COMPOSITION AND PHYSICAL PROPERTIES OF BREWER'S CONDENSED SOLUBLES. PELLET BINDING USE IN FORMULA FEEDS.

by

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INTRODUCTION

Brewer's condensed solubles (BCS) are the concentrated water-soluble by-products from the manufacture of beer. BCS contains primarily the residual sugars recovered from the mashing vessels after wort production, together with solubles obtained from waste beer and rinse streams, and pressing operations. The production of BCS requires concentration from approximatly four percent solids to fifty percent solids in multiple-effect evaporators. The upper solids level that can be reached is limited by viscosity developement in the product. By enzymatic reduction of the starting material, it is hoped that a very high solids (80%) product or a completely dry product can be produced.

It appears that BCS can be utilized as a feed ingredient. However, at the present time more information is needed to determine the full utilazation of this material. The first step in developing BCS for feed markets is to understand it's composition and physical properties. Specific objectives of this investigation include: 1) a determination of BCS' chemical composition, 2) it's physical properties, 3) it's thermal properties, 4) studies into BCS' storage stability, 5) viscosity and viscosity reduction in BCS, and 6) the potential of BCS as a pellet binder in formula feeds.

LITERATURE REVIEW

Composition of BCS Related Products

Barley

Barley with husks is used in brewing to protect the kernel from mechanical injury during handling, to obtain a more uniform germination during malting, and to aid in filtering the wort. The composition of whole barley (db) is: starch, 63 to 65%; sucrose, 1 to 2%; other sugars, less than 1%; soluble gums, 1 to 1.5%; hemicellulose, 8 to 10%; lipid, 2 to 3%; protein, 8 to 13%; ash, 2 to 2.5%; and other constituents, 5 to 6% (Pomeranz, 1973).

Forrest, et al.(1977) reported that the hemicellulose fraction is mostly cell wall material and 75% of the cell wall material is beta-glucan. They also found that the beta-glucan exists as high-molecular weight polymers with firmly linked peptide sequences. These peptide bonds are ruptured in the malting process thus releasing free beta-glucans in the malt.

The impact of beta-glucan upon viscosity in barley products was demonstrated by Gohl, et al.(1978). In this work, it was demonstrated that as barley ripens beyond maturity, the viscosity of a ground barley suspension decreases. This decrease in viscosity parallels a decrease in barley beta-glucan. In another study (Gohl, et al., 1977), they showed the reverse in barley as the kernels approach maturity; the suspension visosity increased as barley beta-glucan content increased. Arabinoxylan, which is also a part of the hemicellulose fraction was found to be responsible for part of the viscosity. Beta-glucan and arabinoxylan are both water soluble; it is possible that they are responsible for part of the viscosity problem in BCS.

Distiller's Feeds

Included in this review will be Condensed Distiller's Solubles (CDS) and Distiller's Dried Grains with Solubles (DDGS). Many factors influence the nutrient composition of distiller's feeds (Carpenter, 1970). Of greatest importance are the raw materials (grains) used in the fermentation. Other important variables include the grinding procedure, the cooking conditions (specially temperature), the fermentation organisms, the type of distillation, and the processing of the waste stillage and solubles.

Bauernfeind, et al in 1944 presented the proximate analysis for several of the distiller's feeds. Their results are given in Table I of the Appendix. Carpenter's (1970) analysis for a typical corn mash waste product (Appendix, Table II) was quite comparable to that of Bauernfeind's. Both studies noted these by-products are good sources of minerals and B-vitamins. Of particular importance is the amount of selenium contained in the barley malt and rye feeds because of their absence in normal feed grains. Barley and rye are particularly high in selenium when they are grown in the Northern Plains area where that mineral is abundent in the soil.

Potential Uses of BCS

The literature presented two major areas where BCS related products have been used successfully. The first is in animal feeds (cattle, swine and poultry), and the second is in feed stock for some type of fermentation processes, such as single-cell protein, organic acids production, or alcohol production.

Animal feeds

As a result of research and experience in the field, distiller's by products have become well established in a wide variety of livestock

rations. The foremost application is in ruminant feeds, especially for cattle (Little, et al. - 1970). The use of distiller's by-products in ruminant feeds has the advantage that it stimulates rumen digestion. Distiller's feeds seem to be a potent source of some factor(s) that increases the cellulolytic activity of the rumen microorganisms, thereby stimulating rumen digestion.

A study conducted by Beeson and Hatch (1971) showed that a liquid supplement fortified with 9.3% CDS (27% dry matter) produced increased daily gains and feed efficiency in the feedlot. Metabolism trials were also conducted and indicated superior performance. Three reasons were given for the improvement; decreased ammonia concentration in the rumen, decreased plasma urea, and an increased retention of nitrogen. No explanation was given, however, for the fact that an 18.6% fortification produced no increase in feed efficiency or daily gain.

Dairy cattle have also benefited from the addition of distiller's feed to their diets. Warner and Loosli (1968) demonstrated that the addition of Corn Distiller's Dried Grains with Solubles increased the milk-fat percentage when added to a concentrate mixture that was known to depress fat in milk. Thus CDDGS should be a useful ingredient in pelleted dairy feeds to help prevent milk-fat depression.

Warner (1970) conducted 14 separate dairy feeding studies with over 150 cows using three types of distiller's by-products. He ranked the fat-elevating effect of the by-products in the following order from most effective to least: distiller's dried grains (DDG), DDGS, and distiller's dried solubles (DDS). In none of the tests did any other feed ingredient produce more fat-corrected milk (FCM) than the distiller's feeds.

Distiller's dried solubles (DDS) seem to have no nutritional properties commending them as a milk replacer ingredient in calf rations. However,

they were found to be a useful ingredient in such rations up to the 35% level by virtue of cost savings, and they gave no adverse effects (Warner - 1970).

The use of distiller's feeds in nonruninant rations has been based, to a large extent, on the presence of unidentified growth factors (UGF) for those species (Little, et al. - 1970). Those UGFs were found to be very important in stimulating the growth of poultry and in some instances swine (Couch, 1971).

Jensen (1977) reported, from a study of 64 gilts, that distiller's dried grains with solubles (DDGS) could be used to replace an appreciable amount of soybean meal in swine gestation diets. In the study, soybean meal was replaced by DDGS in the amounts of 17.7% and 44.2% (maintaining 0.42% lysine in all diets). Average gestation gain, litter size, and birthweight were the same for all three treatments, and nitrogen balance did not differ significantly amoung the diets. The nutritive value of distiller's feeds for swine is mainly due to their high phosphorous and B-vitamin content. Phosphorous is not only one of the most expensive minerals to add to a diet, but the majority of the phosphorous from plant sources is poorly utilized by swine since the phosphorous is present as phytate (Berger, 1981). However, distillers feeds (especially DDS) are quite low in phytate, thus the phosphorous present is more utilizable. The high B-vitamin content of DDS is due to the presence of yeast cells. Thus DDS in rations give improved performance in young pigs.

According to Runnels (1968) the major contributions of distiller's feeds to poultry rations are energy, essential amino acids, and unidentified growth factors. Normally, levels of distiller's feeds in poultry rations should be between 3 and 8%, depending upon the feed's nutritional contribution to the diet. No undesirable effects have been reported from feeding poultry diets containing up to 20% of either DDS of

DDGS. Distiller's feeds have shown significant improvement in growth, egg production, feed utilization and hatchability in turkey retions (Harms, et al. - 1977), layers (Harms, 1970 and Jensen, 1973) and breeder replacement pullets (Couch and Abbot, 1972).

Fermentation Substrate

In recent years a large number and variety of fermentation processes have found commercial application for the production of antibiotics, enzymes, food and feed ingredients, vitamins and similar products. There are four major factors to consider when choosing substrates in such fermentation (Hall, 1955); 1) the substrate must be a good source of essential nutrients for the microorganism used, 2) be available in multi-ton quantities, 3) be fairly uniform in composition, and 4) be reasonably priced. Distiller's feeds meet the above criteria. The first such use for distiller's feeds was performed (and patented) by Woolner and Lassloffy in 1909 when they cultivated A. oryzae in stillage under submerged, aerobic conditions to produce saccharifying enzymes. Bacterial (Beckord, et al. - 1946) and fungal amylases (LeMense, et al. - 1949, and Van Lanen and Smith - 1968) have also been produced from grain stillage, as has riboflavin (Smiley and Stone - 1955). A listing of products, microorganisms and references can be found in Table III of the Appendix.

Other types of fermentations tried included the production of single-cell protein from the stillage of a white wine distillery (Mangey et al., 1977). The organism grown was Penicillium spinulosum, and it not only produced 13,400 mg/L of biomass (41% protein), but reduced the COD of the waste from 44,000 to 4,000 mg/L. Other studies include the possibility of growing Candida on spent potatoe mash (Il'ina and Evseichik, 1976) and vinasse (Karova et al., 1976; Troitskii et al., 1976; Tauk,

1976), Torulopsis on vinasse (Tauk, 1976), and food yeasts on vinasse (Isik, 1977). The yield of high protein yeast were considered very good in these studies, although in the case of the Tauk (1976) study, results were not good enough for full-scale production.

Another area of application could be in the production of organic acids. Karova, et al. (1976) was able to produce acetic acid in suitable quantities from distillery wastes using Acetobacter aceti. Brewery wastes, on the other hand, were found to be suitable for the production of citric acid by Aspergillus foetidus (Hang et al., 1977). Ninety-six percent of the reducing sugars in the brewery waste was consumed, while the yield of citric acid lay between 0.44 and 0.58 g of citric acid formed per gram of reducing sugar consumed.

Liquid Supplements

Liquid supplements are commonly used today in the cattle industry. The term "liquid supplement" is defined as any supplement that is made up of a liquid carrier, to which a nitrogen source and other essential nutrients are added (Wornick, 1969). Liquid supplements provide several advantages in livestock feeding, including ease of handling, increasing the patatability of urea in a ration, and the diversity in it's feeding application. The "carrier" of a liquid supplement can be any of several materials. Molasses from beet, cane or corn are the most common carriers, but others are used as well. These include hemicellulose extract or wood molasses, distiller's solubles, fish solubles, corn solubles, fermentation liquors and propylene glycol. Nitrogen in the supplement can be supplied from protein or from a non-protein nitrogen source such as urea (the most common), ammonium polyshosphates and diammonium phosphate. Other additives include a phosphorous source, vitamins, minerals, antibiotics, drugs and any other nutrient needed by the animal.

Nutritionally, liquid supplements, in most cases, have been reported to be comparable dry supplements, although not necessarily surpassing them. In a lactation study (Huber et al., 1968 and Van Horn et al., 1969) comparing a liquid supplement to a dry supplement, wherein both were added to corn silage, both were equal in performance. Thus, the decision of whether or not to use a liquid supplement must depend upon cost, ease of application, adaptation to the individual's operation and other factors (Huber, 1972).

The wide use of molasses as a carrier has evolved because of three reasons; first, the sugars in molasses provide a readily available energy source; second, molasses increases the palatibility of the ration; and third, the addition of molasses decreases dustiness of complete feeds and keeps the feed components from separations.

Enzymes for Viscosity Reduction in BCS

Based on the composition of BCS related products, there are perhaps six commercial enzymes that might reduce the viscosity of BCS; beta-glucanase, cellulase, hemicellulase, pectinase, gluco-amylase and alpha-amylase. The first four enzymes are endo enzymes, which will reduce viscosity quite rapidly.

Beta-Glucanase

The beta-D-glucans in barley exists as a linear polymer with beta-1,3 and beta-1,4 linked D-glucose units. It occurs at the level of 6.5% in the barley kernel (Flemming, et al., 1977). Degredation of beta-glucans in BCS would require an endo-beta-(1,3),(1,4)-glucanase. Such an enzyme is present in barley malt and other cereal sources, but not in high enough activity nor in thermally stable enough form to treat BCS (Reed, 1975). The end-product of beta-glucanase hydrolysis of barley beta-glucans is generally oligosacharides with 3 to 4 glucose

units. Bacillus subtilis is the principle source of commercial beta-glucanase. This beta-glucanase is almost identical to that produced from cereals except that it is much more heat stable. Beta-glucanse from B. subtilis has a pH optimum range of 4.0 to 5.5, is optimally active at temperatures of 58 to 60°C and is inactivated at temperatures above 70°C. Commercial enzyme preparations vary somewhat in optimum conditions depending upon the mutant strain used in the enzyme's production. The preparations are usually contaminated to differing degrees with amylase and protease activities.

Cellulase

Barley husks contain nearly two-thirds of the cellulosic material in the kernel (Pomeranz, 1973), so the amount of cellulose in BCS must be very low. However, the viscosity of BCS might still be reduced by treatment with cellulase. The beta-glucans and other polymers in the cell walls of barley have structures related to that of cellulose. Furthermore, commercial cellulases contain other enzymes and exhibit broad substrate specificity.

Cellulase is a multicomponent system (Wood, 1975). It consists of three major components: a C_1 component, a C_x component and a beta-glucosidase component. The C_1 component initiates attack upon native cellulose such as cotton fiber. The specific role of this component in cellulose hydrolysis is still in controversey. There are two schools of thought upon this subject, the first being that the C_1 component carries out a non-hydrolytic function (Wood, 1975) and the second being that it is a cellobiohydrolase (Halliwell and Griffin, 1974, Pettersson et al., 1972 and Berghem et al., 1976). Proponents of the non-hydrolytic function of C_1 claim that this component disaggregates the cellulase chains for attack by the C_x component. However, evidence backing the

proponents of C_1's cellobiohydrolase function appears to be convincing. These investigators found that the C_1 component is a hydrolytic enzyme that removes cellobiose units according to an exo-wise mechanism. They conclude that C_1 is a beta-1,4-glucan cellobiohydrolase.

The C_x component has been researched extensively and it's function has been established (Wood, 1975). The C_x component attacks at random (endo-beta-1,4-glucanase) when it's approach is not hindered. Cellobiose and-triose are the end products of C_x attack. Some enzymes in the C_x fraction also act by removing successive units of glucose from the non-reducing end of cellulase (exo-beta-1,4-glucanase). Hydrolysis of cellulose by the C_x component alone is very slow and not complete.

The beta-glucosidase component hydrolyzes cellobiose and short-chain cello-oligosaccharides to glucose (Wood, 1975). This component has no activity on cellulose and it's activity decreases as chain length increases. Overall, cellulase is inhibited if short chain cello-oligosaccharides build up in the system.

Most cell-free cellulase preparations contain C_x and beta-glucosidase activity, but only preparations from Trichoderma koningii and T. viride contain appreciable amounts of C_1 component activity. Therefore, preparations from one of these two sources would be preferable. The most common commercial enzyme of the two types is that from Trichoderma viride. The optimum conditions for cellulase activity from T. viride were established by Ghose, et al. (1973), and are a temperature of 30°C and a pH between 4.5 and 5.0. Ghose was working with purified cellulase when he established those conditions and commercial preparations may have different optimum conditions. A commercial prepartion from Miles Laboratories (Miles Enzyme Products Div., P.O. Box 932, Elkhart, Ind., 46515) called Cellulase Tv Concentrate has an optimum activity at a

temperature between 40 and 45°C and a pH of 4.0 to 5.0. Such cellulase preparations also have contaminating activities of beta-glucanase, hemicellulase, pentosanase, pectinase and xylanase in that order of activity.

Hemicellulase

Hemicellulose is a poorly defined group of plant materals consisting of polymers of mainly, D-xylose, D-galactose, D-mannose, L-arabinose, D-glucose and D-gluconic acid, sometimes in conjunction with protein, pectin, and gluco-polysacharides (Fennema, 1976). Hemicellulase demonstrates a high specificity for these classes of subtrate, with particularly high activity against galactomannans. Commercial prepartions are usually produced from Aspergillus niger and contain contaminating activities of cellulase, beta-glucanase and pectinase. Optimum conditions for hemicellulase are as follows (for Miles Labs. hemicellulase - 100,000): temperature ranges from 50 to 60°C, and pH ranging from 3.5 to 4.5.

Pectinase

The pectic substances we will have to deal with are most probably the methyl esters of polygalacturonic acids since we are dealing with a water-soluble system (Fennema, 1976). Thus, the pectinase needed would be polygalacturonase of which most are produced by mutant strains of Aspergillus niger. Polygalacturonases from A. niger have both endo-and exo-action (Rombouts and Pilnik, 1972). All enzyme fractions of polygalacturonase have a temperature optimum of 45 to 60°C. Endopolygalacturonase A has a pH optimum of 4.0 to 4.2, gives 60% hydrolysis (of polygalactuonic acid) and forms end products of mono and digalacturonic acids. Endo-polygalacturonase C has a pH optimum of 5.5 with a 48% hydrolysis giving end products of trigalacturonic acids. Exo-polygalacturonase I has a pH optimum of 4.4 to 4.6 with a complete hydrolysis

to galacturonic acid. Finally exo-polygalacturonase II has a pH optimum of 5.0 to 5.1 giving a 28% hydrolysis to galacturonic acid. Commercial preparations are about the same as far as optimum conditions are concerned; Miles' Spark-L HPG has an optimum temperature of 50°C and optimum pH of 3.5 to 4.5. Also included in commercial polygalacturonase preparations are trace contaminating activities of cellulase, hemicellulase and protease.

Alpha-Amylase

The enzyme alpha-amylase (or alpha-1,4-glucan-4-glucanohydrolase) occurs commonly in most plants, mammals and microorganisms (Reed, 1975). Alpha-amylase acts upon starch (both amylase and amylopectin) in an essentially random (endo) manner, with the production of reducing sugars. The degredation of amylose occurs in two stages. First, there is a complete, rapid reduction of the amylose to maltose and maltotriose by random attack of the enzyme upon the alpha-1,4 bonds of the polymer. The second step, which is much slower than the first, is a slow hydrolysis of the maltose and maltotriose into glucose and maltose.

The degredation of amylopectin (branched starch) yields glucose, maltose and alpha-limit dextrans (oligosaccharides of 4 or more glucose residues containing an alpha-1,6-glycosidic branch point). Alpha-amylase can not attack withing two glucose residues of the alpha-1,6 branch points of amylopectin.

The optimum conditions for alpha-amylase depends greatly upon the enzyme source. The use for which such an enzyme would be used in this work, would require the use of a highly stable bacterial amylase. Purified alpha-amylase from Bacillus subtilis have an optimum pH between 5.0 and 7.0, with temperature optimum being between 50 and 70°C. Optimum conditions for a commercial alpha-amylase from Bacillus subtilis

(from Miles Enzymes Prod.) are as follows: pH - 6.0 to 7.0 and temperature - 65 to 75°C. Such commercial preparations contain very little contaminating activities of other enzymes.

Glucoamylase

Glucoamylase (alpha-1,4-glucan glucohydrolase) is an exo-splitting enzyme that removes glucose units consecutively from the non-reducing end of starch polymers, oligosaccharides or shorter chain glucose polymers (such as maltose or maltotriose)(Reed, 1975). This enzyme has been called by several other names, including amyloglucosidase and glucamylase. This enzyme has a low degree of specificity having the ability to cleave, alpha-1,4, alpha-1,3 and alpha-1,6 bonds. The rate of hydrolysis of the three bonds varies considerably, with the 1,4 bond being broken easiest followed by the 1,3 and finally the 1,6 bonds.

Optimum activity of purified glucoamylase occurs in the pH range of 4.0 to 5.0 and a temperature range of 50 to 60°C. Commercial preparations of glucoamylase are usually from a variant of Aspergillus niger or Rhizopus. A commercial preparation from Miles Enzymes (Diazyme from Aspergillus niger) has the following optimum conditions: pH - 4.0 to 4.5 and temperature - 50 to 60°C. Such commercial preparation contain very little contaminating activities by other enzymes.

Total, Suspended and Soluble Solids

10.00 g of BCS was placed into a 100 mL volumetric flask and diluted to 100 mL. To determine total solids duplicate 5 mL aliquots were taken and placed into tared 50 or 100 mL beakers which were heated at 100°C for 12 hours. The remaining material (90 mL) was centrifuged at 3100 rpm for 30 minutes. Both the precipitate and supernatant (measure volume) were saved. The precipate was resuspended in distilled water (40 mL) and centrifuged again. The supernatant containing the soluble solids (5.0 mL aliquot) and the total centrifugate (suspended solids) were then dried to constant weight at 100°C (for 12 hours).

