

Wort Clarity: Effects on Fermentation

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ABSTRACT

The positives and negatives of wort clarity have been the subject of considerable debate. Wort solids have been shown to confer nutritive value during fermentation in both the brewing and Scotch whisky industries. The rate of fermentation is faster in the presence of solid material. The presence of insoluble material in wort is associated with high levels of lipid material, particularly unsaturated fatty acids and sterols. Both of these materials are essential membrane components of industrial yeast strains and can only be synthesized in the presence of oxygen, but they can be obtained from the surrounding medium. In addition, in the presence of wort solid material, carbon dioxide removal can be enhanced because the insoluble material acts as a nucleator. However, wort solids can impede beer filtration and cause flavor problems. The influence of all these factors in brewing and distilling on fermented wort quality are considered.

Keywords: esters, fatty acids, fermentation, higher alcohols, solids, wort

SÍNTESIS

Se ha debatido bastante sobre las consideraciones positivas y negativas de la claridad del mosto. Se ha demostrado que los sólidos del mosto le confieren valor nutritivo durante la fermentación, tanto en la elaboración de cerveza como de whisky escocés. La velocidad de la fermentación es mayor en la presencia de material sólido. La presencia de material insoluble en mosto es asociada con altos niveles de material lípido, particularmente ácidos grasos no saturados y esteroides. Ambos materiales son componentes esenciales de las membranas de cepas de levadura industrial y sólo pueden ser sintetizados en la presencia de oxígeno, pero pueden ser también obtenidos del medio circundante. Es más, la remoción de gas carbónico puede ser realizado en la presencia de material sólido del mosto, dado que el material insoluble actúa como formador de núcleos. Sin embargo, los sólidos del mosto pueden dificultar la filtración y causar problemas de sabor. Se tesen consideraciones sobre la influencia de todos estos factores en la calidad del mosto fermentado, tanto para cerveza como para licores destilados.

Palabras claves: esteroides, ácidos grasos, fermentación, alcoholes de alto P.M., sólidos, mosto

Introduction

The fermentation stage is very important in the production of alcoholic beverages. It is here that many flavor and aroma compounds are produced by yeast during sugar and nitrogen-compound consumption. In beer and malt whisky production,

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Presented at the 116th Convention of the Master Brewers Association of the Americas, Milwaukee, WI, October 2003.

Publication no. T-2003-1203-01

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the fermentation stages are similar in many ways. However, fundamental differences do exist, such as the omission of hops and a wort-boiling stage in malt whisky worts. Of importance to both fermentations is the level of solids carried over from the mashing stage. Much work concerning the effect of wort solids has been carried out in both the brewing and wine industries, with relatively little research conducted concerning the effects on whisky fermentation.

Research carried out in brewing has shown that solid particles are important as nucleation sites for the release of evolved CO₂ dissolved in fermenting wort (23,26). Enological research has shown that musts ferment more quickly in the presence of solid particles (28). Indeed, particle size has been shown to be an important determinant in the quality of the final product (1). Relatively little research has been conducted regarding the presence of solid materials during the fermentation of all-malt distiller's wort. Research 30 years ago revealed that spent grains have a stimulatory effect on the initial fermentation rate of distiller's wort, with concomitant increases in higher alcohols and glycerol concentrations (20). In malt distilling, although the wort is not boiled and a real attempt is made to clarify it before fermentation, there has been a gradual change in the mashing and clarification vessels employed in distilleries. There has been a move toward a lautering system or a mash filter and away from the traditional mash tun. This change is thought to have had an effect on the concentration of solids present in distillery worts (7).

This paper reports on the role of solids present in fermenting wort, the effect of certain fermentation parameters, and the role of various nutritional compounds on clarified worts.

Materials and Methods

Wort Production

All-malt wort was produced in the International Centre for Brewing and Distilling pilot brewery (2-hL capacity) and frozen at -20°C until use. Typically, for use in whisky fermentation experiments, the wort was not boiled prior to freezing and no hops were added. The original gravity of the wort used in these experiments was always 15°Plato .

Fermentations

Fermentations (1-L volumes) were carried out in 2-L tall tubes at 27°C . The yeast employed was *Saccharomyces cerevisiae* 'M' strain (Quest International, Menstrie, Scotland) and it was pitched at 0.3% (w/v). Fermentations for CO_2 estimation were carried out on a smaller (400-mL) scale. Wort was clarified by centrifuging cloudy wort at $2,500 \times g$ for 3 min. Fatty acids (C16, C16:1, C18:1, C18:2, and C18:3; obtained from Sigma Chemical Co., St. Louis, MO) were added to wort, and polyoxyethylene 20 acetyl ether (5 g/L, Brij58; Sigma Chemical Co.) was added to act as a dispersant. Zinc was added to wort from a stock solution (88 mg of ZnCl_2 per L) to give the final desired concentration. Silica particles used in CO_2 evolution studies were a gift from Ineos Silica (Warrington, England). All fermentations were carried out in triplicate.

Total Wort Sugar Consumption During Fermentation

Evaluation of wort sugar consumption during fermentation was carried out on 1-L static fermentations. This involved measuring the gross weight of each fermenter at regular intervals during the fermentation. As the fermentation of wort samples progressed, the gross weight of each fermenter decreased as sugar was consumed. Because the molecular weight of ethanol is similar to CO_2 , the decrease in mass (due to CO_2 liberation) approximates the amount of wort sugars consumed. This method permits a rapid and simple evaluation of the rate and extent of sugar consumption during fermentation.

