Structural Changes during the Retrogradation of Legume Starches Modify the *in Vitro* Fermentation

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The fermentation of native and retrograded starches from white beans (*Phaseolus vulgaris*), lentils (*Lens culinaris medicus*), chickpeas (*Cicer arietinum L.*) amylose, and native amylopectin has been studied. Starches were extracted from legumes, and a portion was made resistant to amylolysis (retrograded starch) by repeated autoclaving and cooling cycles. The retrograded fraction was isolated by treatment with heat-stable α-amylase, protease, and amyloglucosidase. Samples were fermented using an *in vitro* batch-culture technique under anaerobic conditions with rat cecal contents as inoculum. Short-chain fatty acids, lactic acid, cell growth, and starch-degrading enzymes were analyzed after 3, 6, 18, 24, and 48 h of fermentation. The rate of production of total acids (acetic, propionic, butyric, and lactic acids) was different between each of the retrograded starches at all times studied. Retrograded amylose and bean starch were fermented at a slower rate than retrograded lentil and chickpea starches. Significant differences were observed after 18 and 24 h of fermentation of native starches. Scanning-electron micrographs of the resistant starches after the autoclaving–cooling treatment and the *in vitro* enzymatic digestion showed changes in the physical appearance between starches. Such physical changes could have affected the accessibility to bacterial enzymes and therefore the fermentability of the starches.

INTRODUCTION

The composition and the properties of native dietary starches vary with the plant source (Stark and Lynn, 1991). However, starches can be modified during technological processing. Fractions more or less resistant to amylases can be formed (Ring *et al.*, 1988; Bjorck *et al.*, 1986), which results in changes in the nutritional properties of the final products.

Starch modifications depend on processing conditions and interactions between starch molecules and other food components such as lipids, proteins, and other carbohydrates (Biliaderis, 1991). Food-processing techniques at relatively high temperature and moisture levels, such as baking (Bjorck *et al.*, 1986) and autoclaving-cooling (Siljestrom *et al.*, 1989), result in the retrogradation of starch. Enzyme-resistant starches (RS) are formed which consist of highly crystalline retrograded amylose chains (Russel *et al.*, 1989; Siljestrom *et al.*, 1989).

Animal (Bjorck *et al.*, 1987) and human (Englyst and Cummings, 1985) experiments have shown that resistant starches can escape digestion in the small intestine and are fermented in the colon with the production of short-chain fatty acids (SCFAs), CO_2, and H_2. The degree of amylolytic hydrolysis in the small intestine determines the amount of resistant starch reaching the colon. However, measuring this fraction and the factors that allow starch to resist pancreatic amylase is difficult. Important factors are the food source (Englyst and Cummings, 1985), the starch intake (Shetty and Kurpad, 1986), the amylose/amylopectin ratio (Hoover and Sosulski, 1985; Holm and Bjorck, 1988), the percentage of retrograded starch (Ring *et al.*, 1988), the amylose chain length (Jood *et al.*, 1988), and the extent of molecular association between starch components and degree of crystallinity (Hoover and Sosulski, 1991). Among food products, legume starches, and especially bean starch, have being found to be resistant to digestion (Würsch *et al.*, 1986; Wong *et al.*, 1985). Differences in susceptibility to amylase activity between various pulses and within varieties of pulses have also been reported (Tovar *et al.*, 1990).

As starches show differences in their susceptibility to digestive enzymes, they may also be degraded at different rates by bacteria within the colon. Previous work on the fermentability of native (McBurney *et al.*, 1990) and processed starches (Wyatt and Horn, 1988) has suggested that not only the amount of starch escaping digestion in the small intestine but also the starch source may have a significant effect on SCFA production in the colon. This could have potentially important effects on colonic metabolism and health.

The aim of the present paper is to determine the rate and the degree of fermentation of retrograded starches from different legume sources, using an *in vitro* closed batch-culture technique with rat cecal contents as inoculum.

MATERIALS AND METHODS

Sources of Starch. White beans (*Phaseolus vulgaris*), lentils (*Lens culinaris medicus*), and chickpeas (*Cicer arietinum* L.) were used as starch sources. Amylose and amylopectin (both from corn starch) were purchased from Sigma Chemical Co.

Extraction of Starches. Seeds (250 g) were washed and left to soak overnight at 6 °C. After being washed, the softened kernels were blended for 2 min in approximately 1.5 L of sodium chloride solution (20 g/L). The slurry was washed through a 125-μm sieve to separate the fiber from the starch, and the filtrate was left to settle for 2 h at room temperature. The resulting supernatant was decanted, and the insoluble material was suspended in ethanol (absolute), filtered under vacuum, and dried (Wyatt and Horn, 1988). Lipids were extracted from starches using methanol/chloroform, 1:2 v/v, as the solvent. The protein contents of all samples were less than 1%.

Determination of Total Starch Contents. The method is based on the procedure described by Siljeström and Asp (1985).

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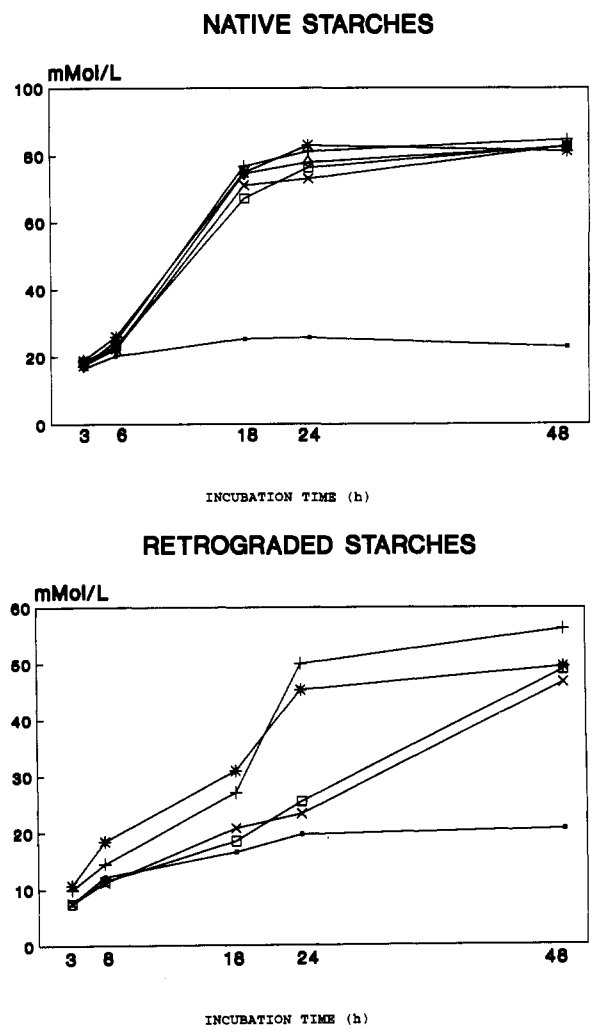

