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Effects of Mash Acidification

Acidification can be useful not only for mash or wort, but also for the decarbonation of brewing liquor. According to the Provisional Beer Law in Germany, an acidification in the brewhouse is permitted, when the lactic acid is obtained biologically. This work gives an overview about the effects of mash acidification at different mashing-in temperatures. Laboratory trials were performed at 58-61-64 °C, on the one hand with and on the other hand without mash acidification (pH-lowering of 0.2 units). Industrial-scale tests, in which a total of over 19,000 hl wort was produced, showed that mash acidification up to a mashing-in temperature of 61 °C has a significant influence on the nitrogen content in wort. Formula for the calculation of the decarbonation are shown.

Descriptors: water, biological acidification, lactic acid, mashing, enzymatic solution, softening, decarbonisation, wort

1 Introduction

Acidification in the brewhouse area has been applied in the breweries for years. According to the regulations of the beer tax law, mash or wort with lactic acid bacteria, which can anyway be found on malt and are mostly enriched in wort, may be applied for the production of pale beers.

Acidification can be useful not only for mash or wort, but also for the decarbonation of brewing liquor. For this purpose mineral acids, as sulphuric acid, phosphoric acid or hydrochloric acid and organic acids, such as lactic acid, are used. In breweries with soda alkaline water mash acidification is already being successfully applied for water treatment. In case of water with a total german hardness over 10 °d, however, calcium precipitations in warm water can occur.

According to the Provisional Beer Law in Germany, an acidification in the brewhouse is permitted, when the lactic acid is obtained biologically. It can be added to mash as well as to wort. In former times mash acidification was generally applied in case of badly dissolved malt and intensive mash work. Because of the continuous bettering of malt quality, the mash acidification was more and more repressed. Nowadays wort acidification is widely-used.

Target of this work is to give an overview about the effects of mash acidification at different mashing-in temperatures. Laboratory trials were performed at 58-61-64 °C, on the one hand with and on the other hand without mash acidification (pH-lowering of 0.2 units). A comparison to practice was carried out in large-scale tests, in which a total of over 19,000 hl wort was produced. Mashings took place, on the one hand at 58 °C without mash acidification and on the other hand at 62 °C with and without mash acidification. The comparison of wort analyses between laboratory trials and large-scale tests and the results of the beer analyses are shown below.

Table 1 Laboratory trial water analyses

Municipal water	
Total hardness [°d]	18.9
Carbonate hardness [°d]	15.7
Non-carbonate hardness [°d]	3.2
$NaHCO_3$ [mg/l]	0
Hardness of Ca^{2+} [°d]	12.1
Hardness of Mg^{2+} [°d]	6.8
Total alkalinity [°d]	15.7
Rest alkalinity [°d]	11.3
pH	7.92
Soda-alkaline water	
Total hardness [°d]	2.6
Carbonate hardness [°d]	2.6
Non-carbonate hardness [°d]	0.0
$NaHCO_3$ [mg/l]	276
Hardness of Ca^{2+} [°d]	1.4
Hardness of Mg^{2+} [°d]	1.2
Total alkalinity [°d]	11.8
Rest alkalinity [°d]	11.2
pH	6.28
Brewing water from an brewery (mashing-in water)	
Total hardness [°d]	4.4
Carbonate hardness [°d]	0.4
Non-carbonate hardness [°d]	4.0
Hardness of Ca^{2+} [°d]	2.8
Hardness of Mg^{2+} [°d]	1.6
Rest alkalinity [°d]	–0.6

2 Material and methods

Raw material

The waters mentioned below were used for the laboratory trials (Table 1). Analysis are according to MEBAK [1]:

In the large-scale test the brewing water mentioned above is used for mashing-in. The sparging liquor is additionally used with the following analysis (Table 2):

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Figures see Appendix

Table 2 Water analysis: Sparging liquor for large-scale tests

Total hardness [°d]	3.8
Carbonate hardness [°d]	0.4
Non-carbonate hardness [°d]	3.4
Hardness of Ca^{2+} [°d]	2.4
Hardness of Mg^{2+} [°d]	1.4
Rest alkalinity [°d]	–0.5

No hop is needed for the laboratory trial: In order to be able to evaluate the mashing procedure, only lautered wort is analysed. The following hop extract is applied in large-scale tests in breweries: ethanol extract, Hallertau Taurus, Harvest 2002, 51.7 % bitterness value of conducting meter.

Malt 1 and 2 were used for the laboratory trials, Malt 3 and 4 for the large-scale tests (Table 3):

Table 3 Malt analysis Malt 1, 2, 3 and 4

Malt	**1**	**2**	**3**	**4**	
water content	4.70	3.70	3.90	4.10	%
Extract	78.5	79.6	80.5	79.0	%
	82.4	82.7	83.8	82.4	% d.m.
friability	84.3	92.8	94.4	96.7	%
total glassy kernels	0.7	1.1	1.3	1.3	%
partial glassy kernels	1.5	1.0	2.7	1.7	%
saccharification time	< 10	< 10	< 10	< 10	min
Course	clear	opale-scent	clear	clear	
colour fotometer	2.6	4.0	2.9	20	EBC
boiled wort colour foto-meter	5.0	4.9	5.5	-	EBC
pH value	5.99	5.97	6.02	5.58	
crude protein	10.6	10.3	9.8	10.5	% d.m.
soluble nitrogen	651	662	655	814	mg/ 100g d.m.
Kolbach index	38	40	42	49	%
viscosity (8.6 %)	1.546	-	-	-	mPas
viscosity 65 °C (8.6 %)	1.621	-	1.555	1.505	mPas
β-glucan congress wort	-	150	131	61	mg/l
β-glucan Hartong 65 °C	-	150	206	103	mg/l

Wort production

Only the mashing process is to be observed in laboratory trials. The worts are therefore produced through the congress mashing method according to the analyses specifications of MEBAK [2]. Simply the temperature conditions are changed in some tests, the equipment or other process steps, however, remain unchanged. The large-scale test is performed in a large German brewery. Its brewhouse comprises a hammer mill, 2 brewing lines, each with one mash filter (Meura 2001), one boiling system with an external wort boiler and a whirlpool, as well as one wort cooler. The malt charge consists of 12 tons per brew: 9.5 t Malt 3 and 2.5 Malt 4. Three mashing programs with different temperature conditions are applied for the test brews (Table 4):

Table 4 Mashing programs of the large-scale tests

Mashing program „58“
mashing-in temperature 58 °C

	temp. [°C]	**period [min]**
mashing-in	58	
rest	58	15
heating-up	64	
rest	64	30
heating-up	72	
rest	72	5
heating-up	78	
final mash pumping	78	

Mashing program „62“
Mashing-in temperature 62 °C

	temp. [°C]	**period [min]**
mashing-in	62	
rest	62	15
heating-up	64	
rest	64	22
heating-up	72	
rest	72	5
heating-up	78	
final mash pumping	78	

Mashing program „62 LA“
mashing-in temperature 62 °C + LA
temperature conditions:
identical to mashing program „62“
+ 10 hl biological lactic acid (LA) at mashing-in

Analyses methods

The chemical-technical analyses are performed according to the MEBAK [4]. The chromatographic analyses are carried out in the laboratory of gas chromatography/high pressure liquid chromatography (HPLC) of the Lehrstuhl für Technologie der Brauerei I, Weihenstephan. The chromatography determination methods are described in the testing method Nr. GC005/96, GC011/96 und GC007/96 of the Chair.