Yeast Cell Number in Suspension

Cell counts of yeast cells in suspension during fermentations were conducted by using an Improved Neubauer Haemocytom-

eter (Fisher Scientific, Loughborough, England) at $\times 400$ magnification with a light microscope.

Particle Size Analysis

Particle size characteristics of all-malt solids, diatomaceous earth (DE), and bentonite were determined by using a Malvern Mastersizer and associated application software (Malvern Instruments Inc., Malvern, England). Measurements are based on the principle of laser-light scattering, with a capability of measuring sizes down to $0.05 \mu\text{m}$. Size distribution is expressed in terms of the volumes of equivalent spheres. Data involving surface area and number distribution were derived by numerical manipulation from the volume distribution.

Environmental Scanning Electron Microscopy (ESEM) of Solids

Samples for ESEM were suspended in a small volume of distilled water before analysis. Bentonite and DE were simply added to distilled water, whereas wort solids were collected following centrifugation of cloudy wort at $2,500 \times g$. The pellet was washed once with distilled water and resuspended in a small volume of distilled water.

Analysis of Fatty Acids in Wort

The concentration of fatty acids in wort was measured by using direct injection gas chromatography of derived methyl esters. By using an on-line derivitization technique, fatty acid methyl esters (C8–C18:3) were quantified with a Hewlett Packard 5890 Series II GC split/splitless injector with flame ionization detection (Stockport, England). The column was a BTR-CW fused silica glass capillary column ($0.32 \text{ mm} \times 50 \text{ m}$, $1\text{-}\mu\text{m}$ film thickness; Quadrex Corp., Woodbridge, CT).

Analysis of Free Amino Nitrogen (FAN) in Wort

The concentration of FAN in wort during fermentation was measured by using the EBC ninhydrin method (18).

CO_2 Evolution Determinations

To assess the CO_2 concentration in fermenting wort, it was necessary to convert dissolved CO_2 into Na_2CO_3 . This was achieved by adding 13.8 mL of 40% NaOH to 400 mL of fer-

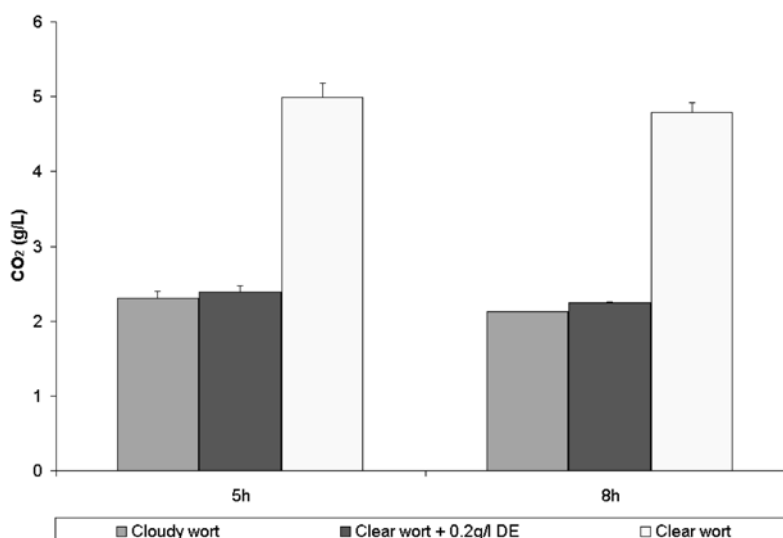


Figure 1. Concentration of CO_2 present during fermentation. DE = diatomaceous earth.

menting wort. Fifty-microliter samples were placed in the reaction chamber of a Corning 965 Carbon Dioxide Analyser (Corning, Loughborough, England), and a release agent (lactic acid) liberated the CO₂. The released CO₂ concentration was measured by means of a thermal conductivity detector and expressed as grams of CO₂ per liter.

Headspace Analysis of Esters and Higher Alcohols

Ethyl acetate, isoamyl acetate, propanol, isobutanol, 2-methyl butanol, and 3-methyl butanol concentrations were determined with a Hewlett Packard 5890 Series II GC split/splitless injector with flame ionization detector and Chrompack CP-Wax-57-CB column (0.25 mm × 60 m, 40-μm film thickness; Chrompack, London, England). 3-Heptonone was used as the internal standard.

Results

Factors Affecting the Concentration of CO₂ During Fermentation

The concentration of CO₂ in wort during fermentation was measured (Fig. 1). After 5 h of fermentation, there was a

greater concentration of CO₂ in clear wort than in cloudy wort. However, when clear wort was fermented in the presence of 0.2 g of DE per L, the resulting CO₂ concentration was similar to that in cloudy wort. A similar situation was seen after 8 h of fermentation. The ability of bentonite to decrease the CO₂ concentration during wort fermentation was also investigated. At the same concentration as DE (0.2 g/L), the level of CO₂ in fermenting wort was similar to that in clear wort after 5 h of fermentation (Fig. 2). As in the initial fermentations (Fig. 1), the addition of DE abrogated excess CO₂ to levels similar to that found in naturally cloudy wort. Differences in the ability of DE and bentonite to release excess CO₂ from fermenting wort at different concentrations were investigated. DE was added to clear wort at concentrations between 0.05 g/L and 0.2 g/L, and bentonite was added at concentrations from 0.2 g/L to 1 g/L. After 5 h of fermentation, the wort CO₂ concentration with different DE concentrations was similar, irrespective of DE levels. In contrast, the CO₂ concentration in clear wort with different added levels of bentonite was only slightly decreased when a relatively high bentonite concentration (1 g/L) had been added (Fig. 3).

