# Fuel Alcohol Production from Hull-less Barley

W. M. INGLEDEW,[1] A. M. JONES,[2] R. S. BHATTY,[3] and B. G. ROSSNAGEL[3]

ABSTRACT

Hull-less barleys were easily milled, mashed, and fermented to ethanol. Problems experienced with viscosity were quickly eliminated by addition of β-glucanase, but even without enzyme, viscosity decreased over the duration of fermentation. Barley mashes prepared by adding 0.33 kg grain to 1 L of water fermented slightly faster than did corresponding wheat mashes, and over 10% v/v alcohol was obtained (94% of theoretical). Distillers' hull-less barley grains collected at end-fermentation had protein contents similar to dried grains from wheat fermentations and were higher in protein than dried grains obtained from hulled barley. They were correspondingly lower in nondigestible fiber.

Cereal Chem. 72(2):147-150

Increased interest in hull-less barley production for food and feed use has resulted in the development of new cultivars such as Scout and Tupper in the early 1980s (Rossnagel et al 1981, 1983), Condor in 1988 (Helm et al 1992) and CDC Buck and CDC Richard in 1990 (Agriculture Canada registered 3302 and 3303, respectively). The primary feature desired for these new barley types was a digestible energy comparable to that of wheat and maize—and an increase over that found in hulled barley (Bhatty et al 1975, 1979). Barley is agronomically suited to production in much of Western Canada, where the growing season is short and often stressful (Rossnagel et al 1988). Hull-less barley yields are comparable to yields of hulled barley varieties when the weight of lost hulls is added to the yield of hull-less barley kernels (Rossnagel et al 1985, 1988). However, less secondary processing is required to prepare the lower fiber, hull-less varieties for food or industrial usage.

On the Canadian prairies, a number of wheat varieties are used as raw material for fuel alcohol production. Efficient production of ethanol requires a feedstock with a high level of degradable starch (Ingledew 1993). As hull-less barley varieties often have high digestible energy due to elevated starch and reduced fiber contents, they would appear ideally suited for the alcohol industry. Hulled barley has been used in the past as a starch source in fuel alcohol manufacture, and in beer brewing as an adjunct to lower the cost associated with the use of malted barley. However, viscosity problems and the comparatively low starch content of hulled barley have limited its use by the alcohol industry. The availability of hull-less barleys for fuel alcohol production has several potential advantages over wheat and hulled barley. Hull-less barleys mature up to two weeks earlier than hard red spring wheat, and yields are as much as 25% higher under Saskatchewan conditions. In addition, its pricing is projected to be lower than wheat (although higher than hulled barley), and it has the potential of producing a distillers grain with a higher protein (and lysine) content for livestock feed. This study was therefore undertaken to assess the fermentability of hull-less barley cultivars compared to commercial hulled barley (Harrington) and hard red spring wheat. Hull-less barleys have not been evaluated for fuel alcohol production.

## MATERIALS AND METHODS

### Materials

Samples of hulled barley (cv. Harrington), hull-less barley (CDC Richard, SB 89528, SB 90354) and wheat (CDC Makwa) were grown in 1992 (except Harrington, grown in 1990) at the Kernen Crop Research Farm, University of Saskatchewan. Harrington is a registered two-rowed malting barley cultivar; CDC Richard, a two-rowed registered hull-less cultivar; and CDC Makwa, a registered cultivar of hard red spring wheat. SB 89528 and SB 90354 are hull-less, experimental barley genotypes; SB 89528 contains waxy (low amylose) starch with high viscosity and β-glucan, while SB 90354 is high viscosity, high β-glucan with normal starch. CDC Richard has normal starch and relatively low β-glucan and viscosity.

### Wheat and Barley Mash Preparation

Mashes were prepared in small scale but in standard fuel alcohol industry concentrations of 20 US gal/bu concentrations, essentially as described by Thomas and Ingledew (1990). Distilled water (3 L) was combined with 37 ml of 100 m*M* $CaCl_2$, heated to 60° C with constant stirring, whereupon 1 kg of the appropriately ground grain (setting 5 on a S.500 Disk Mill, Glen Mills Inc., Clifton, NJ) was added. Sieve analysis showed that 83% of the ground barley had a particle size between 20 and 60 mesh, while 17% of the particles were finer than 60 mesh (K. C. Thomas, personal communication). A 4.7-ml aliquot of high temperature (HT) α-amylase (Alltech Biotechnology Co. Ltd., Nicholasville, KY) with a typical specific activity of 1.14 g starch/min/mg protein (10 mg/ml) was added and, after 5 min, the temperature was increased to 90–95°C, during which time the containers were covered with foil to prevent evaporation. Gelatinization proceeded for 45 min at 90–95°C. The temperature was then decreased to 80°C, and a further 4.7 ml of α-amylase were added. Liquefaction proceeded for 30 min at 80°C. This procedure mimics industrial batch mash production and yielded mashes of ~20° Plato (~21.6 g dissolved solids per 100 ml).

