Influence of beer ethanol content on the wort flavour perception

Philippe Perpète and Sonia Collin[1]

Laboratoire de Brasserie et des Industries Alimentaires

Université Catholique de Louvain, Place Croix du Sud, 2 bte 7

B-1348, Louvain-la-Neuve - Belgium

ABSTRACT

The sensorial threshold value of 3-methylthiopropionaldehyde appears to be influenced by the beer matrix. We have shown that, in typical lager beers, ethanol significantly increases aldehyde retention, leading to lower perception of the worty character. In alcohol-free beers, both the absence of ethanol and the higher level of mono- and disaccharides such as maltose intensify such off-flavours. As improving Strecker aldehyde reduction by yeast seems difficult for alcohol-free beer production without using genetically modified yeasts or adding steps in the process, another interesting approach could be to add "ethanol-mimics" to increase off-flavour thresholds.

1. Introduction

Wort aroma compounds in alcohol-free beers are Strecker aldehydes derived from leucine, isoleucine and especially methionine (Perpète and Collin, 1999[a]). Although viable yeasts can reduce these carbonyle compounds enzymatically to less flavouring alcohols (Peppard and Halsey, 1981; Debourg *et al.*, 1994; Perpète and Collin, 1999[b]), residual concentrations are often enough to impart the worty taste to alcohol-free beers (Perpète and Collin, 1999[c]). Flavanoids recently emerged as responsible for aldehyde retention hindering complete enzymatic reduction by yeast at low temperature (Perpète and Collin, 1999[d]).

According to literature, 3-methylthiopropionaldehyde shows threshold values depend strongly on the matrix: 0.4 ppb in sunflower oil (Grosch, 1994), 1.7 ppb in water (Grosch, 1994) and 250 ppb in

regular beer (Meilgaard, 1975). We therefore logically suspected that worty flavours could be perceived more easily in alcohol-free beers owing to the absence of ethanol.

Comparing alcohol-free and lager beers, however, is complex. Alcohol-free beer has no more than 0.1 % ethanol but contains dextrins and fermentable sugars up to 8 °Plato (1°Plato is equal to 1 g of sugar per 100 g beer). Lager beers usually contain around 5 % ethanol, residual dextrins up to 3°Plato, and no more fermentable sugars if attenuation is complete.

The effect of ethanol on sweet and bitter tastes has been tested previously (Pokorny *et al.*, 1996). Some retention has been evidenced above 20 % ethanol for long chain esters (Piggott *et al.*, 1996). Interactions of flavour compounds, mainly alcohols, ketones and esters, with sugar and glucanes have also been investigated (Nawar, 1971 ; Louant and Dufour, 1981 ; Nahon *et al.*,1998).

As improving Strecker aldehyde reduction by yeast seems difficult for alcohol-free beer production without using genetically modified yeasts or adding steps in the process, another interesting approach could be to add "ethanol-mimics" to increase off-flavours thresholds.

The aim of this study was thus to assess the how various beer constituents or additives influence aldehyde perception. For experimental reasons, mainly 3-methylbutanal and 2-methylbutanal were monitored by dynamic headspace analysis in retention experiments but 3-methylthiopropionaldehyde was selected as Strecker aldehyde reference for triangular tests in model media.

2. Materials and methods

2.1. Reagents

3-Methylthiopropionaldehyde (+95 %) was purchased from Acros Chemika (Belgium). 2-Methylbutanal (95 %) and 3-methylbutanal (98%) were purchased from Janssen Chimica (Belgium). Ethanol (99 %) was from Merck (Germany). Glycerol (98 %) was purchased from Aldrich Chemicals (Belgium). Flolys C4776 S (sugar syrup, see Table 1) was from Roquette (France) and Maldex 20 (dextrin powder, see Table 1) was from Amylum S.A.(Belgium).

