M. Schütz and W. Back

The influence of mash acidification on long-chain fatty acid content in wort determinated by a new developed method

Lipids and their degradation products are well known as negative impact factors of flavour, flavour stability and foaming properties of the beer. On the other hand, lipids can have positive effects on yeast metabolism, because they support the fermentation under anaerobic conditions. They also limit the synthesis of flavour active esters. By investigations of different mashing and mash separation systems it has been shown that the majority of malt lipids remain with the spent grains and the majority of wort and hop lipids can be removed with hot break. But large quantities of lipids can be oxidised during the mashing process and the degradation products can damage the flavour and foam stability. Lipase and lipoxygenase as well as other enzymatic lipolytic factors act during the mashing process. Because of the development of numerous new mash and wort boiling systems (e. g. gentle wort boiling systems) it is very interesting to analyse the long-chain fatty acid content during wort production and to detect the lipid degradation products. Free long-chain fatty acids in wort and beer were detected by a new developed method using gas chromatography. The investigations demonstrate the effect of the mash-pH on free long chain fatty acid content in wort.

Descriptors: Free fatty acids, lipids, lipolytic enzymes, wort, mash acidification

1 Introduction

The analysis of free fatty acids is a very important application in a large number of food analyses. In the brewing field a lot of previous works showed that free fatty acids in small concentrations have a great influence on beer foam, foam stability and flavour stability (1, 2, 6, 7, 8, 9, 15). But there seems to be no direct relationship between the lipid content of wort and beer. A relevant impact factor on the long-chain fatty acid content in beer is the influence of the fermentation process as well as the condition of the yeast. Further investigations showed that a higher content of long-chain fatty acids in pitching wort (especially C18:2) supports the fermentation and could be used as an alternative to wort aeration (3, 4, 5). However, this can negatively affect the synthesis of esters, which are important as aroma constituents of beer (3, 5).

On the basis of the raw material malt, the free long-chain fatty acids palmitic acid and linoleic acid are dominant in wort (10, 17). In context with the long-chain fatty acid content of malt or wort it is important to mind the lipolytic factors during wort production, especially the lipolytic enzymes (e. g. lipase, lipoxygenase and hydroperoxy fatty acid degradation catalysing enzymes). These enzymes are active during the milling and mashing process (1, 11, 15, 19). If there are more long-chain fatty acids as start source in malt, more degradation products caused by enzymatic pathway or autoxidation can result depending on milling, mashing and lautering process. The enzymatic degradation depends on malt quality and mashing process and can mainly be influenced through the temperature program and mash concentration (1, 8, 11, 12, 15, 19). Most of the long chain fatty acids are removed almost completely during wort separation and trub removal, only a small amount remains in the pitching wort (12, 15).

Although it is possible to detect the free fatty acids directly, derivatization can be used to increase sensitivity and chromatographic performance for specific compounds. Derivatization can improve volatility and stability of the substances as well as reduce the analyte adsorption in the GC system (14). Less material wear, reproducible methods and shorter analysis times are obtained, if the free fatty acids are derivatized to the fatty acid methyl esters. In several applications fatty acids methyl esters are analysed on columns coated with polar stationary phases, allowing separation of fatty acids according to carbon number and according to the degree of unsaturation. Polar stationary phases allow to differ the cis- and trans isomers of the fatty acids methyl esters with very strong dipole characteristics (22).

In this application the free long-chain fatty acids have been detected by solid phase extraction, methylation and GC/MS analysis. Saturated and unsaturated fatty acids from C14 through C18:3 have been analysed. For derivatization it has been used a methylation apparatus of the Sigma-Aldrich Company for preparing of diazomethane, which guarantees a simple and safe handling.

2 Materials and Methods

2.1 Reagents

Pentadecanoic acid (Sigma-Aldrich Chemie GmbH, Germany) was dissolved in ethanol (100 mg/l), 1:50 diluted and used as internal standard (ISTD) of the gaschromatographic analysis. Fatty acids (myristic acid/*A* (C14:0); palmitic acid/*B* (C16:0); stearic acid/*F* (C18:0); oleic acid/*C* (C18:1); linoleic acid/*D* (C18:2) and linolenic acid/*E* (C18:3) were purchased from Sigma-Aldrich.

