

## Rye and Triticale as Feedstock for Fuel Ethanol Production

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### ABSTRACT

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Normal gravity rye and triticale mashes, containing 20–21 g of dissolved solids per 100 mL of mash liquid, were fermented with active dry yeast at 27°C. Fermentations were completed within 48 hr for rye, and within 72 hr for triticale. Supplementation of mashes with urea at a concentration of 8 mM accelerated rates of sugar consumption and fermentation, and reduced fermentation time from 48 to 36 hr for rye, and from 72 to 48 hr for triticale. Rye fermented faster than triticale, due to its higher level of free amino nitrogen. Ethanol yields were 356–363 L/tonne

of 14% moisture rye grain, and 362–367 L/tonne of 14% moisture triticale. Fermentation efficiencies, which were 90–91% for triticale, and 91–93% for rye, and ethanol yields were comparable to those obtained from wheat and were not affected significantly by urea supplementation. The replacement of wheats by less expensive crops such as rye and triticale would provide good economic opportunities and alternatives for the fuel alcohol industry.

The current commercial practice in western Canada is to use wheat as the main basic raw material for fuel alcohol production. Considerable laboratory studies, pilot plant trials, and literature reviews have been conducted on the fermentation of wheat for fuel alcohol production. Representative published scientific papers include those of Thomas and Ingledew (1990), Ingledew (1993), Jones and Ingledew (1994a), Sosulski and Sosulski (1994), and Thomas et al (1996). When wheat prices increased significantly in recent years, the need to use alternative, less expensive crops became more and more pressing for industry in order to reduce the expense of feedstock and to remain competitive. Corn, the major feedstock used by most fuel ethanol plants operated in the United States, is not generally available in Western Canada.

Recently, barley (Ingledew et al 1995, Thomas et al 1995, Sosulski et al 1996) and oats (Thomas and Ingledew 1995) have been thoroughly investigated as feedstock for fuel ethanol. The results show that barley and oats on a starch basis are comparable to wheat in terms of ethanol yields and fermentation efficiencies.

Rye and triticale are generally 2–5% (db) lower in starch than wheat, but their prices are significantly lower. However, little is known about the fermentability and efficiency of rye and triticale for fuel alcohol production. Rye has a much higher content of hemicelluloses or pentosans than other cereals, and water-soluble pentosans tend to form viscous solutions in concentrated flour-water slurries (Hoseney 1986, Bengtsson and Aman 1990, Bedford et al 1991, Boros et al 1993). Even though rye is used as the major substrate in at least one Canadian distillery, no published data have been found on viscosities of mashes made from rye or triticale, the latter a cereal produced by crossing wheat (*Triticum*) and rye (*Secale*). The magnitudes of mash viscosity must be quantified, as viscosity will influence ease of stirring and pumping during mashing and fermentation, energy consumption, and the extent of starch hydrolysis to dextrins and low molecular weight sugars during mashing. A laboratory study to address these questions could provide the alcohol industry with more alternatives to choose the most economic raw materials available to the region. The current study was undertaken to investigate the fermentability

of rye and triticale by normal gravity fermentation (20–21 g of dissolved solids per 100 mL of mash liquid) at 27°C. Fermentation parameters, including ethanol yields, fermentation rates and efficiencies, yeast cell growth rates and viabilities, dextrins and low molecular weight sugar composition of fermenting worts, nutritional levels, pH values, and the effect of urea supplementation as an external nitrogenous source, were studied.

### MATERIALS AND METHODS

#### Rye and Triticale Samples

Fall rye (Prima) and triticale (AC Copia) were both developed by, and obtained from, Agriculture and Agri-Food Canada (Saskatoon, SK). The fall rye had a moisture content of  $12.26 \pm 0.01\%$  and a starch content of  $63.11 \pm 0.79\%$  (db). Triticale had a moisture content of  $10.19 \pm 0.01\%$  and a starch content of  $64.95 \pm 0.10\%$  (db).