The sensory analyses are performed according to 4 methods:

DLG verification scheme for beer: The fresh as well as the forced aged beer are analysed according to the DLG verification scheme. For an exact differentiation half grade steps for the evaluation of each test feature is chosen. The forced ageing of the beer is carried out according to a standardized method: the bottled beer is shaken 24 hours, so that its transport is simulated, afterwards

stored 4 days in a heating cabinet at 40 °C and then at 0 °C till the taste testing is performed.

Ageing taste testing according to Eichhorn [3]: In order to exactly evaluate the aged conditions of the beer, the taste testing scheme according to Eichhorn is drawn on. For an exact differentiation half grade steps for the evaluation of each test feature are chosen.

Taste testing scheme with evaluated intensities [4]: For the exact determination of sensory changes a taste testing scheme with evaluated intensities is prepared. Special flavour and aroma impressions of the terminology system according to EBC is chosen, such as “hoppy”, “malty”, “phenolic”, “astringent”, “solvent-like”, “acidic” or “oxidized”.

Extended triangle test: The triangle test is applied to determine small differences between 2 test samples. A series of three consists of 2 identical samples and 1 divergent sample. The taster should try to find the divergent sample. In case of the extended triangle test with preference, the taster should additionally show his preference either for the single or for the twin sample.

3 Results and discussion

The laboratory trials are divided in:

- water decarbonation with acids;
- water hardening by means of calcium sulphate and calcium chloride;
- mash acidification and mashing-in temperatures.

Water decarbonation with acids

The procedure of water decarbonation by means of acids is often called „ester interchange“. The carbonates of water are transformed into non-carbonates. Only a change of carbonate hardness follows; the hardness of calcium or magnesium and the concentration of Ca^{2+} and Mg^{2+}, however, remain unchanged. Therefore the carbonate hardness is taken into account for the following considerations.

The following rule should be applied:

$$1° \text{ d} = 10 \text{ mg/l CaO} \quad [2]$$

10 mg CaO correspond to an amount of substance of 0.1783 mmol, since CaO has a molecular weight of 56.08 mg/mmol. The concentration of the amount of dissolved substance does not always correspond to the concentration of the amount of substance of particles the reaction depends on [5]. Therefore the concentration of amount of substance has to be converted into the equivalence concentration according to following formula:

$$\text{equivalent concentration} = \frac{\text{equivalent amount } n_{eq}}{\text{volume V}} = \frac{\text{amount of substance n} \cdot \text{valence z}}{\text{volume V}}$$

In case of acids the valence z corresponds to the quantity of dissociated protons (resp. hydronium ions), in case of bases it corresponds to the amount of dissociated hydroxide ions, in case of oxidants and reductives to the change of the oxidation number [6].

The amount of substance 0.1783 mmol/l CaO (=1 °d) corresponds with $z_{CaO} = 2$:

equivalent concentration $c_{CaO\ (eq = ½)} = 0.3566$ mmol/l.

To reduce 1 °d carbonate hardness per liter by means of hydrochloric acid, the equivalent concentration of hydrochloric acid HCl must also be $c_{HCl\ (eq = 1/1)} = 0.3566$ mmol/l. According to the formula for the calculation of the equivalent concentration and $z_{HCl} = 1$, the following amount of substance per litre water result:

$$n_{HCl} = \frac{c_{HCl\,(eq=1/1)}}{z_{HCl}} \cdot 1\text{l} = \frac{0.3566\text{mmol/l}}{1} \cdot 1\text{l} = 0.3566\text{mmol}$$

With $M_{HCl} = 36.46$ mg/mmol a mass of $m_{HCl} = 13.00$ mg result.

For the reduction of carbonate hardness 13.00 $\frac{\text{mg HCl}}{\text{l} \cdot ° \text{d}}$ are necessary.

The calculation for further acids achieves following brewing-technical results:

For a reduction of carbonate hardness of 1 °d in one litre water:

hydrochloric acid HCl: 13.00 $\frac{\text{mg HCl}}{\text{l} \cdot ° \text{d}}$

phosphoric acid H_3PO_4: 11.65 $\frac{\text{mg } H_3PO_4}{\text{l} \cdot ° \text{d}}$

sulphuric acid H_2SO_4: 17.49 . $\frac{\text{mg } H_2SO_4}{\text{l} \cdot ° \text{d}}$

lactic acid H_3C-CHOH-COOH: 32.12 $\frac{\text{mg lactic acid}}{\text{l} \cdot ° \text{d}}$ are necessary.

On the basis of the determined quantity of acids, the municipal water is decarbonated. An exact calculated quantity of each acid is added to the water in order to cause a reduction of the carbonate hardness of 10 °d.

Table 5 shows the results of the decarbonation test.

The confirmation of the theory of the ester interchange is clearly recognizable: Carbonate hardness is reduced nearly by the calculated 10 °d, while the total, calcium and magnesium hardness remain unchanged. Thus a decrease of the rest alkalinity to brew-technological values takes place.

Only phosphoric acid does not react according to this pattern: It reduces the carbonate hardness only by 5.6 °d instead of the calculated 10 °d. This can probably be ascribed to the molecular structure: While salt, sulphur and lactic acid consist of one proton, phosphoric acid consists of three protons. Depending upon dissociation, different phosphates, which still contain separable protons, are developed. A complete dissociation of phosphoric acid is not achieved through these attempts [5].

Table 5 Laboratory trial: Decarbonation of the municipal water

municipal water	untreated	HCl	H_2SO_4	H_3PO_4	lactic acid
Total hardness [°d]	18.9	18.9	18.6	18.8	18.8
Carbonate hardness [°d]	15.7	6.6	6.3	10.1	6.4
Non-carbonate hardness [°d]	3.2	12.3	12.3	8.7	12.4
$NaHCO_3$ [mg/l]	0	0	0	0	0
Hardness of Ca^{2+} [°d]	12.1	11.4	12.0	12.1	12.1
Hardness of Mg^{2+} [°d]	6.8	7.5	6.6	6.7	6.7
Total alkalinity [°d]	15.7	6.6	6.3	10.1	6.4
Rest alkalinity [°d]	11.3	2.3	1.9	5.7	2.0
pH	7.92	6.12	6.27	6.51	5.86

The decarbonation of the soda-alkaline water with the same acids offers the following results (table 6):

Table 6 Laboratory trial: Decarbonation of a soda-alkaline water

soda-alkaline water	untreated	HCl	H_2SO_4	H_3PO_4	lactic acid
Total hardness [°d]	2.6	2.5	2.6	2.5	2.7
Carbonate hardness [°d]	13.3	3.2	3.8	8.3	5.5
Non-carbonate hardness [°d]	–10.7	–0.7	–1.2	–5.8	–2.8
$NaHCO_3$ [mg/l]	276	21	36	174	84
Ca^{2+} hardness [°d]	1.4	1.3	1.6	1.7	1.6
Mg^{2+} hardness [°d]	1.2	1.2	1.0	0.8	1.1
Total alkalinity [°d]	11.8	3.2	3.8	8.3	5.5
Rest alkalinity [°d]	11.2	2.7	3.2	7.7	4.9
pH	6.28	5.42	5.47	5.85	5.63

In turn, hydrochloric and sulphuric acid reduce the carbonate hardness by 10 °d, while the total, calcium and magnesium hardness remain unchanged. Also phosphoric acid reacts as in the preceding attempt: For the same reasons it lowers the carbonate hardness only by 5 °d instead of the calculated 10 °d. However lactic acid cannot remove the carbonate hardness completely. This can be traced back to the fact that lactic acid, as a weak acid, cannot be dissociated completely and the carbonates cannot be converted entirely.

