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Title

Malolactic and alcoholic fermentations in black currant juice

Key Words: Berry wines, malolactic fermentation, alcoholic fermentation, black currant, *Ribes nigrum*

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Abstract

Wines and juices made from berries of the northern climates are often characterized by excess acidity. Therefore malolactic fermentation by *Oenococcus oeni* (ATCC 39401) was tested for its applicability in reduction of acidity in black currant juice. The juice was used both before and after malolactic fermentation by *Saccharomyces cerevisiae* (Lalvin V1116). Throughout the fermentations changes in acid, sugar and alcohol contents were monitored. In both yeast-fermented and non-alcoholic juices the malolactic bacterium converted malic acid to lactic acid prior to sugar or citric acid consumption. Thus, sequential utilization of the substrates by *O. oeni* offers a basis for acidity reduction without sugar consumption in production of berry juices and berry wines. Moreover, slightly different product composition can be obtained depending on which of the two fermentations is carried out first. Eventhough only malic acid was eliminated by the malolactic bacterium a noticeable reduction in acid taste of black currant juice and wine was observed.

1. Introduction

Berries of the northern climates, both cultivated and wild berries, have a high content of fibre, vitamins and minerals, and wild berries particularly are rich in flavonoids (1,2). The organic acid composition in berries also affects the organoleptic properties of the product. Being a constituent of the sourness group, each acid conveys a characteristic flavour, aroma or taste (3,4).

However, in making wine from berries, acidity tends to become a problem especially in products intended to have low sugar content. The main acids in most berries are citric and malic acid. Especially, the latter causes in high concentrations undesirable acidity in most wines (3,4). Thus the acceptability of berries of the northern climates would be markedly enhanced if the acid composition of the juices, especially that of malic acid, could be controlled.

The content of malic acid in wine can be selectively reduced by malolactic fermentation (MLF), which converts L-malic acid to weaker L-lactic acid and CO_2 (5-11). However, little is known about the applicability of MLF to berry juices (12) and even less about how both alcoholic fermentation (AF) and MLF can be conducted consecutively in the production of berry wines. Therefore, the aim of the present study was to determine the main changes in organic acids and sugars that take place when both AF and MLF are applied one after another to a berry juice.

A selected strain of *Oenococcus oeni* (ATCC 39401) and *Saccharomyces cerevisiae* (Lalvin V1116) were used as starter cultures. Black currant (*Ribes nigrum*) was chosen as the representative berry. Its high content of flavonoids, vitamin C (1.5–3.0 g/kg) (ascorbic acid) (13) and sugar (12) makes it an ideal candidate for low-alcoholic, low sugar wine.

2. Materials and Methods

Micro organisms

The yeast used was *Saccharomyces cerevisiae* obtained from Lallemand Inc. (Lalvin V1116, Danmark). Dry yeast (3 g) was rehydrated 25 min in 27 mL of physiological saline before inoculation. The concentration of living cells in the inoculum was 3×10^6 CFU/mL.

The malolactic organism was *Oenococcus oeni* (formerly *Leuconostoc oenos*) obtained from the American Type Culture Collection (ATCC 39401). The strain was stored in 10 % (v/v) glycerol at –60 °C until used. The inoculum was prepared by first growing the thawed bacterium in MRS medium (LabM, UK) supplemented with 2 g/L citric acid and 2 g/L malic acid. The strain was grown without shaking at 30 °C for 3 days. The second cultivation medium was a 1:1 diluted and autoclaved black currant juice (pH 3.0) supplemented with 5 g/L yeast extract. The incubation was carried out at 27 °C for 2 days. The final concentration of living cells in the second cultivation medium was about 3×10^8 CFU/mL.

Black currants

The ripe Finnish black currants (*Ribes nigrum*) species 'Öjebyn' were obtained frozen (-20 °C) from Pakkasmarja Oy (Suonenjoki, Finland). The berries were allowed to thaw 3 d at +4 °C before use. The thawed berries were first minced (ENOL OM 10 – 0976, G Wein GmbH + Co., Germany) and treated with 0.4 mL/kg pectinase (Panzym Super E, Novo Nordisk Ferment Ltd., Switzerland) for 3 h in +30 °C. The mash was extracted to juice in a hydraulic press (ENOL OP 20 – 442, G Wein GmbH + Co., Germany).

