Brewers Condensed Solubles. II. Viscosity and Viscosity Reduction of Brewers Condensed Solubles by Cellulase and Beta-Glucanase[1]

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ABSTRACT

The viscosity of brewers condensed solubles (BCS) at 20% solids was reduced by removing suspended solids (7% of total solids) and treating the clarified solution with cellulase or β-glucanase. Compared to a control of BCS at ~65% solids, removal of suspended solids and enzyme treatment reduced viscosity by a factor of 12–15 and 2.5–3.5, respectively. The viscosity of enzyme-thinned BCS (65% solids) was comparable to that of cane molasses (66% solids) or wood molasses (54% solids).

Brewers condensed solubles (BCS) are the concentrated suspended and water-soluble by-products from the manufacture of beer. The average composition of BCS from one brewery was determined to be 44.4% total solids, 8.85% protein (db), 74.8% carbohydrate (db), 1.43% fat (db), and 2.5% ash (db). When calculated by difference, the carbohydrate content was 87% (Sebree et al 1982).

To recover BCS, a waste stream at ~4% solids is concentrated to ~50% solids in multiple-effect evaporators. The objective of this work was to reduce the viscosity of BCS, because lowered viscosity with high solids improves the biological stability of BCS and lowers its shipping, handling, and storage costs.

MATERIALS AND METHODS

Absolute Viscosity of Untreated BCS

Viscosity, reported as absolute viscosity, was measured using a model LVT Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Standards of known viscosity were used to construct a standard curve. Measurements were made daily on 12 consecutive samples at the solids level supplied by the brewery. Each sample was then freeze-dried, reconstituted to 60% solids, and viscosity measurements recorded from 0 to 45°C. After the 60% solutions were diluted to 20% solids, viscosity was again measured. In another experiment, a composite sample was made from the 12 individual samples, and viscosity was measured over a range of 73 to 20% solids.

Reduction of Viscosity of BCS with Enzymes

Six commercial enzymes were tested for their ability to decrease the viscosity of BCS. The enzymes were α-amylase, β-glucanase, hemicellulase, cellulase, glucoamylase, and pectinase. Their activities are given in Table I. The methods to determine specific activities of the various enzymes were obtained from the suppliers.

A composite sample of BCS was prepared by combining equal volumes of the 12 individual samples and mixing for several hours. The composite was diluted to 20% solids; then 2 L of the solution was centrifuged at 2,500 × *g* for 30 min. The sediment, which was dried to constant weight (28 g), contained 15% protein (db).

To test each enzyme, a 100-ml portion of the supernatant was adjusted near the pH optimum of the enzyme (Table I), using sodium hydroxide pellets or 12*M* hydrochloric acid. An aliquot of the solution (25 ml) was placed into each of three test tubes (25 × 200 mm), and the tubes warmed to near the optimal reaction temperature (Table I) in a water bath. Distilled water (1.0 ml) was added to the control tube; enzyme solution (1.0 ml) was added to the duplicate sample tubes. When the commercial enzyme was supplied in liquid form, it was added as supplied. When the enzyme was supplied in solid form, the solid (1.0 g) was mixed gently with 50 ml of water, and a 1.0-ml aliquot was used. The enzyme units, pHs, and temperatures used in the reactions were as follows: Tenase ~408,000 modified Wohlgemuth units, 6.75 and 70°C; Diazyme-100, ~120 Diazyme units, 4.5 and 60°C; Cellulase Tv, 542.5 cellulase units, 4.5 and 50°C; Glucanase GV-L, ~2,400 glucanase units, 4.5 and 65°C; Bio-Glucanase, ~240 units, 5.5 and 70°C; hemicellulase 100,000, 2,000 hemicellulase units, 4.0 and 60°C; and Spark-L HPG, ~12,000 apple-juice depectinization units, 4.0 and 60°C. The BCS solution was allowed to react with each enzyme for 30 min, placed in a boiling water bath for 15 min, filtered, and frozen. After thawing, relative viscosities were measured using an Oswald capillary viscometer at 25°C.