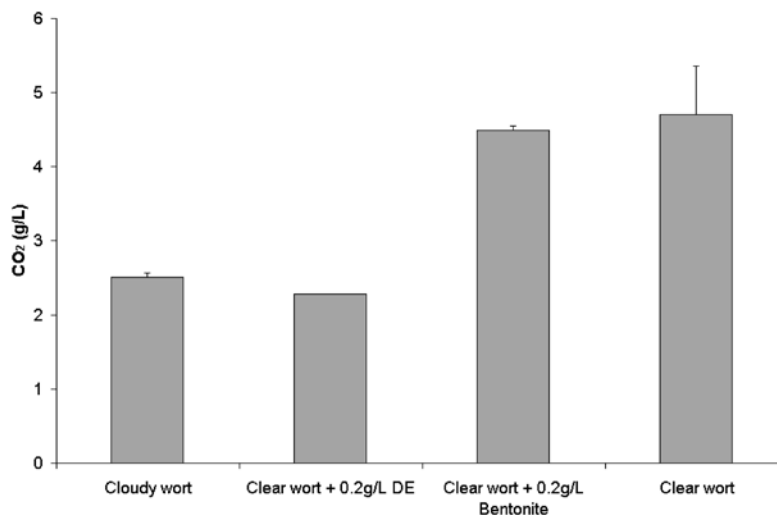


Figure 2. Effect of solid type on abrogation of excess CO₂ during fermentation. DE = diatomaceous earth.

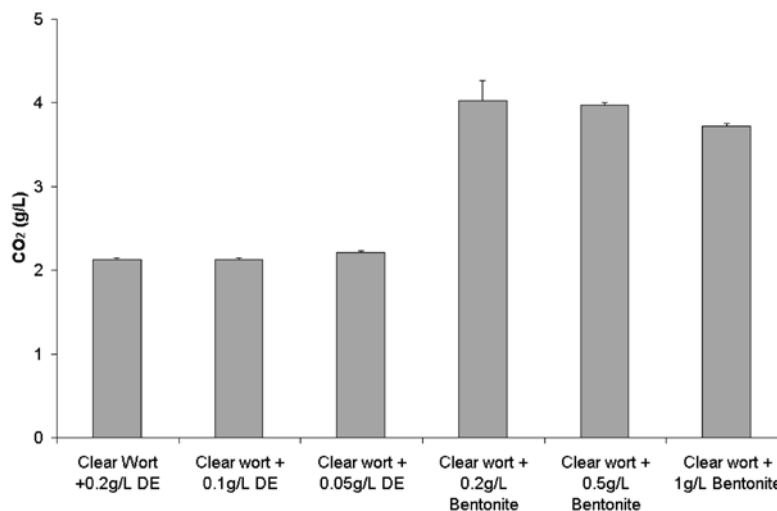


Figure 3. Effect of different concentrations of diatomaceous earth (DE) and bentonite on the concentration of CO₂ in fermenting clear wort.

Table 1. Specifications of silica particles used in experiments to determine the effect of particle dimensions on CO₂ concentration of fermenting clear wort

Silica particle designation	Particle diameter (µm)	Surface area (m ² /g)
SD2909	10–13	725
SD2910	8–12	412
SD2911	9.4–11.3	295

To assess whether the surface area of particles was an important factor in the release of CO₂ from fermenting wort, particles of known dimension (surface area and diameter) (Table 1) were added to clear wort. After 5 h of fermentation, the concentration of CO₂ was determined. There was no discernible difference in the concentration of wort CO₂ with any of the particles added. In all cases, the concentration of CO₂ was high (Fig. 4).

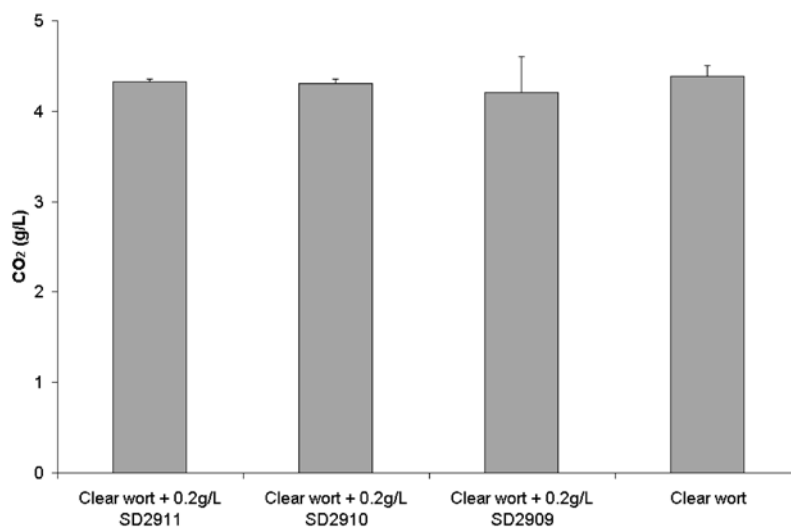


Figure 4. Effect of the addition of particles of different dimensions (Table 1) on the concentration of CO₂ in fermenting clear wort.

