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SUGAR UPTAKE AND SUBSEQUENT ESTER AND HIGHER ALCOHOL PRODUCTION BY *SACCHAROMYCES CEREVISIAE*

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The production of a number of esters and higher alcohols by brewing strains of *Saccharomyces cerevisiae*, in synthetic media containing only glucose, fructose or maltose as sole carbohydrate source, has been investigated. Results indicated that production of most volatiles was generally lower when maltose was the sugar being fermented, despite maltose-grown cells having higher viabilities and vitalities than glucose or fructose-grown cells. There was no significant difference in the levels of esters and higher alcohols produced during fermentation when glucose or fructose was metabolised, although strain variation was observed. Similar results were obtained when wort was supplemented with either glucose, fructose or maltose. Wort supplemented with maltose produced fewer volatiles, especially higher alcohols, than that which had a hexose sugar added. The activity of ester-synthesising enzymes present in glucose or maltose PYN grown cells was also examined. Similar levels of ethyl acetate and isoamyl acetate were obtained when cells grown in either glucose or maltose PYN were disrupted and ester production monitored. The implications of these results for the fermentation of high-gravity worts are discussed.

Key Words: *Esters, higher alcohols, sugar uptake, fermentation,* Saccharomyces cerevisiae, *wort sugars.*

INTRODUCTION

The level of esters and higher alcohols produced by yeast during fermentation has a significant effect on the flavour of the alcoholic beverage. Shorter chain-length esters impart fruity flavours with soapier flavours being associated with increasing chain length, while alcohols can add a characteristic solvent-like flavour[13]. Esters can be formed from a simple condensation reaction between a carboxylic acid and an alcohol but the rate of this reaction is too slow to account for the levels of esters found in beer[15]. Esters are believed to be formed from an activated fatty-acylCoA molecule and an alcohol[22,29] in a reaction catalysed by an acyltransferase enzyme. It is not known whether each activated fatty-acylCoA molecule has its own unique acyltransferase or if several such enzymes can catalyse the formation of all esters, although there are believed to be at least two acyltransferases present in yeast[17,27]. Esterases hydrolyse esters into their constituent carboxylic acid and alcohol[19,40,44]. However, these enzymes are also believed to be able to function "in reverse", and catalyse the formation of esters from acids and alcohols[23,37]. Two pathways exist for the production of higher alcohols, both of which involve amino-acids (which can be formed via carbohydrate), with each amino-acid corresponding to a different alcohol[2]. The biochemical significance of the production of these volatiles is still unclear and there are a number of well documented factors which can influence their synthesis.

Different yeast strains will produce varying levels of esters and alcohols when the same wort is fermented[31,32,33]. Varying fermentation parameters such as temperature[16,32], pitching-rate[28], oxygenation[1], pressure[24,31,32] and wort gravity[34] will also result in different levels of volatile production. Wort nitrogen levels[1,14,33], fatty-acids[42,43] and metal ions[20] can also influence the production of volatiles. Additionally, amino-acid concentration and composition of the wort is significant for the synthesis of higher alcohols[36], as these volatiles' formation is linked to yeast protein synthesis, and amino-acids are also essential for yeast growth. The production of these volatiles is believed to affect the subsequent production of esters[5].

Carbohydrate source is also believed to influence the production of esters and higher alcohols. Jenard and Devreux[24] found that wort supplemented with sucrose led to an increase in higher alcohol production, as did Drews and Riemann[10], although conflicting results have also been cited. It has been reported that fermentation with fructose produced greater levels of higher alcohols than glucose[14,35], with the converse result having also been recorded[45]. Regarding ester production, it has not been established whether metabolism of glucose or fructose results in the highest levels, although several reports suggest that wort supplemented with maltose leads to the production of lower levels of these volatiles[14,30,35,45]. The differences between these reports are most probably due to different fermentation conditions and yeast strains being employed.

The purpose of this study was to determine and compare the levels of production of several esters and higher alcohols, under constant fermentation conditions using several brewing strains of *Saccharomyces cerevisiae*, when either glucose, fructose or maltose was employed as the sole carbohydrate source in shaking flask fermentations. Wort fermentations were also undertaken. Levels of ester-synthesising enzymes present in glucose and maltose grown cells were also investigated.

MATERIALS AND METHODS

Yeast strains and maintenance

Brewing strains of *Saccharomyces cerevisiae* (ale and lager) were employed during this study. All strains were maintained on 10% glucose peptone-yeast extract-nitrogen (PYN) agar slopes and plates and stored at 4°C.

Media

Synthetic (PYN) media was employed, which comprised (g/L): bacteriological peptone (Oxoid) 5.25; yeast extract (Oxoid) 4.0; KH_2PO_4 (Sigma) 3.0; $(NH_4)_2SO_4$ (BDH) 1.5; $MgSO_4.H_2O$ (BDH) 1.5; glucose (Sigma) 100 (for agar plates and slopes). Fermentation media was as above except the carbohydrate source was either glucose, fructose or maltose at 20 or 40 g/L.

All-malt wort was produced using the Centre's 2 hL per brew pilot brewery[8].

Fermentations

For biomass production, one yeast colony was removed from a plate and placed into 5 mL of sterile liquid PYN media

containing 2% or 4% of either glucose, fructose or maltose. After 24 h at 25°C, 1 mL was transferred to 100 mL of similar media in a 250 mL conical flask for a further 48 h at 25°C and shaken at 150 rpm. The yeast was then pitched at 1×10^6 cells/mL, into 300 mL of similar media in a 500 mL conical flask and continuously shaken at 150 rpm. All fermentations were performed in duplicate at least and maintained at 25°C. Samples were removed every 24 h until the fermentation was complete in order to determine: cell number and viability, vitality, specific gravity, pH, ethanol concentration, sugar utilisation, ester and higher alcohol production.

Cell count, viability and biomass

Cell number was determined using an Improved Neubauer Haemocytometer. Cell viability was estimated using methylene blue staining[12]. Biomass was determined by washing the pellet obtained from 8 mL of media with 5 M ammonium sulphate (×2) and distilled water. After centrifugation (900 *g* for 5 min) the pellet was resuspended in ethanol and poured into a weighed aluminium dish. After evaporation of ethanol, the dish was placed into an oven at 105°C for 24 h after which it was allowed to cool before re-weighing.