Experimental conditions

The experiments were carried out in diluted (1:1) black currant juice. The juice was filtered with a disc filter (ENOL F20Z, G Wein GmbH + Co., Germany) equipped first with a coarse filter (Seitz-800, cut size 7.0 µm) and sterilized with a sterile filter (Seitz EK, cut size 0.45 µm). Fermentations were carried out in pre-

sterilized steel fermentation tanks (Tankki Oy, Finland) equipped with a water seal and stirring (77 rpm/min). The final volume of each juice batch was approximately 10 L. No extra additives, except dissolved sucrose (100 g/L) prior AF, were added to the diluted juices.

Two different experimental procedures were used. One set of fermentations was performed by first fermenting the juice with *S. cerevisiae* and after that with *O. oeni* (tanks 1a and 1b). The other fermentation was carried out *vice versa*, first the MLF and then the AF (tanks 2a and 2b). After both AF and MLF the juice was filter sterilised with a Seitz EK sterile filter. Two parallel-inoculated fermentation tanks were used for each combinations.

The AF was carried out at 20 ^{o}C for 14 days. The MLF of both the filter sterilised juice and the filter sterilised wine were carried out simultaneously. The MLF were carried out at 25 ^{o}C for 48 hours. After the first 24 hours a second inoculum of the bacterium was added to one set of duplicates (tanks 1b and 2b).

Sample preparation and analytical methods

Samples were taken from the juice prior to fermentation and during the AF and MLF. The juices and wines were subjected to sensory evaluation for acidity by a panel of six assessors. The content of organic acids (citric, malic, acetic and lactic acids), sugar (sucrose, fructose and glucose) and ethanol as well as pH values were measured from each sample. Organic acid content was determined by using a high-pressure liquid chromatograph (HPLC) (Series 1100, Hewlett Packard, USA) equipped with a UV-detector (Hewlett Packard, USA). An Aminex HPX-87 H^+ column (300 x 7.8 mm, 9 µm, Bio-Rad Laboratories, USA) was used under the following conditions: column temperature, 35 ^{o}C; mobile phase, 5 mM H_2SO_4; and flow rate, 0.6 mL/min. Column effluents were monitored at 214 nm. Quantification was based on peak height measurements. Soluble sugars and ethanol were determined by a HPLC (Series 200, Perkin Elmer, USA), equipped with a RI-detector (1047A, Hewlett Packard, USA). An Aminex HPX-87C column (300 x 7.8 mm, Bio-Rad Laboratories, USA) was used under the

following conditions: column temperature, 60 ^{o}C; mobile phase, degassed Milli-Q water; and flow rate, 0.6 mL/min. Quantification was based on peak area measurements using xylitol as internal standard. Prior to sugar and ethanol analyses, the samples were treated with a strong anion exchange column (Bond Elut SAX, Varian, USA) to eliminate the acidic compounds. All samples were filtered through 0.2 µm membranes before the HPLC analyses.

3. Results and Discussion

Composition of black currant juice

The main acids in the juice were, as in most of the berries of the northern climates, citric acid (28 g/L) and malic acid (5 g/L). The juice had a pH of 3.0. The literature data shows great variation in the organic acid contents of black currant. Citric acid concentration between 13 and 40 g/L and malic acid concentration between 1.7 and 14 g/L have been reported (14-19). The present values fall within these ranges but the total concentration of acids (32 g/L) appeared to be lower than previously reported in the literature. For example, Matala (19) reported the total acid concentration for black currant to be 46 g/L. Regardless of variations between the reported and present data, malic acid and citric acid are invariably considered to be the main acids in black currant. Thus, these two acids and their conversions play a quality-related role in fermentation of black currant juice.

