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Release of Long-Chain Fatty Acids and Zinc from Hot Trub to Wort

The addition of hot trub to the fermenting wort has often been reported to enhance fermentation performance, while on the other hand, some of its components and their degradation products are thought to be detrimental to the quality of the resulting beer. In order to gain more knowledge of a potential improvement of yeast supply by nutrients like long-chain fatty acids (C14-C18:3) and zinc, release (re-dissolution) trials were carried out by adding hot trub to wort, both derived from industrial production. Free long-chain fatty acids were released mainly as palmitic and linoleic acid in a total amount of 0.9 mg/L additional to the content of 1 – 2 mg/L usually found in cold worts, while the hot trub particles showed a total reservoir of fatty acids in the order of 4 mg/L, which may be further released during fermentation. Zinc was released at an average concentration of 0.14 mg/L solely due to hot trub addition, this being relevant for yeast nutrition. For both nutrients, the maximum release was observed after a contact time of 4 h, while an extended period of up to 7 days did not lead to a further release. An increase of ethanol concentration to 5 %v/v, and/or a decrease of pH value to 4.0 in order to simulate some of the changes occurring during fermentation, did not impact on the release of neither fatty acids nor zinc in wort. These trials confirm a high bio-availability of zinc and to a lower extend also of long-chain fatty acids, thus, hot trub addition may improve nutrient supply of yeast, particularly when dealing with deficient worts, which might be the reason for the increase of fermentation performance reported earlier.

Descriptors: bio-availability, hot trub, long-chain fatty acids, lipids, solubility, zinc

1 Introduction

Several components of wort turbidity and, more specifically, of hot trub are believed to have an influence on the brewing process. In particular, lipids such as long-chain fatty acids, zinc as well as the particle characteristics of hot trub have been discussed lively among researchers and practical brewers in the past decades until today. Trub addition to the pitching wort was found to increase yeast vitality [73] and fermentation performance [30, 34, 35, 41, 49, 71, 73, 75, 76, 90]. For example, trials with turbid wort (3.5 – 4 %v/v trub solids; determined by centrifuging) and clarified wort (0.1 %v/v) showed that yeast fermented faster with trub containing wort compared to clear wort in three subsequent fermentations [44]. Additionally, the yield of yeast was found to be higher when wort containing trub was fermented [1]. Moreover, trub may influence the aroma profile of the resulting beer due to its impact on the formation of higher alcohols and esters [1, 11, 44, 69]. On the other hand, components of hot trub are reported to be precursors of staling substances [23, 60] and may damage foam stability in case of excess dosage [81], so that a complete trub separation has been recommended to ensure a high quality of the resulting beer [51, 94]. Since the chemical composition and formation of hot trub have sufficiently been described elsewhere [9, 44, 54, 63], this investigation focuses on the components of hot trub which are thought to be most important for yeast nutrition and improvement of fermentation performance: long-chain fatty acids and zinc. The particle characteristics of hot trub are being dealt with by various current investigations and will later be reported.

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Figures see Appendix

1.1 Lipids in hot trub

Considering all trub components, the lipid fraction seems to have the biggest positive influence on yeast activity and flavor development [10, 45, 72, 75, 83, 86]. The addition of lipids to fermentation, particularly of unsaturated long-chain fatty acids and ergosterol, has a pronounced effect on the growth and metabolism of yeast [8, 83, 93] since unsaturated long-chain fatty acids form an integral part of plasma membrane, where they are reported to regulate the exchange of various compounds into and out of the yeast cell; they improve inter alia the uptake of amino acids and maltotriose [45, 76, 83], enhance the ability of yeast to resist high ethanol concentrations [43, 45, 56, 76, 83, 84] and regulate the activities of membrane-bound enzymes [74]. Among wort lipids, free linoleic acid (C18:2) was often considered to be the most important component due to its comparable high concentration, its physiological functions, its high rate of incorporation into the yeast cell and the fact that linoleic acid, like other polyunsaturated acids, cannot be synthesized by Saccharomyces cerevisiae itself, but has to be taken up from the medium [2, 13, 58, 72, 83]. In this context, an increasing uptake of linoleic acid into the cell by continued contact of yeast with trub was reported, giving an indication of the bio-availability of C18:2 adsorbed to trub, while contact with clarified wort causes a depletion of linoleic acid concentration in the cell membrane [44]. In terms of uptake rate Thurston et al. found that 70 % of C18:2 added to wort in a concentration of 50 mg/L at the start of fermentation (growing cells) was taken up within 4 h, while 100 % was taken up within 18 h after addition [85]. Similarly, when re-suspending stationary-phase cells (non-growing) in fresh wort around 67 % of the supplemented C18:2 (60 mg/L) was taken up within 24 h [59].

Beside its function in improvement of yeast growth and fermentation performance linoleic acid plays an important role in terms of flavor stability of the resulting beer. It has been discussed extensively in the literature that linoleic and linolenic acid (C18:3) could lead to the formation of E-2-nonenal which is supposed to be a main component of staling flavor and may affect the flavor already in traces [15, 18, 53, 87]. Beside E-2-nonenal, a wide

range of C6-C12 aldehydes, ketones and unsaturated alcohols were identified as off-flavors in beer due to degradation of these fatty acids [87]. Moreover, fatty acids seem to be the only part of lipids which is flavor-active in bottled beer [7] and more particularly, unsaturated long-chain fatty acids are the only one reacting with oxygen, e.g. linolenic acid reacts 3-4 times faster than linoleic acid and the latter reacts 30 times faster than oleic (C16) and palmitic acid (C16:1). Fatty acids bound as esters react up to 50 % slower compared to free fatty acids [52, 88]. On the other hand, the oxidation of saturated fatty acids is meaningless due to their slow reaction rate [15]. In contrast, Graf could not detect an influence of the addition of higher fatty acids in terms of sensory analysis of fresh and aged beers. Further, a degradation of linoleic and linolenic acid was not accompanied by increased carbonyl levels [23]. Ahvenainen et al. recommend an optimal concentration of 10 – 20 mg/L of long-chain fatty acids (C16-18:3) in wort which causes an intensive fermentation, while unwanted aroma compounds are not formed excessively [1].