Table 1. Composition (% on dried matter) of Flolys C4776 S and Maldex 20

	Flolys C4776 S	Maldex 20
Dextrose	7%	1.5%
Maltose	48-53%	7%
Maltotriose	17%	10%
Superior polysaccharides	23-28%	81.5% with: 51.9 % DP <25 19.9 % DP 25-60 25.6 % DP 60-300 1.9 % DP 300-600 0.7 % DP > 600

DP = degree of polymerisation

2.2. Sensory analysis

Triangular tests were conducted with two model systems: either a sugar syrup (alcohol-free-beer-like medium) with 6 % (w/v) sugars (Flolys) or a 5 % (v/v) ethanol solution containing 3 % (w/v) Maldex 20 (lager-beer-like medium) (see Table 1). For each medium, 11 concentrations of 3-methylthiopropionaldehyde (from 0.025 to 100 ppb) were given in 4 sessions to a panel of 10 assessors.

2.3. Dynamic headspace sample preparation

Each sample was prepared just before dynamic headspace analysis by adding increasing amounts of aldehydes (from 0 to 300 ppb) to volatile-free deionized water (Milli-Q water purification system, Millipore, Bedford, MA) containing either ethanol, glycerol, Maldex 20 or Flolys sugar syrup. Purge and trap injection was used for samples with less than 1 % ethanol (v/v), thermal desorption cold trap injection for other samples.

2.4. Purge and trap extraction (low ethanol samples)

A Hewlett Packard Model 5890 gas chromatograph equipped with a Chrompack Purge and Trap Injector, a flame ionisation detector and a Shimadzu CR3A integrator was used. 9 ml samples were injected into the chromatographic column in three steps as follows: (1) precooling of the trap (CPSIL 8 CB capillary column, 0.53 mm internal diameter ; film thickness, 5 µm) : the trap was cooled (-95°C) for 2 minutes in a stream of liquid nitrogen; (2) purging of the sample : the temperature of the purge vessel was set at 50°C. The sample was purged with helium gas (12 ml/min) for 15 minutes. The gas stream was passed through a condenser kept at -15 °C by means of a cryostat (Colora WK 15) to remove water vapour and then through an oven at 200°C. The volatiles were finally concentrated in the cold trap maintained at -95°C (liquid nitrogen); (3) desorption of the volatiles: cooling was stopped, and the surrounding metal capillary was immediately heated to 220°C for 5 minutes. The carrier gas swept the trapped compounds into the analytical column.

2.5. Thermal desorption cold trap extraction (high ethanol samples)

250 ml of sample were poured into a 500 ml flat-bottomed flask fitted with a sintered Drechsel head. The flask was placed in a thermostatic bath maintained at 30°C. A Tenax cartridge (90 mg, 25-30 mesh) was fitted to the gas vent branch of the Drechsel head, another attached to the purge unit.

Volatiles were purged to the Tenax phase for 60 min with a 30 ml/min nitrogen flow. The Tenax cartridge was then dried with an inversed 15 ml/min nitrogen current for 10 min and transferred to the Chrompack TCT/PTI 4001 GC unit for analysis. Desorption/injection was carried out in four steps: (1) precooling of the cold trap (see above) for 4 minutes; (2) first desorption: the Tenax cartridge was heated to 230°C, remaining at this temperature for 10 min with an helium gas flow of 10 ml/min; (3) second desorption: cooling of the cold trap was stopped, and the surrounding metal capillary was immediately heated to 200 °C for injection into the analytical column; (4) the Tenax cartridge was heated to 275°C for 45 min, with a 10 ml/min inversed helium flow for reconditioning.

2.6. Chromatographic analyses

GC analyses were carried out on a 50 m x 0.32 mm, wall-coated, open tubular (WCOT) CP-Sil5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness, 1.2 µm). Oven temperature, initially kept at 36°C for 15 minutes, was programmed to rise from 36 to 120°C at 5°C/min then to 200°C at 10°C/min, remaining at the maximum temperature for 10 minutes thereafter. Helium carrier gas was used at a flow rate of 1.0 ml/min. Injection and FID detection temperatures were 200 and 220°C, respectively. All analyses were done in duplicate. The assessment of the technique reproducibility has been previously described (coefficients of variation under 10 % for five analyses of the same standard mixture; Collin *et al.*, 1993).

3. Results and discussion

The dynamic headspace method was used to quantify interactions between aldehydes and various beer components in a model aqueous system.

