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# RELATIONSHIPS BETWEEN THE CHEMICAL COMPOSITION AND SENSORY EVALUATION OF LAGER BEERS

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## ABSTRACT

*This paper examines how several typical components of lager beers affect appreciation of the product. Sensory analyses clearly show a preference in bitterness for isohumulone (17·5–25 EBU), as compared to isocohumulone. As for esters, isoamyl acetate was found to be the most appreciated compound (2·4 ppm). In terms of flavour units, only ethyl caproate can reach a value of 4 without exhibiting an unpleasant aroma. The optimal dimethylsulphide concentration is in the range of 50 ppb.*

*Keywords: lager beer; flavour; esters; sensory analysis; bitterness.*

## INTRODUCTION

In the brewing field, aroma control is of prime importance in producing a constant-quality beverage. It is possible to modify the aromatic profile at various production steps: malt kilning, mashing, wort boiling, and fermentation. During process operations, both the nature of the raw materials (malt, hop, yeast, etc.) and the experimental conditions (oxygen, fermentation tank design, temperature, pressure, etc.) can be controlled.

In a preliminary step aimed at finding the ideal flavour profile of lager beers, we initially observed that the response of tasters to aroma changes was difficult to predict, even when compounds with a nice smell or taste were considered. While studying their actual effect, we therefore added several typical components such as bitter acids, esters, and DMS to a beer produced on an industrial scale, without hops. A panel of 27 tasters was selected to give a hedonic description of the beers.

## EXPERIMENTAL

### Material

A bottom-fermented beer was produced on an industrial scale (70 hl) from a wort with an original gravity of 11·2° Plato. Mashing was done with 70% Pils malt and 30% rice. No hops were added during boiling. Fermentation was started at 8°C with *Saccharomyces cerevisiae var. carlsbergensis* (MUCL 20463). For the bitterness and sulphury note studies, the beer was stored in kegs with additional yeast, in order to obtain a carbon dioxide concentration of 4·8 g/litre in the final beer. For ester analysis, the beer was refermented in bottles until the $CO_2$ concentration reached 6 g/litre.

### Adding of chemical compounds

Iso-$\alpha$-acids were prepared by $\alpha$-acid isomerization at pH 8·0, separated on a preparative scale and purified by Buckee's (1985) method allowing two fractions to be isolated: the first one was isohumulone/isoadhumulone (roughly 9:1) while the second one was pure isocohumulone.

Dimethylsuphide (99%) and ethyl acetate (99%) were purchased from Fluka. Isoamyl acetate (99%), ethyl caproate (99%), and ethyl caprylate (99·5%) came from Sigma. All these compounds were directly added in the kegs or bottles, prior to filling them up with beer.

### Analytical methods

The bitterness has been quoted in EBUs on the basis of the added amounts (in ppm) of isohumulone and isocohumulone. Nine kegs of both series were analysed by applying the EBC method, which determines the absorbance of an acid isooctane extract at 275 nm. The expected concentrations were measured in the first

**TABLE 1.** Example Form Filled in by the Tasters

Name of the taster: ........................................................................ Date: ..................................
You receive 3 or 4 glasses of beer with a typical taste.
Are you able to describe it: ..................................................................
Classify the glasses, placing the best beer first: ...........................................................
Please give points between 0 and 10 for the 4 samples, according to the following scale:
0: undrinkable; 4: drinkable without asking for another one; 7: good; 10: excellent, and taking account the reference which is given in this test

| Points: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Xi: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Xj: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Xk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Xl: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

series (isohumulone/isoadhumulone) while only 90% (±2%) were recovered for isocohumulone. A smaller p$K_a$ value might explain that isocohumulone is less extracted by acidified isooctane than isohumulone/isoadhumulone.

Regarding the DMS and ester levels, one bottle of each concentration was analysed with the help of a headspace technique combined with gas chromatography (Malcorps *et al.*, 1991).