Figure 1. Production of short-chain fatty acids (acetic, propionic, and butyric acids) and lactic acid during 48 h of fermentation. The total concentration (mmol/L) is expressed as the sum of the concentrations of individual acids: (A) native starches and (B) retrograded starches; (■) control, (+) lentil, (*) chickpea, (□) bean, (×) amylose, and (◊) amylopectin.

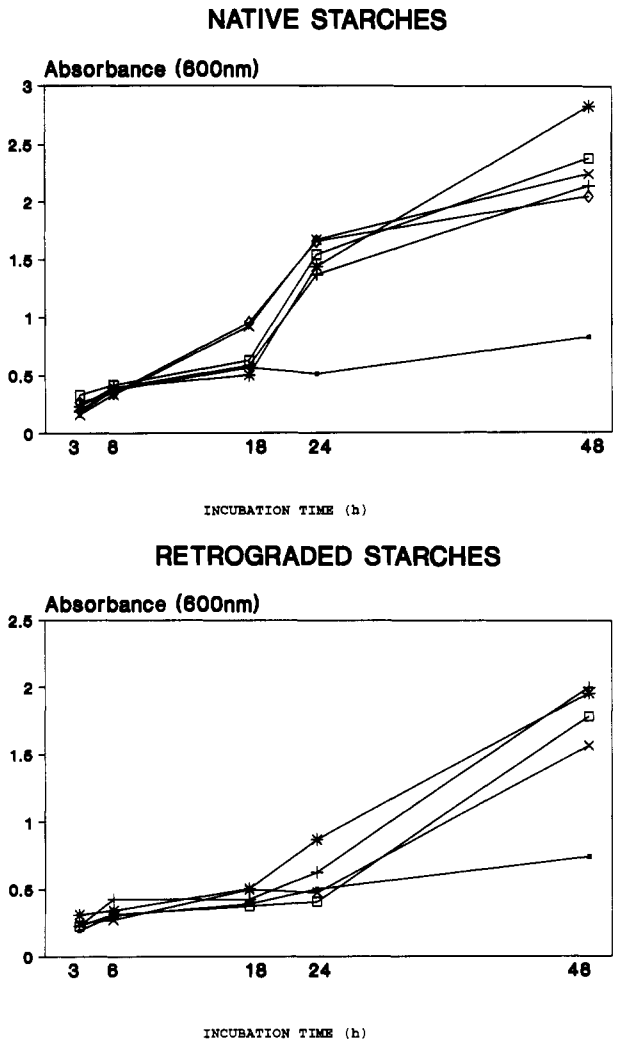

Figure 2. Bacterial growth on (A) native starches and (B) retrograded starches: (■) control, (+) lentil, (*) chickpea, (□) bean, (×) amylose, and (◊) amylopectin.

Approximately 100 mg of each sample was dispersed, with constant mixing, in 20 mL of 2 M KOH for 30 min at room temperature. After complete solubilization, a 2-mL aliquot was taken and adjusted to pH 4.75 by using 1 mL of an acetate buffer (0.4 M, pH 4.75, containing 20 mM $CaCl_2$) and about 1.5 mL of 2 M HCl. Samples were incubated for 30 min at 60 °C with 60 μL of amyloglucosidase (10 mg/mL) (Boehringer Mannheim No. 102857). The amount of glucose liberated was measured with a glucose–peroxidase reagent (Boehringer Mannhein No. 676543). Pure soluble starch (BDH Chemicals) was used as a reference. Results are expressed as polymer weight (glucose × 0.9).

Preparation of Retrograded Starches. Starches were suspended in distilled water (1:4 w/v) and heated at 80 °C for 20 min. The gelatinization of starches was completed by autoclaving at 121 °C for 20 min after which the sample was left at 6 °C for 24 h. After five cycles of autoclaving and cooling, the samples were washed with ethanol, filtered under vacuum, freeze-dried, and ground (Sievert and Pomeraz, 1989).

To obtain the retrograded fraction, 1 g of autoclaved–cooled material was suspended in 50 mL of Na_2HPO_4/NaH_2PO_4 buffer (pH 6.0, 0.08 M) and treated with heat-stable α-amylase (A-3302, Sigma Chemical), 200 μL (100 °C, 30 min), protease (P-3910, Sigma Chemical), 100 μL (50 mg/mL in Na_2HPO_4/NaH_2PO_4 buffer) (60 °C, 30 min), and amyloglucosidase (*Aspergillus niger*), 6100 units/mL, 300 μL (60 °C, 30 min) (A-9913, Sigma Chemical). After incubation, the suspensions were centrifuged (2500*g*) for 15 min. The pellet was washed with ethanol and distilled water, freeze-dried, and finely ground. This assay followed the AOAC method for the determination of dietary fiber (Prosky *et al.*, 1988).

