Wheat Milling By-products Fermentation: Potential Substrate for Bioethanol Production

Marcos Antonio das Neves[1], Naoto Shimizu[1], Toshinori Kimura[2], Kiwamu Shiiba[3]

1. Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Japan

2. Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo. Hokkaido, Japan

3. Nisshin Flour Milling Co. Ltd. 25, Kanda-Nishiki-cho 1-chome, Chiyoda-ku, Tokyo, Japan

Abstract: An overview on the potential application of wheat milling by-products for bioethanol production is made. The fermentation performance of low-grade wheat flour (LG) and wheat bran (WB) was evaluated and compared to wheat flour (WF). α-amylase or cellulase was used for liquefaction, followed by simultaneous saccharification and fermentation (SSF) by glucoamylase and *Zymomonas mobilis*. The final ethanol concentration, overall productivity and yield obtained from LG (51.4 g ethanol/L, 2.72 g ethanol/L·h and 0.17 g ethanol/g flour, respectively) were considerably higher compared to WB (18.1 g/L, 1.09 g/L·h and 0.02 g/g). High LG fermentation rates, reaching the highest ethanol productivity (4.4 g/L·h) within 6 h of SSF, indicated considerable savings on fermentation time, compared to current industrial processes. [Life Science Journal. 2006;3(2):83-87] (ISSN: 1097-8135).

Keywords: wheat; flour; by-product; bran; fermentation; ethanol

Abbreviations: LG: low-grade wheat flour; μ: growth rate; P: ethanol production; Q: ethanol productivity; Q_V: overall volumetric ethanol productivity; R_P: solid residue; SSF: simultaneous saccharification and fermentation; WB: wheat bran; WF: wheat flour; Y_L: liquefaction yield; $Y_{P/S}$: ethanol yield

1 Introduction

With the search for alternative renewable energy sources, biofuels are becoming a viable solution, as they are non-fossil fuels from renewable sources. Of all biofuels, ethanol is already produced on a fair scale worldwide. The bulk of the production is located in Brazil and the USA.

Various studies on ethanol conversion systems from wheat products have been conducted based on the utilization of raw wheat flour or damaged wheat grains (e.g. Montesinos & Navarro 2000, Suresh et al. 1999). However, only a few reports on wheat milling by-products fermentation are available (e.g. Adrados et al. 2005). Thus, the objectives of this study were to develop a simultaneous saccharification and fermentation (SSF) process in batch mode for wheat milling by-products and to evaluate the fermentation performance of low-grade wheat flour (LG) and wheat bran (WB), compared to wheat flour (WF).

2 Materials and Methods

2.1 Materials

Raw material: LG, WB and WF. Typical composition was summarized in Table 1.

Bacterial cells: *Zymomonas mobilis* NBRC 13758.

Enzymes: α-amylase (51 U/mg, Sigma, USA); glucoamylase (23 U/mg, Sigma, USA); cellulase (106 U/mg, MP Biochemicals).

Liquefaction: One liter slurries containing 200 g dried matter/l of LG (pH 6.1), WB (pH 5.9) or WF (pH 5.7) were hydrolyzed separately using 400 U α-amylase/g flour (in the case of LG or WF) or cellulase (for WB) at 55 ℃, 100 rpm for 2 h.

SSF: 200 U glucoamylase/g flour and 100 ml starter culture (3.1×10^8 viable cells/ml) were added to the liquefied slurry and the SSF was conducted in a previously sterilized 2 liter jar fermentor (Marubishi) at 35 ℃, 100 rpm, pH 4.5 in anaerobic environment sparging N_2 gas (100 ml/min).

2.2 Analytical methods

Glucose, maltose, and ethanol were deter-

mined using HPLC (Shiiba et al. 1993). The reducing sugars were calculated based on the sugar distribution obtained by HPLC. They were composed of monosaccharides (glucose, arabinose and xylose) or disaccharides (maltose and cellobiose). The liquefaction yield (Y_L) was calculated as the quotient of the amount of maltose released during liquefaction by the initial substrate.

The fermentation performance was evaluated based on ethanol concentration (P), productivity (Q), overall volumetric productivity (Q_V), yield ($Y_{P/S}$), and solid residue (R_P). Q_V was calculated as the quotient of the total ethanol production by the fermentation time. $Y_{P/S}$ was defined as the quotient of the ethanol produced by the initial substrate. An aliquot of the final product was centrifuged (4, 000 rpm, 20 min), and the solid residue dried in order to evaluate the residue formation (R_P).