3.1. Aroma retention in ethanolic model solutions

For each ethanol content (0.00; 0.25; 0.50; 0.75; 1.00; 1.50 and 5.00%), increasing amounts of the flavouring substances were added prior to chromatographic analysis. The linear relationship between the measured chromatographic area of each substance and the aroma concentration was plotted for each ethanol content (Figure 1, a and c). The slope of each line includes both the detector response coefficient and the aroma recovery factor (Piraprez & Collin, 1995). From these results, the relative recovery was calculated by dividing the slope of the straight line obtained for the ethanolic

solution by the slope of the alcohol-free samples. These data were plotted versus the ethanol content as shown in Figure 1, b and d.

Surprisingly, an ethanol level as low as 0.5% was sufficient to induce slight retention (8 - 12%) (see statistical data in the figure caption). In a usual 5 % ethanol beer, 32 - 39% of the aldehydes seem to be retained by the medium, suggesting that differences in threshold values could occur, depending on the matrix.

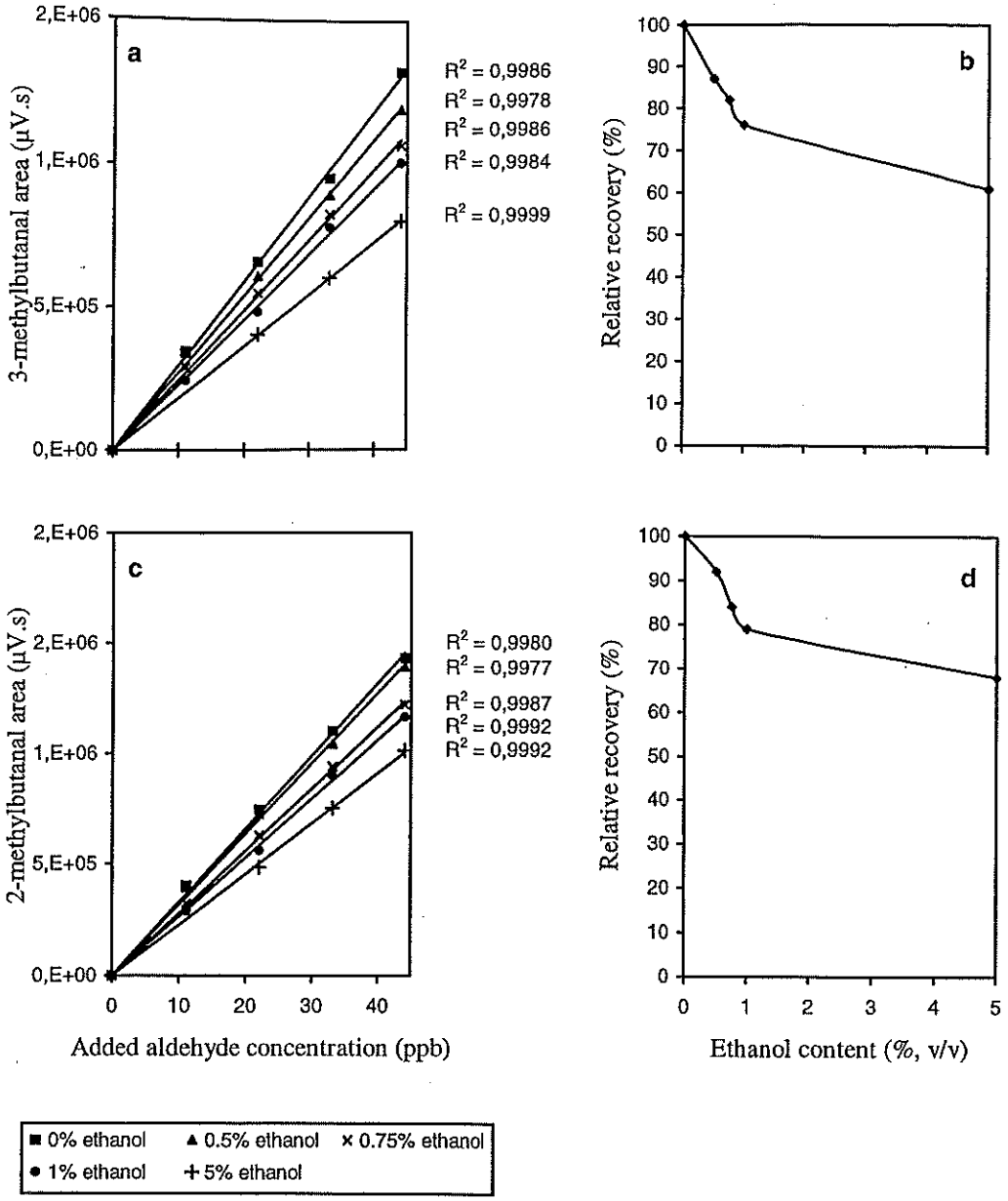

Figure 1. (a) (c) Linear relationships between chromatographic areas and aroma aldehyde concentrations at different percentages of ethanol. ($p<0.05$ for all comparisons; F-tests for equality of slopes of several regression lines executed in the SAS system; Sokal & Rohlf, 1969). (b) (d) Relationship between the relative recovery (%) and the ethanol content (%).

In order to determine which kinds of interactions are likely to take place between aldehydes and ethanol, three successive dichloromethane extractions (1:3 vol./vol.) were applied to a 5% ethanol solution containing 10 ppm 3-methylbutanal. An aldehyde recovery factor around 100% was calculated from GC-FID analysis

data (area of 76243 μV.s for the sample versus 76151 μV.s for the control), excluding significant formation of acetals in such dilute aqueous systems. Hence, probably, only low energy interactions are involved.

3.2. Aroma retention in sugar model solutions

