Karl Wackerbauer, Stefan Meyna and Sascha Marre

Hydroxy fatty acids as indicators for ageing and the influence of oxygen in the brewhouse on the flavour stability of beer

The concentration of oxygenated fatty acids, especially of trihydroxy fatty acids, increase noticeable during the storage of the brewing raw materials barley and malt. Measurement of these lipid oxidation products could therefore contribute to the detection of barley and malt freshness and could additionally be a helpful tool for an assessment of storage conditions. By different pilot plant brews the influence of oxygen in the brewhouse on lipid oxidation and flavour stability was measured: A noticeable connection to brewhouse conditions was found, but in some cases this effect was overlaid by yeast and fermentation influences.

BC 25 Beer / 22 Brewhouse / 11 Barley

(Descriptors: Lipid oxidation, hydroxy fatty acids, flavour stability.

Deskriptoren: Lipidoxidation, Hydroxyfettsäuren, Geschmacksstabilität)

1. Introduction

Although there are a lot of publications about beer flavour stability with special regard to lipid oxidation respectively degradation, the real background of beer staling is not revealed yet. That is why terms like "never ending nonenal story" are common and it is also possible to introduce a new corresponding expression called "hydroxy fatty acid story".

The occurrence of lipid oxidation by enzymes and radical reactions in barley and malt and their possible relevance for beer flavour is known since the 1960s (1). Special products of such lipid oxidative reactions are hydroxy fatty acids which are detectable by GC/MS – done for the first time by *Eglinton* and *Hunneman* (2). It were *Drost* et al. who began the "hydroxy fatty acid story" by firstly assuming a connection between Burger's, Glenister's and Becker's term "cardboard flavour" and such acids at an EBC Congress 32 years ago (3, 4). Since that time a lot of papers about these oxygenated linoleic acids in all states of beer production were published, but in all cases the research was focussed on *free* hydroxy fatty acids.

Recently *Tressl* et al. and further on our institute presented another group of such possible stale flavour precursor – the triglyceride-bonded hydroxy fatty acids in barley and malt – and confirmed the results of *Holtman* et al. who found that lipoxygenase can oxidise esterified storage lipids in germinating barley (5 – 8). The identification of such triglyceride-bonded oxygenated linoleic acids started a new chapter in the "hydroxy fatty acid story". However, newest research results indicate that furthermore there may be a third group of hydroxy fatty acids in barley and malt and probably – because of their high polarity – in finished beer, too: phospholipid-bonded ones (5). Maybe this group of possible stale flavour precursor opens a new door in flavour stability research on the base of lipid oxidation.

The paper consists of two main parts: The first section gives information about the purposes the measurement of hydroxy fatty acids can be used for in general, the second one deals with the role of oxygen in the brewhouse with special regard to lipid oxidation and beer flavour stability.

2. Experimental

2.1 Raw material ageing

In order to give a closer view on the effect of raw material ageing in relation to lipid oxidation, the amount of hydroxy fatty acids in the free and triglyceride-bonded state were measured in different fresh and corresponding six month old barley and malt samples according to a former described analytical preparation method (6).

2.2 Influence of milling

Additionally the influence of the milling process (damaging of the grain) on the lipoxygenase pathway as plant wound response – described for example by *Noordermeer* et al. – was examined (9). Therefore the concentration of free and triglyceride-bonded hydroxy fatty acids (products of lipoxygenases action) in barley and malt after conventional dry milling and grinding at -20 °C under argon atmosphere were compared. The last mentioned procedure should prevent any enzymatic reaction of lipoxygenases.

2.3 Oxidation in the brewhouse

Two different malts, Alexis with a high amount of hydroxy fatty acids and Barke with a marked lower content of these oxylipins, were used for beer production in a 1 hl pilot plant. To influence lipoxygenases activities both malts were mashed in at 40 °C (high enzymatic activities) and 60 °C (fast inactivation of lipoxygenases) using a traditional infusion mash method. Furthermore the gas