### Fermentation

Dextrinized wheat and barley mashes (with undissolved solids) were transferred in 500-g quantities to sterile jacketed 500-ml Celstir bioreactors (Wheaton Scientific, Millville, NJ). The mashes were treated with 0.02% (v/w) diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, MO), a chemical sterilant, and stored at 4°C for 64 hr. The bioreactors were then connected to a 30°C circulating water bath, and the contents mixed with a magnetic stirrer for the duration of the fermentation. Either 10 ml of sterile distilled water or 10 ml of filter-sterilized urea, a common and effective yeast food (Jones and Ingledew, 1994) were added to the bioreactors to a final concentration of 8 m*M*. Agitation of the mash continued for the duration of saccharification and subsequent fermentation. Mash dextrins were saccharified to fermentable sugars at 30°C by adding 0.7 ml of glucoamylase (Allcoholase II, Alltech), which we determined to have a specific activity of 2.1 mg glucose/min/mg protein (152 mg/ml). After 30 min, the wheat mashes were inoculated with preconditioned (38°C for 20 min in prewarmed 0.1% peptone) active dry yeast (*Saccharomyces*

[1]Department of Applied Microbiology and Food Science, University of Saskatchewan, Canada.
[2]Biotechnology Research Institute, National Research Council Canada, Montreal.
[3]Crop Development Center, College of Agriculture, University of Saskatchewan, Canada.

*cerevisiae,* Alltech) providing ~21 million cells per gram of mash in recommended inoculum levels (Casey and Ingledew 1985). Fermentations were conducted at 30°C, and samples were taken over time for determination of total dissolved solids, free amino nitrogen (FAN), and ethanol concentrations.

### Analyses

Subsamples of the grains were ground in a Udy cyclone mill to pass a 1.0-mm screen. Grain samples were analyzed for moisture, protein, ash, ether extract, and acid-detergent fiber using official methods (AOAC 1990). β-Glucan was determined by the method of McCleary and Glennie-Holmes (1985), and starch was determined by the procedure of Holm et al (1986) on samples boiled with 80% ethanol for 30 min and centrifuged at 2,000 × *g* for 10 min. Aliquots of fermenting mashes were centrifuged (10,300 × *g*; 15 min) to collect clear supernatant for assay. Total dissolved solids were determined by measuring specific gravities at 20°C with a digital density meter (DMA-45, Anton Parr KG, Graz, Austria). Specific gravity was converted into grams of dissolved solids (expressed as sucrose) per 100 ml using appropriate tables. The FAN concentration of the supernatant liquid was determined colorimetrically by the ninhydrin method of the EBC (European Brewery Convention 1987) with glycine as the standard. Ethanol was measured enzymatically using alcohol dehydrogenase (Sigma Chemical Company, St. Louis, MO) with known concentrations of ethanol as standards. Total soluble carbohydrate was measured by the anthrone assay (Herbert et al 1971), and glucose concentration using the glucose oxidase-peroxidase method of Sigma (procedure 510). The latter two assays employed known concentrations of glucose as standards. All analyses were conducted in duplicate.