Fermentation of Starches. The *in vitro* fermentation was carried out by a closed batch-culture technique under strict anaerobic conditions. The medium (pH 7.0) contained tryptone (2.5 g/L) and minerals (Na_2HPO_4 1.4 g/L, KH_2PO_4 1.6 g/L, $MgSO_4 \cdot 4H_2O$ 0.2 g/L, $(NH_4)HCO_3$ 1 g/L, $NaHCO_3$ 8.7 g/L, $CaCl_2 \cdot 2H_2O$ 16.5 mg/L, $MnCl_2 \cdot 4H_2O$ 12.5 mg/L, $CoCl_2 \cdot 2H_2O$ 1.25 mg/L, and $FeCl_3 \cdot 6H_2O$ 1 mg/L) as described by Goering and Van Soest, 1979.

Rat cecal contents were used as inoculum. Approximately 12 g of fresh rat cecal contents was removed from rats previously fed a CRM(×) diet (Chow Labsure, Croydon, England; composition (g kg^{-1}): protein 183, fat 29, calcium 8, phosphorus 6, salt 7, water 169, total carbohydrate 598 of which starch 465, total nonstarch polysaccharides 133 of which soluble 31 and insoluble 102 (Englyst *et al.*, 1982), trace elements, and vitamins). The cecal contents were homogenized in 150 mL of sterile anaerobic medium to give a 8% (w/v) cecal slurry. The slurry was mixed at 37 °C for 45 min to break up the cecal fiber matrix and filtered through a 51-μm sieve. The filtrate was retained for inoculation. Anaerobic conditions were maintained during the process using oxygen-free carbon dioxide. Medium, 30 mL, and 1 mL of reducing solution (cysteine hydrochloride 6.2 g/L, NaOH 1.6 g/L, and $Na_2S \cdot 9H_2O$ 6.2 g/L) were dispensed into 150-mL Medical Flat bottles followed by starch samples (200 mg) and 10 mL of inoculum. The bottles were gassed with CO_2, closed with sterile suba-seal stoppers, and laid flat in an incubator at 37 °C for 48 h. (This method has previously been shown to give similar results whether the solution is agitated or stationary). Controls were

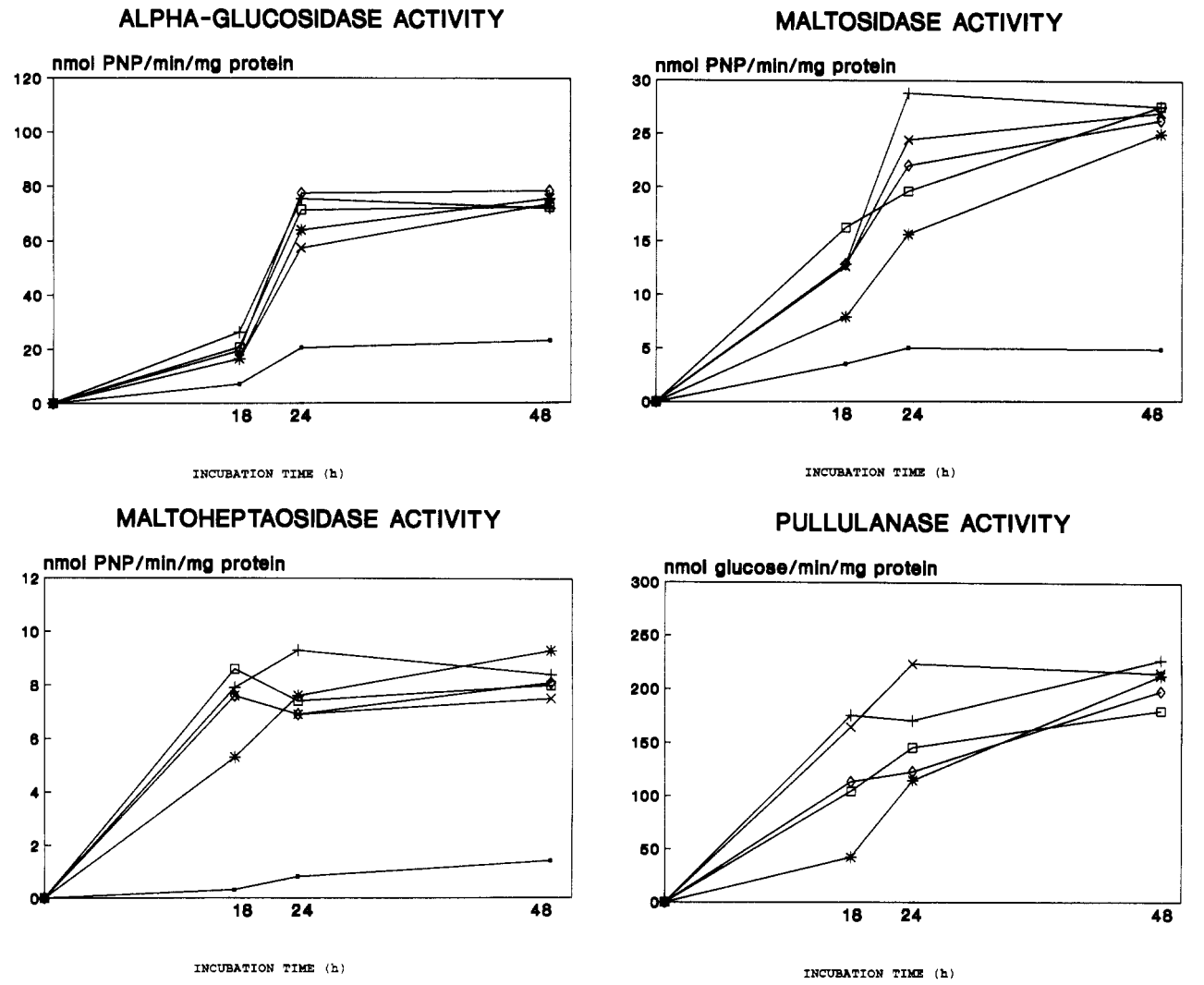

Figure 3. Bacterial starch-degrading enzymes produced during the fermentation of native starches: (A) α-glucosidase activity, (B) maltosidase activity, (C) maltoheptaosidase activity, and (D) pullulanase activity; (■) control, (+) lentil, (*) chickpea, (□) bean, (×) amylose, and (◊) amylopectin.