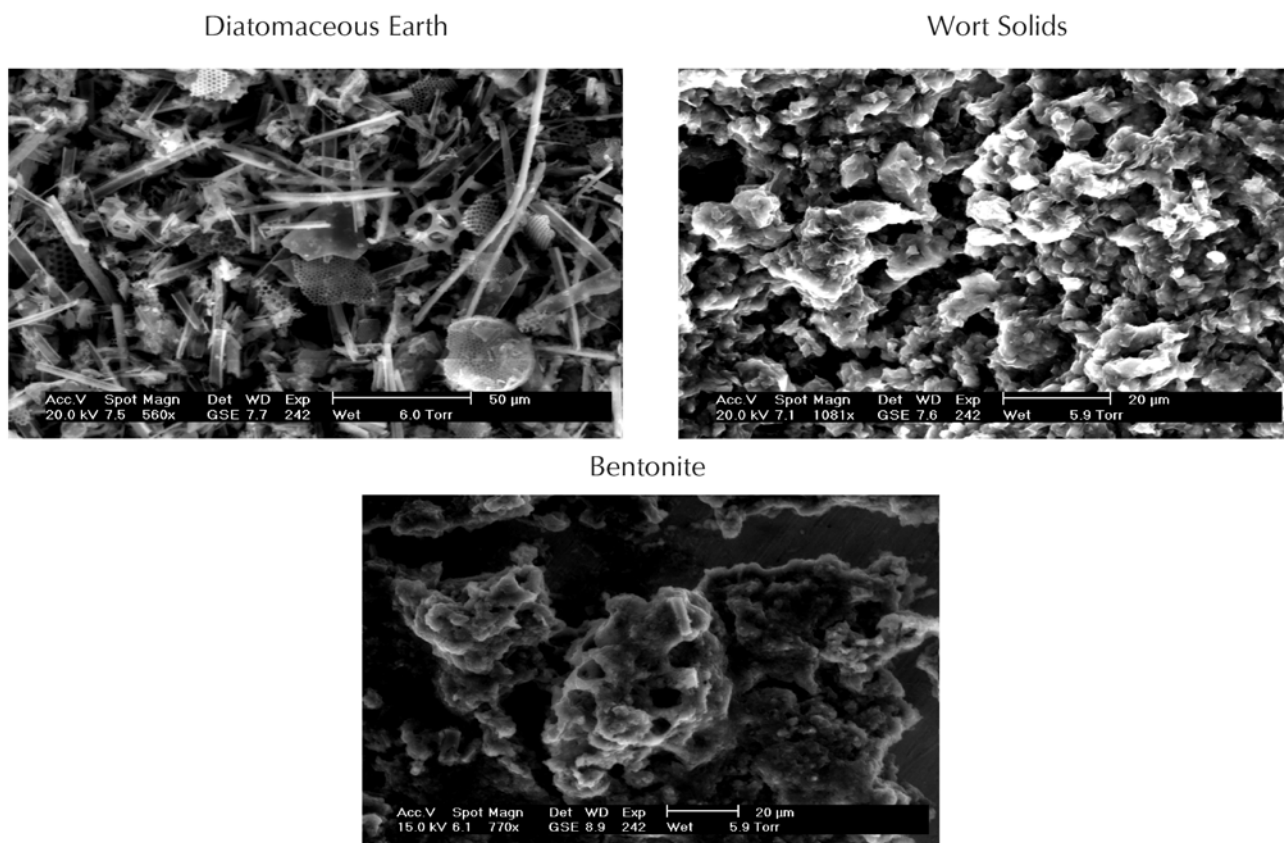


Figure 5. Environmental scanning electron microscopy of different solid materials.

Particle Size Analysis of Cloudy Wort, DE, and Bentonite

Analysis of the particle size profiles of the different worts employed in these experiments was conducted by using a Malvern Mastersign and associated computer software. Particle sizes in cloudy wort, DE, and bentonite (0.2 g/L) in distilled water were assessed. In cloudy wort, there was a wide volume distribution of particles (<0.1 to 100 μm in diameter), with the largest percentage of particles being around 7 μm in diameter. There was also a wide surface area distribution of particles, with sizes ranging from <0.1 to 40 μm in diameter. The largest percentage of particles within the surface area distribution was approximately 0.3 μm in diameter. The largest number of particles in cloudy wort was very small (<1 μm in diameter).

The particle size distribution of DE in distilled water was also measured. The overall distribution was similar to the distribution measured in cloudy wort. However, the volume distribution revealed that there were some large particles present in DE that were up to 250 μm in diameter. Also, the surface area distribution covered a wider range than that in cloudy wort (<1 to 100 μm in diameter). The number distribution of particles in DE was similar to that in cloudy wort, with the majority of particles being less than 1 μm in diameter.

