

The Effects of Linoleic Acid Supplementation of Cropped Yeast on its Subsequent Fermentation Performance and Acetate Ester Synthesis

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ABSTRACT

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For beer wort fermentation the addition of unsaturated fatty acids has sometimes been suggested as an alternative to wort oxygenation. This can however negatively affect the synthesis of acetate esters and consequently beer flavour. This work investigates the effect of supplementing a cropped yeast with an unsaturated fatty acid on the fermentation performance of the pitching yeast. Cropped yeast is in a different physiological state to yeast pitched in unfermented wort. Using a synthetic medium for the fermentations, it was found that the incubation of cropped yeast with linoleic acid resulted in two important changes in the yeasts composition: (1) the ratio of unsaturated fatty acids to total fatty acids increased from 0.53 to 0.66 and (2) the ratio of trehalose to glycogen increased from 0.17 to 0.49. The performance of this yeast in subsequent fermentations was compared to unsupplemented yeast under three conditions: medium pre-aeration, de-aerated medium and de-aerated medium with newly added unsaturated fatty acid. It was found that the supplemented pitching yeast showed growth, attenuation and ethanol formation profiles similar to those obtained with unsupplemented yeast in pre-aerated medium, which simulated the normal brewing practice. Compared to fermentations with unsaturated fatty acids added to the medium, the supplemented cropped yeast did not induce a reduction in acetate ester synthesis. Results indicated that the supplementation of cropped yeast with unsaturated fatty acids could be an interesting alternative to wort oxygenation to restore the optimal membrane fluidity of the yeast.

Key words: Acetate ester synthesis, unsaturated fatty acids, wort oxygenation.

INTRODUCTION

It is well known that the yeast *Saccharomyces cerevisiae* requires oxygen to synthesize its essential membrane components, sterols and unsaturated fatty acids^{2,3,4,18,20,28}. Insufficient levels lead to an alteration of membrane structure and membrane-linked biochemical processes. In the

traditional batch brewing practice, cropped yeast is reused in successive fermentation cycles¹ but its physiological condition may be poor because, under fermentation conditions, it becomes depleted of sterols and unsaturated fatty acids. Therefore, the yeast from a previous fermentation must be revitalized under conditions allowing the synthesis of appropriate levels of these essential membrane compounds. To meet this requirement oxygen is again needed and the brewer must aerate the wort for a short period of time prior to pitching. Using endogenous carbon sources, essential lipids are synthesized and growth proceeds^{24,25}. The control of wort aeration is difficult. Under-aeration can lead to the sub-optimal revitalization, growth defects and low subsequent fermentation rates²⁹ and over-aeration results in more biomass at the expense of ethanol and more yeast that requires disposal⁷. An alternative approach proposed by Ohno and Takahashi²⁵ and described by Boulton *et al.*⁶ relies on the aeration of cropped yeast suspended in water or in wort. The uptake of lipids from the surrounding medium^{9,13} or the addition of lipids to wort, especially unsaturated fatty acids, can

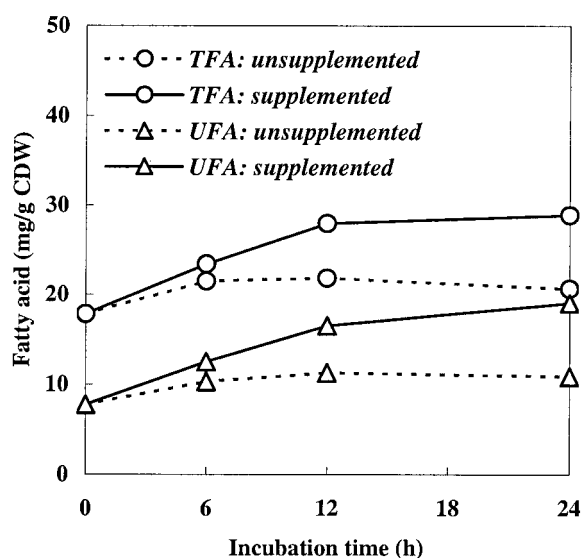


FIG. 1. Changes in levels of fatty acids (TFA: total fatty acids and UFA: unsaturated fatty acids) during pre-incubation with and without linoleic acid supplementation.

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eliminate the requirement for aeration^{12,31}. Wort lipid enrichment and wort aeration drastically reduce the synthesis of volatile esters important for beer flavour^{14,17,27,32,33} which again emphasizes the need for careful control of aeration, wort lipids and subsequent effects on flavour. A general review of the problems was published by Moonjai *et al.*²³.

In this study we investigated the effects of addition of the unsaturated fatty acid, linoleic acid, to cropped yeast, prior to pitching, on fermentation and volatiles synthesis.

MATERIALS AND METHODS

Yeast strain and maintenance

All experiments were carried out with an industrial lager brewer's strain of *Saccharomyces cerevisiae* (*carlsbergensis*) KUL-CMBS 12 (Katholieke Universiteit Leuven, Centre for Malting and Brewing Science Collection, Kasteelpark Arenberg 22, 3001 Heverlee, Belgium) maintained on wort agar (Difco Laboratories, Detroit, MI) and stored at 4°C.

Growth and fermentation media

To avoid the effects of changes in wort composition, a synthetic medium (SM) was used for all experiments. This was a citrate buffer (0.04 M, pH 5.2) containing per L: 80 g of glucose (Sigma Chemical Co., St. Louis, MO), 6.5 g of yeast extract (Difco Laboratories, Detroit, MI), 2.6 g of (NH₄)₂SO₄, 2.72 g of KH₂PO₄, 0.5 g of MgSO₄·7H₂O, 0.5 g of CaCl₂·2H₂O, 0.42 mg of ZnCl₂, 1.5 g of citric acid and 6 g of sodium citrate. Polypropylene glycol 2000

(Sigma-Aldrich Chemie GmbH, Germany) (200 mg/L) was added as an antifoaming agent. The medium was sterilized by autoclaving at 1.0 kg/cm² and 121°C for 15 min. The glucose solution was sterilized separately and mixed with the yeast extract-salt solution while hot.