Table I. Individual Acids Produced after 48 h of Fermentation of Native and Retrograded Starches[a]

	acids (mmol/L)			
	acetic	propionic	butyric	lactic
lentil starch				
native	57.9 (0.2)	12.7 (0.5)	11.6 (0.3)	2.5 (0.2)
retrograded	38.0* (1.1)	4.5* (0.2)	8.2* (0.3)	5.6* (0.5)
chickpea starch				
native	55.5 (2.8)	11.9 (0.4)	11.2 (0.7)	2.8 (0.2)
retrograded	33.3* (2.1)	4.5* (0.3)	7.0* (0.3)	4.8* (0.2)
bean starch				
native	56.3 (2.6)	12.5 (0.7)	11.2 (0.2)	2.8 (0.3)
retrograded	33.9* (2.4)	3.6* (0.5)	5.5* (0.3)	6.0* (0.2)
amylose				
native	57.4 (1.4)	12.4 (0.2)	8.6 (0.2)	4.6 (0.3)
retrograded	31.7* (0.5)	3.6* (0.3)	5.7* (0.2)	5.8* (0.2)
amylopectin				
native	54.4 (2.4)	13.5 (0.2)	12.9 (0.3)	1.9 (0.3)

[a] Individual acids (acetic, propionic, butyric, and lactic acids) are expressed as mmol/L. Values are the mean of six assays. An asterisk denotes a significant difference between native and retrograded starches ($P < 0.001$). Standard deviations are indicated in parentheses.

prepared as above without the addition of starches. Samples were removed aseptically and anaerobically for analysis at 3, 6, 18, 24, and 48 h.

Analysis of Carboxylic Acids. Short-chain fatty acids and lactic acid (LA) were analyzed by gas–liquid chromatography (Carlo-Erba 4200, equipped with a flame ionization detector). Samples were separated using a column containing 10% SP/1200/1% H_3PO_4 chromsorb W-AW 80-100 mesh. SCFAs were determined as described by Spiller *et al.* (1980) and LA by a method of Holdeman *et al.* (1977).

Measurement of Bacterial Cell Growth. Samples were centrifuged for 10 min at 500*g* to remove large particulate material, and the cell growth was determined by measuring the optical density at 600 nm using a UV–vis spectrophotometer (Pye Unicam Ltd., Cambridge, England).

Enzymatic Assay. Samples were sonicated at 5 Å for 3 min (3 × 1 min, with 1 min of cooling after each sonication), and the homogenate was centrifuged for 10 min at 2500*g* to remove unbroken cells and cell debris. The soluble fraction was assayed for pullulanase, α-glucosidase, maltosidase, maltopentaosidase, and maltoheptaosidase as follows.

Pullulanase was assayed by measuring the reducing sugars released from pullulan. Pullulan (P-4516, Sigma Chemical) (10 mg/mL in distilled water) was solubilized by autoclaving for 10 min before use in the enzymatic assay. Pullulan, 0.5 mL, 1% (w/v), was added to 0.5 mL of an acetate–acetic acid buffer (0.1 M, pH 6.0) (Brown *et al.*, 1990) followed by the addition of 100 μL of fermentation fluid. The samples were incubated at 37 °C for 45 min, and the reaction was stopped by freezing. The amount of reducing sugar released was determined by using the PHBAH method (Fry, 1988) with glucose as the standard. One unit of enzyme activity was defined as the amount of enzyme that released 1 nmol of reducing sugar min^{-1} (mg of protein)$^{-1}$. α-Glucosidase activity was determined by using *p*-nitrophenyl α-D-glucopyranoside (PNPG) as the substrate. A 0.5-mL portion of PNPG (10 mM in 10 mM phosphate buffer (pH 7.5)) was

