Journal of Industrial Microbiology (1995) 15, 125–130

Production of fuel alcohol from oats by fermentation

KC Thomas and WM Ingledew

Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8

Very high gravity (>30 g dissolved solids per 100 ml) mashes were prepared from hulled and hulless oats and fermented at 20° C with active dry yeast to produce ethanol. Excessive viscosity development during mashing was prevented by hydrolyzing β-glucan with crude preparations of 'β-glucanase' or 'Biocellulase'. Both these preparations possessed endo-β-glucanase activity. By using these enzymes and by decreasing the water to grain ratio, very high gravity mashes with low viscosity were prepared. Unlike wheat and barley mashes, oat mashes contained sufficient amounts of assimilable nitrogen to promote a fast rate of fermentation. The free amino nitrogen (FAN) content of oat mash could be predicted by the equation, mg FAN L^{-1} = 8.9 *n* where *n* is the number of grams of dissolved solids in 100 ml of mash supernatant fluid. Ethanol yields of 353.2 ± 3.7 L and 317.6 ± 1.3 L were obtained per tonne (dry weight basis) of hulless (59.8% starch) and hulled (50.8% starch) oats respectively. The efficiency of conversion of starch to ethanol was the same in normal and very high gravity mashes.

Keywords: oats fermentation; mash viscosity; β-glucanase; very high gravity; fuel alcohol

Oats have not received serious attention as a viable raw material for fuel alcohol production because of their low starch and high β-glucan contents. Moreover hulls which may be as high as 34% of the grain weight [5] cannot be economically converted to fermentable substrates by the technology available today. Some of the above concerns have been overcome with the development of hulless oats although they have yet to become a widely cultivated crop. The starch content of hulless oats is comparable to that of wheat and the absence of hulls facilitates the preparation of mash and significantly increases fermentor throughput. The composition of hulless oats is expected to be similar to that of oat groats (dehulled oats). Analysis of groats from a number of oat varieties has shown that β-glucan contents range between 4.5 and 5.5% [7]. They are rich in pentosans [2] which in some grains are known to be a primary cause of viscosity [4]. Pentosan content of oat groats is usually higher than their β-glucan content [2]. Analysis of 19 genotypes has shown that starch content of oat groats varies between 49.0 and 75.2% [2]. Similarly the protein content of oat groats has been reported to range from 15 to 20% [15].

One of the practical difficulties in using oats is the development of viscosity during mashing through solubilization of β-glucan and pentosans and the subsequent formation of gels. Although mashes with low dissolved solid contents (15–20 g per 100 ml) can be prepared and fermented, these mashes have high viscosities and they yield very low concentrations of ethanol (6–9% v/v). Considerable difficulties are experienced in handling mashes of higher specific gravity (pumping) and in fermentation (trapping of CO_2 in viscous mash and consequent mash lifting) because of high viscosity. The presence of large amounts of residual hull materials in the mash aggravates these problems. Raising the dissolved solids contents to high levels and applying very high gravity (VHG) fermentation technology for fuel alcohol production can be achieved only if the viscosity of the mash can be reduced. In this communication we report that both hulled and hulless oats can be processed to yield VHG mashes with dissolved solids contents in excess of 30 g per 100 ml and that such mashes can be easily fermented to completion.

Correspondence: Dr WM Ingledew, Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8
Received 8 December 1994; accepted 15 May 1995

Materials and methods

Oats

Hulled feed oats were purchased from a local store and hulless oats (variety Terra) were obtained from Dr PD Brown, Agriculture Canada, Winnipeg. The proximate analysis of these oats is listed in Table 1.