Total Carbohydrates

The method used was that of Dubois, et al. Two mL of sample (diluted appropiately) was pipetted into a colorimetric tube, then 2.0 mL of 5% phenol was added. Next 5.0 mL of concentrated sulfuric acid was added rapidly (directed against the liquid surface to obtain good mixing). The tubes were then allowed to stand for 10 minutes, shaken and placed for 10 to 20 minutes in a water bath at 25° to 30°C. Absorbance was read at 490 nm and sample carbohydrate determined from hexose standard curves prepared from maltose and glucose.

Fats

The method of exctraction was that of Folch, Lees and Stanley [2]. 1 g of sample (10 mL of 1/10 diluted BCS) was mixed with 19 mL of chloroform:methanol (2:1) mixture. To the resulting mixture was added, with mixing, 10% by volume of 0.1M aqueous potassium chloride (the KCl was used because it aids in the breaking of the lipid-solvent-water emulsion, thus facilitating phase separation). The mixture was then

centrifuged to yield two phases, the top of which contains non-lipid material and gangliosides. That layer was discarded. The residual non-lipid material in the chloroform layer was removed by addition of methanol and water and recentrifugation. The top layer was again discarded. The final lipid solution was then placed into a tared beaker and dried over a steam bath. The entire extraction procedure was repeated twice to remove any other non-lipid material. After the final steam drying, the residue was oven dried at 100°C for 12 hr. and then reweighed.

Ash

Because of the viscous nature of BCS the normal procedure for ash was modified. The following procedure was used:

Ten g of sample was diluted to 100 mL total volume as in the solids testing. Duplicate aliquots (5 mL) of this mixture were placed in tared ceramic dishes, which were dried by heating for 12 hours at 45°C and then ashed by conventional means.

Carbohydrate Profile

Subsamples of the 12 daily BCS samples were submitted to Anheuser-Busch (Jerry Tutor) for HPLC Analysis of the principle sugar fractions.

Minerals

The various minerals analyzed for were nitrogen, phosphorous, potassium, calcium, magnesium, copper, maganase, iron, zinc and sodium. Work was performed by the KSU Soils Laboratory (Dr. D. A. Whitney in charge).

Amino Acid Profile

Subsamples of the 12 daily BCS samples were submitted to Dr. Lynn Bates (Department of Grain Science and Industry, KSU) Amino Acid Laboratory for amino acid analysis.

Viscosity

Viscosity was measured using a model LVT Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, Mass.). Standards of known viscosity were used to construct a standard curve. Viscosities reported for BCS samples are given as absolute viscosity. Measurements were made at 20% solids, "as is" solids, and 60% solids versus temperature (from 0 to 45°C). First, viscosity was measured on an "as is" solids content (as received from the plant). Then each individual sample was freeze-dried (shell and vacuum drier) and reconstituted to 60% solids. Viscosity was measured for each at 60% solids (versus temperature). Then the 60% solutions were diluted to 20% solids where viscosity was again measured. For the composite sample, the same was done, except viscosity was measured over a range of 20% to 73% solids.

Freezing Point

Approximately 4 mL of BCS was taken from a composite sample and placed into a test tube (18 x 150 mm). A thermocouple (copper-constantan) connected to an EMF recorder (Varian G-2000) was placed in the middle of the sample and supported in place by a rubber stopper. The sample was then immersed in an acetone bath contained in an insulated calorimeter well. The acetone bath was stirred with a magnetic stirrer and kept at -50° C by addition of solid carbon dioxide. The sample was gently shaken (manually) to insure uniform freezing. After freezing, the sample was moved to a 25°C water bath for melting. An EMF vs. time chart (strip chart recorder) was obtained for each trial. Each sample was done in duplicate. From each chart an inflection point, where the temperature remained constant for a short period of time, was taken as

the freezing point of the sample in question.

Specific Heat

ASTM C351-54T, "Tentative Method of Test for Mean Specific Heat of Thermal Insulation" was modified to measure the specific heat of the daily composite sample of BCS. The method involves the classical method of mixtures. That method consists of adding a known mass of water at a known low temperature to a known mass of sample at a known higher temperature in a thermal capsule and determining the equilibrium temperature that results. The heat absorbed by the water and its containing vessel can be calculated and this value equated to the heat given up by the hot material and its capsule. Knowing the heat given up by the sample, its specific heat can be calculated.

The equipment used was a specific heat calorimeter (model CS-129, manufactured by Custom Scientific Instruments, Inc., 13 Wing Drive, Whippany, New Jersey 07981). The unit consits of a stand, magnetic stirrer, Dewar flask (insulated), differential thermometer, heater, variable transformer, capsule, thermocouple (copper-constantan) and specific heat standard (bus bar copper). An EMF recorder and conversion chart was used to determine the temperature of the sample. Specific heat was determined on individual daily samples at only a single temperature. A composite sample was used to determine specific heat versus solids content and temperature. In this investigation, BCS was slowly dehydrated in a low temperature (38°C) forced-draft oven to 73% solids. The material was then diluted with distilled water to obtain 60% and 20% solids contents.

Density

Density analysis was performed by weighing a volume of the BCS composite sample (same material as was used in the specific heat analysis) at varying solids concentration. A 25 mL graduated cylinder

was weighed (for tare weight), then 25 mL of BCS was added and the cylinder reweighed. Density was calculated from weight and volume (g/mL).

Water activity

A composite sample of BCS (same material as used for specific heat and density analysis) was analyzed for water activity versus solids content and temperature. Each sample in turn was placed into the measuring capsule and the temperature of the filled capsule was equilibrated by holding the capsule inside an insulated container. The equilibrium Relative Humidity (ERH) was then measured by use of a Beckman Water Activity Meter (model SJT-02-08-0, Beckman Instruments, Inc., Cedar Grove, N.J.).

Thermal Conductivity

The instrument used was a C-Matic Thermal Conductance Tester, model TCHM-DV (Dynatech R/D Corp., Cambridge, Mass.). This instrument is designed to test low moisture solids at temperatures between 75 and 400°F. The basis of the determination is a simple one-dimensional heat flow analysis, with the sample sandwiched between a cold plate and hot plate. The heat flow per unit area from the hot side to the cold is measured. The principle of operation used by this instrument is very close to that found in ASTM specifications C518.

A composite BCS sample was freeze-dried and measurements were made on this material (at three temperatures). Thermal conductivities of pure water at different temperatures were obtained from the CRC Handbook of Chemistry and Physics (53rd edition). Thermal conductivities were then computed for BCS at varying solids concentration and temperatures.

Storage Stability Test

The objective of the storage test was to investigate the effects of solids concentration, propionic acid (as a chemical mold inhibitor) and storage temperature on the storage stability of BCS. The stability of

the samples was determined visually by looking for evidence of mold growth or evolution of carbon dioxide. Five daily composites, #7, #12, #14, #17 and #21, were examined out of the total of twelve samples, because those five represented the range of viscosity and solids observed for all the samples.

For each sample, twelve subsamples were prepared using four propionic acid levels (0, 0.25, 0.50 and 0.75 weight %, "as is"). The samples were then stored at 70, 80 and 90° F.

A daily sample (composite) was placed in a mechanical blender and stirred for 5 minutes. Approximately 5 mL of each sample was placed in an 8 dram vial giving approximately a 1/2 inch depth of BCS. The screw caps of the vials were then closed tightly and the level of BCS in the vials were marked. Twice daily the samples were checked for any volume change or mold growth. The vials and transfer equipment were sterilized prior to use.

In another experiment the effect of shaking the samples on storage stability was also examined. An additional three subsamples were taken from each of three composite samples (#7, #17 and #21) using the same propionic acid levels. These samples were shaken continuously at room temperature (about 80° F) on a rotational (mechanical) shaker.

Oven Drying Test

Composite samples of BCS were oven-dehydrated at low temperature to 50% solids. These samples were then dried in a forced-draft oven at four temperatures (54.4, 65.6, 76.7 and 87.8°C, 3 replications each) until noticeable browning occurred. Every few hours these samples were checked for solids content (by weight loss from sample, for drying rate calculation) and browning (color change of BCS). Color change point (solids content of BCS where the color of the BCS sample darkened)

was judged by a panel of graduate students by visual observation.

Total Tritatable Acidity

20.00 g of BCS was placed into a 150 mL beaker and diluted with 50.0 mL of distilled water. The resulting solution was mixed and allowed to stand at room temperature for 30 minutes. Each sample in turn was placed onto a magnetic stirrer set to adequately mix the solution. A Beckman Expandomatic IV pH meter (Beckman Instruments, 2500 Harbor Blvd., Fullerton, Ca., 92634) was used to measure the pH of the solution continuously as base was added. The base used was sodium hydroxide (0.0948N) and was added until the pH of the BCS solution reached a pH of 7.00. Initial results were reported in milliequivalents (Meq) of base needed to neutralize each solution, but final results were reported in Meq of base needed per Kg of BCS solids.

Pellet Binding Test

For a completely randomized block design, the treatment trials were all run in a block. That is, the three treatments were all run in order through each repetition. This was done to dampen the effects of external forces upon the pelleting trials that may change through the course of a day.

The type of feed pelleted was dairy concentrate with 3% by weight (as is) of three different binders, molasses, Masonex, and BCS. The pellets were 3/16" in size. Pellet ingredients and their quanites in a dairy concentrate are tabulated in Table XXIII of the Appendix.

Fines are measured by the weight of material that is separated (by seiving) from the pellets and recorded as a percentage of total final weight.

Pellet Durability Index (PDI) is calculated by use of a pellet hardness tester. The test we employed made use of rotational impacting of the pellets against one another and a metal surface for a specified

amount of time.

Production rate is computed by taking the total amount of product (pellet weight plus fines) and dividing by the time spent in the pelleting operation. Unit energy consumption is computed by measuring the amount of energy used in the operation divided by the total amount of product.

Preliminary Enzyme Screening

A series of six classes of enzymes were tested for their ability to decrease the viscosity of BCS. The enzymes used were α-amylase, β-glucanase, hemicellulase, cellulase, glucoamylase and pectinase. The specific enzyme preparations used and their sources are shown in Table XXL. In this preliminary study, the enzymes were tested on BCS which had its susended solids removed and its pH changed to match each individual enzyme's pH optimum.

An overall composite sample of BCS was prepared by mixing equal volumes of each daily composite sample. This was then mixed for several hours using a Talboy Laboratory stirrer to insure a uniform sample. Total solids was then determined for the sample and entire sample diluted to 20% solids. The composite ($\sim$ 2 L) was divided into 8 large centrifuge bottles (250 mL in each bottle), and the solids were removed by centrifugation. From the resulting supernatant, 700 mL was withdrawn and placed into seven 150 mL beakers (100 mL per beaker) and each solution's pH was adjusted by addition of sodium hydroxide pellets or concentrated hydrochloric acid to the pH optimum of the enzyme to be used.

In this testing, each enzyme was used at its optimum temperature, as well as its optimum pH. Information on the properties of the enzymes used in this study is summarized in Tables XXI and XXII of the Appendix.

An aliquot of each BCS solution (25 mL) was placed into a 25 x 200 mM

reaction tube and brought to reaction temperature in a water bath. A control and two reaction tubes were used. Into the control, 1.0 mL of distilled water was added, while to the reaction samples 1.0 mL of enzyme was added. In the case of liquid enzyme preparations, the enzyme was added as it was received, whereas those enzymes in solid form (1.0 g each) were placed in a 50 mL volumetric flask, made to volume, and an aliquot used. The BCS solution was allowed to react with an enzyme for 30 minutes and then placed into a boiling water bath for 15 minutes. The solutions were then sealed and frozen until their viscosities could be measured using an Oswald capillary viscometer.

Second Enzyme Study

This second study consisted of two major parts. In the first part, a study was done to determine the reduction of viscosity in BCS by Cellulase Tv and Bio-Glucanase (a beta-(1,3),(1-4)-glucanase). In the second part, a study was performed to investigate the effects of multiple-enzyme treatments upon the viscosity of BCS. The enzymes used were Cellulase Tv, Bio-Glucanase and Hemicellulase 100,000. Information on these enzymes can be found in Tables XXI and XXII of the Appendix. In both of these studies the enzymes were tested on BCS which had it's suspended solids removed and it's pH changed to match the pH optimum of each individual enzyme. In the case of multiple-enzyme systems, the reactions were run at pH 4.5 and at the temperature matching the least heat stable of the enzymes present.

A composite sample of BCS was prepared in the same way as was done for the preliminary screen test with enzymes, except that after the suspended solids were centrifuged out of the BCS, the supernatent was divided into two containers. One container (1.5 L) was adjusted to

pH 4.5 by addition of sodium hydroxide pellets, and the other (0.5 L) was adjusted to pH 5.5. Both BCS test solutions contained approximately 20% solids.

An aliquot (25.0 mL) of BCS solution was placed into a 25 x 200 mm reaction tube, brought to reaction temperature in a water bath, and 1.0 mL of enzyme solution added. When the commercial enzymes were liquid, 1.0mL of the solutions were used directly, but when the commercial enzymes were solid, 1.0 g of enzyme was dissolved into 50 mL of water and a 1.0 mL aliquot of that solution was used. Control solutions were made by adding 1.0 mL of distilled water. The enzymatic reactions were run for 5, 10, 15, 30, 45 and 60 minutes. Immediately upon completion, the tubes were placed in a boiling water bath for 15 minutes to denature the enzyme. The resulting solution was then placed in a flask (50 mL), and frozen until tested for viscosity using an Oswald capillary viscometer. A control and duplicates were used for each test.

In the second part of this enzyme study, the following mixtures were tested: Bio-Glucanase (BG) and Cellulase Tv(C), Bio-Glucanase and Hemicellulase 100,000(H); Cellulase Tv and Hemicellulase 100,000; and Bio-Glucanase, Cellulase Tv and Hemicellulase 100,000. All reactions were run for 60 min. under the optimum conditions of the least stable enzyme. In the BG - C test, enzyme solutions were tested in the proportion, respectively, of 0.5 and 0.5 mL, and 0.25 and 0.75 mL and 0.75 and 0.25 mL, against a 1.0 mL Bio-Glucanase standard. The BG-H and C - H tests were both run by adding 0.5 mL of each of the three enzyme solutions were used (control contained 1.5 mL water). In all tests with more than one enzyme solution, each enzyme solution was added separately to the reaction tube. The reactions were terminated

and the viscosities of the samples measured as previously described.

COMPOSITION

The results of the proximate analysis of the BCS samples are shown in Table 2. The solids content varied considerably with a mean and standard deviation of 44.4 ± 7.4%. The protein content of the 12 daily samples of BCS had a mean and standard deviation of 8.85 ± 1.14% (d.b.). Carbohydrate content was rather constant with a mean and standard deviation of 74.81 ± 5.88% (d.b.). On the other hand, the fat content varied considerably with a mean and standard deviation of 1.43 ± 0.303% (d.b.). The ash content of BCS showed a mean and standard deviation of 2.54 ± 0.207. Gross energy contained in the BCS solids varied with a mean and standard deviation of 4073.29 ± 53.12 Cal/g (d.b.). Values are also given in the table for corn, molasses and Masonex.

Table 3 gives some information on the type of solids in BCS. The suspended solids accounted for 6.90 ± 1.91% of the total solids, whereas soluble solids (soluble or colloidal solids) accounted for 93.10 ± 1.91% of the total solids. It appears that as total solids in BCS increase, suspended solids generally decrease.

Partial carbohydrate profiles of the average sample of BCS are given in Table 4. The BCS samples contained 9.36 ± 0.79% (d.b.) glucose, 38.03 ± 4.19% (d.b.) maltose and 11.65 ± 0.80% (d.b.) maltotriose. When the average levels of dextrose, maltose and maltotriose in BCS are summed, those three sugars constituted 59% of the dry matter in BCS. However, total carbohydrate in BCS averaged 74.8%, which leaves 15% carbohydrate unaccounted for. It is probable that the unaccounted portion is mostly maltodextrin and β-glucans together with traces of cellulose, hemicellulose and pectins.

THERMAL AND PHYSICAL PROPERTIES

The results of the thermal and physical testing of BCS is found in Table 5. The pH of the daily samples of BCS varied only slightly with a mean and standard deviation of 4.14 ± 0.22. This acidic pH and the buffering capacity of the protein in BCS implies that alkali will be needed if BCS is treated with enzymes that are active at or near neutral pH. In addition, the acidity of BCS will impose some limitations on the choice of construction material used to store and process BCS.

The results of the freezing point analysis for individual daily samples are shown in Figure 5. Figure 6 shows a typical freezing and melting curve for a sample of BCS. An inverse, exponential relationship was observed between freezing point of BCS and its solids content. A regression equation was computed for this relationship which is as follows:

$$Y = -0.00133\ X^{2.3316}$$

where Y is the sample's freezing point (^{o}C) and X is the sample's solids content expressed in percentage. The correlation coefficient (r) for the expression was 0.959 and it is statistically significant below the 0.01% confidence level.

The specific heats of the twelve daily samples of BCS are given in Figure 7. The specific heats did not vary much with an average and standard deviation of 3.093 ± 0.237 J/Kg-^{o}K. A negative linear relationship was derived between the specific heat and the solids content of the daily samples of BCS. A regression equation was computed as follows:

$$Y = -0.02899\ X + 4.3804$$

where Y is the sample's specific heat (in J/Kg-K) and X is the sample's solids content expressed in percentage. The correlation coefficient (r) was computed to be equal to 0.911.

Data on the specific heat of a composite sample of BCS vs. solids

concentration and temperature is depicted in Figure 8. It was found (Table XV of the Appendix) that the specific heat of a composite BCS sample did not vary with temperature between 43 and 77°C. A statistical analysis of variance showed that any interaction effect of temperature in that range is negligible. However, a good inverse relationship was found between the solids contant of a composite sample of BCS and its specific heat. The equation is as follows:

$$Y = -0.02781\ X + 4.4879$$

where Y is the composite sample's specific heat (in J/Kg-K) and X is the sample's solids content expressed in percentage. The correlation coefficient (r) was computed to be 0.9997. It will be noted that the two equations derived to relate specific heat to solids content are almost identical.

The densities of a composite sample of BCS at various solids contents are shown in Figure 9. As expected, the density of a composite sample of BCS is a linear function of solids content. The equation relating density to solids content is as follows:

$$\rho = (1 + 0.4134\ X) \times 10^3$$

where ρ is the composite sample's density (in Kg/m^3) and X is the solids content in decimal form.

The data for water activity vs. temperature and solids content is shown in Figure 10. Surprisingly, water activity did not change as temperature was increased from 15°C to 60°C. This indicates that BCS is a strong humectant. One can contrast the isotherm for hard red winter wheat with the isotherm of BCS. Wheat gives the normal increase in water activity (ERH) with increasing temperature.

The water activity of BCS between 10 and 60°C is a function of solids content as described by the following expression:

$$ERH = (3.981\ X - 6.003\ X^2 + 3.202\ X^3)(100)$$

where ERH is the equilibrium relative humidity (%) and X is the moisture content in decimal form (1- dry matter, decimal form). This equation is valid between the temperatures of 10 and 60°C and solids contents of 30 to 73%.

The results for thermal conductivity vs. temperature and solids content of the dry sample of BCS are shown in Tables (5, and XVII to XIX of the Appendix). The instrument used to determine thermal conductivity was not able to analyze BCS directly because the BCS contained too much moisture. Our approach was to determine the thermal conductivity of the BCS solids (Table XVII.) and then to calculate a theoretical value assuming a linear relationship between thermal conductivity and the solids content at a given temperature. The thermal conductivities of pure water at various temperatures are given in Table XVIII.

The thermal conductivity of a composite sample of BCS did not vary according to any simple relationship with temperature for the range given (Table XIX of the Appendix). A definite relationship existed between solids content and thermal conductivity and an expression was derived as follows:

$$k = -0.2342\ X + 0.6343$$

where k is the composite sample's thermal conductivity (in W/m-K)(between 30 and 55°C) and X is solids content in decimal form.

