Wort Trub Content and Its Effects on Fermentation and Beer Flavor[1]

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ABSTRACT

Pilot-scale fermentations were conducted to determine the impact of various trub levels on fermentation kinetics and beer flavor. Trub content of the worts ranged from 0.00 to 2.66% (v/v). Clarified worts were produced either by filtration or by whirlpool treatment. Worts with high trub content were produced by omitting clarification treatment altogether, or by clarifying wort and then adding predetermined amounts of trub to the fermentor during fill. Results indicated that trub stimulated yeast activity, and subsequently, the rate of fermentation. In addition, elevated trub levels depressed the formation of esters and slightly increased production of fusel alcohol. Flavor analyses of the finished product demonstrated a preference for beers produced from clarified worts. The relationship between trub and fermentation kinetics appeared to be related to high lipid and zinc contents in the trub, which may have contributed important growth factors to the yeast. These findings demonstrate the importance of utilizing a wort processing technique that achieves an optimum and consistent trub level in production wort.

Key words: *Esters, Fermentation, Trub*

Fresh wort typically contains a substantial amount of suspended solids when dropped from a brew kettle. The solids may be comprised partly of hop leaves, depending on the hopping method used by the individual brewer. The remaining solids are actually precipitates of variable composition that are commonly referred to as "trub." Other terms frequently used to describe precipitates are hot break and cold break. These are more specific terms and are used by some brewers to distinguish precipitates that form in wort at different temperatures.

Brewers typically remove trub solids from wort prior to fermentation. Some common wort clarifiers are the whirlpool, the lauter tun, and the centrifuge. The primary reasons for clarifying wort seem to be the desire for a product that will filter well and for a clean yeast that is suitable for repitching. Little attention has been given to possible interactions between trub and yeast during fermentation, but Ahvenainen et al (1) described the effects of hot break and cold break in stimulating the rate of fermentation. Taylor et al (11) and Äyräpää et al (4) recorded similar findings, although they studied the effects of spent malt grains rather than trub in stimulating yeast activity. Variations in ester synthesis were noted by the latter authors, who reported consistently lower ester level production from worts containing large amounts of spent malt grains (or liquids expressed from them).

The intent of this work was to further characterize the relationship between trub and yeast activity during fermentation. Particular attention was given to the rate of fermentation, the production of esters and fusel alcohols, and overall beer flavor and acceptability. A mechanism is proposed to explain, at least in part, of the role of trub in yeast metabolism during fermentation.

EXPERIMENTAL

Five pilot-scale fermentations were conducted as part of a two-phase investigation. The experimental design in phase I called for an evaluation of extremes in wort clarity. Two fermentors were filled, one with a clarified wort and the other with a high-trub wort. The clarified wort was produced from a mixture of whirlpool-treated and sheet-filtered wort (70/30). The result was a wort that was somewhat turbid but that had particles too small to be measurable by centrifugation. The second fermentor was filled with wort that was pumped directly to the fermentor without clarification treatment. This wort contained a trub content of 2.66% (v/v) as determined by centrifugation at 4,000 rpm for 5 min. Wort oxygenation levels were 8–9 mg/L for both tests.

The second phase of testing comprised a series of three fermentations with worts ranging in trub content from 0 to 1% (v/v). One fermentor was filled with wort run through a whirlpool using a 30-min settling period. Wort oxygen levels for this test were reduced by approximately 40%. A second fermentor was filled in a similar manner, except that trub was harvested from the bottom of the whirlpool and added back to the fermentor at a rate of 1% (v/v). Wort oxygenation levels again were reduced by approximately 40%. The third fermentor was filled with a clear wort, achieved by whirlpool treatment followed by filtration through a small

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Enzinger filter. A diffuser was placed immediately ahead of the fermentor in the fill line, and the oxygen flow rate was adjusted to achieve saturation. Variations in wort oxygen levels were instigated for these tests to determine whether a relationship exists between wort oxygen and trub levels in stimulating the rate of fermentation. All test worts were chilled just before entering the fermentors and were maintained at 8.5°C throughout the fermentation cycle. A summary of test variables for the five fermentations is given in Fig. 1.

Pertinent kinetic parameters measured throughout fermentation included wort attenuation values and suspended yeast-cell counts. The yeast counts were determined with a hemocytometer.