Enzymes and reagents

All routine chemicals were of reagent grade and purchased from local suppliers. Active dry yeast, High-T™ (high-temperature α-amylase) and Allcoholase II™ (glucoamylase)

Table 1 Major constituents of hulled and hulless oats on a per cent dry weight basis

Constituents	Oats	
	Hulled[a]	Hulless[b]
Starch	50.8 ± 1.5	59.8 ± 2.0
Protein	10.6 ± 0.4	16.3 ± 0.5
Lipids	5.0 ± 0.2	6.4 ± 0.2
β-glucan	3.0 ± 0.1	5.7 ± 0.2
Ash	2.9 ± 0.1	1.9 ± 0.1

[a]Originally contained 6.65 ± 0.1% moisture
[b]Originally contained 8.05 ± 0.1% moisture

were supplied by the Alltech Biotechnology Center, Nicholasville, KY, USA. The high-temperature α-amylase preparation contained 11.4 mg protein per ml and had a specific activity of 1.4 g starch (hydrolyzed) min^{-1} mg^{-1} protein at 80° C. The glucoamylase preparation contained 106 mg protein per ml and had a specific activity of 1.9 mg glucose (produced) min^{-1} mg^{-1} at 30° C (10.6 μmol glucose min^{-1} mg^{-1} protein). The 'β-glucanase' was a crude enzyme preparation derived from *Aspergillus niger* (GNC Bioferm, Saskatoon SK, Canada) and it was formulated as a powder. 'Biocellulase' was a liquid enzyme preparation (designated as Biocellulase TRI, Quest International, Sarasota, FL, USA) with considerable 'β-glucanase' activity. The activities of these latter two enzymes were compared by an arbitrary method based on viscosity reduction of a 0.5% solution of purified β-glucan (described below).

Grinding and mashing of the oats

Oat mashes were prepared by the same procedure described previously for the preparation of wheat mashes [17] with minor modifications. The oats were ground with a plate grinder (Disk Mill S.500, Glen Mills Inc, Clifton, NJ, USA) at a setting of 5. Eighty per cent of the ground oat endosperm had a particle size in the range of 20–80 mesh; the rest was smaller than this size range. Hull materials had a greater particle size and their large size facilitated the preparation of mash. The detailed procedure involved warming the required amount of water to 45° C and adding either a preparation of β-glucanase or Biocellulase to the water. In most mashings, 0.2 g of the crude enzyme preparation (β-glucanase or Biocellulase) was added per kilogram of oats. Immediately after the dispersal of the enzyme, ground oats were added to the water and mixed, with continuous stirring. After 30 min, the temperature of the slurry was raised to 60° C. Ten millilitres of 100 mM calcium chloride solution were added for each liter of water used for mashing, followed by 5.0 ml of high-temperature α-amylase per kg of oats. The starch was then gelatinized by raising the temperature of the slurry to 95° C and holding it for 45 min. The volume lost through evaporation was made up by adding sterile distilled water. Little or no make-up water was added when the dissolved solids contents were to be raised beyond the level possible with the original water to grain ratio. After gelatinization of the starch, the temperature of the mash was lowered to 80° C and the liquefaction continued by the addition of another 5.0 ml of high-temperature α-amylase per kg of oats. The quantity of high-temperature α-amylase used and the liquefaction time chosen were such that all of the starch in the mash was converted to soluble dextrins and oligosaccharides. After 30 min, the stirring was stopped and the extract which separated on standing was removed by decantation. The residue was sparged with water and the dissolved solids adhering to the residue recovered. Alternatively the whole mash could be filtered and the residue rinsed. Mashes prepared from hulless oats were fermented directly without separation of insoluble materials.