Table 5 also contains the results of the total titratable acidity tests. Total titratable acidity averaged 208 $\pm$ 42.2 Meq of base/Kg of BCS solids. The individual samples varied quite widely (20.3% variation) and followed no discernable pattern. By assuming a price of $350.00/Ton for 98% sodium hydroxide (caustic soda, 76% Na_2O basis), as found in the May 18, 1981 issue of the Chemical Marketing Reporter, one can put a price of 0.28 cents/Kg of BCS solids upon it's neutralization.

MINERAL ANALYSIS

Results of the mineral analysis of BCS are shown in Table 6. Included in the table are literature values for the mineral contents of corn and molasses (sugarcane). Orchid leaves were used as a standard of comparison in this testing. The concentrations of the minerals in BCS solids remained quite constant from one sample to the next. As can be seen in Table 6, BCS is a better source of phosphorous, calcium, magnesium, manganese, iron, zinc and sodium than is corn, but is a poorer source of nutrient minerals than molasses.

AMINO ACID PROFILE

The amino acid profiles for BCS and yellow-dent corn are shown in Tables 7 and 8. These data were obtained by analysis on the same instrument at the Department of Grain Science to minimize analytical errors and insure a more accurate comparison. The amino acid composition of the corn determined in our laboratory is within the range reported by the National Academy of Sciences for U.S. No. 2 yellow-dent corn. The majority of the essential amino acids in BCS are present at the same or higher concentration as in corn. In the case of lysine, which is the first limiting amino acid in corn, BCS contains almost 1.5 times the amount present in corn. Table 7 does not contain values for tryptophan since it is destroyed by the hydrolytic conditions used in our method of analysis. Tryptophan, however, is important since it is the second limiting amino acid in corn. In the future, we plan to analyze corn and BCS for typtophan, and to recalculate the amino acid profiles.

Table 9 shows a comparison of the amino acid profiles of BCS and corn to the ideal profile pattern for swine. BCS does provide much more of both lysine and isoleucine than does corn, but provides lower amounts of methionine than corn.

VISCOSITY

Figures 1 - 4 show the results of viscosity vs. temperature of BCS at different solids content. Figure 1 contains data on BCS with its solids as received from the plant, Figure 2 at 20% solids, and Figure 3 at 60% solids. Figure 4 contains viscosity data on a composite sample at varying solids contents.

The viscosities of the twelve daily samples (as received) varied widely from day to day. Each sample exhibited a good exponential relationship between viscosity and temperature. In order to determine if the solids content controlled the differences in viscosity between the various samples, each of the daily samples was freeze-dried and reconstituted to 60 and 20% solids. The curves in Figures 2 and 3 clearly show that the viscosity of a given sample of BCS cannot be predicted from its solids content. Obviously, this failure is due to the daily variation in the composition of BCS. It should be mentioned that sample # 17 was lost during freeze-drying. The viscosity of the composite sample (Figure 4) was examined at solids levels of 73, 60, 46 and 20% between 0 and 45°C. Figure 4 shows that no single relationship exists between the viscosity, solids content and temperature of the composite sample of BCS. In general, we noted the viscosity of BCS is higher than that of molasses, at an equal solids content, but lower than that of Masonex (Table XXIV of the Appendix).

STORAGE STABILITY

Results for the three month storage stability test are summarized in Table 10. Five of the daily samples were chosen for study because of their wide levels of solids contents. Two of those samples, #14 and #21, which had relatively low viscosities and low solids, were not microbiologically stable. Addition of as little as 0.25% propionic acid to those two samples made them microbiogogically stable. The remaining

samples (#7, #12 and #17) which contained 41 to 58% solids, were stable over three months at three temperatures with or without the addition of propionic acid. The temperature did not appear to affect storage stability of BCS between 70 and 90°F(21 - 32°C).

BROWNING OF BCS DURING DRYING IN A FORCED CONVECTION OVEN

Results of the oven-drying tests at different drying temperatures are shown in Tables 11 - 13 and Figures 11 - 12. The critical concentration in the oven test is defined as the concentration of solids in BCS when the BCS first begins to visibly brown. For each drying temperature the critical concentrations and drying times are as follows: 54°C, 86.1% dry matter, 168 hours; 66°C, 83.4% D.M., 45 hours; 77°C 81.3% D.M., 8 hours; and 88°C, 81.4% D.M., 4 hours. The endpoint for this quality test was sometimes difficult to determine. For this reason, a panel of several graduate students was used to judge the quality change. It is likely that absorbance at 550 nm could probably be used to obtain a more accurate endpoint.

PRELIMINARY ENZYME SCREEN

Results of the preliminary screening of enzymes to thin BCS are contained in Table 14. On a cost per 1% change in viscosity, the cellulase Tv was by far the most effective, followed by Hemicellulase 100,000, Tenase, Bio-Glucanase and Glucanase GV-L. When comparing the enzymes on the total possible reduction in viscosity, cellulase Tv was once again the most effective with a 44% change. The two glucanases were second with around a 25% change and Hemicellulase 100,000 was third with a change of 15%. Tenase, (α-amylase), Spark-L-HPG (pectinase) and Diazyme L-100 (glucoamylase) gave only a small change in BCS viscosity (at 20% solids the viscosity difference between mono and disaccharides would be very small, however, at elevated solids the difference could be larger). Perhaps a greater reduction in viscosity could be accomplished by using two or maybe three enzymes. 31

Second Enzyme Study

Results for this enzyme study are contained in Tables 15 and 16, with a graphical representation of viscosity reduction rates (for cellulase Tv and Bio-Glucanase) in Figure 13. As can be seen from Figure 13, both Cellulase Tv and Bio-Glucanase decrease the viscosity of BCS in an exponential manner. This exponential curve, however, is not a logrithmic relationship. Bio-Glucanase is the fastest acting of the two enzymes, but seems to begin slowing down after around 15 minutes of reacting. Cellulase, on the other hand, reacts more slowly but in the end decreases viscosity the most. This led us to believe that perhaps a dual enzyme system of Cellulase Tv and Bio-Glucanase might decrease viscosity even more. But, as can be seen from Tables 15 and 16, this is not the case. All combinations of the two enzymes fell well short of what the Cellulase Tv did on it's own. However, the enzyme system did reduce viscosity more than the Bio-Glucanase standard. The standard was lower than previously run possibly because of the lower reaction temperature ($50^{\circ}C$) and pH (4.5) used. Additions of Hemicellulase-100,000 did not affect the reaction considerably. The reason for this failure could be due to several factors: First, the different enzymes could have been denaturing each other in the reaction, or it could have been due to a competitive inhibition of one on the other, or possibly it could have been due to part of the enzyme system not having been at optimum conditions. The cellulase was always at optimum conditions (pH 4.5 and $50^{\circ}C$), but the other two enzymes were not (although each was well within it's operating range - see Table XXII in the Appendix). I believe that it was probably a mixture of all three reasons that the test failed. With 0.5 mL of Cellulase Tv and a 60 minute reaction time, the results should equal those of the 1.0 mL and 30 minute reaction. There was about a

4% difference between the two which was probably due to a reduced activity of the Cellulase Tv in the multiple testing for some reason. Further testing in this area could be a distinct possibility for the future.

PELLET BINDING WITH BCS

Tables 17 and 18 contain results and statistics for the pellet binding study comparing BCS with molasses and Masonex.

The results showed that there was no significant difference in pellet durability index between the three binders tested (95.7% for molasses and Masonex, and 95.4% for BCS). There was also no significant difference in production rate between the three binders. In the analysis of percentage of fines, no significant difference was observed between the BCS and molasses or molasses and Masonex, but a significant difference was found between BCS and Masonex. The analysis of energy consumption (KWH/Ton) showed no significant difference between BCS and molasses. In general, one may conclude that the performance of BCS as a pellet binder is equal to that of either of the two other binders tested.

It should be noted that the BCS from the Williamsburg plant (used in pellet tests) was different from the BCS obtained from the Merrimac plant, which has been undergoing extensive analysis. The BCS used in the pelleting trials was fermenting while in storage. It also had a lower viscosity and lower solids when compared to the other BCS samples received from the Merrimac plant. Another difference was color. The BCS used in the pelleting trials had a much lighter color than the BCS from the Merrimac plant. Those factors could have had an adverse effect on its pelleting properties.

Fermenting would make little difference once the BCS is incorporated into pellets. At the 3% level, little water would be available for the microbes to grow since the water is absorbed by the rest of the feed

ingredients. Also, the heat treatment in the pelleting operation would kill the majority of the microbes in the BCS. Nevertheless, another trial with a better sample of BCS should probably be conducted. It should be also noted that no problems were encountered with the BCS during the mixing and pelleting operations.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

Table 1. Daily Subsamples Received From the Merrimac, Mass. Plant

Date (Nov.) (1979)	6	7	8	9	10	12	13	14	15	16	17	19	20	21
Day of Week	Tu	W	Th	F	Sa	M	Tu	W	Th	F	Sa	M	Tu	W
No. of Subsamples Taken	2	3	3	3	2	3	3	3	3	3	3	3	3	3

Note: Daily samples were not used for the days Nov. 6 and 10 because only two subsamples were provided.

Table 2. Average Composition and Gross Energy of BCS, Corn, Molasses and Masonex

	BCS	Corn[1]	Molasses[2]	Masonex[3]
Total Solids(%)	44.4 ± 7.4	90	66.0	54.1
Protein (%d.b.)	8.85 ± 1.14	10.14	5.90	1.1
Carbohydrate(%d.b.)[4]	74.81 ± 5.88	81.1[5]	84.0[5]	93.8[5]
Fat(%d.b.)	1.43 ± .303	4.4	0.0[5]	0.2[5]
Ash(%d.b.)	2.54 ± .207	2.0	10.10	5.00
Gross Energy (cal/g-d.b.)	4073 ± 53	4393	3768	6750

1) U.S. #2 Yellow Dent Corn.

2) Sugarcane Molasses.

3) Wood Molasses or Wood Hemicellulose Extract.

4) For Corn, Molasses and Masonex the measurement is Nitrogen - Free Extract.

5) Literature values were obtained from the Atlas of Nutritional Data on U.S. and Canadian Feeds; NAS; Washington, D.C.; 1972.

Table 3. Average Composition of BCS Solids

Analysis	Value
Total Solids	44.4 ± 7.40
Suspended (%T.S.)	6.9 ± 1.91
Soluble (%T.S.)	93.1 ± 1.91

Table 4. Average Carbohydrate Composition in BCS

Analysis	Value
Total Carbohydrate(%d.b.)	74.8 ± 5.88
Dextrose(%d.b.)	9.4 ± 0.79
Maltose(%d.b.)	38.0 ± 4.19
Maltotriose(%d.b.)	11.7 ± 0.80

Table 5. Average Thermal and Physical Properties of BCS

Property	Value
pH	4.14 ± 0.222
Freezing Point (°C)	-8.6 ± 2.75
Specific Heat (J/Kg-K)	3.093 ± 0.237
Density (Kg/m^3)	1.196 ± 10^3
Thermal Conductivity (W/m-K) (d.b.)	0.349 ± 0.027
Total Titratable Acidity (Meq/Kg Solid)	208 ± 42.2

Table 6. Average Mineral Analysis of BCS, Corn and Molasses[1]

	BCS	Corn[2]	Molasses[2]
Nitrogen(%)	1.403 ± .212	1.62	0.18
Phosphorous(%)	0.382 ± .022	0.32	0.11
Potassium(ppm)	1097 ± 72	3300	40,200
Calcium(ppm)	1539 ± 206	200	10,500
Magnesium(ppm)	1456 ± 121	1200	4700
Copper(ppm)	3.07 ± 1.76	3.7	80.2
Manganese(ppm)	13.55 ± 2.50	6.1	57.2
Iron(ppm)	84.98 ± 22.42	30	240
Zinc(ppm)	12.56 ± 2.64	--	---
Sodium(ppm)	1349 ± 248	100	2000

1) All values are on a Dry Matter Basis.

2) Literature values were obtained from the Atlas of Nutritional Data on U.S. and Canadian Feeds; NAS; Washington, D.C.; 1922.

Table 7. Essential[1] Amino Acid Profile for Average BCS Sample and Corn

Amino Acid	BCS[2]	Corn[2]
Threonine	3.39 ± 0.14	3.47
Valine	4.42 ± 0.23	3.13
Methionine	1.47 ± 0.14	1.87
Isoleucine	2.70 ± 0.17	1.94
Leucine	6.02 ± 0.27	12.01
Phenylalanine	4.32 ± 0.16	4.39
Histidine	4.78 ± 0.37	4.02
Lysine	3.33 ± 0.28	2.34
Argenine	4.87 ± 0.66	4.83

1) Essentiallity is based on the growing pig (20-35 Kg).

2) Units are in g of amino acid/ 100 g protein (corrected to 100% recovery).

Table 8. Non-Essential[1] Amino Acid Profile for Average BCS Sample and Corn

Amino Acid	BCS[2]	Corn[2]
Aspartic Acid	8.10 ± 0.33	7.09
Serine	4.39 ± 0.17	5.38
Glutamic Acid	20.83 ± 1.21	19.97
Proline	12.51 ± 1.01	9.53
Glycine	5.16 ± 0.22	4.22
Alanine	6.89 ± 0.43	9.25
Half Cystine	1.09 ± 0.16	1.38
Tyrosine	3.50 ± 0.34	3.56
Ammonia	2.24 ± 0.40	1.61

1) Essentiality is based on the growing pig (20-35Kg).

2) Units are in g of amino acid/100 g protein (corrected to 100% recovery).

Table 9. Essential Amino Acid Pattern for Pigs,(1) Comparing BCS to Corn.

Essential A.A.	Average BCS (g.A.A./100g. Protein)	Corn - (g.A.A./100g. Protein)	Ideal Reference Pattern (g.A.A./100g. Protein) for Pigs(3)
Arginine	4.87 ± 0.66	4.83	1.25
Histidine	4.78 ± 0.37	4.02	1.13
Isoleucine	2.70 ± 0.17	1.94	3.13
Leucine	6.02 ± 0.27	12.01	3.75
Methionine	1.47 ± 0.14	1.87	3.13
Phenylalamine	4.32 ± 0.16	4.39	3.13
Threonine	3.39 ± 0.14	3.47	2.81
Tryptophan	——(2)	——(2)	0.81
Valine	4.42 ± 0.23	3.13	3.13
Lysine	3.33 ± 0.28	2.34	4.38

1) Growing Pigs 20-35 kg.

2) Not included in analysis, destroyed by hydrolysis.

3) From Nutrient Requirements of Swine: National Academy of Sciences; 1973. Values changed from % diet to % protein.

Table 10. Three Month Storage Stability Test of BCS, Summary

Storage Period	Samples with mold growth or fermentation
5th Day	#14-0% - 70°F, #14-0%-80°F and #14-0%-90°F showed some apparent bubble formation in the specimen.
9th Day	#21-0%-70°F showed a pink fungal growth.
10th Day	#21-0%-80°F showed a pink fungal growth.
14th Day	#21-0%-80°F (on shaker) showed mold growth.

Note: The storage trial started on March 11, 1980, and was finished August 15, 1980. The symbol #14-0%-70°F means sample #14, 0% propionic acid added and 70°F storage temperature.

Temperatures in °Centigrade are: 70°F = 21.1°C, 80°F = 26.7°C and 90°F = 32.2°C.

Table 11. Oven Drying Test of BCS Composite Sample - Drying Time vs. Weight Change at 54.4°C

Time (Hr.)	Replication #1 (g)	#2 (g)	#3 (g)	Ave. (g)	Remarks
0	4.62577	3.98410	3.58680	4.06556 (50.00%)	Solids contents given in parenthesis
2	3.79136	3.14758	2.79153	3.24349 (62.67%)	
4	3.50225	2.90045	2.58468	2.99579 (67.85%)	
6	3.34770	2.78000	2.48164	2.86978 (70.83%)	
25	2.92407	2.47948	2.21548	2.53968 (80.04%)	
28	2.90377	2.46423	2.20351	2.52384 (80.54%)	
52	2.84377	2.40162	2.15507	2.46682 (82.40%)	
72	2.79202	2.37430	2.13343	2.43325 (83.54%)	
78	2.77667	2.36130	2.12153	2.41983 (84.01%)	
96	2.75337	2.34351	2.10648	2.40112 (84.66%)	
101	2.74949	2.34118	2.10405	2.39824 (84.76%)	
120	2.73361	2.32814	2.09348	2.38508 (85.23%)	
125	2.73605	2.33060	2.09479	2.38715 (85.16%)	
144	2.71573	2.31488	2.08200	2.37087 (85.74%)	
150	2.71567	2.31311	2.08111	2.36996 (85.77%)	
168	2.70364	2.30506	2.07405	2.36902 (86.10%)	Color starts to change.
174	2.67977	2.30370	2.07283	2.35210 (86.42%)	Completely changed.

Table 12. Oven Drying Test of BCS Composite Sample - Drying Time vs. Weight Change at 65.6°C

Time (Hr.)	Replication #1 (g)	#2 (g)	#3 (g)	Ave. (g)	Remarks
0	5.38345	4.17183	4.66027	4.73852 (50.00%)	Solids contents given in parenthesis
1	4.69371	3.51715	3.98407	4.06398 (58.30%)	
2	4.43682	3.28243	3.73397	3.81774 (62.06%)	
3	4.23100	3.12102	3.55041	3.63414 (65.19%)	
19	3.41499	2.59631	2.92036	2.97722 (79.58%)	
21	3.39454	2.58313	2.90380	2.96049 (80.03%)	
25	3.36103	2.56755	2.87993	2.93617 (80.69%)	
45	3.24384	2.48965	2.78807	2.87052 (83.41%)	*Color starts to change.
49	3.23342	2.48080	2.77822	2.83081 (83.70%)	Color became dark. Completely changed.
72	3.17044	2.43824	2.72734	2.77867 (85.27%)	From here, condition is caramel.
92	3.13404	2.41135	2.70049	2.74863 (86.20%)	"
98	3.11438	2.39876	2.68247	2.73187 (86.73%)	"

Table 13. Oven Drying Test of BCS Composite Sample - Drying Time vs. Weight Change at 87.3 and 76.7°C

	87.8°C	76.7°C	
Time (hr)	Average	Average	Remarks
0	2.98970 (50.00%)	2.95489 (50.00%)	Solids contents given in parenthisis
2	2.00020 (74.43%)	2.07496 (71.21%)	
3	1.91264 (78.15%)	1.98212 (74.53%)	
4	1.85861 (81.43%)	1.92186 (76.88%)	87.8°C sample starts to change
5	1.81910 (82.18%)	1.88487 (78.39%)	87.8°C sample was completely changed
8		1.81863 (81.25%)	76.7°C sample starts to change (9 hrs; completely changed)

Note: Each value is the average of two replications.

Table 14. Results of the Preliminary Enzyme Screen

Enzyme	change (%)	Cost per 1% change in Viscosity, ($x10^{4})[1]	Cost per Kg. Dry Solid, ($)
Cellulase Tv (15 min.)[2]	25.24%	$0.349	$0.176
(30 min.)[2]	43.49	0.203	0.176
Glucanase GV-L	27.48	7.744	4.256
Bio-Glucanase	23.98	2.508	1.202
Hemicellulase 100,000	14.77	1.104	0.326
Tenase [3]	7.86	2.005	0.315
Spark-L HPG[4]	6.06	18.000	2.182
Diazyme L-100[5]	0	0	0.019

1) Cost is in 10^{-4} dollars ($). Thus the figure for Cellulase Tv (15 min) is actually 0.349×10^{-4} dollars.

2) For the Cellulase Tv, two different reaction times were used (15 and 30 minutes). For all other enzyme preperations a reaction time of 30 minutes was used.

3) Tenase is an α-amylase preparation.

4) Spark-L HPG is a pectinase preparation.

5) Diazyme L-100 is a glucoamylase preparation.

Table 15. Viscosity Degredation by Cellulase Tv and Bio-Glucanase of a 20% Solution of BCS

Reaction Time	Change in Viscosity %	
	Bio-Glucanase	Cellulase Tv
5 min.	18.0[1]	15.7[2]
10	28.8	20.9
15	30.8	26.9
30	31.1	31.7
45	33.5	37.7
60	33.5	38.6

1) Initial Viscosity was 20.1 abs. cps.
Reaction Temperature = 70°C, pH = 5.5
Units of enzyme used = 200 u.

