

Effect of Oil Extraction Method on Enzymatic Digestibility of Corn Germ Arabinoxylan

Timothy D. Leathers^{1,2} and Neil P. J. Price¹

Cereal Chem. 84(3):243–245

Corn (maize or *Zea mays* L.) residues are abundant renewable sources of lignocellulosic biomass. In a biorefinery concept, some of these materials will become substrates for conversion to fuels and chemicals (Johnson and May 2003). A variety of corn residues have been investigated as sources of fermentable sugars including corn fiber, bran, cobs, and stover. However, very little work has been reported on the utilization of defatted corn germ. More than one million tons of corn oil are produced annually in the United States, producing over a half million ton of defatted germ as a by-product (Ash and Dohman 2006). Currently, this material is folded into relatively low-value animal feeds such as corn gluten feed (Johnson and May 2003). More than 90% of corn oil is produced by the wet-milling process that separates the germ from the kernel. The germ contains 45–50% oil (Orthofer et al 2003), which is extracted using solvents, expelling, or a combination of these methods. Defatted corn germ contains high-quality protein, arabinoxylan, residual starch, and cellulose (Lawton and Wilson 2003; Watson 2003). Hespell et al (1997) reported that purified arabinoxylan from corn germ is resistant to digestion by commercial enzymes. However, crude enzyme preparations from *Aureobasidium* sp. strain NRRL Y-2311-1 were effective in producing monosaccharides from defatted corn germ (Leathers 2004). Moreover, defatted corn germ was more susceptible to enzymatic digestion than corn fiber (pericarp), even without chemical pretreatment (Leathers and Gupta 1996). It was suggested that the superior digestibility of corn germ arabinoxylan was either due to its chemical structure or that the oil extraction process itself served as an efficient pretreatment (Leathers 2004). To test this second possibility, whole unextracted corn germ and germ extracted by three different processes were compared for digestibility.

MATERIALS AND METHODS

Materials

Corn germ and defatted germ were the kind gift of Corn Products International (Westchester, IL). In the fully expelled process (no solvent), germ becomes very hot and produces a dark brown oil and a cake (defatted germ). This method is useful for small operations because capital costs are low. Preexpelling is a gentler process in which half of the oil is removed by expelling before hexane extraction. In an expander process, the germ is turned into a mash that is directly solvent-extracted, resulting in a spent flake cake. By proximate analysis, samples

used in this study contained whole germ, 50% fat; preexpelled then solvent-extracted germ, 2% fat; directly solvent-extracted germ, 5% fat; and fully expelled germ, 10% fat. Samples were ground to consistent powders in a coffee mill before analysis or digestion.

Sugar Analysis

Total neutral sugars were estimated after hydrolysis with 2M trifluoroacetic acid (TFA) at 110°C for 2 hr (Morrison 1988). Sugars were measured by HPLC using a Resex 8% calcium column (Phenomenex, Torrance, CA) eluted with water at 0.6 mL/min at 75°C and detected by refractive index. Results are presented as means with standard errors.

Enzymatic Digestions

Samples were suspended in deionized water at 1% (w/v) and boiled for 5 min. Sodium azide (0.02%, w/v) was added as a preservative. Concentrated crude enzymes were prepared from *Aureobasidium* sp. strain NRRL Y-2311-1 as previously described (Leathers and Gupta 1996). Cultures were grown in a defined basal medium on 1% (w/v) corn fiber pretreated with alkaline hydrogen peroxide. Supernatants contained ≈ 90 IU endo- β -1,4-xylanase/mL and lower activities of β -xylosidase, α -L-arabinofuranosidase, acetyl xylan esterase, amylase, cellulase, and protease (Leathers and Gupta 1996). Supernatants were clarified by centrifugation and concentrated 10-fold by ultrafiltration using Centricon filter devices with 10,000 MWCO membranes (Millipore, Bedford, MA). Enzymes were added to 0.8 IU of endoxylanase/mg of germ substrate and digested at 37°C and 220 rpm as previously described (Leathers 2004).

RESULTS AND DISCUSSION

Whole corn germ, preexpelled then solvent-extracted germ, directly solvent-extracted germ, and fully expelled germ were digested as described above with enzymes from *Aureobasidium* sp. strain NRRL Y-2311-1 (Fig. 1). Directly solvent-extracted germ (Fig. 1C) produced ≈ 100 mg each of xylose and arabinose/g of germ, consistent with values previously reported (Leathers 2004). Germ defatted by other methods was similarly digestible, producing ≈ 120 – 140 mg each of pentose sugar/g of germ (Fig. 1B and D). Glucose yields from defatted samples varied at ≈ 200 – 300 mg/g of germ. Most of this glucose is likely derived from starch, the levels of which may vary depending on extraction methods and other factors. Xylose and arabinose levels from whole corn germ (Fig. 1A) were about half those of extracted samples, which is reasonable since whole germ contains $\approx 50\%$ oil. In all cases, the kinetics of sugar production were similar; the bulk of digestion occurred within 24 hr. Thus, the overall conclusion is that all samples were similarly digested by enzymes, suggesting that oil extraction methods do not have a major effect on digestibility.