Fermentation

The liquefied mash was saccharified with glucoamylase and fermented at 20° C as described for wheat [17,19]. In most of the experiments, 500-g samples of oat mashes free of hull materials were fermented at 20° C with or without 16 mM urea as the nutrient supplement. Mashes were transferred to sterile Celstir fermentors (Wheaton Scientific, Millville, NJ, USA) which contained 10 ml distilled water or 10 ml solutions of urea. To each fermentor, the required amount of glucoamylase proportional to the dissolved solids contents of the mash (1.2 ml per 500 g mash of 20° P) was added to saccharify dextrins to fermentable sugars. Thirty minutes after the addition of glucoamylase, the temperature of the mash was lowered to 20° C and inoculated with active dry yeast. The active dry yeast was preconditioned by suspending 11 g of active dry yeast in 99 ml of sterile 0.1% peptone water and incubating in a water bath at 38° C for 20 min. Appropriate volumes of the yeast suspension were transferred so that the inoculation rate was 1.0×10^8 cells per g of dissolved solids in the mash. This level of inoculation is equivalent to the recommended pitching rate of 10^6 cells per ml per °P used in the brewing industry [6].

Fermentation progress

Progress of fermentation was monitored at regular intervals by measuring the disappearance of dissolved solids from the mash. Samples collected at various times were centrifuged ($10300 \times g$) for 15 min, and the specific gravity of the clear supernatant liquids at 20° C was measured with a digital density meter (DMA-45, Anton Paar Graz, Austria). With the aid of appropriate tables [14], dissolved solids contents of the supernatant phases were estimated from the specific gravities.

Viscosity measurements

Viscosities of the mashes at 30° C were measured with a starch tester (Model AV 30, Haake Inc, Saddle Brook, NJ, USA) and the relative viscosity expressed in Brabender Units (BU) [16]. Liquefied mash prepared from hulled oats was strained through a double layer of cheese cloth to remove large particulate materials and 450 g of the liquid portion was used for the viscosity determination. In the case of hulless oat mash, viscosity measurements were made without the removal of particulate materials. Hydrolysis of β-glucan by 'β-glucanase' and 'Biocellulase' was tested by measuring reduction in viscosity of a 0.5% β-glucan solution in water (w/w). The final concentrations of enzymes in the test solutions were 0.02% (w/v). A sample of β-glucan prepared from oats by the method of Bhatty [3] was dissolved by heating to boiling. The β-glucan solution was then cooled to 40° C and filtered through Whatman 1 filter paper. The viscosity at 40° C was measured with a Cannon-Fenske Viscometer (Viscometer no 100, Cannon Instrument Co, State College, PA, USA) and the results expressed in centistokes (cs).

Analyses

Total free amino nitrogen (FAN) in the supernatant liquid was determined by the ninhydrin method of the European Brewery Convention [1]. Ethanol was measured enzymatically by the alcohol dehydrogenase assay (Sigma Bulletin no 331, Sigma Chemical Co, St Louis, MO, USA) [17]. Free amino acids, glycerol, sugars and dextrins were meas-

ured by high performance liquid chromatographic methods described previously [20,21].

Results

Viscosity of oats mash can be lowered by enzyme treatment

The viscosity of the hulless oat mash prepared with a water to grain ratio of 3 : 1 by weight and without the aid of viscosity-reducing enzymes was considerably higher than that observed with wheat mash prepared under similar conditions. A typical mash prepared in 3 kg (2.25 kg water + 0.75 kg ground oats) quantities and without making up for the water lost through evaporation had a dissolved solids content of over 28 g per 100 ml and a relative viscosity between 1180 and 1220 BU. Treating the mash with 0.2 g β-glucanase or Biocellulase per kg of oats reduced the relative viscosity to 120 BU at rates of 6.5–7.2 BU per second. Both enzymes possessed β-glucanase activity as shown by the reduction in viscosity of a β-glucan solution. The β-glucan solution was prepared by dissolving 0.5 g of purified β-glucan in water and making up the weight of the solution to 100 g. The β-glucan solution at 40° C had a viscosity of 1.213 centistokes (cs) and the final viscosities after treatment with 0.02% (w/w) β-glucanase or Biocellulase for 60 min were 0.343 and 0.344 cs respectively. The viscosity of distilled water at this temperature was 0.323 cs.