As shown in Figure 2, the Flolys sugar syrup caused, unlike ethanol, a "salting out" effect for 3- and 2-methylbutanal when the density of the analysed sample was under 6° Plato. Similar results have been mentioned for ketones (Nawar, 1971) and esters (Kieckbusch & King,1979 ; Nahon, 1998) with mono-and di-saccharides. The wort-like composition of our syrup and the present data on ethanolic solutions confirm that aldehyde perception will be higher in low-alcohol beers.

Sensory analyses were then performed on the Flolys sugar syrup medium (alcohol-free beer-like medium) and the 5 % ethanolic Maldex 20 medium (regular beer-like medium). Table 2 shows that when the 3-methylthiopropionaldehyde concentration was below 50 ppb, a higher number of assessors usually detected the compound in the alcohol-free beer-like medium. Even at 0.1 ppb, 80 % of the panel still identified the spiked glass without ethanol. As only a few number of samples were tested around the 0.025–0.25 ppb range, no threshold value has been proposed. However, it can be already pointed out that this value will be much lower than 250 ppb. On the other hand, in the case of regular beers, we can assume that fruity flavours imparted by higher alcohols and esters will significantly decrease the worty taste in comparison with our alcoholic model solution.

Table 2. Rate (%) of 3-methylthiopropionaldehyde detection in triangular tests

Concentration added (ppb)	0.025	0.05	0.1	0.25	0.5	2	6	10	20	50	100
matrix used :											
alcoholic model solution	20	20	50	60	70*	80*	80*	90*	90*	100*	100*
alcohol-free model solution	40	50	80*	90*	100*	100*	100*	100*	100*	100*	100*

* Significant with a 5 % threshold (Sauvageot, 1991)

Surprisingly, dynamic headspace analysis yielded contrasting results when a higher level of the Flolys sugar syrup was used: at 12° Plato, 20 % of 3-methylbutanal and 40 % of 2-methylbutanal were no longer extracted, compared to the control (Figure 2). Retention by dextrins (Kieckbusch and King, 1979) most probably balances in this case the first effect, which is also already attenuated by too-high levels of small sugars (salting in effect in that case, as shown by Nawar, 1971).