In terms of foam stability there seems to be a significant negative correlation between free long-chain fatty acids and foam stability since unsaturated fatty acids (C16:1, C18:1, C18:2, C18:3) are efficient at disrupting foams when added to beer in certain concentrations (> 0.6 mg/L) [40]. It has also been reported that long-chain fatty acids such as oleic acid, linoleic acid and linolenic acid only show a detrimental effect to foam when present in a 40 to 100-fold concentration usually found in beer [31, 36, 88].

In terms of the non-biological stability, the role of hot trub and fatty acids does not seem to be completely clear by now. According to Nadzeyka, the main factors involved in non-biological stability are: protein amount, polyphenol, and oxygen content of the final beer [61]. Schuster reported that unsaturated fatty acids do not seem to lower non-biological stability of the resulting beer [76], while an adverse effect of hot trub in wort at the fermenting stage was reported, which might, however, have been due to the low molecular polyphenols rather than to lipids [48, 68].

1.2 Long-chain fatty acids in brewhouse wort and hot trub

The destiny of lipids in mash and wort during brewhouse operations has been described in detail in several publications [2, 3, 6, 24, 45, 89, 92] and are briefly summarized here: During mashing, a part of the total fatty acids contained in malt, particularly in fine grist [60], is released to mash as free acids and modified due to the activity of several enzymes, such as lipase and lipoxygenase [25, 40, 67, 89]. When high temperature, short time mashing is applied, higher concentrations of fatty acids are noted compared to one mash or infusion mash method [89]. While decoction mashing is reported to cause a higher amount of fatty acids (C12-C18:3) by 10 – 25 % in the resulting mash [89], more recent investigations could not detect a significant difference between one-mash method and infusion mashing in terms of the free fatty acid content in mash [38]. A high amount of fatty acid release was found at high mashing-in temperatures in the range of 65 – 68°C, while up to 65°C only small amounts were released [40, 60]. Within the first 30 min of mashing linoleic and linolenic acid are strongly degraded due to the activity of lipoxygenase, while other fatty acids are not attacked [60]. Further, during lautering the concentration of free fatty acids is reduced due to adsorption to the spent grains filter bed [60]. Here the applied technique (lauter tun or mash filter) as well as the process parameter (filter bed height, racking, deep cuts) play an important role in determining the fatty acid content of the kettle-full wort [31, 60, 89]. Indeed, there seems to be a significant correlation between lauter turbidity and fatty acid content of wort [63, 65, 76, 89]. Also, a drawing-off of wort from the top layer of pre-run caused high fatty acid concentrations in kettle-full worts [24, 89]. Owing to the removal of the solid spent grain particles, the total fatty acid concentration is decreased by more than 90 % for all lautering systems with lauter tuns being stronger in reductive action than mash filters. During wort boiling coagulation of proteins takes place due to the physical breakdown of hydrogen bonds and disulfide bridges causing a lower hydration and solubility of protein molecules [4]. This is favored by the presence of polyphenols and the decrease in pH meaning the medium comes closer to the isoelectric point of proteins [4]. The most intensive precipitation takes place at a pH value of 5.2 [77]. On the other hand, other authors doubt whether there is an impact of polyphenols on hot trub formation [33, 78]. The coagulation reactions and factors affecting those are described in detail in [4]. In terms of the amount of hot trub Just et al. reported an average amount of 500 mg/L for a 12°P wort [32], Narziß reported 400 – 800 mg/L (extract free d.m.) [62], while Eils determined an amount of 600-800 mg /L (d.m.) as being typical for lauter tuns [20].

Due to their hydrophobic character long-chain fatty acids adsorb to trub particles [37, 40, 60, 66], while the overall fatty acid concentration is reported to remain unchanged during wort boiling [76]. In contrast to this, oxidation of fatty acids during wort boiling in the presence of oxygen is reported to lead to the formation of degradation products or intermediates [1, 66], which finally affect the flavor stability of the resulting beer due to carbonyl formation. The loss of lipids and particularly of free long-chain fatty acids during wort boiling was found to be in the range of 74 – 90 % [21, 31, 89]. Applying different boiling temperatures, e.g. atmospheric boiling (100°C) vs. high temperature wort boiling (140°C), did not make a difference in fatty acid behavior [66]. Since protein coagulation is most intense within the first 25 min of boiling [19], the adsorption of fatty acids is expected to be most intensive within this period, too. Beside technical aspects such as boiling system and duration, the reduction depends on the chemical properties of the particular fatty acid such as the number of double bonds, e.g. C18:1 was reported to decrease by 73 %, C18:2 by 80 %, and C18:3 by 90 % [89]. Because of hot trub separation during whirlpool operation the total linoleic acid content is reduced by 75 to more than 92 % while the removal of cold trub by flotation may cause another reduction of 33 % [37, 60]. In contrast to this, according to Narziß et al. the application of hot trub centrifugation causes only a reduction of short-chain fatty acids but not of long-chain fatty acids, while a sedimentation of 20 min is a suitable measure for removing more than 90 % of long-chain fatty acids [66]. Finally, a concentration of around 0.7 – 1.6 mg/L of free fatty acids (C14-C18:3) is reported for pitching wort [89]. According to the findings of several authors the dominating acids are linoleic acids as well as palmitic acid among the long-chain fatty acids, while the others such as myristic acid (C14), oleic acid and linolenic acid play only a minor role [1, 31, 60, 89].

During fermentation the unsaturated long-chain fatty acids are mainly assimilated by yeast, thus the resulting beers contain mostly much less than 0.1 mg/L [40, 57, 95]. In detail following final beer values are reported: 8 – 15 µg/L of C14, 20 – 50 µg/L of C16, 10 – 30 µg/L of C18, 1 – 2 µg/L of C18:1, 2 – 8 µg/L of C18:2, and 0.5 – 3 µg/L of C18:3 [37, 39]. Some authors also observed higher values in final beers: according to Anness a fatty acid content of 1 – 2 mg/L seemed to be typical [2], while Jones et al. found 1.3 – 3.3 mg/L C14-C18 in commercial ales [31].