β-glucan hydrolyzing enzymes can decrease viscosity during the preparation of oat mash

For the mashings reported here, only β-glucanase was used but Biocellulase and a number of other similar enzyme preparations can be substituted for β-glucanase. By adjusting the water to oats ratio it was possible to select a particular dissolved solids content for the prepared mash. A few examples are given in Table 2. A mash prepared with a water to hulled oats ratio of 3 : 1 had a dissolved solids content very similar to normal gravity wheat mash [17,21]. These mashes were prepared in 3-L quantities with or without adding water to make up for the volume lost through evaporation. The ground oats contained large amounts of hull materials not ground to the same particle size as the other parts of the grain during milling. Hulls were more or less cleaved longitudinally into thin fibrous strands big enough to be seen with the naked eye. It was possible to grind the hull materials to finer particles (smaller than 100 mesh size) by using another size setting on the grinder. This, however, would not have improved alcohol yield. The presence of larger hull materials aided the mashing procedure, since the whole mash became easy to filter or even to decant, thereby removing unwanted hull materials before the extract was transferred to fermentors. The viscosities of the resulting enzyme-treated extracts were usually less than 10 BU even when the mash was prepared with a reduced water to oats ratio of 2 : 1. This also suggested that filtration removed residual amounts of viscosity-causing components present in hulled oat mash. Hulless oat mashes were not filtered. This and the fact that hulless oats contained 1.9 times more β-glucan than hulled oats (Table 1) may account for the slightly increased viscosities of these mashes. Even at the highest viscosity observed (520 BU) mashes had a consistency that was not difficult to handle. The concentration of dissolved solids in a mash was a function of water to grain ratio, the starch content of the grain and the amount of water added to make up for the volume lost through evaporation.

Carbohydrate composition of oats mash

Carbohydrate analysis of a hulled oat mash showed that 90% of the dissolved solids in the mash were carbohydrates (Table 3). Mashes prepared from hulless oats had similar carbohydrate contents and sugar percentages. The residual amount of dextrin present in the mash was resistant to enzyme hydrolysis and its concentration therefore remained unchanged during fermentation. Glucose constituted 76% of the dissolved solids. During fermentation, virtually all of the glucose was consumed and a small quantity of glycerol was produced.

Fermentation of oat mash

Three oat mashes listed in Table 4 were used in this study. When the initial dissolved solids content of mash did not exceed 33 g per 100 ml, fermentations at 20° C were competed in a time proportional to the initial dissolved solids contents (Figure 1a). Fermentation was incomplete when

Table 2 Dissolved solids contents and viscosities of mashes prepared from hulled and hulless oats processed with the aid of β-glucanase

Type of oats	Water to grain ratio	Water made up[a]	Viscosity (BU)	Dissolved solids in the mash (g per 100 ml)
Hulled	3 : 1	yes	<10	15.9 ± 0.5
	3 : 1	no	<10	21.6 ± 0.4
	2 : 1	yes	<10	23.9 ± 0.5
	2 : 1	no	<10	29.4 ± 0.6
Hulless	3 : 1	no	110	28.4 ± 0.4
	2.5 : 1	no	280	33.6 ± 0.3
	2 : 1	yes	100	26.9 ± 0.6
	2 : 1	no	520	42.3 ± 0.4

[a]Volume lost through evaporation was made up by adding sterile water

Table 3 Carbohydrate and glycerol contents of a mash at the beginning and at the end of fermentation[a]

Component	Initial (g per 100 ml)	Final (g per 100 ml)
Dextrin	1.6 ± 0.01	1.4 ± 0.11
Maltotriose	1.1 ± 0	0
Maltose	0.3 ± 0.10	0.4 ± 0.11[b]
Glucose	16.4 ± 0.66	0.5 ± 0.13[b]
Glycerol	0.05 ± 0	0.9 ± 0.19
Total	19.5	3.2

[a]The initial dissolved solid content of the mash prepared from hulled oats was 21.6 g per 100 ml (liquid portion)
[b]Residual amounts are not reducing sugars. They are probably compounds that co-elute with maltose and glucose respectively

oat mash (Table 5). The oat mash was prepared from hulled oats, and contained 29.4 g dissolved solids per 100 ml. Total free amino acid content of this mash was 10.9 mmol L^{-1} (152.6 mg α-amino nitrogen per liter). On the basis of total nitrogen available (α-amino nitrogen plus other catabolizable nitrogen available in certain amino acids), arginine was the most important amino acid in the mash. Arginine initially constituted about 30% of the total assimilable nitrogen in the mash.