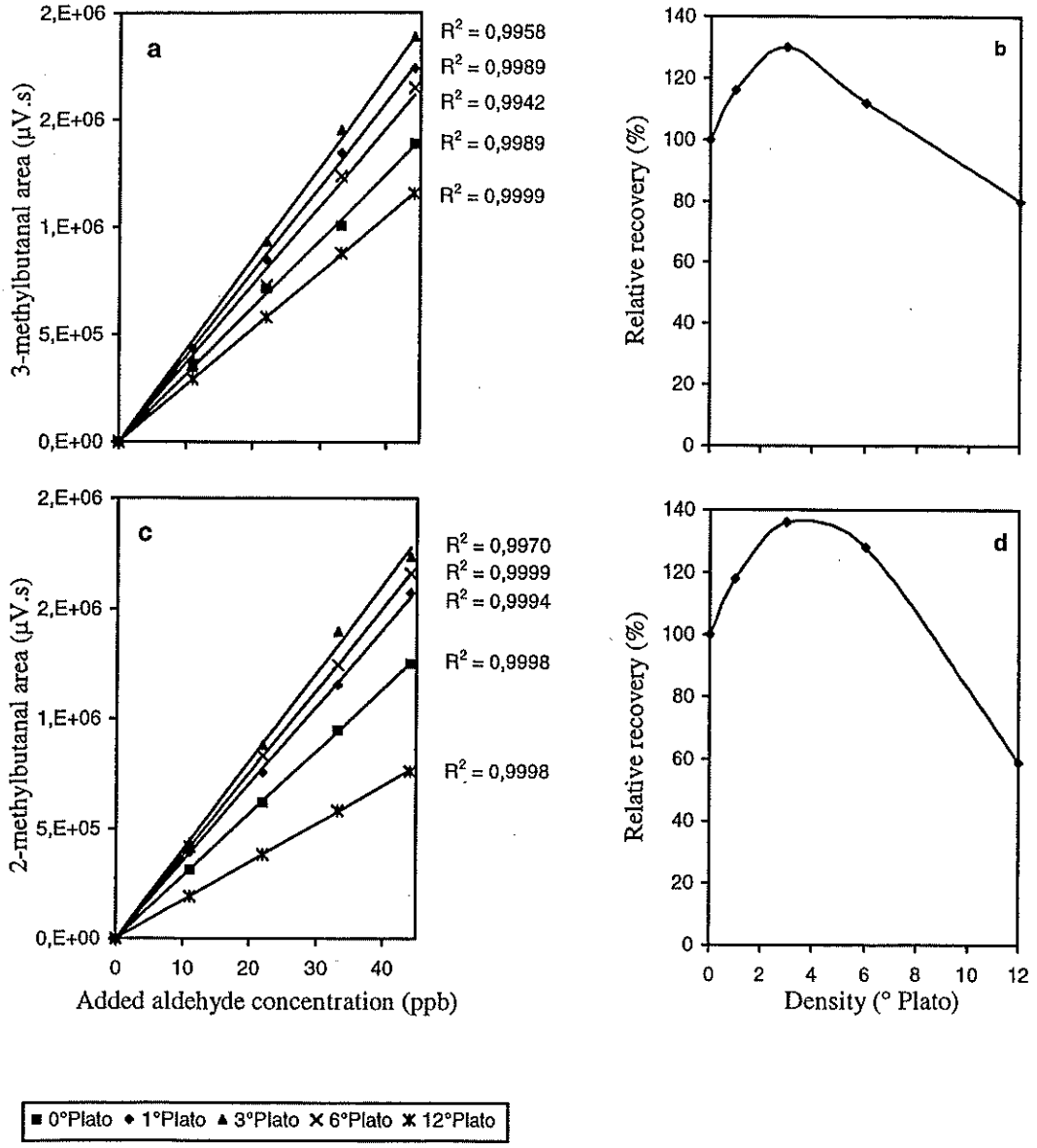

Figure 2. (a) (c) Linear relationships between chromatographic areas and aroma aldehyde concentrations at different concentrations of Flolys sugar syrup ($p<0.01$ for all comparisons except 1°Plato against 6°Plato; F-tests for equality of slopes of several regression lines executed in the SAS system; Sokal & Rohlf, 1969). (b) (d) Relationship between the relative recovery (%) and the solution density (° Plato).

3.3. Aroma retention in "ethanol-mimic" solutions

As ethanol-mimics might advantageously restore higher aldehyde flavour thresholds, dynamic headspace analyses were performed in presence of glycerol. As reported by Nawar (1971), a flavour-binding effect of glycerol is obvious when glycerol is used at 50 %. Since a glycerol level around 1.5 % could be obtained naturally by using selected yeasts (Scanes *et al.*, 1998), we investigated retention by glycerol at such low concentrations. As with ethanol, glycerol level as low as 0.5% proved sufficient to cause retention, amounting to up to 40% in the medium containing 4.5% glycerol (Figure 3).