Vitality

Yeast cell vitality was determined throughout the fermentation using the modified acidification power test[21]. The results were expressed as the pH at time 10 min minus that at 25 min, and thus the pH of the water need not be taken into account when determining the vitality of the yeast.

Ethanol determination

Ethanol content was determined using a Hewlett Packard 5890 Series II GC with a split/splitless injector and Chrompack CP SIL 5CB column (10 m×0.32 mm, 1.2 µm film thickness). Butanol was used as the internal standard.

Sugar utilisation

The removal of sugars from the growth medium was followed using a PAAR Model DMA 46 Digital Density Meter. The concentration of glucose, fructose or maltose was monitored using High Performance Liquid Chromatography (HPLC) with a Dionex Carbopak PA-100 Guard Column (4×50 mm), a Dionex Carbopak PA-100 Column (4×250 mm) and a Dionex PAD electrochemical detector. HPLC grade water and 500 mM sodium hydroxide were used as eluents, and cellobiose was employed as the internal standard.

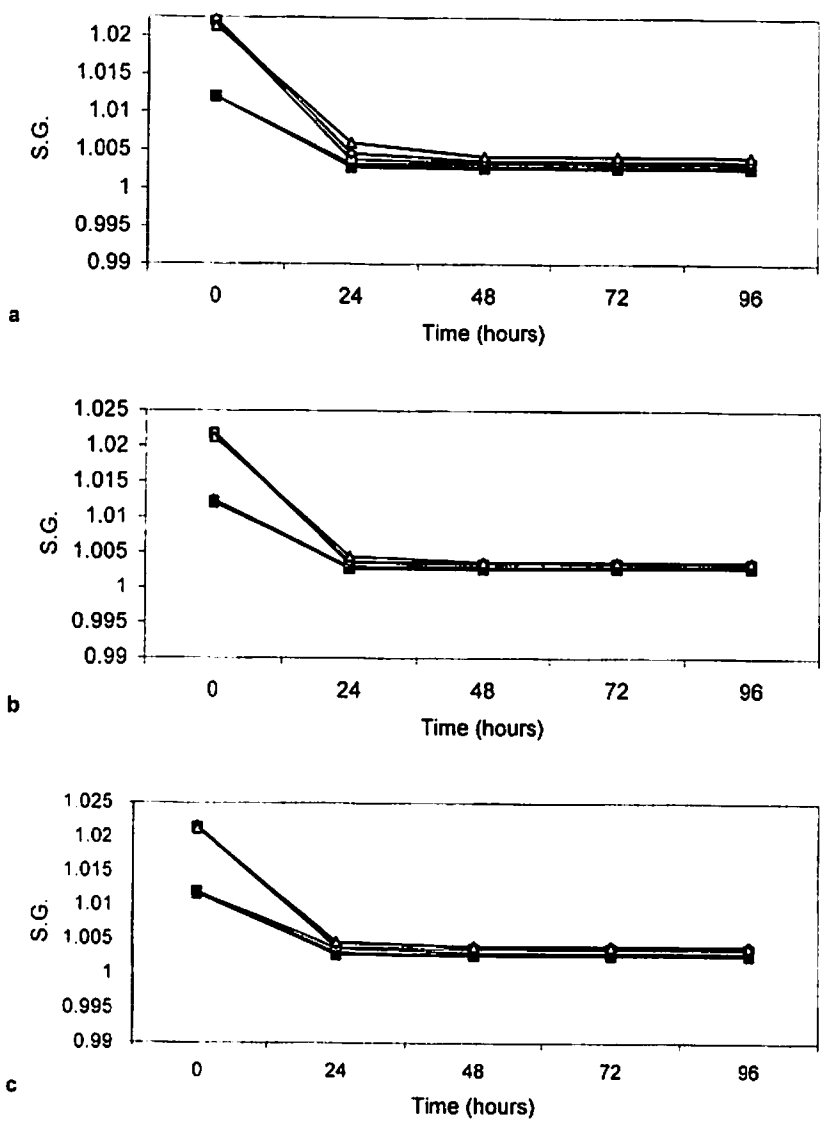

FIG. 1. The decrease in gravity produced by *Saccharomyces cerevisiae* ale strains. (a) #1006; (b) #3; (c) #70. ■, 2% glucose PYN; ◆, 2% fructose PYN; ▲, 2% maltose PYN; □, 4% glucose PYN; ◇, 4% fructose PYN; △, 4% maltose PYN.