2.2 Apparatus

The GC/MS-analysis was carried out using a Hewlett-Packard model 5890 equipped with Hewlett-Packard mass selective detec-

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tor 5970A (scan parameter: mass range: 40 – 240 m/z). Helium at a flow rate of 28 cm/s was used as carrier gas (split vent flow: 14 ml/min). The GC/MS analysis was done with an Optima-5-MS fused silica capillary column (Macherey-Nagel; length = 50 m; film thickness = 0.2 µm; i.d. = 0.2 mm) and as precolumn a fused silica tube (Chromatographiehandel Müller; length = 1 m, i.d. = 0.25 mm).

2.3 Temperature program

Injection port was set at 250 °C and the detector temperature was 280 °C. Column temperature was initially maintained at 120 °C for 2 min and then increased to 260 °C at a rate of 8 °C/min, it was maintained at this level for 15 min, again increased to 280 °C at a rate of 8 °C/min and held at this level for another 5 min. The total run time comes to 42 minutes.

2.4 Analysis procedure

Beer was degassed (23) for analysis and cloudy wort was centrifugated with 2500 × g for 20 min at 4 °C. To 50 ml tempered (20 °C) wort or beer 1 ml of internal standard (pentadecanoic acid) was added.

A C18 solid phase column (Strata C18-E, Phenomenex, Germany) was conditioned with each 4 ml diethyl ether, methanol and distilled H_2O. After conditioning, 20 ml of wort or beer samples was loaded on the extraction column. After the sample loading step, the column was washed with 5 ml distilled H_2O. After the washing step, the long-chain fatty acids were eluted with 5 ml diethyl ether. HPLC grade solvents were used for the long-chain fatty acid analysis.

2.5 Methylation

The long-chain fatty acid-diethyl ether solution was methylated with the Aldrich Mini Diazald® apparatus (Sigma-Aldrich Chemie GmbH, Germany). The unit is designed for preparation of 1-50 mmoles of diazomethane from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald®, Sigma-Aldrich) and consists of a reaction vessel and condenser in one compact piece. The handling is described in the Sigma-Aldrich Technical Information Bulletin AL-180 (13). Figure 1 describes the components of the apparatus.

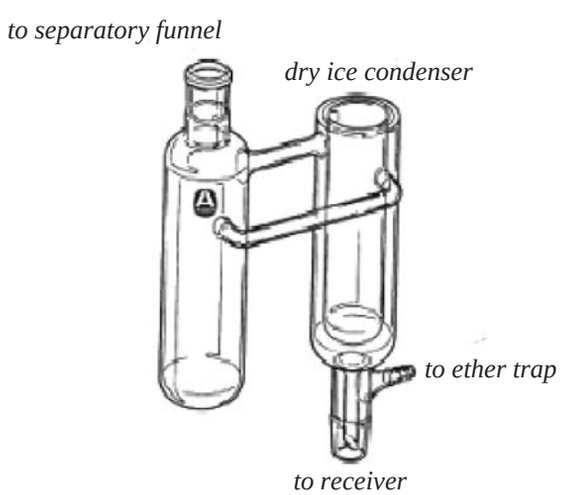

Fig. 1 Mini Diazald® apparatus (13)

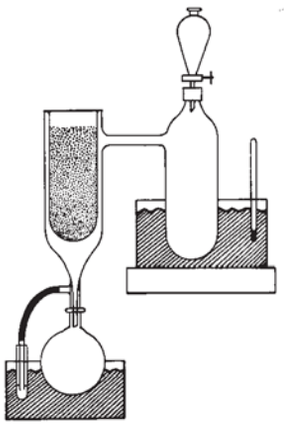

Fig. 2 Set-up using Mini Diazald® apparatus (13)

2.6 Procedure for an alcohol-containing ethereal solution (13)

The experiment set-up is shown in Figure 2. The funnel was charged with a solution of Diazald® (2 g in 25 ml ether). When all the Diazald® has been used up, slowly 20 ml of ether were added and the distillation was continued until the distillate had been colourless. The solution of potassium hydroxide (5 g in 8 ml water) and 10 ml ethanol was replaced after each second methylation. After methylation the solution was evaporated to dryness and the residue was dissolved in 2 ml ethanol. The solution was then subjected to gas chromatographic analysis. Injection volume was 2 µl.