The main sugars in the undiluted black currant juice were fructose (43 g/L), glucose (35 g/L) and sucrose (3 g/L). The literature data shows also great variations in their concentrations. Values for fructose vary between 20.9 and 64 g/L, for glucose between 16 and 47 g/L and for sucrose between <1 and 18 g/L (14-22). Again, the concentrations of individual sugars of the present juice fall within the reported ranges but their sum is notably higher (81 g/L) than reported by some authors. For example, according to Haila (14) the total sugar content of black currant is only 46.4–57.0 g/L. The scattering data in both acid and sugar concentrations probably reflect large seasonal and geographical variations (23). However, regardless of these variations, the reduction of acidity while maintaining the fermentable sugars for alcohol production remains a major challenge.

Alcoholic fermentation of native juice

In the two parallel fermentation tanks 1a and 1b AF with *S. cerevisiae* was carried out first. The juice (1:1 dilution) was supplemented with 100 g/L sucrose prior to fermentation. As expected, the sugars were fermented to ethanol. The changes in

sugar, acid and ethanol concentrations during the course of fermentation are presented in figure 1. During the first 14 days the sucrose concentration decreased from the initial 92.7 to 0.6 g/L, glucose concentration from 22.0 to 0.5 g/L and fructose concentration from 26.2 to 8.7 g/L. Simultaneously, the ethanol concentration increased from 1.1 to 68.0 g/L. The acid concentrations remained almost unchanged. Slight increases in citric acid and acetic acid concentrations were noticed. The pH increased from the initial 3.0 to 3.1.

At the end of the AF, the total sugar concentration was approximately 10.0 g/L, the total acid concentration 18.1 g/L and the ethanol concentration 68.0 g/L. Thus, the intermediate products after the AF were red-coloured, low-alcoholic (6.8 %) black currant wines. These were the fermentation liquors for the malolactic fermentation (MLF) by *O. oeni* in the tanks 1a and 1b.

Malolactic fermentation of native juice

In the two parallel fermentation tanks 2a and 2b, MLF was carried out in the native 1:1-diluted juice without any glucose supplementation. During MLF malic acid concentration decreased and lactic acid concentration increased while the sugar concentrations remained practically unchanged (figure 2). Reduction of acidic taste was considered obvious by all panel members. This is in accordance with the measurements indicating that malic acid concentration decreased from 2.3 to 1.1 g/L, lactic acid concentration increased from 0.3 to 2.1 g/L, and the pH increased by approximately 0.1 pH-units. Simultaneously, citric acid concentration decreased only from 13.9 g/L to 13.2 g/L and acetic acid concentration increased from 0.1 g/L to 0.5 g/L. The addition of a second inoculumn after the first 24 hours to one of the fermentation tanks had practically no further influence on these malolactic reactions. Towards the end of the MLF, slight decreases in sugar concentrations were noticed indicating the initiation of a shift from malic acid utilization to sugar fermentation by the malolactic bacterium. This points to the importance of accurately timed cessation of MLF.

After 40 hours of MLF the total acid concentration was about 16.9 g/L, the total sugar concentration was reduced to 30.7 g/L due to its degradation during the last 5 hours. Ethanol concentration was increased to 5.9 g/L. The final pH was 3.1. Thus, the intermediate product after MLF was a juice with very low ethanol content and diminished acidity but still containing the original sugar. This was the fermentation liquor for the subsequent AF for the tanks 2a and 2b.

Malolactic fermentation after alcoholic fermentation

After the primary AF in tanks 1a and 1b, the wine was filter-sterilised and inoculated with *O. oeni* to initiate MLF. During 40 hours of MLF the concentration of malic acid decreased from 2.1 to 1.0 g/L, that of lactic acid raised from 0.3 to 1.8 g/L and the pH increased by about 0.1 pH-units (figure 1). Slight increase in acetic acid concentration was also noticed. MLF did not alter the sugars remaining after AF of the native juice. The ethanol already produced during AF did not disturb the malolactic reactions. A second inoculation with *O. oeni,* added after 24 h from the first one, did not further influence the profiles of acids or sugars.