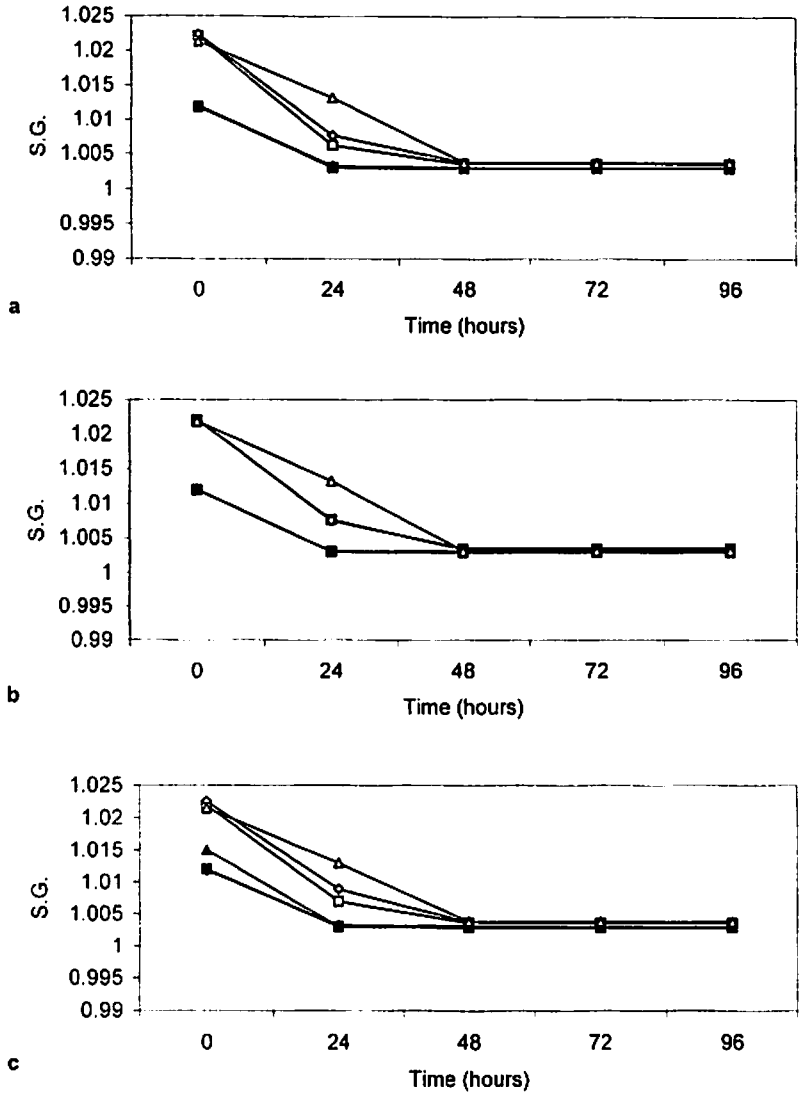

FIG. 2. The decrease in gravity produced by *Saccharomyces cerevisiae* lager strains. (a) #22; (b) #15; (c) #1056. Symbols as in Figure 1.

Headspace analysis of esters and higher alcohols

Production of ethyl acetate, isoamyl acetate, ethyl butyrate, propanol, isobutanol, 2-methyl butanol and 3-methyl butanol was monitored using a Hewlett Packard 5890 Series II GC split/splitless injector with Flame Ionisation Detector and Chrompack Cp-Wax-57-CB column (0.25 mm×60 m, film thickness 40 µm). 3-heptanone was used as the internal standard.

Assay of ester-synthesising enzymes

Ester-synthesising enzymes were assayed using the method described by Malcorps and Dufour[26]. The reaction was carried out at pH 7 and incubated at 30°C for 30 minutes.

Whole cell homogenate was obtained by harvesting the yeast (900 g for 5 min) and washing three times with distilled water. Pelleted yeast was resuspended in homogenisation buffer A (2.2 mL/g). PMSF (10 µL, 100 mM stock solution in dimethyl-sulphoxide) and glass beads (Ballotini from Jencons, Grade 9, 0.29–0.42 mm diameter) were added to 1 mL of suspension in 1.5 mL Eppendorf tubes and vortexed eight times for 1 minute (cells were stored once for 1 minute between successive vortices).

Ethyl acetate and isoamyl acetate were measured using a Hewlett Packard 5890 Series II GC with a split/splitless injector and Chrompack CP SIL 5CB column (10 m×0.32 mm, 1.2 µm film thickness).

RESULTS

Fermentation performance

Figures 1 and 2 show the decrease in gravity produced by the six yeast strains employed in this study. The three ale strains all attained attenuation within 24 hours for fermentations in 2% sugar PYN media, with the fermentations in 4% sugar requiring slightly longer to do so. However, the lager strains took subsequently longer to attenuate when grown in 4% sugar PYN, although attenuation had been reached by 24 hours when fermented in 2% sugar. These results all applied regardless of the carbohydrate source being used and were corroborated by HPLC analysis (data not shown).

The biomass of the six strains after 96 hours of fermentation is shown in Figure 3. All strains produced similar levels when grown in either glucose, fructose or maltose, with an increase in growth when the cells were grown in 4% sugar PYN.

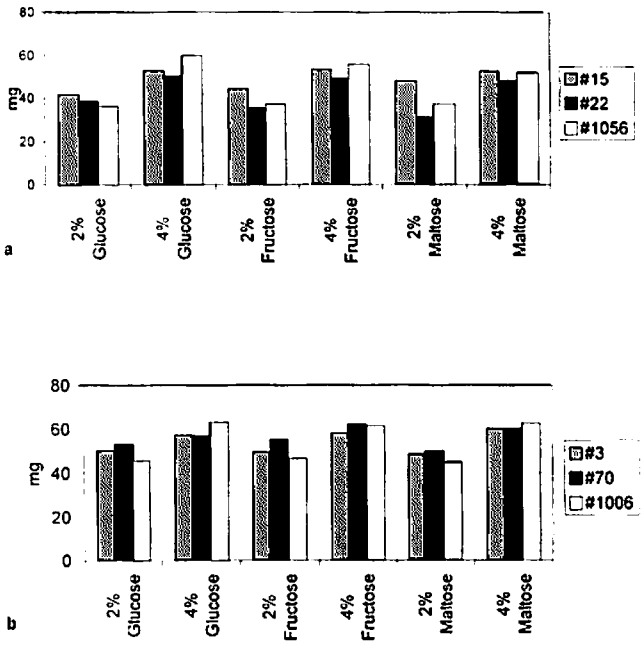

FIG. 3. The final biomass of *S. cerevisiae* obtained from 8 mL media after 96 hours of fermentation. (a) lager strains; (b) ale strains.

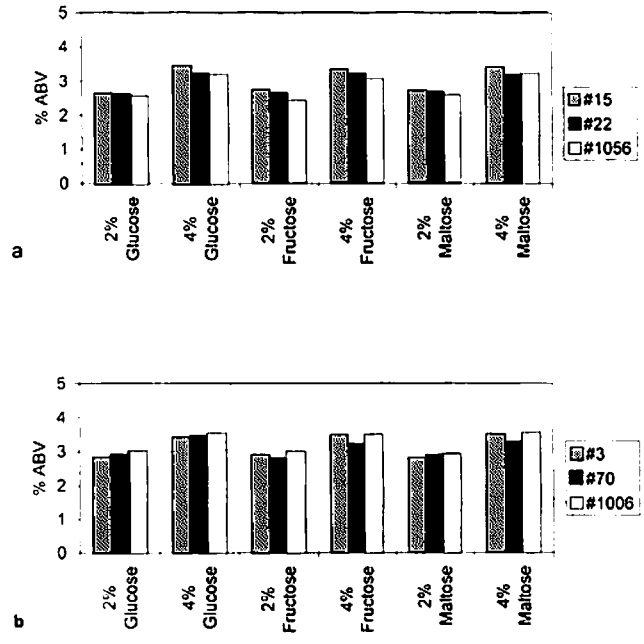

FIG. 4. Ethanol produced by *S. cerevisiae* after 96 hours of fermentation in synthetic media. (a) lager strains; (b) ale strains.

