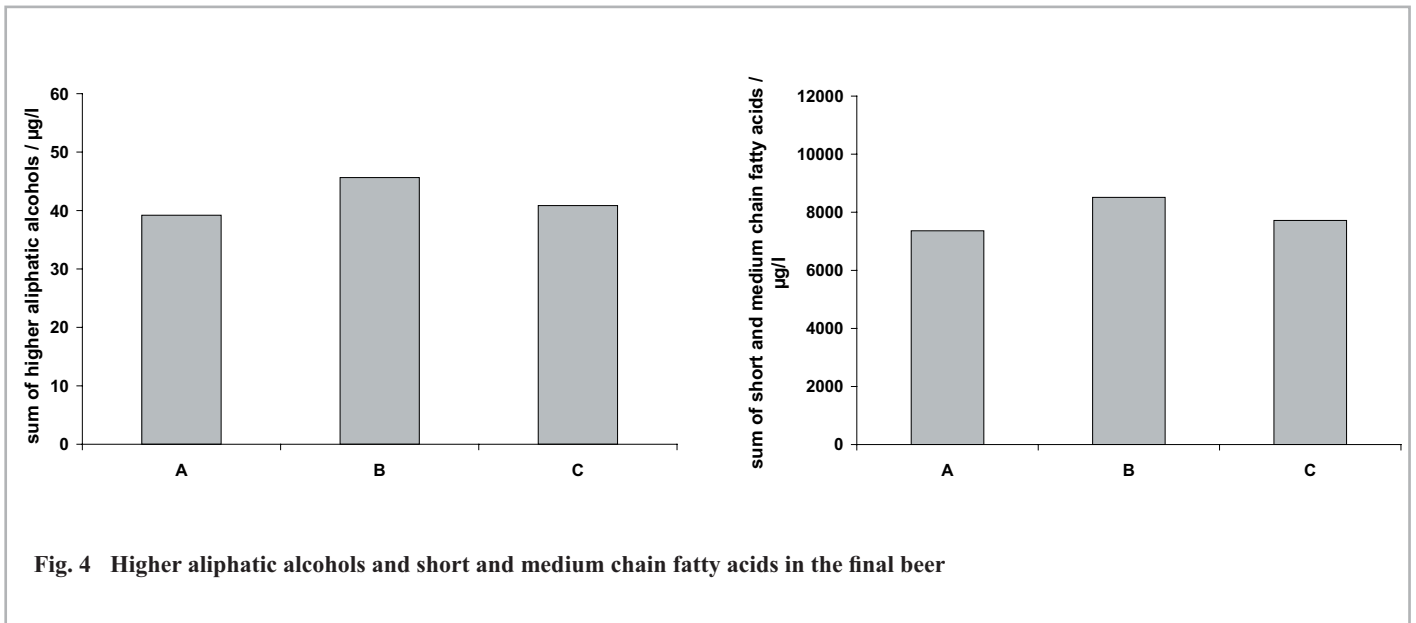
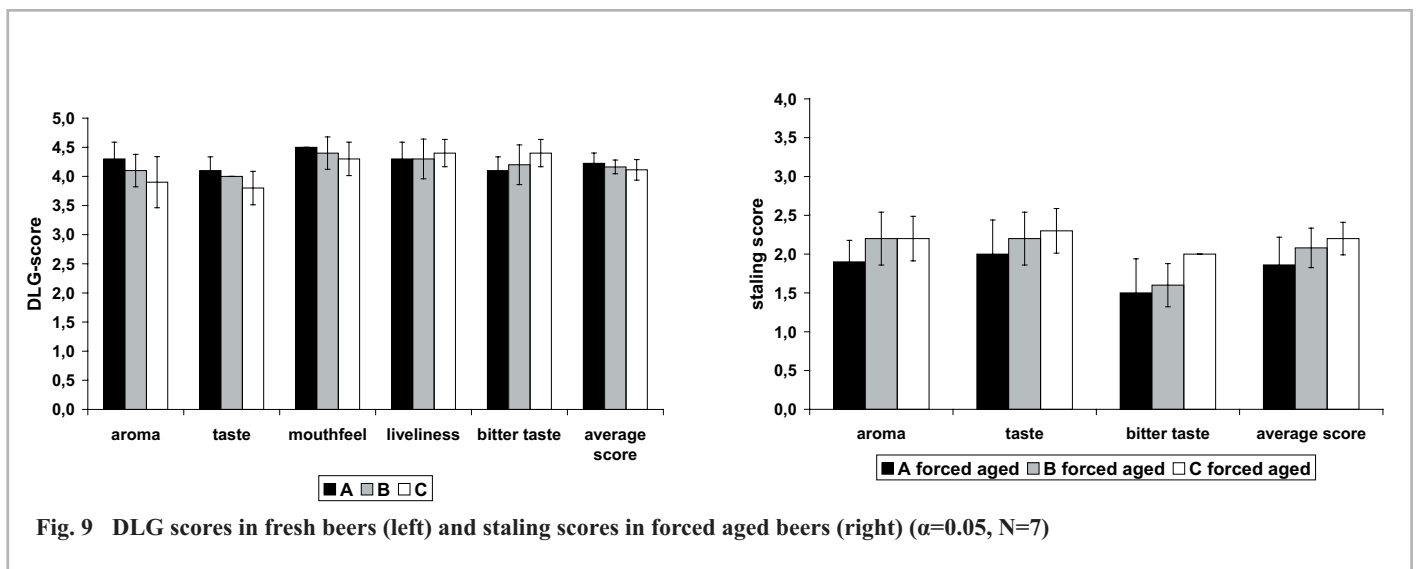