#### Enzymes, Reagents, and Chemicals

A powdered fungal enzyme preparation from *Trichoderma viride*, Roxazyme G, was provided by Hoffmann-La Roche (Mississauga, ON). The Roxazyme G contained cellulase activity of 8,000 IU/g and xylanase activity of 43,350 IU/g. High-temperature  $\alpha$ -amylase (High-T), glucoamylase (Allcoholase II), and active dry yeast were all obtained from the Alltech Biotechnology Center, Nicholasville, KY. The high-temperature  $\alpha$ -amylase preparation contained 10 mg of protein/mL and had a specific activity of  $\approx 1.14$  g of starch (hydrolyzed)/min/mg of protein between 80 and 90°C. The glucoamylase preparation contained 98.5 mg of protein/mL and had a specific activity of  $\approx 1$  mg of glucose (produced)/min/mg of protein at 30°C. All other chemicals and enzymes were purchased from Sigma Chemical Co., St. Louis, MO, or obtained locally. All chemicals were reagent grade.

#### Grinding and Mashing

Rye and triticale were ground with a plate grinder (Glen Mills Inc., Clifton, NJ) at setting number 5. For mashing, 100 g of ground whole grain (as-is basis) was dispersed with constant agitation in 300 mL of prewarmed (40°C) distilled water containing 1 mM calcium chloride. For rye mashes, 0.0002 g of Roxazyme G was added per gram of ground cereal mashed. The slurries were incubated with stirring at 40°C for 30 min to break down viscosity-causing nonstarch polysaccharides. Such an enzyme pretreatment was not necessary for triticale mashes as viscosity of the mashes were as low as 102 BU. To the mash, 0.5 mL of high temperature  $\alpha$ -amylase was added. After 5 min, the temperature was raised to  $\approx 96^\circ\text{C}$  and held at this temperature for 45 min with continuous agitation. The temperature was then lowered to 80°C. The

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gelatinized starch was liquefied further by the addition of another 0.5 mL of high temperature  $\alpha$ -amylase and incubated at 80°C for 30 min. The volume lost because of evaporation was made up by the addition of sterile distilled water, and part of the water was used at the end of mashing to transfer and rinse the mash to a jacketed Celstir fermentor (Wheaton Instruments, Millville, NJ). The mash was cooled to 4°C by connecting the fermentor to a D3-G waterbath circulator (Haake Inc., Saddle Brook, NJ). Then 40  $\mu$ L of the chemical sterilant, diethyl pyrocarbonate (DEPC) (Sigma), was added to the fermentor. The fermentor was placed in a refrigerator at 4°C for  $\approx$ 68 hr before fermentation.

### Viscosity Measurements

Viscosities of the mashes were measured with a starch tester (model AV 30, Haake). Prepared mashes (400 g) were transferred to the starch tester vessel equilibrated to 40°C, and the relative viscosity was expressed in Brabender units (BU).

### Fermentation

The fermentor was connected to a waterbath circulator set at 30°C. Either 10 mL of sterile distilled water or 10 mL of filter-sterilized urea solution, a common and effective yeast food, was added to the fermentor to a final concentration of 8 mM. Then, 0.8 mL of Allcoholase II preparation was added to saccharify the dextrins to fermentable sugars. After 30 min, the temperature of the fermentor was lowered, maintained at 27°C throughout the fermentation, and inoculated with 2 mL of active dry yeast inocula (inoculated at  $\approx 10^7$  viable cells/g of mash). Fermentations were conducted until all sugars were consumed.

### Preparation of Inocula

Active dry yeast (11 g) was dispersed in 99 mL of 0.1% sterile peptone water prewarmed at 38°C. The yeast solution contained

$\approx 2 \times 10^9$  viable cells/mL. After 20 min at 38°C, the required amount of inoculum was added to the fermentor.

