# High-Gravity Brewing: Production of High Levels of Ethanol Without Excessive Concentrations of Esters and Fusel Alcohols

**Gregory P. Casey,[1] E. C.-H. Chen,[2] and W. M. Ingledew,** *Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0*

ABSTRACT

The influence of nutritional supplementation and the degree of anaerobiosis on the production of esters and fusel alcohols in very high gravity brewing was examined. Although fermentation in semianaerobic conditions led to a drastic reduction in ester production, acceptable ester levels were found under anaerobic conditions where worts were supplemented with lipids and assimilable nitrogen. Such fermentations were rapid, and resultant beers may be organoleptically acceptable to the consumer.

Key words: High-gravity brewing, Esters, Fusel alcohols, Ethanol

In traditional brewing, worts of 11–12° Plato (P) are fermented to produce beers of 4–5% (v/v) ethanol. Recently, high-gravity brewing, at a limit of 16–18° P (19,25,29,37,38), has been practiced because of numerous product quality and economic advantages (19,36).

The first reports on the fermentation of worts above 18° P described problems with yeast viability and slow and incomplete fermentations (15,33). Both ethanol toxicity (15,33) and high osmotic pressure levels (25) were implicated as the limiting factors. Recently, research in this laboratory has demonstrated that a combination of increased pitching rate (9) and nutritional supplementation (10,11) can facilitate very rapid fermentation of worts as high as 31% (w/v) dissolved solids. Specifically, supplementation of these worts with yeast extract (or other sources of usable or assimilable nitrogen) and ergosterol-Tween 80 (to provide a sterol and oleic acid) at a pitching rate near $2 \times 10^7$ colony-forming units (CFU)/ml resulted in the production of 16.2% (v/v) ethanol within normal brewing times at 14°C. The improvements were a result of prolonged and increased production of yeast cell mass arising from supplementation (10,11). Such yeast could be repitched over at least five "generations" at these levels of ethanol (11), and this work demonstrated that normal lager brewer's yeasts, without genetic manipulation or strain improvement, are tolerant to ethanol concentrations of 16–17% (v/v) (22).

For the above results to have any application in the brewing industry, however, and for there to be further economic advantages of high-gravity brewing, it is absolutely essential that the beers resulting from nutritionally supplemented fermentations have balanced organoleptic qualities similar to traditionally brewed products. The flavor of a beer is largely determined by the type and concentration of secondary metabolites (especially esters) that occur during a fermentation. The most significant esters in brewing (along with their characteristic aromas) are: ethyl acetate (fruity), isoamyl acetate and isobutyl acetate (banana), ethyl caprylate (apple), and 2-phenylethyl acetate (fruity, flowery) (17).

On a quantitative basis, the most significant esters are ethyl acetate and isoamyl acetate. These are normally found in beer at concentrations below their flavor thresholds of 30 mg/L and 2 mg/L, respectively (13,20,23). The level of these two esters is profoundly influenced by wort original gravity, and there are numerous reports of exponential increases in the production of ethyl and isoamyl acetate in wort fermentations above 16° P (2,5,16,26–29,32,34,37). When beers resulting from these fermentations were diluted to an original gravity of 10–12° P, the levels of these esters significantly exceeded traditional concentrations, dramatically altering beer flavor. Consumers generally disliked the result (28). Excessive ester production is therefore viewed as a contributing factor in the self-imposed limitation of commercial wort gravities to 16–18° P.

In this report, beers resulting from the fermentations of 27% (w/v) dissolved solids worts were analyzed for concentrations of esters and fusel alcohols. In particular, the influence of nutritional supplementations and the degree of anaerobiosis on the production of these compounds were examined. The impact of these on the potential adaptation of this technology to the brewing industry is discussed.

## MATERIALS AND METHODS

### Brewing Yeast

A production strain of *Saccharomyces uvarum* was used. Fresh slurries were collected from a brewery just before use.

### High-Gravity Worts

An 11.5° P (22.5% corn grit adjunct) commercial wort was used as a base to prepare the 27% (w/v) dissolved solids wort. The additional extract came from the addition of Casco syrup no. 1636U (Canada Starch Co. Ltd.). The manufacturer's carbohydrate specifications for this syrup are 19.4% DP4+, 10.7% DP3, 39.0% DP2, and 30.9% DP1. The syrup is virtually nitrogen free, containing only 0.03–0.06% (w/v) protein.