Maximal enzymatic sugar yields were compared with total neutral sugar compositions as determined by the TFA analysis

¹ National Center for Agricultural Research, USDA, Peoria, IL 61604. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

² Corresponding author. Fax: 309-681-6040. E-mail: leathetd@ncaur.usda.gov

(Table I). TFA is believed to hydrolyze starch and hemicellulose but leave cellulose largely intact (Morrison 1988). However, TFA provides minimal estimates for sugar composition because sugars are degraded to varying extents by the process.

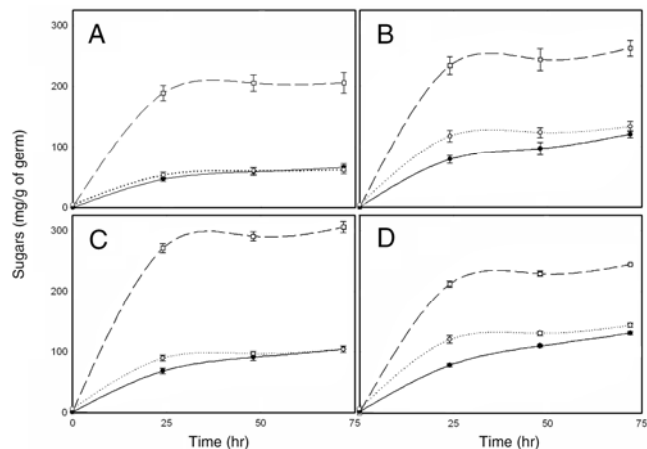


Fig. 1. Digestion of corn germ samples by enzymes from *Aureobasidium* sp. strain NRRL 2311-1. **A**, Unextracted germ; **B**, preexpelled then solvent-extracted germ; **C**, directly solvent-extracted germ; **D**, fully expelled germ. □, Glucose; ●, xylose; ○, arabinose.

Furthermore, pentose sugars are believed to be more susceptible to degradation than hexose sugars (Morrison 1988). As shown, standard errors were generally <10% of the mean, reflecting acceptable variability in the assay methods. For each type of corn germ sample, sugar composition and enzymatic digestion estimates were generally similar. Direct comparisons between samples are not appropriate because they vary in oil composition. This is most apparent in the relatively low pentose sugar composition of unextracted germ (Table I). Therefore, sugar data were corrected based on proximate analysis of fat composition (Table II). TFA composition values for defatted germ samples were 190–259 mg/g of germ for glucose, 138–148 mg/g of germ for xylose, and 262–273 mg/g of germ for arabinose (fat-free basis). TFA sugar composition values for whole germ were slightly higher for as yet unknown reasons. It is possible that the high oil composition of whole germ affects both TFA analysis and enzymatic digestions. Nevertheless, enzymatic digestion yields of xylose plus arabinose were excellent for all samples with 84–115% of composition values (Table II). Values within each column of Table II are not significantly different (*t*-test, *P* = 0.05). This confirms that corn germ is readily digestible regardless of oil extraction treatments. Although not statistically significant, fully expelled germ was slightly more digestible than the other samples, producing >300 mg of pentose sugars/g of germ (fat-free basis). This result was somewhat unexpected because fully expelled germ has a dark, almost burned

TABLE I
TFA Composition Estimates^a and Enzymatic Digestion Yields^b

	Sugar Composition (mg/g of germ)			
	Glucose	Xylose	Arabinose	Xylose + Arabinose
Whole corn germ (unextracted)				
TFA composition	182 ± 1	80 ± 2	67 ± 7	147 ± 9
Enzymatic digestion	206 ± 17	66 ± 6	62 ± 7	129 ± 13
Preexpelled then solvent-extracted				
TFA composition	211 ± 1	145 < 1	123 ± 3	268 ± 3
Enzymatic digestion	262 ± 13	121 ± 6	133 ± 8	254 ± 14
Directly solvent-extracted				
TFA composition	246 ± 12	141 ± 6	108 < 1	250 ± 6
Enzymatic digestion	306 ± 9	105 ± 5	104 ± 5	209 ± 10
Fully expelled (no solvent)				
TFA composition	170 ± 9	124 ± 10	114 ± 14	239 ± 24
Enzymatic digestion	244 ± 1	131 ± 3	144 ± 3	275 ± 6

^a Estimated by HPLC after hydrolysis with 2M trifluoroacetic acid at 110°C for 2 hr.