After 40 hours of MLF the final concentrations of the products were, 18.2 g/L total acids, 15.3 g/L total sugars, 60.6 g/L ethanol and the pH was 3.2. Thus, the end product of the consecutive fermentations, where AF was the primary and MLF the secondary fermentation, was a red-coloured berry wine, with reduced acid concentration and with low concentration of sugars left. Consequently, the traditional order of fermentations used for grape juices is applicable also to black currant juice at least with the present organisms.

Alcoholic fermentation after malolactic fermentation

After the primary MLF in tanks 2a and 2b, the malic acid free black currant juice was filter sterilised and inoculated with *S. cerevisiae*. At this point the juice was supplemented with 100 g/L sucrose. The conversion of sugars to ethanol was clearly evident (figure 2). During the 14 days of AF, total sugar concentration decreased from 106.3 g/L to 16.9 g/L while ethanol concentration increased from

4.4 g/L to 54.7 g/L. Degradation of sucrose to glucose and fructose was also noticed. Changes in acid concentrations and pH were insignificant.

At the end of AF the final concentration of total acids was 12.6 g/L and the pH was 3.1. The end product of these consecutive fermentations, was a deep red-coloured low-alcoholic berry wine, where the composition of acids was changed and their total concentration reduced while some sugars still remained.

Comparison of the end products

Independent of in which order the two fermentations were carried out, the main alcoholic and malolactic reactions occurred. Differences in final sugar concentrations or pH of the wines were minor. The only clear difference between the two wines was in the remainder citric acid. When AF was carried out before MLF, citric acid concentration was even slightly higher than in the original juice. As the fermentations were carried out *vice versa* (MLF before AF), citric acid concentration diminished from the initial 13.8 g/L to 10.0 g/L. In the latter case also the degradation of malic acid was more complete and less lactic acid and acetic acid was formed. Thus, the content of total acids in the case of MLF→AF was over 6 g/L lower than when the fermentations were carried out in the order AF→MLF. Irrespective of the order of the fermentations it seems that the first fermentation rendered the juice more unfavourable for the second one.

4. Conclusion

MLF proved to be applicable to reduction of malic acid, and thus acidity, of berry juices such as black currant (12). Present work gives further an example that berry juices treated with a malolactic bacterium can be subjected thereafter to alcoholic fermentation. MLF of non-alcoholic berry juice enables more efficient conversion of malic acid into lactic acid than MLF of AF-fermented juice. The reduced acidity is probably advantageous also for the survival of the fermenting yeast. MLF before AF enables the use of low ethanol tolerant strains of malolactic organisms (9) and thus, wider options for flavour development would be available by strain selection. Previous data (12, 24) also show that MLF preserves the natural fermentable sugars when the duration of the process is controlled by malic acid measurements. Consequently, berry wines low in malic acid can be produced without addition of extra sugar for the subsequent yeast fermentation. Alternatively, the alcohol content of MLF-fermented juice can be increased by sugar additions. On the other hand, MLF in sugar-containing juices requires far greater vigilance to avoid rapid and serious spoilage (25). Especially, as malic acid is used up relatively rapidly in berry juices by the malolactic organism, the fermentation of sugars into volatile acids (acetic acid) may be initiated.

Present data shows also that MLF can be applied to berry wines in a traditional way, immediately after the yeast fermentation, for the elimination of malic acid. Then the malolactic process is however slower, probably due to reduction of nutrients by the yeast. From a practical point of view, optimisation of malolactic process is probably easier in juices already fermented by yeast, as the initiation of the sugar metabolism by the heterofermentative *O. oeni* would then be of less concern.