Yeast propagation

Single yeast colonies were taken from stock plates and streaked on wort agar slants. After incubation for 48 h at 27°C, the slants were stored at 4°C. When required, 5 mL of SM was added to slant cultures and the cells were brought into suspension by gently shaking. The suspension was inoculated into 150 mL of SM in cotton wool plugged 250-mL Erlenmeyer flasks incubated at 20°C for 48 h, on an orbital shaker at 150 rpm. The cells were harvested by centrifugation (2500g, 5 min) and pitched into fresh SM at a level of 15×10⁶ cells/mL. This initiated the first fermentation cycle.

First fermentation cycle and unsaturated fatty acid supplementation

A first fermentation cycle was started using 1-L Erlenmeyer flasks with one way valves containing 500 mL of SM. For the sake of repeatability the media were first aerated to reach a dissolved oxygen concentration of 8 ppm. After pitching nitrogen gas was passed over the media during 5 min at a flow rate of 1.5 L per min. Fermentation was carried out at 20°C for 72 h with magnetic stirring at 150 rpm. To study the effects of adding unsaturated fatty acids to cropped yeast, before using the yeast for a next fermentation cycle, the yeast was not separated from the fermented medium and linoleic acid (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 60 mg/L using 0.5 mL of an ethanolic solution per 500 mL of fermented medium. Ethanol (0.5 mL without linoleic acid) was added to a parallel fermented medium as a control. After a contact time of 12 h, with or without linoleic acid, the cells were collected by centrifugation, washed twice with cold water (4°C) and then used for a second fermentation cycle. This procedure for adding linoleic acid was adopted to simulate the addition of the unsaturated fatty acids to cropped yeast suspended in a fermented medium. As *S. cerevisiae* cannot synthesize lin-

TABLE I. Unsaturated fatty acid content of cropped yeast obtained after pre-incubation with and without linoleic acid supplementation.

Incubation time (h)	Unsupplemented condition	Linoleic acid supplemented condition	
	UFA (% of total fatty acids)	UFA (% of total fatty acids)	Linoleic acid (% of total UFA)
0	43.5	43.5	0
6	47.9	53.4	21.4
12	51.6	59.0	31.7
24	52.6	65.9	30.4

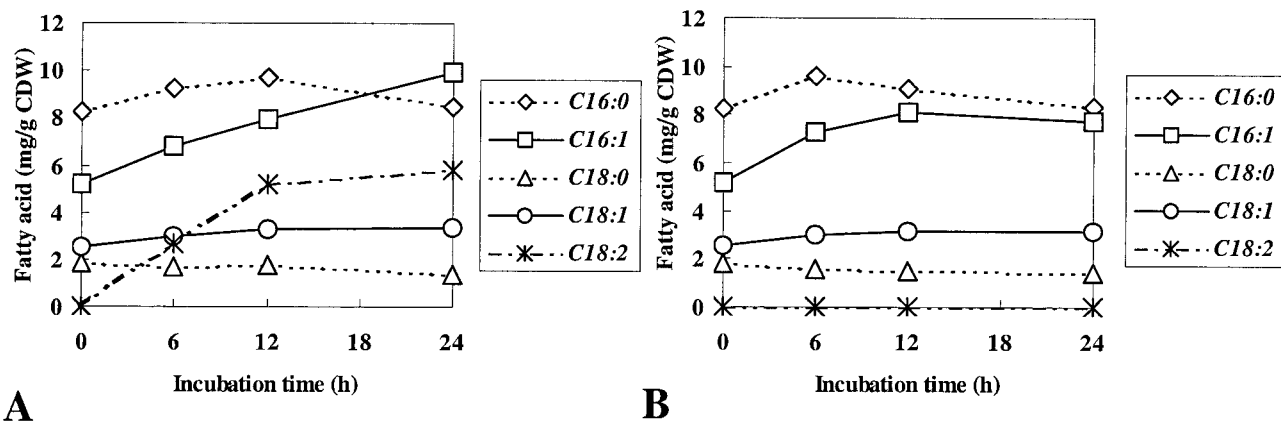


FIG. 2. Changes in levels of fatty acids (C-16 and C-18) during pre-incubation with linoleic acid (A) and without linoleic acid (B) supplementation.

oleic acid, this acid was chosen to study the effects of unsaturated fatty acid supplementation as it facilitates the study of its further distribution into cell lipids.

Second fermentation cycle

Yeasts from the first fermentation cycle were used for subsequent fermentations of 3 L batches of SM in 5-L fermentors (BioFlow III, New Brunswick Scientific, Edison, NJ) with stirring at 100 rpm at 15°C and a pitching rate of 15×10^6 cells/mL. Four fermentation conditions were studied (1) unsupplemented cells pitched in pre-aerated medium, (2) unsupplemented cells pitched in de-aerated medium, (3) unsupplemented cells pitched in de-aerated medium with added linoleic acid and (4) linoleic acid supplemented cells pitched in de-aerated medium. For pre-aerated conditions the medium, before pitching, was oxygenated to contain 8 ppm dissolved oxygen, by flushing the vigorously stirred medium with sterile air for 30 min at 15°C. After pitching nitrogen gas was passed over the medium as for the first fermentation cycle. For de-aerated conditions, oxygen, before pitching, was removed from the medium by flushing the medium with nitrogen gas for 10 min at 15°C and stirring at 500 rpm. In all fermentations nitrogen gas was passed continuously over the media at a flow rate of 30 mL per min to avoid any further entry of oxygen. The content of dissolved oxygen was measured by means of a dissolved oxygen meter (Oxi 340-A/SET, Weilheim, Germany). For de-aerated conditions with added linoleic acid, the unsaturated fatty acid in 3 mL of ethanol was added to a final concentration of 15 mg/L. An equal amount of ethanol (3 mL) was added into the other fermentations. At regular time intervals 100 mL samples were removed for further analysis.