In conclusion, it can be said that an acidification of the water can replace water decarbonation in brewery practice. This alternative is already being used in some breweries in case of soda-alkaline waters with low total hardness. Within the German purity law, biologically won lactic acid is used for this purpose. However if the total hardness exceeds approx. 10 °d, technical problems can occur in the hot water area due to calcium carbonate sediments.

Abridged two-mash method

To which extent the differently softened waters influence mashing work is to be analysed below. Three different waters are used in this test series:

Raw water: natural water, supply water
Ion exchange: softened water by ion exchanger
Lactic acid: decarbonated water by addition of lactic acid

Rest alkalinity of both treated waters (softened and decarbonated) amount 1 °d each. As proved in the laboratory trial of the decarbonation attempt, lactic acid must be added in larger quantity as 32.12 $\frac{\text{mg lactic acid}}{\text{l} \cdot \text{°d}}$ so that the desired rest alkalinity is reached. By using Malt 1 the mashing process takes place in a congress mash facility after temperature adjustment of the abridged two-mash method (Table 7).

Table 7 Temperature adjustment of the abridged two-mash method in the laboratory trial

„abridged two-mash method“	Temp. [°C]	Period [min]
Mashing-in	62	
Rest	62	45
Heating-up	70	8
Rest	70	62
Final mash pumping	70	

In order to make sure the analyses data are statistically correct, each analysis is performed five times. The real value is calculated according to the following formula [7]:

$$\mu = \bar{x} \pm z \cdot \frac{\sigma}{\sqrt{n}} = \bar{x} \cdot a$$

with μ = real value,
x = average value of the measured data,
z = tabulated parameter,
σ = real distribution,
n = quantity of measured data,
a = confidence interval,
at a specified security F.

In the following illustrations the confidence interval is marked around the average value of the measured data.

In Figure 1 the extract contents and final attenuation of the worts are represented. With acidified water the worts show a notably higher value of extract. Also the final attenuation with waters with low rest alkalinity increases significantly.

As expected, the pH values of the worts with treated waters are lower than in case of natural water. However the acidified water supplies lower values than the water softened by ion exchangers (Fig. 2). The wort colour decreases significantly in case of acidified mash.

Figure 3 shows that viscosity can be considerably lowered by water decarbonation. Also the zinc content rises notably by mash acidification; however it tends to increase only slightly in comparison to water softened by ion exchangers.

The content on soluble nitrogen (Fig. 4) rises not only by the employment of an ion exchanger, but, to a large extent, particularly by a mash acidification. The same significant increase is also identifiable in the content on free amino nitrogen.

Although the water softened by ion exchangers has the same rest alkalinity as the water charged with lactic acid, the secondly mentioned water showed a strengthened proteolysis and cytolysis during mashing.

Water hardening by means of calcium sulphate and calcium chloride

During brewing liquor conditioning calcium sulphate $CaSO_4$ and calcium chloride $CaCl_2$ are applied for the increase of the calcium hardness. The concrete quantities required for brewery practice are calculated as follows:

Increase of calcium hardness by 1 °d

One degree calcium hardness corresponds:

1° d = 10 mg/l CaO

With M_{CaO} = 56.08 mg/mmol follows:

Concentration of the quantity of material n_{CaO} = 0.1783 mmol/l

With z_{CaO} = 2 of Table 8 follows:

Equivalent concentration $c_{CaO\ (eq = ½)}$ = 0.3566 mmol/l.

Table 8 Molecular weights and valences of calcium sulphate and calcium chloride

	M [g/mol]	z
$CaSO_4$	136.14	2
$CaCl_2$	110.98	2

In order to increase 1 °d calcium hardness per litre water by means of calcium sulphate, the equivalent concentration of calcium sulphate $CaSO_4$ must amount to $c_{CaSO_4\ (eq = 1/2)}$ = 0.3566 mmol/l too. According to the formula for the calculation of the equivalent concentration and z_{CaSO_4} = 2, the amount of material per litre water results as follows:

$$n_{CaSO_4} = \frac{c_{CaSO_4\ (eq=1/2)}}{z_{CaSO_4}} \cdot 1l = \frac{0.3566\ mmol/l}{2} \cdot 1l = 0.1783\ mmol$$

With M_{CaSO_4} = 136.14 mg/mmol the mass m_{CaSO_4} = 24.27 mg result.

In order to increase calcium hardness by 1 °d, 24.27 $\frac{mg\ CaSO_4}{l \cdot °d}$ respectively 2.4 $\frac{mg\ CaSO_4}{l \cdot °d}$ are necessary.

In order to increase 1 °d calcium hardness per litre water by means of calcium chloride, the equivalent concentration of calcium chloride $CaCl_2$ must amount to $c_{CaCl_2\ (eq = 1/2)}$ = 0.3566 mmol/l. According to the formula for the calculation of the equivalent concentration and z_{CaCl_2} = 2, the amount of material per litre water results as follows:

$$n_{CaCl_2} = \frac{c_{CaCl_2\ (eq=1/2)}}{z_{CaCl_2}} \cdot 1l = \frac{0.3566\ mmol/l}{2} \cdot 1l = 0.1783\ mmol$$

With M_{CaCl_2} = 110.98 mg/mmol a mass m_{CaCl_2} = 19.79 mg result

In order to increase calcium hardness by 1 °d, 19.79 $\frac{mg\ CaCl_2}{l \cdot °d}$ respectively **2.0** $\frac{mg\ CaCl_2}{l \cdot °d}$ are necessary.

Decrease of rest alkalinity by 1° d

According to Kolbach, the acidity-changing characteristics of a water are characterized by the term "rest alkalinity" [2].

$$\text{Rest alkalinity} = \text{total alkalinity}[°d] - \frac{\text{calcium hardness}[°d] + 0.5 \cdot \text{magnesium hardness}[°d]}{3.5}$$

By adding calcium sulphate $CaSO_4$ and calcium chloride $CaCl_2$ only the calcium hardness is changed. Total alkalinity and magnesium hardness, however, remain unchanged. The acidity-destroying effect of 1 °d total alkalinity is compensated through 3.5 °d calcium hardness. In order to decrease the rest alkalinity of a water by 1 °d, a calcium hardness of 3.5 °d must be added.

The following results from the above determined addition of Ca^{2+}:

For the decrease of rest alkalinity by 1 °d, 3.5 · 24.27 $\frac{mg\ CaSO_4}{l \cdot °d}$ = 84.95 $\frac{mg\ CaSO_4}{l \cdot °d}$ respectively

8.5 $\frac{g\ CaSO_4}{hl \cdot °d}$ are necessary.