Ethanol yield

Ethanol yield from oats was determined by preparing mashes from hulled oats with a water to grain ratio of 2 : 1, and fermenting the whole mash without sampling or removing of insoluble materials. The fermentation was carried out in duplicate at 20° C with 500-g quantities of mashes. The mashes were inoculated with preconditioned active dry yeast as described in Materials and Methods. At the end of fermentation (72 h) the total fermented mash was distilled and the distillate collected and made up to a known volume. The ethanol yield was calculated by determining the concentration of ethanol in a measured volume of distillate. On a dry weight basis, the hulled oats which contained 50.8% starch (Table 1) yielded 317.6 ± 1.3 L of ethanol per tonne (296.5 ± 1.2 L per tonne of moist grain). Ethanol yield from hulless oats was determined in a similar manner. Two kinds of mashes were prepared, one at a 'normal' gravity (19.9 g dissolved solids per 100 ml) and the other at very high gravity (37.2 g dissolved solids per 100 ml). The respective ethanol yields obtained from the normal gravity and very high gravity mashes were 357.4 ± 2.1 and 353.7 L per tonne of oats (dry weight basis). On a moist (harvested) grain basis these values corresponded to ethanol yields of 328.6 ± 2.0 and 323.7 ± 3.4 L respectively.

Table 4 Ethanol yields and FAN utilization from oat mashes of various dissolved solids contents

Initial dissolved solids (g per 100 ml)	Dissolved solids consumed[b] (g per 100 ml)	Ethanol % (vol/vol)	Initial FAN[a] (mg L^{-1})	FAN utilization[a,b] (mg L^{-1})
21.6[c]	21.6 (100)	10.5	190	156 (82)
33.6[d]	33.6 (100)	16.0	282	198 (70)
42.3[d]	34.2 (81)	16.0	373	195 (52)

[a]FAN values of unsupplemented mashes only are given. The FAN uptake was the same in urea-supplemented samples except when the dissolved solids content was 42.4 g per 100 ml
[b]Amounts in parentheses represent the amount consumed expressed as % of the original substrate
[c]Mash from hulled oats
[d]Mash from hulless oats

the dissolved solids content of the mash was very high (42 g per 100 ml). Supplementing mashes with 16 mM urea did not stimulate fermentation (Figure 1a). This is in contrast to the dramatic stimulatory effect of assimilable nitrogen supplementation on fermentation of barley and wheat mashes [8,17,18]. Normally supplementing nitrogen-deficient mashes with 16 mM urea stimulates fermentation by increasing the yeast growth. The amount of dissolved solids (sugars) consumed and the volumes of ethanol produced were proportional to the initial dissolved solids concentration up to a maximum value of 33–34 g per 100 ml (Table 4).

Increasing the mash dissolved solids concentration to 42 g per 100 ml did not appreciably increase the sugar consumption or the ethanol yield over those observed for a mash with 33 g dissolved solids per 100 ml (Table 4). The FAN contents of oat mashes were proportional to their dissolved solids contents. The percentage of FAN taken up by the yeast, however, decreased with increasing gravity of the mash (Table 4). The rate and extent of FAN uptake were less in urea-supplemented VHG mashes than in the unsupplemented controls. The FAN content of all urea-supplemented samples slightly increased towards the end of fermentation (Figure 1b), a phenomenon reported previously [21].