Fermentation monitoring

Yeast growth was followed by cell biomass measurements. Cells were also counted with the Thoma counting chamber and viability was determined by methylene blue staining¹⁶. For analysis, yeast cells were harvested by centrifugation (2500g, 10 min), washed with 30 mL of 1% tergitol (Sigma Chemical Co., St. Louis, MO), then with 30 mL of 0.5% tergitol, and finally with 30 mL of distilled water to remove adsorbed fatty acids. All manipulations were done under nitrogen flushing conditions. Yeast pellets were weighed and stored at -20°C for further analysis. On the clarified media the apparent attenuation and the ethanol content were measured using a digital density meter (Paar DSA 48 + SP-1, Anton PAAR KG, Graz, Austria). Five mL amounts were also quickly collected in 20-mL vials, capped and frozen at -20°C.

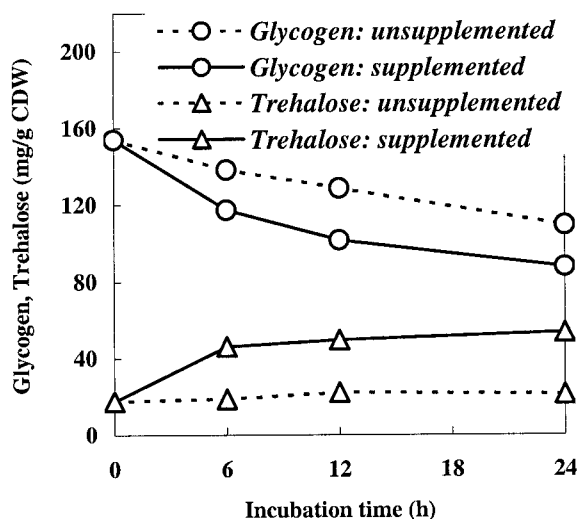


FIG. 3. Changes in levels of glycogen and trehalose during pre-fermentation with and without linoleic acid supplementation.

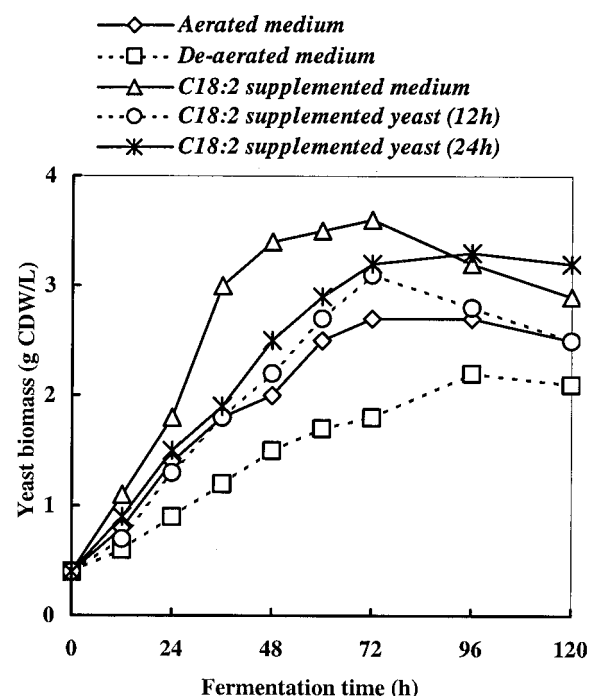


FIG. 4. Changes in yeast biomass during fermentations under different conditions.

TABLE II. The uptake of FAN and the synthesis of volatiles in mg/g CDW at 40 % and 80 % attenuation.

Fermentation conditions	At 40 % attenuation			At 80 % attenuation		
	FAN (mg/g CDW)	Acetate esters (mg/g CDW)	Higher alcohols (mg/g CDW)	FAN (mg/g CDW)	Acetate esters (mg/g CDW)	Higher alcohols (mg/g CDW)
Aerated medium	48.0	7.0	43.2	43.0	12.5	71.3
De-aerated medium	47.3	11.1	59.6	51.8	20.2	113.1
C18:2 supplemented medium	31.5	3.7	34.8	25.5	8.2	51.8
C18:2 supplemented pitching yeast	37.3	9.3	62.0	38.9	13.4	69.6

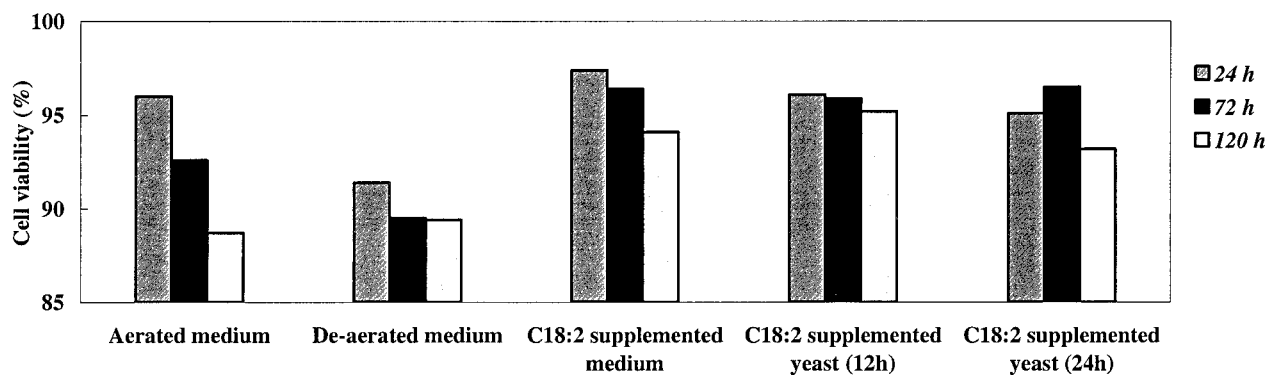


FIG. 5. Comparison of cell viability after 24 h, 72 h and 120 h of fermentation under different conditions.