The volume distribution of bentonite particles was relatively narrow, with particle sizes ranging from <0.4 to <50 μm in diameter. The peak particle diameter of the volume distribution was approximately 8 μm in diameter. In terms of the surface area distribution of particles, the range was much narrower than those of cloudy wort and DE, it being between 0.4 and 20 μm in diameter. Whereas the surface area distributions of cloudy wort and DE particles

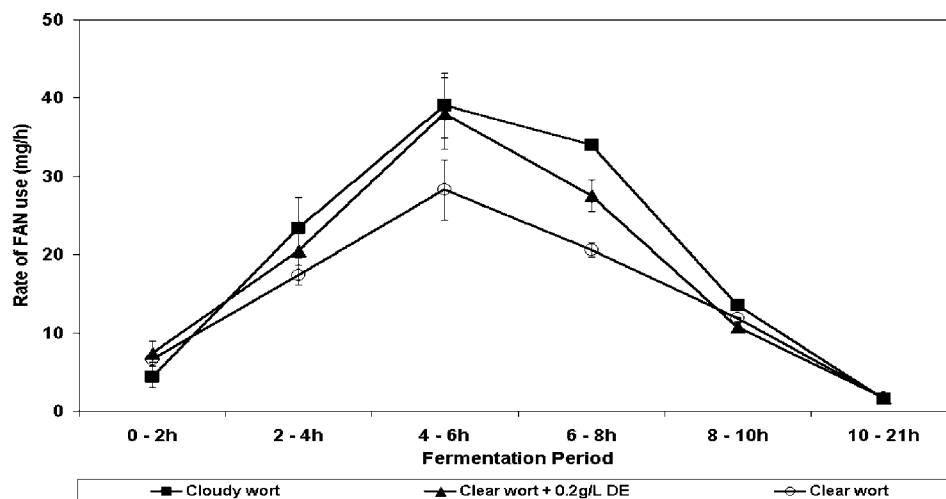


Figure 6. Rate of free amino nitrogen (FAN) consumption during wort fermentation. DE = diatomaceous earth.

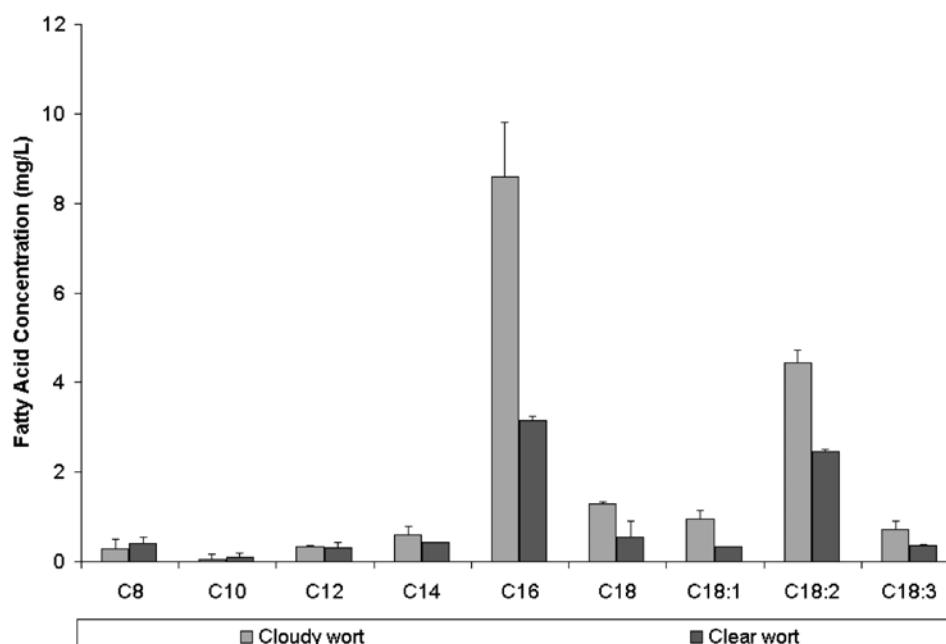


Figure 7. Concentration of fatty acids in clear and cloudy worts.

appeared bimodal, for bentonite, the distribution was monomodal. The number distribution of bentonite particles was also different, and distribution ranged between 2 and 6 µm in diameter.

ESEM of Solid Particles

In order to visualize each type of particle and obtain data on their individual surface characteristics, ESEM analysis of the three was conducted (Fig. 5). In DE, there was a heterogeneous mix of shapes and sizes of particles. The surface of most of the particles revealed an extremely porous structure, which would be expected. The micrograph of cloudy wort solids showed a mix of different structures, again porous in nature. Bentonite had a more homogenous structure. In addition, it possessed a much different surface topology. It did not appear to possess the same porous nature as DE or wort solids.

Fermentation Characteristics of Clear Wort, Cloudy Wort, and Clear Wort Supplemented with DE

The rate of FAN consumption by yeast during the initial stages of fermentation was determined (Fig. 6). Between 0 and 6 h, yeast in cloudy wort and clear wort with 0.2 g of DE per L added utilized FAN at similar rates, whereas fermenting clear wort utilized FAN at a reduced rate. After 6 h of fermentation, FAN was consumed at an increased rate in cloudy wort compared with that in clear wort with 0.2 g of DE per L. Since the concentration of CO₂ in fermenting cloudy wort was comparable to that of clear wort with added DE (Fig. 1), it has to be assumed that there must be additional factors that contribute to the dynamics of fermentation.