For the decrease of rest alkalinity by 1 °d, 3.5 · 19.79 $\frac{mg\ CaCl_2}{l \cdot °d}$ = 69.27 $\frac{mg\ CaCl_2}{l \cdot °d}$ respectively **7.0** $\frac{g\ CaCl_2}{hl \cdot °d}$ are necessary.

Mash acidification and mashing-in temperature

In this test series raw materials of the large brewery are worked with: fine grist is made of Malt 2 by means of a DLFU mill, in order to approach as close as possible the refinement of the hammer mill in practice. The mashing-in water is brewing liquor from the large brewery. The casting conditions and the quantity of acidification are applied identically as in the preliminary test. This time, the final mash pumping temperature is kept at 76 °C. However different mashing-in temperatures are selected: 58, 61, 64 °C. The temperature conditions are as follows:

Triple regulations are performed in all tests. The lactic acid addition is carried out according to the process of the large brewery. 10 hl 1.5 % biological lactic acid is given to 120 dt malt during mash acidification. This corresponds to 10.8 ml 1.5 % technical lactic acid on the laboratory throw 130 g and/or 2.7 ml 6.0 % technical lactic acid. Lactic acid is added immediately after mashing-in.

Chemical-technical analyses

The target of the mash acidification - the decrease of the pH value - is exactly reached in the test series. On average the acidified worts are lower by 0.22 units in comparison to not acidified worts (Fig. 6). This is supported by results in literature: For a pH sinking of 0.1 units, 6 l biological acid with a lactic acid content of 0.8 % per 100 kg is necessary [8]. The 2.7 ml 6.0 % technical lactic acid given on 130 g in this test series correspond then to a theoretical pH sinking by 0.26. This is supported again by practical results,

which suggests again a stable matrix. When analysing wort colour the results from the preliminary tests and of the literature could be confirmed: Worts, which are acidified during mashing, show a lower colour. In turn, a dependence of the wort colour on the mashing-in temperature cannot be observed.

The contents of soluble nitrogen in case of the not acidified worts cannot be differentiated statistically (Fig. 7). The acidified worts show that the content of soluble nitrogen tends to decrease when mashing-in temperature rises. The content of free amino nitrogen (FAN) can be associated to the flavour stability of the finished beer. According to Back, flavour stability is facilitated by FAN-content of the worts [9]. Figure 7 shows that the FAN-contents of worts without mash acidification are not significantly dependent on the mashing-in temperature. This is confirmed by the thesis of Riis, which states that only 1.5-4.5 % of the endoproteases EP-A and EP-B are solved and active during mashing process [10]. No protein decomposition takes place, but rather only one dilution of the protein already reduced during malting.

In contrast, the worts with mash acidification show significantly higher FAN contents. The mashing-in temperature of these worts is apparently important too, since the FAN-contents tend to decrease when mashing-in temperatures rises. The higher content of free amino acids with acidified mashes could be caused by the fact that amino acids and peptides are solved in this acidic medium in a larger amount and/or faster. The enzyme activity could also be a reason: It is well-known that sulfhydryle additives, such as β-mercapto ethanol or cysteine activate the endoproteases, by dissolving the enzymes from the protease inhibitors of malt [10]. Therefore it could be possible that lactic acid and in particular biologically complex assembled biological acid work similar as sulfhydryle additives and possibly activate the endoproteases. This means for flavour stability: If the positive proved influences of the biological mash acidification are not waived, a mashing-in at temperatures > 61 °C must take place with regard to the FAN content. Otherwise too many amino acids would be dissolved and flavour stability would be reduced.

HPLC amino acid analyses

In determining the sum of all amino acids (Fig. 8), similar results are found as in case of the evaluation of free amino nitrogen (Fig. 7). During a mashing-in at a temperature of 58 °C and a simultaneous acidification of the mash, the content of amino acids tends to rise, in case of some amino acids even significantly. According to Back a degradation of flavour stability is caused [9]. A higher mashing-in temperature would be of advantage here. However the absolute FAN content is mainly influenced by the malt solution beside the mash process.

Large scale test

The samples for the wort analyses are taken at different times of the production process:

Kettle-full wort: Sampling takes place 5 minutes after beginning of boiling. This can guarantee a good mixing of the kettle content and thus a homogeneous sample.

Cast-out wort: Sampling takes place after boiling process.

Mid of cooling: Sampling takes place on the intake side of the wort cooler, when half of the hot wort was cooled down.

Beer: fermented, filtered and bottled sales beer

Chemical-technical analyses

The pH ratio of the kettle-full worts and mid of cooling worts are shown in Figure 9. While the differences of pH values, which were caused by mash acidification in the kettle-full wort, are still clearly recognizable, the values of the mid of cooling worts assimilate. The strong pH decrease of the kettle-full worts to the mid of cooling worts is caused by wort acidification. The pH value is not influenced by mash acidification in the bottled beer. Along with an increase of the buffer capacity of the wort, a stronger proteolysis was observed when the mash acidification is highly intensified. In such case the pH decrease during fermentation is lower and the pH of the beer is higher (data not shown)

The free amino nitrogen runs similarly to the laboratory trial. An increase of the mashing-in temperature causes a decrease of the free nitrogen; it increases strongly, however, during mash acidification (Fig. 10). The increased contents of nitrogen in the acidified worts are reflected in the bottled beers too, despite filtration and stabilisation.

The sum of the amino acids of Group I was regarded as follows: aspartic acid, glutamic acid, asparagine, glutamine, threonine, serine, lysine and arginine. These are the first to be absorbed by the yeast during fermentation.

The sums of amino acids are shown in Figure 11. Parallel to previous tests, it becomes apparent that an increase of temperature causes a slight decrease of concentration of amino acids. However, the concentration still strongly increases at 62 °C due to mash acidification. The concentration of amino acids remains unchanged during boiling. The thesis that high molecular protein fractions react during coagulation is confirmed here.

In comparison to the mid of cooling worts, the percentage of amino acids of Group I in beer is very low, i. e. they are almost completely metabolized by yeast. Due to fermentation, filtration and stabilisation the content sinks to 120-122 mg/l. In comparison to the not acidified worts, however, the increased nitrogen contents of worts „62 °C lactic acid“ are also found in the bottled beer.

Wort flavour components

The effect of mash acidification on typical wort flavour components are analysed below. According to Pfenninger, free dimethyl sulphide (DMS) in concentrations over 100 µg/l causes off-flavour in beer [11]. DMS is developed during thermal decomposition of DMS-precursor (S-methyl-methionine). During wort boiling

DMS should be evaporated to a great extent. The contents on free dimethyl sulphide (DMS) and DMS-precursor (S-methyl-methionine) of the test brews are shown in Figure 12. Between two brewhouses clear fluctuation can be observed in the kettle-full worts. But all values were equalized after wort boiling. The content on free DMS was evaporated to 68-87 µg/l and DMS-P was split to 49-72 µg/l. The technology of wort boiling can therefore be regarded as ideal. The values of free dimethyl sulphide in the bottled beer are far below the flavour threshold level of 100 µg/l. An influence of mash acidification or mashing-in temperature could not be observed.

The ageing components determined in the complete beer production process is shown in Figure 13. The 62 °C kettle-full worts show clearly increased values. However, after wort cooling the values equalize, only the acidified mid of cooling worts show increased values of Strekker aldehydes. On the one hand, the finished product is analysed as fresh aged beer, on the other hand as forced aged beer. There is no relevant difference between the trial beers with and without mash acidification, neither in the fresh nor forced aged beers. All values stand in their normal range, a fact that indicates that all beers have a high flavour stability.