Esters and fusel alcohols were determined by headspace gas chromatography using a Perkin-Elmer Sigma II gas chromatograph equipped with an HS-6 headspace sampler. Each beer was analyzed after secondary fermentation using an isothermal program with a flame ionization detector. Temperature setpoints for the gas chromatograph included an injector temperature of 110°C, an oven temperature of 60°C, and a detector temperature of 200°C. Headspace samples were injected into a 12 ft × ⅛ in. stainless steel column packed with 10% 1-2-3 tri-2-cyanoethoxypropane on 80/100 chromosorb W-AW. The carrier gas was nitrogen at a flow rate of 30 ml/min. Each analysis required 30 min for completion. Ethanol analyses were determined by distillation, and vicinal diketones were determined by an ASBC method (2).

Total lipids were measured in trub and in centrifuged wort by the method of Bligh et al (5). Specific fatty acids were extracted from trub using an adaptation of a method designed for whole milk (10). Trub was recovered from wort by centrifugation. The liquid portion was decanted off, and the wet trub was qualitatively analyzed for fatty acids. Seven grams of the trub material was mixed with 80 ml of distilled water. Ten milliliters of the trub solution was then added to a separatory funnel, followed by 10 ml of ethanol, 3 ml of 28% ammonium hydroxide (w/w), 25 ml of petroleum ether, and 25 ml of diethyl ether. The funnel was shaken for 5 min and left standing for 20 min. The bottom phase was drained off, and the ether phase was carefully poured out the top of the funnel and dried under a stream of nitrogen gas. After drying, the residue was dissolved in 0.5*N* NaOH in methanol and transferred to a separatory funnel. Five milliliters of distilled water was added, followed by enough 2*N* HCl to adjust the pH to 2.0. Fatty acids were then extracted with 5 ml of petroleum ether and 5 ml of diethyl ether. The ether layer was drawn off and dried under a stream of nitrogen, and the sample was taken up in approximately 1 ml of methylene chloride. The fatty acids were analyzed by gas-liquid chromatography, using an isothermal program with an oven temperature of 190°C. Other temperature setpoints included an injector temperature of 250°C and a flame ionization detector temperature of 250°C. One-milliliter samples were injected into a 6 ft × 2 mm (i.d.) glass column packed with 5% DEGS-PS stabilized on 100/120 Supelcoport. Each analysis required 7 min for completion.

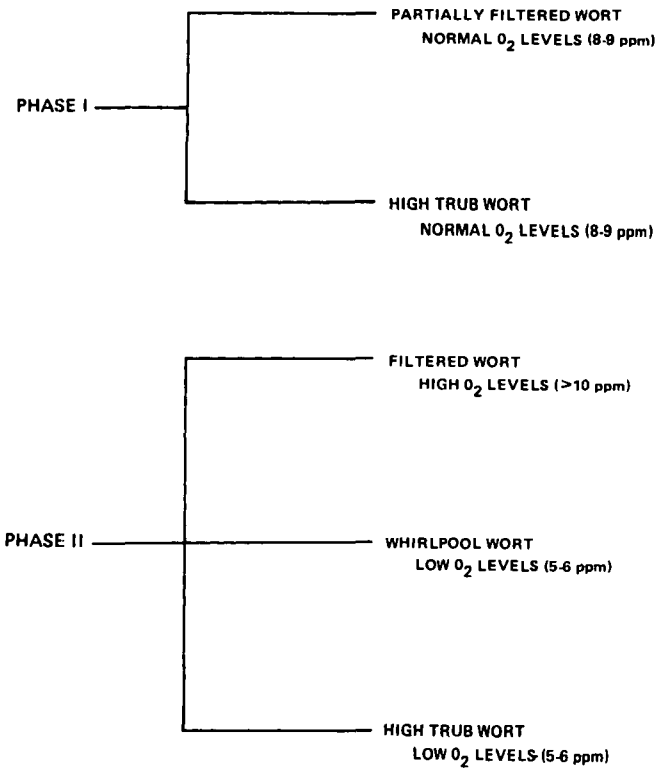

Fig. 1. Experimental variables in five fermentations.