2) Initial viscosity was 19.8 abs. cps.
Reaction Temperature = 50°C, pH = 4.5
Units of enzyme used = 540 (CMC)u.

Table 16. Results from Multiple-Enzyme System Testing on Viscosity Degredation of a 20% Solution of BCS

Enzymes in System	Composition of Enzyme System[1] 3/4-1/4	1/2-1/2	1/4-3/4	B-G std.[2]
Bio-Glucanase Cellulase Tv	27.5%[3]	27.9%	27.0%	26.1%
Bio-Glucanase Hemicellulase	-	28.8	-	27.8
Cellulase Hemicellulase	-	27.7	-	-
Bio-Glucanase Cellulase Hemicellulase	-	22.9[4]	-	-

1) Composition refers to the amount of each enzyme in the system, according to the order the enzymes are given in the first column.

2) Bio-Glucanase standard needed to be run at the system temperature used.

3) Results are in % change of BCS viscosity.

4) System is actually 1/3-1/3-1/3, but was reacted as 1/2-1/2-1/2 of a 1.50mL amount of enzyme.

Table 17. Average results of the Pellet Binding Tests Comparing BCS to Molasses and Masonex

Binder	Fines (%)	PDI (%) [2]	Prod. Rate (#/Hr) [3]	Energy ($\frac{Kw\text{-}Hr}{Ton}$) [4]
Molasses	3.07	95.7	3113	10.52
Masonex	2.80	95.7	3047	10.65
BCS	3.2	95.4	3003	10.95

1) Fines means the % Fines collected during the pelleting process.

2) PDI stands for Pellet Durability Index.

3) Prod. Rade stands for Production Rate.

4) Energy means the amount of energy used by the pelleting process.

Table 18. Duncan's Test on Means for the Pellet Binding Tests

Fines (%)			PDI[1] (%)			Prod. Rate (#/Hr)			Energy ($\frac{Kw-Hr}{Ton}$)		
Treat.[2]	Mean	Group[3]	Treat.[2]	Mean	Group[3]	Treat.[2]	Mean	Group[3]	Treat.[2]	Mean	Group[3]
3	3.20	A	1	95.4	A	1	3113	A	3	10.95	A
1	3.07	A B	2	95.7	A	2	3047	A	2	10.65	A B
2	2.80	B	3	95.7	A	3	3003	A	1	10.50	B

[1]PDI stands for Pellet Durability Index

[2]Treat. stands for Treatment: 1 = Molasses; 2 = Masonex; 3 = BCS

[3]Group. stands for letter grouping; Means with the same letter are not significantly different (at a 95% confidence level)

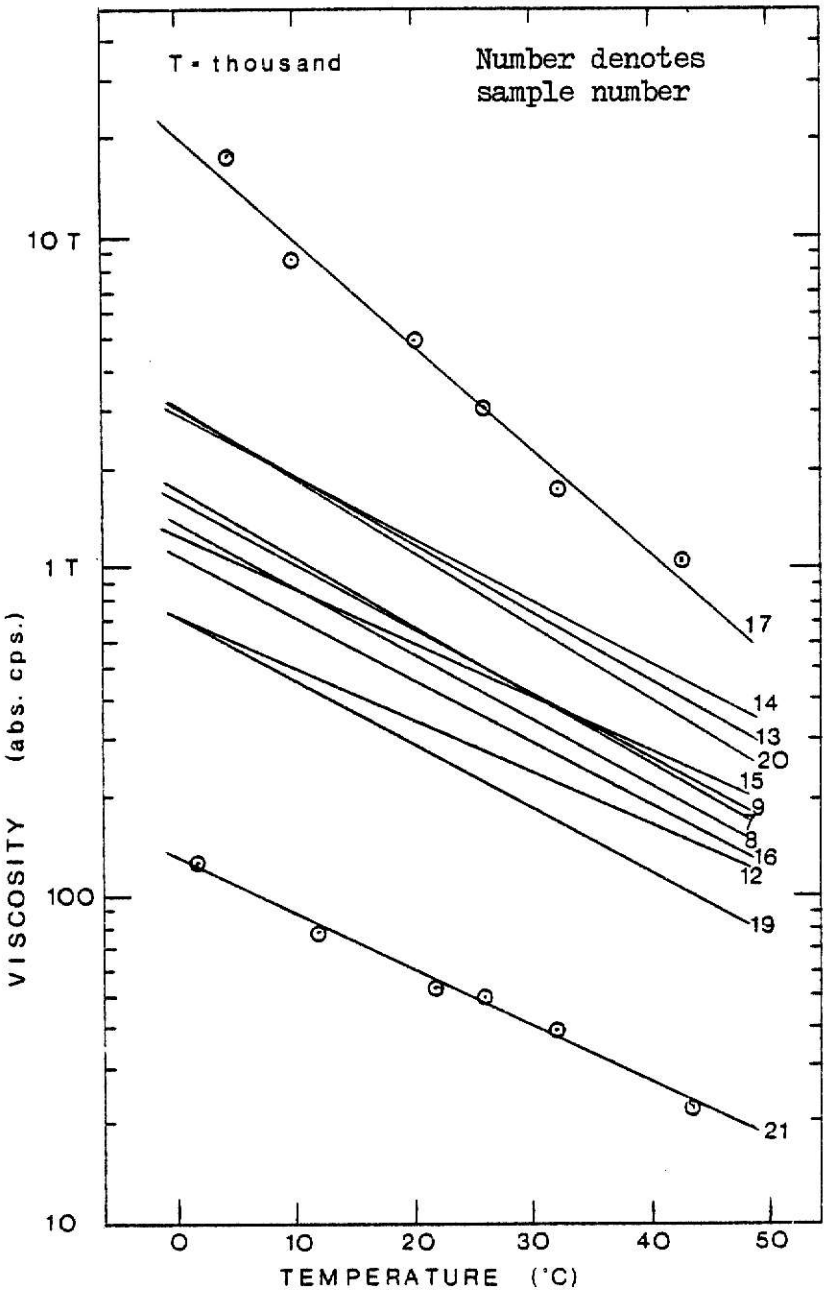

Figure 1. Viscosity vs. Temperature of BCS Daily Samples "as recieved" (solids content ranged from 27 to 58%). Most experimental points were left off for clarity.

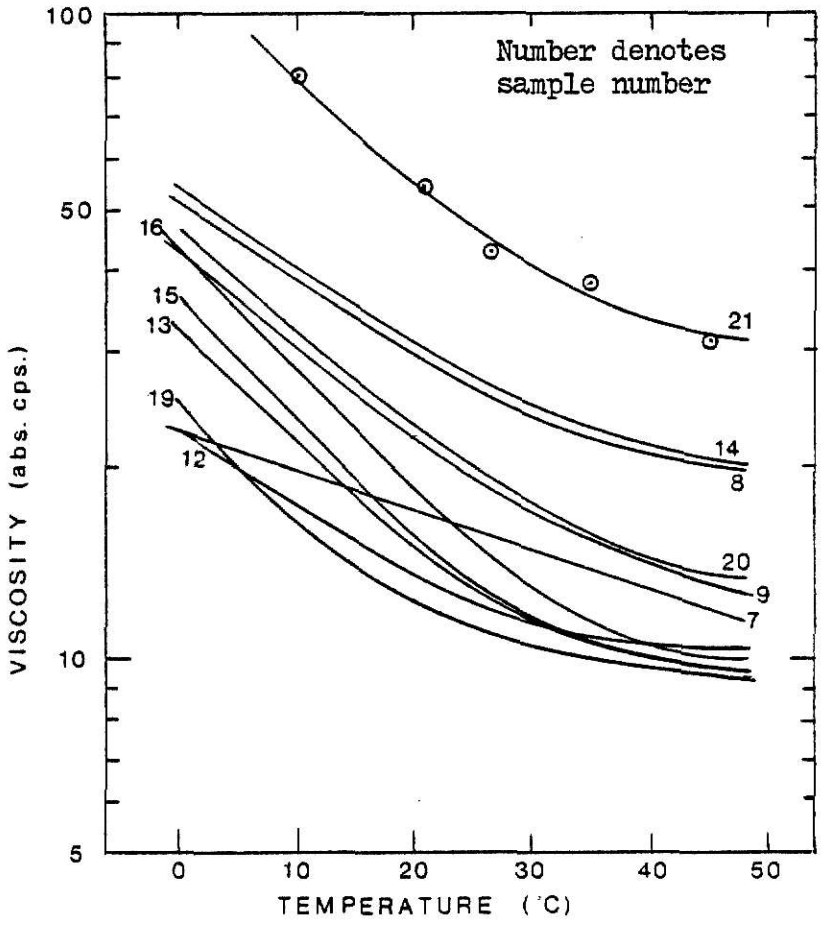

Figure 2. Viscosity vs. Temperature for Reconstituted Solutions of Freeze-Dried BCS Daily Samples Rehydrated to 20% Solids. Most experimental points were left off for clarity.

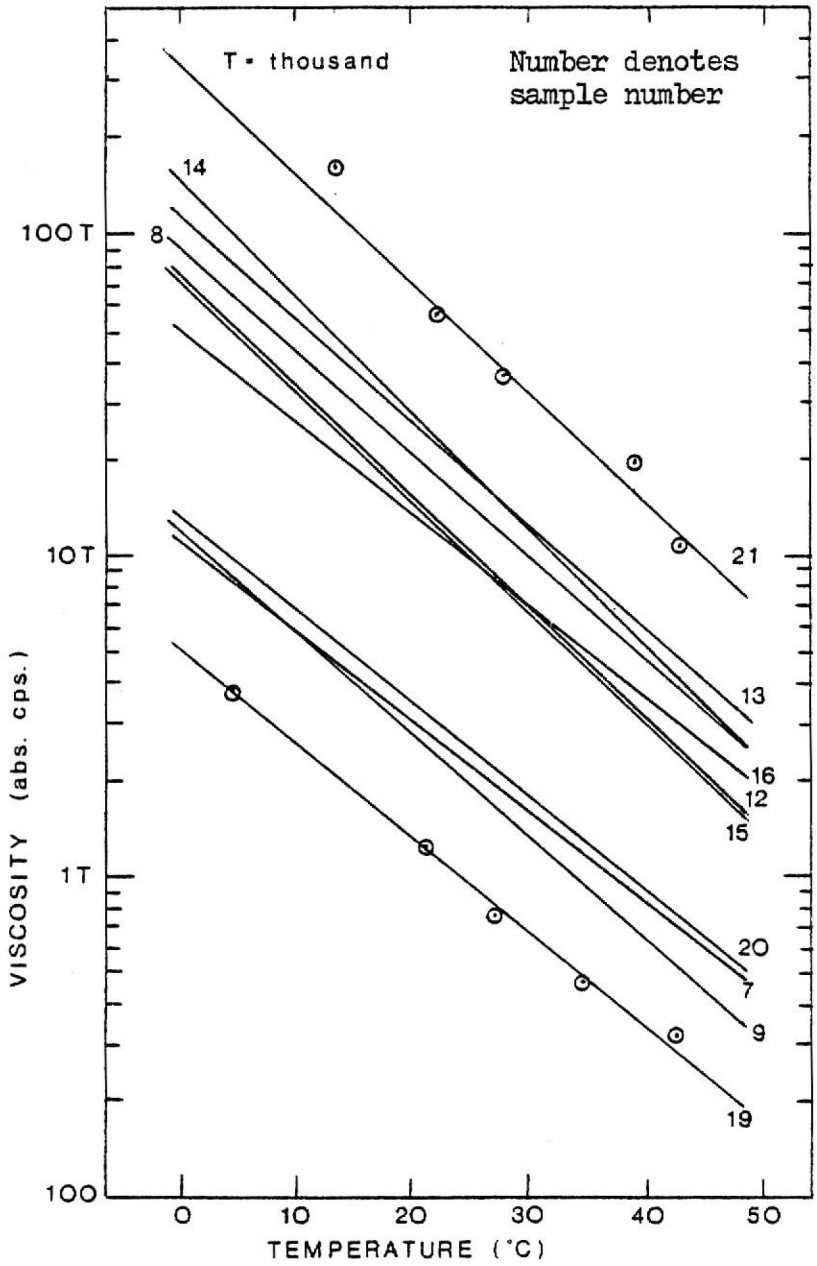

Figure 3. Viscosity vs. Temperature for Reconstituted Solutions of Freeze-Dried BCS Daily Samples Rehydrated to 60% solids. Most experimental points were left off for clarity.

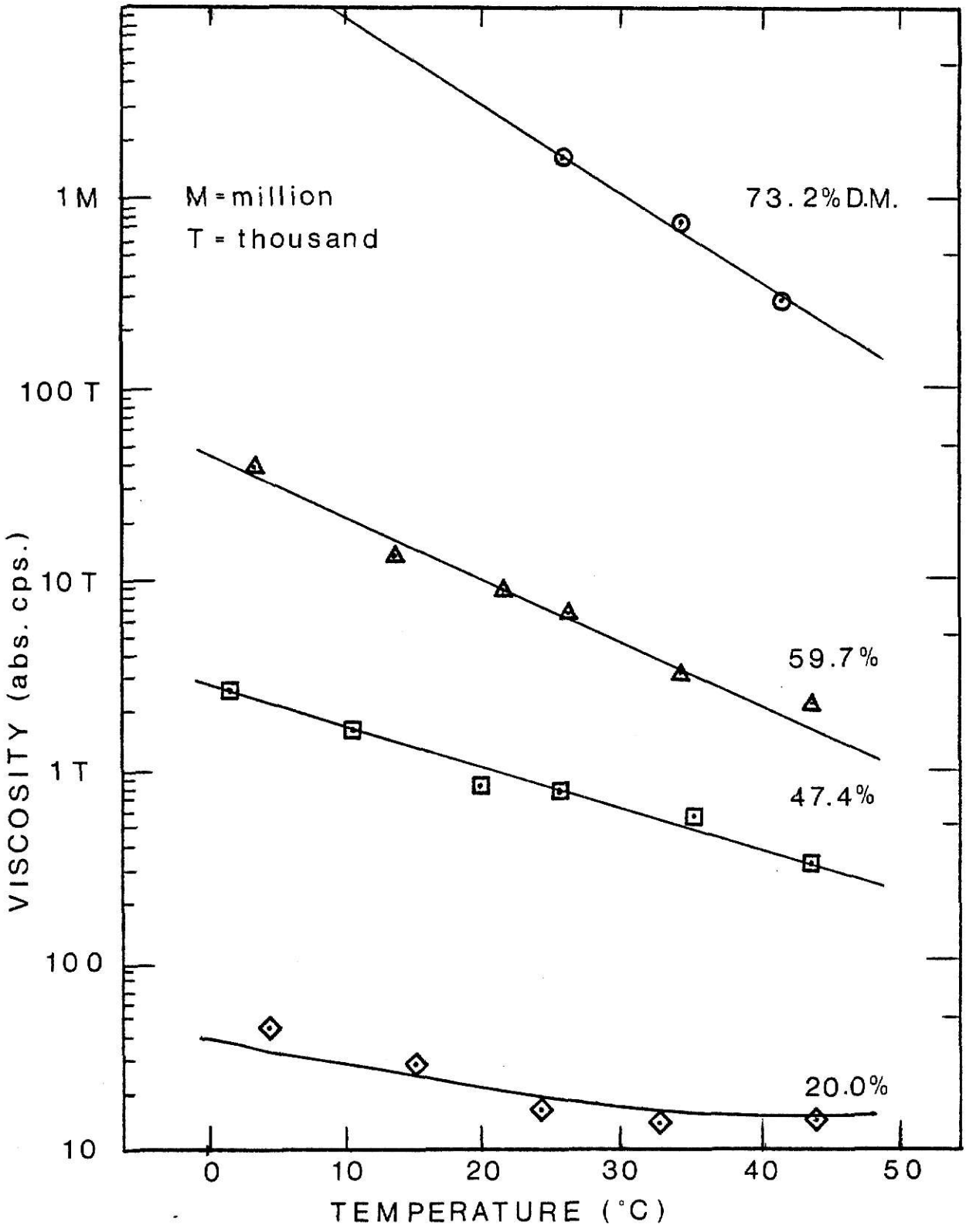

Figure 4. Viscosity vs. Temperature and Solids for Reconstituted Solutions of Freeze-Dried Composite Sample of BCS

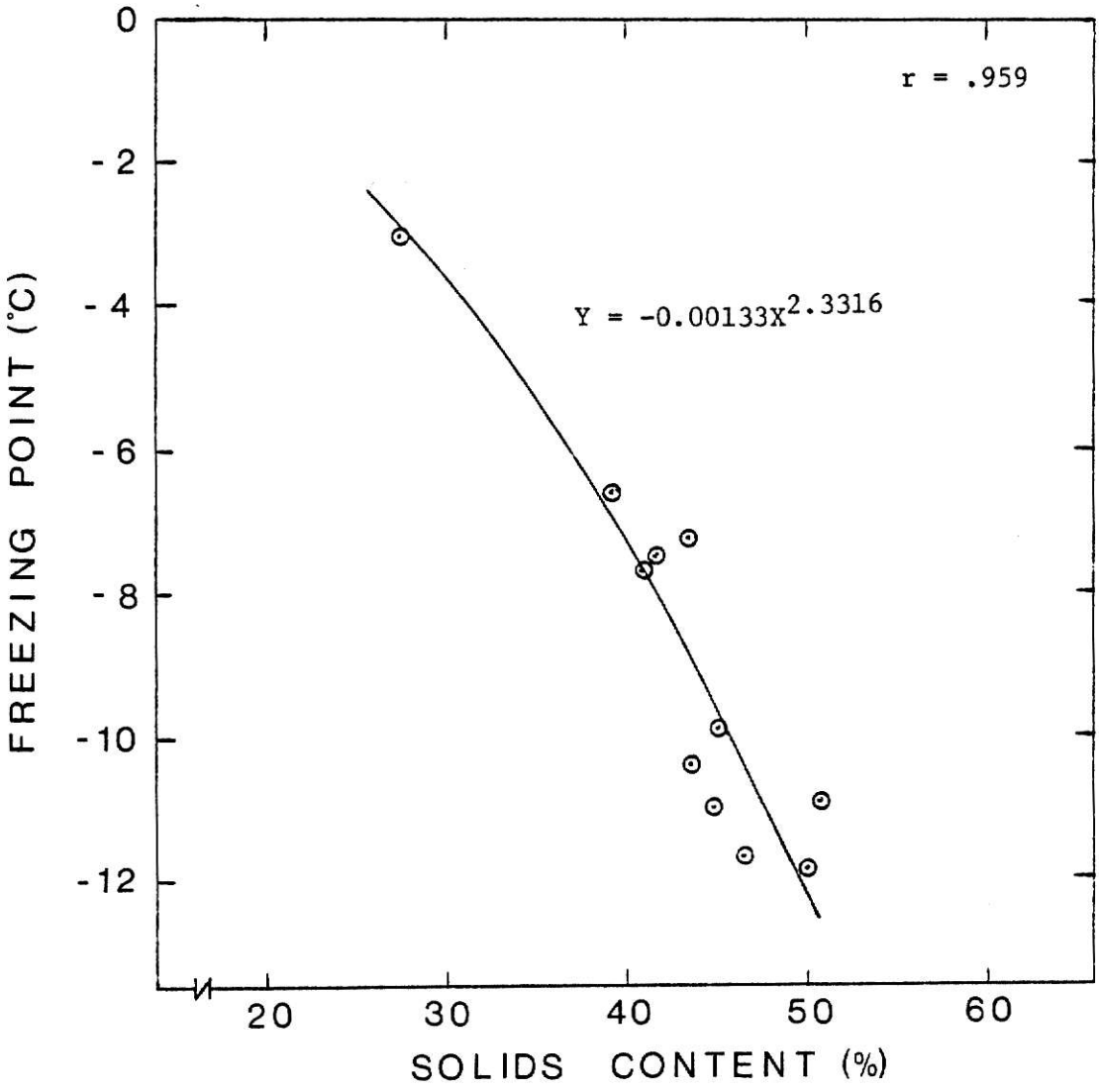

Figure 5. Freezing Point vs. Solids Content of BCS Daily Samples "as recieved"