Table 1. Fermentation performance of various wheat milling products

Substrate	LG	WB	WF
Raw material			
Substrate concentration (g/L)[a]	200	200	200
Protein (%) (w/w)[b]	15.0	13.3	10.4
Starch (%) (w/w)[c]	15.6±0.09	11.7±0.25	62.0±0.04
Moisture (%) (w/w)	14.0±0.15	12.2±0.19	13.1±0.01
Ash (%) (w/w)	2.7±0.01	5.6±0.04	0.6±0.15
Final product			
Ethanol concentration (P)(g/l)[d]	51.40±0.37	18.10±3.17	68.10±1.43
Supernatant (ml)	600	320	760
Solid residue (R_P) (g dry matter)	28.50±2.69	111.20±8.31	8.30±0.93
Ash (%) (w/w)	3.90±0.04	18.70±0.21	7.70±0.42
Ethanol yield ($Y_{P/S}$) (g ethanol/g flour)	0.17±0.01	0.02±0.08	0.30±0.03
Overall volumetric ethanol productivity (Q_V) (g/L·h)	2.72±0.04	1.09±0.21	3.64±0.08
Growth rate (μ)(1/h)[e]	0.119	0.142	0.043

[a] 1 liter slurries were prepared for each substrate, separately

[b] Protein content (total nitrogen×5.7); provided by Nisshin Flour Milling Co. Ltd.

[c] Composition assays performed as described elsewhere (Neves et al. 2006). Mean±S.D. of three experiments

[d] Before centrifugation (5,000 rpm for 30 min)

[e] Growth rate was calculated by linear regression of microbial growth curves (Slope=μ/2.303)

3 Results and Discussion

3.1 Liquefaction

The LG liquefaction yield was 0.065 ±0.002 g maltose/g flour (Mean ± S.D., n = 3 repetitions), followed by WB (0.010 ±0.005 g/g), compared to the reference substrate WF (0.126 ± 0.021 g/g).

The Y_L from LG was nearly six fold that from WB, evidencing the lower initial starch content in WB. Besides releasing glucose (the major product of WB hydrolysis using cellulase), arabinose and xylose may also be produced, as mentioned in previous literature (Adrados et al. 2004; Shiiba et al. 1993). The sugar content during WB liquefaction fluctuated considerably between replicates. This behavior was likely caused by WB pentosans. They are known to have low water solubility, thus decreasing their availability for enzymatic hydrolysis (Adrados et al. 2004). For instance, while amylopectin is soluble in water, the starch granules itself as well as amylose are insoluble in cold water.

In the case of LG, maltose concentration was nearly stable after 2 h liquefaction, indicating the end of starch hydrolysis. This is in line with the results reported by Montesinos et al. (2000). They demonstrated that liquefaction during 2 h was necessary for efficient hydrolysis of glucose polymers when slurries containing 300 g raw wheat flour/L were hydrolyzed at 95 ℃ using thermostable α-amylase.

3.2 Simultaneous saccharification and fermentation

The results of SSF using different wheat products are depicted in Figure 1. The increase in glucose at the SSF onset implies that glucoamylase ac-

tivity was sufficient to release more glucose than *Z. mobilis* could metabolize. However, in the case of LG, after 2 h SSF the glucose level decreased continuously, disappearing at *c. a.* 9 h. Meanwhile, ethanol increased gradually, stabilizing at nearly 18 h.