^b Enzymes from *Aureobasidium* sp. strain NRRL Y-2311-1 at 0.8 IU of endoxylanase/mg of germ substrate and 37°C for 72 hr.

TABLE II
TFA Composition Estimates^a and Enzymatic Digestion Yields^b (fat-free basis)^c

	Sugar Composition (mg/g of germ)			
	Glucose	Xylose	Arabinose	Xylose + Arabinose
Whole corn germ (unextracted)				
TFA composition	364 ± 2	160 ± 4	134 ± 14	294 ± 18
Enzymatic digestion	411 ± 34	133 ± 13	125 ± 13	257 ± 26
Enzymatic composition	113%	83%	93%	87%
Preexpelled then solvent-extracted				
TFA composition	215 ± 1	148 < 1	125 ± 3	273 ± 3
Enzymatic digestion	267 ± 13	123 ± 6	136 ± 8	260 ± 14
Enzymatic composition	124%	83%	108%	95%
Directly solvent-extracted				
TFA composition	259 ± 12	148 ± 6	114 < 1	262 ± 6
Enzymatic digestion	321 ± 9	110 ± 6	110 ± 5	220 ± 11
Enzymatic composition	124%	74%	96%	84%
Fully expelled (no solvent)				
TFA composition	190 ± 10	138 ± 12	127 ± 15	265 ± 27
Enzymatic digestion	271 ± 1	145 ± 3	160 ± 4	305 ± 7
Enzymatic composition	143%	105%	126%	115%

^a Estimated by HPLC after hydrolysis with 2M trifluoroacetic acid at 110°C for 2 hr.

^b Enzymes from *Aureobasidium* sp. strain NRRL Y-2311-1 at 0.8 IU of endoxylanase/mg of germ substrate and 37°C for 72 hr.

^c Determined by proximate analysis: whole germ, 50% fat; preexpelled then solvent-extracted, 2% fat; directly solvent-extracted, 5% fat; fully expelled, 10% fat.

appearance due to the high-heat of the process. Fully expelled germ is not solvent-extracted, so it is possible that it is slightly more digestible than other defatted germ because organic solvent dehydrates arabinoxylan, making it more difficult to rehydrate and thus less accessible to subsequent enzymatic digestion.

CONCLUSIONS

Whole corn germ and germ extracted by three different processes were all excellent substrates for arabinoxylan digestion by crude enzymes from the *Aureobasidium* sp. strain NRRL Y-2311-1. Thus, oil extraction does not serve as a pretreatment to enhance digestibility.

ACKNOWLEDGMENTS

We thank Corn Products International, Inc. for the kind gift of corn germ and defatted corn germ samples. Expert technical assistance was provided by Melinda S. Nunnally and Trina Hartman.

LITERATURE CITED

Ash, M., and Dohlman, E. 2006. Oil Crops Situation and Outlook Yearbook. U.S. Department of Agriculture, Economic Research Ser-

vice, Market and Trade Economics Division: Washington, DC.
Hespell, R. B., O'Bryan, P. J., Moniruzzaman, M., and Bothast, R. J. 1997. Hydrolysis by commercial enzyme mixtures of AFEX-treated corn fiber and isolated xylans. *Appl. Biochem. Biotechnol.* 62:87-97.
Johnson, L. A., and May, J. B. 2003. Wet milling: the basis for corn biorefineries. Pages 449-494 in: *Corn: Chemistry and Technology*, 2nd Ed. P. J. White and L. A. Johnson, eds. AACC International: St. Paul, MN.
Lawton, J. W., and Wilson, C. M. 2003. Proteins of the kernel. Pages 313-354 in: *Corn: Chemistry and Technology*, 2nd Ed. P. J. White and L. A. Johnson, eds. AACC International: St. Paul, MN.
Leathers, T. D. 2004. Enzymatic saccharification of defatted corn germ. *Biotechnol. Lett.* 26:203-207.
Leathers, T. D., and Gupta, S. C. 1996. Saccharification of corn fiber using enzymes from *Aureobasidium* sp. strain NRRL Y-2311-1. *Appl. Biochem. Biotechnol.* 59:337-347.
Morrison, I. M. 1988. Hydrolysis of plant cell walls with trifluoroacetic acid. *Phytochemistry* 27:1097-1100.
Orthofer, F., Eastman, J., and List, G. 2003. Corn oil: Composition, processing and utilization. Pages 671-693 in: *Corn: Chemistry and Technology*, 2nd Ed. P. J. White and L. A. Johnson, eds. AACC International: St. Paul, MN.
Watson, S. A. 2003. Description, development, structure, and composition of the corn kernel. Pages 69-106 in: *Corn: Chemistry and Technology*, 2nd Ed. P. J. White and L. A. Johnson, eds. AACC International: St. Paul, MN.

[Received November 17, 2006. Accepted February 27, 2007.]