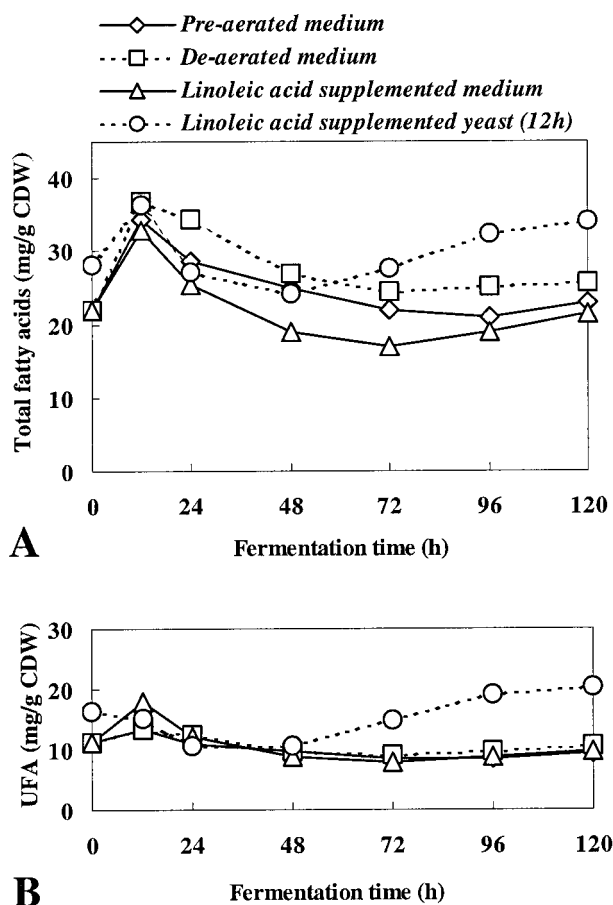


FIG. 6. Changes in levels of total fatty acid (A) and unsaturated fatty acids (B) during fermentation.

Yeast cell analysis

Frozen pellets were suspended in cold (4°C) 0.8% NaCl to obtain 0.1 g wet cells per mL. For dry weight determination, 1 mL of the suspension was centrifuged and the pellet was dried at 105°C for 2 h. Fatty acids, glycogen and trehalose content were determined and the contents were calculated as mg/g cell dry weight (CDW).

Total fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2)) were determined after direct saponification of 0.5 g of wet cells pellet in a 30 mL capped Pyrex tube with 10 mL of an equal-volume mixture of KOH (1N) and methanol at 100°C for 30 min. Heptadecanoic acid (Sigma Chemical Co., St. Louis, MO) was used as an internal standard. After cooling, the saponified mixtures were acidified with 2 mL of HCl (6N) followed by extraction with n-hexane as described by Chen¹⁰. The lipid extract was evaporated to dryness. The methylation of fatty acids was achieved by incubation of the dried extract with 1 mL of boron trifluoride in methanol (14% solution) for 10 min at 100°C. After cooling, 6 mL of NaCl saturated water was added and the fatty acid methyl esters were extracted with 300 µl of toluene for gas-chromatographic analysis. Gas chromatography was done with a Varian 3300 analyzer (Varian Association, Inc., Walnut Creek, CA) equipped with a 30 m length, 0.32 mm internal diameter, 0.25 µm film thickness, Alltech Heliflex AT-225 capillary column (Alltech Associated, Inc., Deerfield, IL) and a flame ionization detector. Conditions were: oven temperature of 150°C to 210°C at a rate of 6°C per min and 3 min at 210°C, injection port at 250°C and detector at 230°C. The carrier gas was helium.

Glycogen was determined according to Quain²⁶. Alkali- and acid-soluble glycogens were extracted from 0.1 g of wet cells pellet with 1 mL of 0.25 M sodium carbonate (100°C, 15 min) and 1 mL of 0.5 M perchloric acid

TABLE III. Total FAN uptake and total volatiles at maximum attenuation (88%).

Fermentation conditions	Time (h)	Total FAN (mg/L)	Total higher alcohols (mg/L)	Total esters (mg/L)	Ratio 'higher alcohols/esters'
Aerated medium	81	118.6	220.6	38.5	5.7
De-aerated medium	126	112.9	258.8	46.5	5.6
C18:2 supplemented medium	67	95.7	197.1	37.0	5.3
C18:2 supplemented pitching yeast	75	124.3	220.6	45.5	4.8

(100°C, 10 min), respectively. The pH of the alkali- and acid-soluble extracts was adjusted to 5.0. To the extracts amyloglucosidase (Boehringer Mannheim, Germany) was added to obtain 1.4 U per mL and the mixtures were incubated for 2 h at 37°C. After neutralization, the resulting glucose was determined spectrophotometrically at 505 nm using a glucose oxidase diagnostic kit (Sigma Diagnostics Inc., St. Louis, MO). Total glycogen represented the sum of alkali- and acid-soluble glycogens.

Trehalose was extracted from a 0.1 g wet cell pellet with 4 mL of cold trichloroacetic acid (0.5 M) for 1 h, according to Trevelyan and Harrison³⁴. The extract was further analysed by the anthrone method of Spiro³⁰. Trehalose was determined spectrophotometrically at 625 nm and the results were compared to a glucose standard solution. Glycogen and trehalose are expressed as mg glucose/g CDW.

Free α -amino nitrogen (FAN)

Free α -amino nitrogen (FAN) was determined by the EBC ninhydrin method¹⁵. Glycine (Fisher Scientific Co., Pittsburgh, PA) was used as a standard, and the results were calculated by linear regression.