## Sensory evaluation

Each sample has been evaluated by a trained, 27-member panel, using the form reported in Table 1. The sessions were organized as follows. Each kind of compound was numbered (1 for isohumulone/isoadhumulone, 2 for isocohumulone, 3 for ethyl acetate, 4 for isoamyl acetate, 5 for ethyl caproate, 6 for ethyl caprylate and 7 for dimethylsulphide) while a letter (alphabetical order) was indicative of increasing concentration. Two different tests were done during a single session. Each test consisted in analysing 3–4 beers, and a reference (either A1 = A2, score fixed at 5, or a beer previously tested, with its former score). For instance, during session 1, test 1, the beers numbered A1, B1, C1, D1 and E1 were proposed. Session 1, test 2: E1/F1/G1/H1. Session 2: A2/B2/C2/D2/E2 and E2/F2/G2/H2. Session 3, G1/I1/J1/K1 and H1/L1/M1/N1, etc.

# RESULTS AND DISCUSSION

The beer composition prior to addition of further compounds is shown in Table 2.

**TABLE 2.** Beer Characteristics Prior to Adding Compounds

| Chemical name | Concentration (ppm) |
|---|---|
| Ethyl acetate | 8 |
| Isoamyl acetate | 1·2 |
| Ethyl caproate | 0·3 |
| Ethyl caprylate | 0·6 |
| Dimethylsulphide | 0·03 |

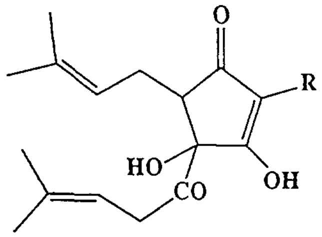
## Bitterness

Bitterness, sweetness, saltiness, sourness, and umami are the five primary tastes. As a general rule, bitterness adversely affects the detection of nutritious foods (Roy, 1992). The bitter principles of hop, however, play an essential role in beer taste. The hop variety seems to be a determining factor in this regard. It mainly influences the relative amounts of humulone, cohumulone, adhumulone, prehumulone, and posthumulone, known precursors of the bitter-tasting substances in beer (Aitken *et al*, 1970; Verzele & De Keukeleire 1991). While the α-acids are almost tasteless, the iso-α-acids, produced by thermal isomerization in the brewing kettle, are extremely bitter. Their threshold value, estimated at *c.* 6 ppm, is comparable to that of quinine sulphate while caffeine is reported to be more than 20 times less bitter. The ability to create a stable and pleasing hop presence in beer remains one of the greatest challenges in the brewer's art. In particular, the impact of each bittering compound on the overall aroma remains unclear so far.

For this reason, we have attempted to quantify the appreciation of isohumulone and isocohumulone in lager beers. Previous results have shown that *cis* and *trans* iso-α-acids exhibit a similar bitter taste and bittering power (Verzele *et al.*, 1970). In this study, a diastereoisomer mixture was used.

The optimal bitterness was found to be 17·5–25 EBC bitter units (EBU), for both kinds of iso-α-acids. However, Table 3 clearly shows that added isohumulone (with traces of isoadhumulone) is preferred to isocohumulone, despite their very similar structure. This assertion is supported by the statistical analysis of the true difference between the highest mean scores obtained for isohumulone (sample J1) and isocohumulone (sample I2), respectively (Student test for HO: $\mu 1 = \mu 2$, *t*-statistic: 7·31; degrees of freedom: 52; significance: 0·0). These compounds differ solely by the nature of the side chain at C(2), due to incorporation of a specific amino acid residue (leucine and valine, respectively) during biogenesis of the corresponding α-acid. The structural differences are responsible for the dif-