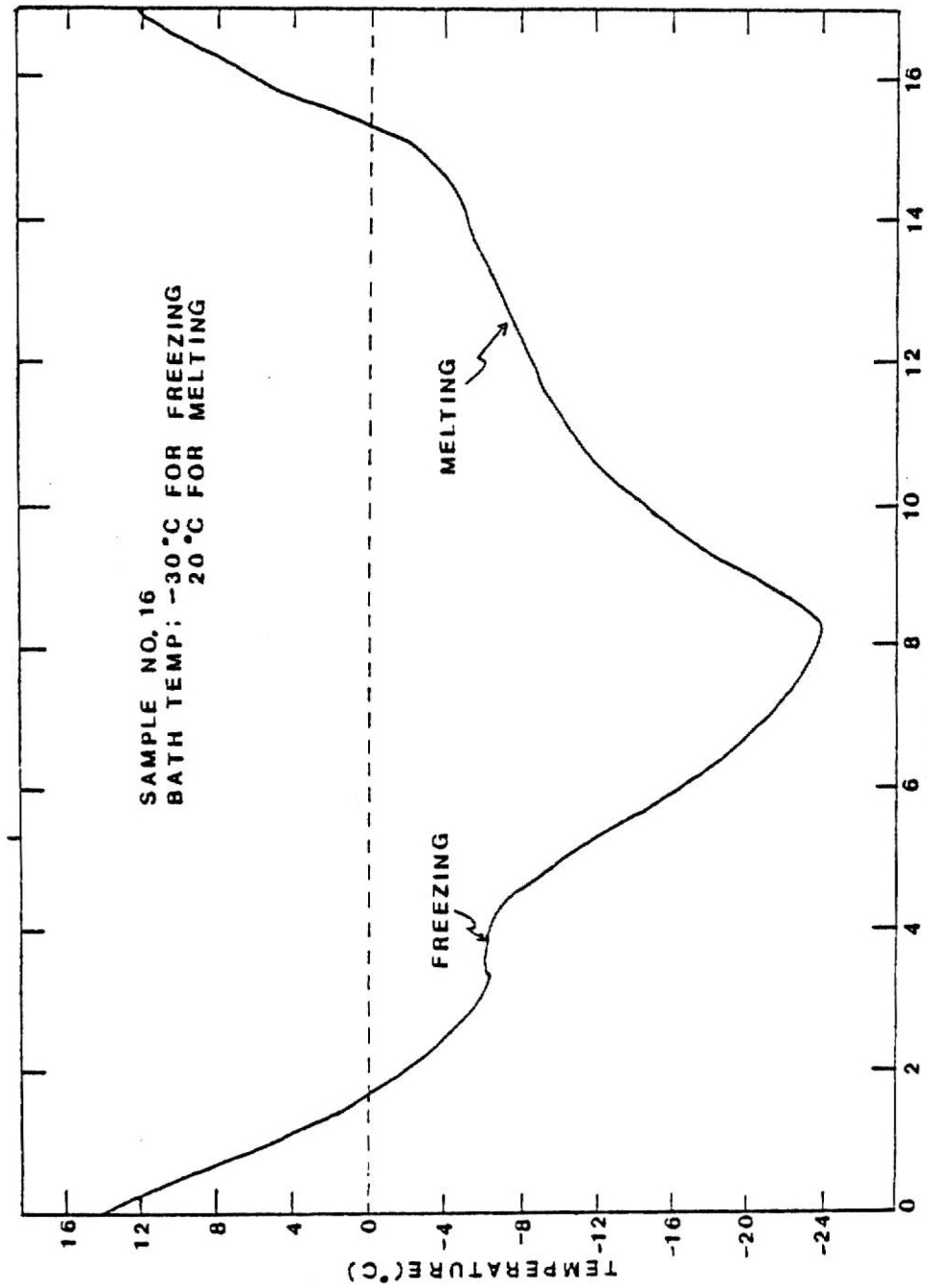

Fig. 6. A typical freezing and melting curve of BCS.

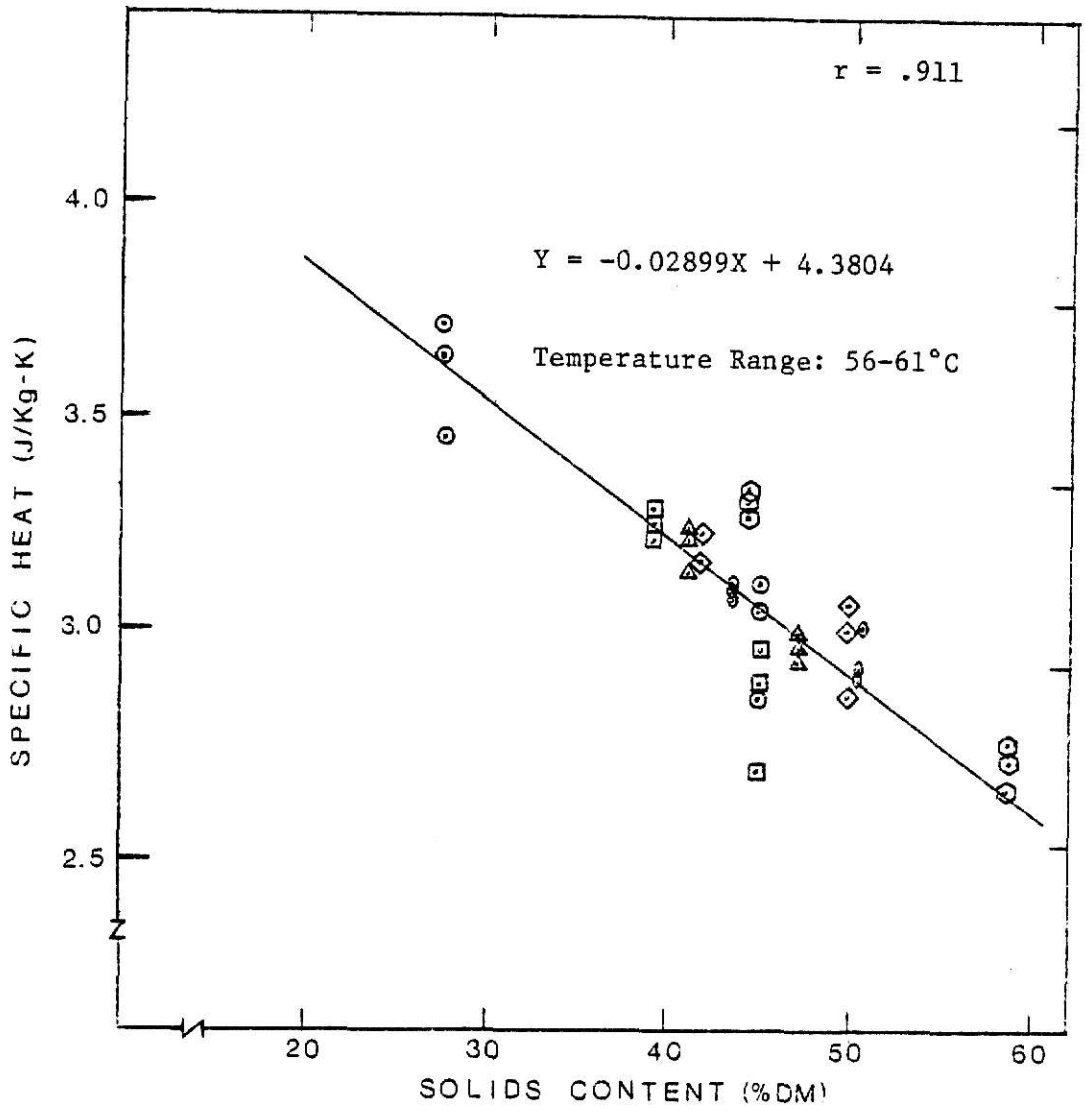

Figure 7. Specific Heat vs. Solid Content of BCS Composite Samples "as received"

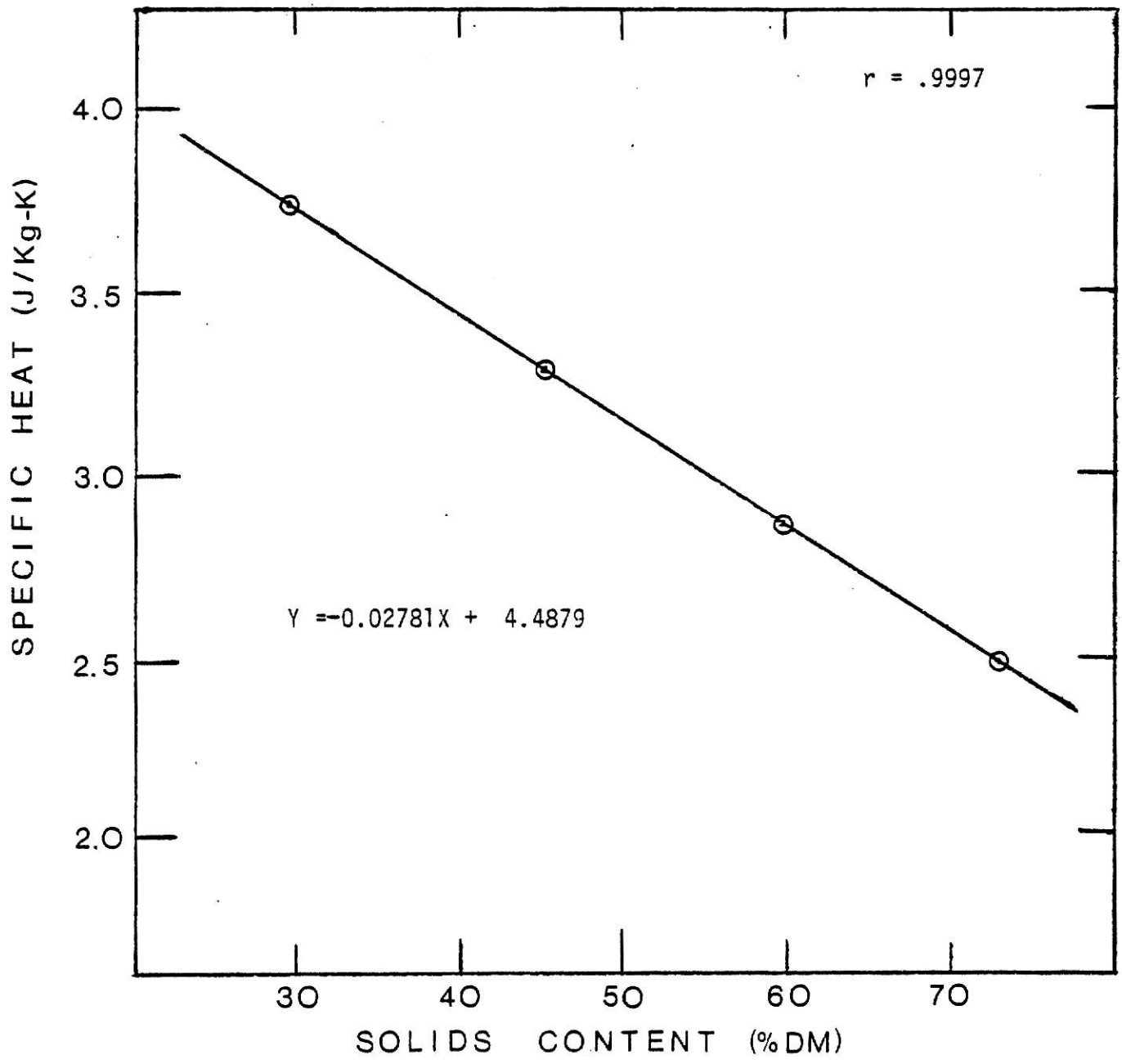

Figure 8. Specific Heat vs. Solids Content for BCS(composite)

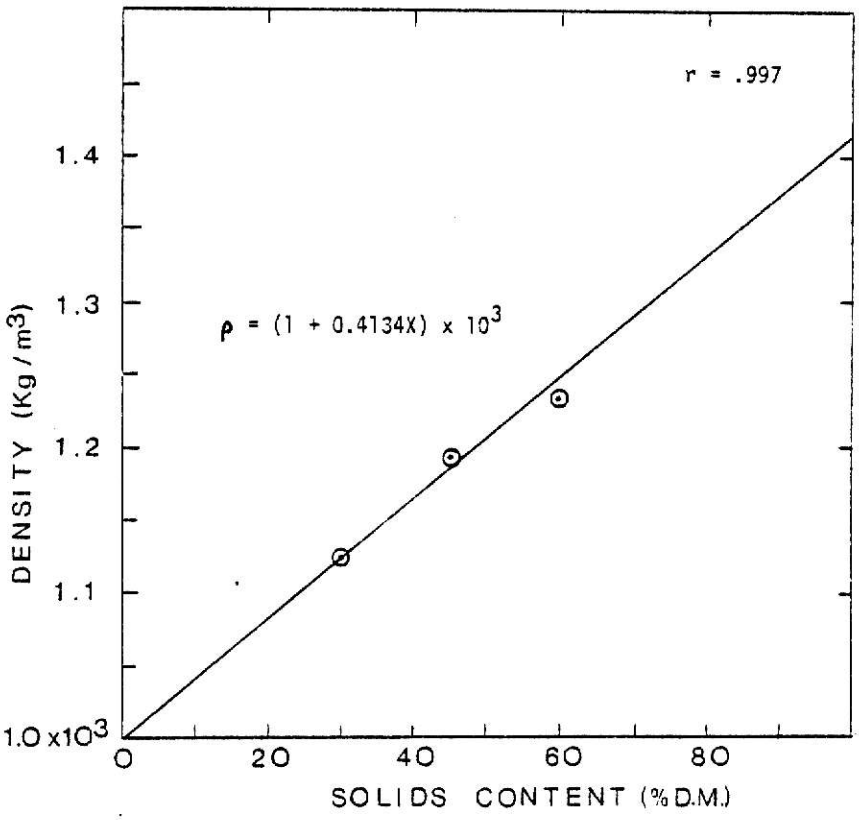

Figure 9. Solids Content vs. Density for BCS Composite

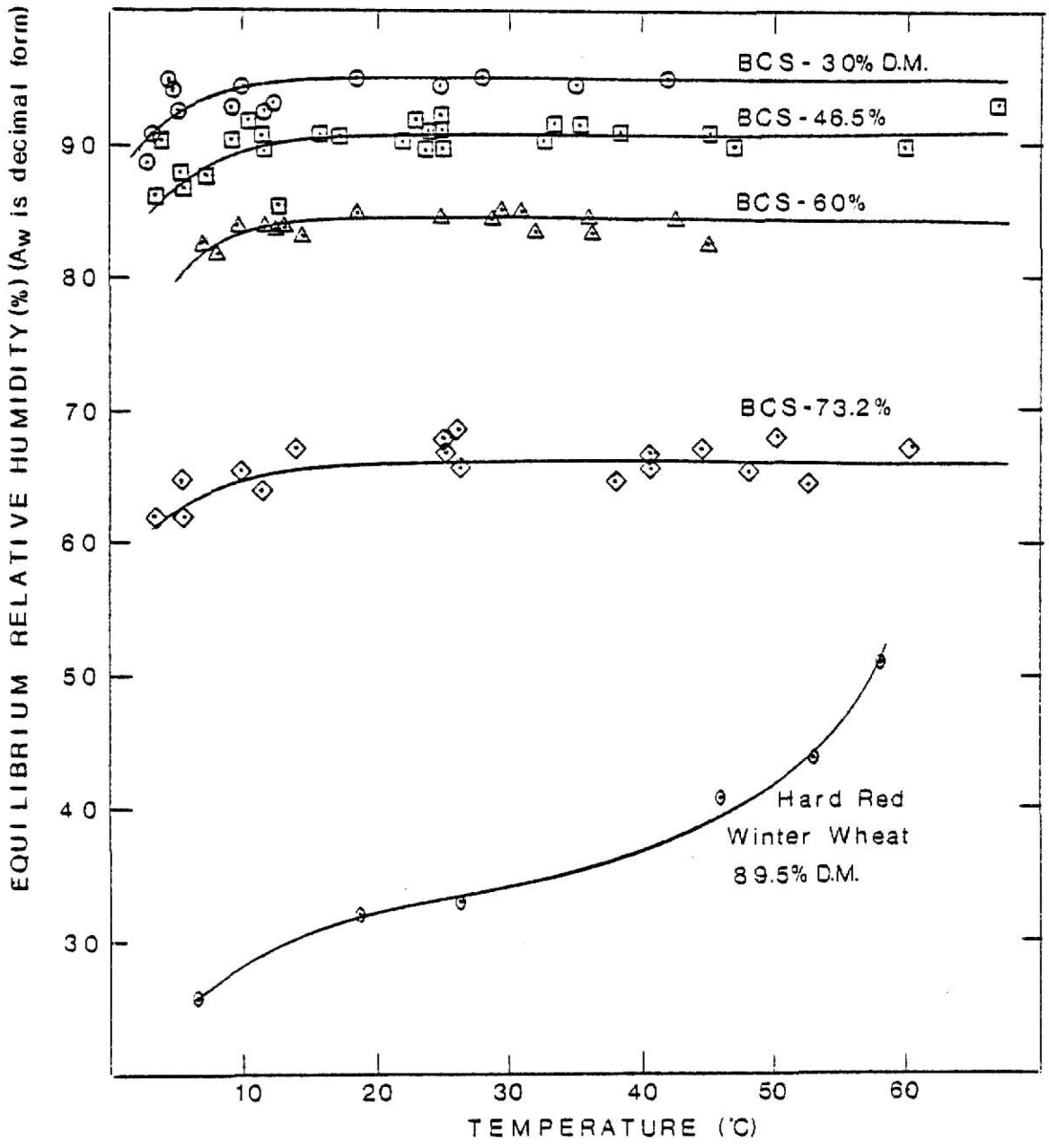

Figure 10. ERH vs. Temperature and Solids for BCS Composite

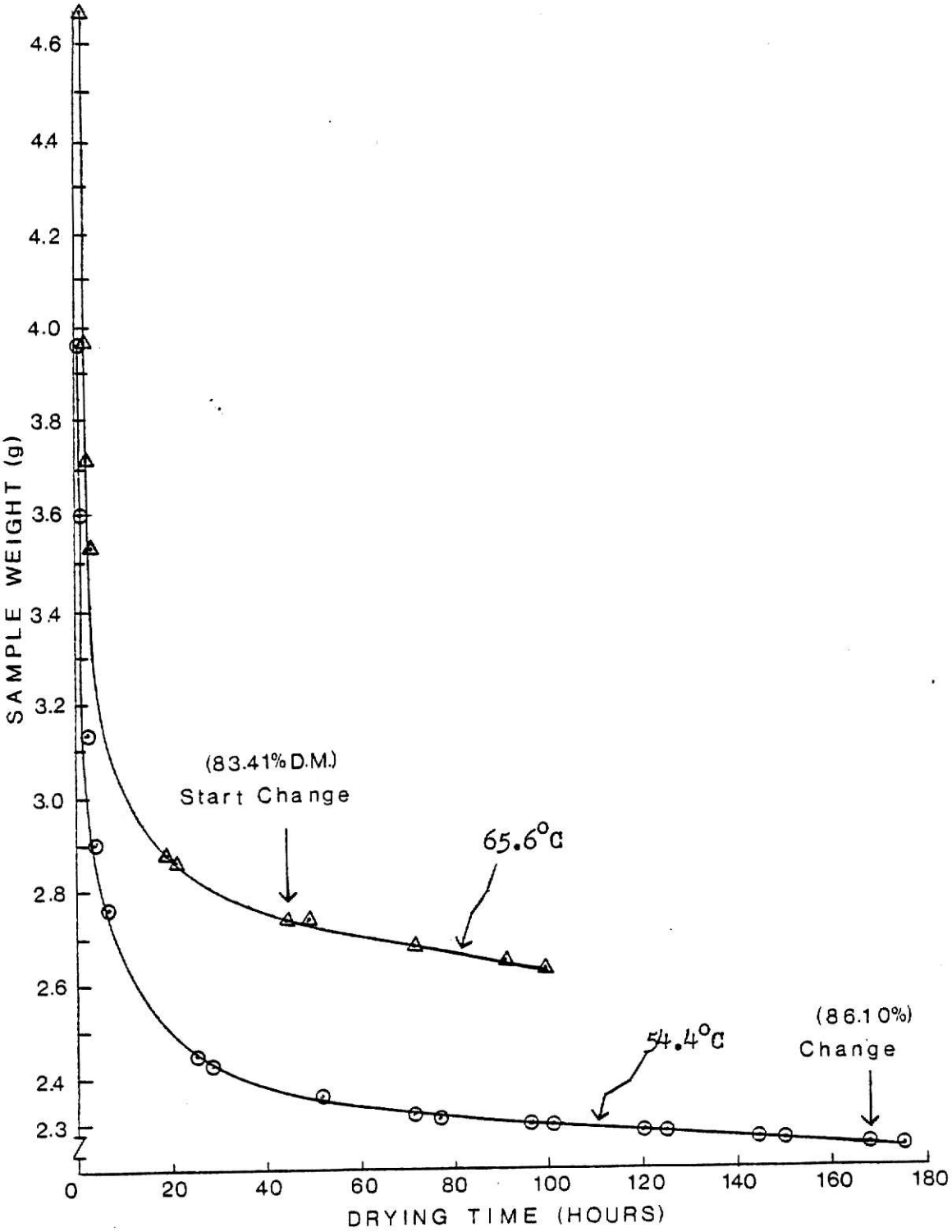

Figure 11. Average Weight Change vs. Drying Time at 54.4 and 65.6°C for BCS Composite Sample.