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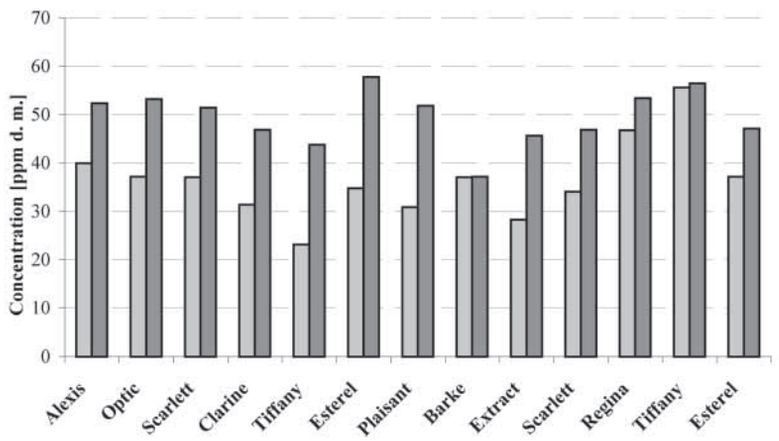

Fig. 1 Effect of barley storage on free and triglyceride-bonded THOE of the crop 1999 (dark colour) and crop 2000 (pale colour), green colour: fresh samples, red colour: stored for six months

atmosphere was varied: Completely brewing under CO_2 to minimize any oxidative reaction (oxygen levels lower than 0.5 ppm in the mash) and brewing with a defined oxygen supply of approximately 3 ppm to 5 ppm, depending on the mash temperature. As base for comparison a standard brew of each malt with mashing-in at 50 °C was done, too. By this experimental procedure it was possible to examine the influence of:

- Oxygen,
- mashing-in temperature and
- the concentration of free and triglyceride-bonded hydroxy fatty acids in malt

on lipid oxidation in the brewhouse and later beer flavour stability. All ten resulting brews were finished after the mashing and lautering process in the same way. The filtrated beers were stored for two and four weeks at 28 °C to simulate staling and were evaluated by our trained taste panel. Beside the standard analyses programme the amounts of free trihydroxy fatty acids (THOE) in all worts and beers were measured by GC/MS (10). Dihydroxy fatty acids (DHOE) and monohydroxy fatty acids (HOD) are of minor importance because they are metabolised by yeast to a great extent, respectively completely.

3 Results

There is a relationship between the sum of free and triglyceride-bonded trihydroxy fatty acids and raw material storage that is shown in Figure 1 for different barley samples. In all cases the sum of the mentioned oxylipins increased during the half year storage period, independent of variety or crop year. This is also true for malt samples, not shown here. THOE therefore may be used as marker for raw material ageing.

It is well known that lipid oxidation especially by lipoxygenases has to do with wound responses in plants. Wounding introduces the lipoxygenase pathway which leads to oxylipins – hydroxy fatty acids belong to this group – with wound healing activities. This process is proven for most of living plants growing in nature on the fields and being attacked for example by insects. Beer production starts with the milling of malt and this is per definition also a form of wounding. Is there an activation of the lipoxygenase pathway in this case, too? Table 1 represents the results of the milling examination, described in the experimental part.

It is obvious that there is no activation of the lipoxygenase pathway due to milling, neither for malt nor for barley. The values for the concentration of different fractions of hydroxy fatty acids are approximately in the same range considering the error of the analysis method. Thus, there is neither enzymatic nor radical lipid oxidation due to milling, because otherwise there would be higher values for the traditional milling in comparison to the lipoxygenase-inhibited one. But nevertheless this is irrespective of the oxylipin formation by the lipoxygenase pathway in plants *growing on the fields* as response to wounding.

To summarize all the features of hydroxy fatty acids it is to say that there is an influence of germination and heat stress, for example during kilning, as we have already shown in former publications (7). Additionally the values of Table 1 indicate that there is no influence of the milling process on the formation of free and triglyceride-bonded hydroxy fatty acids although it is well known that the lipoxygenase pathway is activated by wounding in plants growing on the fields. Furthermore it was shown that the storage of barley and malt leads to an increase in the concentration of free and triglyceride-bonded THOE. Finally it is known that lipid oxidation is initiated by natural pests and diseases of plants.

To examine the influence of brewhouse oxidation and mashing-in temperature on the hydroxygenation of linoleic acid two different malts – Alexis and Barke – with varying amounts of HOD, DHOE and THOE were processed (see experimental part). Figure 2 gives an overview of the contribution of the hydroxy fatty acid fractions in the two malt batches.

Table 1 Influence of milling on lipid oxidation (ppm d. m.)

