an increasing X:A ratio and cell wall degradability. The negative relationship that exists between the A:X ratio and IVDMD is believed to reflect the negative effect that an increase in xylan polymer branching in the hemicellulosic fraction has on forage digestibility.

The percentage of galactose in the cell wall (eq 4) may also be an indicator of polymer branching, since galactose exists predominately in the hemicellulosic fraction of cell wall as a constituent of the side chains of galactoarabinoxylans (Reid and Wilkie, 1969). Reed canary grass samples had the highest A:X ratios as well as the highest galactose contents of the grasses studied. A neative relationship exists between galactose and IVDMD in eq 4.

Research relating the monomeric constituents of plant cell polysaccharides to digestibility is limited. Results presented in this study indicate possible advantages in using these types of parameters as estimators of digestibility and as a way to gain a better understanding of plant-related factors influencing digestibility.

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Registry No. Lignin, 9005-53-2; arabinose, 147-81-9; xylose, 58-86-6; galactose, 59-23-4; silica, 7631-86-9; hemicellulose, 9034-32-6; cellulose, 9004-34-6.

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Composition of the Mucilaginous Spore Matrix of *Colletotrichum graminicola*, a Pathogen of Corn, Sorghum, and Other Grasses

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A mucilaginous matrix produced by the fungus *Colletotrichum graminicola* has several properties which enhance survival of fungal spores. The purpose of this research was to resolve and characterize the compounds of the matrix to facilitate investigation of their function. The major component is a group of high molecular weight glycoproteins composed of oxygen-linked oligomers of rhamnose and mannose and high levels of hydrophobic and hydroxylic amino acids. The amino acid composition and percentage carbohydrate of this viscous material are similar to that of mucins, which may account for the antidesiccant property of the matrix. In addition to the previously reported invertase and esterase, a specific β-glucosidase was identified as a matrix component. Three nonprotein, UV-absorbing components were resolved from the matrix; one of these was identified as uracil.

The anthracnose disease of corn and sorghum is a limiting factor in the production of these crops in the developing nations of the humid and semiarid tropics (Hooker, 1977; Pastor-Corrales and Frederiksen, 1980), and anthracnose has now become one of the most important diseases of corn in the United States where it often reaches epiphytotic proportions resulting in substantial crop loss in localized areas (Hooker, 1977; Lipps, 1983). The potential threat of this disease is further emphasized by the fact that, unlike other fungal pathogens of corn, *Colletotrichum graminicola* (Ces.) Wils. (*Glomerella graminicola* Politis) is capable of attacking all parts of the plant and at any time during the growing season.

The repeated occurrence of the disease in a localized area is associated with the practice of soil conservation through minimum tillage where the fungus survives the winter in infested plant debris on the soil surface (Lipps, 1983).

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However, reasons for the rapid spread of the pathogen across the corn belt of the United States, especially during periods of drought, were not clear since spores of the fungus were assumed to be unable to withstand even limited periods of desiccation. Nicholson and Moraes (1980) then demonstrated that spores are produced in association with a water-soluble mucilaginous material (spore matrix) which protects them against desiccation and aids in their survival and dissemination. The matrix is so protective that spore masses maintain their viability when stored at relative humidities as low as 45% over a period of several weeks. However, if the matrix is removed from spores, they become desiccated and lose viability within hours even at high relative humidities such as 80–90%. Thus, in nature it seems likely that the matrix protects spores from the periodic desiccation and rehydration which would occur due to changes in relative humidity.

Numerous other plant pathogenic fungi produce their spores in association with a mucilage, suggesting that their survival depends on antidesiccant or other properties of the mucilage and that the phenomenon is wide spread in nature. Previous studies showed that antidesiccant activity is associated with a high molecular weight component of the matrix and that the matrix contains carbohydrate, protein, and invertase and esterase activities (Nicholson and Moraes, 1980; Bergstrom and Nicholson, 1981). Characterization of the antidesiccant property of the *C. graminicola* matrix first requires identification of matrix components and that is the purpose of this investigation.