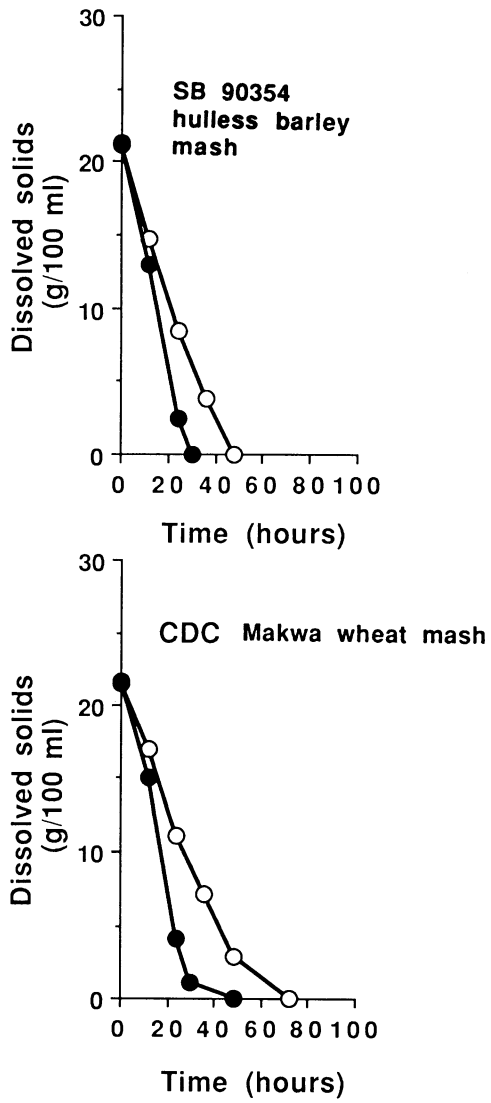


**Fig. 1.** Comparative fermentation velocities of a selected hull-less barley mash and a CDC Makwa wheat mash. ○ = unsupplemented fermentations; ● = fermentations supplemented with 8 m*M* urea.

### β-Glucanase and Viscosity Trials

The β-glucanase enzyme used was an *Aspergillus niger* enzyme (GNC Bioferm, Inc., Saskatoon, SK). It is formulated as a powder with a suggested application rate at 45°C of 1 g/kg of grain (10% moisture) in mashes made with 1 kg of grain in 3 L of water (~0.025%, w/w, of enzyme in grain mash). In a preliminary trial using SB 89528 hull-less barley mash (8.8% β-glucan), the suggested application rate extensively reduced the viscosity of the mash within only 2–3 min at 45°C. When the application rate was decreased to ~0.008% (w/w, in prepared mash), the change in viscosity of the mash with time could be more accurately measured. β-Glucanase-treated samples were also tested to determine whether glucose was liberated due to the action of the enzyme.

A Haake viscoamylograph (starch tester model AV30, Karlsruhe, Germany) was used to monitor the viscosity in the presence of β-glucanase at a constant temperature, and to determine mash viscosity over the 20–80°C temperature range of concern to industry (in the absence of β-glucanase). The mash sample volume used for all measurements was 200 g. A probe was inserted into the sample and the viscosity recorded on chart paper in Brabender Units (BU).

### Distillers' Grain

Distillers' grains collected from the fermentors were oven-dried (120°C for 60 min) and analyzed for protein, β-glucan, neutral detergent, and acid detergent fiber fractions using methods described previously.

## RESULTS AND DISCUSSION

### Grain Analysis

The results of grain analysis are shown in Table I. Two of the hull-less barleys, CDC Richard and SB 90354, contained higher levels of starch than the hulled, control Harrington barley. These levels were comparable to the CDC Makwa wheat sample included in this study. Average starch values of 63% have been used to calculate yields of ethanol from wheat (Ingledew 1993).

**TABLE I**
**Chemical Composition of Grain[a]**

| Sample | Moisture | Protein | Ash | Ether Extract | Acid Detergent Fiber | β-Glucan | Starch |
|---|---|---|---|---|---|---|---|
| Harrington | 9.8 ± 0.1[b] | 17.7 ± 0.2 | 2.5 ± 0.1 | 2.5 ± 0.0 | 5.7 ± 0.0 | 6.0 ± 0.0 | 64.7 ± 2.1 |
| CDC Richard | 10.2 ± 0.0 | 18.5 ± 0.1 | 1.9 ± 0.0 | 2.6 ± 0.0 | 2.3 ± 0.0 | 5.5 ± 0.0 | 68.7 ± 3.1 |
| SB 89528 | 9.6 ± 0.0 | 18.7 ± 0.4 | 2.0 ± 0.1 | 2.9 ± 0.0 | 2.4 ± 0.0 | 8.8 ± 0.0 | 62.7 ± 1.9 |
| SB 90354 | 10.3 ± 0.1 | 15.5 ± 0.2 | 1.8 ± 0.0 | 2.3 ± 0.0 | 2.0 ± 0.0 | 6.8 ± 0.1 | 70.0 ± 1.4 |
| CDC Makwa | 11.1 ± 0.1 | 17.7 ± 0.0 | 1.7 ± 0.0 | 1.8 ± 0.1 | 3.9 ± 0.1 | 1.0 ± 0.0 | 71.9 ± 1.2 |

[a] All results on a % dry basis, except moisture.
[b] Means ± standard deviation of duplicated measurements.

Also noteworthy were the high β-glucan contents of the barleys compared to wheat, and the low fiber levels of the hull-less varieties.