2.7 Calibration

Calibration levels: 0.2; 0.5; 1.0; 2.0; 4.0 mg/l (cost wort, pitching wort)/additional level 8.0 mg/l (first wort, pfannevoll) or dilution of the samples

- fatty acids stock solution 1 g/l
- dilution series of the stock solution
- addition of 1 ml diluted stock solution to 49 ml wort or beer and 1 ml ISTD
- 12 °P standard wort prepared from unhopped malt extract "Ba varian Pilsner" (Weyermann Speciality Malting Co., Germany)
- characteristic beer type for beer analysis

Figure 3 and 4 show the calibration curves with the correlation coefficient each of the substances.

Coefficient of variation with n = 7:

- C14 5 %
- C16 3 %
- C18 4 %
- C18:1 3 %
- C18:2 6 %
- C18:3 4 %
- Σ 6 %

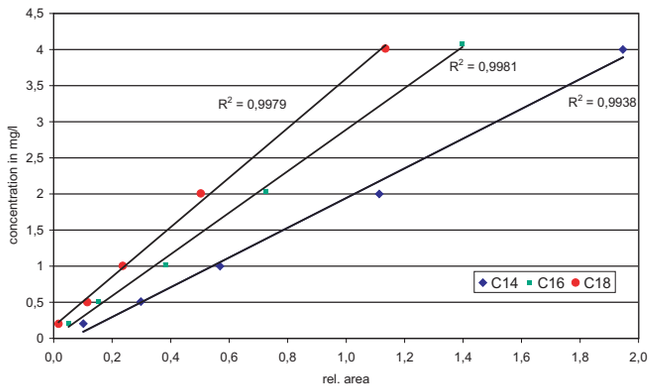

Fig. 3 Calibration curve of the saturated fatty acids C14; C16 and C18

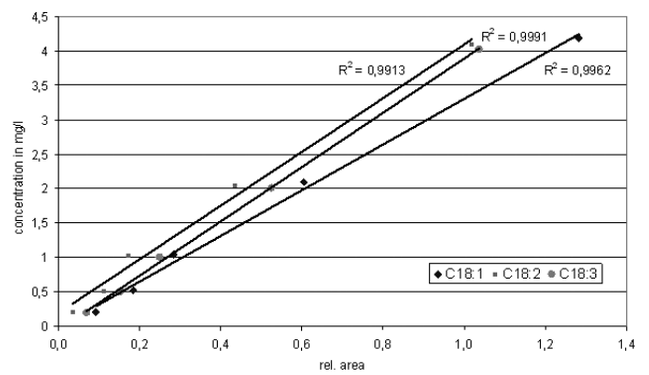

Fig. 4 Calibration curve of the unsaturated fatty acids C18:1; C18:2 and C18:3

2.8 Wort production

Genuine malt samples (2 varieties, 2 proveniences) were produced for the "Berliner Programm" of the Arbeitsgemeinschaft zur Förderung des Qualitätsgerstenanbaus im Bundesgebiet e. V. (Lehrstuhl für Technologie der Brauerei I, Weihenstephan/Germany). The malt varieties of provenience 2 have approximately 1% less crude protein and a higher soluble nitrogen rate as the malts of provenience 1.

The wort production was carried out in a 10-litre scale pilot plant. Malt was milled with a MIAG laboratory mill (0.8 mm). The temperature program of the infusion mash is shown in Figure 5.