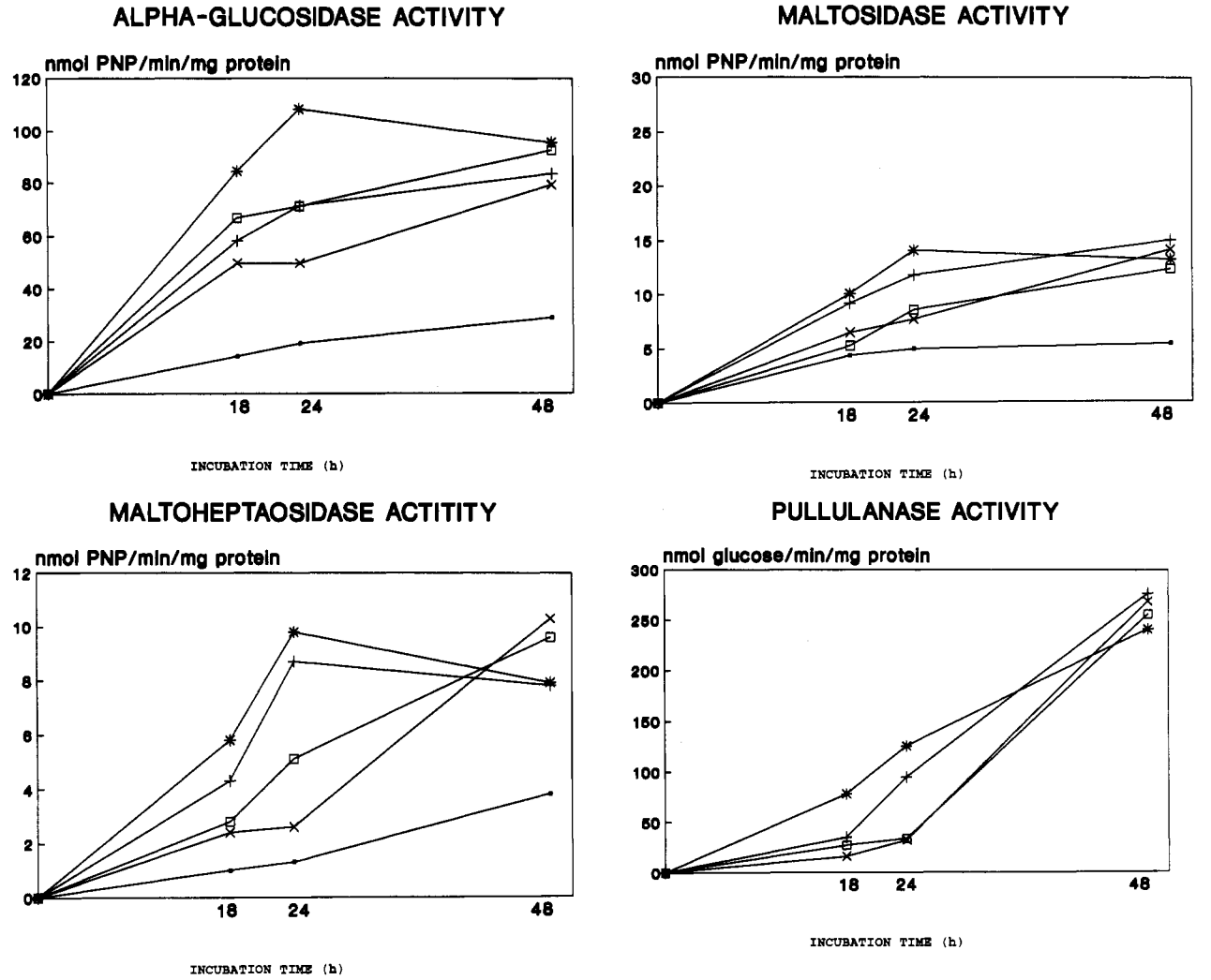

Figure 4. Bacterial starch-degrading enzymes produced during the fermentation of retrograded starches: (A) α-glucosidase activity, (B) maltosidase activity, (C) maltoheptaosidase activity, and (D) pullulanase activity; (■) control, (+) lentil, (*) chickpea, (□) bean, and (×) amylose.

added to 0.5 mL of the sample, and then sample was incubated at 37 °C for 2 h. The reaction was stopped by adding 1 mL of 1 M sodium carbonate, and the samples were centrifuged at 2700*g* for 10 min. The optical density was determined by reading at 415 nm. Known concentrations of *p*-nitrophenol (PNP) (N-0503, Sigma Chemical) in 0.5 M Na_2CO_3 were used to calculate the amount of PNP released. A blank containing no sample was used to correct for nonenzymatic release of PNP (Halnorson and Ellias, 1958). One unit of α-glucosidase activity was defined as the amount of enzyme releasing 1 nmol of PNP min^{-1} (mg of protein)$^{-1}$.

Maltosidase, maltopentaosidase, and maltoheptaosidase activities were measured by using respectively *p*-nitrophenyl α-D-maltoside (0.4 mM) (N-1884, Sigma Chemical), *p*-nitrophenyl α-D-maltopentaoside (0.3 mM) (N-1519, Sigma Chemical), and *p*-nitrophenyl α-D-maltoheptaoside (0.3 mM) (N-1894, Sigma Chemical) as substrates. A 0.4-mL volume of each substrate in 10 mM phosphate buffer, pH 7.5, was added to 0.1 mL of the sample and then incubated at 37 °C for 2 h. The reaction was stopped by adding 1 mL of 1 M sodium carbonate, and the samples were read at 415 nm; PNP was used as a standard.

Scanning-Electron Microscopy. Scanning-electron microscopy (SEM) was carried out using a Cambridge stereoscan S250 microscope. Starch samples were placed on double-stick carbon adhesive disks mounted on specimen stubs supplied by Agar Scientific Ltd., Cambridge, England. The samples were gold coated using a Emscope SC500A sputter coater.

Protein Determination. Protein was estimated by the Bio-Rad protein assay (Bio-Rad Laboratories Ltd., Hertfordshire, England). A standard curve was prepared using bovine serum albumin (Sigma Chemical Co., Dorset, England).

Statistical Analysis. The results were tested for significance using analysis of variance (one-way ANOVA). Means of individual starches were compared using a two-tailed Student's *t* test. Differences of $P < 0.05$ were considered significant (Snedecor and Cochran, 1980). A statistical package (Minitab, release 7.1, 1989, State College, PA) was used.

RESULTS

Determination of Total Starch. The total starch content in samples after extraction from pulses was 94% in beans, 94% in lentils, and 89% in chickpeas.

Fermentation of Samples. *Short-Chain Fatty Acids and Lactic Acid Formation.* The production of acids (acetic, propionic, butyric, and lactic acids) during 48 h of fermentation of native and retrograded samples is shown in Figure 1. One-way ANOVA showed no differences among native starches after 3, 6, and 48 h of fermentation. However, significant differences were found after 18 and 24 h ($P < 0.005$) (Figure 1A).

The rate of production of SCFAs differed among retrograded starches at all times ($P < 0.001$). A two-sided t test showed significant differences between bean starch and both lentil and chickpea starches at 3 ($P < 0.05$), 6, 18, and 24 h of fermentation (lentil $P < 0.001$, chickpea $P < 0.005$) and with chickpea starch at 48 h ($P < 0.05$). Retrograded lentil and chickpea starches showed significant differences ($P < 0.05$) at 6 and 48 h of fermentation (Figure 1B).