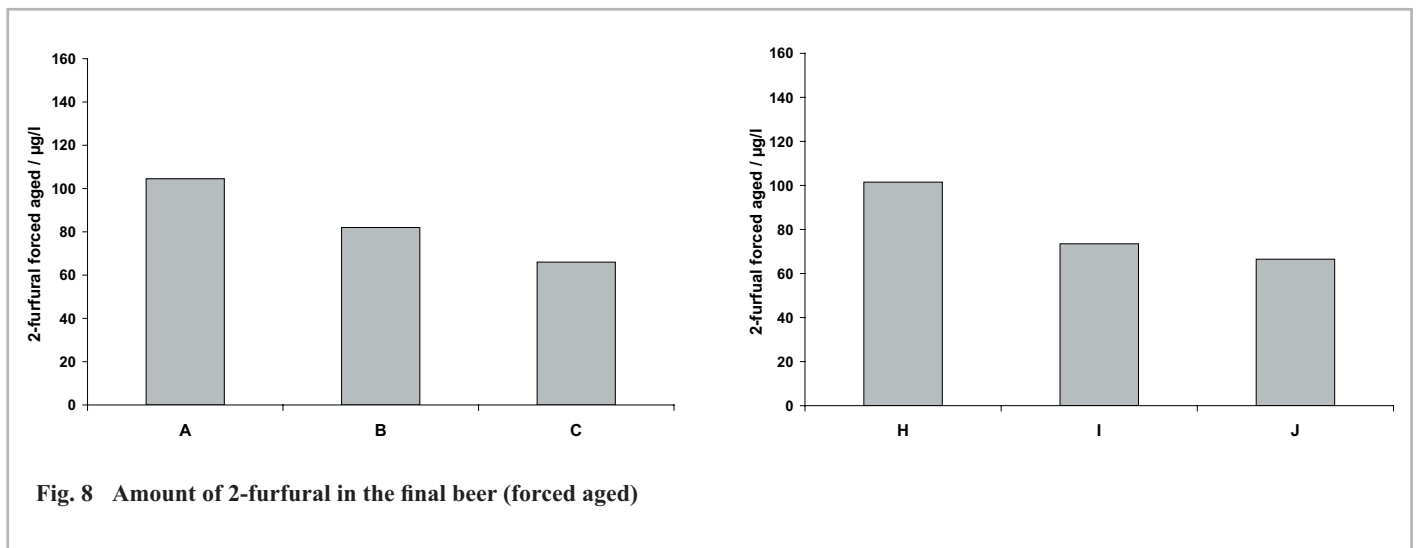
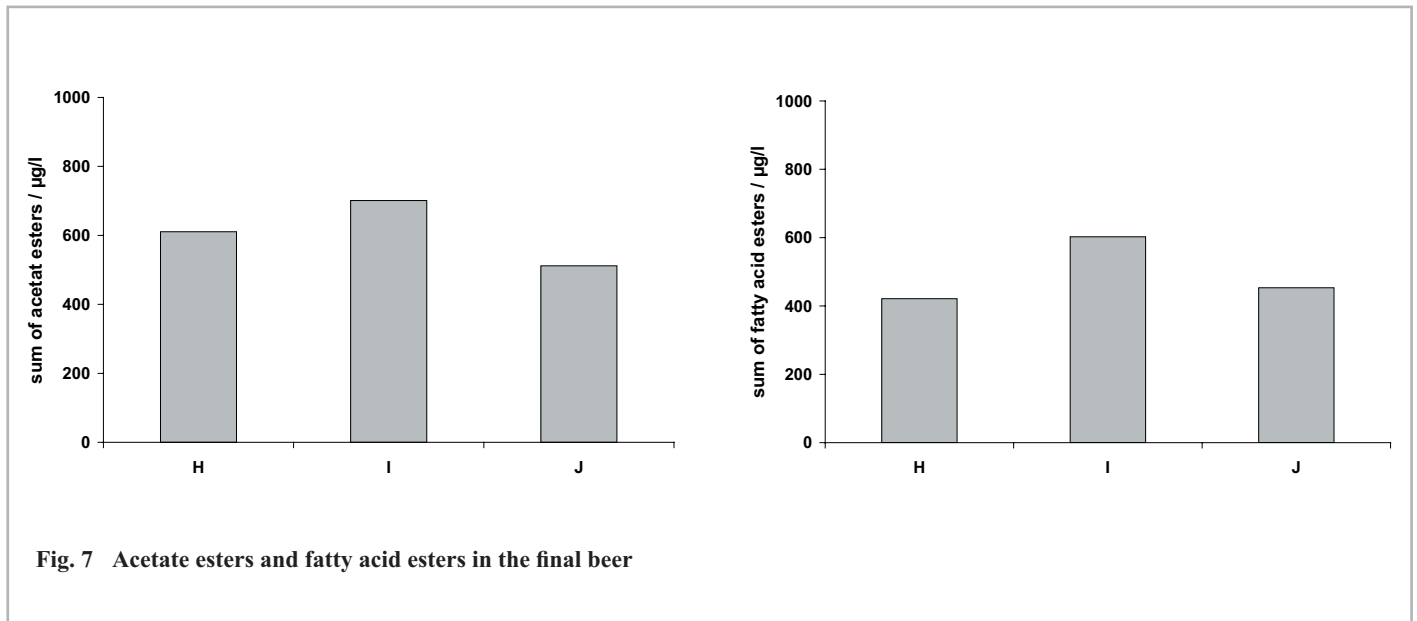
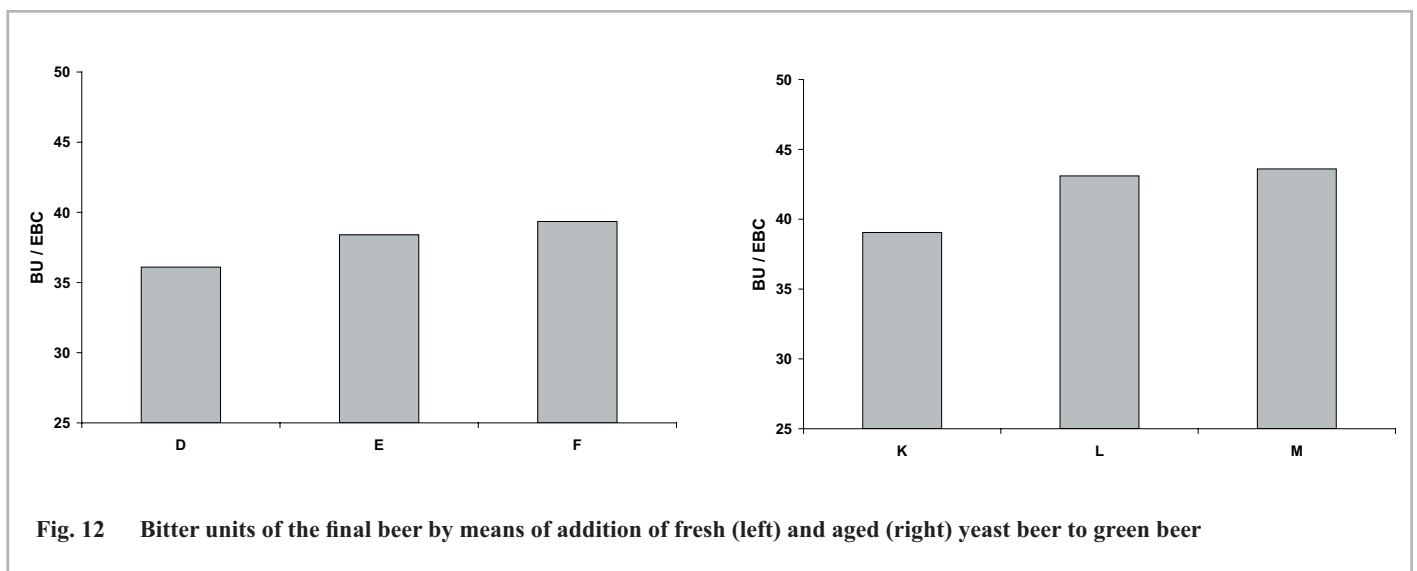