**TABLE 3.** Average Scores with Standard Deviation (ESD) Obtained after the Addition of Isohumulone (A–Q 1) and Isocohumulone (A–Q 2)




| EBU | Isohumulone $R{=}CO{-}CH_2{-}CH(CH_3)_2$ | | | Isocohumulone $R{=}CO{-}CH(CH_3)_2$ | | |
|---|---|---|---|---|---|---|
| | **No.** | **Score** | **ESD** | **No.** | **Score** | **ESD** |
| 0 | A1 | 5·00 | 0·00 | A2 | 5·00 | 0·00 |
| 2·5 | B1 | 5·00 | 0·00 | B2 | 5·00 | 0·00 |
| 5 | C1 | 5·26 | 0·44 | C2 | 5·26 | 0·44 |
| 7·5 | D1 | 5·44 | 0·63 | D2 | 5·70 | 0·53 |
| 10 | E1 | 6·15 | 0·70 | E2 | 6·04 | 0·51 |
| 12·5 | F1 | 6·70 | 0·71 | F2 | 6·41 | 0·56 |
| 15 | G1 | 7·48 | 0·99 | G2 | 7·04 | 0·64 |
| 17·5 | H1 | 8·22 | 0·92 | H2 | 7·55 | 0·68 |
| 20 | I1 | 8·70 | 0·97 | I2 | 7·78 | 0·64 |
| 22·5 | J1 | 9·11 | 0·69 | J2 | 7·67 | 0·72 |
| 25 | K1 | 8·04 | 1·23 | K2 | 6·63 | 0·73 |
| 27·5 | L1 | 6·85 | 1·32 | L2 | 5·37 | 1·09 |
| 30 | M1 | 5·33 | 1·22 | M2 | 4·00 | 1·02 |
| 32·5 | N1 | 4·37 | 1·16 | N2 | 2·85 | 0·97 |
| 35 | O1 | 2·81 | 1·09 | O2 | 1·92 | 1·01 |
| 37·5 | P1 | 2·18 | 1·31 | P2 | 1·11 | 1·06 |
| 40 | Q1 | 1·59 | 1·19 | Q2 | 0·33 | 0·61 |

ferent ionization constants (a $pK_a$ of 5·5 for humulone and 4·7 for cohumulone). As previously suggested (Verzele & Keukeleire, 1991), non-ionized iso-α-acids favour a mild bitterness.

## Fruity note

Among the compounds formed as by-products during brewery fermentations, the acetate esters are widely regarded as the most flavour-active. The flavour thresholds and amounts of ethyl and isoamyl acetate in beer make it clear that these esters significantly contribute to beer flavour. Ethyl caproate and ethyl caprylate are two additional fruity aromas in beer, often described as more delicate than the former.

The amounts of esters can be altered within ample limits by varying the fermentation conditions. Due to the fact that the production of acetate esters and fatty-acid esters involves different enzymes (Malcorps *et al.*, 1991), proportions of these are also subject to variation. When seeking to control ester formation, choosing an appropriate yeast strain is paramount (Ramos-Jeunehomme *et al.*, 1991). Ester synthesis is also closely linked to all parameters that influence yeast growth, such as temperature, original gravity, carbon dioxide pressure, oxygen, wort lipid content, or stirring. This relation is rather complex as, for example, oxygen and unsaturated fatty acids stimulate growth but inhibit acetate ester synthesis (Malcorps *et al.*, 1991). Higher carbon dioxide pressures lead to lower acetate ester levels (Reid, 1989; Kruger *et al.*, 1992), while they may increase the concentrations of free acids and their ethyl esters (Posada *et al.*, 1977). Norstedt *et al.* (1975) have shown that the ester with the lowest perception threshold, ethyl hexanoate, is only slightly influenced by most fermentation parameters. In their experiments, only the amount of assimilable nitrogen was found to be important in this respect. When an immobilized cell reactor was used for secondary fermentation, losses of acetate esters, particularly isoamyl acetate, were observed (Pajunen *et al.*, 1987). This could provide a good means of controlling acetate ester levels in high-gravity brewing with excess ester production during the primary fermentation.