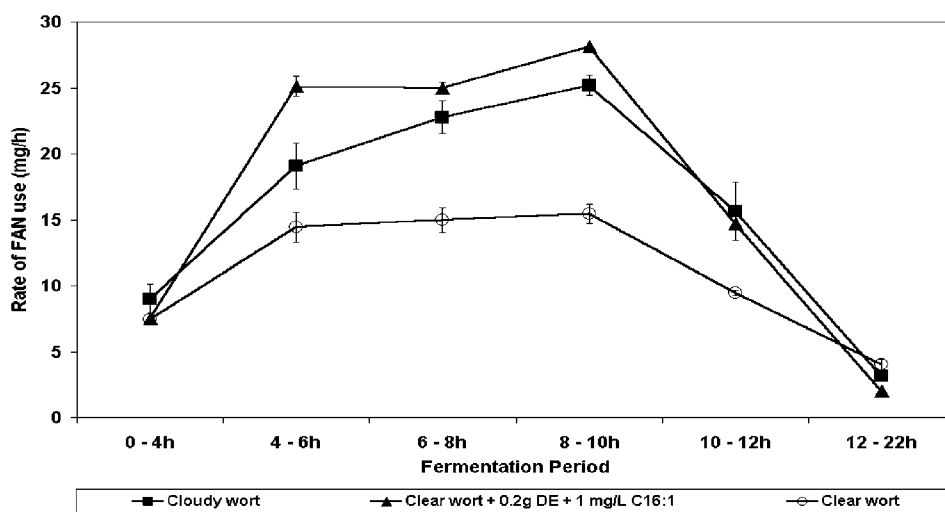


Figure 8. Effect of palmitoleic acid (C16:1) addition to clear wort with diatomaceous earth (DE) during fermentation. FAN = free amino nitrogen.

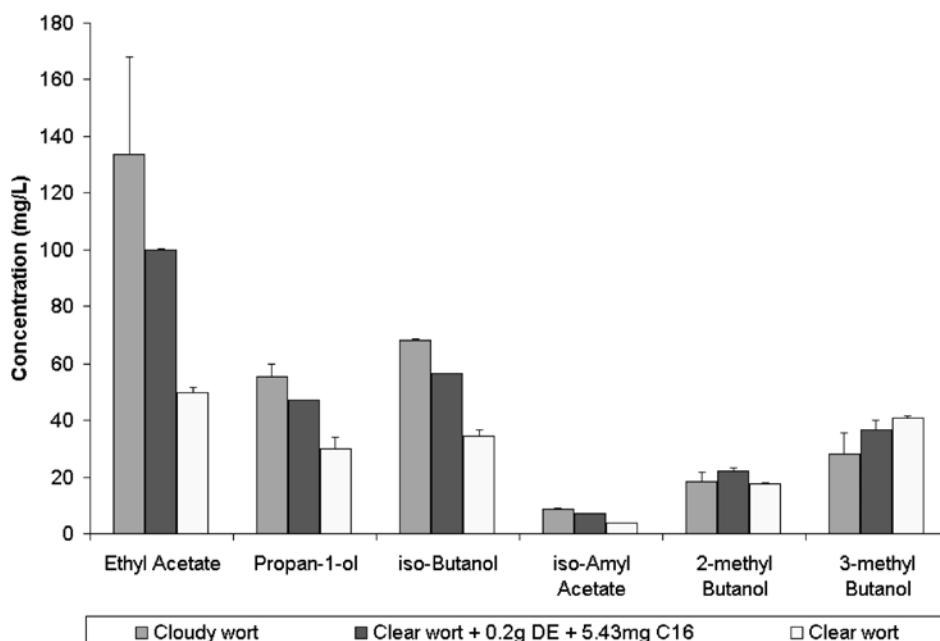


Figure 9. Production of volatile flavor compounds in cloudy wort, clear wort with diatomaceous earth (DE) and palmitic acid (C16), and clear wort.

Concentration of Fatty Acids in Cloudy and Clear Worts

Fatty acids are important factors that contribute to overall wort fermentation rate and extent (10,14,17,29). Consequently, the effect of fatty acids on the rate of FAN uptake was investigated. The concentration of a range of fatty acids was determined in cloudy and clear worts (Fig. 7). There were only small concentrations of short- to medium-chain fatty acids (C8–C14), whereas there were much larger concentrations of palmitic acid (C16) and long-chain fatty acids (C18–C18:3). Linoleic acid (C18:2) was the most abundant unsaturated fatty acid (UFA). Following clarification of cloudy wort, the concentration of long-chain fatty acids reduced to levels found in clear wort (data not shown).

Fermentation Effects of Palmitic Acid (C16) Addition to Clear Wort with DE

The effect of palmitic acid (C16) addition to clear wort during fermentation on FAN utilization is shown in Figures 8 and 9. During the initial 6-h fermentation period, cloudy wort and clear wort with DE and added C16 (5.43 mg/L) utilized FAN at a similar rate. However, clear wort used FAN at a reduced rate. After 6 h of fermentation, cloudy wort used FAN at a greater rate than did clear wort with added C16. There was also a considerable increase in the cell number in suspension in cloudy wort and in clear wort with DE and added C16 during the initial 10 h of fermentation. There were fewer yeast cells in clear wort during the same fermentation period. The number of yeast cells in suspension was similar in cloudy wort and clear wort with DE and added C16.