Reduction in Viscosity of BCS Using Cellulase or β-Glucanase

A composite sample was prepared by thoroughly mixing equal amounts of the 12 daily samples of BCS and diluting to 20% solids. The mixture was centrifuged at 2,500 × *g* for 30 min to remove suspended solids. One portion of the composite was adjusted to pH 4.5, and 25 ml of the solution was added to each of six test tubes held at 50°C. Cellulase solution was prepared as described previously, and 1.0 ml of the enzyme solution was added to each tube. A control mixture was prepared using 1.0 ml of water. Hydrolysis was allowed to proceed 5, 10, 15, 30, 45, and 60 min, and the relative viscosity of each reaction mixture determined as

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previously described, using an Oswald viscometer. The hydrolysis of BCS over time was examined in a similar manner, using β-glucanase (Bio-Glucanase) at pH 5.5 and 70° C.

In another experiment, a second aliquot of either enzyme (1.0 ml) was added after the initial 30 min of hydrolysis. The reactions were then allowed to proceed another 30 min, and relative viscosities were recorded.

In a third experiment, 250 ml of BCS at 20% solids was thinned for 60 min with cellulase or β-glucanase, and the thinned reaction mixtures were concentrated at 68° C in an oven to 64-66% solids. The viscosities of the syrups were determined using the Brookfield viscometer.

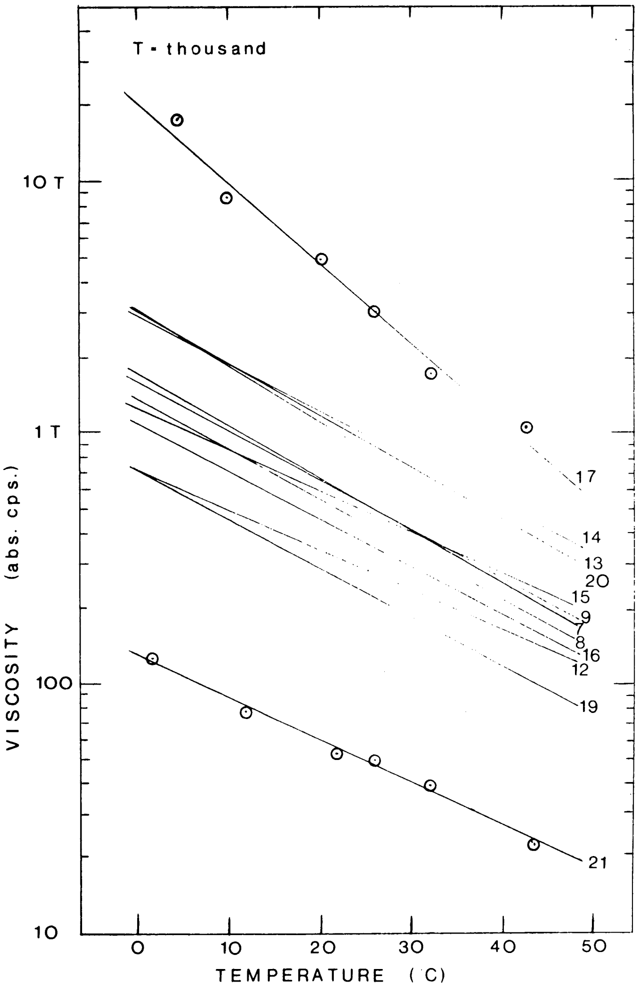

Fig. 1. Absolute viscosity vs temperature of brewers condensed solubles daily samples "as received." Solids content ranged from 27 to 58%. Most experimental points were left off for clarity. The sample number on a curve was the date the sample was collected in December 1979.