		Traditional milling	Milling at -20 °C under Argon atmosphere
Barley variety "Barke"	HOD	61	60
	DHOE	51	41
	THOE	35	28
Barley variety "Pasadena"	HOD	65	69
	DHOE	49	48
	THOE	29	32
Commercial malt	HOD	67	73
	DHOE	35	30
	THOE	33	31

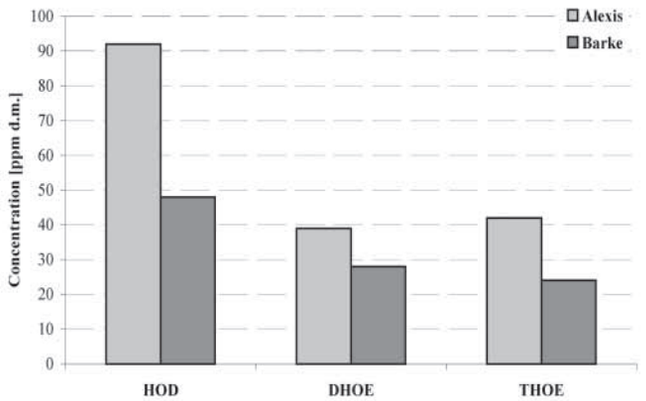

Fig. 2 Free and triglyceride-bonded hydroxy fatty acids in the processed malt batches

Alexis has approximately twice as much HOD, 10 ppm more DHOE and 20 ppm more THOE than Barke. The following Figure 3 represents the measured concentration of free trihydroxy fatty acids for the brews made from the Alexis malt under the already described conditions. First it is to be mentioned that the gassing with oxygen at the 40 °C brew has led to the highest results for free THOE in wort. On the other hand it can be seen that mashing-in at 60 °C in combination with a CO_2 treatment resulted in the lowest concentration of these acids. The same is true for the resulting beers: Again highest content of free THOE in the case of using oxygen and mashing-in at 40 °C and lowest values for the combination CO_2 and 60 °C.

A uniform influence of mashing-in temperature and gas atmosphere can be seen: Mashing-in at 60 °C in comparison to 40 °C gave lower amounts of free THOE in wort and beer and additionally mashing under an excessive oxygen supply has led to higher values of these oxylipins in comparison to a CO_2 atmosphere. These results fulfil all the common expectations but a closer look at the following Figure 4, the corresponding concentrations for the Barke brews, diminishes the common theory.

Of course the brew done at 60 °C mashing-in temperature and under CO_2 atmosphere again has the lowest amounts of free THOE in wort and beer, but furthermore it can be seen that the higher mashing-in temperature in the case of the two oxygen brews did not lead to a lower concentration of free THOE in wort and beer. Moreover we measured higher amounts of free THOE in the wort of the brew mashed-in at 40 °C under CO_2 in comparison to the standard brew. These results are in contradiction to the Alexis brews. To summarize, it can be concluded from these experiments that the influence of oxygen and mashing-in temperature on lipid oxidation is not absolutely uniform, although we found lowest values for the combination 60 °C mashing-in temperature and CO_2 atmosphere for both test series.

For the last years the measurement of the ESR "lag-time" is very common as a further tool for the prediction of beer flavour stability

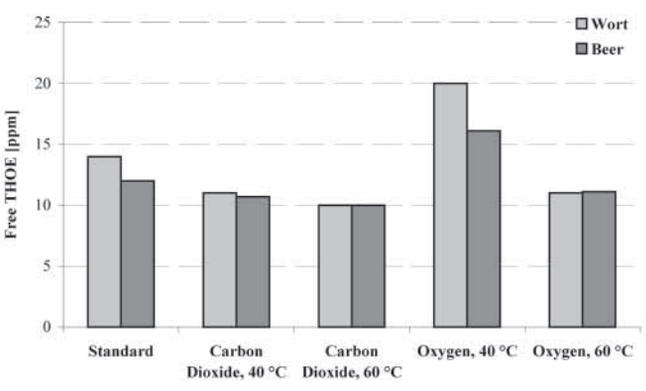

Fig. 3 Influence of mashing-in temperature and oxygen on the concentration of free THOE (Alexis, calculated to 12 % original gravity)

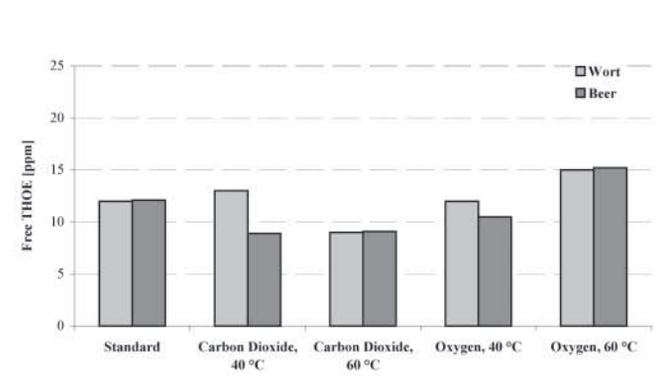

Fig. 4 Influence of mashing-in temperature and oxygen on the concentration of free THOE (Barke, calculated to 12 % original gravity)