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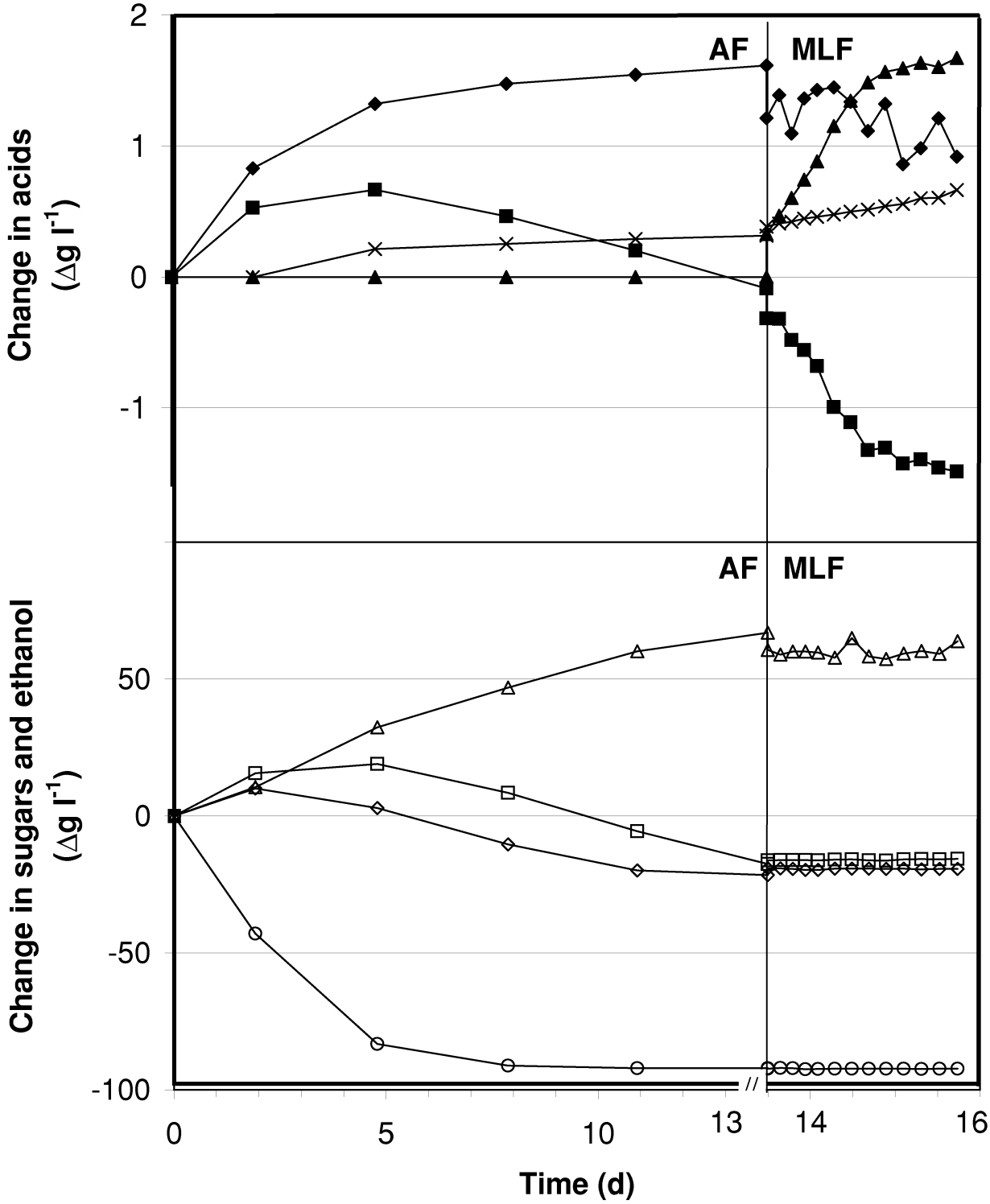
Legends for figures

Fig. 1: Changes in acid, sugar, and ethanol concentrations in black currant juice during consecutive alcoholic (AF) and malolactic fermentations (MLF). The vertical line on the time scale axis indicates the point at which filtration was performed. ♦, citric acid; ■, malic acid; ×, acetic acid; ▲, lactic acid; ◊, glucose; □, fructose; o, sucrose; Δ, ethanol.

Fig. 2: Changes in acid, sugar and ethanol concentrations in black currant juice during consecutive malolactic (MLF) and alcoholic fermentations (AF). The vertical line on the time scale axis indicates the point at which filtration was performed. ♦, citric acid; ■, malic acid; ×, acetic acid; ▲, lactic acid; ◊, glucose; □, fructose; o, sucrose; Δ, ethanol.