### Fermentation Conditions

These have been previously described for both anaerobic (10,11) and semianaerobic (11) fermentations. Final ethanol concentrations were in the order of 9.0% (w/v) in the undiluted beers.

### Ester and Fusel Alcohol Analyses

These volatile compounds were analyzed by using a dynamic headspace sampling technique in conjunction with capillary gas chromatography under conditions described earlier by Chen (12).

*Purge-and-trap system.* A Hewlett-Packard model 7675A purge-and-trap sampler was used (Hewlett-Packard Analytical Instruments Co., Avondale, PA). The automated unit performs three basic functions: purging the volatile sample components, entraining the components on a suitable adsorbent, and back flushing the desorbed compounds into the gas chromatography column while rapidly heating the absorbent-trap. The trap consists of a stainless steel tube (6 × 100 mm) packed with Tenax GC (60–80 mesh; 2,6-diphenyl-*p*-phenylene oxide) and held at room temperature. One of the features of the sampler is a postdesorb cycle in which the trap flow is switched to vent, and the trap temperature is elevated an additional 50°C for a predetermined time to eliminate accumulation of materials of low volatility. This is followed by a cooling period to prepare the trap for succeeding samples. All of the above functions, and their timings, were controlled by a Hewlett-Packard model 5800 microprocessor.

*Gas chromatographic conditions.* All gas-liquid chromatographic analyses were performed with a Hewlett-Packard model 5840A chromatograph equipped with a capillary inlet system and a flame ionization detector at 250° C. Injector temperature was 200° C. The column (DB-5, J & W Scientific, Rancho Cordova, CA) was a 30-m fused silica open tubular (0.25 mm internal diameter) wall coated

[1] Carlsberg Laboratory, Department of Physiology, GL Carlsberg VEJ 10, DK 2500, Copenhagen, Valby, Denmark.
[2] Molson Breweries of Canada, Ltd., Technical Center, 2486 Dunwin Drive, Mississauga, Ontario, Canada L5L 1J9.

with a stationary film consisting of 95% dimethyl- and 5% diphenylpolysiloxane (film thickness was 0.25 μm). The carrier gas was helium (UHP grade) with flow rates of 1 ml/min through the column and 30 ml/min through the detector. The capillary inlet system was operated at a 1:100 split mode. The interface between the purge-and-trap sampler and the gas-liquid chromatograph injection port was facilitated by a needle assembly (Hewlett-Packard model 7675-8009). The transfer line was insulated to prevent sample condensation. During a chromatographic run, the column temperature was held initially at −20°C for 5 min, and increased 5°C/min to 185°C, 10°C/min to 250°C, and then held at this temperature until completion. Liquid carbon dioxide was used for oven cooling.

*Sampling procedure and conditions.* Undiluted beer samples were cooled to 0°C before analysis. Without unduly disturbing the sample, 5 ml of beer was pipetted into a purge vessel that contained a magnetic stirring bar. The contents of the vessel were stirred so that a vortex was created to prevent foaming and then purged for 30 min with a helium flow of 50 ml/min. At the end of the purge time the trap was quickly heated to 200°C while the desorbed compounds were back flushed for 3 min into the chromatographic column with the same purge gas. Of the desorbed material, 1/100th was admitted to the capillary column through the needle assembly.

**TABLE I**
**Compounds Used as External Standards and Their Chromatographic Retention Times**

| Compound | Retention Time (min) |
|---|---|
| *n*-Propanol | 14.7 |
| Ethyl acetate | 17.6 |
| Isobutanol | 18.3 |
| *n*-Butanol | 20.1 |
| Ethyl propionate | 22.4 |
| Amyl alcohols[a] | 23.5 + 23.7 |
| Ethyl isobutyrate | 24.6 |
| Ethyl butyrate | 26.5 |
| Isoamyl acetate | 29.7 |
| Ethyl hexanoate | 34.6 |
| Phenyl ethanol | 39.2 |
| Ethyl octanoate | 41.6 |
| Phenyl ethyl acetate | 43.8 |
| Ethyl decanoate | 46.7 |

[a]2-Methyl-1-butanol and 3-methyl-1-butanol.