Table 2 ESR "lag-time" of the Alexis beers (fresh and stored at 28 °C)

Gas atmosphere	Mashing-in temperature [°C]	ESR "lag-time" [min]		
		fresh	2 weeks	4 weeks
Standard	50	109	97	80
Carbon dioxide	40	119	120	110
Carbon dioxide	60	190	142	130
Oxygen	40	140	125	122
Oxygen	60	200	170	160

Table 3 ESR "lag-time" of the Barke beers (fresh and stored at 28 °C)

Gas atmosphere	Mashing-in temperature [°C]	ESR "lag-time" [min]		
		fresh	2 weeks	4 weeks
Standard	50	84	70	50
Carbon dioxide	40	159	160	160
Carbon dioxide	60	151	143	138
Oxygen	40	110	106	70
Oxygen	60	126	112	82

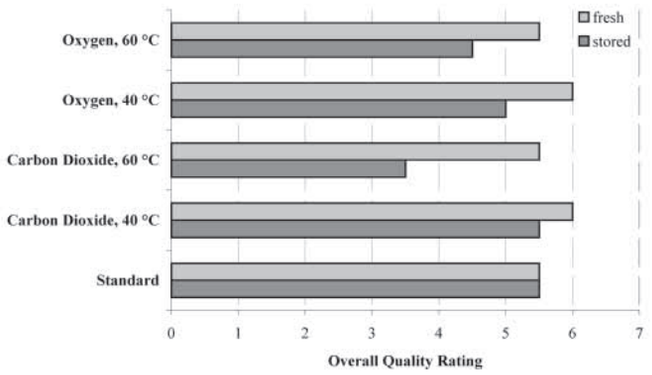

Fig. 5 Sensory analysis of Alexis beers on a scale from 0 to 9 (best quality)
(fresh vs. stored for 4 weeks at 28 °C)

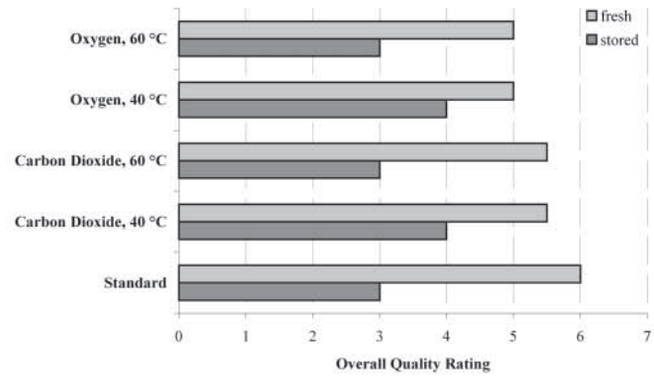

Fig. 6 Sensory analysis of Barke beers on a scale from 0 to 9 (best quality)
(fresh vs. stored for 4 weeks at 28 °C)

(e. g. 11, 12). According to theory a beer sample with a long "lag-time" offers a good flavour stability. Tables 2 and 3 show the "lag-time" of the ten fresh beers from the brewhouse experiment and also the "lag-time" from the same beers stored for two and four weeks at 28 °C.

It is obvious that there is no expected connection between the ESR "lag-time" and the mashing-in temperature respectively the gas atmosphere. In the case of the Alexis beers the brew done with an excess of oxygen at 60 °C mashing-in temperature has the longest "lag-time" in the fresh state, while the sample brewed under CO_2 at 40 °C has the shortest one. In the case of the beers from Barke we found that brewing under CO_2 led to a longer "lag-time" in comparison to the corresponding oxygen brews, but the higher mashing-in temperature gave no longer "lag-time" within the two CO_2 brews. Figures 5 and 6 show the results of the sensory analysis; Figure 5 gives information about the Alexis beers and Figure 6 about the Barke brews.