As can be seen from Figure 4, the production of ethanol was again similar for all six strains when they were grown in glucose, fructose or maltose. As would be expected, higher levels of ethanol were obtained when the strains are fermented in 4% sugar PYN as opposed to 2%. However the increase in ethanol production was not as large as would be expected. This was most probably due to the fact that the fermentations were taking place in shaking (aerobic), as opposed to static, flasks. Strain #1006 produced marginally higher levels than the other ale strains, as did lager strain #15 compared with lager strains #22 and #1056.

Viability and vitality

The viabilities of the cells after 96 hours of fermentation are shown in Figure 5. It can be seen that in all strains there was a considerable difference in final viability when the strains are grown in maltose PYN, either 2% or 4%. This was most notable in strain #22 in which approximately 92% and 93% of cells were viable when grown in 2% and 4% glucose or fructose PYN, respectively, when grown in 2% or 4% maltose PYN this figure rises to 98% and 99% of viable cells. For all strains there were

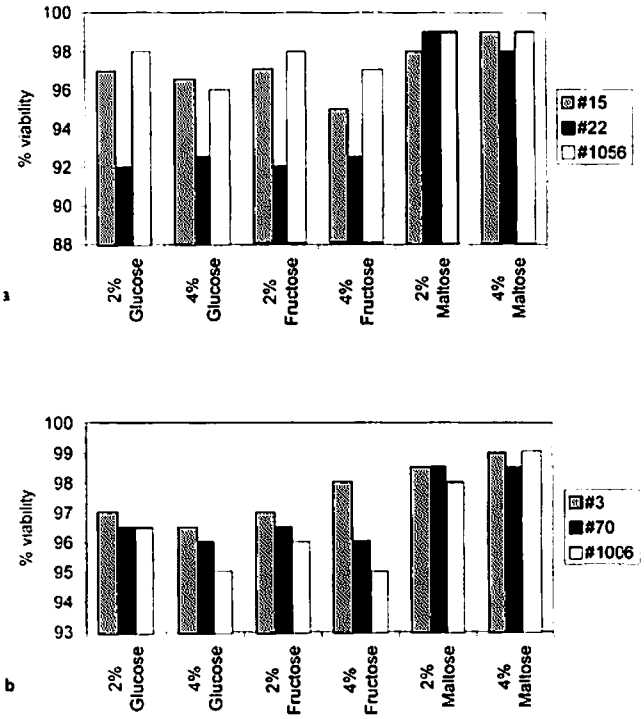

FIG. 5. Viabilities from methylene blue staining of *S. cerevisiae* after 96 hours fermentation in synthetic media. (a) lager strains; (b) ale strains.

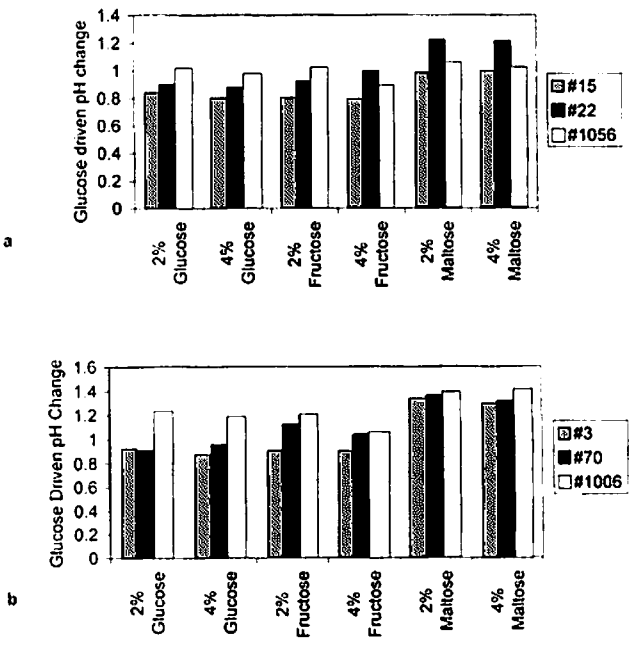

FIG. 6. Acidification power of *S. cerevisiae* after 96 hours fermentation in synthetic media. (a) lager strains; (b) ale strains.

TABLE I. The maximum level of ethyl acetate produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	1.1	1.21	1.01	4.13	3.91	2.79
70	1.35	1.52	1.22	2.97	3.11	2.59
1006	1.17	1.1	0.95	3.13	3.38	2.71
15	0.94	0.99	0.86	6.0	6.07	5.8
22	1.29	1.25	1.05	3.75	3.75	3.28
1056	1.36	1.39	1.19	4.6	3.88	3.5

TABLE II. The maximum level of isoamyl acetate produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	0.037	0.047	0.033	0.138	0.147	0.136
70	0.031	0.035	0.027	0.059	0.055	0.043
1006	0.026	0.028	0.017	0.05	0.065	0.039
15	0.053	0.052	0.046	0.22	0.219	0.21
22	0.054	0.063	0.062	0.288	0.3	0.25
1056	0.07	0.066	0.056	0.1685	0.17	0.165

TABLE III. The maximum level of ethyl butyrate produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	0.06	0.06	0.05	0.161	0.17	0.1
70	0.094	0.094	0.091	0.155	0.158	0.107
1006	0.112	0.119	0.1	0.119	0.131	0.108
15	0.11	0.119	0.092	0.22	0.27	0.162
22	0.246	0.232	0.21	0.261	0.284	0.257
1056	0.123	0.119	0.105	0.230	0.190	0.15

no significant differences in the viabilities of the yeast when they were grown in either glucose or fructose PYN.