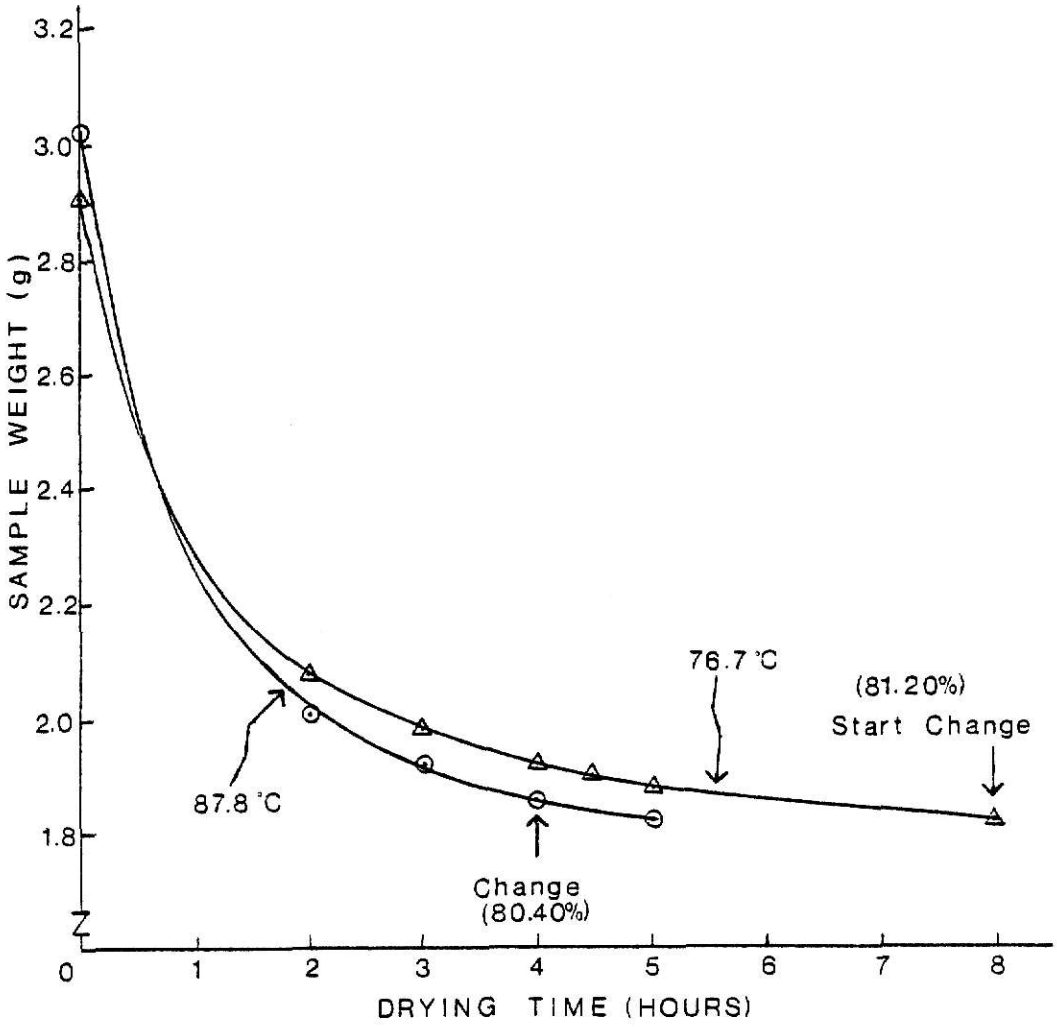

Figure 12. Average Weight Change vs. Drying Time at 76.7 and 87.8°C for BCS Composite Sample.

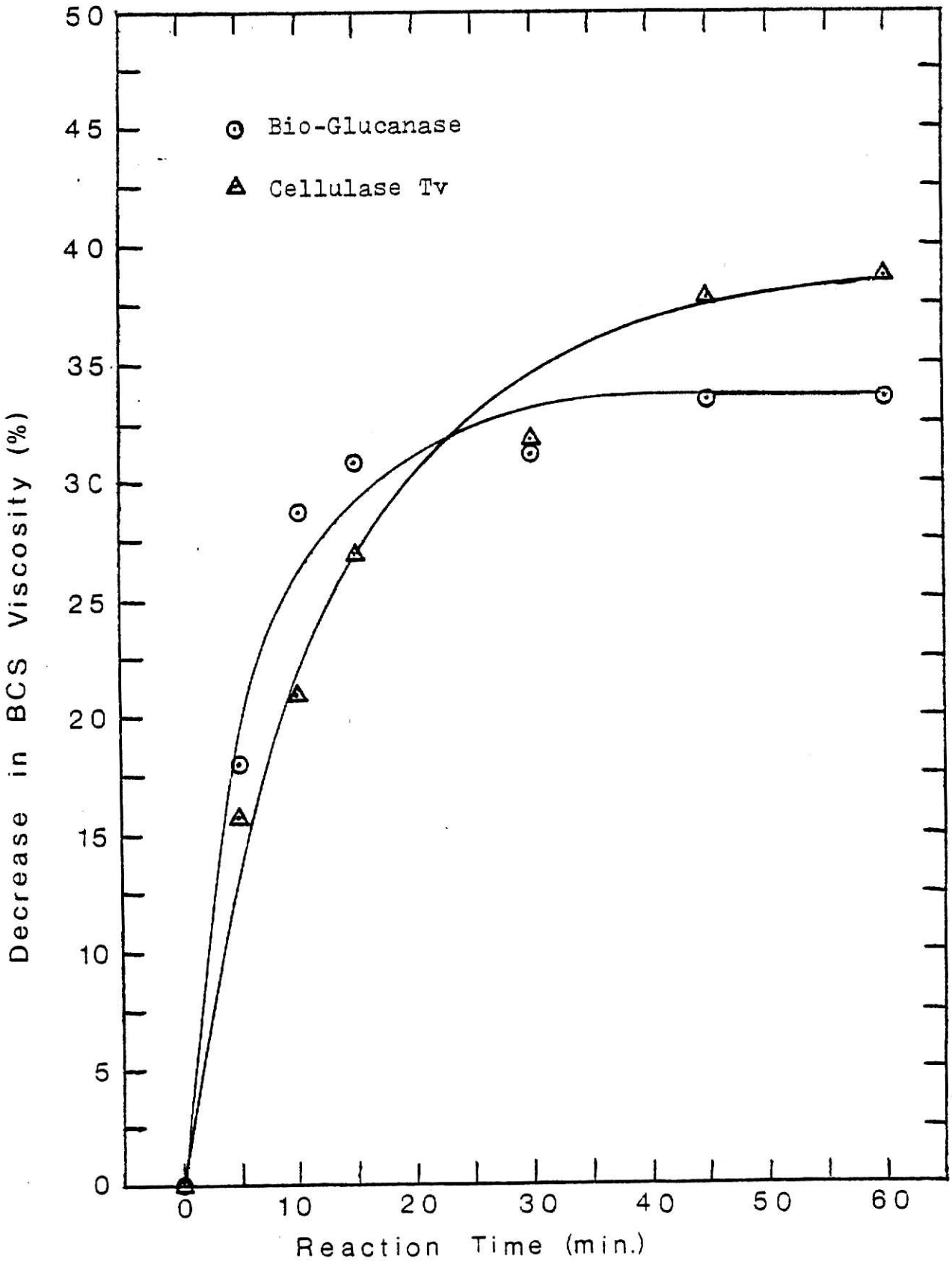

Fig. 13. Reduction of Viscosity in a BCS Composite Sample using Cellulase Tv and Bio-Glucanase.

SUMMARY

Three subsamples of BCS were collected daily from Anheuser-Busch's Merrimac Mass. plant as the BCS left the evaporators. The three subsamples were pooled to give a daily sample, and twelve daily samples were collected. The daily samples were assayed for proximate analysis (including total solids, protein, carbohydrate, fat and ash), types of solids (including total, suspended and soluble solids), pH, gross energy, glucose and oligosaccharides, mineral analysis, amino acid analysis, viscometric analysis, freezing point analysis, specific heat, density, water activity and thermal conductivity. Also included are the results of other studies: storage stability of BCS, oven drying (critical point temperature), enzymatic breakdown of BCS (two studies),(feed) pellet binding and total titratable acidity.

Results of the testing are as follows: total solids in the daily samples ranged from 27.6 to 58.3% with an average of 44.4 $\pm$ 7.4%; crude protein varied from 7.29 to 10.94% (d.b.) and averaged 8.85 $\pm$ 1.14% (dry basis): The range of values for the carbohydrate analysis were 67.30 to 87.48% (d.b.), with an average of 74.81 $\pm$ 5.88% (d.b.); crude fat ranged from 0.82 to 1.96% (d.b.), averaging 1.43 $\pm$ 0.30% (d.b.); ash content varied from 2.09 to 2.85% (d.b.), with an average of 2.54 $\pm$ 0.21%; suspended solids ranged from 4.80 to 10.48% of total solids, averaging 6.90 $\pm$ 1.91% (T.S.); soluble solids varied from 89.52 to 95.20% (T.S.), with an average of 93.10 $\pm$ 1.91% (T.S.); pH had a range of 3.75 to 4.53 and averaged 4.14 $\pm$ 0.22; gross energy varied from 3996.06 to 4155.26 cal/g (d.b.) with an average of 4073.29 $\pm$ 53.12 cal/g (d.b.); dextrose in the samples ranged from 8.2 to 10.3% (d.b.), with an average of 9.36 $\pm$ 0.79% (d.b.); maltose was found to vary from 32.1 to 43.9% (d.b.) and averaged 38.03 $\pm$ 4.19% (d.b.) in the samples; and maltotroise

ranged from 10.7 to 13.7% (d.b.), with an average of 11.65 ± 0.80% (d.b.).

The results of the mineral analysis are nitrogen: range = 1.08 to 1.75% (d.b.), average = 1.40 ± 0.21% (d.b.); phosphorous: range = 0.340 to 0.425% (d.b.), average = 0.382 ± 0.22% (d.b.); postassium: range = 964 to 1218 ppm (d.b.), average = 1097 ± 72 ppm (d.b.); calcium: range 1061 to 1794 ppm (d.b.), average = 1539 ± 206 ppm (d.b.); magnesium: range = 1198 to 1595 ppm (d.b.), average = 1456 ± 121 ppm (d.b.); copper: range = 0.98 to 6.90 ppm (d.b.), average = 3.07 ± 1.76 ppm (d.b.); manganese: range = 8.58 to 17.20 ppm (d.b.), average = 13.55 ± 2.50 ppm (d.b.); iron: range = 49.78 to 117.88 ppm (d.b.), average = 84.98 ± 22.42 ppm (d.b.); zinc: range = 8.58 to 16.81 ppm (d.b.), average = 12.56 ± 2.64 ppm (d.b.); and sodium: range = 1107 to 1855 ppm (d.b.), average = 1349 ± 248 (d.b.).

Values for the amino acid analysis are as follows (results are in g of amino acid/100g protein, corrected to a 100% protein recovery basis for all values); aspartic acid: range = 7.62 to 8.54, average = 8.10 ± 0.33; theronine: range = 3.11 to 3.56, average = 3.39 ± 0.14; serine: range = 4.13 to 4.61, average = 4.39 ± 0.17; glutamic acid: range = 18.43 to 22.93, average = 20.38 ± 1.21; proline: range = 11.12 to 14.12, average = 12.51 ± 1.01; glycine: range = 4.87 to 5.66, average = 5.16 ± 0.22; alanine: range = 6.42 to 7.75, average = 6.89 ± 0.43; (half) crystine: range = 0.81 to 1.42, average = 1.09 ± 0.16; valine: range = 4.01 to 4.92, average = 4.42 ± 0.23; methionine: range = 1.34 to 1.85, average = 1.47 ± 0.14; isoleucine: range = 2.49 to 3.15, average = 2.70 ± 0.27; tyrosine: range = 2.83 to 3.97, average = 3.50 ± 0.34; phenylaline: range = 4.10 to 4.63, average = 4.32 ± 0.16; histidine: range = 4.28 to 5.32, average = 4.78 ± 0.37; lysine: range = 2.98 to 3.97, average = 3.33 ± 0.28; arginine: range = 4.01 to 6.44, average = 4.87 ± 0.66;

and also included in the analysis was ammonia: range = 1.96 to 2.98, average = 2.27 $\pm$ 0.40.

Results of the viscosity work clearly showed that the viscosity of an unknown sample could not be determined from the sample's solids content and temperature. There was no definite relationship that held for all samples tested. Freezing points for BCS samples ranged from -3.1°C to -11.9°C (with an average of -8.9 $\pm$ 2.75°C). For one sample there was no freezing point found, even at -47.8°C. The relationship equating freezing point to BCS' total solids was found to be

$$Y = -0.00133X^{2.3316}$$

where Y is the freezing point of BCS (in °C) and X is the solid's content of BCS (in percentage) for the range of values of solids from 27.6 to 50.6%.

Specific heat of the BCS samples ranged from 2.72 to 3.60 J/Kg-K with an average of 3.09 $\pm$ 0.237 J/Kg-K. For individual daily samples of BCS, the relationship between specific heat and solids content was found to be

$$Y = -0.02899X + 4.3804$$

where Y = specific heat (in J/Kg-K) and X is solids content (in % dry matter). For the composite BCS sample, the relationship was found to be

$$Y = -0.02781X + 4.4879$$

Specific heat did not vary with temperature in the temperature range of 43 to 77°C.

The density of BCS was found to vary with solids content according to the following equation:

$$\rho = (1 + 0.4134\ X) \times 10^{-3}$$

where ρ is density in Kg/m^3 and X is solids content (in decimal form).

Thermal conductivity of BCS varied with solids content (for the temperature range of 32.2 to 54.4°C) as follows:

$$k = 0.2842\ X + 0.634$$

where k is thermal conductivity in W/m-K and X is solids content (in decimal form). A relationship could not be derived between thermal conductivity and temperature (between 32 and 55°C).

Water activity did not change, with an increase in temperature, indicating that BCS must have strong humectant properties. The results did show a relationship between water activity and solids content between 10 and 60°C as follows:

$$ERH = (3.981X - 6.003X^2 + 3.202X^3)\ X\ (100)$$

where ERH is equilibrium relative humidity (water activity) in % and X is moisture content in decimal form (1-dry matter).

The results from the BCS storage stability study showed that with as little as 0.25% (by weight) propionic acid added, BCS will be microbiologically stable for over three months. The oven drying tests provided data on the rate at which BCS will dry at different drying temperatures, as well as the time and % solids at which the BCS is visibly browned at a given temperature. At 54.4°C it took 168 hours for the change in quality to occur, (BCS is at 86.10% solids); at 65.5°C, it took 45 hours (83.41% solids); at 76.7°C, it took 8 hours (81.25% solids); and at 87.8°C, it took 4 hours (76.88% solids).

Results from the pelleting trial, showed that BCS compared favorably to both molasses and Masonex as a pellet binder. There was no significant (statistical) difference between the three treatments in the pellet durability tests or the production rate. However, there was a significant difference between BCS and Masonex in the % fines after pelleting (BCS having more fines). Also, pelleting with BCS

consumed more energy than did molasses.

The study of BCS's titratable acidity showed a range of 147 to 283 Meq/Kg of BCS solids, with an average of 208 $\pm$ 42.2 Meq/Kg. There seemed to be no relationship between titratable acidity of BCS and it's solids content.

Results from the enzyme screen show that the cellulase preparation was the most effective enzyme at decreasing the viscosity of BCS at 20% solids. With a 30 minute reaction time (at optimum pH and temperature) the results for total decrease in viscosity were, cellulase - 43.49% change; beta-(1,3), -(1,4)- glucanase - 27.48 and 23.98% (two preparations tried, Glucanase GV-L and Bio-Glucanase); hemicellulase - 14.77%, alpha-amylase - 7.86%; pectinase - 6.06% and glucoamylase - 0%. Using cost (of enzyme) per 1% change in BCS viscosity, cellulase was most effective with a cost of $\$0.203 \times 10^{-4}$ per 1% change, the hemicellulase was $\$1.104 \times 10^{-4}$, the alpha-amylase was $\$2.005 \times 10^{-4}$ and the pectinase was $\$18.000 \times 10^{-4}$.

In the second enzyme study, the rate of viscosity reduction was studied for Cellulase Tv and for Bio-Glucanase. Also, work was done with multiple enzyme systems. Both the cellulase and beta-glucanase gave an exponential reduction in viscosity of BCS. After a rapid 25% reduction in viscosity, the enzyme reaction abruptly stopped. Either the substrate was depleted or the enzyme denatured or inhibited. In the multiple enzyme system using combinations of cellulase Tv, Bio-glucanase and Hemicellulase 100,000, no advantages were observed using any combination of the three.

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APPENDIX

Table I. Composition of Distiller's Feeds [1]

Fermentation by-product	Cooking Temperature	Composition (dry-matter basis) Protein	Fat	Ash	Fiber
Wheat Distiller's Dried Solubles	68.3°C	46.5%	0.5%	8.8%	2.2%
	100.0	39.0	0.8	10.1	2.5
	137.8	37.0	0.9	10.0	2.4
	155.6	35.8	0.6	10.1	2.3
Corn Distiller's Dried Solubles	100.0	21.7	5.2	9.7	2.9
	137.8	28.5	6.6	9.5	3.8
	155.6	24.7	-	9.3	3.1
Rye Distiller's Dried Solubles	68.3	40.4	0.7	8.0	2.6
	137.8	36.4	0.7	8.5	2.8
Granular Wheat Flour Distiller's Dried Solubles	68.3	46.0	1.1	5.3	2.2
	137.8	42.8	1.3	4.9	2.9

1) From Bauernfeind, et al, 1944.