Ageing tasting according to Eichhorn

In order to evaluate the forced aged beers exactly, a ageing tasting according to Eichhorn is performed. The notes are assigned as follows:

1 = „Fresh beer, without ageing character“
2 = Beer with a slight ageing character “
3 = „Beer with a strong ageing character “
4 = „Beer with an extremely pronounced ageing character“.

The acceptance corresponds to the ageing of the beer:

1.0 = „no ageing detectable“
0.0 = „strong ageing detectable“.

With respect to taste the beers are close to each other. The ageing intensity of smell and drinking increases parallel to proteolysis, i. e. the increased configuration of free amino nitrogen influences negatively smell and taste of the forced aged beers. Therefore the trial beer „62 °C” performs better as the other beers (Fig. 14). The lower ageing taste is reflected in a higher acceptance of the aged beer. Regarding flavour stability, the beer with the mashing-in temperature of 62 °C without acidification was better as the beers with acidification or reduced mashing-in temperature (58 °C).

4 Conclusion

In the described trials the effects of the mash acidification at different mashing-in temperatures were analytically comprised in laboratory and large-scale tests.

The employment of a mash acidification as instrument of the preparation of different waters can be calculated exactly. The calculations are presented in detail. The quality of the wort produced of acidified brewing liquor is comparable to worts produced with waters prepared by means of ion exchangers. From the technological point of view, better enzymatic solution processes take place during mashing with acidified brewing liquor.

In laboratory mashing-in trials took place at 58 °C, 61 °C und 64 °C, once with, once without addition of lactic acid. Mash acidification up to a mashing-in temperature of 61 °C was realised to have a significant influence on the nitrogen content in wort. Despite high temperatures proteolytic degradation and solution processes still take place, both always running more intensely in an acidified mash.

The results from the laboratory worts were confirmed in a large-scale test:

The nitrogen conditions in the acidified test brews are always increased, a fact that can be proved analytically up to the bottled beer. Mash acidification has no relevant influence on beer pH, colour, bitter units, final attenuation, polyphenols, β-glucane values, wort viscosity, free dimethyl sulphide and foam. Flavour stability, considering ageing components, stability index and organoleptic were not altered by mash acidification. In respect of beer flavour, the tasters reported on differences in the beer character, although this could only be a matter of nuances. In order to evaluate the effects on beer flavour definitely, more trial beers would be necessary.

It can be concluded that systematic coherences can be deduced from the data of wort analyses, which can be followed analytically up to the beer.

5 Acknowledgement

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Appendix

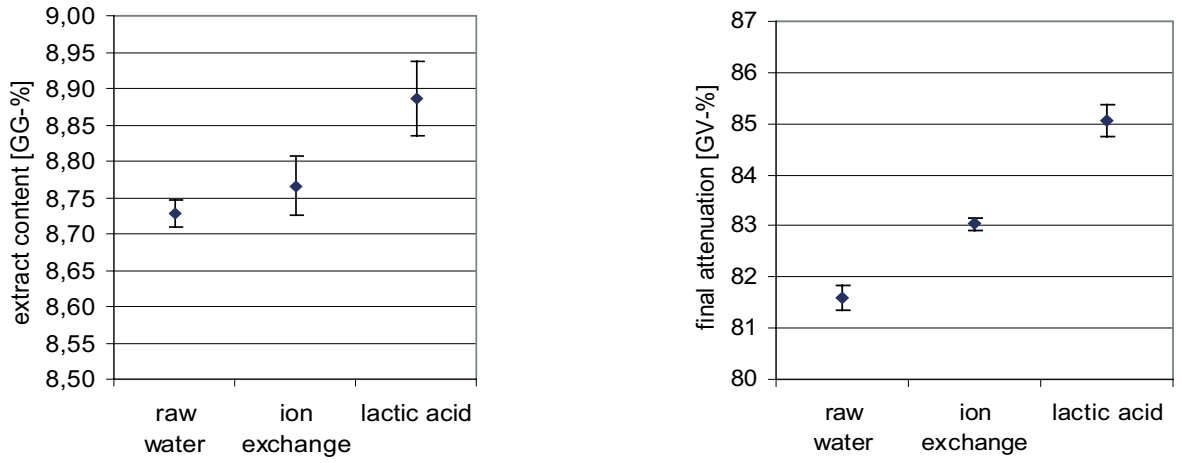

Fig. 1 Laboratory trial reproduction: Extract and final attenuation (n = 5, α = 0,05)

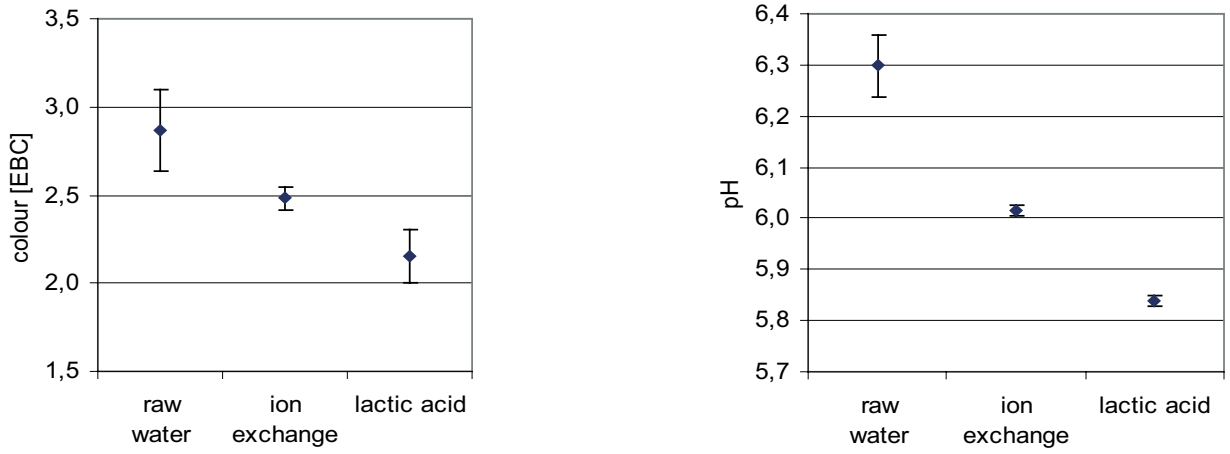

Fig. 2 Laboratory trial reproduction: Wort pH and colour (n = 5, α = 0,05)