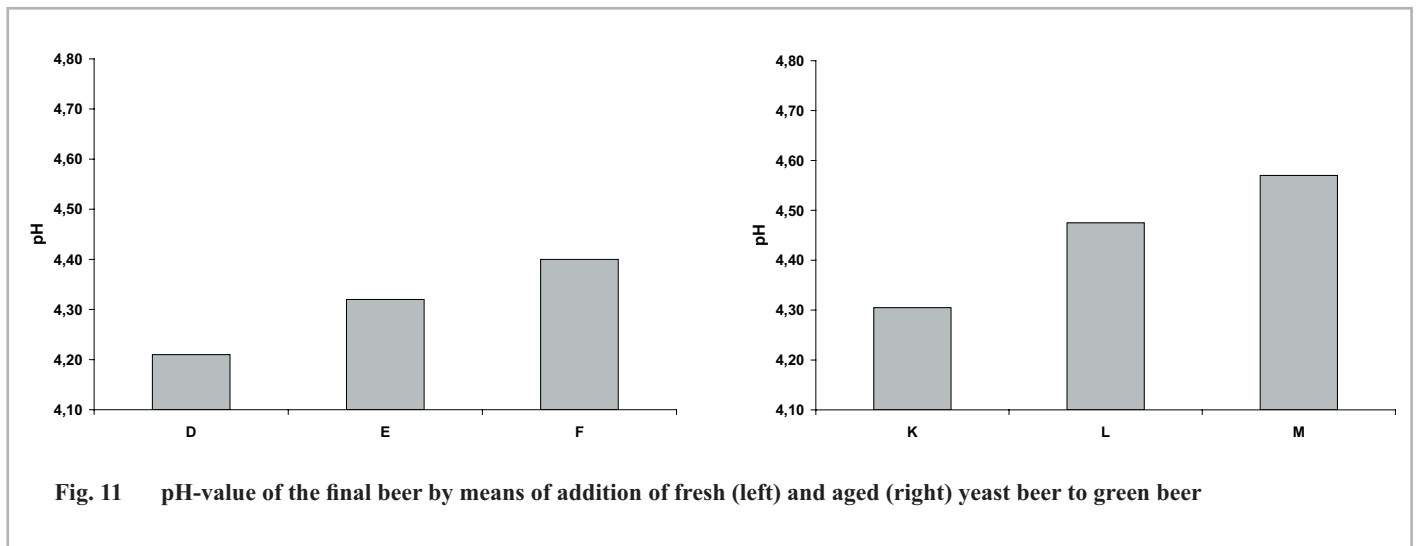
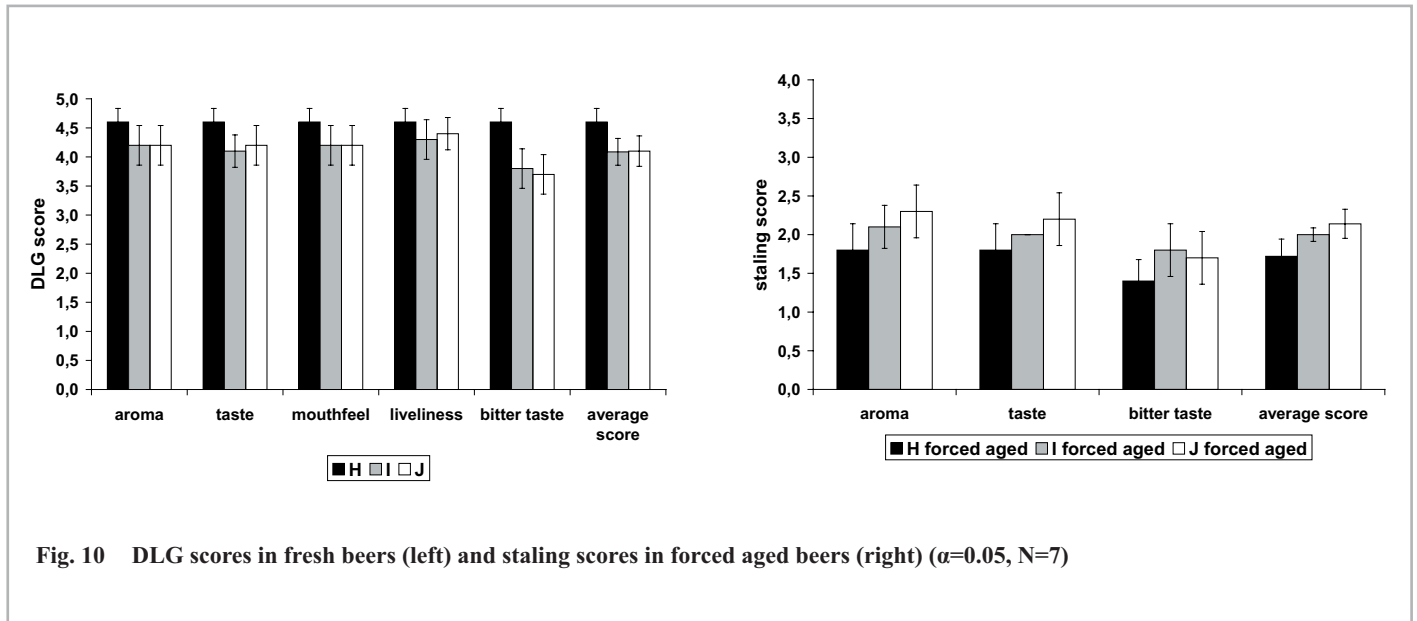