Analysis of volatile esters and higher alcohols

Volatile compounds were determined by headspace gas chromatography (Perkin Elmer Autosystem XL) and flame ionization detection. Samples of 5 mL were heated for 16 min at 60°C in the headspace autosampler (Perkin Elmer Headspace Sampler HS-40). Esters and higher alcohols were separated using a 50 m WCOT fused silica capillary column coated with CP-Wax 52CB, with 1.2 μ m film thickness and 0.32 mm internal diameter. The following conditions were applied: injection temperature 180°C; oven temperature 75°C for 6 min, increase at 25°C/min to 110°C, hold for 3.5 min; detector temperature 250°C. The carrier gas was helium.

RESULTS AND DISCUSSION

Effects of linoleic acid supplementation on cropped yeast composition

Figs. 1 and 2 and Table I show the changes in the fatty acid content of yeast obtained during the pre-incubation with linoleic acid. In both conditions, with and without linoleic acid supplementation, the total yeast cell fatty

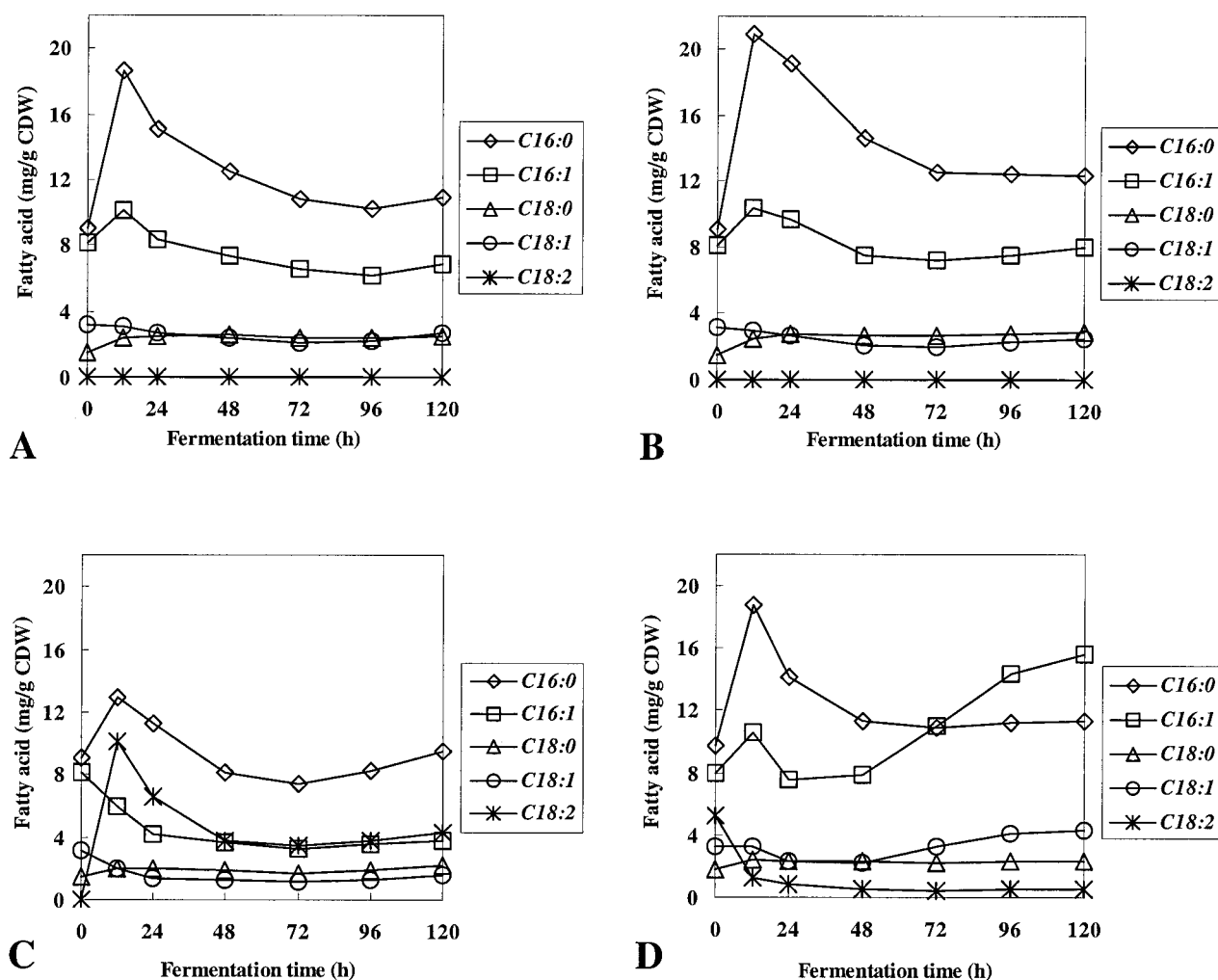


FIG. 7. Changes in levels of fatty acids in yeast cells during fermentation under different conditions. (A) Pre-aerated medium, (B) De-aerated medium, (C) Linoleic acid supplemented medium, (D) Linoleic acid supplemented yeast.

acids (TFA) increased. In the linoleic acid supplemented condition, the TFA increased by 57.3% after 12 h and the UFA increased by 114.3 %, from 7.7 to 16.5 mg/g CDW, and the increase was mainly due to the uptake of linoleic acid (C18:2, Fig. 2), which corresponds to 31.7% of total UFA. The percentage of UFA to total fatty acids increased from 43.5% to 59.0% (Table I). The increase in UFA content was also observed in linoleic acid unsupplemented conditions, however to a lesser extent, from 7.7 to 11.3 mg/g CDW after 12 h incubation. The increase in UFA was mainly due to palmitoleic (C16:1) and oleic (C18:1) acids. The synthesis of unsaturated fatty acids in unsupplemented conditions could have been due to the presence of residual oxygen. However with linoleic acid supplementation the final content in palmitoleic acid was higher (Fig. 2). The increase in UFA from 7.7 to 16.5 mg/g CDW after 12 h linoleic acid supplementation was comparable to an increase from 7 to 16 mg/g CDW found by Ohno and Takahashi²⁵ after 4 h oxygenation of cropped yeast suspended in wort. Fig. 3 shows that the glycogen content

decreased under the supplemented condition but that the trehalose content more than doubled. A similar increase in trehalose at the expense of glycogen has also been reported by Callaerts *et al.*⁸ when cropped yeast was oxygenated. This was explained as a yeast response to new (stress) conditions^{22,35}.