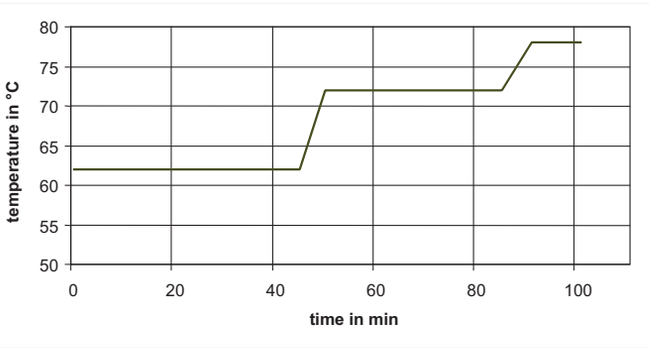

Fig. 5 Temperature program of mashing procedure

The malt/liquor ratio was 1:4. Before mashing-in a defined amount of technical lactic acid (18 w/w%) was added in the brewing water to set on the mash-pH at pH 5.7 or 5.4. The wort has been boiled for 70 minutes at atmospheric pressure. 10 minutes before end of boiling aroma hop pellets (12 g Hallertauer Perle, 5.9 % α) were added. The cast wort was weighed up to 12 °P.

3 Results

Each measurement of free long-chain fatty acids was performed in duplicates. Therefore the relative standard deviation (RSD) of the method was used for an error discussion.

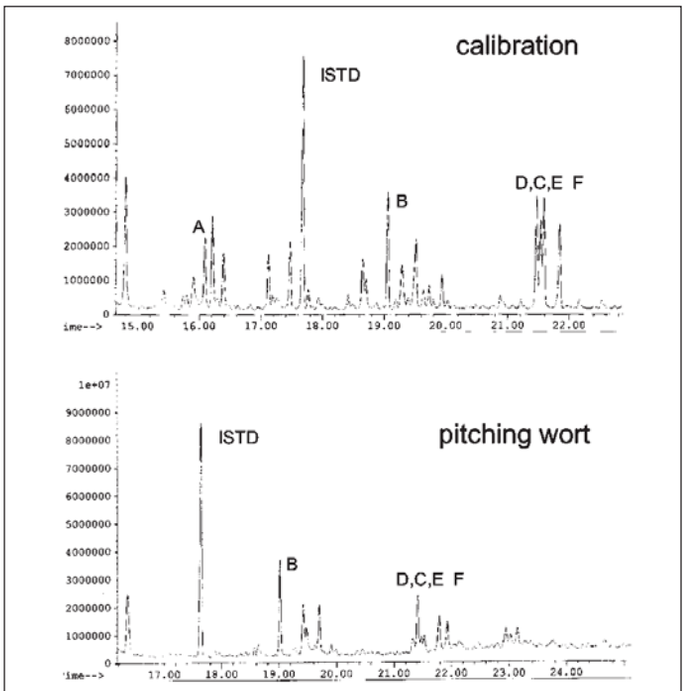

Fig. 6 Gas chromatogram of calibration and pitching wort sample

The influence of mash acidification and therefore the mash-pH during the mashing process on the content of free long-chain fatty acids has been investigated. Figure 6 shows a typical gas chromatogram of the analysis of free long-chain fatty acid methyl ester in a calibration and pitching wort chromatogram.

Table 1 shows the pH-developing of first wort, pfannevoll and

Table 1 pH of the wort with and without mash acidification

first wort	no acidification	adapted pH 5,7	adapted pH 5,4
V1 P1	5,87	5,55	5,65
V1 P2	5,78	5,59	5,59
V2 P1	5,74	5,62	5,64
V2 P2	5,81	5,64	5,60
pfannevollwürze	**no acidification**	**adapted pH 5,7**	**adapted pH 5,4**
V1 P1	5,93	5,66	5,76
V1 P2	5,85	5,70	5,69
V2 P1	5,85	5,73	5,73
V2 P2	5,94	5,72	5,67
pitching wort	**no acidification**	**adapted pH 5,7**	**adapted pH 5,4**
V1 P1	5,77	5,84	5,56
V1 P2	5,69	5,66	5,61
V2 P1	5,76	5,69	5,63
V2 P2	5,73	5,63	5,63