1.3 Zinc

Besides lipids, the physiological impact of the zinc content of trub on yeast is a matter of discussion. Zinc is an essential element for yeast growth and influences proteolytic activity, protein biosynthesis, and carbohydrate metabolism of the yeast cell [49, 50, 91]. Therefore, a sufficient supply increases fermentation performance [30, 41] and causes a large yeast crop [22, 49]. As an optimum, zinc concentration in pitching wort (12°P) a range of 0.08 – 0.15 mg/L is reported in order to attain a final zinc content of 6 mg per 100 g of yeast d.m. [16, 17]. A latest publication recommended a concentration in the range of 0.15 – 0.3 mg/L in wort [80]. Investigations of Jacobsen showed that an addition of zinc (0.2 mg/L) increased fermentation rate when the original wort was zinc deficient (0.05 mg/L). A positive effect of the addition could be detected for original zinc concentrations of up to 0.2 mg/L. When the concentration in wort exceeded a level of 0.2 mg/L, an addition did not cause a further improvement of fermentation [30]. On the other hand, when EDTA was added (1mM), zinc and other metal ions became unavailable for yeast due to chelation causing a very poor fermentation performance [30]. In brewery worts zinc is often present in suboptimal concentrations [29], thus if concentrations drop below 0.1 mg/L, yeast cells become deficient in metallo-enzymes [49] and slow fermentations result [26]. Similarly, if concentrations exceed 0.6 mg/L, yeast growth is depressed unless the concentration of manganese ions is of a similar level [26, 27]. It was observed that zinc was taken up rapidly and at an early stage of fermentation, mainly in the lag- and late-exponential phases [47]. For example, a concentration of ca. 0.36 mg/L was reduced to below 0.08 mg/L within 2 h after inoculation in a stirred fermentation at 12°C, while the concentrations of Mn and Fe ions hardly changed within this period [47]. After fermentation hardly any zinc could be detected in beer, but almost 100 % was taken up by yeast [41].

1.3.1 Zinc in hot trub

Similar to the binding affinity of hydrophobic fatty acids Zn^{2+} ions tend to be chelated by components of trub [46, 75] which means a decrease in availability of zinc for yeast since the trub remains in the whirlpool almost completely [44]. Therefore, in case of hot trub separation, the zinc content is already lost prior to pitching. Moreover, according to Lentini et al., the strong binding ability of trub for ions further reduces the bio-availability, so that zinc may not be easily available to the yeast during fermentation [44]. They conclude that trub bound zinc is not directly responsible for the stimulation of fermentation performance due to its lower bio-availability while direct addition of zinc to the fermenting wort is more effective [44]. In this context Kreder disagrees observing a positive effect of zinc bound to trub on yeast. When placing dialysis bags filled with cold trub into zinc deficient wort for seven days, the zinc content in wort increased from 0.02 to 0.18 mg/L. According to Kreder this illustrates that zinc is bound to trub only loosely and should be bio-available to yeast during fermentation. In fermentation trials he showed that both, zinc bound to trub or alternatively added as zinc salt, can be assimilated by yeast and therefore stimulate fermentation. Indeed, 84 % of the zinc in trub contained in the dialysis sacs was utilized by yeast during fermentation, supporting his thesis of a high bio-availability of zinc bound to cold trub [41].

1.3.2 Zinc in brewhouse wort

During mashing zinc is extracted from malt as the only important raw material source for zinc in the brewing process [28] resulting in a concentration of 0.37 – 0.56 mg/L at the end of mashing [14]. Due to the removal of solid mash particles [5] and dilution during lautering, a concentration of 0.07 – 0.16 mg/L remains in the kettle-up wort [14]. The lautering technique and procedure applied were reported to have a direct impact on the zinc content of the resulting wort [12]. Thus, Eils observed an increase of the zinc content in wort if turbid lautering was applied compared to clear lautering [20]. The zinc content further decreased during wort boiling to 0.04 – 0.10 mg/L in cast wort after 30 min of boiling [14]. When boiling was extended to 90 min, no further reduction was observed [14]. An addition of high zinc amounts (resulting in 0.52 mg/L in wort) only 5 min prior to end of boiling (and subsequent hot trub remove) caused a loss of the dissolved zinc by 36 % [14]. As in case of fatty acids the strong reduction of dissolved zinc and other metals during wort boiling is closely connected to the formation of hot trub [82]. Thus, around 50 % of the total zinc content in wort is deposited in trub [41], resulting in high zinc concentrations of 32.2 mg/kg in trub compared to 6.3 mg/kg of the corresponding clear wort [75]. Daveloose observed an even more extreme ratio of 197 ppm in whirlpool trub vs. 0.11 ppm in wort [14]. Quite a variety of measures has been offered to increase the zinc content in wort during brewhouse operations, such as the use of well modified malts, a low mashing-in temperature, and mash acidification (pH 5.4 – 5.5) [16, 64].

Considering this background it is the aim of this investigation to follow the free fatty acid concentration along the different stages in an industrial scale brewhouse operation and to observe the release properties of a hot trub derived from industrial production in terms of long-chain fatty acids and zinc, when added back to wort.

2 Material and Methods

2.1 Fatty acid survey in an industrial brewhouse

2.1.1 Brewhouse equipment and procedure in brewery A

For mashing a one-mash decoction method with a mashing-in temperature of 61°C and a final mashing temperature of 75°C was used. A grist amount of 6,900 kg per brew (Pilsen type malt) was mashed in to a total mash volume of 340 hL. Lautering took 3 h 10 min in a classic lauter tun with a false-bottom load of 350 kg/m². Turbid wort pumping was undertaken for 10 min, lautering of first wort for 85 min was followed by sparging and deep raking. The post run lautering took 80 min. An external boiler was used to boil the wort at ca. 102°C for 60 min. First and second hopping were done at the start of boiling and 10 min before end of boiling. The hot trub was removed by whirlpooling with a stand of 5 min. After cooling the wort to 8°C, a final volume of ca. 480 hL was obtained per brew.

2.1.2 Sampling

Mash samples were taken at the final mash temperature right before end of mashing. First wort samples were taken 45 min after start of first wort run-off. Post-run samples were taken 45 min after start of post run lautering. The kettle-up worts were taken 5 min after start of boiling, while the whirlpool samples were taken 5 min after the whirlpool stand was completed. The cooled wort samples were taken 30 min after start of cooling. All samples were quickly chilled to below 10°C after sampling and were stored at 0°C for later analysis.