### Fermentation Progress and Analyses

Progress of fermentation was monitored by a number of fermentation parameters at 0, 6, 12, 24, 36, 48, and 72 hr of fermentation. Total yeast cell counts and viable counts were determined by the direct microscopic method at a magnification of 400 $\times$  with an improved Neubauer counting chamber. Samples collected at various times were centrifuged (10,300  $\times$  g) at 4°C for 15 min, and the pH of clear supernatant liquids was measured. Dissolved solids (g of sugar/100 mL of fermenting liquid) were measured at 20°C with a digital density meter (DMA-45, Anton Paar, Graz, Austria). Total free amino nitrogen (FAN) in the supernatant liquid was determined by the ninhydrin method of the European Brewery Convention. Ethanol was measured by the alcohol dehydrogenase assay (Sigma Bulletin no. 331 U.V.). A standard solution of ethanol (0.08%, w/w) was used as a control. Glycerol, sugars (maltotriose, maltose, and glucose), and dextrans were measured by HPLC. Clear supernatant liquids obtained by centrifugation were diluted with distilled water and injected into a column (FAM-PAK, Waters Chromatographic Div., Milford, MA) maintained at 65°C. This column separates sugars, organic acids, and alcohols. These components were eluted from the column with HPLC-grade water containing orthophosphoric acid at a concentration of 1.5 mM. The separated components were detected with a differential refractometer (model 410, Waters), and the chromatographic data processed with a computer program (Maxima 810, Waters). The elution rate was 1 mL/min and methanol was used as internal standard. Parallel fermentations without sampling were set up simultaneously, and the total alcoholic mashes were distilled at the end of fermentation for the determination of ethanol yields and fermentation efficiencies. Analyses were conducted in duplicate, except for mash viscosity measurements, and the results were presented as the mean of the two duplicate analyses.

## RESULTS AND DISCUSSION

### Viscosity of Mashes

The viscosity of triticale mashes prepared with a water-to-grain ratio of 3:1 (w/w) was 102 BU (40°C) at the end of mashing. Such low mash viscosity indicated that enzyme pretreatment for viscosity reduction was not necessary for triticale mashes. However, rye mash prepared with a water-to-grain ratio of 3:1 (w/w) had a viscosity of 600 BU (40°C) at the end of mashing.

When a freshly prepared rye mash was treated after mashing with 0.0002 g of Roxazyme G/g of ground cereal mashes (as-is basis), the relative viscosity decreased from 600 to 130 BU in 5 min. A larger dose of 0.0004 g/g of ground cereal led to a faster initial rate of viscosity reduction and a 5-min value of 120 BU. When Roxazyme G was added as a pretreatment before mashing and starch liquefaction at 0.0002 (g/g of cereal) at 40°C for 30, 60, and 120 min, the relative viscosity of rye mashes were 260, 200, and 182 BU, respectively, as measured at the end of mashing. A 30-min Roxazyme G enzyme pretreatment was considered suffi-

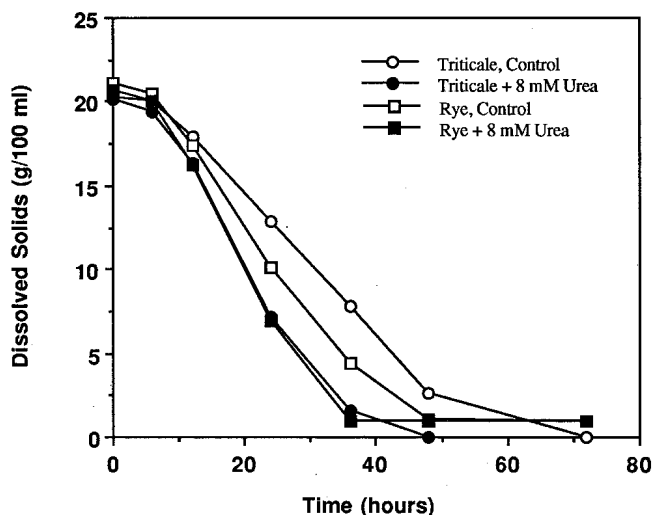


Fig. 1. Fermentation rates (utilization of dissolved solids) of rye and triticale mashes.