Table II. Typical Nutrient Composition of Corn Distiller's Feeds

	Distillers Dried Solubles	Distiller's Dried Grains with Solubles
Water, %	4.5	9.0
Protein, %	28.5	27.0
Fat, %	9.0	8.0
Fiber, %	4.0	8.5
Ash, %	7.0	4.5

Table III. Fermentation Products Produced from Distiller's Feeds as Feedstock

Product	Microorganism	
Riboflavin	*Ashbya gossyppi*	Wickerham et al., 1946
Riboflavin	*Eremothecium ashbyii*	Phelps, 1949
Vitamin B_{12}	*Streptomyces olivaceus*	Hall, 1951, Hall et al., 1958; Pfeiffer et al., 1954
Streptomycin	*Streptomyces grisens*	Woodruff and McDaniel, 1954
Chloramphenicol	*Strep. venezuelae*	Ehrlich et al., 1947
Actidione	*Strep. grisens*	Peterson and Petersen, 1954
Oleandomycin	*Strep. antibioticus*	Ratajak and Nubel, 1958
Xanthan gum	*Xanthomonas campestris*	Rogovin et al., 1961

Table IV. Proximate Analysis of BCS Daily Samples

Sample	Total Solids (% D.M.)	Protein (% d.b.)	Carbohydrate (% d.b.)	Fat (% d.b.)	Ash (% d.b.)
Corn[1]	90	10.14	81.1[4]	4.4	2.0
Molassis[2]	66.0	5.9	84.4[4]	0.0[4]	10.1
Masonex[3]	54.1	1.1	93.8[4]	0.2[4]	5.0
BCS - 7	50.6	8.63	79.72	1.00	2.61
8	43.5	9.34	71.12	1.62	2.55
9	51.0	8.82	69.20	1.57	2.46
12	41.1	8.89	72.21	1.59	2.48
13	45.0	10.94	78.81	1.9	2.76
14	43.6	10.11	74.49	1.28	2.85
15	41.6	8.52	72.00	1.51	2.53
16	39.1	10.22	77.77	1.55	2.37
17	58.3	7.45	87.48	0.821	2.09
19	45.0	7.29	78.91	1.24	2.70
20	46.4	7.74	68.65	1.50	2.71
21	27.6	8.19	67.30	1.53	2.38
Average	44.4	8.35	74.81	1.43	2.54
Std. Dev.	7.4	1.14	5.88	0.303	0.207
Range	58.3 to 27.6	10.94 to 7.29	87.48 to 67.30	1.96 to 0.821	2.85 to 2.09

1) U.S. #2 Yellow Dent Corn

2) Sugar Cane Molasses

3) Wood Molasses or Wood Hemicellulose Extract

4) Values are taken from the Atlas of Nutritional Data on U.S. and Canadian Feeds; NAS; Washington, D.C.; 1972; NFE was used as carbohydrate for the table.

Table V. Proximate Analysis for Solids in BCS Daily Samples

Sample	Solids (% D.M.)	Suspended Solids (% Total Solids)	Colloidal and Soluble Solids (% Total Solids)	pH	Gross Energy (Cal/g. d.b.)
Corn[1]	90	-	-	-	4392.61
Molasses[2]	66.0	-	-	-	3767.91
Masonex[3]	54.1	-	-	-	6750.16
BCS - 7	50.6	5.40	94.60	4.02	4067.32
8	43.5	6.77	93.60	4.20	4155.26
9	51.0	5.09	94.91	3.86	4049.61
12	41.1	5.22	94.78	3.75	4124.64
13	45.0	10.48	89.52	4.09	3996.06
14	43.6	7.19	92.81	4.13	4000.60
15	41.6	6.20	93.80	4.02	4032.73
16	39.1	8.05	91.95	4.35	4154.97
17	58.3	5.95	94.05	4.185	4081.52
19	45.0	4.80	95.20	4.11	4096.37
20	46.4	7.31	92.69	4.53	4043.20
21	27.6	10.32	89.68	4.42	4077.21
Average	44.4	6.90	93.10	4.14	4073.29
Std. Dev.	7.4	1.91	1.91	0.222	53.12
Range	58.3 to 27.6	10.48 to 4.80	95.20 to 89.52	4.53 to 3.75	4155.26 to 3996.06

(1) U.S. #2 Yellow Dent Corn

(2) Sugar Cane Molasses

(3) Wood Molasses or Wood Hemicellulose Extract

Table VI. Carbohydrate Profile for BCS Daily Samples

Sample Number	Total Carbohydrate (% d.b.)	Sugar Profile		
		Dextrose % d.b.	Maltose % d.b.	Maltotriose % d.b.
7	79.72	8.2	32.1	11.1
8	71.12	8.7	32.9	10.7
9	69.20	10.3	35.3	10.9
12	72.21	8.5	33.8	11.5
13	78.81	10.1	35.4	11.2
14	74.49	9.7	36.4	11.8
15	72.00	8.7	39.4	11.7
16	77.77	10.1	41.1	11.2
17	87.48	8.5	43.3	12.0
19	78.91	9.3	43.9	11.6
20	68.65	10.1	40.5	12.4
21	67.30	10.1	42.2	13.7
Ave	74.81	9.36	38.03	11.65
S. Dev.	5.88	0.791	4.913	0.804
Range	87.48 to 67.30	10.3 to 8.2	43.9 to 32.1	13.7 to 10.7

Table VII. Mineral Analysis of BCS Daily Samples - dry basis

Sample	N%	P%	K (ppm)	Ca (ppm)	Mg (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Na (ppm)
7	1.31	0.393	1139	1348	1521	6.90	11.90	88.03	9.90	1650
8	1.43	0.380	1079	1599	1554	1.15	16.11	60.99	11.51	1461
9	1.39	0.384	1120	1449	1578	0.98	14.70	67.63	11.76	969
12	1.46	0.377	1091	1494	1323	3.65	12.16	105.31	14.59	1477
13	1.73	0.400	1218	1686	1502	4.44	15.55	76.65	13.33	1467
14	1.61	0.425	1218	1776	1526	4.59	17.20	57.30	16.05	1154
15	1.42	0.379	1031	1609	1595	2.40	13.21	99.66	15.81	1312
16	1.75	0.388	1087	1385	1460	2.56	10.24	89.58	12.80	1312
17	1.19	0.340	964	1061	1198	1.72	8.58	49.78	8.58	1171
19	1.21	0.360	1092	1639	1324	4.44	13.33	94.42	15.55	1855
20	1.08	0.399	1061	1629	1396	2.15	15.07	111.97	10.77	1107
21	1.25	0.359	1059	1794	1496	1.81	14.51	117.88	9.07	1248
Ave	1.403	0.382	1097	1539	1456	3.07	13.55	84.98	12.56	1349
St. Dev.	0.212	0.022	71.9	206	121	1.76	2.50	22.42	2.64	248
Range	1.75	0.425	1218	1794	1595	6.90	17.20	117.38	16.81	1855
	1.08	0.340	964	1061	1198	0.98	8.58	49.78	8.58	1107
Corn[1]	-	0.32	3300	200	1200	3.7	6.1	30	-	100
Molasses[2]	-	0.11	40,200	10,500	4700	80.2	57.2	240	-	2000

(1) U.S. #2 Yellow Cent Corn

(2) Sugarcane Molasses, mn 48% Invert Sugar

NOTE: Values for corn and molasses were obtained from the "Atlas of Nutritional Data on U.S. and Canadian Feeds", National Academy of Sciences, Washington, D.C., 1971.

Table VIII. Amino Acid Profile of BCS Daily Samples as Compared to Corn[1]

Amino Acid	Grams of Amino Acid/100 Grams Protein[2] Sample 7	8	9	12	13	14	15	16	17	19	20	21	AVE.	Std. Dev.	Corn[3]
Aspartic Acid	8.47	7.67	8.20	7.93	8.29	8.49	7.97	8.54	8.38	7.68	7.98	7.62	8.10	0.33	7.09
Threonine[4][6]	3.42	3.47	3.56	3.46	3.43	3.45	3.43	3.50	3.16	3.11	3.27	3.37	3.39	0.14	3.47
Serine[6]	4.50	4.40	4.42	4.61	4.61	4.52	4.43	4.41	4.14	4.13	4.18	4.27	4.39	0.17	5.38
Glutamic Acid	20.45	20.06	18.43	20.15	22.93	21.11	21.37	21.27	22.26	21.73	20.42	19.78	20.83	1.21	19.97
Proline	14.12	13.80	11.48	11.80	12.36	11.95	11.64	11.19	12.93	13.56	13.48	11.84	12.51	1.01	9.53
Glycine[6]	5.05	5.21	4.99	5.26	5.66	5.44	5.02	5.10	4.94	5.21	5.12	4.87	5.16	0.22	4.22
Alanine	6.81	7.05	7.75	7.42	6.49	6.93	7.41	6.49	6.51	6.73	6.68	6.42	6.89	0.43	9.25
Half Cystine[6]	1.04	0.97	0.81	0.96	1.00	0.99	1.20	1.14	1.06	1.18	1.26	1.42	1.09	0.16	1.38
Valine[4][6]	4.38	4.12	4.92	4.36	4.01	4.45	4.41	4.53	4.52	4.61	4.46	4.31	4.42	0.23	3.13
Methionine[4][6]	1.35	1.43	1.85	1.54	1.34	1.41	1.43	1.51	1.46	1.35	1.44	1.57	1.47	0.14	1.87
Isoleucine[4][6]	2.60	2.64	3.15	2.16	2.49	2.64	2.66	2.83	2.77	2.61	2.62	2.74	2.70	0.17	1.94
Leucine[4][6]	5.91	6.06	6.61	6.08	5.86	6.04	5.97	6.44	6.00	5.71	5.70	5.81	6.02	0.27	12.01
Tyrosine[6]	3.61	3.87	3.97	3.90	3.56	3.48	3.22	3.42	2.83	3.26	3.19	3.72	3.50	0.34	3.56
Phenylalanine[4][6]	4.28	4.37	4.63	4.49	4.10	4.24	4.16	4.54	4.21	4.26	4.27	4.27	4.32	0.16	4.39
Histidine[4][6]	4.34	4.37	4.77	4.38	4.46	5.01	4.92	5.17	4.28	4.46	5.32	4.88	4.78	0.37	4.02
Lysine[4][5][6]	3.11	3.33	3.97	3.54	3.09	3.30	3.20	3.23	2.98	3.31	3.40	3.71	3.33	0.28	2.34
Ammonia	1.89	1.78	1.69	1.98	2.13	2.16	2.81	2.18	2.55	2.40	2.34	2.98	2.24	0.40	1.61
Arginine[4][6]	4.67	5.42	4.99	5.52	4.19	4.40	4.76	4.52	4.01	4.70	4.87	6.44	4.87	0.66	4.83
Protein(% "As is")	4.36	4.06	4.50	3.66	4.93	4.41	3.55	3.99	4.34	3.28	3.59	2.26	3.91	0.71	9.12

1) Dept. of Grain Science and Industry - KSU; Dr. Bates Lab

2) Corrected to 100% Recovery Protein Basis

3) #2 Yellow Dent, also analyzed in this lab

4) Amino Acids essential for the human infant and growing pig (20-35 kg.)

5) Limiting Amino Acid in Corn

6) Amino acids essential for the chicken and turkey

Table IX. Viscosity of BCS Daily Samples at Various Temperatures on an "As Recieved" Basis

Sample Number	Temp.$_1$ °C	Visc.$_1$ (abs. cps.)	Temp.$_2$ °C	Visc.$_2$ (cps.)(1)	Temp.$_3$ °C	Visc.$_3$ (cps.)	Temp.$_4$ °C	Visc.$_4$ (cps.)	Temp.$_5$ °C	Visc.$_5$ (cps.)	Temp.$_6$ °C	Visc.$_6$ (cps.)
7	1.0	1158	11.0	944	20.5	654	26.0	539	33.0	342	43.0	216
8	1.5	1138	11.0	835	20.5	593	26.0	462	33.0	284	43.0	201
9	2.0	1486	10.5	938	21.0	670	26.0	610	32.0	359	43.0	237
12	1.0	633	10.0	494	21.0	351	26.0	279	32.0	221	43.0	139
13	1.0	3197	10.0	1674	20.5	1058	26.0	835	33.0	761	43.0	423
14	1.5	2944	10.5	1484	21.0	1028	26.0	870	33.0	779	43.0	397
15	2.0	1179	10.0	735	21.0	474	26.0	446	32.0	380	43.0	256
16	2.0	1096	11.0	642	21.5	405	26.0	347	32.0	243	43.0	192
17	4.0	17,891	10.0	8986	21.0	4900	26.0	2946	32.5	1617	43.0	1002
19	1.0	705	10.0	446	21.0	236	26.0	202	32.0	186	42.5	115
20	1.0	3002	10.5	1494	21.0	941	26.0	885	32.5	670	42.5	357
21	1.0	129	11.0	79	20.5	52	26.0	48	32.5	38	43.0	26.5

(1) cps. - stands for absolute centipoise.

Table X. Viscosity of BCS Daily Samples at Various Temperatures at 20% Solids

Sample Number	Solids Content	T_1 °C	V_1 (abs. cps.)	T_2 °C	V_2 (abs. cps.)	T_3 °C	V_3 (abs. cps.)	T_4 °C	V_4 (abs. cps.)	T_5 °C	V_5 (abs. cps.)
7	20%[1]	8.5	19.7	19.7	17.4	26.5	16.8	37.5	13.0	42.5	12.4
8	20%	6.5	42.0	18.5	32.0	26.5	25.8	38.0	21.4	44.0	20.6
9	20%	10.0	30.0	20.5	22.5	26.5	17.6	34.0	15.0	43.5	13.5
12	20%	10.0	17.5	21.0	14.5	26.5	11.7	36.5	10.5	42.5	10.2
13	20%	9.5	21.0	20.5	15.5	26.5	12.0	36.5	10.7	43.5	10.6
14	20%	8.0	41.9	18.5	35.5	26.5	26.4	35.5	23.9	43.5	20.7
15	20%	11.0	20.6	21.0	16.0	26.5	11.6	34.5	10.6	44.5	10.2
16	20%	5.5	29.6	20.0	19.5	26.5	14.0	35.0	11.2	44.0	10.6
19	20%	5.5	19.1	21.5	13.5	26.5	10.6	35.5	10.0	46.5	9.5
20	20%	11.0	31.1	20.5	23.0	26.5	19.4	39.5	14.9	43.5	13.5
21	20%	10.0	79.9	20.5	53.5	26.5	42.4	36.5	36.8	44.5	33.0

1) All samples were at approximately the same solids content - 20%.

Table XI. Viscosity of BCS Daily Samples at Various Temperatures at Approximately 60% Solids

Sample Number	Solids Content	$Temp_1$ (°C)	$Visc_1$ (abs. cps.)	T_2 (°C)	V_2 (abs. cps.)	T_3 (°C)	V_3 (abs. cps.)	T_4 (°C)	V_4 (abs. cps.)	T_5 (°C)	V_5 (abs. cps.)
7	57.3%	9.0	6,880	21.5	1,830	27.5	1,518	34.5	1,737	42.5	860
8	57.0	8.0	55,040	22.0	13,800	27.5	11,480	35.0	5,960	42.0	4,800
9	56.2	11.0	4,660	21.0	2,084	27.5	1,274	36.0	1,292	43.0	490
12	58.5	6.0	43,040	20.5	12,400	27.0	6,670	35.5	5,580	42.5	2,840
13	56.7	9.0	59,840	18.5	23,480	27.0	11,680	36.5	7,180	43.0	4,620
14	58.8	8.0	68,400	19.5	27,200	27.5	13,200	36.0	11,780	41.0	5,820
15	57.4	10.0	22,720	20.0	11,340	27.0	6,170	36.5	4,950	44.5	3,028
16	56.0	9.0	26,160	18.0	14,000	27.5	8,780	35.0	4,960	36.0	4,600
19	55.5	5.5	3,745	21.0	1,213	27.5	736	36.0	466	42.0	340
20	55.3	10.0	6,790	19.0	4,210	27.5	1,820	36.0	1,036	43.5	800
21	57.0	13.5	117,600	22.0	56,800	27.5	30,400	38.0	21,750	44.5	12,260

Table XII. Viscosity of BCS Composite Sample vs. Tempature and Solids Content

Temperature and Viscosity

Solids Content (% D.M.)	T_1 °C	V_1 (abs. cps.)	T_2 °C	V_2 (abs. cps.)	T_3 °C	V_3 (abs. cps.)	T_4 °C	V_4 (abs. cps.)	T_5 °C	V_5 (abs. cps.)	T_6 °C	V_6 (abs. cps.)
20.0	4.5	36.3	15.5	25.0	24.0	19.7	32.0	15.5	43.5	14.5	--	--
46.4	2.0	2600	11.0	1735	20.0	854	26.0	712	35.0	594	43.0	356
59.7	4.0	39,600	12.0	14,400	21.0	8,720	26.0	6,380	35.0	3,060	43.0	2,110
73.2	--	--	--	--	--	--	26.0	1,708,000	34.0	721,000	42.0	325,600

Table XIII. Freezing Points of the Daily Samples of BCS

Sample #	Solids % D.M.	Average Freezing Point °C	°F
#7	50.6%	-11.0°C	(12.2 °F)
#8	43.5	- 7.3	(18.9)
#9	51.0	-11.9	(10.6)
#12	41.1	- 7.7	(18.1)
#13	45.0	- 9.8	(14.4)
#14	43.6	-10.3	(13.5)
#15	41.6	- 7.5	(18.5)
#16	39.1	- 6.1	(21.0)
#17	58.3	None [a]	
#19	45.0	-11.0	(12.2)
#20	46.4	-11.7	(10.9)
#21	27.6	- 3.1	(26.4)

[a] No inflexion point was observed until the specimen temperature reached -47.8°C (-54°F)

Table XIV. Specific Heats of the BCS Daily Samples

Sample #	Solids (% D.M.)	Specific Heat (J/Kg-K)	Temperature range (°C)
#7	50.6	2.93	58 to 60
#8	43.5	3.31	58 to 60
#9	51.0	2.97	56 to 61
#12	41.1	3.18	56 to 60
#13	45.0	2.85	56 to 59
#14	43.6	3.10	58 to 61
#15	41.6	3.22	59
#16	39.1	3.26	58 to 60
#17	58.3	2.72	58 to 59
#19	45.0	3.01	58 to 60
#20	46.4	2.97	57 to 61
#21	27.6	3.60	56 to 60
Range	58.3 / 27.6	3.60 / 2.72	-- --
Average	44.4	3.093	--
Std. Dev	7.4	0.237	--

Table XV. Specific Heat* of a Composite** Sample of BCS vs. Solids Concentration and Temperature

Solids Content %	43.3°C	60°C	76.7°C	Average
30	3.70	3.64	3.61	3.65
46	3.18	3.25	3.18	3.20
60	2.92	2.84	2.85	2.85
73	2.56	2.50	2.44	2.50
Average	3.08	3.06	3.03	

* Units for specific heat are in J/Kg-K.

** This sample is a composite of the 12 daily samples.

Note: Values are the average of 3 replications.

Table XVI. Density of a BCS Composite Sample vs. Solids Concentration

Solids Content*	Average Density (Kg/m^3)
30.00%	1.124×10^3
46.39%	1.196×10^3
60.00%	1.242×10^3

$\rho = (1 + 0.4134X) \times 10^3$ **

*A composite sample was used in this experiment, "as is" solids were 46.39%

**Regression equation showing relationship between density (ρ) and solids content (X)(in decimal form) of BCS.

Note: Temperature during tests was $25^{\circ}C$ ($77^{\circ}F$).

Table XVII. Thermal Conductivity (W/m-K) of Dry Solids from a BCS Composite Sample* vs. Temperature

Replication	Temp. °C 32.2	43.3	54.4
1	0.367	0.324	0.343
2	0.384	0.322	0.348
3	0.393	0.315	0.346
Ave.	0.381	0.320	0.346

*Condition of BCS is 100% dry matter obtained by freeze-drying.

Table XVIII. Literature Values of Thermal Conductivity (W/m-K) of Water vs. Temperature*

Temp (°C)	32.2	43.3	54.4
Thermal Cond. (W/m-K)	0.621	0.635	0.647

*Values were taken from the CRC Handbook of Chemistry and Physics; 53rd Ed.; 1972-3 E-11.

Table XIX. Thermal Conductivity* (W/m-K) of a BCS Composite Sample vs. Temperature and Solids Concentration

	Solids Concentration			
°C	30%	50%	60%	75%
32.2	0.549	0.502	0.478	0.441
43.3	0.540	0.478	0.447	0.400
54.4	0.557	0.497	0.467	0.422

$k = -0.2342X + 0.6343$**

*Computed values.

**Regression equation showing relationship between thermal conductivity (k) and solids content (X) (in decimal form) of BCS for temperature range of 32.2 to 54.4°C.

Table XX. Total Titratable Acidity of BCS Daily Samples

Sample #	[1]Meq/20g sample	[1]Meq/Kg Solid
7	2.26	222
8	1.75	201
9	2.76	266
12	2.33	283
13	2.18	232
14	1.94	221
15	1.87	220
16	1.43	181
17	1.98	169
19	1.85	205
20	1.37	147
21	0.83	150
Ave.	1.88	208
Std. Dev.	0.506	42.2
Range	2.76 / 0.83	283 / 147

1) Meq stands for milli-equivalents of base (NaOH) needed to neutralize the BCS.