2.2 Hot trub

Hot trub samples were taken manually from the whirlpool in the industrial scale brewing procedure of brewery B. The samples were immediately deep-frozen after sampling. Before use they were

gently defrosted and mechanically homogenized without further treatment. For determination of dry matter (d.m.) a sample of hot trub was heated at a temperature of 105°C to a constant weight. The d.m. was calculated by relating the dry to the wet weight.

2.3 Wort preparation

A lager type wort was obtained as chilled wort after whirlpool operation from another industrial scale brewing procedure (brewery C). To 20 L of cold wort around 100 mL of pulpy cropped yeast (ca. $4 \cdot 10^9$ cells/mL; bottom fermenting, strain: W34) was added and homogenized. The resulting suspension was shaken every 30 min. After a total contact time of around 1.5 h, the suspension was filtered in a sheet frame filter (Pall-Seitz, Bad Kreuznach/Germany) applying deep filter sheets SEITZ KS-80 (Pall-Seitz, Bad Kreuznach/Germany) at a pressure difference of 2.5 bars. Prior to filtration, the filter including the filter sheets was rinsed with hot water (80°C) for 20 min and cooled by rinsing with cold de-aerated water. To the resulting clear wort benzoic acid (puriss., p.a., 33047, Riedel-de Haen, Seelze/Germany) was added at a concentration of 500 mg/L for microbiological stabilization.

2.4 Design of release trial

As a control a volume of 800 mL of the obtained wort was filled into a Erlenmeyer flask (2 L) and sealed with Parafilm (Pechniney Plastic Packaging, Menasha WY/USA). Further, an amount of 6.4 g homogenized hot trub as is was added to a volume of 1,600 mL of the obtained wort and thoroughly mixed by shaking. This was at a final concentration of wet hot trub of 4,000 mg/L, which corresponds to 800 mg d.m./L. In order to disperse the trub, the suspension was shaken thoroughly and then divided in two equal portions, each of which was filled into an Erlenmeyer flask (2 L) and sealed with Parafilm. All flasks were placed on a shaker (Guwina-Hoffmann, Berlin/Germany) and gently agitated (intensity: 75 units) at room temperature to keep the suspension permanently in motion. In order to vary experimental conditions, an ethanol content of 5 % by vol. was achieved by addition of 85 mL Ethanol (puriss., p.a., 32205, Reidel-de Haen, Seelze/Germany) to 1,600 mL of the wort prior to trub addition. In another trial, the wort pH value of 4.0 was achieved by adjusting the pH value to 4.0 by adding diluted hydrochloric acid (puriss., p.a., 84409, Fluka, Buchs SG/Switzerland). Finally, in a third trial the wort was adjusted to 5 %v/v ethanol and pH 4.0.

2.5 Sampling and sample preparation

Samples of the homogenized suspensions (ca. 200 mL) were taken at defined periods after the hot trub addition: 0.5, 4, 24 and 48 h. In another trial, additional sampling was carried out after 120 and 168 h. The samples were paper filtered (522 032, Machery-Nagel MN 514 1/4, Düren/Germany), since centrifugation (9,000 rpm, 20 min) was not sufficient to completely remove particles. The clarified samples were chilled to 0°C (fatty acids, pH, extract) or deep frozen (Zn, Mg) until analyses. When the fatty acid content of the suspension (including trub particles) was the subject of investigation, the filtration step was skipped.

2.6 Analyses

Wort analyses were carried out according to MEBAK II 2.14 (pH), 2.10.2.3 (extract) [55], MEBAK III 4.6 (Zn) and 4.8 (Mg) [70]. The content of long-chain fatty acids (C14-C18:3) in wort samples was detected by solid-phase extraction, diazomethane methylation and GC/MS analysis according to a method published earlier [79].

3 Results and discussion

3.1 Long-chain fatty acids in wort throughout the brewhouse procedure (brewery A)

Due to degradation processes of mashing a high amount of long-chain fatty acids (C14-C18:3: >25 mg/L) was released to mash (Fig. 1). The mash values represent the highest overall values during brewhouse procedure, i.e., during the following steps the concentrations were subsequently lowered due to further mash and wort treatment. As a filtration step, lautering did not only remove solids from the mash, since the spent grain layer also functions as a filter bed in order to partly adsorb dissolved and dispersed substances, confirming the observations of Mück [60]. This resulted in lower free fatty acid concentrations of the first wort of ca. 15-20 mg/L. The concentrations in the post runs as well as in the kettle-full worts were similar to this value confirming former findings [42]. However, if the extract content was considered the free fatty acid content related to extract increased dramatically as the extract value of the run-off decreased toward the end of lautering (not shown). When wort boiling was completed after 60 min, an amount of only ca. 5 mg/L remained in the wort. That is, during wort boiling the loss of free fatty acids was highest throughout the entire process with a decrease of 72 % in this brewhouse. This corresponds very well to the findings of Wackerbauer et al., Jones et al. and Forch discussed above. Since the sample preparation only allows a detection of dissolved free fatty acids, the binding of the great majority of fatty acids to hot trub after its formation during boiling or a degradation [1, 66] caused the free fatty acids to disappear to a significant extend after boiling, while the amount adsorbed to trub probably increased. Further on, the total free fatty acid content slightly decreased to around 3 mg/L in the cooled wort. The fact that there was hardly any difference in the detected concentration before and after the whirlpool operation showed that most of long-chain fatty acids were already bound to trub particles at that stage or degraded when entering the whirlpool. The value of 3 mg/L was quite high due to the old-fashioned equipment of the observed brewhouse. For more modern brewhouses and particularly more modern lauter systems, total long-chain fatty acid concentrations of 1 – 2 mg/L are rather typical in chilled worts and closer to the values published by Wackerbauer et al. [89].

When detecting the fatty acid profile of a wort prior to boiling, e.g. a first wort (Fig. 2), a dominance of linoleic acid (C18:2) and palmitic acid (C16) was typically observed, while all other fatty acids played only a minor role. In this case C18:2 concentrations (ca. 6 mg/L) are higher compared to the C16 concentrations (ca. 4 mg/L). This confirms the findings published earlier [1, 31, 60, 89].