MATERIALS AND METHODS

Culture Conditions and Collection of the Crude Spore Matrix. Cultures of *C. graminicola* were maintained on oatmeal agar under constant fluorescent light (60 μE m^{-2} s^{-1} across the range 400–700 nm) to induce sporulation (Nicholson and Moraes, 1980). Spore masses with their associated matrix were scraped from the surface of 10-day-old cultures, and the spores were separated from the viscous matrix by centrifugation (20 000*g* for 15 min). Thus, the viscous spore matrix was never exposed to any solvent during the collection process. The resulting clear, yellow-brown supernatant is subsequently referred to as the crude matrix. Microscopic examination showed that no mycelial fragments were present in the crude matrix. The conditions of centrifugation had no deleterious effect on spore germination and viability.

Gel Filtration and DEAE Cellulose Chromatography. Crude matrix (8–9 mL) was applied to a column of Sephacryl S-200 (Pharmacia Fine Chemicals, 2.5 × 84.0 cm), which had been equilibrated with 50 mM sodium phosphate buffer, pH 6.5. The column was eluted with the same buffer at a flow rate of 1.0 mL/min maintained by a peristaltic pump. Fractions of 5.0 mL were collected and monitored at 280 nm. The peak I (PI) fraction was concentrated to about 5% of the original volume by ultrafiltration (Amicon PM-10 membrane). The concentrated, viscous material was dialyzed overnight against two changes of a 50 mM sodium phosphate buffer (pH 6.8).

The concentrated, dialyzed PI fraction was then applied to a column of DEAE cellulose (Whatman DE-52) (2.5 × 7.0 cm) previously equilibrated with 50 mM sodium phosphate buffer at pH 6.8. The column was washed with the equilibrating buffer and eluted with a 300-mL linear gradient of sodium chloride (0–0.5 M) in the same buffer. Fractions (7.0 mL) were collected and assayed for carbohydrate and enzyme activities.

SDS-Polyacrylamide Gel Electrophoresis. Crude matrix and fractions from the Sephacryl S-200 and DEAE cellulose separations were subjected to SDS-polyacrylamide gel electrophoresis. The procedure was essentially that described by Laemmli (1970). Samples were concentrated with a microconcentrator (Centricon 10, Amicon Corp) and then mixed with sample buffer and placed in a boiling water bath for 5 min before electrophoresis. Gels were run at a constant current of 30 mA, and protein bands were visualized by the silver stain procedure of Merril et al. (1979). Glycoproteins were detected after SDS-polyacrylamide gel electrophoresis by the periodic acid–Schiff (PAS) staining procedure (Fairbanks et al., 1971).

Enzyme Assays. Esterase activity was assayed as described previously with *p*-nitrophenyl propionate as the substrate (Huggins and Lapides, 1947). B-Glucosidase and β-galactosidase activities were measured with *p*-nitrophenyl β-D-glucoside and *p*-nitrophenyl β-D-galactoside, respectively. Assay mixtures containing 0.09 M acetate buffer (pH 4.7), 0.45 mM substrate, and 10–100 μL of sample containing 10–50 μg of crude matrix protein in a total volume of 2.2 mL were incubated at 37 °C for 5 min, and the reaction was stopped by addition of 1.0 mL of 16% Na_2CO_3. *p*-Nitrophenol was measured at 400 nm after subtraction of a reagent blank. One unit of enzyme activity was defined as the liberation of 1 μmol of *p*-nitrophenol per min for both glycosidase and esterase activities. Protein determinations were by the method of Lowry et al. (1951).

Amino Acid Analysis. Protein samples (0.1–0.8 mg) were flushed with nitrogen and hydrolyzed in 1 mL of 6 N HCl at 110 °C for 21 h under vacuum. The hydrolysates were then dried under a stream of nitrogen. Amino acid analysis was performed in the laboratory of Dr. M. Laskowski, Chemistry Department, Purdue University, on a Durrum analyzer using single column methodology with norleucine as the internal standard.

Carbohydrate Analysis. Neutral sugars were estimated by the phenol–sulfuric acid procedure (Dubois et al., 1956) with glucose as a standard. Carbohydrate composition was determined by gas chromatography of the alditol acetates. One micromole of sugar, as estimated by the phenol–sulfuric acid procedure, was hydrolyzed in 1 mL of 2.5 N trifluoroacetic acid at 100 °C for 4 h. Inositol (200 μmol) had been added as an internal standard. Alditol acetates were prepared by the procedure of Blakeney et al. (1983). Acetylation was terminated with the addition of 1.5 mL of 1 N H_2SO_4. The alditol acetates were partitioned into 3 mL of dichloromethane and washed according to Weber and Carlson (1982). The alditol acetates were analyzed with a 25-m capillary column (Supelco SP2100) in a Varian 3700 gas chromatograph. Separations were made isothermally at 200 °C and peaks were integrated with a Varian CDS-111.