Fig. 3 Stability index of trials A-C and H-J







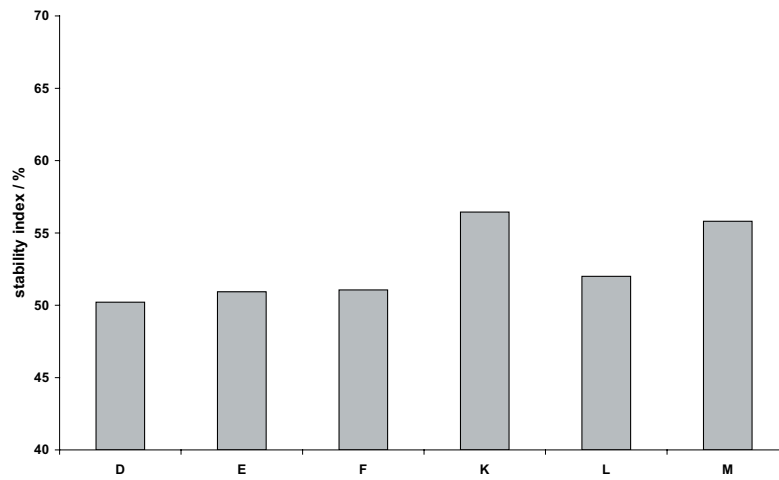


Fig. 13 Stability index for trials D–F and K–M

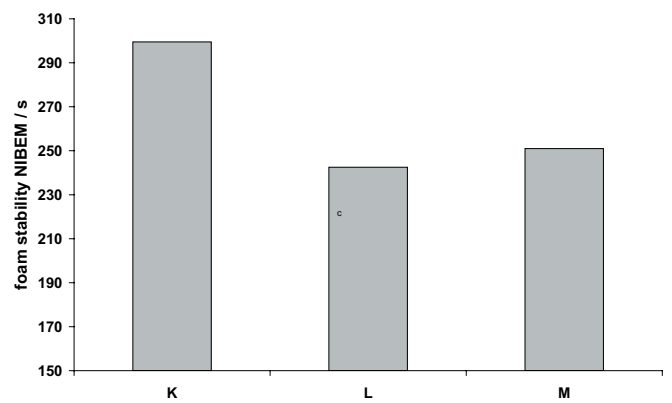
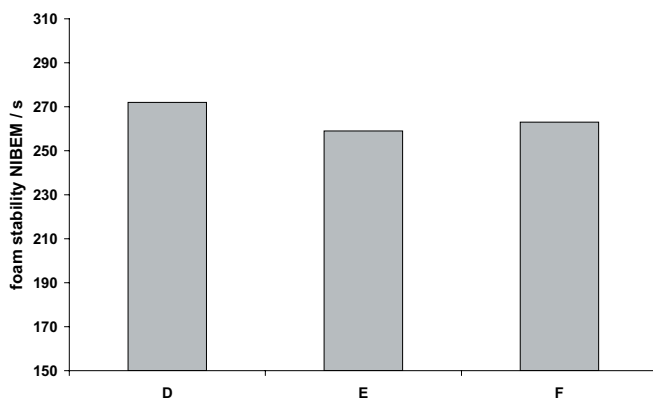