Table 2 Content of the summary of long-chain fatty acids in wort samples in mg/l [mean ± RSD]

summary of long-chain fatty acid content in wort in mg/l (mean ± RSD)

first wort	no acidification	pH 5,7	pH 5,4
V1 P1	24,84 ± 1,49	21,19 ± 1,27	20,08 ± 1,20
V1 P2	22,62 ± 1,36	20,98 ± 1,26	19,94 ± 1,20
V2 P1	23,78 ± 1,43	22,50 ± 1,35	18,17 ± 1,09
V2 P2	23,96 ± 1,44	21,81 ± 1,31	18,36 ± 1,10
pfannevollwürze	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	18,79 ± 1,13	16,72 ± 1,00	14,97 ± 0,90
V1 P2	18,30 ± 1,10	17,06 ± 1,02	15,43 ± 0,93
V2 P1	16,67 ± 1,00	16,44 ± 0,99	13,10 ± 0,79
V2 P2	16,58 ± 0,99	18,04 ± 1,08	14,20 ± 0,85
pitching wort	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	4,70 ± 0,28	3,64 ± 0,22	4,09 ± 0,25
V1 P2	4,34 ± 0,26	3,50 ± 0,21	3,94 ± 0,24
V2 P1	4,27 ± 0,26	2,81 ± 0,17	2,76 ± 0,17
V2 P2	2,86 ± 0,17	2,77 ± 0,17	2,34 ± 0,14

summary of long-chain fatty acid content in wort in mg/l (mean ± RSD) related to 12 °P

first wort	no acidification	pH 5,7	pH 5,4
V1 P1	19,51 ± 1,17	16,38 ± 0,98	15,95 ± 0,96
V1 P2	17,42 ± 1,05	16,10 ± 0,97	15,63 ± 0,94
V2 P1	18,38 ± 1,10	17,43 ± 1,05	14,19 ± 0,85
V2 P2	18,27 ± 1,10	16,35 ± 0,98	14,25 ± 0,86
pfannevollwürze	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	23,13 ± 1,39	19,81 ± 1,19	18,47 ± 1,11
V1 P2	21,59 ± 1,30	19,77 ± 1,19	18,25 ± 1,10
V2 P1	20,23 ± 1,21	19,53 ± 1,17	15,96 ± 0,96
V2 P2	20,14 ± 1,21	21,20 ± 1,27	16,89 ± 1,01
pitching wort	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	4,70 ± 0,28	4,06 ± 0,24	4,19 ± 0,25
V1 P2	4,40 ± 0,26	3,66 ± 0,22	4,04 ± 0,24
V2 P1	4,35 ± 0,26	2,88 ± 0,17	2,86 ± 0,17
V2 P2	2,86 ± 0,17	2,84 ± 0,17	2,44 ± 0,15

pitching wort measured by the different mash conditions. The pH levels of pH 5.4 and 5.7 by mashing-in offer similar values in first wort, pfannevoll and pitching wort. Table 2 shows the changes of content of long-chain fatty acids in wort (first wort, pfannevollwürze, pitching wort) produced by the mashing procedure with and without mash acidification. It compares also the fatty acid content of the mash depending on the malt characteristics of two varieties (V1, V2) and proveniences (P1, P2).

The results in Table 2 show a decrease of the summary of long-chain fatty acids in connection with the mash-pH by mashing-in in absolute concentrations [mg/l] and related to 12 °P. The results confirm the influence of malt conditions on the content of long-chain fatty acids in wort and the influence of the mash and lautering conditions. Although there is a significant decrease in first wort and pfannevollwürze, the pitching wort shows similar concentrations depending on barley variety. Within the specified error margin V1 has significant higher fatty acid content in pitching wort as V2. One reason could be the different lipid degradation during malting and mashing as a consequence of the different lipolytic enzymatic factors, another reason could be the unequal potential

Table 3 Concentration of C18:2 in wort samples in mg/l [mean ± RSD]