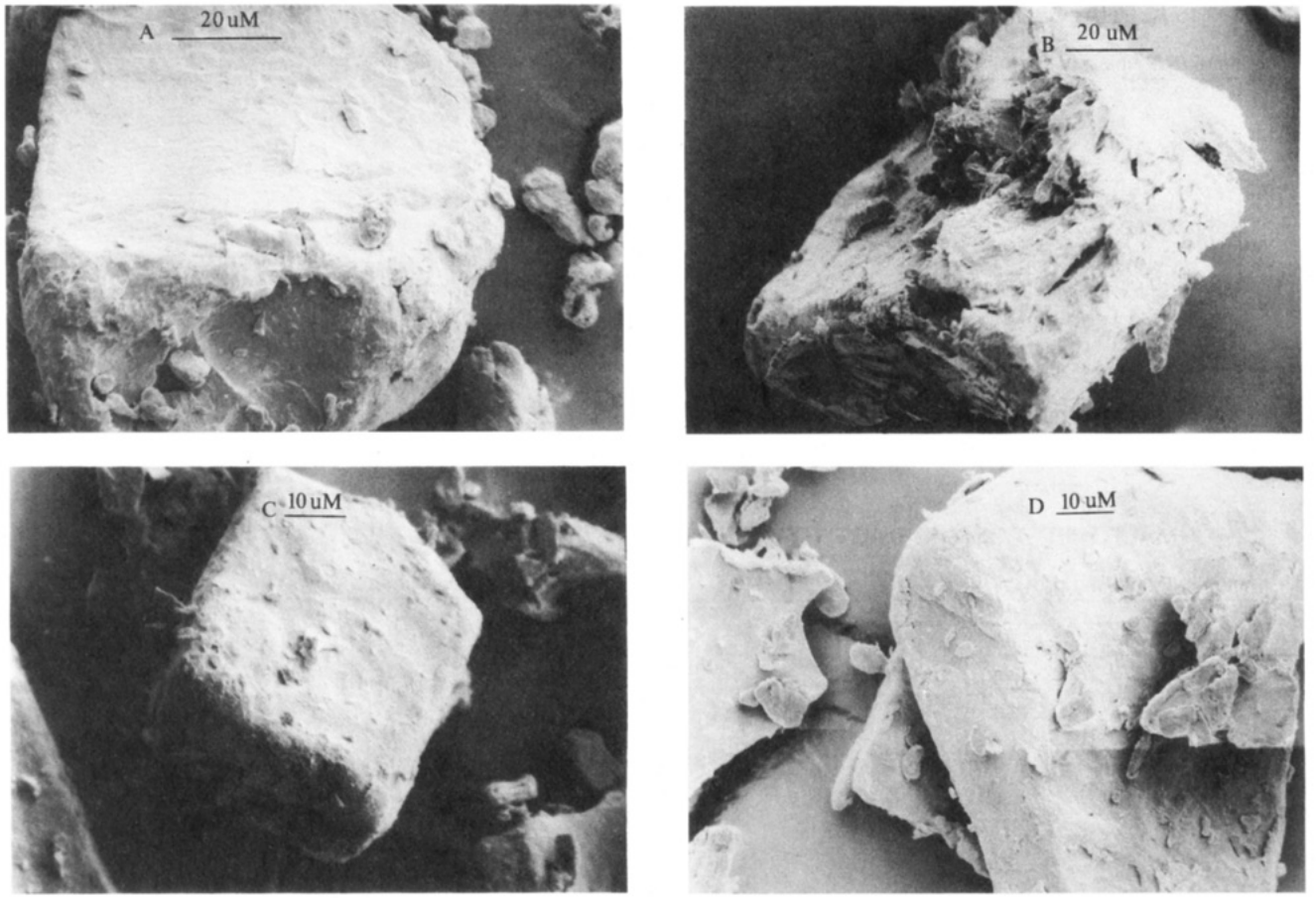

Figure 5. Scanning-electron micrographs of resistant starches prior to enzymic treatment: (A) amylose, (B) bean, (C) chickpea, and (D) lentil.

Retrograded amylose and bean starch were degraded at a slow constant rate compared to retrograded lentil and chickpea starches. After 18 h of fermentation, retrograded amylose and bean starch produced only 29% and 27% (of the SCFAs obtained during fermentation by their respective native samples) and retrograded lentil and chickpea starches 35% and 41%. After 24 h, the SCFA production during retrograded lentil and chickpea fermentation increased to 59% and 56%, while only a small increase to 28% and 30% was detected for retrograded amylose and bean starch. Overall, retrogradation of starches lessened the production of SCFAs during fermentation to 30–40%.

The individual acids calculated as millimoles per liter of acetic, propionic, butyric, and lactic acids after 48 h of fermentation of native and retrograded starches are shown in Table I. In all cases, the production of individual acids during fermentation of native starches was significantly different ($P < 0.001$) from those during fermentation of the retrograded starches.

Bacterial Cell Growth. The rates of cell growth on raw and retrograded starches are shown in Figure 2. The growth of cecal microorganisms on native starches followed the expected exponential pattern of bacterial growth (Figure 2A); however, growth on retrograded samples was much slower (Figure 2B). When native starches were fermented an 18-h lag phase was observed. The lag phase was prolonged to 22 h with retrograded lentil and chickpea starches and to 24 h with retrograded amylose and bean starch. The final optical density was slightly lower for growth on resistant starches.

Production of Bacterial Starch-Degrading Enzymes. Total starch-degrading enzyme activities are shown in Figures 3 and 4. The rate of production of α-glucosidase, maltosidase, and maltoheptaosidase was similar among native starches throughout the fermentation (Figure 3). The patterns of α-glucosidase and maltosidase paralleled the results obtained for cell growth (Figure 2A).

The rate of production of the enzymes was slow and differed between samples when resistant starches were fermented (Figure 4). Resistant amylose and bean starch had a slower and steadier production of maltosidase, maltoheptaosidase, and pullulanase than retrograded lentil and chickpea starches.

Pullulanase production (Figure 4D) followed the development of cell growth (Figure 2B) in all retrograded samples. However, a similar pattern of activity was only observed for the production of maltosidase and maltoheptaosidase activities (Figure 4B,C) during growth on retrograded amylose and bean starches. α-Glucosidase production was continuous throughout growth on all the retrograded substrates (Figure 4A). Similar results were found for maltopentaosidase (data not shown).

Characterization of Starches by SEM. Scanning electron micrographs of autoclaved samples before and after enzymatic treatment are shown in Figures 5, 6, and 7. Native samples showed the typical granular structures. After the autoclaving and cooling cycles, irregularly shaped particles with an apparent compact and dense formation were found in all samples (Figure 5).

Higher magnification of these structures showed that all particles were porous. Lentil and chickpea starches had a layered structure (Figure 6A), while amylose, and to a lesser extent bean starch, had a vermiform structure which persisted even after *in vitro* enzymatic treatment (Figure 6B).