Eight amino acids (asp, glu, asn + gln, ala, arg, pro and leu) constituted over 70% of the free amino acids in the

Discussion

Oats have always been considered less suitable for the production of fuel alcohol because of their low starch contents. However, the yield of oats per acre is 25–30% higher than that of wheat (Saskatchewan Agriculture and Food Report no 27, October 1994) and the yield per acre of oat starch is therefore comparable to, if not higher than that of wheat

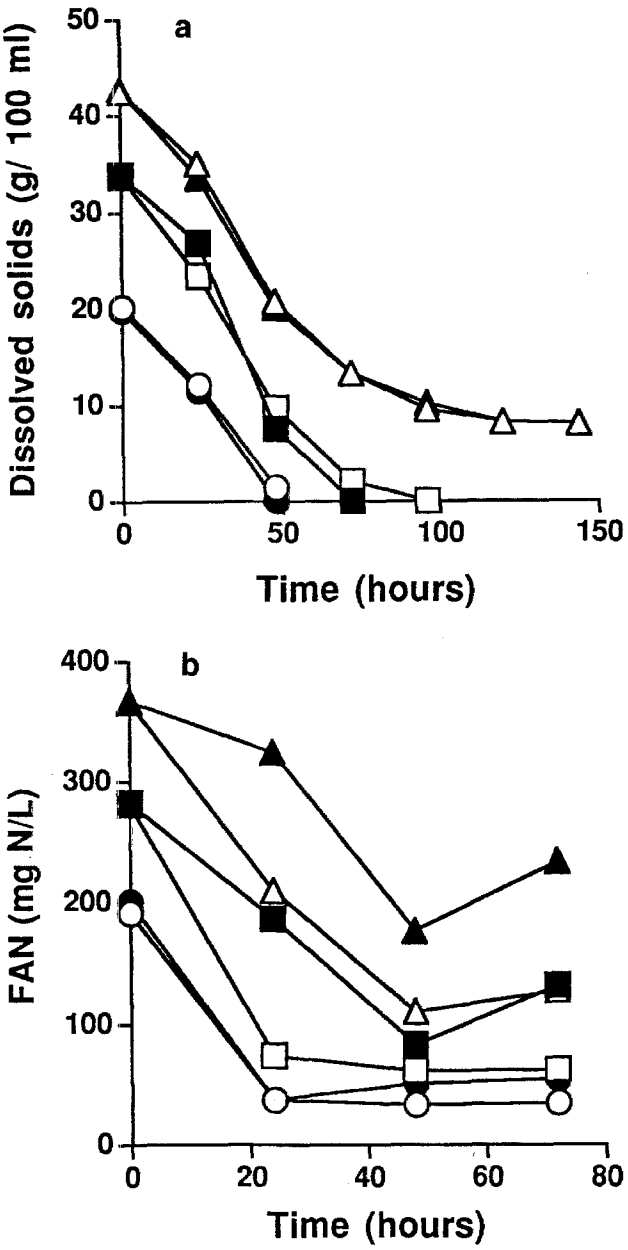

Figure 1 Rates of utilization of dissolved solids (sugars) (a), and rates of uptake of free amino nitrogen (FAN) (b) by *Saccharomyces cerevisiae* during fermentation of normal and very high gravity oat mashes. Mash gravities are expressed as g of dissolved solids per 100 ml. ○,● 21.6 g; □,■ 33.0 g; △,▲ 42.3 g. Open symbols denote unsupplemented mashes and closed symbols denote mashes supplemented with 16 mM urea

starch. In addition, unlike wheat, oats can be grown on poorer soils and under mildly adverse conditions such as drought. The results reported here clearly show that the main objections against the use of oats as a raw material for fuel alcohol production can be overcome. While both hulled and hulless oats can be used to prepare VHG mashes, hulled oats offer certain advantages during mash preparation. The hulls, along with other suspended particles, can be easily removed by filtration or decantation and then rinsed to remove and recover dissolved solids from the wet grains. Removal of the hull materials significantly increases the fermentor output (amount of grain that can be handled and alcohol produced per unit time in each fermentor).

