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Amylodextrins containing β-glucan from oat flours and bran

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Amylodextrins with soluble β-glucan contents as high as 10% were prepared from milled oat flours and bran. The level of enzymatic conversion was measured by high performance liquid chromatography and gel permeation chromatography. The large molecular weight amylodextrin fragments containing the β-glucan from low level amylolytic conversions were measured by gel permeation chromatography. The different elution times of the two separate enzyme conversion mixture components suggest different modes of enzyme actions. Lower molecular weight fractions are produced by the action of *Bacillus licheniformis* α-amylase in contrast to the action of *B. stearothermophilus* α-amylase, due possibly to dissimilar enzyme dosages. Comparisons of the amylodextrin compositions produced by *B. stearothermophilus* α-amylase and *B. licheniformis* α-amylase systems at intermediate conversion levels revealed that the *B. stearothermophilus* α-amylase gave much larger quantities of the maltohexaose component than the *B. licheniformis* α-amylase.

INTRODUCTION

Amylodextrins are produced generally from corn starch by acid, enzyme, or a combination to give a variety of products called maltodextrins or corn syrup solids with properties dependent on the degree of conversion. Acid conversions are known to give uniform distribution of hydrolyzate fragments because of the random cleavages of the starch molecule, whereas enzymic treatments result in variations in amounts of the different oligomer fragments (Inglett, 1987, 1990*a*). Various amylolytic enzymes and acids are used in the thinning or liquefaction of corn starch and in the production of starch hydrolyzates (Morehouse *et al.*, 1972). Some other sources of starch for commercial hydrolyzates are tapioca, potato and rice.

Amylodextrins with low levels of conversion are used as fat substitutes based on their fatty consistency and mouthfeel (Inglett, 1990*b*, 1991*a,b*). The use of these amylodextrins can reduce caloric intake as well as replace fat in the diet. The caloric value of a 25% maltodextrin gel is about 10% of the caloric value of fat. Because of the increasing need for lowering blood cholesterol levels, this study was undertaken to obtain oat flour amylodextrins which can be used as fat substitutes.

MATERIALS AND METHODS

Materials

Commercially available amylases were used in these studies: *Bacillus stearothermophilus* α-amylase, 'G-zyme G995' (Enzyme Bio-Systems Ltd, International Plaza, Englewood, NJ); and *B. licheniformis* α-amylase, 'Taka Therm L-340' (Solvay Enzymes, Elhart, IN). Oat bran was provided by National Oats Company (Cedar Rapids, IA) and Quaker Oats Company (Chicago, IL). Whole oat flour (Lab 16) and debranned oat flour (Lab 120) were provided by National Oats Company.

Methods

Proximate analyses for moisture, protein, and ash were obtained by official procedures of the AACC (1983) and for total lipid by AOAC (1980). The automated high performance liquid chromatographic (HPLC) system used to analyze the hydrolyzates as degree of polymerization (DP) was reported earlier (Inglett, 1987, 1990a). β-Glucan analysis was determined using the procedure of McCleary and Glennie-Holmes (1985) using a barley β-glucan assay kit provided by Biocon

† The mention of company names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other companies or similar products not mentioned.

Table 1. Oatrim β-glucan–amylodextrins

Oat substrate	α-Amylase	Enzyme dosage	Conversion level	Soluble β-glucan (%)
Bran flour	*B. stearothermophilus*[a]	1	Low	10·2
Bran flour	*B. licheniformis*[b]	816	Low	9·0
Whole flour	*B. stearothermophilus*[a]	24	Intermediate	3·1
Whole flour	*B. licheniformis*[b]	1 632	Intermediate	3·6
Bran flour	*B. stearothermophilus*[a]	24	Intermediate	8·4
Bran flour	*B. licheniformis*[b]	1 632	Intermediate	8·7

[a] Units, μg/g.
[b] Units, MWU.

Pty Ltd, 31 Wadhurst Drive Boronia, Victoria 3155, Australia.

General procedures for β-glucan–amylodextrin preparations from oat products

Four kilograms of an oat product was slurried in 28 liters of water containing 50 ppm of calcium. The pH of the slurry was 5·8. After gelatinization by passage of the mixture through a steam injection cooker, the slurry was collected in a 30-gal steam-heated kettle. α-Amylase preparation was added to the slurry as shown in Table 1. After 5 min of stirring at 80–90°C, the enzyme was inactivated by passing the slurry through a steam injection cooker or heating at pH 4·0 for 10 min. The slurry was centrifuged at 15 000 rpm by a large 'Sharples' centrifuge to separate the soluble and insoluble components. The soluble fiber–amylodextrin fraction was dried and weighed. The oligomer composition was measured by degree of polymerization (DP).