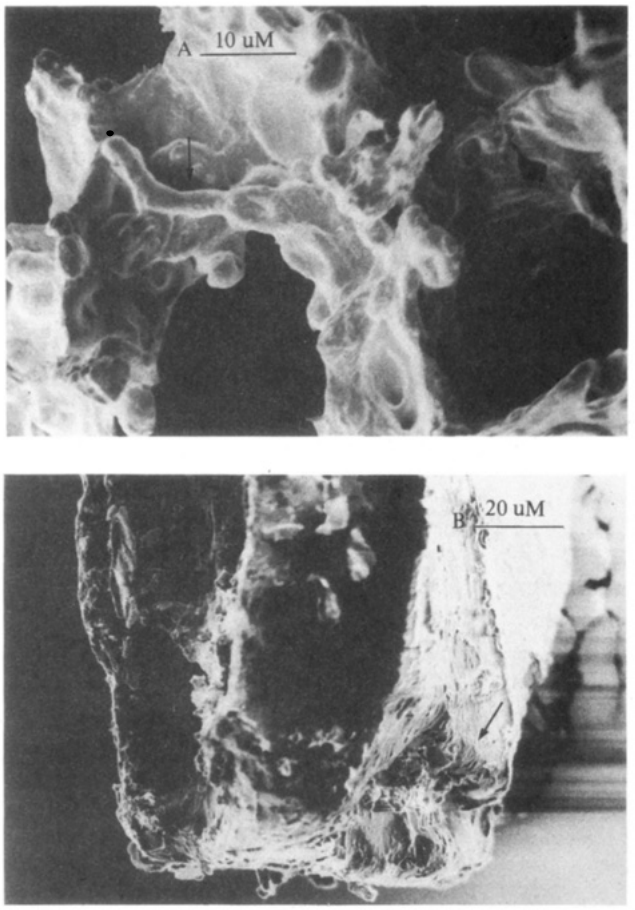

Figure 6. Scanning-electron micrographs (at higher magnification): (A) resistant amylose (after enzymatic treatment) and (B) resistant lentil starch (before enzymatic treatment).

After enzymatic treatment, retrograded lentil and chickpea starches showed many pitted zones due to erosion and the formation of numerous canals into the starches, resulting in a spongelike structure (Figure 7C,D). Amylose and bean starch were less eroded (Figure 7A,B).

DISCUSSION

Starch is a dietary nutrient which is very sensitive to the method of processing. The chemistry, the source of starch, and the processing alter the resistance of such starches to mammalian and bacterial amylolytic enzymes. The fermentation of three sources of legume starches has been studied in their native and retrograded form.

Using an *in vitro* system, we have obtained similar rates of fermentation and cell growth for three native legume starches, amylose, and amylopectin. Significant differences in total SCFA and lactic acid production were found between the three starches after 18 and 24 h. These findings are not in complete agreement with those of McBurney *et al.* (1990), who found different profiles of SCFAs and gases throughout the fermentation (using a human fecal slurry as the inoculum) of six kinds of starch (wheat, kidney bean, rice, corn, amylopectin, and amylose). The individual SCFA and total production per g of starch fermented at 24 h did not differ with the starch source. This difference could be due to variations in the source and the preparation of the inoculum. The results for individual acids showed differences mainly in butyric and lactic acid production during fermentation of amylose and amylopectin. Therefore, differences in fermentation products between starch samples may result from different ratios of amylose/amylopectin.

Retrograded starches were fermented more slowly than native starches, which was also observed by Wyatt and Horn (1988). The reduced fermentation rate appears to be a consequence of the reduced rate of bacterial growth and/or enzyme production. This is presumably due to the inaccessibility of the starch to enzyme activity or the reduced bacterial attachment.

Individual SCFA and lactic acid measurements after 48 h of fermentation of retrograded starches showed a decrease in acetic, propionic, and butyric acid production compared to those of the native starches. However, the concentration of lactic acid was increased. Increased production of lactic acid was associated with fermentation of retrograded amylose (Table I). Therefore, the high values of lactic acid found during fermentation of starches could be due to the formation of retrograded amylose during processing.

Despite all of the resistant samples being slowly fermented, amylose and bean starch were degraded at a slower and steadier rate than lentil and chickpea starches. The production of maltoheptaosidase, maltosidase, and pullulanase was less in retrograded amylose and bean starch fermentations than in retrograded lentil and chickpea starch fermentations.

The slow rate of fermentation of retrograded amylose is consistent with recent *in vivo* studies where low breath hydrogen responses were obtained in subjects given a diet containing high amounts of retrograded amylose compared to those in diets containing equivalent amounts of either β-glucan or amylopectin. These findings therefore suggest that retrograded amylose is fermented more slowly by the large intestinal microflora (Gee *et al.*, 1992; Edwards and Gee, 1992).

Recent *in vivo* studies (Tovar *et al.*, 1992) using rats fed on precooked green coat lentils and red kidney beans, containing both retrograded and physically inaccesible starch fractions, have shown that indigestible starch in lentils is less susceptible to fermentation than that in red kidney beans. The different botanical source of the legumes and preparation of the samples may account for these apparent discrepancies. This may suggest that the physical changes occurring during food processing, which seem to depend on the chemical composition of the native samples, could influence the fermentability of starches from the same botanical source.

Scanning-electron micrographs of retrograded starches showed structural differences between the samples before and after *in vitro* treatment with amylase, protease, and amyloglucosidase (Figures 5, 6, and 7). The structures of the retrograded starches are dependent on molecular interactions occurring during gelation and retrogradation of the samples. It has been shown that these interactions depend on the time and temperature of processing (Sievert *et al.*, 1991). The concentration of starch and its botanical source (Miles *et al.*, 1985; Orford *et al.*, 1987; Leluop *et al.*, 1991a) may also affect gelation and retrogradation.