TABLE I  
Carbohydrate Composition, Glycerol, and Ethanol Concentrations<sup>a</sup> in Triticale Fermentations Without Urea Supplementation

Hr	Dextrins <sup>b</sup>	Maltotriose	Maltose	Glucose	Glycerol	Ethanol
0	5.57 $\pm$ 0.11	1.88 $\pm$ 0.11	8.67 $\pm$ 0.09	4.91 $\pm$ 0.08	0	0
6	4.88 $\pm$ 0.05	0	5.86 $\pm$ 0.04	10.65 $\pm$ 0.15	0.04 $\pm$ 0.0	10.34 $\pm$ 0.01
12	3.79 $\pm$ 0.14	0	1.68 $\pm$ 0.20	11.73 $\pm$ 0.14	0.16 $\pm$ 0.02	1.40 $\pm$ 0.02
24	1.23 $\pm$ 0.03	0.97 $\pm$ 0.02	0.11 $\pm$ 0.00	10.83 $\pm$ 0.06	0.47 $\pm$ 0.01	4.02 $\pm$ 0.06
36	1.45 $\pm$ 0.02	0.15 $\pm$ 0.00	0.17 $\pm$ 0.00	7.21 $\pm$ 0.01	0.64 $\pm$ 0.00	6.34 $\pm$ 0.01
48	1.29 $\pm$ 0.09	0	0.23 $\pm$ 0.00	2.76 $\pm$ 0.10	0.80 $\pm$ 0.01	8.72 $\pm$ 0.02
72	1.08 $\pm$ 0.08	0	0.28 $\pm$ 0.11	0.08 $\pm$ 0.00	0.78 $\pm$ 0.00	10.03 $\pm$ 0.06

<sup>a</sup> All expressed in g/100 mL of fermenting supernatant except ethanol (mL of ethanol/100 mL).

<sup>b</sup> Includes maltotetraose, maltopentaose, and other soluble dextrans of higher molecular weight.

cient for rye mash viscosity reduction. The effectiveness of Roxazyme G enzyme for the reduction of rye mash viscosity was probably due to its xylanase activity breaking down arabinoxylans. Boros et al (1993) have identified arabinoxylans as the most responsible components (rather than starch, protein, or  $\beta$ -glucan) for high viscosity rye extracts. Also, the Roxazyme G led to greater mash viscosity reduction when it was added after the mashing was completed. In all probability, nonstarch carbohydrates are more susceptible to Roxazyme G attack after they are subjected to the heating regime during mashing.

### Rate of Fermentation

Mashes were fermented with and without 8 mM urea as a nitrogenous supplement. All fermentations, with or without urea supplementation, were completed within 72 hr for mashes initially containing 20–21 g of sugars/100 mL of fermenting liquid (Fig. 1). The supplementation of mashes with urea at 8 mM accelerated sugar consumption (rate of fermentation) and reduced fermentation time by  $\approx$ 24 hr for triticale and by  $\approx$ 12 hr for rye. Without supplementation, rye mashes fermented faster than triticale mashes. The fermentation rates were similar in rye and triticale mashes when both were supplemented with urea. About 1 g/100 mL more of residual dissolved solids, some of which may be non-fermentable carbohydrates, remained unused in rye fermentations than in triticale mashes. Urea is an inexpensive and acceptable yeast food for fuel alcohol, but it would not be acceptable for potable alcohol production due to possible production of ethyl carbamate. In such a case, diammonium phosphate or other more

complex nitrogen sources can be used (Jones and Ingledew 1994b).