### Fermentation

In fermentation studies, hull-less barley mashes fermented to completion at least as quickly as wheat mash fermentations, and these rates were accelerated by the presence of urea, a nitrogen source well-used by *Saccharomyces* yeasts (Fig. 1). When urea was added under the conditions described, all barley and wheat fermentations finished ~18 hr earlier than the control fermentations without urea (~30 hr rather than 48). Ethanol concentrations were determined by alcohol dehydrogenase enzyme, and yields were calculated based on starch analysis of grain and starch concentration per liter of mash. Yields averaged 94.3% of theoretical; ethanol concentrations (which depend on the initial sugar content of each mash) in all cases exceeded 10.6%, v/v (data not shown). From 12 to 15% of the total solids in the initial mashes were unfermentable. Some of this material was soluble minerals, some was anthrone positive carbohydrate which appeared to be fibrous or cellulosic in nature.

The FAN in mashes made from hull-less varieties exceeds that found in Makwa wheat and Harrington barley by 20–50% (Table II). Regardless of the presence of added urea nitrogen, the yeast appears to take up all the usable FAN from these mashes. Variations in usable FAN levels appeared to contribute to the differences in fermentation rates between wheat and barleys, and between genotypes of hull-less barleys. Unusable FAN consists of peptides (larger than tripeptides) and small proteins that are not metabolized by yeasts (Patterson 1995).

### β-Glucan

The barley mashes were more viscous than wheat mashes of equivalent sugar content, especially when held at 4°C during chemical sterilization. Barley mashes ranged from 260 (CDC Richard) to 800 BU (SB 90354), while the viscosity of the CDC Makwa wheat mash was only 90 BU when measured at 30°C (Table III). Figure 2 compares a SB 90354 hull-less barley mash to a Makwa wheat mash and shows mash viscosity versus temperature over 20–80°C. In the absence of β-glucanase, viscosity reduction of barley mashes proceeded slowly during fermentation—perhaps this was a function of the enzymic conversion of dextrins to sugars with subsequent fermentation to ethanol, of the partial hydrolysis of proteins, of the precipitation of proteins and β-glucans by ethanol, and of the possible action of the yeast itself on the β-glucan. Viscosity reduction by the end of fermentation was remarkably complete, yet initially, viscous mash tends to trap carbon dioxide and the fermentor volume expands rather dramatically. When 0.008% β-glucanase was added to the mashes (Table III), the approximate time (in minutes) needed to reduce viscosity to levels of 40–70 BU, which we consider normal for nonviscous mashes, was short. Although β-glucan degradation by enzymatic means is not novel, most of the industry is not confident enough in the use of β-glucanase to adopt full-scale production of ethanol from barley.

Apart from viscosity reduction, this β-glucanase treatment did not seem to offer any other advantage. Liberation of greater amounts of fermentable sugars was expected due to the nature of the crude enzyme preparation used (β-glucanase and β-glucosidase activities), but increased yields were not detected. In the absence of yeast, no perceptible increases in glucose over background values (glucoamylase-treated mash) were noted in centrifuged, heated samples, where glucoamylase enzyme had been denatured. Although it is possible that end-product inhibition of β-glucosidase or β-glucanase can occur, no incremental increase in alcohol was expected by the addition of this particular enzyme preparation, and no increase in alcohol was produced in fermentations where the enzyme was used. Other preparations and procedures for mash preparation, which may result in liberation of additional glucose and reduce viscosity, are under consideration.

### Distillers' Grains

Analysis of oven-dried distillers' grains from these experiments (Table IV) suggested that hull-less barleys show promise for the

**TABLE II**
**Utilization of Free Amino Nitrogen (FAN) During Fermentation of Various Wheat and Barley Mashes**

| Grain | 8 m*M* Urea | FAN/L (mg) Initial | Final | Utilized | % FAN Utilized |
|---|---|---|---|---|---|
| Harrington | − | 81.9 | 20.6 | 61.3 | 74.8 |
| | + | 80.0 | 19.9 | 60.1 | 75.1 |
| CDC Richard | − | 125.6 | 20.4 | 105.2 | 83.8 |
| | + | 122.7 | 21.1 | 101.6 | 82.8 |
| SB 89528 | − | 122.7 | 27.6 | 95.0 | 77.5 |
| | + | 124.0 | 22.9 | 101.2 | 81.6 |
| SB 90354 | − | 97.3 | 26.1 | 71.3 | 73.2 |
| | + | 95.5 | 24.4 | 71.2 | 74.5 |
| CDC Makwa | − | 80.4 | 25.4 | 55.0 | 68.4 |
| | + | 78.7 | 27.1 | 51.6 | 65.6 |