Gel permeation chromatography

Number average molecular weights (M_n) of the amylodextrin compositions prepared herein were estimated by gel permeation chromatography. Results are given in Table 2. System hardware and operating parameters were as follows: pump, 'SpectraPhysics 8810'; autosampler, 'SpectraPhysics 8780'; detector, 'Waters 410 refractive index' (35°C); integrator, 'SpectraPhysics 4270'; column, 'Waters Linear Ultrahydrogel' (45°C); mobile phase, unbuffered nanopure water, 0·5 ml/min (149 psig); sample, 20 μl loop injection, 5 g/liter concentration. Water soluble polysaccharide (dextrans) standards from Polymer Laboratories, Inc. (Amherst Fields Research Park, Amhurst, MA 01002) were used for the calibration of columns.

RESULTS AND DISCUSSION

Water-soluble dietary fiber compositions were recovered from such milled products as oat bran and oat flour after enzymatic hydrolysis of these substrates with α-amylase (Inglett, 1990*a*, 1991*a*,*b*). The slurried substrate was gelatinized prior to enzymatic treatment or converted during gelatinization. The ungelatinized slurry or the gelatinized dispersion was adjusted to about pH 6·0, with sodium hydroxide or other alkali before gelatinization and α-amylase addition.

Among the wide selection of α-amylases that could be used, the commercial α-amylases referred to as 1,4-

Table 2. *B. stearothermophilus* α-amylase and *B. licheniformis* α-amylase action on oat bran for low conversion products measured by gel permeation chromatography[a]

Retention time (min)	*B. stearothermophilus* α-amylase, 1 unit, 5 min			*B. licheniformis* α-amylase, 1 unit, 5 min		
	Peak area (%)	Molecular weight × 1000	DP[b]	Peak area (%)	Molecular weight × 1000	DP
9·28				4	>1 000	5 556
9·52	25	>1 000	5 556			
10·30	12	>1 000	5 556			
10·63				7	>1 000	5 556
15·26	42	289	1 606			
16·15				32	124	689
17·63				24	28	157
18·00	11	20	111			
19·91				31	3	17
21·50	10	0·6	3			
22·35				2	0·2	2

[a] Calibration curve is log (MW) = 12·092 80 − 0·433 43 for the retention time using dextran standards with molecular weights of 180, 5 800, 12 200, 23 700, 100 000, 380 000, and 853 000.
[b] Degree of polymerization.

α-D-glucan glucanohydrolases which have the essential enzymatic characteristics of those produced by the *Bacillus stearothermophilus* strains were found to be satisfactory. Other commercial sources of this enzyme included organisms such as *B. subtilis* and its genetically modified form to express the thermostable α-amylase of *B. stearothermophilus*. Other suitable α-amylases were those having the essential enzymatic characteristics of these produced by *B. licheniformis*. Any α-amylase suitable for starch liquefaction could be used.

The conditions of enzyme conversion, including the enzyme concentration and the time and temperature of reaction, were selected to achieve liquefaction of the starch in the substrate to the extent that the soluble fiber bound by the cellular matrix was substantially liberated into the water. When thermostable α-amylase was used, a preferred conversion temperature was in the range 70–100°C, preferably about 95°C. At these temperatures, gelatinization of the starch was complete. For concurrent hydrolysis, it would be necessary to add the enzyme prior to heating above 50°C. Better control of the degree of hydrolysis is obtained by adding the enzyme to the gelatinized starch at about 95°C for the desired time of conversion. Other α-amylase could be used at lower temperatures. The duration of the treatment at the desired conversion temperature depended on the desired product properties and generally can range from about 1 to 60 min.

After completion of the enzymatic hydrolysis, the enzyme was inactivated, e.g. by passing the mixture through a steam injection pressure cooker at a temperature of about 140°C. Alternatively, the enzyme could be inactivated by acidification (pH 3·5–4·0) at 95°C for about 10 min. Optional neutralization with alkali increases the salt concentration of the product. After inactivation, the soluble fraction contains the soluble oat fiber, mainly β-glucan, and amylodextrins. Their soluble fraction was separated from the insoluble residue by centrifugation. Water was then removed from the soluble fraction in the centrifugate by freeze-drying, drum-drying, or spray-drying to obtain the β-glucan–amylodextrins.

In addition to the amylodextrins and β-glucans in the water-soluble material, some protein, lipid, and minerals are found in various amounts depending on conversion techniques. Amylodextrin compositions from oats are generally white and smooth-textured without any undesirable flavours. Since the soluble-fibres of oats are principally β-glucan, these compositions are referred to as oat β-glucan–amylodextrins. Their preparations are shown in Table 1.

When *B. stearothermophilus* α-amylase and *B. licheniformis* α-amylase were allowed to act on oat flour and bran for controlled low levels of conversion, the degree of conversion was measured by HPLC for determining the amount of oligomers between DPs of 1 and 8 and by gel permeation chromatography for determining the large oligomer fragments with molecular weights between several hundred and greater than several million daltons. Nearly all low conversion products had compositions with 98% or higher of fragments greater than DP8. These products gave gel permeation chromatographs revealing different fragmentation between the *B. stearothermophilus* α-amylase and the *β. licheniformis* α-amylase systems.