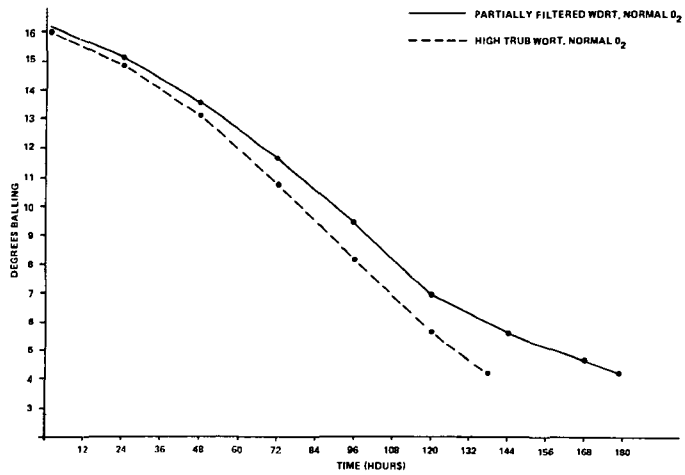

Fig. 2. Attenuation profiles for Phase I fermentations.

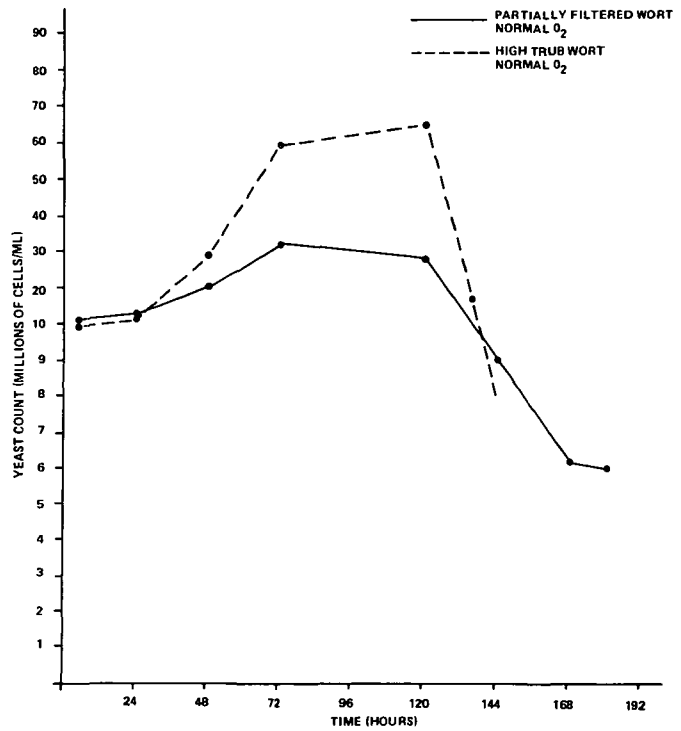

Fig. 3. Suspended yeast count profiles for Phase I fermentations.

Zinc levels in yeast, clarified wort, and trub were determined by atomic absorption spectrophotometry. Yeast samples obtained from production pitching facilities were centrifuged and washed three times with distilled water. Wort was separated from trub by centrifugation and decantation in a manner identical to that used for the fatty acid analysis. Each sample was digested using a modification of a published method (9). One gram of a well-mixed sample was transferred to a 250-ml beaker. Concentrated nitric acid (10 ml) was added, and the beaker was placed on a hot plate and evaporated without boiling until nearly dry. The beaker was then cooled, and 5 ml of concentrated nitric acid was added. After covering with a watch glass, the beaker was returned to the hot plate and again evaporated to near dryness. This process was repeated three times with 5-ml additions of concentrated nitric acid and, when necessary, twice with 5-ml additions of 30% hydrogen peroxide. Complete digestion was indicated by a light-colored residue. The residue was dissolved in distilled water, with care being taken to wash the watch glass and beaker. In some cases, filtration was necessary to remove silicate and other insoluble material that would interfere with the atomizer. The volume was then adjusted to 25 ml in a volumetric flask. Samples were prepared in duplicate along with one blank. Analysis for zinc was completed using a Perkin-Elmer model 703 atomic absorption spectrophotometer at a wavelength of 2139A. Zinc values were calculated based upon the solids portion of the 1-g sample. Flavor testing, which included preference tests and descriptive analyses, was conducted on the finished, filtered beers.

RESULTS

Attenuation rates and suspended yeast counts during fermentation were clearly affected by the trub content of the wort. In the first phase of testing, the high-trub wort fermented in 138 hr, substantially faster than its low-trub counterpart, which fermented in 180 hr (Fig. 2). Yeast activity was more vigorous in the high-trub wort, as evidenced by the suspended yeast count profiles (Fig. 3). Phase II results were similar but were influenced by the diverse wort oxygenation rates established at fermentor fill. An examination of attenuation curves (Fig. 4) indicates that the filtered wort with a high level of dissolved oxygen fermented at virtually the same rate as the high-trub wort with a low level of dissolved oxygen. The fermentation residence times for these tests were 152 and 158 hr, respectively. By contrast, the clarified wort with a low level of dissolved oxygen fermented very slowly and required 312 hr to reach drop balling. Suspended yeast counts followed an expected pattern based upon the rate of fermentation. High densities of suspended yeast developed in the clear, well-oxygenated wort and in the high-trub wort with reduced oxygen levels. Conversely, very low densities of yeast developed in the clarified wort with reduced oxygen levels (Fig. 5).