Fig. 14 Foam stability for trials D–F and K–M

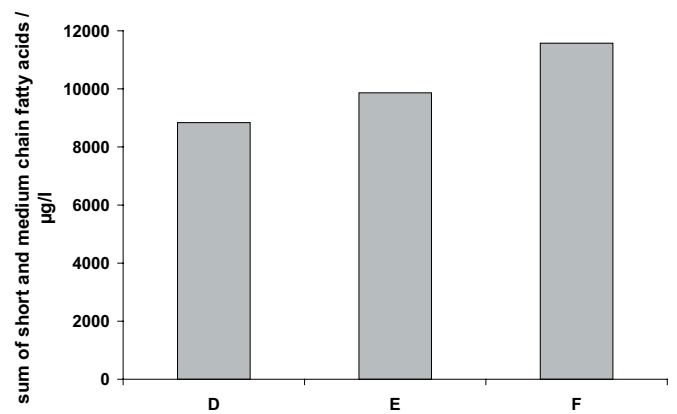
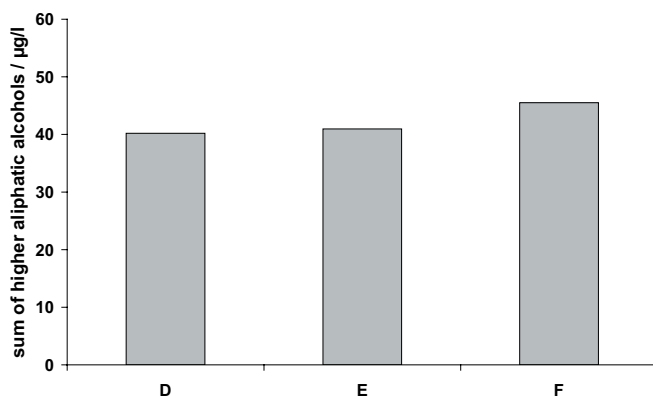
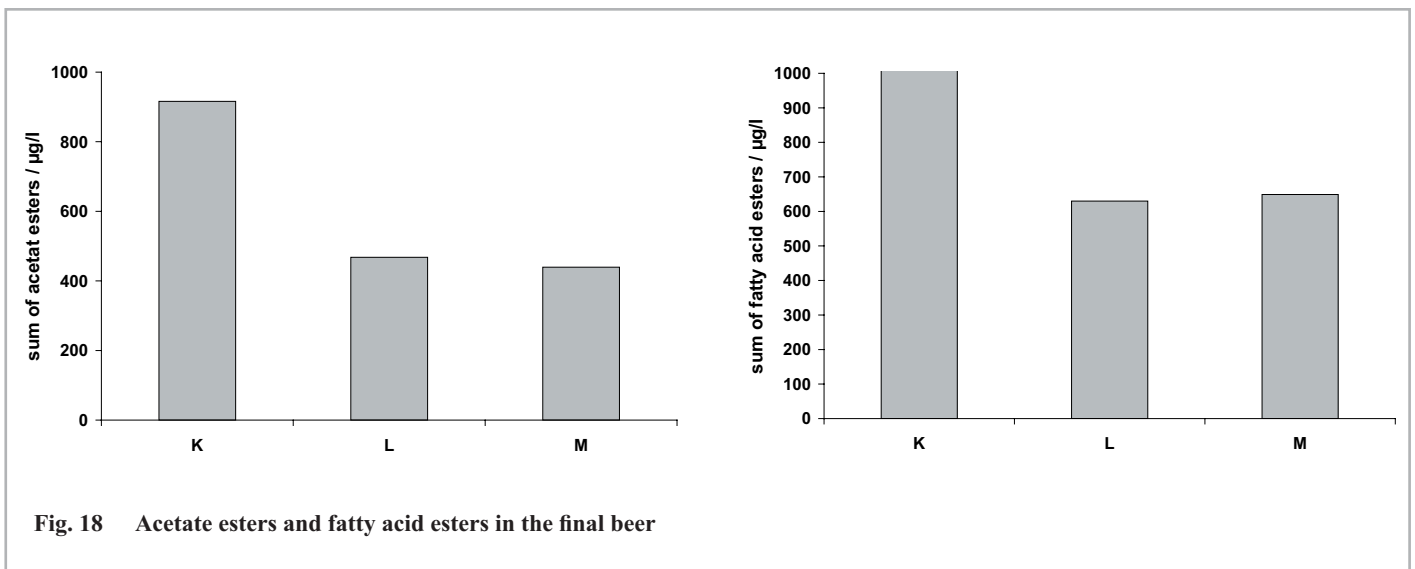
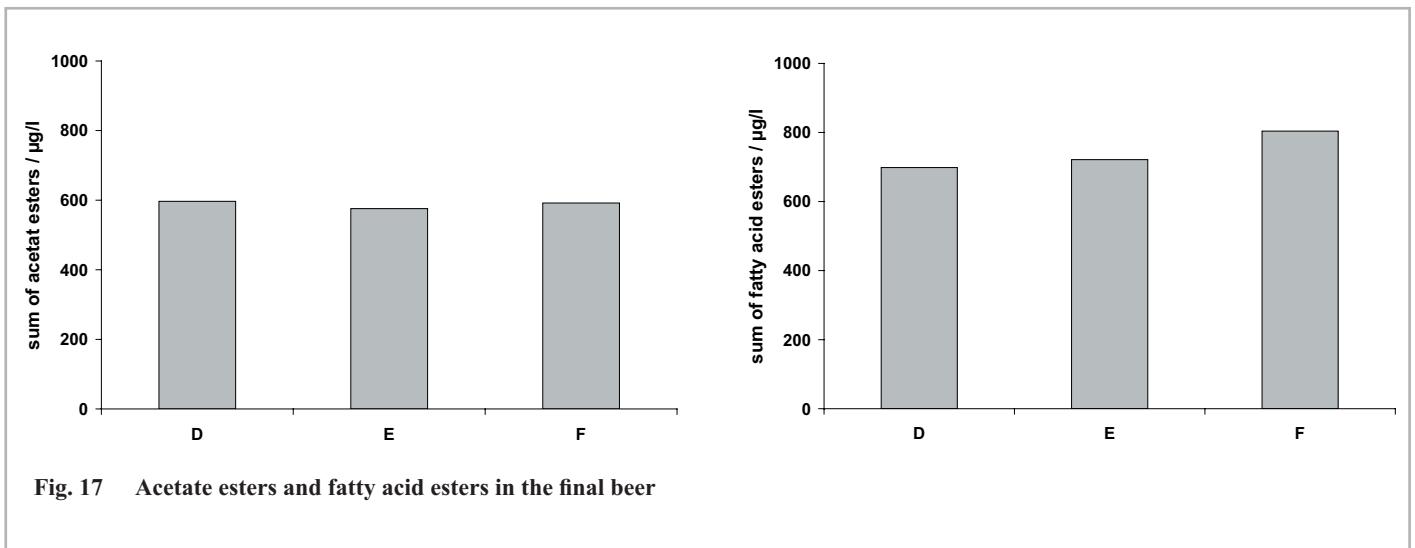
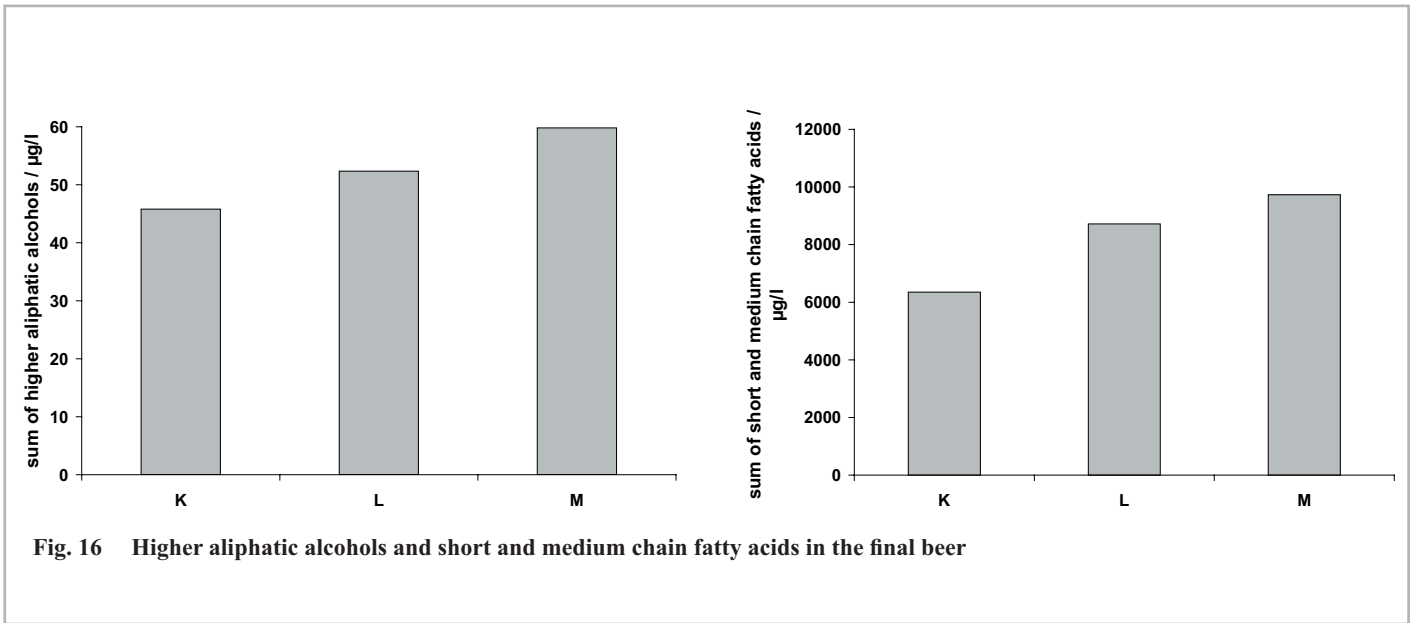