In this study, appreciation of the main fruity notes was evaluated by adding various concentrations of ethyl acetate, isoamyl acetate, ethyl caproate, and ethyl caprylate. Surprisingly, isoamyl acetate gives the highest mean score (9·3 for 2·4 ppm), with a very small standard deviation (0·73). In this beer, the respective optimal concentrations for ethyl acetate, ethyl caproate and ethyl caprylate are 32 ppm (mean score: 8·4), 0·8 ppm (mean score: 8·3) and 1·1 ppm (mean score: 7·1). Just a few tasters liked ethyl caprylate while others

**TABLE 4.** Optimal Concentrations in our Study, Detection Thresholds (Meilgaard, 1975) and Olfactive Notes (Meilgaard, 1975) of Various Esters

| | Optimal concentrations (ppm) | Thresholds (ppm) | Olfactive notes |
|---|---|---|---|
| Ethyl acetate | 28–40 | 33 | Solvent, fruity |
| Isoamyl acetate | 2·0–2·8 | 1·6 | Banana, apple, pear |
| Ethyl caproate | 0·5–0·8 | 0·23 | Fruity, apple, sweetish |
| Ethyl caprylate | 1·1 | 0·9 | Fruity, apple, sweetish |

**TABLE 5.** Average, Maximum and Minimum Values of Ester and Dimethylsulphide Content in 33 Commercial Lager Beers

| | Mean | Maximum | Minimum |
|---|---|---|---|
| Ethyl acetate (ppm) | 19·26 | 33·80 | 8·00 |
| Isoamyl acetate (ppm) | 1·77 | 3·80 | 0·30 |
| Ethyl caproate (ppm) | 0·13 | 0·19 | 0·05 |
| Ethyl caprylate (ppm) | 0·19 | 0·53 | 0·04 |
| Dimethylsulphide (ppb) | 33·8 | 81·9 | 5·0 |

found it quickly negative. This variability is expressed by the standard deviation of 1·53 at the optimum concentration of 1·1 ppm.

On the basis of Meilgaard's (1975) detection thresholds, only ethyl caproate can reach 4 flavour units (0·8 ppm) without exhibiting an unpleasant aroma (Table 4). However, we do not think it judicious to use these threshold values in this study. Indeed, as shown in Fig. 1(A), ethyl acetate can be smelled before reaching 33 ppm (detection threshold). This clearly indicates that the nature of a beer significantly influences these limits.

Our results suggest that the brewer must mainly check the isoamyl acetate content. We must bear in mind, however, that ethyl acetate and isoamyl acetate can be found in commercial beers in the range of our