### Carbohydrate Composition, Glycerol, and Wort Ethanol Concentration

HPLC analyses further illustrated that, at the beginning of fermentation, worts consisted mainly of dextrans (5.2–6.2 g/100 mL), maltose (7.8–9.4 g/100 mL), and glucose (4.9–6.5 g/100 mL), with small amounts of maltotriose (0–1.9 g/100 mL) (Tables I–IV). Through the action of glucoamylase, dextrans were gradually hydrolyzed to low molecular weight (LMW) sugars that were subsequently consumed by the yeast to produce yeast cells, ethanol, and minor fermentation by-products such as glycerol and organic acids. Consequently, reductions in dextrin and maltose concentrations were observed (Tables I–IV). Increases of maltotriose in midfermentation, and peak concentrations of glucose at  $\approx$ 12 hr of fermentation, were due to hydrolysis of dextrans to LMW sugars. The rates of hydrolysis of dextrans and the rates of sugar consumption were higher when urea was added. Glycerol concentrations were 0.5–0.8 g/100 mL at the end of fermentation, which represented 3–4% (w/w) of total carbohydrate used. Ethanol concentrations in fermenting liquids ranged from 9.4–10.0 mL/100 mL at 72 hr. Twice the amount of residual dextrans were found in rye fermentation, compared to those in triticale fermentations. However, the dextrans estimated by HPLC measurement could include other unknown dissolved solids as well, while loss of (or reduced) glucoamylase activity toward the end of fermentation could also lead to unhydrolyzed dextrans. More study is needed to

**TABLE II**  
Carbohydrate Composition, Glycerol, and Ethanol Concentrations<sup>a</sup> in Triticale Fermentations Supplemented with 8 mM Urea

Hr	Dextrans <sup>b</sup>	Maltotriose	Maltose	Glucose	Glycerol	Ethanol
0	5.26 ± 0.11	1.58 ± 0.07	8.38 ± 0.18	5.22 ± 0.44	0	0
6	4.24 ± 0.04	0	4.77 ± 0.15	10.17 ± 0.70	0	0.25 ± 0.04
12	2.45 ± 0.20	0.48 ± 0.06	0.74 ± 0.05	10.32 ± 1.50	0.18 ± 0.04	1.72 ± 0.23
24	1.08 ± 0.07	0.52 ± 0.07	0.27 ± 0.06	6.27 ± 0.50	0.57 ± 0.02	6.12 ± 0.30
36	0.99 ± 0.01	0	0.44 ± 0.01	1.90 ± 0.02	0.74 ± 0.01	8.71 ± 0.06
48	0.98 ± 0.00	0	0.32 ± 0.02	0.07 ± 0.00	0.71 ± 0.01	9.66 ± 0.08
72	0.96 ± 0.00	0	0.27 ± 0.01	0.08 ± 0.00	0.70 ± 0.00	9.67 ± 0.00

<sup>a</sup> All expressed in g/100 mL of fermenting supernatant except ethanol (mL of ethanol/100 mL).

<sup>b</sup> Includes maltotetraose, maltopentaose, and other soluble dextrans of higher molecular weight.

**TABLE III**  
Carbohydrate Composition, Glycerol, and Ethanol Concentrations<sup>a</sup> in Rye Fermentations Without Urea Supplementation

Hr	Dextrans <sup>b</sup>	Maltotriose	Maltose	Glucose	Glycerol	Ethanol
0	6.24 ± 0.30	0	9.41 ± 0.69	6.50 ± 0.36	0	0
6	4.94 ± 0.03	0	4.72 ± 0.03	9.30 ± 0.06	0.04 ± 0.00	0.41 ± 0.02
12	3.44 ± 0.01	0.11 ± 0.00	0.52 ± 0.02	10.24 ± 0.12	0.15 ± 0.00	1.53 ± 0.01
24	2.29 ± 0.18	0.16 ± 0.00	0.23 ± 0.03	6.86 ± 0.03	0.41 ± 0.01	5.01 ± 0.02
36	2.16 ± 0.00	0	0.34 ± 0.01	3.16 ± 0.10	0.54 ± 0.00	7.55 ± 0.02
48	2.04 ± 0.00	0	0.26 ± 0.00	0.21 ± 0.02	0.58 ± 0.00	9.44 ± 0.04
72	1.94 ± 0.04	0	0.24 ± 0.01	0.18 ± 0.04	0.61 ± 0.01	9.59 ± 0.09

<sup>a</sup> All expressed in g/100 mL of fermenting supernatant except ethanol (mL of ethanol/100 mL).

<sup>b</sup> Includes maltotetraose, maltopentaose, and other soluble dextrans of higher molecular weight.