As already suggested in Figure 2, a high level of carbohydrates could also mimic ethanol. As discussed above, dextrins are most probably involved. Dynamic headspace extractions were therefore applied to the Maldex 20 medium (see Figure 4). As with Flolys syrup, the relative recovery of 3-methylbutanal was only 60% in 12° Plato Maldex 20 medium. Hence, by adapting the mashing diagram in the brewhouse (short stands around 60°C), it should be possible to improve the organoleptic properties of low-alcohol beers. However, a comparison of the 12° Plato Flolys and 4° Plato Maldex 20 systems (both around 3 % dextrins) indicates that the structure of dextrins present is probably also of prime importance.

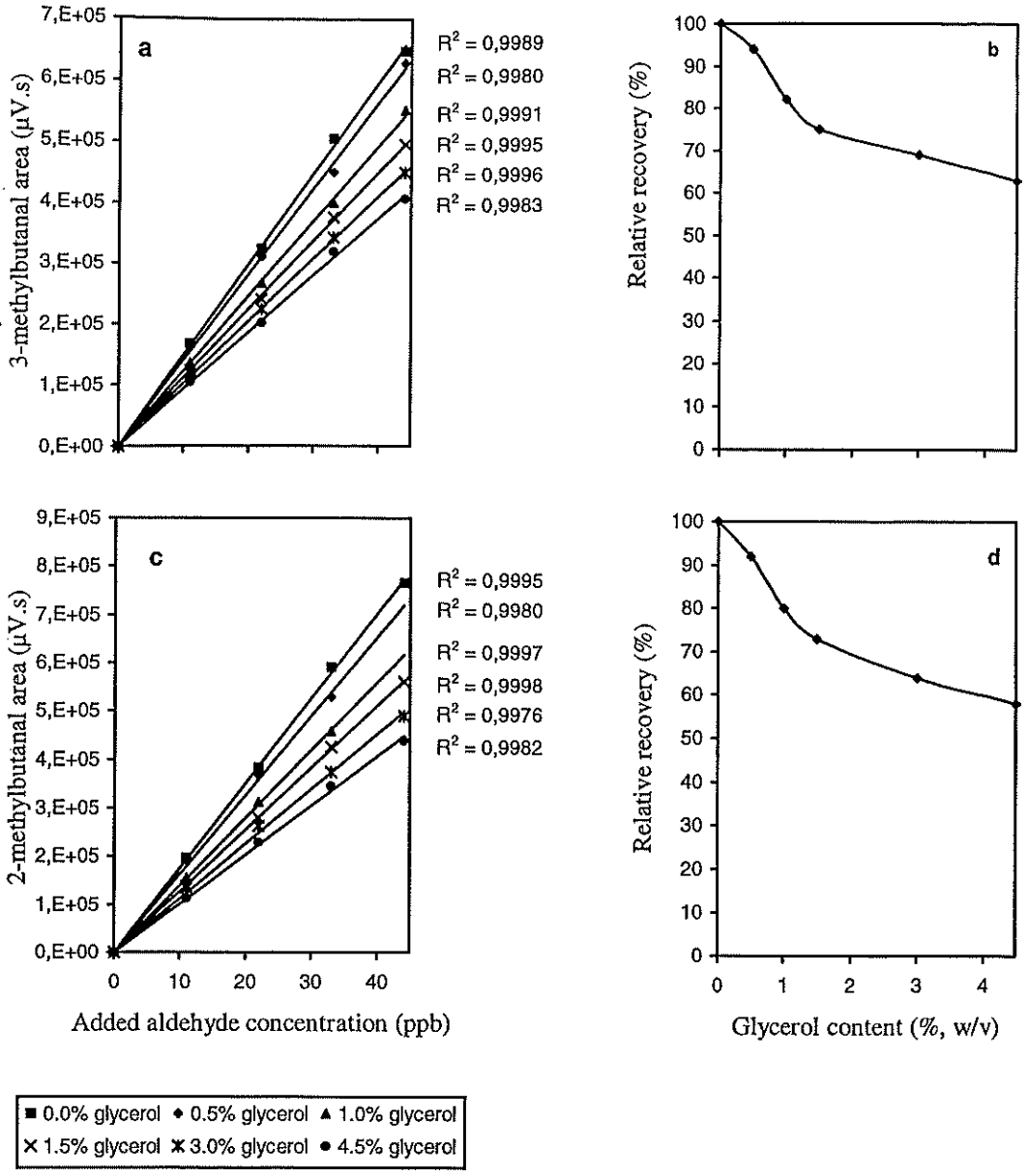
a
b
c
d
3-methylbutanal area (µV.s)
2-methylbutanal area (µV.s)
Relative recovery (%)
Added aldehyde concentration (ppb)
Glycerol content (%, w/v)
R² = 0,9989
R² = 0,9980
R² = 0,9991
R² = 0,9995
R² = 0,9996
R² = 0,9983
R² = 0,9995
R² = 0,9980
R² = 0,9997
R² = 0,9998
R² = 0,9976
R² = 0,9982
0.0% glycerol
0.5% glycerol
1.0% glycerol
1.5% glycerol
3.0% glycerol
4.5% glycerol