The vitality of the strains after 96 hours fermentation is shown in Figure 6. The glucose driven pH change was greatest for all strains when they were grown in maltose supplemented PYN. With the lager strains, the increase in vitality when grown in 2% maltose compared to 2% glucose, varied from 5% (#1056) to 35% (#22). For fermentations in 4% sugar, the increase in vitality again varied from 5% (#1056) to 38% (#22). Fermentations in fructose had similar vitalities as those in glucose. The difference in vitality between cells grown in 2% fructose PYN compared to 2% maltose PYN ranged from 4% (#1056) to 33% (#22). Increases in vitality of 14% (#1056) to 25% (#15) were observed when strains were grown in 4% maltose PYN instead of 4% fructose.

More pronounced changes in vitality were observed when ale strains were grown in glucose or fructose compared to maltose. In 2% glucose PYN, the vitality of the cells ranged from 14% less (#1006) to 54% less (#70) compared to the same strains grown in 2% maltose. Similarly, when grown in 4% glucose, the decreases in vitality ranged from 20% (#1006) to 47% (#3) compared to maltose grown cells. In fermentations with 2% fructose PYN, the cells' vitalities were from 16% less (#1006) to 43% less (#3) than with 2% maltose grown cells. Increases in vitality of between 26% (#70) and 43% (#3) were observed when cells were grown in 4% maltose PYN instead of 4% fructose PYN.

Volatile production

Table I shows the production of ethyl acetate by ale and lager strains in 4% sugar PYN media. In all cases, lower levels of this ester were obtained when maltose was metabolised compared to the hexose sugars, most notably in strains #3 (approximately 45% less) in 4% sugar fermentations (Fig. 7).

Isoamyl acetate production was also lower in all strains (Table II) when maltose was the carbohydrate utilised, with up to 40% less than with glucose or fructose PYN being obtained in both 2% and 4% sugar fermentations with strain #1006 (Fig. 8).

Table III shows the production of ethyl butyrate. The levels of this ester were similar regardless of the carbohydrate source in strain #1006 and #70. However with strain #3 and lager strain #15 fermentation with 4% maltose resulted in up to 70% less and 60% less of this ester, respectively, when compared to levels obtained from fermentations using hexose sugars (Fig. 9).

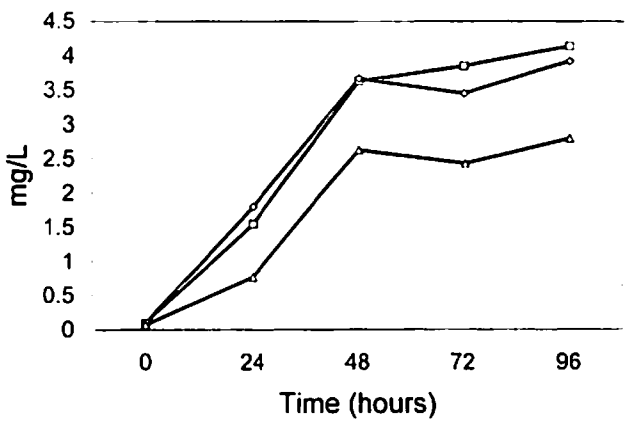

FIG. 7. Ethyl acetate production by *S. cerevisiae* strain #3 in 4% sugar synthetic media. □, glucose PYN; ◇, fructose PYN; △, maltose PYN.

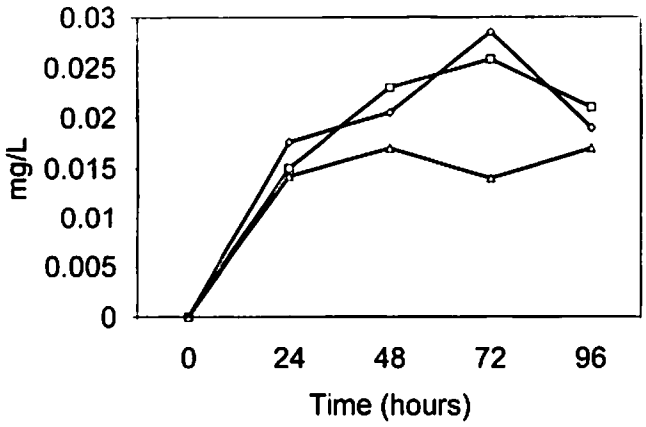

FIG. 8. Isoamyl acetate production by *S. cerevisiae* strain #1006 in 2% sugar synthetic media. Symbols as in Figure 7.

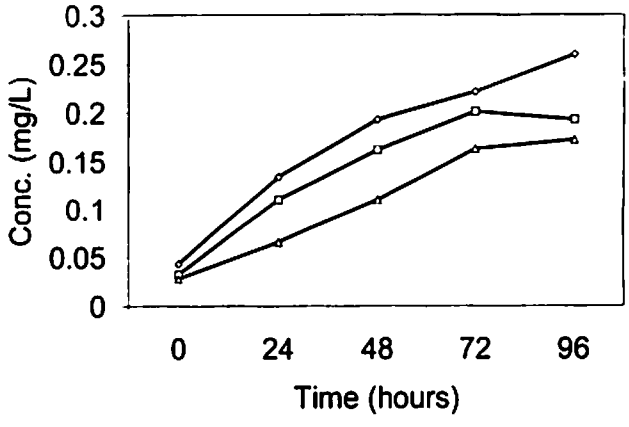

FIG. 9. Ethyl butyrate production by *S. cerevisiae* strain #15 in 4% sugar synthetic media. Symbols as in Figure 7.