**TABLE III**
**Change in Viscosity of Barley and Wheat Mashes After Addition of 0.008% (w/w) β-Glucanase at 30°C**

| Sample | Viscosity (BU) Initial | Final[a] (min) | Final[b] |
|---|---|---|---|
| Harrington | 550 | 60 (16.0) | 12 |
| CDC Richard | 260 | 40 (11.5) | 5 |
| SB 89528 | 280 | 40 (12.5) | 9 |
| SB 90354 | 800 | 40 (19.5)[c] | 12 |
| CDC Makwa | 90 | 50 (10.5) | 20 |

[a] After treatment with β-glucanase at 45°C.
[b] After fermentation, no β-glucanase used.
[c] This sample took only 4.5 min to drop from 800 to 140 BU.

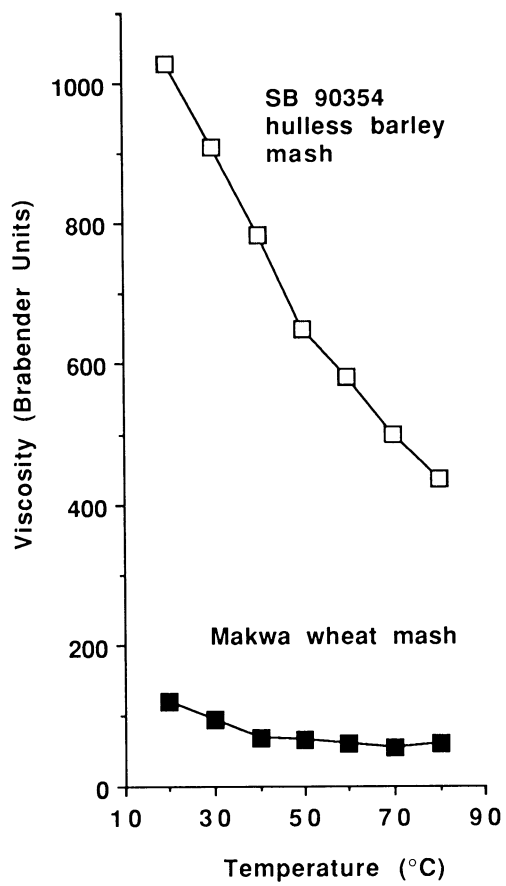


**Fig. 2.** Comparative viscosities of 20° Plato CDC Makwa wheat mash and SB 90354 hull-less barley mash over the range of 20–80°C. No enzyme was added.

TABLE IV
Composition (%) of Distillers Dried Grains

| Sample | Protein (N × 6.25) | β-Glucan | Neutral Detergent Fiber | Acid[a] Detergent Fiber |
|---|---|---|---|---|
| Harrington | 24.2 | 1.7 | 70.1 | 33.9 |
| CDC Richard | 34.5 | 1.9 | 66.1 | 22.6 |
| SB 89528 | 34.8 | 3.2 | 64.3 | 21.4 |
| SB 90354 | 36.4 | 2.6 | 63.4 | 22.7 |
| CDC Makwa | 34.3 | 0.4 | 73.7 | 19.2 |

[a]Acid detergent fiber is a component of the neutral detergent fiber.

animal feed side of the fuel alcohol industry. Protein contents of the hull-less distillers' grains were comparable to that from wheat and exceeded the protein content of Harrington by ~40%. Although residual β-glucan was detected in dried distillers grains (a small amount considering the utilization of the starch component of the grain), the amounts were considerably reduced even without the addition of β-glucanase. Such distillers grains can therefore be assessed as a feed for monogastric animals and ruminants without the concerns of excessive feed viscosity and the problems described by Walsh et al (1993). In addition, lysine content of barley exceeds that of wheat (Bell and Keith 1994), giving the protein in barley distillers grains a higher biological value and thus a nutritional advantage.

## CONCLUSIONS

This study reports encouraging findings in the use of hull-less barley for fuel alcohol production and suggests that the stillage would have nutritional advantages for the feed industry. From a fermentation standpoint, no problems were observed in the conversion of starch to ethanol. Fermentation times appeared to be somewhat better when barleys were compared to wheat. Control of viscosity can be done with β-glucanase enzyme, eliminating any problems with $CO_2$ entrapment and consequential foam expansion in the fermentation vessel. This may help to ease industrial concerns on barley use for alcohol production. We have not yet attempted to remove the β-glucan before liquefaction or to investigate a wide number of other hull-less varieties. Other work is in progress. Our objective at this time was to show merit for the industrial application of hull-less barleys to the fuel alcohol industry. Time will tell whether pricing of contractually grown hull-less varieties for fuel alcohol production will permit exploitation of the hull-less barleys and permit continued diversification of prairie agriculture.