## RESULTS AND DISCUSSION

For quantitative determination of beer volatiles by the purge-and-trap technique, consideration must be given to the difference in purging efficiency between beer and aqueous matrices if the calibration mixture is prepared in aqueous medium. Using *n*-octanol as a test compound, it was found that the recovery of the alcohol from the beer matrix was about 6% less than that from the aqueous matrix. This effect on recovery may vary somewhat for each beer volatile compound and is difficult to assess with accuracy. However, because most beer volatiles are relatively similar in molecular size and volatility, for practical and comparative purposes, it is reasonable to assume that the matrix effect on them does not differ greatly from that on octanol. Therefore, the 6% reduction in recovery for octanol was taken as a general guide in adjusting the actual amount of purgeable volatiles in beer. Octanol emerges from the chromatographic column between ethyl hexanoate and phenylethanol, and thus may be used as an internal standard. For the present application, however, octanol is not suitable, because some of the samples also contain discernable amounts of the alcohol. An aqueous mixture of authentic compound was therefore prepared and used as an external standard. The identities of these compounds and their retention times under chromatographic conditions are listed in Table I.

Table II shows the effect of nutritional supplementation and the degree of anaerobiosis on the level of esters in beers produced from 27% (w/v) dissolved solids wort. The most striking feature in this table is the drastically reduced level of esters under semianaerobic conditions. Regardless of the type of nutritional supplementation, dramatically reduced levels of ethyl acetate, isoamyl acetate, ethyl propionate, ethyl butyrate, and ethyl isobutyrate were seen compared to levels under anaerobic conditions. The most significant difference was in the level of ethyl acetate, found at 35.90 and 2.49 mg/L in the unsupplemented beers from anaerobic and semianaerobic fermentations, respectively. Such low levels of esters in beers from semianaerobic fermentations would certainly result in more bland, less aromatic beers compared to their anaerobically

**TABLE II**
**Ester Content (mg/L) in High-Gravity Brews Fermented With or Without Nutritional Supplements Under Anaerobic or Semianaerobic Conditons[a]**

| | Ethyl Acetate | Ethyl Propionate | Ethyl Isobutyrate | Ethyl Butyrate | Isoamyl Acetate | Ethyl Hexanoate | Ethyl Octanoate | 2-Phenylethyl Acetate | Ethyl Decanoate |
|---|---|---|---|---|---|---|---|---|---|
| Anaerobic[b] | | | | | | | | | |
| Unsupplemented | 35.90 | 0.20 | 0.03 | 0.30 | 3.74 | 0.42 | 0.68 | 0.62 | 0.66 |
| 1% (w/v) yeast extract | 50.98 | 0.25 | 0.03 | 0.78 | 6.82 | 0.34 | 1.01 | 0.71 | 0.44 |
| 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 39.18 | 0.31 | 0.04 | 0.33 | 3.40 | 0.32 | 0.58 | 0.42 | 2.19 |
| 1% (w/v) yeast extract/ 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 26.37 | 0.23 | 0.03 | 0.64 | 4.72 | 0.50 | 4.13 | 0.62 | 48.86 |
| Semianaerobic[b] | | | | | | | | | |
| Unsupplemented | 2.49 | 0.02 | 0.01 | 0.05 | 0.05 | 0.23 | 0.18 | 0.53 | 0.74 |
| 1% (w/v) yeast extract | 8.63 | 0.03 | 0.01 | 0.27 | 1.07 | 0.29 | 1.22 | 0.64 | 14.78 |
| 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 6.81 | 0.06 | 0.03 | 0.20 | 0.53 | 0.50 | 1.55 | 0.69 | 19.22 |
| 1% (w/v) yeast extract/ 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 12.01 | 0.04 | 0.01 | 0.22 | 0.96 | 0.49 | 3.81 | 0.45 | 53.27 |
| Commercial lager[c] | 24.29 | 0.09 | ND[d] | 0.11 | 2.05 | 0.17 | 0.41 | 0.93 | 0.95 |

[a]Each figure represents the average of triplicate analyses.
[b]Undiluted beer, 9% w/v alcohol (see text).
[c]4% w/v alcohol.
[d]ND = not determined.

brewed counterparts. In fact, as beers brewed at traditional gravity normally have ethyl acetate levels ranging from 10–20 mg/L (26,34), the unsupplemented semianaerobic beer would have 10- to 20-fold lower levels of this ester than normal after dilution. A similar pattern would also be seen with the other esters.