3.2 Generation of a fatty acid and zinc-deficient wort by yeast treatment

The interaction of high yeast dosages with chilled wort (final concentration ca. $2 \cdot 10^7$ cells/mL) for a short time (ca. 1.5 h) lead to a slight depletion of long-chain fatty acids (C14-C18:3) from 0.67 to 0.50 mg/L on average. This depletion confirms the findings of Lie [47] and would probably be greater if contact time of yeast and wort had been extended [59, 85]. More distinct was the decrease of the wort zinc concentration from 0.13 to 0.05 mg/L

on average due to yeast contact, which confirms the finding of Kreder who detected a reduction in wort from 0.17 to 0.02 mg/L because of yeast exposure [41]. This observation shows the zinc adsorbing effect of yeast, which can be utilized to deplete a wort prior to a release trial. On the other hand, it suggests that for release experiments either with lipids or metals a contact with yeast should be avoided, since the presence of yeast influences wort composition.

3.3 Release of long-chain fatty acids

Figure 3 shows the average values of the total long-chain fatty acids dissolved in wort versus the time of contact of hot trub with wort. The arrow at the ordinate shows the average level of total fatty acids originally contained in wort without any treatment. Since the levels of fatty acids before and after yeast treatment depend on the level in the original wort and therefore vary, all values have been calculated corresponding to the level after yeast treatment (t = 0) as blank value, which is defined as "0". Already 30 min after the addition of hot trub to wort, a release of fatty acids was observed. The final value was reached after 4 h of contact at a concentration of 0.9 mg/L on average. All samples taken later on, after 24 and 48 h, did not show different values, therefore there is no indication of an increasing release over time. Additional sampling after 120 h (5 days) and 168 h (7 days) in other trials also did not indicate further release (not shown). Considering that growing yeast cells have a demand for fatty acids right from the start of fermentation [59, 85], and the fact that polyunsaturated fatty acids, such as linoleic acid, cannot be synthesized by the yeast cell itself [13, 58], the fast release of long-chain fatty acids seem to improve the supply of yeast with long-chain fatty acids.

In order to simulate the formation of ethanol and the decrease of pH during fermentation and also to investigate their impact on the release of fatty acids, wort with an ethanol content set to 5 %v/v or with the pH adjusted to 4.0 or both was applied. However, none of the variations caused a difference in fatty acid release. In terms of microbiology, the applied experimental set-up seemed to be stable for at least 48 h, while the risk of an infection seemed to increase for trials of longer periods (5 and 7 days), which was easily detectable by a pH decrease and observation of an opalescence in filtered samples.

As mentioned above, the applied experimental method allows only to observe free fatty acids dissolved in wort due to particle separation prior to analysis. Additionally, it might be interesting to investigate the potential of release in the suspension containing hot trub and wort. Therefore, in another trial the particle separation was skipped and the turbid suspensions were eluted without pretreatment. As can be seen from Figure 4, the non-filtered samples showed much higher values of ca. 4 mg/L, which were gained 1 – 2 days after hot trub addition. This means that even after a first contact and release to wort there is still a high proportion of fatty acids bound to trub.

Beside the release of the total fatty acid amount it was also of interest to observe the composition of the released fatty acids. A typical fingerprint of the released fatty acids in filtered wort is presented in Figure 5. Here, as well as in case of the non-filtered wort (not shown), C16 and C18:2 dominate while all other fatty acids were of minor importance. This basically corresponds to the fingerprint of the first wort during brewhouse procedure (Fig. 2) and results published earlier [1, 31, 60, 89]. However, in contrast to these, the dominating released fatty acid observed here is C16, which might be due to differences in solubility in aqueous media.

As discussed above, several authors ascribe the nutrition properties of trub solids to the availability of unsaturated fatty acids in trub [1, 75, 83]. In conclusion of the experimental results presented here the fatty acid supply of yeast cells can be improved with an addition of hot trub to the fermenting wort.

3.4 Release of zinc and magnesium

Figure 6 presents the release of zinc in wort after addition of hot trub. The arrow at the ordinate shows the average zinc level originally contained in wort without any treatment. The value in the origin (t = 0) shows the average value of zinc after yeast treatment and complete separation of yeast. Since the levels of zinc before and after treatment depend on the level in the original wort and therefore vary, all values have been calculated corresponding to the level after yeast treatment as blank value, which is defined as "0".

Similar to the fatty acids, a release of zinc was observed only 30 min after hot trub addition to wort. After a contact time of 4 h the final value was reached at a concentration of 0.14 mg/L in average. All samples taken later on, after 24 and 48 h, did not show higher values, meaning the equilibrium was achieved after only ca. 4 h of intense contact. Even sampling after 120 h (5 days) and 168 h (7 days) in additional trials did not indicate a further release (not shown). Further trials with adjusted ethanol content (5 %v) and pH value (4.0) did not indicate any difference in zinc release, neither single nor in combination.

Beside zinc the release of magnesium was investigated in a pre-trail. Here, a significant depletion due to the yeast treatment could not be observed, which parallels the findings of Lie et al. for Mn and Fe ions [47]. The magnesium concentrations were within the range of 108 – 116 mg/L without indicating any significant difference due to the addition of hot trub. The high magnesium concentration in chilled wort is also an indication that magnesium is one of the metals which is not enriched in hot trub, and therefore, hot trub addition does not increase its level in wort after addition.

In conclusion these results mean that the addition of hot trub to wort can provide an amount of zinc which should be sufficient for yeast nutrition [16, 17, 80], even if wort was completely deficient of zinc.

4 Conclusion

Throughout the brewhouse procedure the content of long-chain fatty acids and zinc in wort decreases stepwise, with the mash having the highest overall values. That is, the fatty acid input into wort is determined by intensity of mashing and lautering, while the decrease of free fatty acid concentration is primarily determined by boiling, where a transfer from the liquid to the forming hot trub takes place. Correspondingly, zinc has a high affinity towards hot trub and is therefore strongly adsorbed. Finally, the hot trub containing high amounts of fatty acids and zinc is removed during whirlpool operation resulting in low final values in chilled wort. Thus the formation and removal of hot trub from wort are the rate-limiting steps for depleting the wort in fatty acids and zinc.