RESULTS AND DISCUSSION

Crude matrix contained carbohydrate (4.1–5.4 mg/mL) and protein (2.0–4.0 mg/mL). The present investigation confirms the presence of invertase and esterase in the matrix (Nicholson and Moraes, 1980) and further identifies β-glucosidase as a matrix component. β-Glcosidase activity was differentiated from invertase activity by competition assays. A 20-fold molar excess of sucrose over that of *p*-nitrophenyl β-D-glucoside had no inhibiting effect on β-glucosidase activity. Similarly, β-D-glucosylamine, a potent inhibitor of β-glucosidase (Lai and Axelrod, 1973), completely inhibited the *C. graminicola* enzyme at a concentration of 0.1 mM, while invertase was unaffected. These results suggest that the two activities probably reside in different proteins. Crude matrix from 10-day-old *C. graminicola* cultures typically contained 0.5–1.5 units of invertase/mg of protein, 0.07–0.18 units of esterase/mg

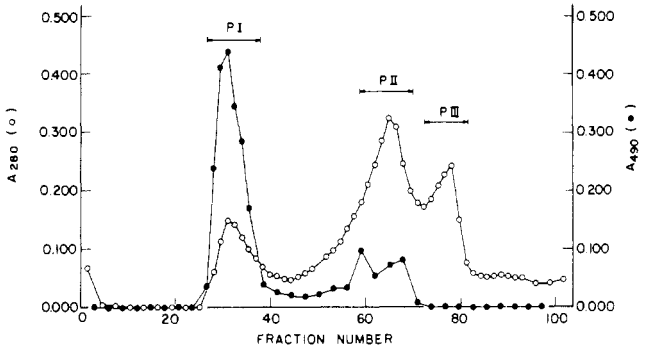

Figure 1. Chromatography of crude matrix on Sephacryl S-200. The PI, II, and III fractions were detected on the basis of absorbance at 280 nm and at 490 nm after carbohydrate analysis (phenol–sulfuric acid procedure).

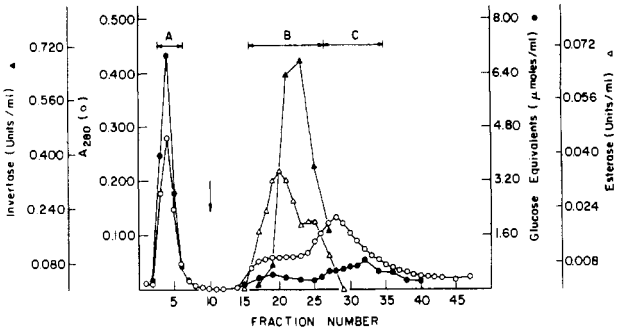

Figure 2. Fractionation of PI on DEAE cellulose. Fractions A, B, and C were selected on the basis of absorbance at 280 nm, carbohydrate content, and enzymatic activities. For clarity β-glucosidase activity, which was found solely in fraction B, is omitted from the figure. The arrow indicates the start of the NaCl gradient.

of protein, and 0.06–0.18 units of β-glucosidase/mg of protein. β-Galactosidase was not detected by the assay system described.

Chromatographic Separation of Matrix Components. Crude matrix was separated by gel filtration into three peaks based on absorbance at 280 nm (Figure 1). Peak I (PI) contained approximately 90% of the total carbohydrate of the crude matrix as well as all enzyme activities. This material was further fractionated and characterized as described below. Peak II (PII) contained oligosaccharides, protein, and two UV-absorbing components, one with an absorption maximum at 310 nm and the other with an absorption maximum at 263 nm. The carbohydrate analysis of PII is shown in Table II. The UV-absorbing components have not yet been identified. Peak III (PiII) contained a component with an absorption maximum at 259 nm and with a spectrum characteristic of pyridine or pyridine bases. The component did not bind to cation or anion exchange resins. It was tentatively identified as uracil on the basis of its spectral shift from 259 nm att pH 7.0 to 284 nm at pH 12.0 (Beaven et al., 1955). The unknown comigrated with a uracil standard on cellulose TLC plates with two solvent systems [ethanol–1 M ammonium acetate pH 7.5 (70:30 v/v) and isobutyric acid–NH_4OH–water (66:1:33 v/v/v)]. The unknown also cochromatographed with the uracil standard on a reverse-phase HPLC C-8 column (LiChrosorb 10 μm, Brownlee Laboratories, Santa Clara, CA 95051) by isocratic elution with a mixture of 70% acetonitrile–30% water at a flow rate of 1 mL/min. Detection was at 254 nm. Based on tentative identification as uracil, its concentration in crude matrix ranged from 0.2–0.3 mM. Except for the possible association of uracil with the synthesis of matrix oligosaccharides (UDP sugars) the reason for its presence at these levels is unknown.