Table 5 Amino acid composition of oat mash

Amino acid	mmol L^{-1}	%
asp	1.209	10.5
glu	0.689	6.0
ser	0.540	4.7
asn + gln	2.096	18.3
gly	0.399	3.5
his	0.174	1.5
thr	0.168	1.5
ala	1.071	9.3
arg	1.285	11.2
pro	0.605	5.3
tyr	0.276	2.4
val	0.617	5.4
met	0.096	0.8
cys	0.014	0.1
ile	0.286	2.5
leu	0.853	7.4
phe	0.421	3.7
trp	0.160	1.4
orn	0.063	0.5
lys	0.450	3.9

Total α-amino nitrogen = 152.6 mg L^{-1}
Total nitrogen available from free amino acids = 250 mg L^{-1}

Much of the difficulty in preparing oat mash is associated with its high β-glucan content, which when heated in water forms a gel resulting in increased viscosity. The development of viscosity during the preparation of mashes can be prevented if the ground oat–water slurry is treated with enzymes such as β-glucanase or Biocellulase prior to starch gelatinization. Both these enzyme preparations are endo enzymes since no demonstrable release of monomers (glucose) was observed (data not shown).

It is possible to prepare VHG mashes from oats simply by adjusting the water to grain ratio and hydrolyzing β-glucan prior to gelatinization of the starch. Decreasing the water to grain ratio to 2 : 1 did not negatively affect gelatinization or hydrolysis of starch although this has been reported to occur during the preparation of malt extract [12,13]. Thus, VHG mashes from oats may be prepared in one step without resorting to the more cumbersome double mashing procedure or to addition of adjuncts [9,11,17].

From a very high gravity mash of 42 g per 100 ml only about 81% of the 42 g of dissolved solids was fermented, suggesting that this amount may be at or near the upper limit of sugar concentration that this yeast can utilize. The amount of sugar that can be fermented in a VHG mash, however, is a function of the initial concentration of dissolved solids, the type of carbohydrate (dextrins or glucose), the availability of all required nutrients for yeast growth [19,21], the fermentation temperature [10,21], and the presence or absence of particulate materials and osmoprotectants [22]. The maximum amount of dissolved solids that can be fermented in oat mash at 20° C without the addition of any osmoprotectants appears to be 37–38 g per 100 ml. This is the same value reported for wheat mash

under similar fermentation conditions [21]. When dissolved solids concentrations were higher than 38% (w/v), the amount that was fermented actually declined, possibly resulting from a combination of the osmotic effects and ethanol toxicity.

One important distinction of oat mash is its high FAN content. An oat mash contained approximately three times as much FAN as a wheat mash of equivalent dissolved solids content. The amount of FAN in a given mash prepared from these oats was proportional to the dissolved solids content; and the FAN content could be predicted by the equation

$$\text{mg FAN L}^{-1} = 8.9\,n$$

where n is the number of grams of dissolved solids per 100 ml of mash supernatant fluid or filtrate. The stimulatory effects of exogenously added yeast extract (data not shown) or urea on fermentation were negligible suggesting that oat mash was not limiting in assimilable nitrogen. This is in sharp contrast to the nitrogen-limiting nature of barley and wheat mashes [8,17] and this is the first report of a grain mash which has not been deficient in yeast-utilizable nitrogenous materials. Nearly 30% of the total available nitrogen was provided by arginine, an amino acid most favored during ethanolic fermentation [18,20]. All these factors suggest that oat mash is an excellent medium for fuel alcohol production by fermentation.

Acknowledgements

We thank Dr R Bhatty and Dr B Rossnagel for helpful suggestions and Dr R Bhatty for analytical help. Anita Dhas and Sandra Hynes are thanked for technical assistance. The Western Grain Research Foundation and the Natural Research Science and Engineering Council provided research support.