From this investigation it may be concluded that fatty acids as well as zinc are released very quickly from hot trub as soon as the trub is in contact with wort with both reaching their final concentrations after only ca. 4 h of contact under this experimental set-up. If the release kinetic in a fermentation tank is assumed to be similar, both nutrients would be available for the growing yeast right from the start of fermentation. The release of free fatty acids in a level of

0.9 mg/L means a supplementation of the chilled wort, whereas the non-filtered samples still containing hot-trub particles showed a potential for fatty acid release of around fourfold compared to the initial release. This potential should not be under-estimated when discussing yeast nutrition and possible quality impact on the resulting beers. The analogy of the fatty acid fingerprints of a brewhouse first wort and the trub loaded wort confirms the conclusion that fatty acids originally contained in the wort bind to hot trub as soon as the latter is formed during wort boiling. In case of adding hot trub to the fermentation tank, these fatty acids are released in a similar composition as they were in the brewhouse wort, to the fermenting wort to support yeast nutrition and increase fermentation performance.

In terms of zinc, the release of 0.14 mg/L from hot trub within 4 h of contact means a rapid and significant contribution to yeast nutrition. Even with cooled wort devoid of any zinc, this contribution would be enough to sufficiently supply yeast for a high fermentation performance. The high amount of released, i.e., dissolved zinc also gives an indication of zinc being loosely bound to the trub particles and therefore having a high bio-availability to yeast which confirms former findings. On the other hand, no additional release of magnesium could be found due to hot trub application.

A good supply of yeast nutrients provides a proper and fast fermentation, which is a requirement for high stabilities of the resulting beer (flavor, non-biological, foam and microbiological stability). Therefore, if long-chain fatty acids or zinc are the limiting factor concerning a proper fermentation performance, the supply of long-chain fatty acids and zinc may be improved by an addition of hot trub to the fermenting wort, as was shown by the release trials in this investigation. On the other hand, when a recommended dosage of hot trub is supposed to be defined the flavor-active degradation products of trub components influencing beer quality and particularly flavor stability have to be considered as well. Finally, the quantification can only be done based on the individual conditions in the particular brewery, such as the applied raw material composition (all-malt or adjuncts addition), the yeast management (yeast vitality, number of generations, fermentation performance), the fermentation conditions (original gravity, temperature, pressure), and the characteristics of the resulting beer (aroma profile, flavor matrix, flavor thresholds).

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Appendix

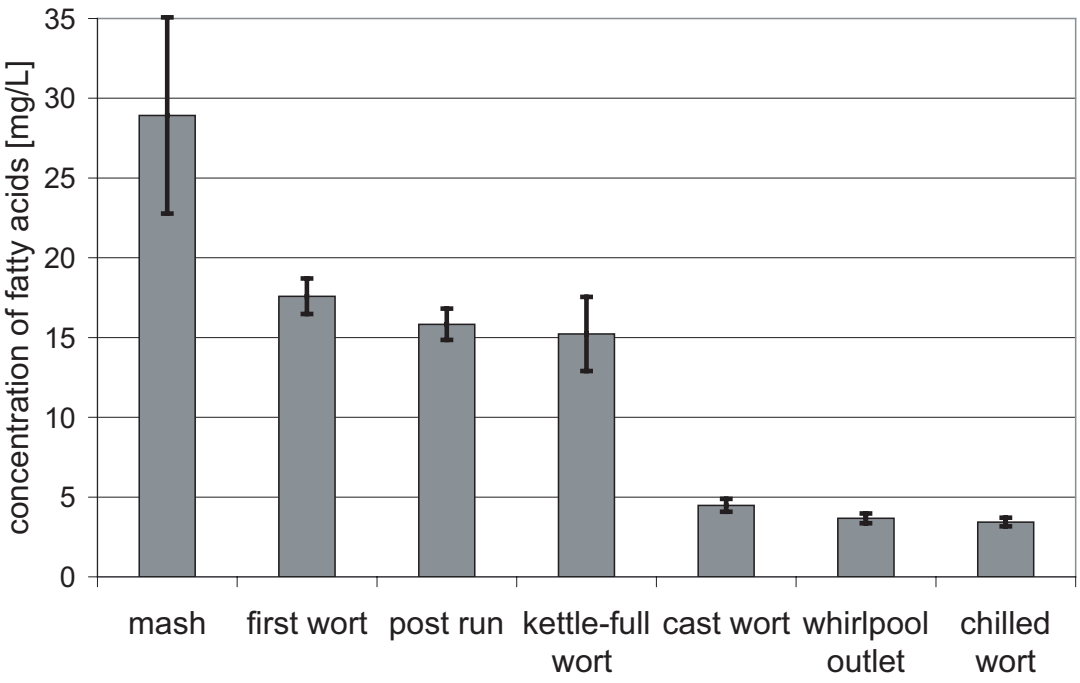

Fig. 1: Total concentration of free long-chain fatty acids (C14-C18:3) in mash and wort throughout an industrial brewhouse procedure (n = 4, p = 0.95)

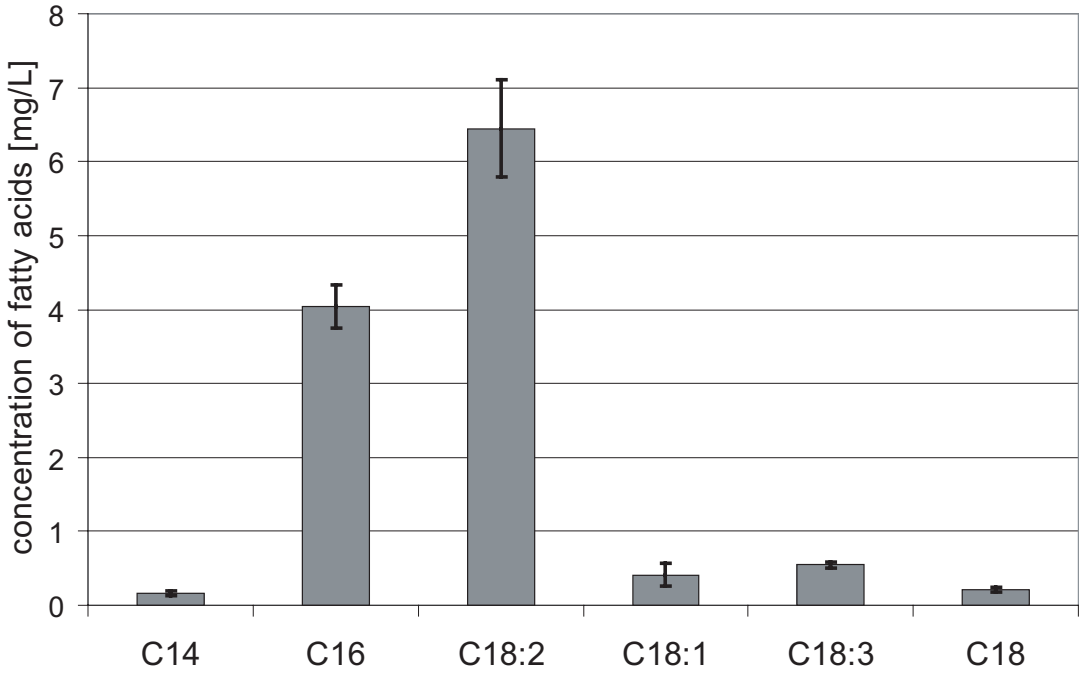

Fig. 2: Fingerprint of free long-chain fatty acids (C14-C18:3) in first wort of an industrial brewhouse procedure (n = 4, p = 0.95)