Production of esters and fusel alcohol during fermentation was also influenced by wort trub levels. In both series of tests, ester levels were higher in beers produced from clarified worts. Conversely, fusel alcohols were higher in beers containing high levels of trub (Table I).

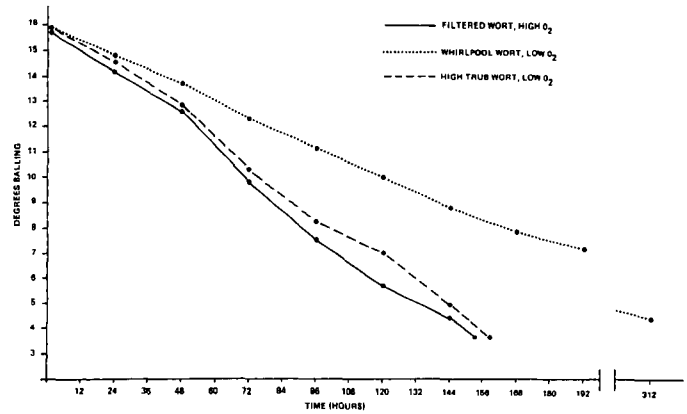

Fig. 4. Attenuation profiles for Phase 2 fermentations.

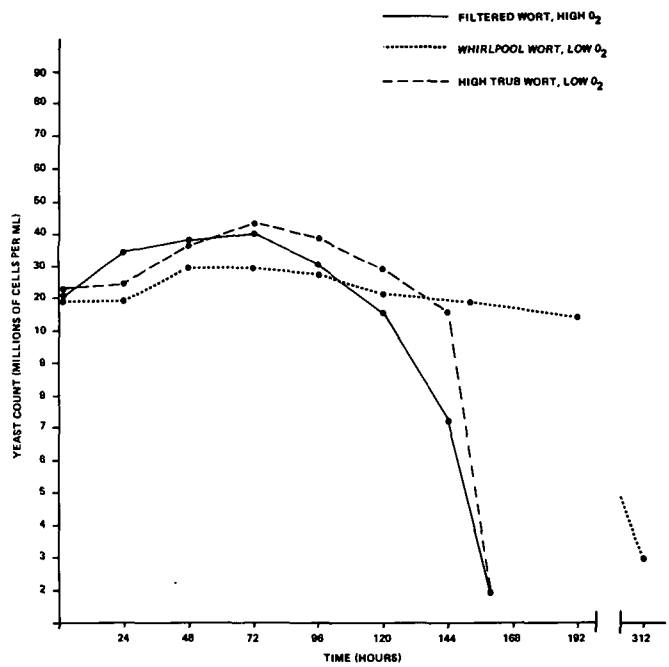

Fig. 5. Suspended yeast count profiles for Phase 2 fermentations.

TABLE I
Esters and Fusel Alcohols (ppm) in Aging-Test Beers

	Tests				
	Phase 1		Phase 2		
	Partially Filtered Wort (Normal O_2)	High-Trub Wort (Normal O_2)	Filtered Wort (High O_2)	Whirlpool Wort (Low O_2)	High-Trub Wort (Low O_2)
Acetaldehyde	4.93	2.01	1.66	0.97	1.02
Ethyl formate	...	...	0.14	0.08	0.24
Ethyl acetate	19.00	15.17	14.03	13.35	8.63
Isoamyl acetate	2.23	1.39	1.39	1.29	0.70
Ethyl caproate	3.05	2.46	2.29	2.50	2.02
Propanol	...	7.71	7.66	7.14	11.34
Butanol	17.28	22.33	21.64	21.08	21.77
Isoamyl alcohol	99.15	97.96	74.76	66.23	81.60

An analysis of wort lipids showed that levels in trub (13.4 mg/g, d wt) were significantly higher than those in clear, centrifuged wort (0.26 mg/g, d wt). A more definitive evaluation of the trub fraction indicated the presence of four predominant fatty acids: lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), and linoleic ($C_{18:2}$, Fig. 6).