Fermentation with linoleic acid supplemented pitching yeast

Yeast composition during fermentation. Pitching yeasts obtained in the first fermentation after a further 12 or 24h pre-incubation with and without linoleic acid were used for a second fermentation under 4 different conditions. Three conditions with unsupplemented pitching yeast were used: (1) pre-aeration to simulate the normal brewing practice, (2) de-aerated conditions and (3) de-aerated conditions with linoleic acid supplementation. These fermentations were compared to (4) fermentations with linoleic acid sup-

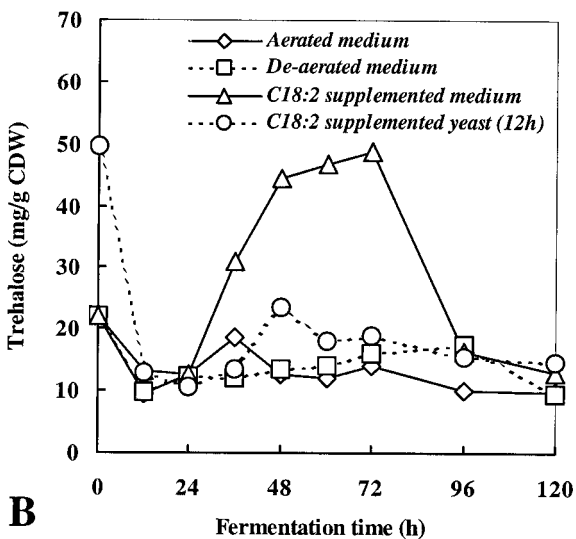
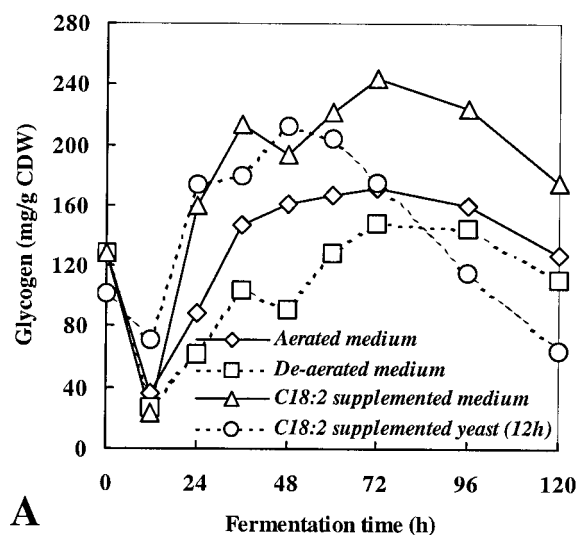


FIG. 8. Changes in levels of glycogen (A) and trehalose (B) in yeast cells during fermentation under different conditions.

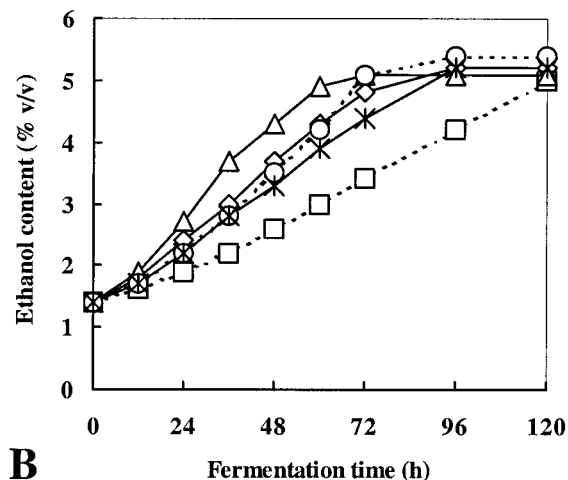
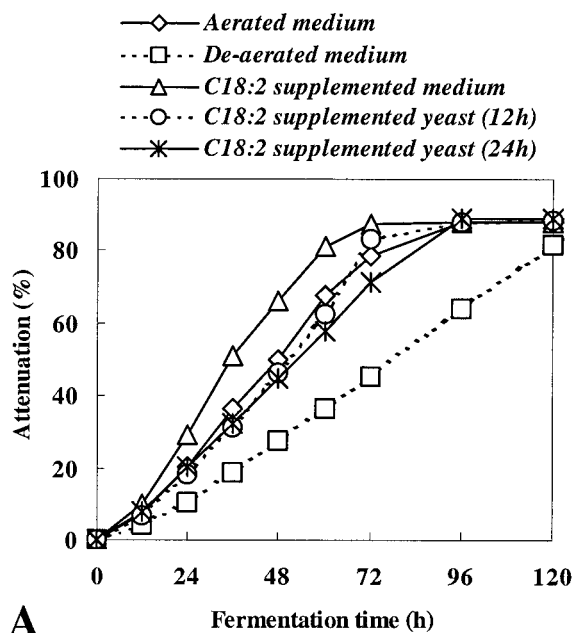


FIG. 9. Changes in apparent attenuation (A) and ethanol content (B) during fermentation.

plemented pitching yeast under de-aerated conditions. Fig. 4 shows the growth of yeast represented by the increase in yeast biomass during the 120 h fermentation time. The lowest growth, as expected, was found under the de-aerated conditions. The highest growth was detected under the de-aerated conditions in the presence of added linoleic acid, confirming previous results³¹. More interesting, however, were the similar growth patterns found for unsupplemented pre-aerated pitching yeast and 12 h linoleic supplemented pitching yeast. Supplementation for 24 h resulted in higher biomass but equal cell numbers. This was a first indication that medium pre-aeration could be replaced by a process using pitching yeast supplemented with unsaturated fatty acids. As shown in Fig. 5 cell viability was lowest under de-aerated conditions. Under pre-aerated conditions the viability was initially high but decreased towards the end of fermentation, as was also reported by Cunningham¹¹. The highest viable cell numbers were found under conditions where linoleic acid was present, either in the medium or in the pitching yeast.