**TABLE IV**  
Carbohydrate Composition, Glycerol, and Ethanol Concentrations<sup>a</sup> in Rye Fermentations Supplemented with 8 mM Urea

Hr	Dextrans <sup>b</sup>	Maltotriose	Maltose	Glucose	Glycerol	Ethanol
0	5.72 ± 0.17	0	7.77 ± 0.23	6.53 ± 0.10	0	0
6	4.99 ± 0.25	0	3.92 ± 0.20	10.75 ± 0.91	0.04 ± 0.00	0.46 ± 0.12
12	3.30 ± 0.04	0.22 ± 0.00	0.32 ± 0.00	10.03 ± 0.08	0.16 ± 0.00	1.67 ± 0.00
24	2.28 ± 0.07	0.07 ± 0.00	0.27 ± 0.06	3.95 ± 0.14	0.49 ± 0.01	6.82 ± 0.06
36	2.10 ± 0.00	0	0.24 ± 0.00	0.24 ± 0.03	0.64 ± 0.00	9.54 ± 0.08
48	1.92 ± 0.04	0	0.22 ± 0.01	0.16 ± 0.07	0.60 ± 0.01	9.18 ± 0.15
72	1.86 ± 0.01	0	0.21 ± 0.00	0.20 ± 0.07	0.53 ± 0.00	9.46 ± 0.12

<sup>a</sup> All expressed in g/100 mL of fermenting supernatant except ethanol (mL of ethanol/100 mL).

<sup>b</sup> Includes maltotetraose, maltopentaose, and other soluble dextrans of higher molecular weight.

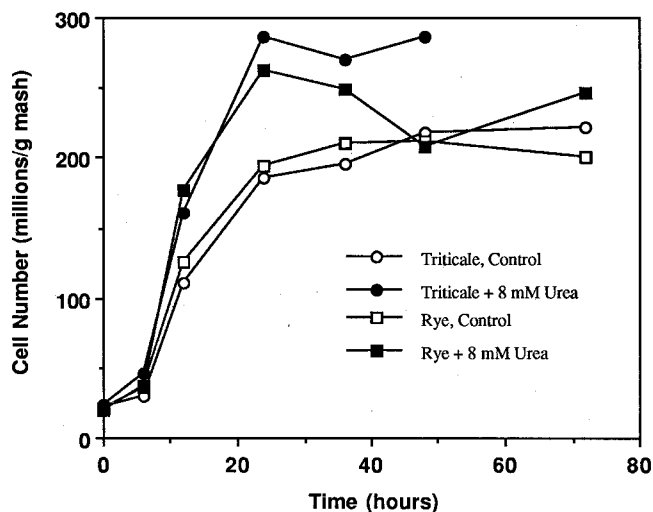


Fig. 2. Yeast cell populations during fermentation of rye and triticale mashes.

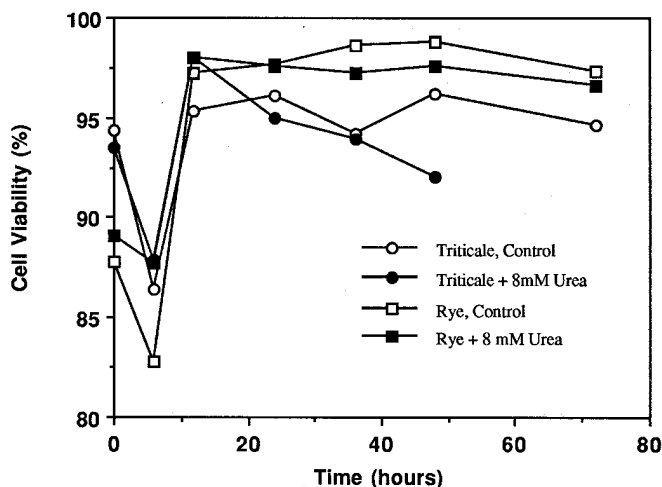


Fig. 3. Yeast cell viability during rye and triticale fermentations.

reveal the composition of the residual dissolved solids. LMW sugars such as maltose and glucose were only found in trace amounts.