Fig. 15 Higher aliphatic alcohols and short and medium chain fatty acids in the final beer



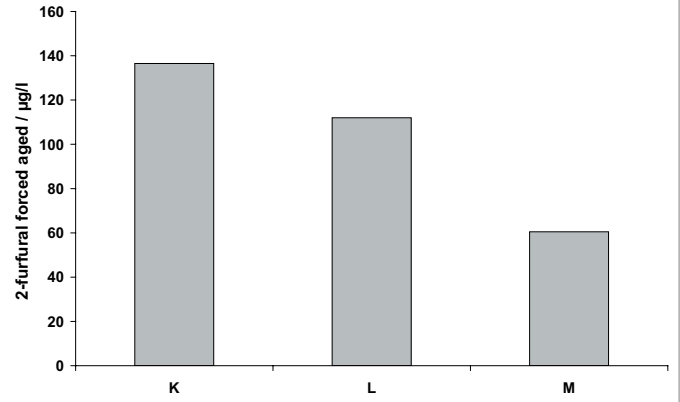
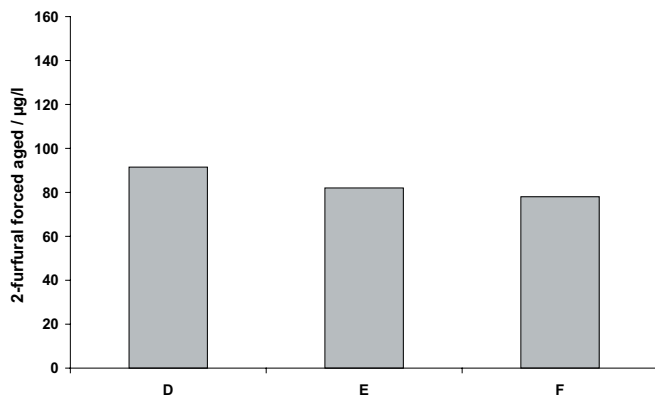


Fig. 19 Amount of 2-furfural in the final beer (forced aged)

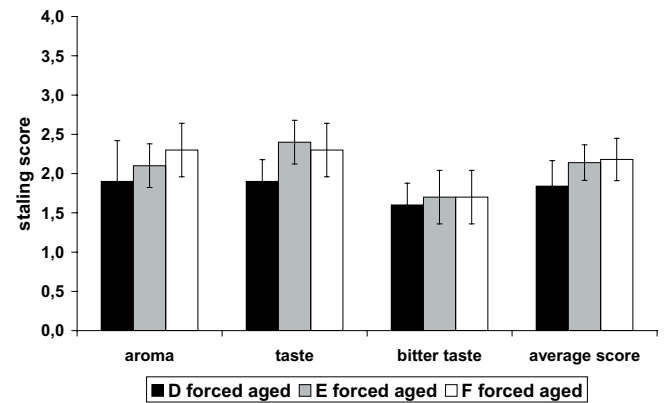
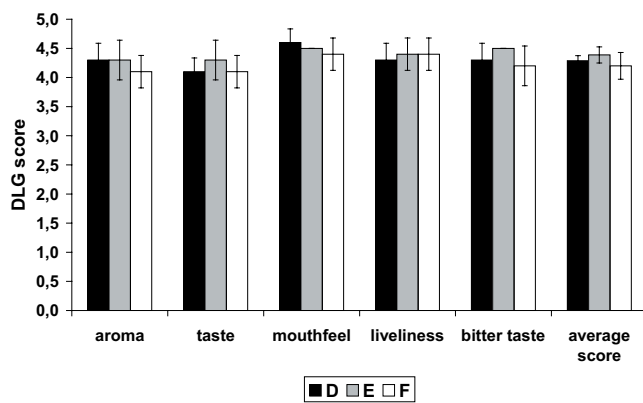