Table XXI. Enzymes for BCS Thinning, Their Sources and Cost

Common Name	Brand Name	Source Micro-organism	Manufacturer	Cost	
				Order Size	Price
α-amylase	Tenase[5]	*Bacillus subtilis*	Miles	2,500 lbs and over[1]	$0.65/lb.
Glucoamylase	Diazyme-100[5]	*Asp. niger*	Miles	50 gal (40 lb)	$77.00[2] per lb.[3]
				200 L. Drum	$0.21/std. unit[3]
				Tank Truck	$0.205/std. unit[3]
Cellulase	Cellulase Tv concentrate	*Trichoderma viride*	Miles	5 lb Drum	$25.00/lb.
				50 lb Drum (110.23 lb)	$22.50/lb.
				50 kg Drum	$20.00/lb.
β-Glucanase	Glucanase GB-L[5]		Grindsted		$9.00/lb.[4]
β-Glucanase	Bio-Glucanase[5]		Biocon (U.S.)		$2.48/lb.
Hemicellulase	Hemicellulase-100,000	*Asp. niger*	Miles	25 lb Drum	$38.00/lb.
				50 lb Drum (110.23 lb)	$37.50/lb.
				50kg Drum	$37.00/lb.
Pectinase	Spark-L[5] HPG	*Asp. niger*	Miles	1-4 Hedpaks[6]	$5.00/lb.
				5-10 Hedpaks	$4.75/lb.
				11 and over Hedpaks	$4.50/lb.

1) Delivered in 500 lb. drums
2) Prices for Miles products include shipping costs
3) Standard unit = 8,000 D.U. (Diazyme-100 has 100 DU/ml. preparation)
4) Prices effective February, 1980
5) Enzyme is in liquid form
6) 1 Hedpak = 40 lbs.

Table XXII. Properties of Enzymes Used for BCS Thinning

Enzyme	Physical Form	Activity	pH Optimum	Temperature Optimum
Tenase (α-amylase)	Liquid	340,000 MWU/g[1]	6.0 to 7.0 (6.75)	65 to 75°C (70)
Hemicellulase 100,000	Powder	100,000 HCU/g[2]	3.5 to 4.5 (4.0)	50 to 60°C (60)
Cellulase Tv	Powder	27,125 (CMC)u/g[3]	4.0 to 5.0 (4.5)	40 to 50°C (50)
Bioglucanase	Liquid	200 u/g	4.5 to 6.5 (5.5)	up to 80°C (70) (70)
Glucanase GV-L	Liquid	2000 Gu/kg[4]	4.0 to 5.5 (4.5)	up to 65°C (65)
Diazyme L-100 (glucoamylase)	Liquid	100 DU/mL	3.5 to 5.0 (4.5)	up to 60°C (60)
Spark-L HPG	Liquid	10,000 AJDU/mL[5]	3.5 to 4.5 (4.0)	50°C

1) MWU stands for Modified Wohlgemuth Unit, information is available upon request from Miles Laboratories, Inc.

2) HCU stands for Hemicellulase units.

3) (CMC)U stands for enzyme activity (in units) arrived at by the carboxymethyl cellulose test. 27,125 (CMC) U/g is equal to 140 u/g by the filter paper method.

4) GU stands for Glucanase Units, information may be available from Grindsted Products.

5) AJDU stands for Apple Juice Depectinization Units. A minimum of 80 PGU (Polygalacturonose Units)/mL is guaranteed.

Note: pH value and temperature value underlined are the values that were used in the study.

Table XXIII. Feed Formulation for Pelleting Trial

Ingredients	Individual	Cumulative
Bulk (pounds)		
Corn (ground)	372	372
Grain Sorghum	372	744
Soybean meal (44%)	200	944
Molasses, Masonex, or BCS	30	974
Premix A (pounds)		
Dical phos.	20	994
Salt	5	999
Trace mineral (Z-10)	0.5	999.5

Table XXIV. Solid Contents and Viscosities of the Three Binders Used in the Pelleting Trial

Binder	Viscosity	Solids (as is %)
Molasses	1063 cps (26°C)	66.03 ± .06%
Masonex	6517 cps (26°C)	54.13 ± .14%
BCS	3667 cps (26°C)	51.34 ± .22%

Table XXV. Individual Results of the Pellet Binding Tests

Treatment	Fines (%)	PDI (%)[1]	Prod. Rate ($\frac{\#}{Hr}$)[2]	Energy ($\frac{Kw-Hr}{Ton}$)
Molasses 1	3.2	95.5	3337	10.03
Molasses 2	3.1	96.0	2968	10.76
Molasses 3	2.9	95.5	3034	10.78
Masonex 1	2.9	95.6	3202	10.38
Masonex 2	2.8	96.0	2901	10.92
Masonex 3	2.7	95.4	3038	10.64
BCS 1	3.3	95.7	3104	10.60
BCS 2	3.0	95.1	2958	11.09
BCS 3	3.3	95.3	2946	11.15

[1]PDI stands for Pellet Durability Index

[2]Prod. Rate stands for Production Rate

LITERATURE REFERENCES

1) Bauernfeind, J.C., Smith, M.B., Carey, J.C., Baumgarten, W, Gustoff, F.H. and Leonard S.,(1944); "Nutrient Content of Alcohol Fermentation By-Products from Various Grains"; Vol. 21; 421-29.

2) Beckord, L.D., Peltier, G.L. and Keen, E. (1946); "Bacterial Amylases - Production in Thin Stillage"; Ind. Eng. Chem.; 38; 232.

3) Beeson, W.M. and Hatch, C.F. (1971); "Condensed Distiller's Solubles in Liquid Supplements for Beef Cattle"; Distiller's Feed Research Council (DFRC) Conference Proceedings; Vol. 26; March 31; 47-52.

4) Berger, L. (1981); "Alcohol By-Product Utilization", Kansas Formula Feed Conference Proc.; 36th; KSU; Jan. 19-20; A-1.

5) Berghem, L.E.R., Petterson, L.B. and Axio-Fredricksson, U.B.(1976); "The Mechanism of Enzymic Cellulose Degredation"; Europ. J. Biochem.; 61; 621.

6) Carpenter, L.E. (1970); "Nutrient Composition of Distiller's Feeds"; DFRC* Conference Proc.; Vol. 25; March 31; 54-61.

7) Couch, J.R. (1971); "Current Status of Unidentified Growth Factors in Distiller's Feeds"; DFRC* Conf. Proc.; Vol. 26; March 31; 67-70.

8) Couch, J.R. and Abbot, W.W. (1972); "Corn Distiller's Dried Grains with Solubles for the Formulation of Feeds for Broiler Breeder Replacement Pullets"; DFRC* Conf. Proc.; Vol. 27; March 29; 50-5.

9) Dubois, Gilles, Hamilton, Rebers and Smith (1956); "Colorimetric Method for Determination of Sugars and Related Substances"; Anal. Chem.; 28(3); 350-6.

10) Ehrlich, J., Bartz, Q.R., Smith, R.M. and Joslyn, D.A. (1947); "Chloromycetin, a New Antibiotic from a Soil Actinomycete"; Science; 106; 417.

11) Fennema, O.R.; "Hemicelluloses and Pentosans"; Principles of Food Science - Food Chemistry; Marcel Dekker Inc; N.Y., N.Y; 1976; pg. 117; Lib. of Congr. Cat.#75-29694.

12) Flemming, M. and Kawakami, K. (1977); "Studies of the Fine Structure of Beta-Glucans of Barley Extracted at Different Temperatures"; Carbohydrate Res.; 57; 15.

13) Folch, Lees and Stanley (1957); "A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues"; J. Biol. Chem.; 226; 497-509.

14) Forrest, I.S. and Wainright, T. (1977); "The Mode of Binding of Beta-Glucans and Pentosans in Barley Endosperm Cell Walls"; J. Inst. Brew.; 83(5); 279-86.

15) Ghose and Pathak (1973); "Cellulases - 1: Sources, Technology"; Process Biochem.; 8(4); 35-8.

16) Gohl, B., Larsson, K., Nilsson, M., Theander, O. and Thomke, S. (1977); "Distribution of Carbohydrate in Early Harvested Barley Grain"; Cereal Chem.; 54(3); 690.

17) Gohl, B., Nilssom, M. and Thomke, S. (1978); Distribution of Soluble Carbohydrate in Barley Grain at the Late Stage of Maturity and Relation to Viscosity"; Cereal Chem.; 55(3); 341.

18) Hall, H.H. (1951); "The Fermentative Production of Vitamin B_{12} in Grain Stillage and Other Materials"; DFRC* Conf. Proc.; Vol.6;37.

19) Hall, H.H. (1955); "The Use of Distiller's Dried Solubles and Some Other Supplements for the Production of Biologicals"; DFRC* Conf. Proc.; Vol. 10; 15.

20) Hall, H.H., Benedict, R.G., Weisen, C.S., Smith, C.E. and Jackson, R.W. (1958); "Studies on Vitamin B_{12} Production with Streptomyces olivaceus"; J. Appl. Microbiol.; 1; 124.

21) Halliwell, G. and Griffin, M. (1974); "Endo cellulase Activity in the Cellulase Complex of T. koningii"; Biochem. Soc. Trans.; 2; 497.

22) Hang, Y.D., Splittstoesser, D.R., Woodams, E.E. and Sherman, R.M. (1977); "Citric Acid Fermentation of Brewery Waste"; J. Food Sci.; 42; 383.

23) Harms, R.H. (1970); "The Use of Distiller's Dried Grains with Solubles in Feeds for Egg Production"; DFRC* Conf. Proc.; Vol. 25; 34-40.

24) Harms, R.H., Manley, J.G. and Voitle, R.A. (1977); "The Influence of Distiller's Dried Grains with Solubles in Turkey Breeder Diets and The Effect of Dietary Calcium and Phosphorous Levels on This Response"; DFRC* Conf. Proc.; Vol. 32; 44-7.

25) Huber, J.T. (1972); "Reasearch on Liquid Nitrogen Supplements for Dairy Cattle"; J. An. Sci.; 34; 166.

26) Huber, J.T., Palan, C.E. and Hillman, D. (1968); "Urea in High Corn Silage Rations for Dairy Cattle"; J. An. Sci.; 27; 220.

27) Il'ina, L.D. and Evseichik, B.I. (1976); "Expediency of Growing Food Yeasts on Spent Mash from Alcoholic Fermentations"; Nauk. Pre.-Udr. Sil's'kogospod. Akad, (USSR); 191; 126: Chem. Abstr.; 86; 153917e (1977).

28) Isik, H. (1977); "The Production of Food Yeast from Vinasse"; Seker (Turkey); 27; 103: Chem. Abstr.; 87; 51602b (1977).

29) Jensen, A.H. (1977); "Dried Distiller's Grains with Solubles as a Supplemental Protein Source in Diets for Gestating Gilts"; DFRC* Conf. Proc.; Vol. 32; 10 - 13.

30) Jensen, L.S. (1973); The Evaluation of Distiller's Dried Grains with Solubles in Layer Rations"; DFRC* Conf. Proc.; Vol. 28; 43-9.

31) Karova, E., Rashkova, Z., Marinov, M. and K'oseva, N. (1976); Possibilities of Using Vinasse in the Production of Feed Yeast and L-Sorbose"; Priroda(Sofia)(Bulgarian); 25(5); 58: Chem. Abstr.; 86; 169299r; 1977.

32) Karova, E., Rashkova, Z., Marinov, M. and K'oseva, N. (1976); "Study of Possibilities for Using Distillery Grains in Some Microbiological Industries"; Lozar Vinar.(Bulgarian); 25(5); 36: Chem. Abstr.; 86; 87652m; 1977.

33) LeMense, E.H., Sohns, V.E., Corman, J., Blom, R.H., Van Lanen, J.M. and Langlykke, A.R. (1949); "Grain Alcohol Fermentations-Submerged Mold Amylase as a Saccharifying Agent"; Ind. Eng. Chem.; 41; 100.

34) Little, C.O., Potter, G.D. and Amos, H.E. (1970); "Distiller's Feeds-Stimulants of Rumen Digestion"; DFRC* Conf. Proc.; Vol. 26; 41-7.

35) Magney, J., Montant, C., Raynaud, P., Gontier, C. and Dardenne, J. (1977); "Treating the Distillation Residues From White Wine Production"; Ger. Offen.(Patent); 2,630,680: Chem. Abstr.; 86; 87690x; 1977.

36) Peterson, W.H. and Petersen, M.S. (1954); "The Broad Spectrum, Polypeptide and Other Antibiotics"; Industrial Fermentations; Vol.II; Chemical Publishing Co., Inc,; N.Y.; Underkofler and Hickey, Ed.

37) Petterson, L.B., Axio-Fredricksson, U.B. and Berghem, L.E.R (1972); "The Mechanism of Enzymic Cellulose Deredation"; Proc. IVth Int. Ferment. Symp.; Osaka; Terui, G. (Ed.); 727-9.

38) Pfeiffer, V.F., Vojnovich, C. and Heger, E.N. (1954); "Vitamin B_{12} Fermentation with Strep. olivaceus"; Ind. Eng. Chem.; 46; 843.

39) Phelps, A.S. (1949); "Production of Riboflavin"; U.S. Patent; #2,473,818.

40) Pomeranz, Y (1973); "Industrial Uses of Barley"; Industrial Uses of Cereals; Symposium Proceedings; AACC - 58th Annual Meeting; St. Louis, Mo.; 371-92.

41) Ratajak, E.J. and Nubel, R.C. (1958); "Oleandomycin"; U.S. Patent; #2,842,481.

42) Reed, G. (Editor)(1975); "Chpt. 16-Beer","Chpt. 6-Carbohydrases"; Enzymes in Food Processing; 2nd Ed.; Academic Press; N.Y., N.Y.; 462, 65 and 74.

43) Rombouts and Pilnik (1972); "Research on Pectin Depolymerases in the 1960's - A Literature Review"; Critical Reviews in Food Tech.; CRC; 3(1); 1-26.

44) Rogovin, S.P., Anderson, R.F. and Cadmus, M. (1961); Production of Polysaccharide with Xanthomonas campestris"; J. Biochem. Microbiol. Technol. Eng.; 3;51.

45) Runnels, T.D. (1968); "Effective Levels of Distiller's Feeds in Poultry Rations"; DFRC* Conf. Pro.; Vol. 23; 15-22.

46) Smiley, K.L. and Stone, L. (1955); "Production of Riboflavin by Ashbya gossypii"; U.S. Patent; #2,702,265.

47) Tank, S.M (1976); "Preliminary Study on Vinasse as a Substrate for Yeast Growth"; Rev. Microbiol.(Brazil); 7(4); 92: Chem. Abstr.; 87; 37345p; 1977.

48) Troitskii, A.S., Razyvaev, N.I. and Zankal, T.V. (1976); "Production of Protein Biomass from Viniculture Discharge"; Sadovod. Vinograd. Vinodel. Mold. (Ussr); 31 (12): 28: Chem. Abstr.; 86; 153927h (1977).

49) Van Horn, H.H., Jacobson, D.R. and Graden, A.P. (1969); "Influence of Levels and Source of Nitrogen on Milk Production and Blood Components"; J. Dairy Sci; 52; 1395.

50) Van Lanen, J.M. and Smith, M.B. (1968); "Starch Hydrolyzing Enzyme for Use in Grain Alcohol Fermentation"; U.S. Patent; #3,418,211.

51) Warner, R.G. (1970); "The Place of Distiller's Feeds in Dairy Cattle Rations - A Review"; DFRC* Conf. Proc.; Vol. 23; 23-6.

52) Warner, R.G. and Looli, J.K. (1968); "The Effect of Corn Distiller's Dried Grains with Solubles and Other Ingredients on Milk-Fat Percentage"; DFRC* Conference Proc.; Vol. 23; 23-6.

53) Wickerham, L.J., Flickenger, L.H. and Johnson, R.M. (1946); "The Production of Riboflavin by Ashbya gossypii"; Arch. Biochem.; 9;95.

54) Wood, T.M., (1975); "Properties and Mode of Action of Cellulases"; Biotech. Bioeng. Symp. #5; Cellulose Conf.; Univ. of Cal. at Berkely; 111-37.

55) Woodruff, H.B. and McDaniel, L.E. (1954); "Streptomycin"; Industrial Fermentation, Vol.II; 264; Chemical Pub. Co., Inc.; N.Y.

56) Woolner, A.Jr. and Lassloffy, A. (1909); Process for Making Alcohol"; U.S. Patent; #923,232.

57) Wornick, R.C. (1969); Liquid Supplements for Livestock Feeding; Chas. Pfizer and Co.

*DFRC stands for Distiller's Feed Research Council.

FUTURE WORK

A number of areas for future investigation have presented themselves during the course of this thesis work. They include:

1) Determination of viscosity of BCS upon concentration to 50% solids after enzyme treatment. This is to determine the full effect of such an enzyme treatment upon the dehydration of BCS.
2) Enzyme thinning - the effect that a second shot of enzyme has upon the degredation of BCS. The purpose of this is to determine whether the enzyme reaction ends because of loss of substrate, or because of enzyme denaturation.
3) An analysis for the essential amino acid tryptophan.
4) A study to identify the concentration of longer-chain oligosaccharides in BCS. There is around 10 - 15% carbohydrate in BCS that has not been identified. This analysis will provide some information on that carbohydrate.
5) A crude fiber analysis.
6) A further mineral analysis, including toxic minerals and metals.
7) An analysis of BCS B-vitamin content.

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COMPOSITION AND PHYSICAL PROPERTIES OF BREWER'S CONDENSED SOLUBLES. PELLET BINDING USE IN FORMULA FEEDS.

by

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B. S., Kansas State University, 1978

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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ABSTRACT

Brewer's Condensed Solubles (BCS), a by-product of the brewing industry, has been examined for a number of chemical and physical properties. Daily samples of BCS were obtained from Anheuser-Busch's Merrimac, Mass. plant. Three subsamples were taken at various times during the day and composited into a single daily sample of around 3 L. Twelve daily samples were collected (36 total subsamples). These were frozen daily, and when all were collected, were sent by air to Manhattan.

The average composition of BCS was found to be: total solids, 44.4%; protein, 8.85%(db); carbohydrate, 74.8%(db); fat, 1.43%(db); and ash, 2.5%(db). Of the total solids it was found that suspended solids accounted for 6.9%, while soluble solids constituted 93.1% of the total solids. Major carbohydrate fractions included dectrose at 9.4%(db), maltose at 38.0%(db) and maltotriose at 11.7%(db).

Mineral and amino acid analyses were done. The mineral analysis showed that BCS was a good source of several minerals, including sodium, phosphorcus, calcium and iron. The amino acid profile demonstrated that BCS contains a high quality protein.

The density of BCS was found to vary with solids content and averaged 1.2×10^3 Kg/m^3. A linear function was found to indentify this relationship. The average freezing point of BCS was -9.0°C and varied from -3 to - 12°C. Freezing point was also found to vary with solids content according to a power function.

Thermal conductivity of BCS averaged 0.349 W/m-K and it's relationship with solids content could be defined by a power function. BCS was also analyzed for specific heat and was found to average 3.09 J/Kg-K

with little variability. A linear function was found to identify this relationship.

BCS was found to have an average water activity of 88% ERH at room temperature. The water activity had a strong relationship (a polynomial power function) with solids content, but no relationship was found with temperature between 10 and 60°C. A storage stability study was also performed using propionic acid as a microbial preservation. It was found that with as little as 0.25% propionic acid (by weight) BCS would be stable for aver 3 months.

Viscosity varied considerably in BCS, following no relationship with solids content. Within each sample there was a strong relationship with temperature. Several types of enzymes were tested to see which could be used to reduce the viscosity of BCS at 20% solids. A commercial cellulase was found to work best, followed by two commercial samples of beta-(1,3),(1,4)-glucanase and by a hemicellulase.

BCS was tested against molasses and Masonex in a study of pellet binding ability. It was found that there was no statistical difference (at the 95% Confidence Level) between the three binders in pellet durability. However, the BCS did use more energy (statisically) that molasses and had a larger % fines after pelleting (statistically) than the Masonex.