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Investigation of Factors That Affect the Solubility of Dietary Fiber, as Nonstarch Polysaccharides, in Seed Tissues of Mung Bean (*Vigna radiata*) and Black Gram (*Vigna mungo*)

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The dietary fiber content of mung bean, as component seed tissues and as the raw and extrusion cooked flour, and of black gram flour has been measured, as nonstarch polysaccharides (NSP), using two methods of fiber analysis. Changes in the distribution of NSP between soluble and insoluble fiber have been used as an indicator of the effect of cooking on the extent of degradation of fiber NSP. The fiber content of mung bean flour and black gram flour was 12.2% and 14.2%, respectively. The soluble NSP from mung bean flour (0.5%) was rich in intracellular, mannose-containing, glycoproteins while the insoluble NSP (11.6%) fraction contained much arabinose-rich pectic polysaccharide, xylose-containing polysaccharides, and cellulose. The arabinose-rich pectic polysaccharides arose mainly from the cotyledon tissue, while xylose in cotyledon fiber was derived from xyloglucan and in hull fiber from xylan. Pectic polysaccharides were particularly susceptible to degradation, and extrusion cooking led to an increase in the solubility of arabinose-enriched pectic polysaccharides. More extensive heat treatment and use of phosphate buffer during fiber preparation further increased fiber solubility, showing that the method of fiber preparation can result in a significant further degradation of the constituent pectic polysaccharides within the fiber matrix. Sequential extraction of pectic polysaccharides from the insoluble fiber under nondegradative conditions showed the extrusion-cooked insoluble fiber was rich in degraded arabinose-rich pectic polysaccharide. This study has shown that a significant degradation of fiber can occur due to cooking and processing of pectic polysaccharide-rich materials, such as legume seeds. The result of degradation is to produce a spectrum of fiber solubility, for example saline soluble and buffer soluble, and this could have important consequences in distinguishing between the nutritional response to soluble and insoluble fiber in the diet.

INTRODUCTION

Legume seeds contain a significant amount of dietary fiber (DF), starch, and protein but require relatively extended cooking times to soften the tissue and to ensure inactivation of constituent antinutritional factors, e.g. lectins (Liener, 1989). It is primarily due to the disruption of the constituent cell walls and the degradation and solubilization of pectic polysaccharides that the tissue softens (Van Buren, 1979). Such changes in pectic polysaccharide solubility may affect the nutritional response to the fiber [e.g. as a hypocholesterolemic agent (Shutler et al., 1987) or to improve glycemic response (Wolever and Jenkins, 1986)] during gut transit.

The glycemic response has been attributed to a reduced rate of glucose absorption, the "slow release" property, and for this a heat-labile soluble component of the fiber matrix, notably the pectic polysaccharides, has been implicated to be important in eliciting the glycemic response. However, multiple regression analysis of fiber content, as nonstarch polysaccharides (NSP), and composition of 25 foods related to their glycemic index (Wolever, 1990) has suggested that the uronic acid content

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of the insoluble fiber, measured by *in vitro* chemical analysis, was the more strongly related factor to glycemic response. Thus the persistence of the fiber matrix, containing cross-linked pectic polysaccharides, hemicelluloses, and cellulose, which resist solubilization during cooking and processing, may be more important than has been hitherto realized (Selvendran and Robertson, 1990).

A complication in identifying the importance of the insoluble fiber matrix in the gut is that during cooking and processing and using methods designed to measure the fiber content of foods (Theander and Aman, 1979; Asp et al., 1983; Prosky et al., 1984; Faulks and Timms, 1985; Theander and Westerlund, 1986; Englyst et al., 1992), the heat treatment required to gelatinize starch can result in the degradation of pectic polysaccharides. Unless the problem of measuring the extent of fiber degradation due to the method of cooking and/or fiber analysis can be resolved and rectified, then the problem of relating the intake of fiber to its nutritional response will also remain unresolved.

To determine how the solubility of fiber is affected by cooking and processing, mung bean, as raw flour and its extruded product, and black gram have been investigated for fiber content and composition, as NSP, using two methods of fiber analysis, slight variations of which are in current use. Solubility differences have been measured and related to the different component seed tissues. Also, a sequential extraction of fiber residues made under nondegradative conditions has been undertaken to determine how different polysaccharide "types" alter on processing and in relation to solubility measured by standard chemical analyses.

MATERIALS AND METHODS

Materials. *Seeds.* Mung bean seed was obtained from Booker Seeds, Sleaford, Lincs, U.K. Black gram seed was obtained locally. Seeds were pin-milled to produce a flour prior to analysis. Mung bean seeds were also manually separated into component cotyledon, hull, and embryonic tissues, after hydrating for 12 h at 4 °C to minimize germination. Tissues were ground in liquid N_2 and freeze-dried prior to analysis. To determine seed and tissue dry weight distribution, a sample of each component tissue from mung bean was dried (16 h) at 105 °C.

Processing. Extruded material was prepared from the mung bean flour using a Baker-Perkins MPF 50D twin-screw extruder (barrel temperature 125–130 °C; moisture content 15%; material pressure 355 psi; feed rate 20 kg/h; screw speed 200 rpm; die size 2 × 3 mm circular die). The extruded product was allowed to cool before being sealed in an airtight container at room temperature. The product (8% moisture) was ground prior to analysis.