#### Yeast Cell Number and Viability

Yeast populations in fermentors inoculated with  $\approx 22$  million cells/g of mash increased 10- to 15-fold within the first 24 hr (Fig. 2). With urea supplementation, the rates of cell multiplication were faster and the final cell numbers were much higher, resulting in faster fermentation rates. After 24 hr, cell numbers remained stable without urea addition and declined slightly in fermentors with added urea. Initial cell viability was  $\approx 88$ –94% but dropped to 83% 6 hr after inoculation (Fig. 3). Such decreases are a common phenomenon (Casey and Ingledew 1993) seldom measured in the early stages of fermentation and presumably caused by stress-mediated leakage of cell membranes. The viabilities returned to 95–97% at 12 hr and remained constant or decreased only slightly during the rest of the fermentation. Supplementation with urea did not significantly affect the cell viability.

#### pH Values of Fermenting Mashes

The pH values of fermenting mashes decreased from an initial 5.6–5.8 to 4.2–4.3 toward the end of fermentation as more acidic products were coproduced during fermentation. The rates of pH decrease were similar with both grains, with or without urea supplementation. However, final pH values of  $\approx 4.2$ –4.3 indicated that no major contamination occurred during the fermentations; pH

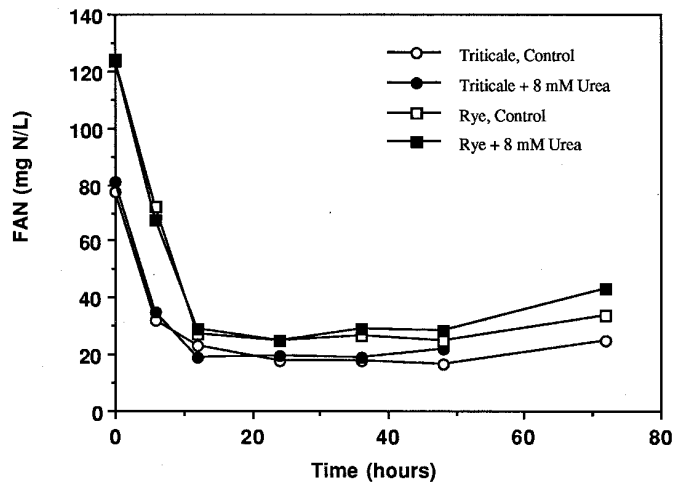


Fig. 4. Changes in the free amino nitrogen (FAN) during fermentations of rye and triticale mashes.

TABLE V  
Ethanol Yields and Fermentation Efficiencies

Cereal Grains	Ethanol Yields (L/Tonne 14% moisture)	Fermentation Efficiencies <sup>a</sup> (% theoretical)
Triticale control	367 $\pm$ 13	91.4 $\pm$ 3.1
Triticale + 8 mM urea	362 $\pm$ 2	90.2 $\pm$ 0.5
Rye control	363 $\pm$ 6	92.9 $\pm$ 1.7
Rye + 8 mM urea	356 $\pm$ 11	91.3 $\pm$ 2.9

<sup>a</sup> Fermentation efficiency was calculated as the ratio between actual ethanol yield and theoretical ethanol yield, assuming all starch was converted to glucose and then to ethanol.

values well below 4 normally indicate severe contamination with lactic acid bacteria.

#### Free Amino Nitrogen

Rye mashes had initial FAN contents of 124 mg of FAN/L, whereas triticale mash had only 78 mg of FAN/L. The higher FAN level in rye explained the faster fermentation rates (when urea was not added), due to the effect of extra amino nitrogen on yeast growth. Also, most of the FAN uptake occurred within 12 hr for both rye and triticale (Fig. 4). This is the period of initiation of rapid cell growth. After 12 hr, the FAN contents did not change, indicating that “usable” FAN was depleted. Near the end of fermentation, there was a slight increase in FAN content, especially when urea was supplemented. This apparently resulted from autolysis of yeast cells, the release of peptidase enzyme from stressed yeast or excretion of FAN from yeasts due to protein degradation inside the cell.