Zinc levels were also much higher in trub (32.2 μg/g) than in centrifuged wort (6.3 μg/g). Washed yeast samples contained slightly higher zinc levels than did trub (37.8 μg/g).

Levels of vicinal diketones at the end of aging showed some variation, but these differences appeared insignificant, considering the precision of the analysis. Ethanol concentrations were consistently higher in beers produced from high-trub worts, and values for real degree of fermentation (RDF) were similar for tests in each phase of the study (Table II).

Descriptive and preference testing demonstrated better flavor characteristics in beers produced from clarified worts. In phase 1 tests, the beer produced from clarified wort was found to be within an established standard flavor profile. Two consecutive evaluations were conducted on this particular beer. No off-flavors were detected in the first test, and only a mild winey flavor and a grapefruit-rind aroma were present in the second. In both flavor and aroma, the high-trub beer exhibited spoiled fruit and caramel characteristics. Both beers were judged acceptable in preference tests, although panelists demonstrated a significant preference for the beer produced from clarified wort at a 99% confidence level. All beers produced during the second phase of testing were outside the standard profile. However, the high-trub beer was notably inferior because of its strong caramel flavor. This beer was omitted from preference testing. Of the remaining two beers, the whirlpool-treated beer, which had a sulfury aroma, was preferred over the beer produced from filtered wort, which had an aroma of caramel. In this instance, the level of significance was less than 95%, but the voting margin still exceeded a 2:1 ratio.

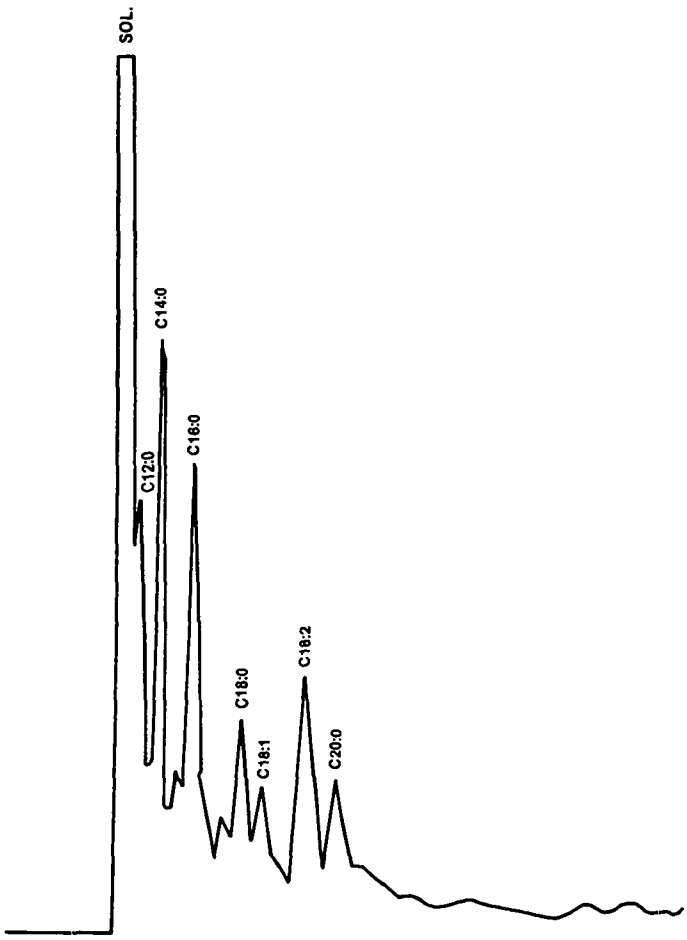

Fig. 6. Gas chromatogram of fatty acids extracted from trub.

DISCUSSION

The findings in this study indicate that trub solids play a dynamic role in yeast metabolism during fermentation. In both series of tests, worts containing substantial amounts of trub displayed more vigorous yeast activity and more rapid fermentation. The relationship appears to be primarily one of nutrition. This conclusion is supported by the findings of other workers in related studies (1,3,7). The concept that an important physical interaction exists between yeast and trub was not indicated in this work but certainly should not be overlooked in future studies.