C18:2 content in wort in mg/l (mean ± RSD)

first wort	no acidification	pH 5,7	pH 5,4
V1 P1	10,58 ± 0,63	9,98 ± 0,60	8,76 ± 0,53
V1 P2	10,22 ± 0,61	9,26 ± 0,56	9,21 ± 0,55
V2 P1	10,32 ± 0,62	10,87 ± 0,65	9,04 ± 0,54
V2 P2	11,14 ± 0,67	12,28 ± 0,74	9,14 ± 0,55
pfannevollwürze	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	9,55 ± 0,57	7,86 ± 0,47	6,37 ± 0,38
V1 P2	9,24 ± 0,55	8,28 ± 0,50	6,88 ± 0,41
V2 P1	8,58 ± 0,51	8,46 ± 0,51	6,09 ± 0,37
V2 P2	9,50 ± 0,57	9,06 ± 0,54	7,04 ± 0,42
pitching wort	**no acidification**	**pH 5,7**	**pH 5,4**
V1 P1	1,25 ± 0,08	1,15 ± 0,07	1,20 ± 0,07
V1 P2	1,57 ± 0,09	1,17 ± 0,07	1,23 ± 0,07
V2 P1	1,50 ± 0,09	0,71 ± 0,04	0,76 ± 0,05
V2 P2	1,09 ± 0,07	1,01 ± 0,06	0,72 ± 0,04

on lipids in barley. Ketterer (10) found out, that the influence of variety on the malt lipid content is higher as the influence of provenience. The investigations confirm that free long-chain fatty acid content in wort depends on malt characteristics.

C16 and C18:2 are the main components in mash and wort made with raw material malt. The first wort and pfannevollwürze show significant differences in the C18:2 concentrations [mg/l] decreasing with the pH level by mashing-in (Table 3). But here the influence of variety is not as clear as in the summary content.

Lipases (E.C. 3.1.1.3) are enzymes which hydrolyse the esters of long-chain aliphatic acids from glycerol at oil/water interface (18). Lipase activity was determinated using a modification of the method of Kwon and Rhee (21) which measures free fatty acids (C18:1; C18:2; C18:3) as copper soaps. Schwarz et al. (20) showed that both barley and rice lipases are active during mashing up to temperatures of 67"70 °C. Boivin (11) detected an increase

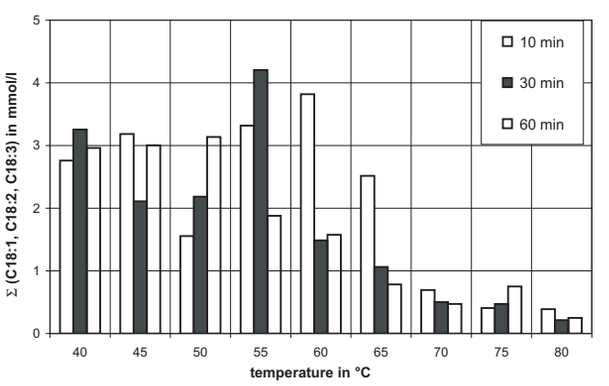

Fig. 7 Determination of Lipase activity by measuring the produced free fatty acids in mmol/l

of free fatty acid to 20 % of the total lipid during mashing and assumed, that Lipase is mainly active during mashing.

The results of this study showed, that a lower mash-pH inhibits the lipase activity. Researches in isothermal congress mashes between 40 and 80 °C in 5 °C-steps demonstrated that concentrations of long-chain fatty acids in wort get to the maximum at 55 °C (Fig. 7). Mash acidification of congress wort during mashing-in (2 ml lactic acid; 5 w/w%) by avoiding oxygen by the use of CO_2-gassing the free fatty acid content decreases up to 1/3. This suggests that lipase activity which is responsible for the liberation of free fatty acids, is lower.

4 Conclusions

The paper describes a method for the analysis of free saturated and unsaturated long-chain fatty acids (C14 – C18:3) in wort and beer. Mash acidification affected a lower long-chain fatty acid content in wort. The lower mash-pH seems to affect the lipase activity.

Basically there are different impact factors of the long-chain fatty acid content in wort as well as for the resulting degradation products:

- malt characteristics;
- grist fineness;
- mashing conditions (temperature program and mash conditions);
- lautering conditions and system;
- wort boiling conditions and system;
- wort separation and trub removal.

The free long-chain fatty acid content in beer is affected by the condition of the yeast and the fermentation process. The content of malt lipids and their degradation by enzymatic factors or autoxidation during wort production is an important aspect because of the negative effects on beer quality.

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