RESULTS AND DISCUSSION

Viscosity

Figures 1–4 show the results of absolute viscosity vs temperature of BCS at different solids content as received from the brewery (Fig. 1), at the constant-solids levels of 20 and 60% (Figs. 2 and 3, respectively), and on a composite sample at various solids contents

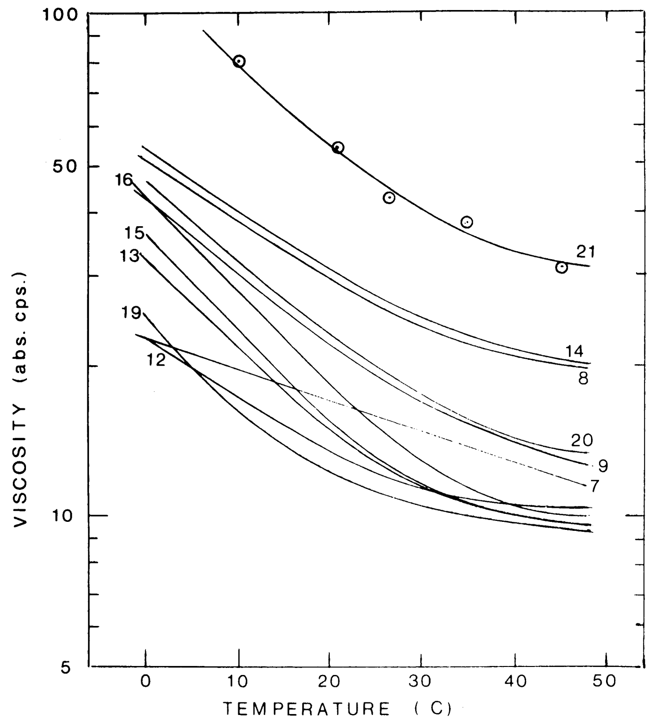

Fig. 2. Viscosity vs temperature for reconstituted solutions of freeze-dried, daily samples of brewers condensed solubles rehydrated to 20% solids. Most experimental points were left off for clarity. The sample number on a curve was the date the sample was collected in December 1979.

TABLE I
Commercial Enzymes for Thinning Brewers Condensed Solubles, Their Properties and Prices

Enzyme	Trade Name and Supplier	Microbial Source	Form	Optimum pH	Optimum Temperature (°C)	Activity[a]	Price[b] (dollars/lb)
α-Amylase	Tenase, Miles	*Bacillus subtilis*	Liquid	6.0–7.0	65–75	340,000 MWU/g	0.65
Glucoamylase	Diazyme-100, Miles	*Asp. niger*	Liquid	3.5–5.0	60	100 DU/ml	0.26[c]
Cellulase	Cellulase Tv concentrate, Miles	*Trichoderma viride*	Powder	4.0–5.0	40–50	27,125 CU/g	25.00
β-Glucanase	Glucanase GV-L, Grindstead	fungal[d]	Liquid	4.0–5.0	65	2000 GU/g	9.00
β-Glucanase	Bioglucanase, Biocon	fungal[d]	Liquid	4.5–6.5	80	200 U/g	2.48
Hemicellulase	Hemicellulase-100,000, Miles	*Asp. niger*	Powder	3.5–4.5	50–60	100,000 HU/g	38.00
Pectinase	Spark-L HPG, Miles	*Asp. niger*	Liquid	3.5–4.5	50	10,000 AJDU/ml	5.00

[a] Activity units of enzymes as described by supplier. MWU = modified Wohlgemuth unit; DU = Diazyme unit; CU = cellulase unit; GU = glucanase units; U = units; HU = hemicellulase units; and AJDU = apple-juice depectinization units.
[b] Price given for total weight of enzyme in the form supplied.
[c] Cost per dextrose unit.
[d] Source is proprietary.

(Fig. 4).