The changes in fatty acids content during fermentation are given in Fig. 6 (TFA and UFA) and Fig. 7 (individual fatty acids). Under all 4 fermentation conditions TFA increased after 12 h of fermentation. In pre-aerated and de-aerated conditions the increases in TFA (Fig. 6A) and UFA (Fig. 6B) were mainly due to palmitic and palmitoleic acids (Figs. 7A and 7B). In the linoleic acid supplemented medium the increase was mainly due to uptake (Fig. 7C). Finally in the linoleic acid supplemented pitched yeast the increase was mainly due to palmitic acid (Fig. 7D). The highest UFA was in linoleic acid supplemented medium with the incorporation of 10 mg/g CDW after 12 h from a starting medium supplementation of 15 mg/L of linoleic acid. This was very high when compared to an incorporation of only 5.2 mg/g CDW in cells of the first fermentation cycle, starting with a fermented medium supplemented with 60 mg/L of linoleic acid. This might be explained by differences in medium composition. Addition of linoleic acid in the second fermentation cycle was done in the original rich medium, while addition in the first fer-

mentation cycle was done in the final fermented medium. Ohno and Takahashi²⁵ reported that medium richness promoted the synthesis of UFA during the aeration of cropped yeast composition. Our results confirm that medium richness may also promote UFA uptake as previously reported³³. The low level of stearic and oleic acids when linoleic acid was taken up can be explained by the inhibition of several enzymes of fatty acid synthesis by supplemented unsaturated fatty acids as reported earlier^{5,19}. After 12 h, TFA and UFA decreased except with supplemented pitching yeast. The decreases are the result of decreases in mainly palmitic and palmitoleic acid, and linoleic acid in fermentations where this UFA was added to the medium. With linoleic acid supplemented pitching yeast, TFA and UFA increased after 24 h of fermentation (Fig. 6), and this was due to an important increase in palmitoleic acid (Fig. 7D) apparently at the expense of palmitic acid and after a strong reduction in the linoleic acid content. An increase in palmitoleic acid was also found in the pre-incubation of pitching yeast with linoleic acid (Fig. 2A). We have no explanation for this unexpected increase. Glycogen and trehalose profiles are given in Fig. 8. After a strong decrease

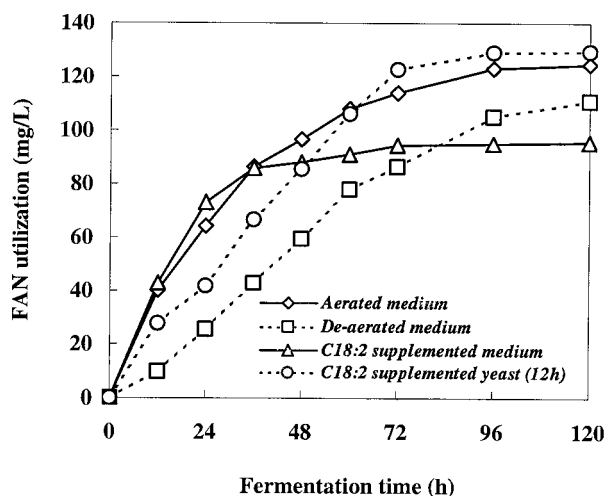
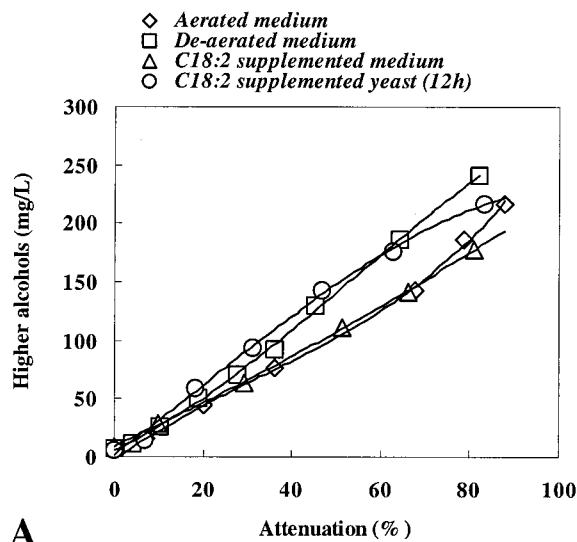
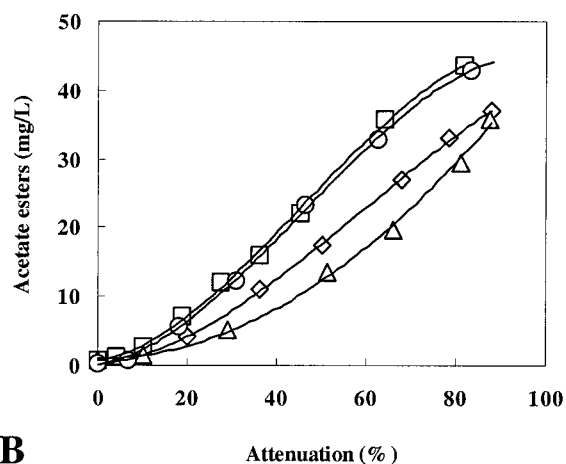


FIG. 10. FAN utilization during fermentation under different conditions.