The overall quality rating on a scale from 0 to 9, where 9 represents best quality, of these beers suggests that there is neither a connection between the gas atmosphere and the taste panel results nor between the mashing-in temperature and the overall quality rating. For example the Alexis beer which was brewed with an excess of oxygen at 40 °C mashing-in temperature together with the corresponding CO_2 beer has the best evaluation in the fresh state. Moreover the CO_2 Alexis beer which was brewed by mashing-in at 60 °C has the poorest flavour stability because of a total decrease of two evaluation points. In the case of the Barke beers the results are comparable. Again the CO_2 beer mashed in at 60 °C has, together with the standard brew, the poorest flavour stability and the oxygen and CO_2 brews mashed in at 40 °C the best one. Once more: Oxidation in the brewhouse or mashing-in at lower temperatures do not in general lead to worse beers or poor flavour stability. Thus, there must be some more factors, e. g. during fermentation, which influence the beer flavour stability. An avoidance of oxygen and an inhibition of lipoxygenases by higher mashing-in temperatures may influence lipid oxidation in some cases but these effects are sometimes overlaid by influences of yeast and fermentation.

4 Conclusions

The presented data assess the free and triglyceride-bonded trihydroxy fatty acids as a helpful tool for the evaluation of raw material storage, because of their noticeable increase during the ageing of barley and malt. Although it is well known that hydroxy fatty acids are synthesized due to wound responding of plants growing on the field via the lipoxygenase pathway, an increase of this oxylipins during milling (also a form of plant wounding) could not be observed.

An intensive study of the technological factors mashing-in temperature and gas atmosphere, especially oxygen, has shown that lipid oxidation in the brewhouse seems not to be exclusively dependent on enzymatic reactions, because in this case all brews done at mashing-in temperatures of 60 °C would have had a lower concentration of free THOE in wort and beer. This result is in accordance with the theoretical thesis of *Bamforth*, who mentioned that "the potential for oxygen consumption in non-enzymic reactions is vastly greater than that for consumption by lipoxygenase" (13). The autoxidation of linoleic acid could be one of these non-enzymic reactions especially in those cases where the lipid oxidation in the raw materials before was not as much intensive (cp. Barke vs. Alexis brews).

Kobayashi et al. assume higher concentrations of free hydroxy fatty acids in the mash with increasing amounts of oxygen and lower mashing-in temperatures (14). Within our experiments we could not find an uniform dependence of free THOE concentration on oxygen availability and mashing-in temperature.

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5 Zusammenfassung / Resumé

Wackerbauer, K., Meyna, S., und Marre, S.: Hydroxyfettsäuren als Indikator der Alterung und der Einfluss von Sauerstoff im Sudhaus auf die Geschmacksstabilität des Bieres — Monatsschrift für Brauwissenschaft 56, Nr. 9/10, 174 – 178, 2003.

BC 25 Bier / 22 Sudhaus / 11Gerste

Es konnte gezeigt werden, daß die Konzentrationen an oxygenierten Fettsäuren, insbesondere an Trihydroxyfettsäuren, während der Lagerung der Brauereirohstoffe Gerste und Malz deutlich ansteigen. Die Messung

dieser Lipidoxidationsprodukte könnte somit eine Beitrag zur Beurteilung der Frische von Gerste und Malz leisten und könnte ferner ein hilfreiches Mittel zur Einschätzung von Lagerbedingungen darstellen. Auf Basis verschiedener Brauversuche im Pilotmaßstab wurde der Einfluß von Sauerstoff im Sudhaus auf die Lipidoxidation und die Geschmacksstabilität des Bieres untersucht: Es konnte ein deutlicher Zusammenhang gefunden werden, welcher allerdings in einigen Fällen durch Hefe- und Gärparameter überlagert wurde.

Wackerbauer, K., Meyna, S., et Marre, S.: Les acides gras hydroxylés en tant qu'indicateurs de vieillissement et l'influence de l'oxygène en salle à brasser sur la stabilité de goût de la bière — Monatsschrift für Brauwissenschaft 56, No. 9/10, 174 – 178, 2003.

BC 25 Bière / 22 Salle à brasser / 11 Orge

On a pu montrer que la concentration d'acides gras oxydés, en particulier les acides gras trihydroxy augmentait sensiblement pendant le stockage des matières premières orge et malt. La détermination des produits d'oxydation des lipides pourrait être une contribution pour l'évaluation de la fraîcheur de l'orge et du malt ; en plus cela représente un moyen pour juger les conditions de stockage. A l'aide de différents essais de brassage, à l'échelle pilote, on a examiné l'influence de l'oxygène en salle à brasser sur la stabilité de goût de la bière. On a trouve une forte relation qui toutefois était biaisée dans quelques cas par des paramètres de levure et de fermentation.

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