The viscosities of the twelve samples, as received, varied from day to day (Fig. 1). Each sample exhibited a good exponential relationship between viscosity and temperature. To determine whether the solids content controlled the differences in viscosity, each of the daily samples was freeze-dried and reconstituted at 60 and 20% solids. As indicated by the curves in Figs. 2 and 3, the viscosity of a given sample of BCS could not be predicted from its solids content. The daily samples of BCS probably varied in composition and in the molecular weights of their components. The viscosity of the composite sample (Fig. 4), when examined at solids levels of 73, 60, 46, and 20% between 0 and 45° C, showed that no single relationship could be derived between viscosity, solids content, and temperature.

Enzyme-Thinning

Six commercially available enzymes were examined for their ability to reduce the viscosity of BCS. Each enzyme was tested near its optimum pH and temperature on 20% solids BCS for 30 min. The results are given in Table II. On a cost basis, the cellulase enzyme was by far the most effective; it was one-fifth as expensive as the second most cost-effective enzyme, the hemicellulase. The α-amylase and β-glucanase were moderately effective, and pectinase and glucoamylase were least effective.

The reduction of viscosity of BCS with time using cellulase or β-glucanase is shown in Fig. 5. The reaction was rapid during the first 15 min, but slowed dramatically after ~30 min. Adding more enzyme after 30 min did not reduce viscosity further, showing that denaturation of the enzyme did not cause the reaction to slow down. Furthermore, combinations of cellulase, β-glucanase, and/or hemicellulase at pH 4.5 and 50° C gave no greater reduction in viscosity than did cellulase alone.

The rapid decrease in the viscosity of BCS observed in Fig. 5 shows that much less cellulase can be used to thin the by-product when the reaction time is extended. It is also more desirable to treat BCS at 5 or 50% solids, rather than at 20% solids. BCS with 5 and 50% solids occur in existing breweries.

The thinning of BCS by cellulase and β-glucanase suggests that the high viscosity of BCS was due mainly to barley β-glucan (Gohl et al 1977, 1978; Greenberg 1974). The structure of the β-glucan consists of long chains of two or three (1→4)-β-D linkages separated by a single (1→3)-β-D linkage (Fleming and Kawakami 1977). In addition, each chain contains several groups of five or more glucose

TABLE II
Enzymic Reduction of Relative Viscosity of BCS[a]

Enzyme	Percent Change in Relative Viscosity[b]	Cost of Enzyme[c] to Thin (1-kg) BCS (dry basis)
Cellulase Tv	44	0.18
Hemicellulase 100,000	15	0.33
Tenase	8	0.32
Bio-Glucanase	24	1.20
Glucanase GV-L	28	4.26
Spark-L HPG	6	2.18
Diazyme L-100	0	...

[a] All reactions were done on brewers condensed solubles (25 ml) for 30 min at 20% solids near the optimum temperature and pH of an enzyme (1 ml). Costs given are for large-scale shipments in 1981.

[b] Change in relative viscosity was determined at 25° C by an Oswald viscometer. Relative viscosity of starting solution was 5.53.

[c] In dollars.