Fractionation of Peak I. Peak I from the S-200 separation was further resolved into three broad fractions (A, B, and C) by anion exchange chromatography on DEAE cellulose (Figure 2). Fraction A, which eluted in the buffer wash, contained approximately 70% of the carbohydrate applied to the column and tested positively for protein. A gradient of NaCl eluted additional carbohydrate and protein which was divided into two fractions, B and C. Fraction B contained 10–13% of the carbohydrate applied to the column and all of the esterase, invertase, and β-glucosidase activities. Fraction C contained from 18–20% of the carbohydrate applied to the column. No enzyme activities were found in fraction A or C.

Amino Acid and Carbohydrate Analyses. Amino acid composition of fractions A, B, and C is presented in Table I. The fractions clearly differ in composition, although each contained approximately 50% hydrophobic amino acids, 23% hydroxylic amino acids (threonine and serine), and very low levels of aromatic amino acids. This amino acid composition is characteristic of mucin glycoproteins found in mammalian species (Reid and Clamp, 1978; Hill et al., 1977; Clamp et al., 1978).

Table I. Amino Acid Composition of DEAE Fractions[a]

amino acid	fraction A	fraction B	fraction C
Asp	5	11	14
Thr	15	10	12
Ser	11	9	11
Glu	5	8	8
Pro	11	6	10
Gly	10	11	11
Ala	9	10	9
Val	11	8	8
Met	0	2	2
Ile	6	5	5
Leu	10	7	7
Tyr	2	3	0
Phe	2	4	0
His	0	2	0
Lys	1	3	4
Arg	1	2	0

[a] Values represent mole percent. Norleucine was used as internal standard. Tryptophan and cysteine were not determined.

Table II. Carbohydrate Composition of the Spore Matrix of *Colletotrichum graminicola*

fractions	rhamnose	mannose	glucose	galactose
crude matrix	11.2*	27.0	24.9	36.9
S-200				
PI	17.9	44.1	5.0	33.0
PII	5.1	9.6	77.2	8.1
PI on DEAE cellulose				
A	22.2	66.2	1.6	10.0
B	10.4	35.0	2.2	52.4
C	2.4	10.6	2.1	84.9

[a] Values presented represent mole percent. Rhamnose was also identified by descending chromatography on Whatman No. 1 paper with butanol–pyridine–water (6:4:3 v/v/v) as the solvent and silver nitrate as the developing reagent.

Carbohydrate analyses of crude matrix and of various column fractions are presented in Table II. Galactose, mannose, glucose, and rhamnose were the major sugar components of the crude matrix. Glucose was only a minor component of the PI fraction (5%) but was a major component (77%) of the PII fraction.

Analysis of fractions separated by anion exchange chromatography revealed that fraction A, the major car-