#### Ethanol Yield, Fermentation Efficiency, and Ethanol Concentration

Ethanol yields and fermentation efficiencies were determined by distilling mashes fermented without sampling and after fermentations had finished (72 hr). Neither ethanol yields or fermentation efficiencies were affected significantly by the supplementation with urea (Table V). The ethanol yields were 356–363 L/tonne of 14% moisture rye grain, and 362–367 L/tonne of 14% moisture triticale grain. Fermentation efficiencies for rye were 91.3–92.9%, while for triticale they were 90.2–91.4%. For 20–21 g of dissolved solids fermentations, the ethanol concentrations in the fermentor were 9.5–9.6% (v/v) for rye and 9.7–10.0% (v/v) for triticale (Tables I–IV). At the end of fermentations, during which no samplings were conducted, entire alcoholic mashes were distilled for the determination of ethanol yields.

TABLE VI  
Chemical Composition of Fermentation Stillages (% db)

Fermentation Stillages	Starch	Ash	Fat	Protein <sup>a</sup>	TDF <sup>b</sup>
Rye, no urea	1.08 ± 0.24	5.35 ± 0.00	2.64 ± 0.00	25.00 ± 0.29	32.84 ± 0.32
Rye + 8 mM urea	1.09 ± 0.56	5.32 ± 0.03	2.29 ± 0.22	25.04 ± 1.08	33.83 ± 0.35
Triticale, no urea	1.01 ± 0.01	4.94 ± 0.03	4.10 ± 0.09	33.48 ± 0.57	34.00 ± 1.25
Triticale + 8 mM urea	1.98 ± 0.39	4.71 ± 0.01	3.55 ± 0.08	34.18 ± 0.66	35.84 ± 0.36

<sup>a</sup> Protein contents were calculated using the nitrogen-to-protein conversion factor of 5.7.

<sup>b</sup> Total dietary fiber.

### Chemical Composition of Stillages

Analysis on the chemical composition of fermentation stillages showed that protein and total dietary fiber each accounted for about one-third of the spent grain (db), while the remaining one-third (db) were ash, fat, residual starch, cell materials, and fermentation by-products (Table VI). Stillages from rye were lower in protein content than those found in triticale stillages; the initial protein content in rye grain was 9.94% (db), which was significantly lower than the initial protein in triticale grain (12.97%, db). Supplementation of urea during fermentation did not alter the chemical composition of the stillages. The results obtained provide information on the nutritional profile of rye and triticale stillages that could be available as animal feeds.

### CONCLUSIONS

The study showed that mashes prepared from rye and triticale containing 20–21 g of dissolved solids/100 mL could be completely fermented within 72 hr regardless of whether external assimilable nitrogenous sources such as urea were introduced. These results are comparable to those for wheat fermentations. The supplementation of mashes with urea to a final concentration of 8 mM accelerated rates of fermentation and reduced fermentation time from 72 to ≈48 hr for triticale, and from 48 to ≈36 hr for rye. Rye mashes fermented faster than triticale, probably due to their higher levels of FAN (≈124 mg of FAN/L). Only ≈80 mg of FAN/L were present in triticale mashes. Most of the FAN was consumed during the first 12 hr of fermentation. The reduction in fermentation time by one-third for triticale and one-fourth for rye, due to supplementation of urea, leads to a shorter production cycle and therefore would lead to an increased output rate for an alcohol plant. The effect of the supplementation with urea is manifested by a significant increase in viable yeast cell numbers, which is vital for achieving faster rates of sugars consumption and fermentation. However, final ethanol yields were not reduced and were calculated for 14% moisture grains at 356–363 L/tonne of rye, and 362–367 L/tonne of triticale. Fermentation efficiencies, which were 90.2–91.4% for triticale and 91.3–92.9% for rye, were also not affected significantly by urea supplementation. The fermentation efficiencies achieved on rye and triticale were similar to those obtained from wheat, which have been widely reported between 90 and 95%. Based on these results, the replacement of wheats by cheaper crops such as rye and triticale for fuel alcohol production should provide good economic opportunities and alternatives for the industry while wheat prices are high. This work may indicate an important step toward regular use of these feedstocks in this industry.

### ACKNOWLEDGMENTS

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