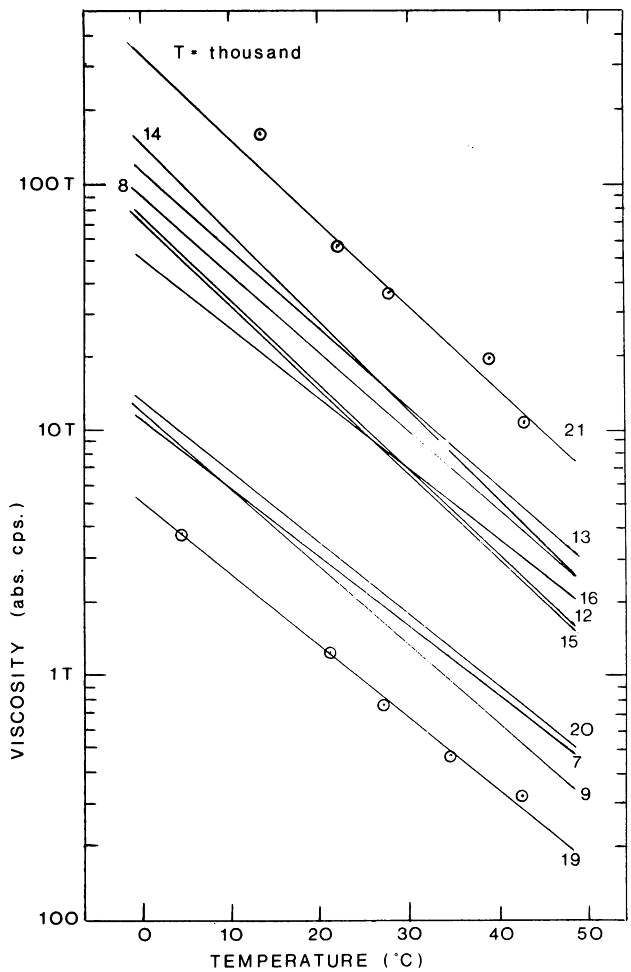

Fig. 3. Viscosity vs temperature for reconstituted solutions of freeze-dried, daily samples of brewers condensed solubles rehydrated to 60% solids. Most experimental points were left off for clarity. The sample number on a curve was the date the sample was collected in December 1979.

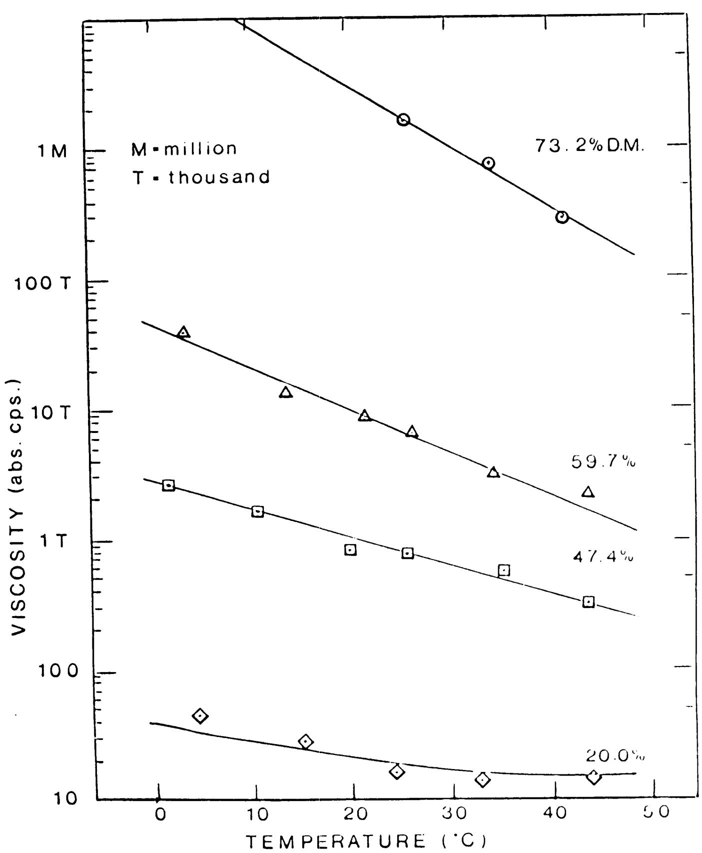

Fig. 4. Viscosity vs temperature and solids for reconstituted solutions of freeze-dried, composite samples of brewers condensed solubles.