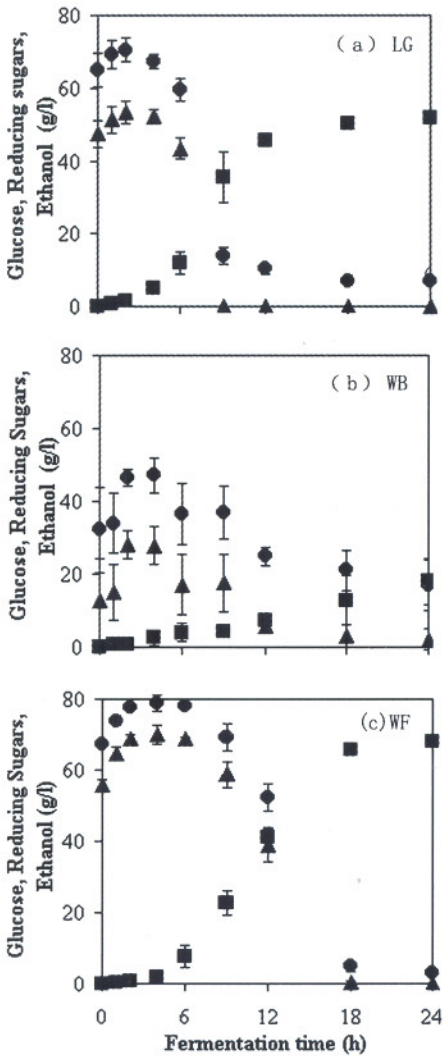

Figure 1. Time-course profiles of simultaneous saccharification and fermentation from various wheat products.
(a) Low grade flour; (b) Wheat bran; (c) Wheat flour.
Symbols: ▲, Glucose; ●, Reducing sugars; ■, Ethanol.
The bars represent the Standard Deviation ($n=3$ replicates)

From Figure 1c it was apparent that besides glucose, other reducing sugars were released during WB hydrolysis, mostly arabinose or xylose, rather than maltose which was rarely detected. These results are in agreement with Shiiba et al. (1993). Those authors reported that hemicellulose (which is mainly consisted of arabinose and xylose) is the major component of WB cell wall polysaccharides. Xylose fermentation involves a metabolic pathway with two enzymes, xylose reductase and xylitol dehydrogenase. *Z. mobilis* is not able to produce these two enzymes, thus the impossibility to ferment pentoses. Some other microorganisms, such as *P. stipitis*, produce these enzymes naturally (Kodaki et al. 2004). Slight amounts of cellobiose, a reducing sugar obtained by partial cellulose hydrolysis, were detected in WB fermentation mash; this is a potential carbon source, since it can be converted to glucose by α-glucosidase, which occurs naturally in wheat products.

Experimental microbial growth rate (μ) values indicated that *Z. mobilis* grew faster in WB- or LG-based substrates, rather than in WF. Low μ values obtained for WF (Table 1) suggested that in this case the sugars contained in the fermentation mash were preferably metabolized into ethanol, rather than used for biomass production, substantiated by high P and low R_P values.

3.3 Fermentation performance

The fermentation performance was evaluated using various parameters (P, Q, Q_V, $Y_{P/S}$, and R_P), summarized in Table 1.

Figure 2 shows the ethanol productivity (Q) profiles throughout the SSF process. Q values for WB were lower compared to LG or WF, which was likely due to the presence of pentose sugars in WB. Incomplete starch hydrolysis during WF liquefaction may have caused delay on ethanol production, as well. This was consistent with the hypothesis that starch hydrolysis is made difficult by the increased viscosity during liquefaction, caused by starch granules swelling as well as water penetration (Montesinos & Navarro 2000).

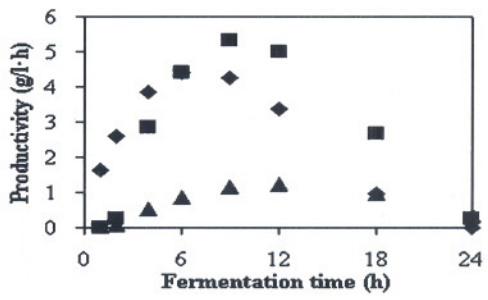

Figure 2. Ethanol productivity (Q) from various wheat products (◆, low grade flour; ▲, wheat bran; ■, wheat flour)
Q was calculated by differentiating the experimental ethanol production data as function of time (Jain et al. 1985).

To gain some perspective on the improvements achieved using different conversion systems and microorganisms, a comparison was provided in Table

2. A combination of high ethanol levels and productivity is desirable in minimizing processing costs. It was apparent that ethanol concentration and yield obtained for LG were relatively low, compared to other systems using WF. Such problems can be overcome, e.g. using fed-batch culture systems, as reported in previous literature (Roble et al. 2003). Those authors were able to reach up to 90 g ethanol/L using a circulating loop bioreactor for SSF of raw cassava starch.

In the year 2000 nearly 6.8 million tons of WF were produced in Brazil (FIBGE, 2001) resulting in about 0.34 million tons of LG. Assuming that this by-product could be fully used as feedstock, the potential bioethanol production from LG becomes 78.2 mega liters.