After 56 h of fermentation, the concentration of a spectrum of volatile flavor compounds was measured. The cloudy wort contained the highest concentration of ethyl acetate, propanol, butanol, and isoamyl acetate (Fig. 10). Clear wort with DE (0.2 g/L) also contained enhanced concentrations of these volatiles compared with unsupplemented clear wort. The concentrations of 2-methyl butanol and 3-methyl butanol were similar in all three wort preparations following fermentations.

Fermentation Effects of Oleic Acid (C18:1) Addition to Clear Wort with DE

Oleic acid (C18:1) is another UFA found in wort. It was added to clear wort in slight excess of the difference between

clear and cloudy worts (Fig. 7) and fermented. The effect of added C18:1 on the rate of FAN utilization during fermentation is shown in Figure 10. Both cloudy wort and clear wort with added C18:1 fermented faster during the initial 12 h of fermentation compared with fermented unsupplemented clear wort. The addition of C18:1 to clear wort with DE resulted in an increase in the rate of FAN uptake between 0 and 8 hr of fermentation compared with that in cloudy wort. There was a greater number of yeast cells in suspension in clear wort with DE and C18:1 than in cloudy wort during the 4- to 22-h fermentation period.

Discussion

The concentration of CO₂ present in wort fermentation is an important factor that will influence optimal yeast fermentation performance (2,8,23,26–28). Because of pH values typically encountered during alcoholic fermentation, the majority of the dissolved CO₂ present will be the aqueous species CO₂ (aq.) (8). The inhibitory effects of CO₂ include effects on biological membranes and cytoplasmic enzymes, particularly those of the tricarboxylic acid (TCA) cycle (6). Research focused on the brewing process has shown that increased concentrations of CO₂ result in the decreased production of flavor-active volatiles, related to reductions in yeast cell growth and overall biomass production (24). Further, it has been shown that increased CO₂ pressures have an effect on the uptake of certain amino acids (15,27). In the studies reported here, the concentration of CO₂ during the early stage of an all-malt fermentation has been measured. There was a greater concentration of CO₂ in clear wort than in cloudy wort. When DE was added to the clarified wort, the concentration of CO₂ decreased to that of cloudy wort. This finding concurs with the report of previous researchers (16,26). The solid particles act as nucleation agents and release CO₂ from solution.

Although DE was efficient at relieving the increased CO₂ concentration, similar effects were not observed with bentonite. It is assumed that the nature of the solid particles is very important in determining its ability to act as an efficient gas nucleator. To study this aspect further, a range of silica particles of known size and surface area were added to clarified wort. These particles, known to have a very porous surface, were assumed to be good gas nucleators. However, the concen-

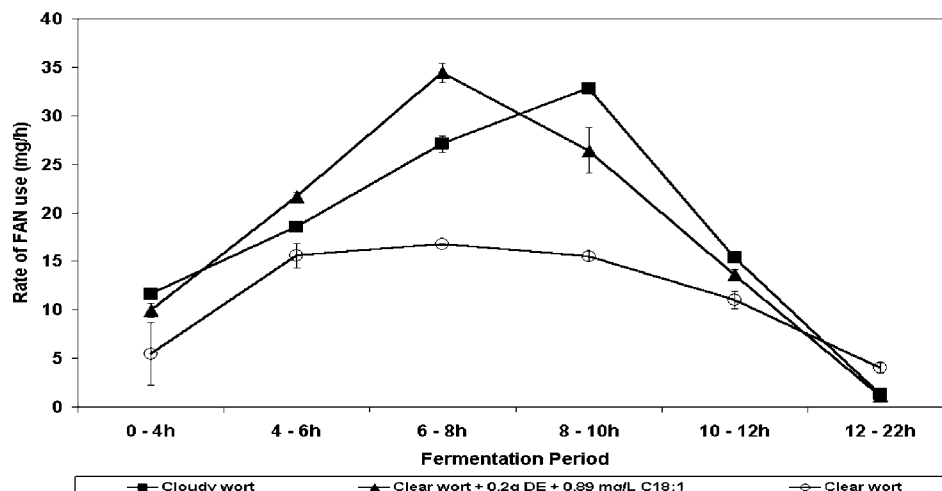


Figure 10. Effect of oleic acid (C18:1) addition to clear wort with diatomaceous earth (DE) during fermentation. FAN = free amino nitrogen.

tration of CO₂ was still high in these worts after 5 h of fermentation. Obviously, the porosity of the particle surface was insufficient to nucleate CO₂. It may have been that these particles were so small that, when CO₂ bubbles evolved, the particles were raised to the surface of the fermentation and out of suspension. This was supported by Delente et al. (5), who showed that most nucleation in a fermenter occurred from particles that sedimented to its bottom. As a consequence, it would mean that, in the experiments reported here, the CO₂ concentration would increase to the levels observed in clear wort.