After gelation and retrogradation, starches develop a weak X-ray diffraction pattern of the B-type (Sievert *et al.*, 1991). The botanical source and therefore the amylose/amylopectin content of the starches used may result in different ratios of ordered and disordered retrograded fragments and a different response to amylolytic attack. It has been reported that amylolytic treatment of retrograded starches leads to hydrolysis of the amorphous and less crystalline structures, leaving the crystalline regions intact (Jane and Robyt, 1984). The porosity of the substrate (Colonna *et al.*, 1992) and the adsorption of

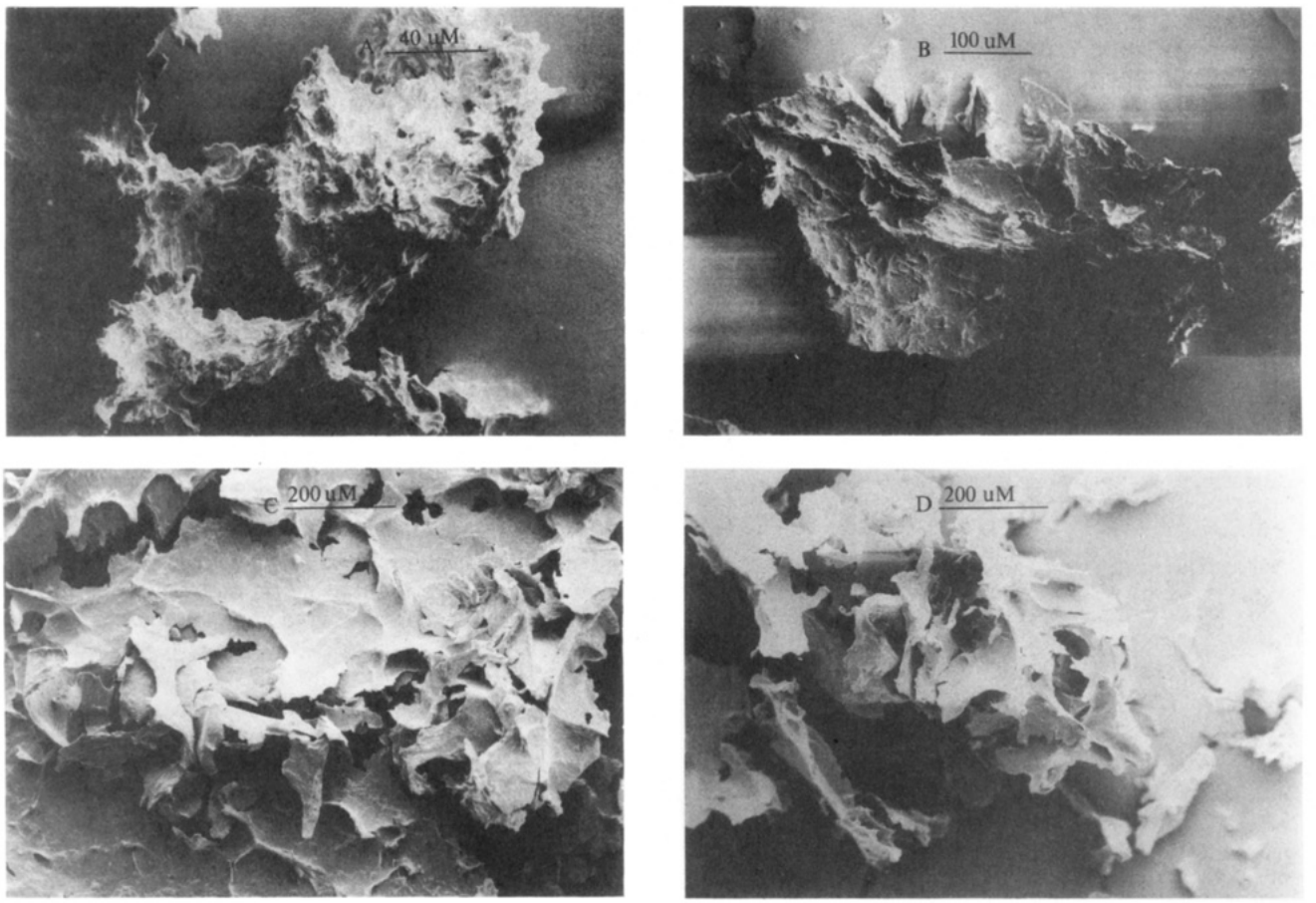

Figure 7. Scanning-electron micrographs of resistant starches after hydrolysis by α-amylase, protease, and amyloglucosidase: (A) amylose, (B) bean, (C) chickpea, and (D) lentil.

enzyme onto the substrate (Leloup *et al.*, 1991b) have been found to be limiting factors in hydrolysis.

After enzymatic treatment, the retrograded starches had different physical structures (Figure 7). Lentil and chickpea starches showed many pitted zones forming spongelike structures, which could have increased the accessibility to bacterial enzymes since they were more readily fermented compared to amylose and bean starch.

In conclusion, this study shows that retrograded starches from different sources are not equally digested *in vitro* by enzymes, resulting in structures of different porosity. This could be a factor affecting the accessibility to bacteria and hence resulting in unequal rates of fermentation of starches from different sources. The rates of fermentation of retrograded starches were slowest for retrograded amylose and bean starch. Such effects may be explained in terms of different structural modifications of the starches during the retrogradation process.

ABBREVIATIONS USED

ANOVA, analysis of variance; PHBAH, *p*-hydroxybenzoic acid hydrazide; PNP, *p*-nitrophenol; PNPG, *p*-nitrophenyl α-D-glucopyranoside; RS, resistant starch; SCFAs, short-chain fatty acids.

ACKNOWLEDGMENT

The financial support of the Spanish Comisión Interministerial de Ciencia y Technología (Project ALI 92-0278) and the Spanish Ministry of Education and Science is acknowledged. We thank Mr. J. Findlay (Science faculty EM facility, University of Edinburgh) for technical assistance in scanning-electron microscopy.

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Received for review May 7, 1993. Revised manuscript received August 20, 1993. Accepted August 30, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1993.