Table 2. Summary of ethanol production using various substrates and microorganisms

Reactor (volume)	Mode	Culture	Substrate (g/L)	Ethanol (g/L)	Productivity (g/L·h)	Yield (g/g flour)	Source
Jar fermentor (2 L)	Batch (40 h)	Commercial α-amylase, glucoamylase and *S. cerevisiae*	Raw wheat flour (300)	67	3.19	0.45[a]	b
Erlenmeyer (500 mL)	Batch (90 h)	α-amylase (from *B. subtilis*) and *S. cerevisiae*	Fine-wheat flour	44	0.49	0.18	c
			Damaged wheat	34	0.38	0.14	
			Damaged sorghum (250)	27	0.30	0.11	
Circulating loop reactor (9 L)	Fed-batch (600 h)	α-amylase (from *A. awamori*) and *S. cerevisiae*	Raw cassava starch (150)	90	1.17	0.45[a]	d
Jar fermentor (2 L)	Batch (48 h)	Commercial α-amylase, glucoamylase and *Z. mobilis*	LG (200)	51	2.72	0.17	This work
			WB (200)	18	1.09	0.02	
			WF (200)	68	3.64	0.30	

[a] Ethanol yield (g ethanol/g starch); [b](Montesinos et al. 2000); [c](Suresh et al. 1999); [d](Roble et al. 2003)

4 Conclusion

In this work, two wheat milling by-products, LG and WB, were utilized as substrate for bioethanol production. High LG fermentation rates, reaching the highest ethanol productivity (4.4 g/L·h) within 6 h of SSF, indicated considerable savings on fermentation time, compared to current industrial processes.

The present system employing commercial enzymes might be too expensive for commercial bioethanol production, in view of the current market prices of most amylolytic enzymes. This process was used to evaluate the fermentation performance of different wheat milling by-products. Industrial application of this system requires modifications in order to decrease the overall cost, e.g. *in situ* α-amylase production as well as immobilizing the ethanol-producing strain, utilizing fed-batch or continuous fermentation process.

In order to use wheat milling by-products for large scale fermentation, a system for collecting these by-products from the milling site has to be developed, which could be done after LG is recognized as a low cost feedstock.

Acknowledgments

We are thankful to Nisshin Flour Milling Co. Ltd. for providing LG, WB and WF samples.

Correspondence to:
Marcos Antonio das Neves
Graduate School of Life and Environmental Sciences
University of Tsukuba
1-1-1 Tennodai, Tsukuba, Japan
Telephone: 81-29-853-7222
Fax: 81-29-855-2203
Email: maneves2000@yahoo.com

References

1. Adrados BP, Chotěborská P, Galbe M, Zacchi G. Ethanol production from non-starch carbohydrate of wheat bran. Biores Technol 2005; 96: 843 – 50.
2. Adrados BP, Juhász T, Galbe M, Zacchi G. Hydrolysis of nonstarch carbohydrates of wheat starch effluent for ethanol production. Biotechnol Prog 2004; 20: 474 – 9.
3. FIBGE Fundação Instituto Brasileiro de Geografia e Estatística. Anuário Estatístico do Brasil 2000 – 2001 (in Portuguese).
4. Jain WK, Diaz IT, Baratti J. Preparation and characterization of immobilized cells of *Zymomonas mobilis* for

ethanol production Biotechnol. Bioeng 1985; 27: 273 – 9.

5. Kodaki T, Watanabe S, Makino K. Baiomasu no E-tanoru Hankan. Purotein Kougakuteki Apurochi. Eco Industry 2004; 9: 38 – 44 (in Japanese).
6. Montesinos T, Navarro JM. Production of alcohol from raw wheat flour by amyloglucosidase and *saccharomyces cerevisiae*. Enzyme Microb Technol 2000; 27: 362 – 70.
7. Neves MA, Kimura T, Shimizu N, Shiiba K. Production of alcohol by simultaneous saccharification and fermentation of low-grade wheat flour. Brazilian Arch Biol Technol 2006; In press.
8. Roble ND, Ogbonna JC, Tanaka HA. novel circulating loop bioreactor with cells immobilized in loofa sponge for the bio-conversion of raw cassava starch to ethanol. Appl Microbiol Biotechnol 2003; 60: 671 – 8.
9. Shiiba K, Yamada H, Hara H, Okada K, Nagao S. Purification and characterization of two arabinoxylans from wheat bran. Cereal Chem 1993; 70: 209 – 14.
10. Suresh K, Kiransree N, Rao LV. Production of ethanol by raw starch hydrolysis and fermentation of damaged grains of wheat and sorghum. Bioprocess Eng 1999; 21: 165 – 8.

Received March 10, 2006