## ACKNOWLEDGMENTS

We thank the Western Grains Research Foundation and the Natural Sciences and Engineering Research Council of Canada for research support; K. C. Thomas for research discussions; and Anita Dhas, C. Brown, and D. Hassard for technical assistance.


## LITERATURE CITED


AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed. The Association: Washington, DC.

BELL, J. M., and KEITH, M. O. 1994. Effects of adding barley hulls and linseed meal to wheat and hulless barley diets fed to growing pigs. Anim. Feed Sci. Technol. 45:177.

BHATTY, R. S., BERDAHL, J. D., and CHRISTISON, G. I. 1975. Chemical composition and digestible energy of barley. Can. J. Anim. Sci. 55:759.

BHATTY, R. S., CHRISTISON, G. I., and ROSSNAGEL, B. G. 1979. Energy and protein digestibilities of hulled and hulless barley as determined by swine feeding. Can J. Anim. Sci. 59: 585.

CASEY, G. P., and INGLEDEW, W. M., 1985. Reevaluation of alcohol synthesis and tolerance in brewers yeast. J. Am. Soc. Brew. Chem. 43:75.

EUROPEAN BREWERY CONVENTION. 1987. Analytica EBC, 4th ed. Free amino nitrogen E141-E142. Brauerei und Getränke Rundschau: Zurich.

HELM, J. H., SALMON, D. F., DYSON, D. H., and STEWART, W. M. 1992. Registration of Condor barley. Crop Sci. 32:278.

HERBERT, D., PHIPPS, P. J., and STRANGE, R. E. 1971. Chemical analysis of microbial cells. Pages 209-344 in: Methods in Microbiology, Vol. 5B. J. R. Norris and D. W. Ribbons, eds. Academic Press: London.

HOLM, J., BJORCK, I., DREWS, A., and ASP, N.-G. 1986. A rapid method for the analysis of starch. Starch/Staerke 38:224.

INGLEDEW, W. M., 1993. Yeasts for production of fuel alcohol. Pages 245-291 in: The Yeasts, Vol. 5, 2nd ed. A. H. Rose and J. S. Harrison, ed. Academic Press: New York.

JONES, A. M., and INGLEDEW, W. M. 1994. Fuel alcohol production: Appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation. Process Biochem. 29:483.

McCLEARY, S. V., and GLENNIE-HOLMES, M. 1985. Enzymic quantification of (1→3),(1→4)-β-D-glucan in barley and malt. J. Inst. Brew. 91:285.

PATTERSON, C. A. 1995. Transport and utilization of dipeptides by *Saccharomyces cerevisiae* NCYC 1324. PhD thesis. University of Saskatchewan: Saskatoon, Canada.

ROSSNAGEL, B. G., BHATTY, R. S., and HARVEY, B. L. 1981. Developing high-energy hulless feed barley for Western Canada. Page 293 in: Barley Genetics IV. Proc. 4th Int. Barley Genet. Symp. R. N. H. Whitehouse, ed. Edinburgh University Press: Edinburgh University.

ROSSNAGEL, B. G., BHATTY, R. S., and HARVEY, B. L. 1983. Scout hulless barley. Can. J. Plant Sci. 63:751.

ROSSNAGEL, B. G., BHATTY, R. S., and HARVEY, B. L. 1985. Performance of hulless barley compared to wheat. Barley Newsl. 29:99.

ROSSNAGEL, B. G., BHATTY, R. S., HARVEY, B. L., CAMPBELL, G. L., and CLASSEN, H. L. 1988. Development of hulless barley for the feed and food industries. Page 77 in: Proceedings of the Alternative End-Uses for Barley Workshop. Waite Agriculture Research Institute: Adelaide, Australia.

THOMAS, K. C., and INGLEDEW, W. M. 1990. Fuel alcohol production: Effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. Appl. Environ. Microbiol. 56:2046.

WALSH, G. A., POWER, R. F., and HEADON, D. R. 1993. Enzymes in the animal-feed industry. Tibtech. 11:424.


[Received June 15, 1994. Accepted October 20, 1994.]