This observation is in agreement with the results of other researchers, where decreased ester synthesis (especially acetate esters) has been described under conditions of increased wort aeration (2–4,14,18,21,26,35). Under anaerobic conditions, it is known that brewer's yeasts require a sterol (6) and an unsaturated fatty acid (7) as growth factors. Wort aeration and other conditions related to yeast lipid metabolism—i.e., adding preformed unsaturated fatty acids (2,26), preaeration of yeast (1), or addition of spent grain pressings (35)—have been hypothesized to decrease ester production by increasing cell production. These practices no doubt influence the availability and/or synthesis of acetyl coenzyme A for acetate ester synthesis (2,24,30,31), as more acetyl coenzyme A would be channeled into growth than into ester synthesis. There is some evidence, however, that direct inhibition of ester synthesis by unsaturated lipids may instead be responsible for this effect (39). Oxygen has already been shown to be a growth-limiting nutrient in these 27% (w/v) fermentations under both semianaerobic (11) and anaerobic (10) conditions, but to a lesser extent under semianaerobic conditions (where it is continually introduced in low levels throughout the fermentation) (11). The decreased ester values under semianaerobic conditions reported in Table II are in agreement with this observation.

As brewery fermentations, with the exception of the first few hours, are carried out under anaerobic conditions, the results from the anaerobic fermentations are most pertinent to the brewing industry. As Table II illustrates, only these beers—after dilution to prescribed alcohol levels—would have ester levels resembling a commercial lager beer. In particular, ethyl acetate, the ester of most concern in high-gravity brewing (2,5,27–29,32,34,37), would be below its flavor threshold of 30 mg/L in all such beers upon dilution. Acetate ester levels did, however, vary with the absence or presence of nutritional supplements.

In the unsupplemented anaerobic wort, ethyl acetate and isoamyl acetate levels were 35.90 and 3.74 mg/L, respectively. In other reports, ethyl acetate levels of 36 (34), 60 (26), 80 (26), and 40–69 mg/L (5); and isoamyl acetate levels of 3.0 (26), 4.2 (26), 4.8 (34), and 2.6–8.6 mg/L (5) have been measured in undiluted 20° P original gravity beer. Lower concentrations of acetate esters were seen in worts with higher levels of adjuncts, as higher C/N ratios in high-gravity worts result in decreased ester production (27,35). For example, in 18° P wort, ethyl acetate levels fell from 54 to 32 mg/L in the undiluted beer when 60% of the all-malt extract was replaced with an adjunct (27). The suggestion has been made that there is increased lipid production in "high adjunct" yeast, resulting in decreased availability of acetyl coenzyme A for ester synthesis (27). As the 27% (w/v) worts used here are 69% adjunct, it is not surprising that the concentrations reported here fall within the lower range. Excessive ester production in the unsupplemented beer, traditionally observed in high-gravity fermentations, would have been more likely had a much lower level of adjunct been used.

In the anaerobic beer supplemented with lipids only, the levels of ethyl acetate and isoamyl acetate (39.18 and 3.40 mg/L, respectively) were virtually identical to the levels seen in the unsupplemented beer. As adding lipids does not significantly alter the C/N ratio in the wort, such a result was not unexpected. However, adding yeast extract does lower the C/N ratio, and in the case of the anaerobic beer supplemented with yeast extract, the levels of ethyl acetate and isoamyl acetate rose to 50.98 and 6.82 mg/L, respectively.