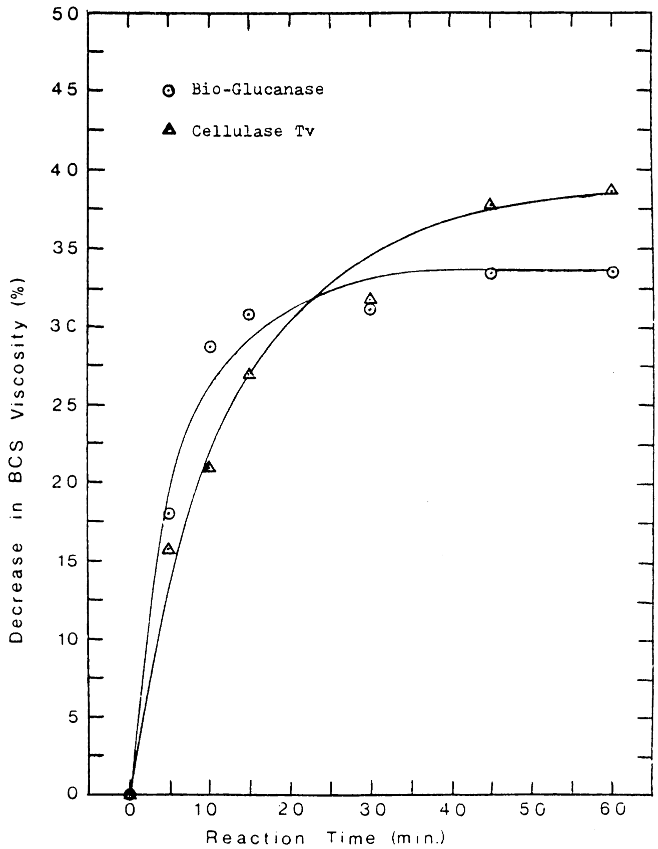

Fig. 5. Reduction of viscosity in a brewers condensed solubles composite sample (25 ml of 20% solids) using cellulase and β-glucanase (Bio-Glucanase). The cellulase reaction was done at 50°C, pH 4.5 and 540 CMC-units of enzyme; the β-glucanase reaction, at 70°C, pH 5.5, and 200 units of enzyme.

units that are linked contiguously by (1→3)-β-D linkages. The β-linkages would be hydrolyzed by β-glucanase and cellulase, enzymes that display broad substrate specificity (Berghem et al 1976, Greenberg 1974, Wood 1975). The hemicellulase would also be expected to contain cellulase activity, which explains its ability to reduce the viscosity of BCS. Combinations of those enzymes are no more effective than cellulase alone because they all degrade the same substrate.

Viscosity and Suspended Solids

Suspended solids have a marked effect on the viscosity of BCS. When the suspended solids (~7% of the total solids) were removed by centrifugation, the viscosity of BCS at ~65% solids was reduced by a factor of 12–15 (Table III). When the supernatant was then treated with cellulase or β-glucanase, its viscosity was further reduced by a factor of 2.5–3.5. Overall, an approximate 40-fold reduction in viscosity was achieved by removal of suspended solids followed by enzyme thinning (Table III). The viscosity of the thinned BCS at 65% solids is less than that of molasses at 66% solids and of wood molasses at 54% solids.

TABLE III
Viscosity (25°C) Reduction of Brewers Condensed Solubles (BCS) by Removing Suspended Solids (S.S.) and Thinning with Enzymes

Sample	Viscosity of Composite[a] (cps)	Viscosity After Removal of S.S. and Reconcentration[b] (cps)	Viscosity After Removal of S.S., Enzyme Thinning, and Reconcentration[c] (cps)
BCS composite 1, 66.0% solids	~45,000	3,825	1,088
BCS composite 2, 64.7% solids	~28,000	1,845	783

[a]Composite samples 1 and 2 were prepared from the same set of 12 daily samples.
[b]Samples diluted to 20% solids, centrifuged to remove suspended solids (~7% of total solids), and the supernatant concentrated to 66.0 or 64.7% solids.
[c]Samples diluted to 20% solids and centrifuged to remove S.S., supernatant thinned with cellulase (composite 1) or β-glucanase (composite 2), and reconcentrated to 66.0 of 64.7% solids. Viscosities of cane (66% solids) and wood molasses (54%) solids were 1,063 and 6,517 cp, respectively, at 25°C.

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