A key to the relationship became evident during the second series of fermentations, in which a filtered wort fermented at virtually the same rate as a high-trub wort and displayed similar suspended-yeast counts. This apparent anomoly was related to a second variable: wort oxygenation. At fermentor-fill, as discussed above, the filtered wort was heavily oxygenated, whereas the high-trub wort contained reduced oxygen levels. This seems to indicate that dissolved oxygen and trub function similarly in stimulating yeast activity. This premise is supported by the lipid characteristics of trub and by the findings of other workers. Aries et al (3) documented the effects of oxygen in stimulating the production of sterols and unsaturated fatty acids. These compounds are vital to yeast growth and to metabolic activities involving the cell membrane. Taylor et al (11) and Ahvenainen et al (1) found that unsaturated fatty acids derived from spent grain stimulates yeast growth and, when present in adequate quantities, permit complete fermentation of deoxygenated wort. Th :se authors also stated that linoleic acid may be the single most important fatty acid in achieving the stimulatory effect. Because the fatty-acid analysis of trub in our study indicated high levels of linoleic acid, the high-trub wort with low levels of oxygen fermented well, due to the availability of unsaturated fatty acids in the trub. The filtered wort, on the other hand, contained no trub but fermented well because the yeast was able to synthesize its own lipid material in the presence of oxygen. The third fermentation featured a clarified wort with insufficient oxygen levels. As a result, the yeast was unable to synthesize lipids by any means and fermented poorly.

Even though wort oxygen levels and wort lipid content in trub seemed to be related in stimulating fermentation, their metabolic pathways were different, as demonstrated by the shift in levels of esters, fusel alcohols, and ethanol produced in worts containing different levels of trub. The reduced ester levels and increased fusel alcohol content in beers produced from high-trub worts were measurable and consistent for both series of tests. Furthermore, the

TABLE II
Vicinal Diketone, Ethanol, and Real Degree of Fermentation for Test Beers at Aging Drop

	Test Phase				
	1		2		
	Partially Filtered Wort (Normal O_2)	High-Trub Wort (Normal O_2)	Filtered Wort (High O_2)	Whirlpool Wort (Low O_2)	High-Trub Wort (Low O_2)
Vicinal diketone (ppm)	0.15	0.11	0.06	0.06	0.07
Ethanol (% wt)	5.49	5.56	5.06	5.07	5.20
Real degree of fermentation (%)	63.6	64.9	62.0	60.8	62.4

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same trend was noted by other workers experimenting with fatty acids in malt fermentations (4,6,13). The mechanism is not fully understood, but the consensus appears to be that unsaturated fatty acids stimulate yeast growth and lipid synthesis within the membrane. The supply of acetyl CoA is reduced substantially in the formation of triglycerides and is therefore unavailable for ester synthesis. A mechanism to explain the shift in fusel alcohols has not been proposed.

The differences in ethanol production have also not been explained. The possibility that wort lipids and zinc contribute to more vigorous fermentation, and thus more efficient conversion of carbohydrates to ethanol, was considered. However, the values obtained for RDF did not support this premise. RDF values for beers within each phase of the study were remarkably similar. A larger proportion of carbohydrates in the clarified worts may have been utilized in yeast biosynthesis, and hence were not available for conversion to ethanol.

The possibility that zinc stimulates yeast activity during fermentation has been discussed (8,12). Evidence suggested that zinc was important in stimulating the high-trub fermentations in the present study. Analytical results indicated that a good portion of the total zinc content in wort was bound up in trub solids. Furthermore, zinc seemed to be an important compound in yeast biosynthesis, as evidenced by high zinc levels measured in washed yeast cells. The other notable test differences (esters, fusel alcohols, ethanol, and beer flavor) do not appear to be related to wort zinc content, based upon available published information.

Flavor panelists did not perceive enhanced fruity characteristics in beers produced from clarified worts, despite measurable differences in esters as determined by gas chromatography. The clarified wort in the first series of tests did exhibit a grapefruit-rind aroma and winey flavor in one evaluation. In another evaluation with the same beer, however, no unusual flavor characteristics were noted. The only reasonable explanation for this is that differences in ester levels were below a flavor threshold and simply could not be differentiated with the human palate. Two beers produced from high-trub worts exhibited a caramel flavor. This trait is often associated with a subthreshold level of diacetyl, although the data in this case are not conclusive. Analysis of aging beers during the first phase of testing indicated slightly higher levels of vicinal diketones in the clarified beer. The second phase of testing revealed virtually no such differences in any of the beers. Given the precision of the vicinal diketone analysis and the fact that it is not specific for diacetyl alone, a possible relationship between subthreshold levels of diacetyl and caramel flavor must remain unsubstantiated.

A trend that seems certain is that fermentation of clarified wort results in beer with preferred flavor characteristics. This was demonstrated by a simple test for reference, as well as in a descriptive flavor analysis.

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