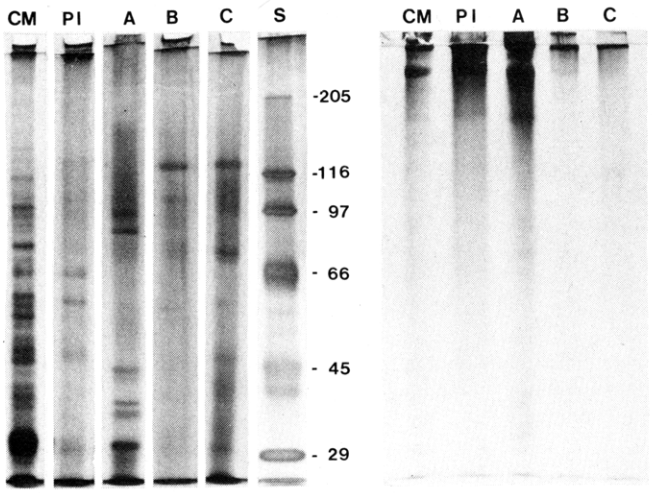

Figure 3. SDS–polyacrylamide gel electrophoresis of crude matrix (CM), peak I (PI), peak I components A, B, and C, and molecular weight standards (S). Molecular weights are $\times 10^{-3}$ and from top to bottom represent myosin, β-galactosidase, phosphorylase B, bovine serum albumin, egg albumin, and carbonic anhydrase. The gel on the left was stained for protein by the silver stain procedure and the gel on the right was stained for carbohydrate by the PAS stain procedure.

bohydrate–containing component of PI, was composed primarily of mannose and rhamnose (molar ratio, 3:1) (Table II) and was 57% carbohydrate by weight. Fraction B, which contained all enzyme activities, contained galactose, mannose, and rhamnose (molar ratio 5:3:1) and was 55% carbohydrate by weight. Fraction C (84% carbohydrate by weight) contained galactose as the major carbohydrate.

SDS–Polyacrylamide Gel Electrophoresis. Electrophoresis of the crude matrix indicated the presence of several protein components of molecular weight 100 000 or less (Figure 3). Protein components could be visualized by silver stain procedure of Merril et al. (1979), but not by the Coomasie procedure (Fairbanks et al., 1971). The PAS stain for carbohydrate (Fairbanks et al., 1971) showed some intensely staining components of each fraction which accumulated at the lane origin, indicating the presence of components containing carbohydrate of very high molecular weight. Fraction A also contained several components which stained positively for carbohydrate of lower apparent molecular weight. These same components did not stain positively for protein, again suggesting a similarity to mucins (Holden et al., 1971).

Pronase Digestion. In order to determine whether the carbohydrates were present as polysaccharides or glycoproteins, a PI preparation was rechromatographed on Sephacryl S-200 gel to confirm the PI elution profile and to ensure the absence of PII components. The rechromatographed PI fraction was digested with pronase and then chromatographed on the same Sephacryl column. Both carbohydrate and protein components of the digested material (Figure 4) eluted at much lower molelcular weights than the original material (Figure 1, PI). Thus, it appears that carbohydrates present in the PI preparation were associated with proteins and are not simply polysaccharides.

Determination of the Sugar–Amino Acid Linkage. Oligosaccharides were removed from the protein components of a PI preparation by the alkaline borohydride β-elimination procedure of Carlson (1968) for oxygen-linked sugars. The procedure for nitrogen-linked sugars (Zinn et al., 1978) gave similar results indicating that the oligosaccharides were oxygen-linked to protein. The liberated oligosaccharides were separated into two fractions by gel filtration on a Sephadex G-25 column, 1.5 × 168 cm, equilibrated with 50 mM ammonium bicarbonate, and the fractions were analyzed for sugar composition. The first fraction was composed of 85% galactose with mannose, glucose, and rhamnose present in the same ratios as in fraction C of the PI component (Table II). The second fraction was composed of mannose (68%), rhamnose (22%), galactose (5%), and glucose (5%), which corresponded to the composition of fraction A of the PI component (Table II).

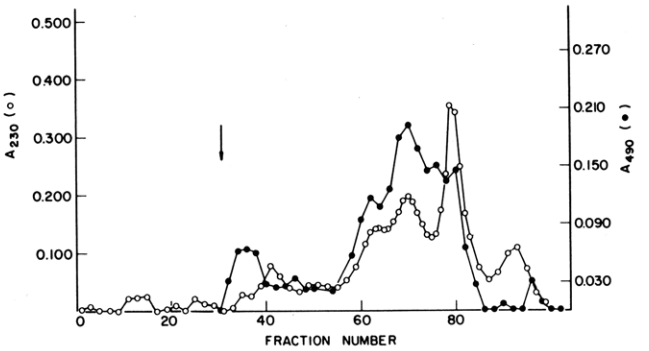

Figure 4. Chromatography of a PI proteolytic digest on Sephacryl S-200. Pronase treated PI was applied and eluted under identical conditions from the S-200 column used in Figure 1. Carbohydrate was detected by the phenol–sulfuric acid procedure and monitored at 490 nm. Peptides were monitored by absorbance at 230 nm. Arrow indicates where PI eluted prior to pronase digestion.