TABLE IV. The maximum level of isobutanol produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	8.78	10	7.23	19.06	18.93	12.3
70	17.31	19.52	16.32	20.2	20.45	16.46
1006	10.74	9.85	7.92	16.62	14.97	12.4
15	6.21	6.28	5.46	15.17	15.2	13.6
22	6.1	6.1	3.76	8.1	7.48	5.02
1056	8.43	8.47	8.11	13.85	12.55	12.3

TABLE V. The maximum level of propanol produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	8.14	8.9	7.3	17.04	18.72	14.1
70	8.03	9.4	7.3	12.88	13.56	11.4
1006	8.51	8.79	6.4	15.8	16.4	12.3
15	7.18	7.51	5.73	22.8	24.07	19.9
22	8.14	8.48	7.5	15.6	16.37	11.7
1056	6.8	6.4	5.8	14.06	13.89	10.2

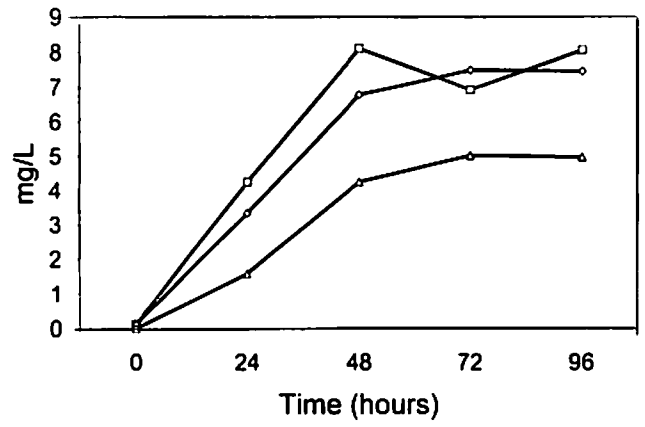

FIG. 10. Isobutanol production by *S. cerevisiae* strain #22 in 4% sugar synthetic media. Symbols as in Figure 7.

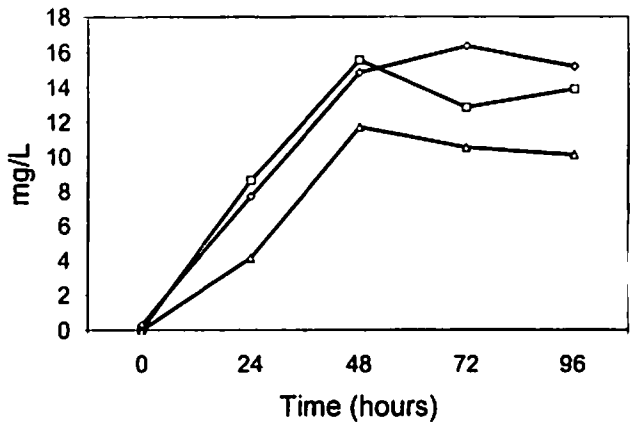

FIG. 11. Propanol production by *S. cerevisiae* strain #22 in 4% sugar synthetic media. Symbols as in Figure 7.

The production of isobutanol during fermentation is shown in Table IV. Significantly lower levels were again obtained when maltose was fermented, although strain to strain differences were once more observed. Strain #22 (Fig. 10) gives 60% more and 50% more of this alcohol when 4% glucose or fructose PYN was fermented, respectively, instead of 4% maltose PYN. In 2% sugar PYN, approximately 90% less is obtained when maltose was fermented compared with the hexose sugars (Table

TABLE VI. The maximum level of 2-methyl butanol produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	7.6	7.99	6.57	14.7	14.4	9.54
70	13.15	12.88	12.3	16.73	18.23	17.3
1006	7.54	7.23	6.6	8.63	9.69	8.48
15	7.61	8.2	7.06	9.81	8.90	9.78
22	8.12	8.56	7.5	11.05	10.5	8.79
1056	10.22	12.12	9.8	14.2	12.6	12.1

TABLE VII. The maximum level of 3-methyl butanol produced (mg/L) by *S. cerevisiae* during fermentation of synthetic PYN media

Strain #	2% Glucose PYN	2% Fructose PYN	2% Maltose PYN	4% Glucose PYN	4% Fructose PYN	4% Maltose PYN
3	29.87	32.5	25.4	60.69	60.72	38.02
70	30.91	32.1	29.66	41.7	42.04	39
1006	19.12	19.47	14.99	29.98	32.55	26.8
15	22.63	24.7	21.41	46.54	43	41.2
22	32.18	34.47	29.43	42.69	42.49	37.63
1056	31.54	34.76	29.08	60.71	60.33	40.63

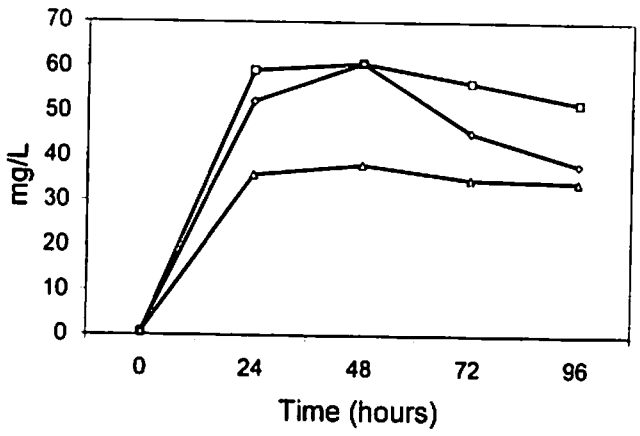

FIG. 12. 3-Methyl butanol production by *S. cerevisiae* strain #3 in 4% sugar synthetic media. Symbols as in Figure 7.

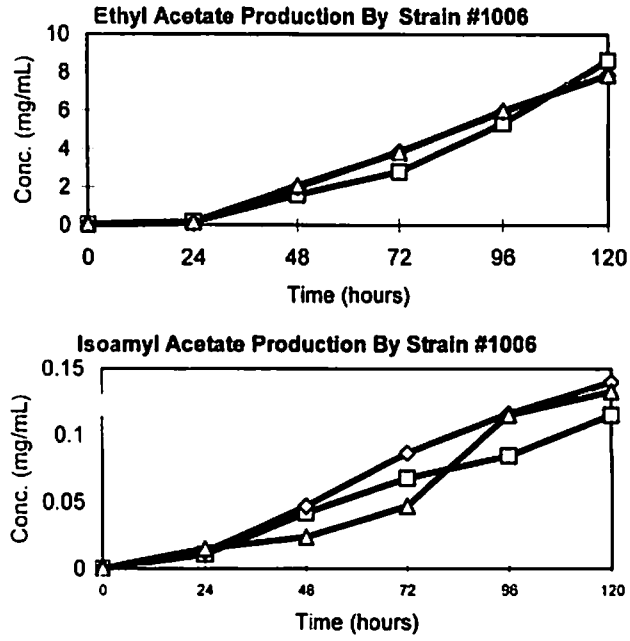

FIG. 13. Production of esters by *S. cerevisiae* ale strain #1006 in 12°P wort supplemented with 4% carbohydrate. □, normal gravity wort plus 4% glucose; ◇, normal gravity wort plus 4% fructose; △, normal gravity wort plus 4% maltose.