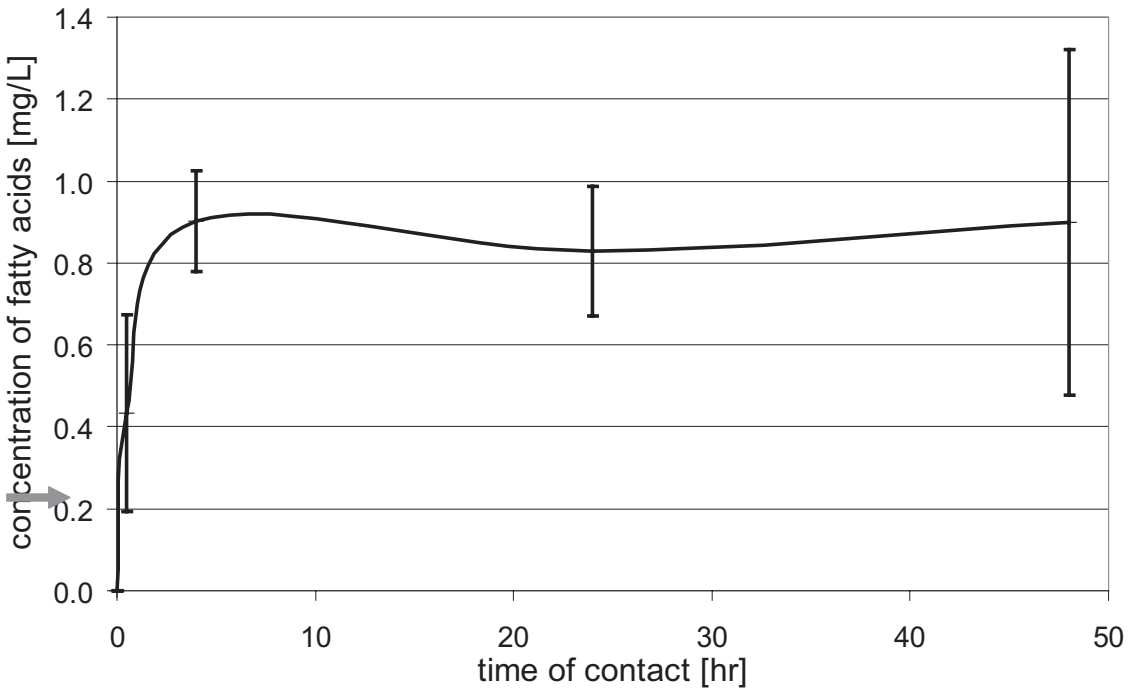

Fig. 3: Release of free long-chain fatty acids (C14-C18:3) after addition of hot trub to wort (800 mg/L (d.m.) at t = 0) vs. contact time of trub and wort (n = 6, p = 0.95). The arrow indicates the original content in wort prior to yeast treatment, while the origin of the diagram (t = 0) represents the value after yeast treatment right before trub addition. For each trial the fatty acid concentration at t = 0 was set to "0" and all other values relate to this set point.

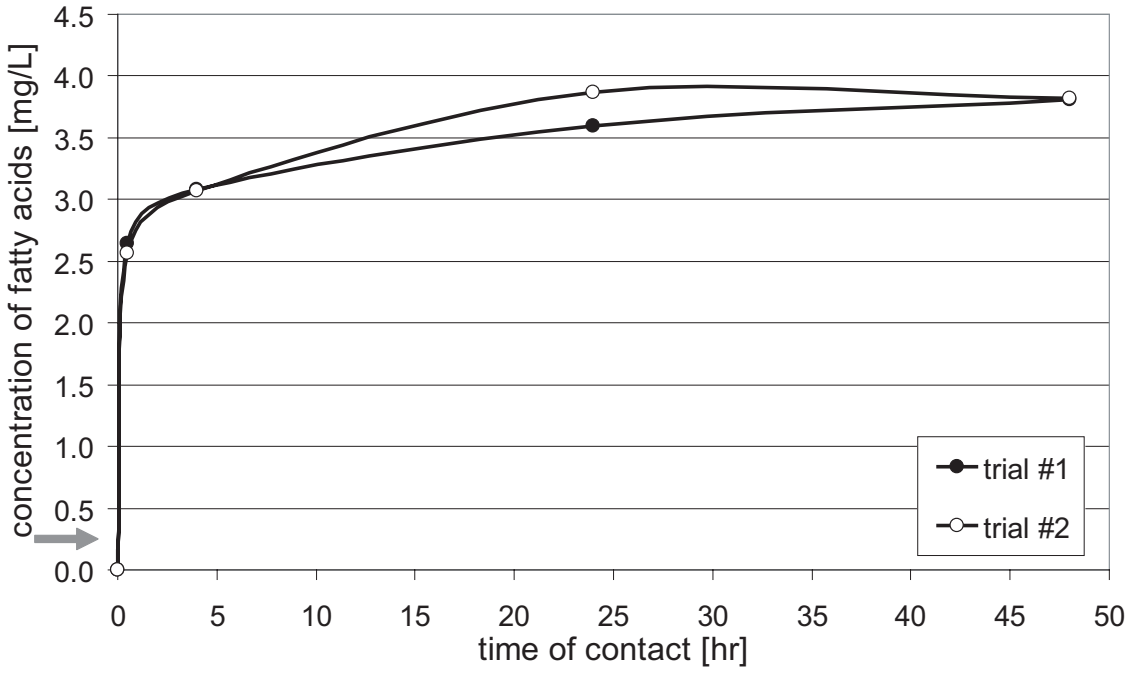

Fig. 4: Long-chain fatty acids (C14-C18:3) in suspension after addition of hot trub to wort (800 mg /L (d.m.) at t = 0; no separation of trub particles) vs. contact time of trub and wort. The arrow indicates the original content in wort prior to yeast treatment, while the origin of the diagram (t = 0) represents the value after yeast treatment right before trub addition. For each trial the fatty acid concentration at t = 0 was set to "0" and all other values relate to this set point.