Particle size analysis revealed that, although there were some relatively large particles in cloudy wort, DE, and bentonite, particles of small diameters were predominant. Cloudy wort and DE had wide surface area and volume distributions of particle diameter, whereas in bentonite, the distributions were narrower. This indicates that bentonite was a more homogeneous compound in terms of particle structure than were cloudy wort particles and DE. These results suggest that particle size is a likely determinant of a particle's ability to be an effective gas nucleator.

ESEM analysis of particles from cloudy wort, DE, and bentonite was carried out to determine differences in surface characteristics. ESEM is a technique that permits biological samples to be analyzed without pretreatment, such as fixing, which results in destruction of the sample. As the samples are scanned, it is possible to analyze the surface topography of each particle type. There were differences in the surface characteristics of these materials. DE had a diverse mix of particles that appeared to be very porous, as did wort particles. Bentonite particles appeared to have a smoother surface. Gas nucleation, which occurs with porous particulate materials, has been classified as type III pseudo-classified by Jones et al. (11). Nucleation of this type refers to the existence of preexisting gas cavities at the surface of suspended particles that, under low supersaturation conditions, can form bubbles of gas. The energy required to form initial gas bubbles is less because of the existence of gas cavities. Because of the surface characteristics of DE, there will be many gas cavities present in the situation similar to wort solids. Bentonite does not have the same porous nature as DE and, therefore, CO₂ is less likely to nucleate under similar supersaturation conditions. This is the situation observed in this study (Fig. 2).

There were fermentation performance differences between clear wort, clear wort with DE, and cloudy wort. The rate of FAN consumption during fermentation was much lower in clear wort than in cloudy wort. This agrees with the reports of other researches (21,22). However, the rate of FAN consumption was similar in cloudy wort and clear wort with DE until 8 h of fermentation, when FAN was consumed at a decreased rate in clear wort with DE. Since the concentration of CO₂ was similar in clear wort with DE and cloudy wort, there were other factors present in cloudy wort that stimulated increased FAN consumption. The association of trub with nutritional components, such as lipids and metal ions, has long been recognized (12). Retention of fatty acid content in vacuum-filtered wine must has also been reported (3). Consequently, the concentrations of fatty acids in cloudy and clear worts were determined. There were small concentrations of short- to medium-chain-length fatty acids present (C8–C14), with slightly larger concentrations of long-chain fatty acids (C16–C18:3). Whereas, with long-chain fatty acids, there were always lower concentrations in clear wort than in cloudy wort, providing indirect evidence for the association of fatty acids with wort solids. These findings agree with the findings of Narziss (22) and others.

The role of some nutritional compounds in the fermentation of all-malt wort has been studied. Different concentrations of fatty acids were added to clear wort with DE (0.2 g/L). In the case of palmitoleic acid (C16:1) and linoleic acid (C18:2), they were added at the concentration equivalent to the difference between cloudy and clear worts. The results showed that C16 and C18:2 were unable to stimulate the rate of FAN usage by yeast to the same extent as cloudy wort. Analysis of volatile compounds showed that clear wort contained, overall, lower concentrations than did cloudy wort. Addition of the UFAs C16:1 and C18:1 had the opposite effect on fermentation of clear wort with DE in that the concentration of acetate esters was slightly higher than that in cloudy wort. Growth of the yeast was also stimulated in these worts. As these fatty acids were available for uptake by yeast, it may have been the case that yeast would have required less cellular palmitoyl-coenzyme A for long-chain fatty acid production. Consequently, there would have been less inhibition of aspartate aminotransferase (AAT), and thus an increase of acetate esters. Rosi and Bertuccioli (25) have reported that yeast fermenting in the presence of C16:1 (which was subsequently consumed) resulted in wines with a higher concentration of acetate and ethyl esters. Ayestarán et al. (3) showed that filtration of wine must caused a reduction of fatty acid content. Groat and Ough (8) found that the addition of grape solids to wine must showed an increase in the total volatile ester content of wine. Fermentation after addition of C18:2 to clear wort with DE resulted in a decreased concentration of acetate esters compared with that in cloudy wort. This suggests an important role for C16:1 and C18:1 as UFA components of yeast cell membranes compared with C18:2. Mauricio et al. (19) found that, after addition of a relatively high concentration of C18:1 to wine must, there was a higher final concentration of ethyl acetate in the final product (4,9). This was attributed to high yeast growth rates in the presence of C18:1. During an assessment of wort preparation and beer stability, Kaneda et al. (13) showed that there were no significant differences in the concentration of volatiles, including acetate esters, after wort clarification. After clarification, there were lower concentrations of fatty acids, particularly UFAs.

Understanding the role of nutritional compounds present in cloudy worts compared with those in clear worts will help in the control of beer and whisky production, leading to more consistent products. Scotch whisky production is governed by legally binding procedures that, for example, similar to the Bavarian Purity Law (21), do not permit the addition of exogenous substances during any part of the process (Scotch Whisky Act/Scotch Whisky Order 1990). Therefore, any attempt to modify the nutritional components of all-malt wort will have to be completely natural and result in some changes in process conditions. The goal of future studies will be to determine the factors that are important during mashing and that contribute to differences in the composition of worts for fermentation.

ACKNOWLEDGMENTS

The authors would like to thank Suntory Limited for financial support and invaluable assistance during the course of this project. Thanks are also due to Graham McKernan and Jim MacKinley for help with technical matters.

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