Thus, the major components of the mucilaginous spore matrix of *C. graminicola* have properties similar to those of mucin glycoproteins, including amino acid composition, percentage carbohydrate, and high apparent molecular weight. The functional significance of other matrix components are under further investigation.

A primary function of the matrix is to protect spores from desiccation (Nicholson and Moraes, 1980); mucins which line mucous membranes exhibit a similar water-binding capacity (Orten and Neuhaus, 1982; Reid et al., 1982). Mucins are also known to protect the gut lining from digestive enzymes. Hasan (1982) recovered viable *C. graminicola* spores from the feces of two locust species and suggested that the long distance migration of the locusts could also account for the long range dispersal of the fungus. Survival of spores in the locust gut would require their protection from gut enzymes and such protection may also be a function of the spore matrix.

Registry No. Esterase, 9013-79-0; β-glucosidase, 9001-22-3; invertase, 9001-57-4.

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Composition of Raw and Parboiled Rice Bran from Common Sri Lankan Varieties and from Different Types of Rice Mills

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The levels of protein, fat, crude fiber, starch, and ash in bran obtained from raw and parboiled rice belonging to six popular Sri Lankan varieties were determined. Also, the contents of these nutrients in raw and parboiled rice bran obtained from three types of rice mills, namely, traditional mill (Engelberg stell huller), semimodern mill (rubber roll sheller and steel huller), and modern mill (rubber roll sheller, separator, and a battery of abrasive and friction type polishers) were compared. Significant varietal influence on the chemical composition of bran was observed. In all varieties the parboiled rice bran contained, on an average, 26% higher fat than raw rice bran. Traditional and semimodern mills produced bran with high fiber and ash contents probably due to contamination with paddy husk. Contamination of bran with husk resulted in a reduction in the levels of protein and fat in both raw and parboiled rice bran. Even though the starch content was also reduced in parboiled rice bran obtained from the traditional mill due to husk admixture, this reduction was not observed in raw rice bran because of its contamination with portions of the starchy endosperm.

INTRODUCTION

Rice bran, which is a byproduct formed during polishing of dehusked rice, consists of the outer most layers of the rice grain, namely fragments of pericarp, tegmen, aleurone layer, the germ, and sometimes portions of the endosperm. The total bran constitutes 8-10% of the weight of rice. It is widely used as an animal feed and for oil extraction. It is also mixed to a limited extent in human food preparations.

The chemical composition of rice bran varies significantly among varieties (Mc Call et al., 1953; Limcango-Lopez et al., 1962). Also, parboiling results in compositional changes in rice bran (Houston et al., 1969). The composition of bran may also vary according to the type of machinery used for milling. In traditional types of rice mills where the operations of dehusking (removal of hulls from paddy) and polishing (removal of bran from brown rice) are not clearly demarcated, the bran gets mixed to varying amounts with paddy husk resulting in a poor quality byproduct with limited usage. On the other hand, in improved modern rice mills, processing is carried out in stages by separate machines and hence contamination of bran with husk is minimal.

Scientific data on the chemical composition of rice byproducts obtained from different sources become important in order to maximize their utilization. This study was undertaken to determine the influence of variety and machinery used for processing on the chemical composition of bran from raw and parboiled rice.

MATERIAL AND METHODS

Freshly harvested paddy free from varietal admixture and foreign matter from six popular Sri Lankan varieties were used in the study. Bran from the different varieties were obtained by dehusking 200 g of samples of paddy by using a "Satake" laboratory dehusker and polishing the brown (unpolished) rice with a Mc Gill miller no. 2 to obtain a bran removal of 8 ± 0.5% by weight of brown rice. To obtain bran from different mills, 100 kg of samples of paddy belonging to the variety BG 276-5 were milled with the following types of machinery to obtain a bran removal of 8 ± 0.5%.

Traditional Mill. The paddy was milled with a steel huller (Engelberg) where both operations of dehusking and polishing were done in one pass. The byproduct formed during the milling operation was collected for analysis.

Semimodern Mill. The paddy was dehusked with a rubber roll sheller with husk aspirator and the brown rice containing 5% unhusked grains was polished with a steel

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