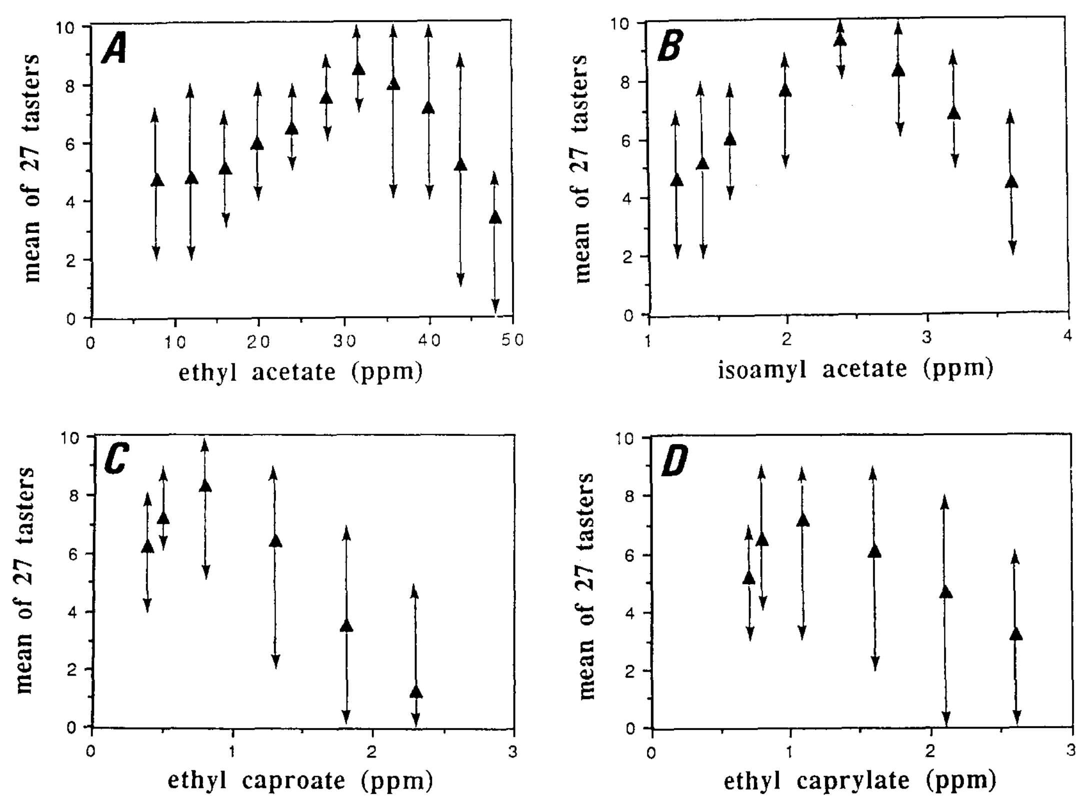



**FIG. 1.** Average, minimum and maximum drinkability scores of 27 tasters; addition of: (A) ethyl acetate; (B) isoamyl acetate; (C) ethyl caproate; (D) ethyl caprylate.

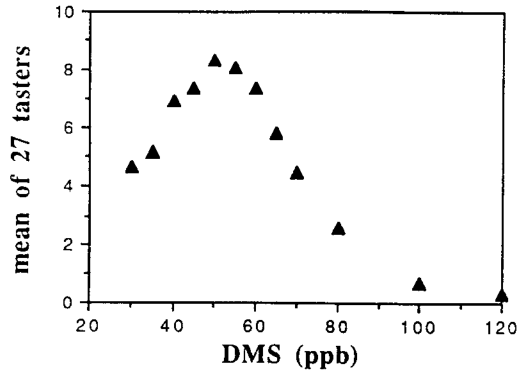



**FIG. 2.** Average drinkability scores of 27 tasters when dimethylsulphide is added.

optimal values, while ethyl caproate and ethyl caprylate are usually deficient (Nykänen & Suomalainen, 1983). The ranges of ester contents of a large series of commercial beers are presented in Table 5.

One ester might influence the others, but this has not yet been quantified. In an effort to improve our knowledge of synergistic and antagonist effects on overall aroma, it would be worth clarifying whether just one olfactory receptor is involved for all the fruity notes mentioned here. An overview of the literature (Meilgaard, 1975) seems to indicate, in support of this hypothesis, that an optimal hydrophobicity is required for the side chain of the ester (6 carbon atoms in ethylic esters give the lowest threshold).

### Sulphury note

Contrary to its effect in other beverages like wine, dimethylsulphide (DMS) in moderate concentrations is an essential flavour constituent of lager beers (Van Den Eynde, 1991). At higher content, when DMS can be smelled as such, it is considered an off-flavour.

S-methylmethionine (SMM) has been identified as the precursor in malt. During malt kilning, SMM is degraded to homoserine and DMS, which escapes with the kilning gases. During brewing, SMM goes into solution and continues to be degraded, with subsequent evaporation from the kettle. On the other hand, during the hot stand of the wort, thermal degradation of SMM continues but DMS no longer evaporates. This quantity of DMS goes into the fermenter and might persist in the finished beer. Moreover, a substantial quantity of DMS is formed in the fermentation vessel through reduction of dimethylsulfoxide by the yeast (Yang *et al.*, 1992). Control of the process including regulation of wort boiling and selection of the appropriate fermentation yeast, can thus modulate the final DMS content.

This study shows that the optimal concentration in the lager beers examined here (Fig. 2) is close to the threshold value published by Meilgaard, i.e. 50 ppb.

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