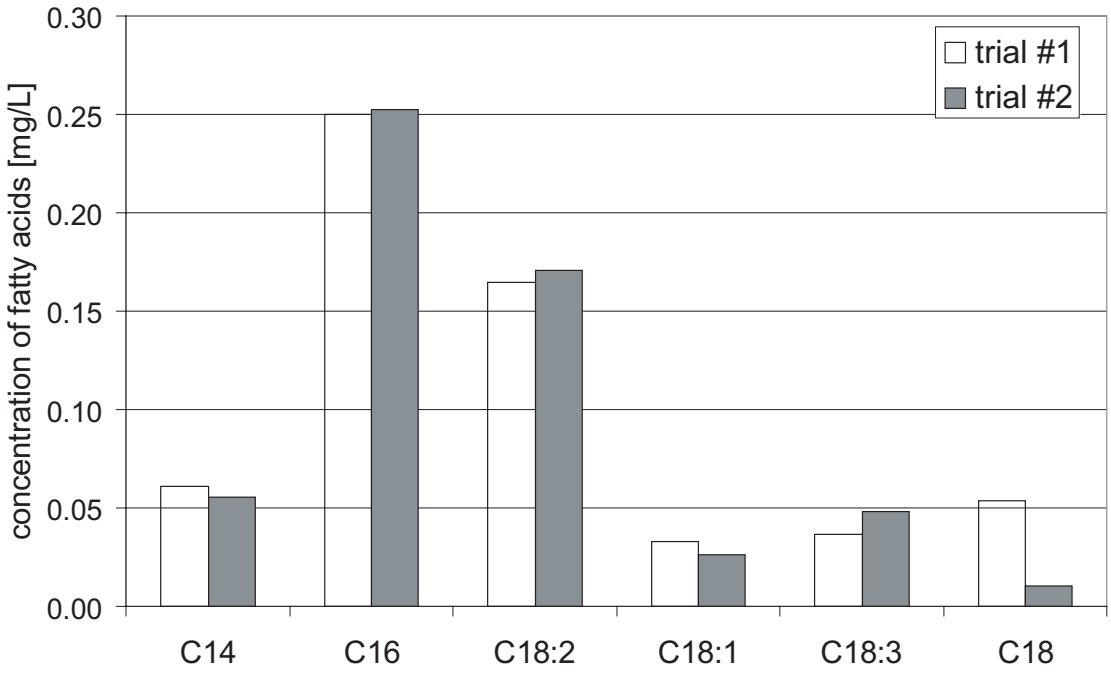

Fig. 5: Fingerprint of released free long-chain fatty acids (C14-C18:3) after addition of hot trub to wort (800 mg/L (d.m.); filtered samples, t = 4 hr)

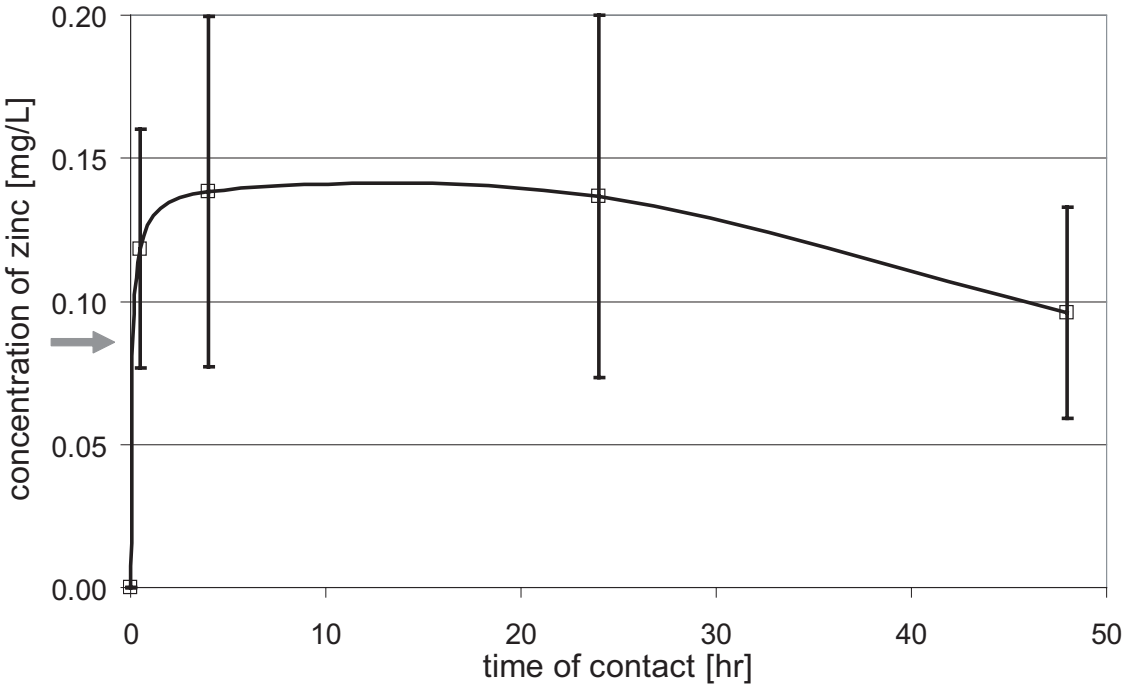

Fig. 6: Release of zinc after addition of hot trub to wort (800 mg /L (d.m.) at t = 0) vs. contact time of trub and wort (n = 6, p = 0.95). The arrow indicates the original content in wort prior to yeast treatment, while the origin of the diagram (t = 0) represents the value after yeast treatment right before trub addition. For each trial the zinc concentration at t = 0 was set to "0" and all other values relate to this set point