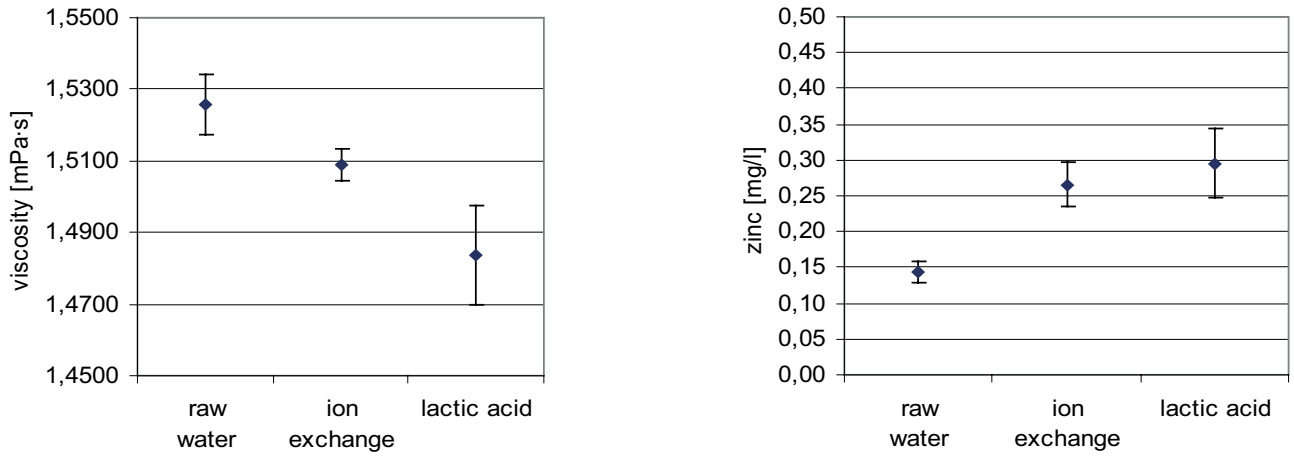

Fig. 3 Laboratory trial reproduction: Viscosity and zinc content (n = 5, α = 0,05)

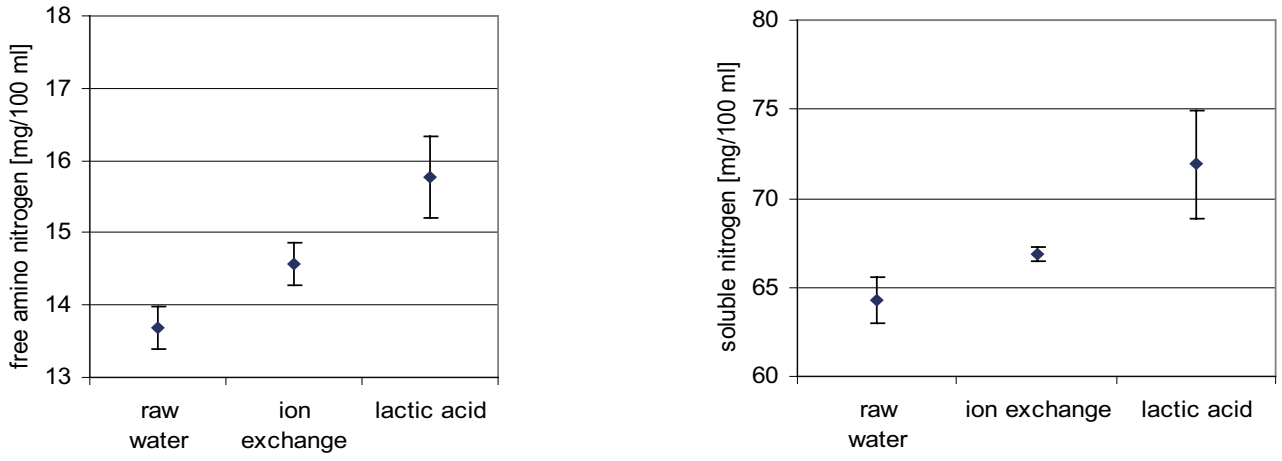

Fig. 4 Laboratory trial reproduction: soluble nitrogen and free amino nitrogen (n = 5, α = 0,05)

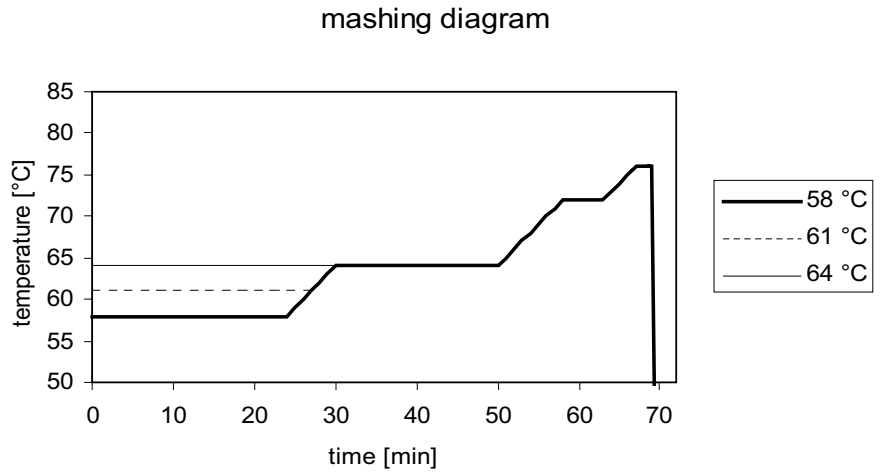

Fig. 5 Laboratory trial mashing-in temperature: Mashing diagram

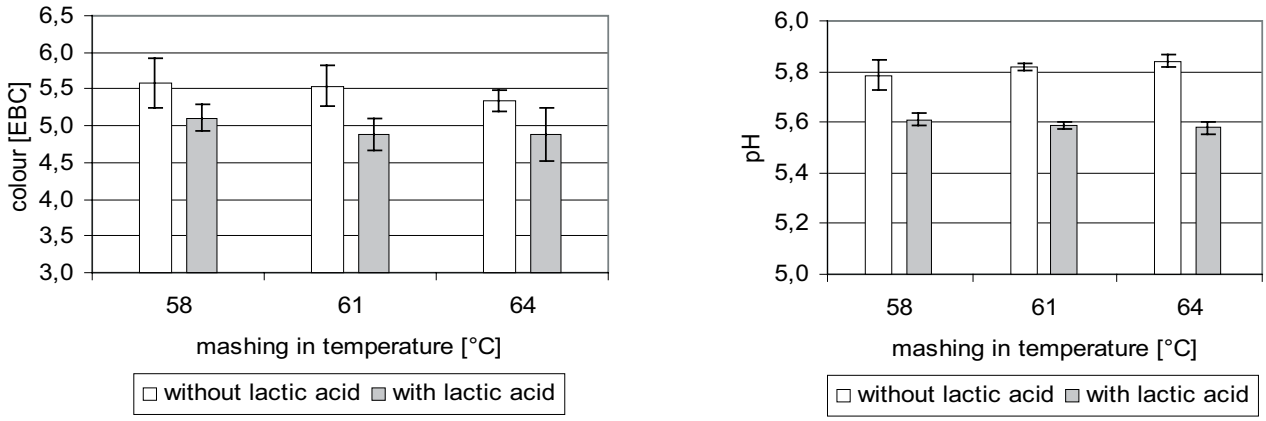

Fig. 6 Laboratory trial mashing-in temperature: pH and colour (n = 3, α = 0,05)