IV). Values ranging from 2% less to 58% less isobutanol were obtained from the other strains when maltose was metabolised rather than glucose or fructose.

Levels of propanol production were lower in all strains when maltose was the carbohydrate employed (Table V). In 4% sugar media, up to 54% less propanol was produced when maltose was fermented instead of glucose or fructose (strain #22; Fig. 11).

The variation in the production of 2-methyl butanol was not as pronounced as the preceding alcohols (Table VI). There were no significant differences in the production of this alcohol regardless of the carbohydrate source in 4% or 2% sugar fermentations using ale strain #70 and lager strain #15, whereas strain #3 shows over a 50% decrease in production when maltose was fermented as opposed to glucose or fructose.

Regarding the production of 3-methyl butanol (Table VII), strain #3 again showed the greatest difference in production depending on the carbohydrate source, with up to 58% less obtained when maltose, rather than glucose or fructose, was metabolised in both 2% and 4% sugar fermentations (Fig. 12). Strains #70 and #15 show the least variation in levels obtained.

Figures 13 and 14 show the production of volatiles by ale strain #1006 in normal-gravity wort supplemented with either 4% glucose, fructose or maltose. Lower levels of all volatiles were generally obtained from the wort supplemented with maltose, although not to the same extent as with PYN media. Similar results were obtained using lager strains (data not shown).

Production of volatiles by disrupted cells

Figure 15 shows the production of ethyl acetate and isoamyl acetate by disrupted cells. There were no significant differences in the levels of either ester produced when cells are pre-grown in 4% glucose or 4% maltose PYN media.

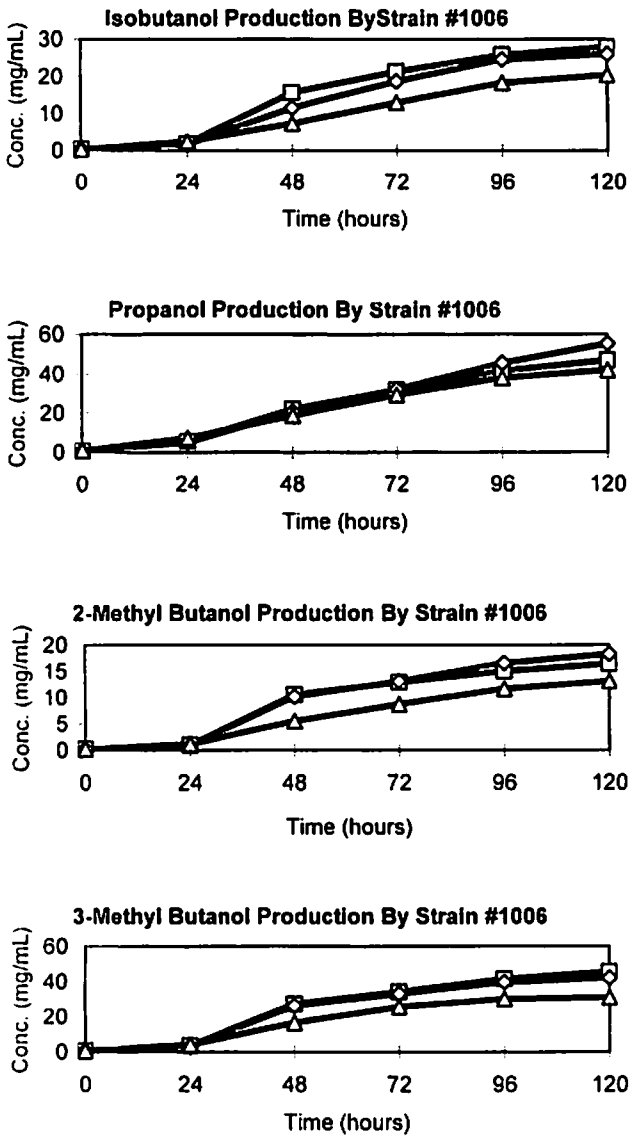

FIG. 14. Production of higher alcohols by *S. cerevisiae* ale strain #1006 in 12°P wort supplemented with 4% carbohydrate. Symbols as in Figure 13.

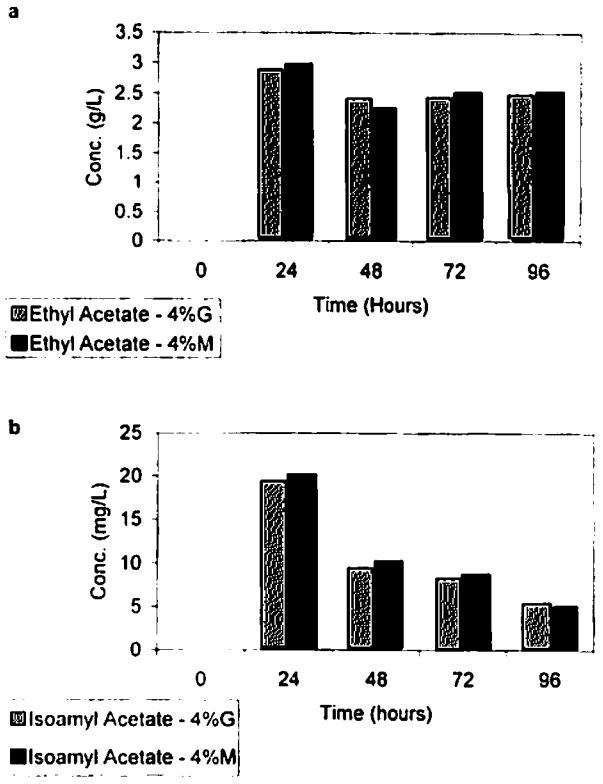

FIG. 15. The production of (a) ethyl acetate and (b) isoamyl acetate using a cell free extract of *S. cerevisiae* strain #3 pre-grown in either 4% glucose or 4% maltose PYN.