Fig. 20 DLG scores in fresh beers (left) and staling scores in forced aged beers (right) ($\alpha=0.05$, N=7)

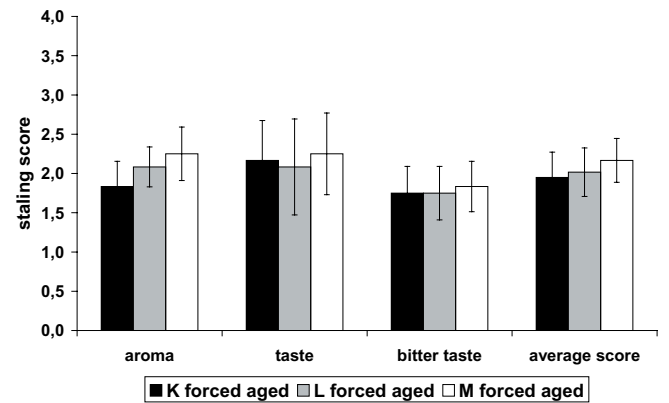
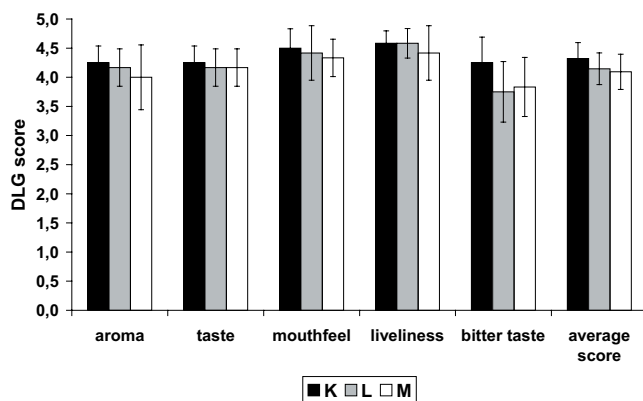


Fig. 21 DLG scores in fresh beers (left) and staling scores in forced aged beers (right) ($\alpha=0.05$, N=7)

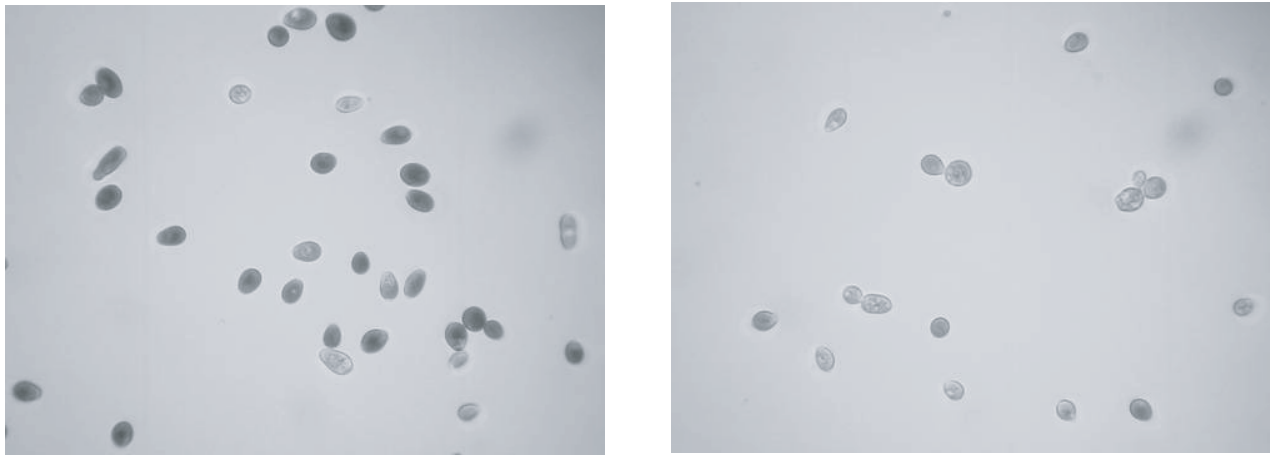


Fig. 22 Microscopic pictures of yeast cells with high (left) and low (right) amounts of intracellular carbohydrates after dyeing with Lugol's iodine