A



B

FIG. 11. Formation of higher alcohols (A) and acetate esters (B) during fermentation as a function of attenuation.

in glycogen content during the first 12 h of fermentation, the content rapidly increased and reached a maximum after 48-72 h, except under de-aerated conditions. The highest levels were obtained when linoleic acid had been present either in the medium or in the pitching yeast. Later, a fast decrease occurred in the fermentation pitched with supplemented yeast. The trehalose content also decreased during the first 12 h, and especially the initial high trehalose level in the supplemented pitching yeast was strongly reduced. Later, a temporary strong increase in trehalose occurred under conditions where linoleic acid was added to the fermentation medium.

Medium composition during fermentation. All fermentations were followed analytically with respect to attenuation, FAN, higher alcohols and acetate esters. Fig. 9 shows the attenuation and ethanol profiles. As expected the fermentation rate was lowest under de-aerated conditions and it took more than 120 h to reach the maximum attenuation. In the linoleic acid supplemented medium the rate was the fastest and a final attenuation of 88% was reached after 67 h. Somewhat lower, but very similar rates, were recorded for the pre-aerated conditions and de-aerated conditions pitched with linoleic acid supplemented yeast for both 12 h and 24 h pre-incubation times. All attenuation profiles reflected the same trend as the cell growth profiles (Fig. 4). Again the results indicate that pitching yeast supplementation with linoleic acid could be an alternative to pre-aeration of the medium. FAN uptake is presented in Fig. 10 and Table II. The original FAN concentration (derived from yeast extract) was 450 mg/L. During the first 72 h, the lowest but almost constant uptake rate was found under de-aerated conditions. A higher and constant uptake rate was detected for the supplemented pitching yeast. The highest and comparable constant uptake rates were found for the pre-aerated conditions and in the supplemented medium, however only during the first 36 h. In the supplemented medium after 36 h the uptake stopped, while it continued at a lower rate under the pre-aerated conditions. After 72 h the highest FAN uptake in mg/L was found under pre-aerated conditions and with the supplemented pitching yeast. The uptake of FAN in mg/g CDW was however highest under de-aerated conditions. In the supplemented medium the uptake in mg/g CDW was high after the first 36 h (~40% attenuation), but low when calculated for cells at 72 h (~80% attenuation) of fermentation. This might have been due to the accumulation of glycogen and trehalose between 36 and 72 h (see Fig. 8). The formation of higher alcohols (propanol, isobutanol, isoamyl alcohol) and acetate esters (ethyl acetate, isoamyl acetate) in terms of the attenuation percentage is given in Figs. 11A and 11B. As expected all volatiles examined increased with attenuation but the rate of increase was lower under pre-aerated and supplemented medium conditions. As a result the concentration of acetate esters at maximum attenuation was approximately 18% lower than under de-aerated condition. In the linoleic acid supplemented medium this could be explained by the inhibitory effects of unsaturated fatty acids on acetate esters synthesis as reported by Thurston *et al.*³³ and the long lag phase in ester synthesis could be related to the high intracellular linoleic acid concentration at 12 h fermentation time (Fig. 8C). The lower ester concentration under the pre-aerated

conditions could be due to the repression effects of aeration on genes involved in ester synthesis as reported by Fujii *et al.*¹⁷ However, the most interesting result was that the ester concentration, reached with the linoleic acid supplemented pitching yeast, did not suffer from a linoleic acid inhibitory effect. This was explained by the lower initial intracellular content of linoleic acid, due to the supplementation of the pitching yeast in the resting stage in an exhausted fermentation medium. Table II shows the uptake of FAN and the synthesis of volatiles in mg/g CDW at 40% and 80% attenuation. Uptake of FAN and synthesis of higher alcohols and esters remained lowest under the supplemented medium conditions. This was also found at maximum attenuation (Table III shows results in mg/L). The highest 'higher alcohol' levels found for the non-aerated conditions may have resulted from continuous FAN uptake and the low 'higher alcohol' formation may correspond to a low FAN utilization from the supplemented medium. In fact, the level of higher alcohols and esters produced by yeast during fermentation has a significant effect on the flavour of beer. For example, concentrations of higher alcohols above 100 mg/L can damage the flavour and acceptability of beer produced from a 12°P wort²¹. However, it is still not clear whether more or less ester in beer is desirable. With regard to the balance of beer flavour it is not the absolute amount, but rather it is the ratio of the higher alcohols to esters, which is particularly important. If one considers the 'higher alcohols/esters' ratio, our results indicate that there was an increase of fruity flavour (ester compounds) in the fermentations with linoleic acid supplemented pitching yeast as indicated by a low ratio of higher alcohols to esters (see Table III).

CONCLUSIONS

The addition of unsaturated fatty acids (UFA) to wort has sometimes been suggested as an alternative to wort oxygenation. This may, however, have negative effects on acetate ester synthesis and beer flavour. This work investigated the addition of an unsaturated fatty acid to cropped yeast. The yeast was in a resting stage, and thus different from the growth stage reached in wort. We found that cropped yeast upon supplementation with linoleic acid showed two important changes in composition: the ratio of UFA to saturated fatty acids increased; and the ratio of trehalose to glycogen increased from 0.17 to 0.49. The supplemented yeast in subsequent fermentations showed growth and attenuation profiles comparable to yeast from pre-aerated medium. More important, acetate ester synthesis was not reduced and around 22 % more esters were produced, compared to fermentations in which UFA was added to the medium as an alternative to oxygenation. The unexpected increase in palmitoleic acid during fermentations with linoleic acid supplemented pitching yeast requires further investigation. In our experiments, a synthetic medium was used to avoid the effects of differences in medium composition. The fermentation behaviour of supplemented cropped yeast using wort, as well as its behaviour in EBC fermentation tubes now requires further study. Additionally sterol synthesis and the fate of the added linoleic acid after it is taken up will be investigated.

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