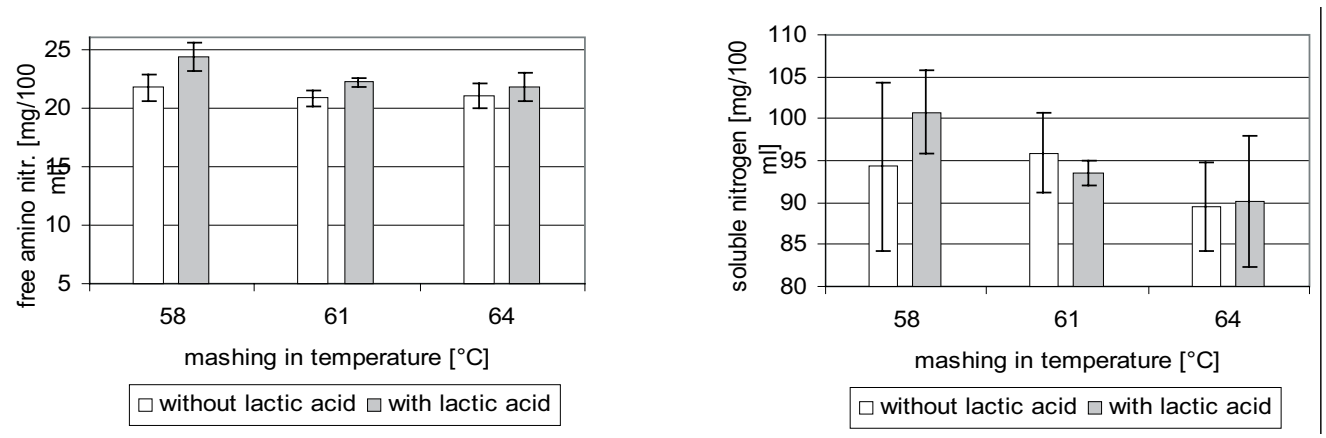

Fig. 7 Laboratory trial mashing-in temperature: soluble nitrogen and free amino nitrogen (n = 3, α = 0,05)

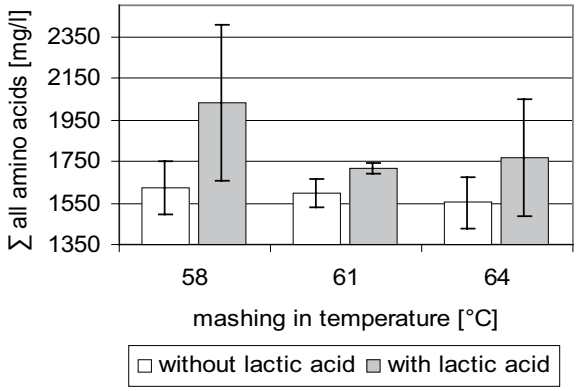

Fig. 8 Laboratory trial mashing-in temperature: sum of all amino acids (n = 3, α = 0,05)