The *B. stearothermophilus* α-amylase produced two fractions with molecular weights greater than one million in approximate percentages of the mixture of 25% and 12% that eluted from the column at retention times of 9·52 and 10·30 min, respectively (Table 2). The corresponding *B. licheniformis* α-amylase action produced two smaller size fractions with molecular weights greater than one million in approximate percentages of the mixture of 4% and 7% that eluted from the column at retention times of 9·28 and 10·63 min, respectively.

The third fraction from the action of *B. stearothermophilus* α-amylase had a molecular weight of about 289 000 in an approximate percentage of the mixture of 42% that eluted from the column at a retention time of 15·26 min. The corresponding *B. licheniformis* α-amylase action gave two fractions that had molecular weights of about 124 000 and 28 000 in approximate percentages of the mixture of 32% and 24% that eluted from the column at retention times of 16·15 and 17·63 min, respectively. The DPs of the fractions show the same decreases as would be expected. Although lower molecular weight fractions are produced by the action of *B. licheniformis* α-amylase than by the action of *B. stearothermophilus* α-amylase, it is possible to explain these differences in part by dissimilar enzyme dosages. However, the elution at different retention times appears to suggest different modes of action.

When these enzyme systems were controlled for intermediate conversion, the compositions revealed that the *B. stearothermophilus* α-amylase gave much larger quantities of the maltohexoase component than the *B. licheniformis* α-amylase as shown in Table 3.

Oat flour and bran treated with *B. licheniformis* α-amylase give a trio product-specificity for the forma-

Table 3. *B. stearothermophilus* α-amylase and *B. licheniformis* α-amylase action on oat flour and bran for intermediate conversion products measured by high-pressure liquid chromatography[a]

Degree of polymerization	*B. stearothermophilus* α-amylase, 24 units		*B. licheniformis* α-amylase, 1632 units	
	Flour 20 min	Bran 5 min	Flour 20 min	Bran 20 min
DP > 8	32·7	35·9	27·7	32·3
DP8	0·6	0·0	0·0	0·0
DP7	1·7	2·8	1·2	1·0
DP6	30·7	27·6	14·2	13·0
DP5	7·5	7·2	15·6	13·9
DP4	4·8	4·2	8·0	6·4
DP3	10·7	10·0	15·3	13·0
DP2	8·2	7·5	14·9	12·1
DP1	2·6	3·3	3·3	5·6

[a] Determined by high pressure liquid chromatography (Inglett, 1987, 1990*a*).

tion of the oligosaccharides, DP5, DP3, and DP2, as found earlier by Inglett (1987) on corn starches. By contrast, under equivalent α-amylolysis conditions, the action of *B. stearothermophilus* α-amylase on these oat products is distinguished by early dominant quantities of DP6, maltohexaose, followed by its erosion and increases in DP5, DP3, and DP2 oligomers. The production of large quantities of maltohexaose with *B. stearothermophilus* α-amylase action in corn starches was reported earlier by Inglett (1990*a*).

In an earlier investigation (Inglett, 1987), α-amylolysis by *B. licheniformis* α-amylase of jet-cooked ordinary, waxy, and high-amylose (70%) corn starches likewise revealed greater activity with high-amylose starch and less activity as proportions of the branched amylopectin increased. The amylolysis was somewhat more extensive as evidenced by the oligomers (DP10 and higher) remaining after 120 min of corn starch α-amylolysis: high-amylose, 1·6%; ordinary, 14·2%; and waxy, 19·2%.

Inglett (1987) found that high concentrations of corn starches (about 30–40%) gave selective formation of DP5, DP3 and DP2 oligomers by the action of *B. licheniformis* α-amylase which was previously reported by Saito (1973) using dilute starch substrates. These results were confirmed and expanded by Nakakuki *et al.* (1984), who found that *B. licheniformis* α-amylase degraded a 1% solution of short-chain amylose at 40°C to form mainly DP5 and DP3 oligomers with slightly smaller quantities of DP2.

Bertoft (1986) reported that the molecular weight distribution resulting from the action of *B, subtilis* α-amylase on waxy corn starch indicated that intermediate products of defined molecular weights were formed as the reaction proceeded toward smaller dextrins. These data, supported by the present research results, also suggest that the α-amylases do not hydrolyze amylopectin in a random manner. A more extensive discussion on the specificity of α-amylase reactions was reported by MacGregor and MacGregor (1985).

CONCLUSIONS

β-Glucan–amylodextrins were prepared by the treatment of oat flour and bran products with α-amylases. Oat starch of the flours and bran was converted into amylodextrins, which go into solution along with the soluble β-glucans. Oat products treated with *B. licheniformis* α-amylase gave a trio product-specificity for the formation of the oligosaccharides: DP5, DP3, and DP2. By contrast, the action of *B. stearothermophilus* α-amylase on these oat products produced dominant quantities of DP6 followed by its erosion and increases in DP5, DP3, and DP2 oligomers during longer conversion. Large molecular weight amylodextrin fragments containing the β-glucan were produced from low level amylolytic conversions.

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