AF
MLF
Change in acids
(Δg l-1)
2
1
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-1
AF
MLF
Change in sugars and ethanol
(Δg l-1)
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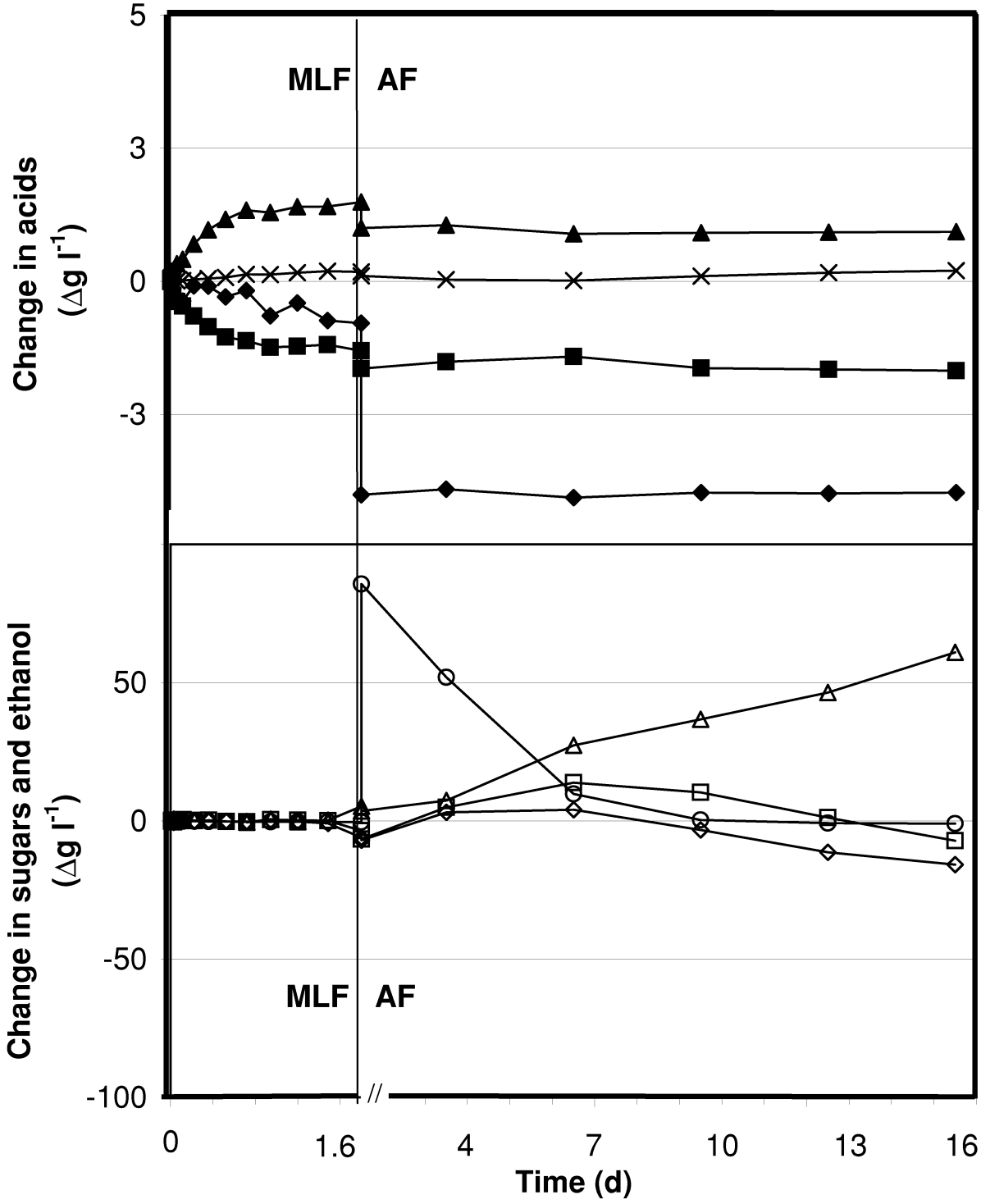
Time (d)


5
3
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MLF
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Change in acids
(Δg l-1)
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MLF
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