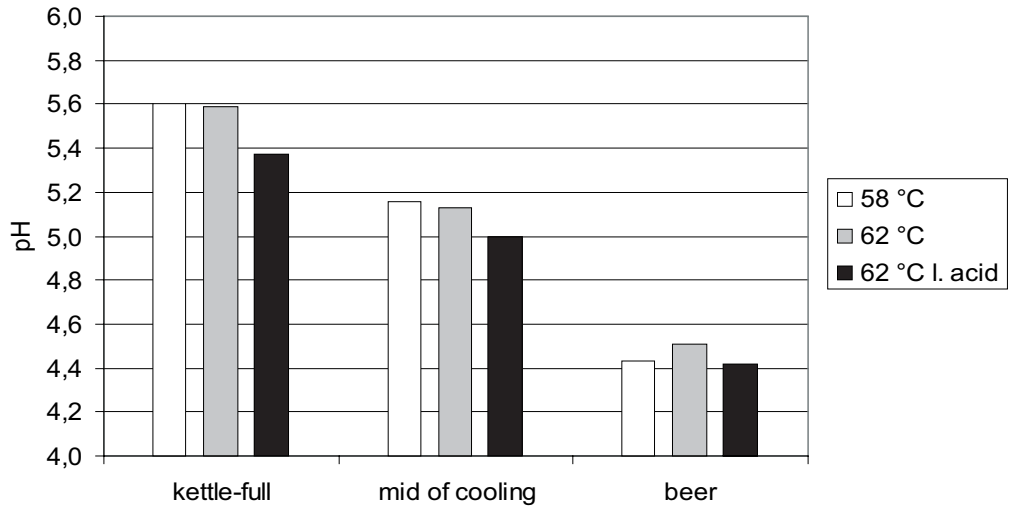

Fig. 9 Large scale test: pH in wort and beer

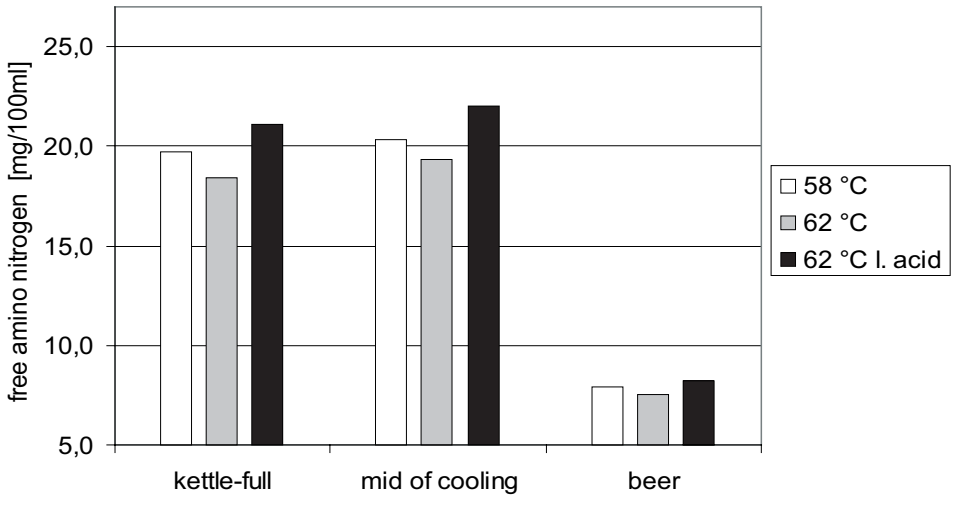

Fig. 10 Large scale test: free amino nitrogen in wort and beer

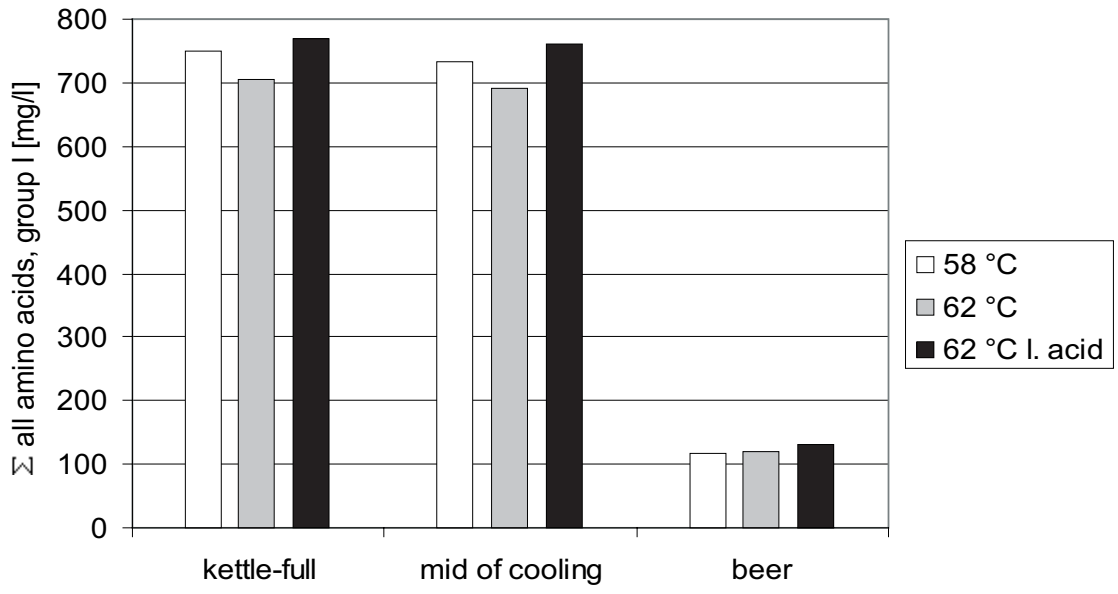

Fig. 11 **Large scale test: sum of all amino acids (group I) in wort and beer**

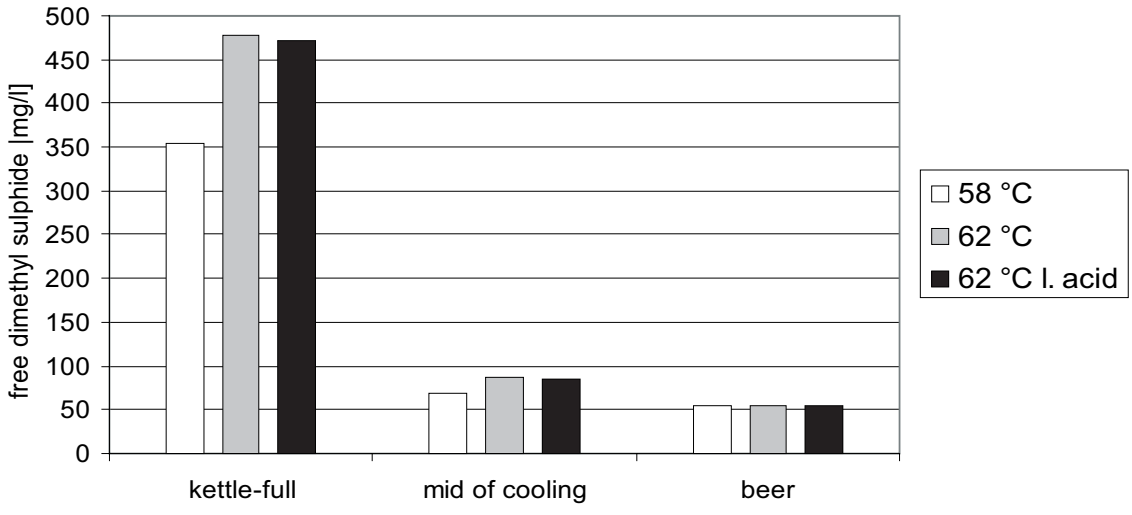

Fig. 12 **Large scale test: free dimethyl sulphide in wort and beer**

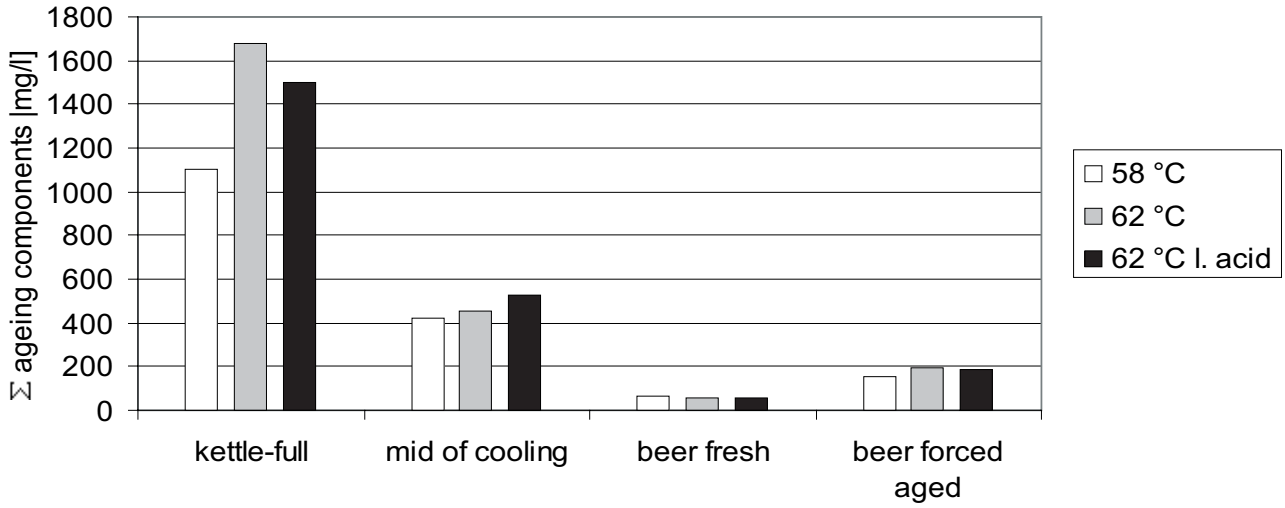

Fig. 13 **Large scale test: sum of ageing components in wort and beer**

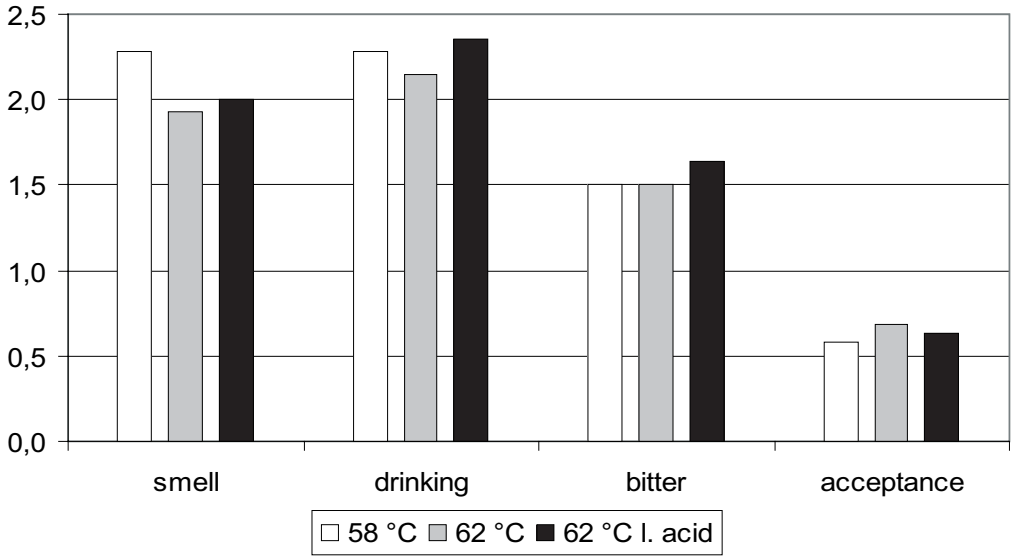

Fig. 14 **Large scale test: Ageing tasting according to Eichhorn**