In the fully supplemented anaerobic beer, the concentration of ethyl acetate was lowest (26.37 mg/L), despite the lowered C/N ratio. However, because there was an increase of 52.2% in yeast cell mass in the fully supplemented fermentation over the amount seen in the unsupplemented fermentation (10), the lowered ester production was not unexpected, based on literature reporting decreased ester production from increased cell mass production (2–4,8). However, the concentration of ethyl decanoate (48.86 mg/L) was very high (as in its semianaerobic counterpart), a 23-fold higher concentration (after dilution) than the level in the reference lager beer. Such a high concentration of this ester could possibly alter the aroma and characteristic flavor of the beer. This would have to be determined by taste test panels. Ethyl decanoate aside, ester levels found in the beer from the fully supplemented anaerobic fermentation (after dilution) compared favorably with those in the reference lager beer. It is not legal in North America to add fatty acids and ergosterol at the levels described here. Addition to the fermentation of the critical amount of oxygen, however, will allow enough yeast growth and metabolism to make it possible to rapidly ferment nitrogen-supplemented "very high gravity worts"

**TABLE III**
**Fusel Alcohol Levels (mg/L) in High-Gravity Brews Fermented With or Without Nutritional Supplements Under Anaerobic or Semianaerobic Conditions[a]**

| | *n*-Propanol | Iso-butanol | *n*-Butanol | Amyl Alcohols | 2-Phenyl Ethanol |
|---|---|---|---|---|---|
| Anaerobic[b] | | | | | |
| Unsupplemented | 7.07 | 27.05 | 0.57 | 184.12 | 23.81 |
| 1% (w/v) yeast extract | 5.50 | 17.91 | 1.76 | 147.65 | 8.95 |
| 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 7.99 | 27.70 | 0.38 | 236.82 | 27.82 |
| 1% (w/v) yeast extract/ 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 6.17 | 22.09 | 1.63 | 189.61 | 28.71 |
| Semianaerobic[b] | | | | | |
| Unsupplemented | 5.13 | 18.28 | 1.02 | 104.92 | 34.97 |
| 1% (w/v) yeast extract | 5.16 | 23.01 | 2.78 | 159.49 | 16.77 |
| 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 7.93 | 30.09 | 0.45 | 223.92 | 39.47 |
| 1% (w/v) yeast extract/ 40 ppm ergosterol/ 0.40% (v/v) Tween 80 | 6.28 | 23.89 | 2.97 | 148.03 | 17.01 |
| Commercial lager[c] | 3.93 | 13.19 | ND[d] | 74.58 | 23.83 |

[a] Each figure represents the average of triplicate analyses.
[b] Undiluted beer, 9% w/v alcohol (see text).
[c] 4% w/v alcohol.
[d] ND = not determined.

and produce beers of 16–17% (w/v) ethanol without excessive ester production.

Unlike ester production, fusel alcohol levels did not appear to be significantly influenced by the use of either anaerobic or semianaerobic conditions (Table III). Levels of *n*-propanol, isobutanol and 2-phenyl ethanol were lower in all beers compared to their levels in the reference lager beer. The exception was *n*-butanol (not normally seen in this lager), which was found at concentrations up to 2.97 mg/L. Only levels of amyl alcohols would, after dilution, significantly exceed those in the reference lager, reaching 236.82 mg/L (compared to 74.58 mg/L). Interestingly, under both conditions, the lipid-supplemented fermentations tended to have the highest levels of all the alcohols. As with ester levels, levels of alcohols in diluted beer from fully supplemented anaerobic conditions would be comparable to normal levels and would not therefore preclude the potential commercial application of fermenting such high-gravity worts.

## CONCLUSIONS

Fermentation of 27% (w/v) dissolved solids worts under semianaerobic conditions results in drastically decreased ester production compared to beers fermented anaerobically. Therefore, such beers would not be of interest to the brewing industry because of their bland flavor. Anaerobically produced beers, however, with the exception of ethyl decanoate, had ester concentrations that would resemble (after dilution) those found in commercially produced beers of similar alcohol level. The final concentration of esters was influenced by the C/N ratio and yeast cell mass production. It would therefore appear that if the exact amount of oxygen needed for cell growth could be determined, brewers could consider rapid fermentation of supplemented high-gravity worts (greater than 18% (w/v) dissolved solids) without incurring the traditionally observed difficulty of excessive ester production. Work on this subject is in progress.

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