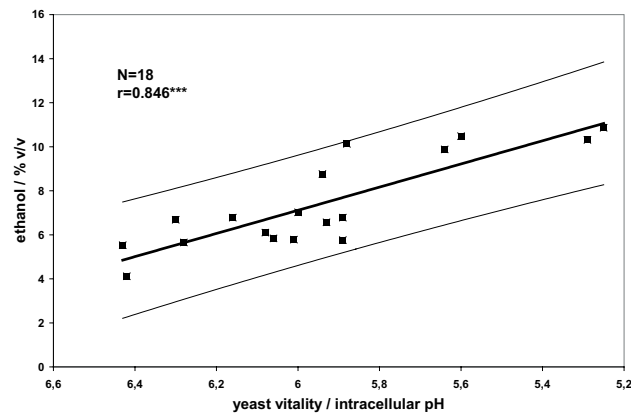


Fig. 23 Correlation between ethanol content in yeast beer and yeast vitality

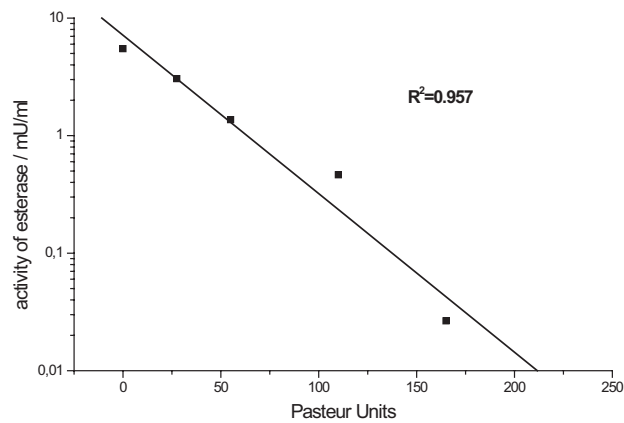


Fig. 24 Influence of Pasteur units on activity of esterase

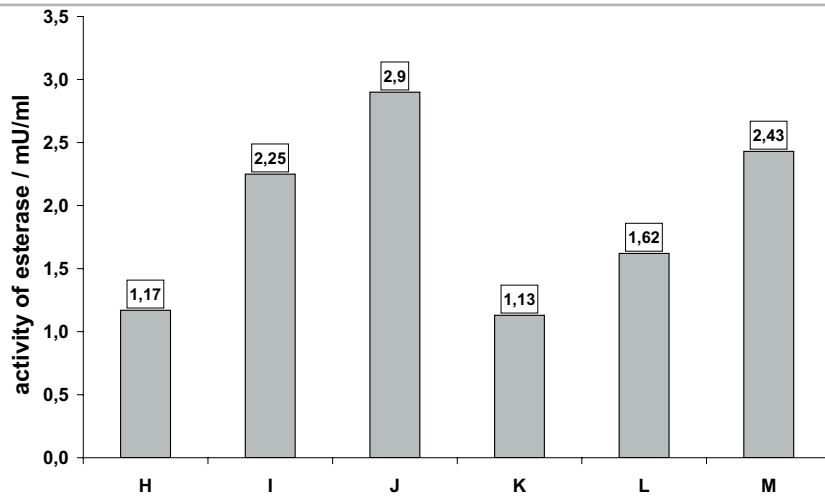


Fig. 25 Activity of esterase in trials H–J and K–M

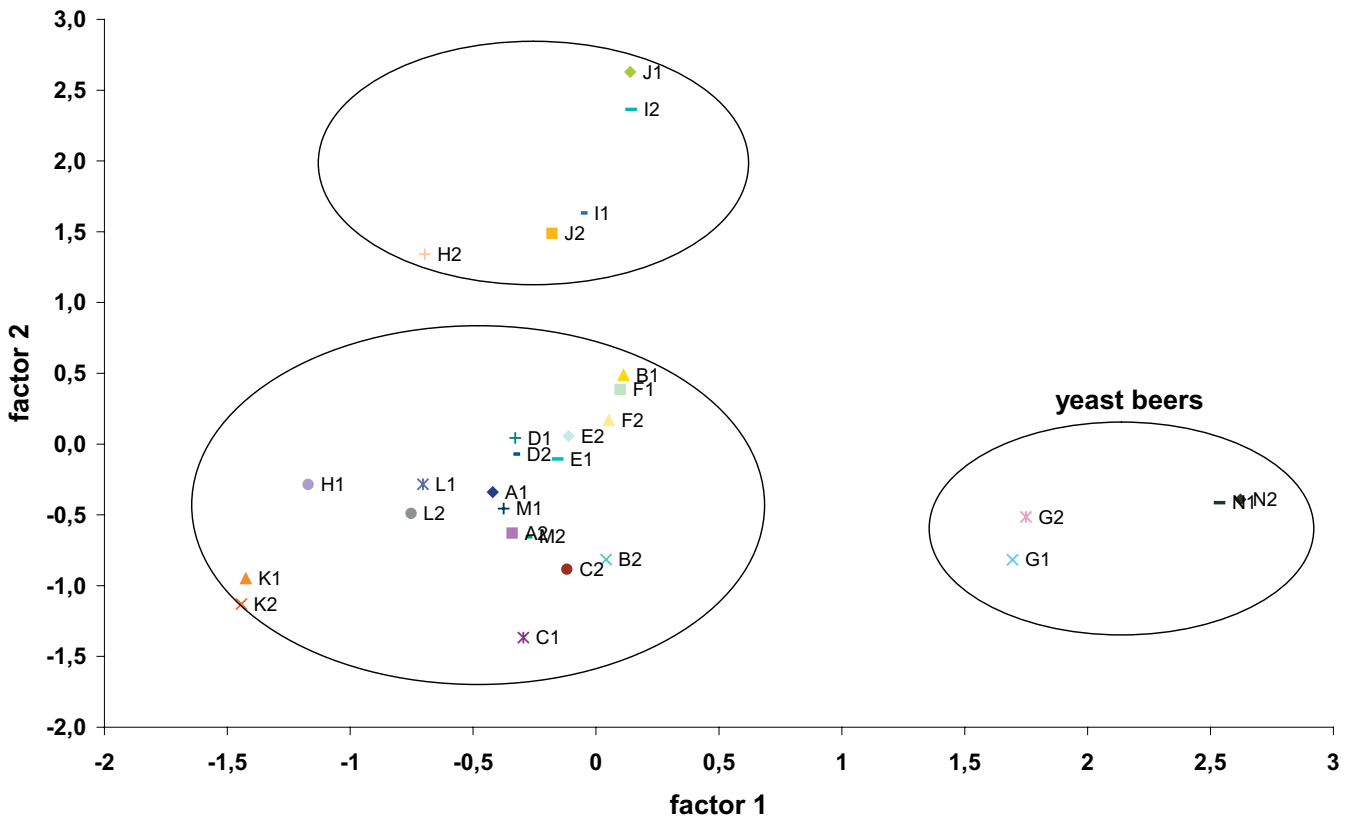


Fig. 26 Principal component analysis of final beers A–N