DISCUSSION

Although brewing fermentations are processes by which carbohydrate is essentially converted to ethanol and carbon dioxide, many more compounds are produced during this process, a number of which have organoleptic properties, and two such groups are esters and higher alcohols. Varying the carbohydrate source is believed to modify the levels of production of these metabolites, although it is not know why. Entry of the hexose sugars, glucose and fructose, into the yeast cell is facilitated by the same protein, although utilisation of glucose occurs more quickly than that of fructose when the two sugars are fermented separately[7] possibly due to the differing affinities of the sugars for the transporter[3]. The disaccharide maltose is internalised by the cell only when 40%–50% of glucose has been removed from the growth media[36] and occurs via a different, active, transporter[38]. In this study, glucose, fructose and maltose were fermented separately, under identical conditions, in order to eliminate any possible inhibition of uptake of sugar or possible interaction, and production of the volatile compounds ethyl acetate, isoamyl acetate, ethyl butyrate, isobutanol, propanol, 2-methyl butanol and 3-methyl butanol was monitored.

The fermentation performance of the six brewing yeast strains employed in this study were very similar regardless of the carbohydrate source being fermented. Ethanol production by yeast is of paramount importance to the brewer and it can be seen from Figure 4 that levels obtained also do not vary greatly among a given strain when any of the three carbohydrates were fermented. As would be expected, 4% sugar fermentations produced higher ethanol concentrations than when 2% sugar was present, although it was not double the amount, which is probably due to the high level of oxygen present in shaking flask fermentations.

Figure 1 shows that all ale strains achieved attenuation within 24 hours when 2% sugar PYN media was used. Slightly longer was needed for all the sugar to be removed from the growth media when 4% carbohydrate was present, although all ale strains reached attenuation at the same point no matter which sugar was being fermented. The lager strains studied also achieved attenuation within 24 hours of fermentation when 2% sugar PYN was used, regardless of carbohydrate source. However, in 4% sugar media, the specific gravity of the fermentations was considerably higher than the ale strains after 24 hours (Fig. 2). The removal of maltose from the 4% sugar growth media was also substantially slower than that of the hexose sugars. This could perhaps explain why the biomass obtained from maltose grown cells was less than that of glucose or fructose grown cells during the early stages of fermentation (data not shown). After 96 hours of fermentation, the biomass was not substantially different when grown in the three sugars (Fig. 3).

Figures 5 and 6 show the viabilities and vitalities of the cells, respectively, following four days of fermentation. For all strains, in both sugar concentrations, cells grown in maltose PYN consistently had greater viabilities and enhanced vitalities compared with their glucose or fructose grown counterparts, and they also had enhanced viabilities following acid-washing (data not shown). This may be as a result of the slower initial

uptake of maltose and consequent reduced growth rate, although a comparison of the glycogen and trehalose content of glucose, fructose and maltose grown cells may explain the resilience of maltose grown yeast.

Despite the apparent sturdiness of the maltose grown cells, the production of volatiles (Figs 7 to 12 and Tables I to VII) was generally lower than that of glucose or fructose grown cells, although strain to strain differences were observed. The production of the esters ethyl and isoamyl acetate and ethyl butyrate was lowest in all strains at both sugar concentrations when maltose was metabolised, which agrees with earlier studies[14,30,35,45]. Ethyl acetate synthesis did not vary greatly amongst the six strains, although the production of the two other esters studied seemed to be greater when lager strains are used, which contradicts previous work[31] (although different fermentation conditions were employed in this study). However, due to the low levels of synthesis of these esters, and the reduced accuracy of the GC at these concentrations, these results should be noted with caution.

There was no distinct difference in the levels of higher alcohols obtained from either fermentation of ale or lager strains. Cells grown in maltose tended to produce lower levels of these volatiles with hexose grown cells giving similar amounts, and the distinction was clearer with higher alcohol production than with ester production. This was reinforced with all-malt wort supplemented with glucose, fructose or maltose (Figs 13–14). Variation in pattern was again found amongst strains, for example there was no significant differences in the levels of 2-methyl butanol obtained from any sugar in strain #15, while other strains showed a marked decrease when maltose was fermented (Table VI). There was also a difference among strains regarding the increase in volatile production when fermented in 4% sugar PYN compared to 2%. Strain #3 gave a respective increase of 54%, 48% and 41% in the production of isobutanol (Table IV) when grown in 4% glucose, fructose and maltose PYN, when compared with 2% sugar, while strain #70 gave only a rise of between 1% and 14% when grown in the richer media. This suggests that the factors which influence the production of volatiles, other than amount of fermentable sugar, vary from strain to strain.

The lower levels of volatiles produced from fermentation with maltose could be due to a number of reasons. It is possible that fermentation with maltose inhibits the transport of volatiles out of the cell, perhaps by altering the plasma membrane, thus giving the impression that fewer volatiles are produced. Another possibility is that metabolism of maltose produces lower levels of acetyl-coA, which has been suggested[39], resulting in fewer esters due to lack of substrate. It has been proposed that ester production is linked to lipid metabolism during fermentation[11,33]. If this is the case, and if for some reason maltose metabolism produces fewer of these toxic fatty acids, then it would be reasonable to assume that fewer esters would also be produced. Lower levels of ester synthesising enzymes (i.e. esterases and acyltransferases) in maltose grown cells would also lead to fewer levels of these volatiles. Figure 15 shows that there was no significant difference in the level of ethyl or isoamyl acetate produced when cells were pre-grown in glucose or maltose, disrupted and then assayed for ester synthesising enzyme activity. This suggests that the lower levels of esters produced when maltose is fermented by non-disrupted cells, must be due to some other aspect other than the amount or activity of ester synthesising enzyme activity of maltose grown cells.

The lower levels of volatiles produced from metabolism of maltose could have significant implications for brewery fermentations. Fermentations using brewers' wort with higher than normal levels of maltose (e.g. 70% of total carbohydrate) would produce fewer volatiles, and this could result in notable flavour changes in the alcoholic beverage being produced. Altering the level of fermentable maltose in the wort could lead to better control of flavour compound production and better flavour-matching when using high-gravity worts. Current work will attempt to determine the amount of maltose needed to supplement high-gravity wort in order to obtain similar levels of volatiles obtained with lower gravity wort, following dilution of the alcoholic product.

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