References

1 Anonymous. 1987. Free amino nitrogen. In: Analytica-EBC (4th edn), European Brewery Convention, pp E141–E142.
2 Bhatty RS. 1992. Total and extractable β-glucan contents of oats and their relationship to viscosity. J Cereal Sci 15: 185–192.
3 Bhatty RS. 1993. Extraction and enrichment of (1→3), (1→4)-β-D-glucan from barley and oat brans. Cereal Chem 70: 73–77.
4 Boros D, RR Marqardt, BA Slominski and W Guenter. 1993. Extract viscosity as an indirect assay for water-soluble pentosan content in rye. Cereal Chem 70: 575–580.
5 Burrows VD. 1986. Breeding oats for food and feed: conventional and new techniques and materials. In: Oats: Chemistry and Technology (Webster FH, ed), pp 13–46, American Association of Cereal Chemists, St Paul, Minnesota.
6 Casey GP and WM Ingledew. 1985. Reevaluation of alcohol synthesis and tolerance in brewers yeast. J Am Soc Brew Chem 43: 75–83.
7 Cho KC and PJ White. 1993. Enzymatic analysis of β-glucan content of different oat genotypes. Cereal Chem 70: 539–542.
8 Ingledew WM, AM Jones, RS Bhatty and BG Rossnagel. 1995. Fuel alcohol production from hulless barley. Cereal Chem 72: 147–150.
9 Jones AM and WM Ingledew. 1994. Fuel alcohol production: appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation. Process Biochem 29: 483–488.
10 Jones AM and WM Ingledew. 1994. Fuel alcohol production: optimization of temperature for efficient very-high-gravity fermentation. Appl Environ Microbiol 60: 1048–1051.
11 Jones AM, KC Thomas and WM Ingledew. 1994. Ethanolic fermentation of blackstrap molasses and sugar cane juice using very high gravity technology. J Agric Food Chem 42: 1242–1246.
12 Muller RE. 1989. The importance of water in gelatinization of starch and amylolysis during mashing. European Brewery Convention. Proceedings of the 22nd Congress, Zurich 22: 283–290.
13 Muller R and E Canterranne. 1994. Activity of amylolytic enzymes in thick mashes. J Am Soc Brew Chem 52: 56–61.
14 Pancoats HM and WR Junk. 1980. Handbook of Sugars. 2nd edn, pp 53–62, AVI Publishing Company, Westport, Connecticut.
15 Peterson DM and AC Brinegar. 1986. Oat storage proteins. In: Oats: Chemistry and Technology (Webster FH, ed), pp 153–203, The American Association of Cereal Chemists, St Paul, Minnesota.
16 Shuey WC and KH Tripples. 1980. The Amylograph Handbook. pp 33–37, The American Association of Cereal Chemists, St Paul, Minnesota.
17 Thomas KC and WM Ingledew. 1990. Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. Appl Environ Microbiol 56: 2046–2050.
18 Thomas KC and WM Ingledew. 1992. Relationship of low lysine and high arginine concentrations to efficient ethanolic fermentation of wheat mash. Can J Microbiol 38: 626–634.
19 Thomas KC and WM Ingledew. 1992. Production of 21% (v/v) ethanol by fermentation of very high gravity (VHG) wheat mashes. J Ind Microbiol 10: 61–68.
20 Thomas KC, SH Hynes and WM Ingledew. 1993. Excretion of proline by *Saccharomyces cerevisiae* during fermentation of arginine-supplemented high gravity wheat mash. J Ind Microbiol 12: 93–98.
21 Thomas KC, SH Hynes, AM Jones and WM Ingledew. 1993. Production of fuel alcohol from wheat by VHG technology: effect of sugar concentration and fermentation temperature. Appl Biochem Biotechnol 43: 211–226.
22 Thomas KC, SH Hynes and WM Ingledew. 1994. Effects of particulate materials and osmoprotectants on very-high-gravity ethanolic fermentation. Appl Environ Microbiol 60: 1519–1524.