Figure 3. (a) (c) Linear relationships between chromatographic areas and aroma aldehyde concentrations at different percentages of glycerol ($p<0.01$ for all comparisons; F-tests for equality of slopes of several regression lines executed in the SAS system; Sokal & Rohlf, 1969). (b) (d) Relationship between the relative recovery (%) and the glycerol content (%).

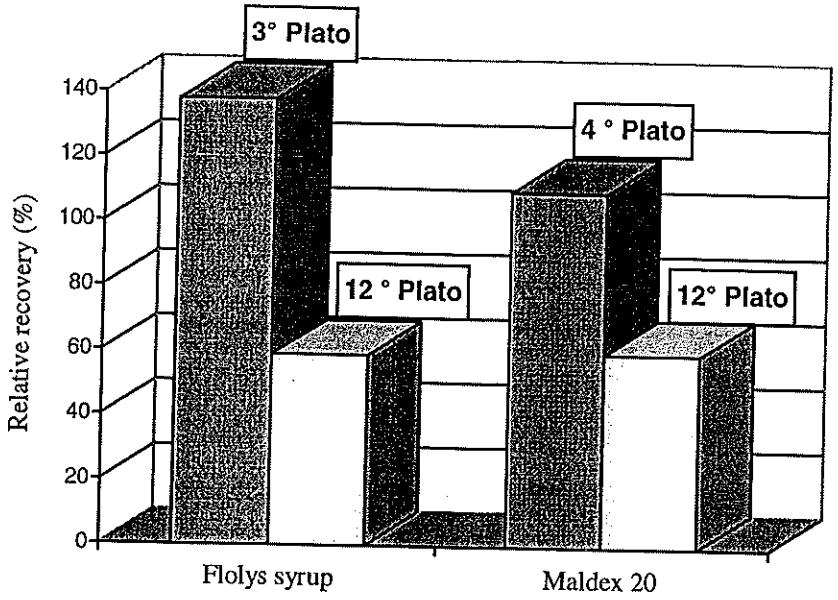

Figure 4. Comparison between relative recoveries of 3-methylbutanal from Flolys and Maldex 20 solutions

4. Conclusions

The worty off-flavour of alcohol-free beers produced by a cold contact process was, until now, mainly attributed to insufficient aldehyde reduction by yeast enzymes, due to a limited fermentation. It appears, however, that perception of the aldehydes responsible for the worty flavour is influenced by the medium. In a typical lager beer, ethanol increases aldehyde retention while in alcohol-free beers, both the absence of ethanol (less than 0.1%) and the higher level of sugars could strengthen worty off-flavours.

Our results suggest that aldehyde retention in a regular beer could be mimicked by increasing the level of dextrins (to 10%) or glycerol (to 4%). In the former case, dextrin concentrations can be enhanced by minimising the β-amylase activity during wort mashing. However, low density worts which are industrially used to avoid ethanol will inevitably decrease the carbohydrate concentration. Increasing glycerol content could be done naturally by using selected yeasts, all the more because glycerol formation and ethanol excretion seem to be inversely related (Prior *et al.*, 1999). Alternatively, addition of sulfites up to 10 ppm would also be enough to decrease by 40% headspace concentration of the aldehyde (Perpète & Collin, 1999[d]).

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5. References

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