Analytical Methods. *Free Sugars and Starch.* Free reducing sugars were determined from an 80% ethanol extract of mung bean flour (Nelson, 1944) with glucose as standard. Starch content was determined following sequential digestion of mung bean flour with Termamyl (Novo Enzymes, Denmark) and amyloglucosidase (Sigma, U.K.). Samples (100 mg) dispersed in 10 mL of H_2O were gelatinized for 15 min in a boiling water bath. Termamyl (0.1 mL; 4% stock solution) was added, and the mixture was then incubated a further 15 min at 100 °C. After cooling (60 °C), 1 mL of acetate buffer (pH 4.5) and 10 μL of amyloglucosidase were added, and the mixture was reincubated (60 °C for 16 h) and then precipitated in ethanol (80% final volume). The supernatant recovered was analyzed for glucose (Boehringer; Test Kit 124036).

Starch Resistant to Hydrolysis during NSP Preparation. Resistant starch was solubilized from the NSP using 4 M KOH followed by enzymic hydrolysis and estimation of glucose released. Samples (20 mg) were extracted (30 min; 20 °C) in 1 mL of 4 M KOH, containing 200 μg of $NaBH_4$, the pH was adjusted to 5.5 (1 M acetic acid), and the supernatant was recovered by centrifugation (1200*g*). The insoluble pellet was resuspended in 2 mL of distilled water and recentrifuged, and the resulting supernatants were combined and dialyzed (1 L deionized water × 3). An aliquot of the dialyzed supernatant (10%) was used for starch estimation using a 0.5-mL Pancrex solution (pH 4.6; acetate buffer for 16 h at 42 °C) for the digestion followed by 0.1 mL of amyloglucosidase (Boehringer 102857) and incubation for a further 30 min at 60 °C. [Pancrex (Paines & Byrne Ltd., Greenford, Middlesex, U.K.) was prepared by suspending 1 capsule in 4.5 mL of water and centrifuging to remove insoluble material.] A 1-mL aliquot of the final incubation mixture was reduced in volume to 0.2 mL and glucose estimated using glucose oxidase as above.

Nonstarch Polysaccharide (NSP) Content. Two procedures were used to determine the NSP content of the samples and to estimate the influence of the method of analysis on NSP solubilization.

In the first procedure [a modification of Faulks and Timms (1985) based on that of Theander and Aman (1979)], samples (300 mg) were dispersed in 10 mL of Tris-maleate buffer (0.2 M; pH 6.7) containing 2.5 mM $CaCl_2$, heated for 10 min at 100 °C to gelatinize starch, and digested with Termamyl (0.2 mL; 4% stock concentration) for 15 min at 100 °C. The sample was treated with ethanol (80% v/v), and the insoluble residue recovered after cooling (50 °C) and centrifugation (2000*g*) was dispersed in 2 mL of DMSO, at 100 °C for 5 min. After cooling (37 °C) and addition of acetate buffer (8 mL, pH 4.6, 0.2 M), the mixture was incubated with 0.1 mL of amyloglucosidase for 35 min at 37 °C and centrifuged (2000*g*), and the water-insoluble pellet was recovered and freeze-dried (buffer-insoluble material). The material was tested for the absence of residual starch using I_2/KI solution. The supernatant was precipitated in ethanol, (80% v/v), and the precipitate was dialyzed against deionized water and freeze-dried (buffer-soluble material).

In the second procedure (Englyst et al., 1992), samples (300 mg) were dispersed in 2 mL of DMSO, heated at 100 °C for 30 min to gelatinize starch, and digested with Termamyl (8.0 mL; 1.25% stock concentration, preequilibrated at 50 °C in acetate buffer; pH 5.2) for 10 min at 100 °C. After the mixture was cooled to 50 °C, 0.5 mL of Pancrex and pullulanase (Boehringer 108944) (0.1 mL of 1% stock) were added, and incubating continued for 30 min at 50 °C. Incubation temperature was finally raised to 100 °C for 10 min, 40 mL of phosphate buffer was added (200 mM; pH 7), and incubation continued for 30 min. Buffer-insoluble material was recovered by centrifugation (2000*g*) and the soluble polymeric material recovered from the supernatant by ethanol precipitation (80% final volume). The ethanolic supernatant was retained. The buffer-insoluble material was dispersed in 50 mL of distilled water followed by recentrifugation and precipitation as above. The ethanolic precipitates were combined and dialyzed against deionized water before freeze-drying to yield a buffer-soluble fiber fraction. The ethanolic supernatants were dialyzed and freeze-dried to provide an ethanol-soluble NSP. The buffer-insoluble pellet was also dialyzed and freeze-dried (buffer-insoluble fraction).

Solubilization of NSP. Saline-soluble NSP from mung bean samples was obtained as follows: Samples, dispersed in saline (0.15 M NaCl; 60 mL/g) containing 0.01% sodium azide as a bacteriostat, were pummeled (30 s; Colworth Stomacher), transferred to a 250-mL conical flask, and incubated, with shaking (100 rpm), at 37 °C for 3 h. The supernatant, recovered by centrifugation (2000*g*), was precipitated in ethanol (80% v/v), dialyzed (5 L × 3) against deionized water, freeze-dried, and analyzed for NSP (Faulks and Timms, 1985).

The insoluble residue, recovered by freeze-drying and depleted of starch using the method of Faulks & Timms, gave a further buffer-soluble and a buffer-insoluble fraction. The buffer-insoluble residue resulting was sequentially extracted using *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) and Na_2CO_3 to solublize mainly pectic polysaccharides under nondegradative conditions (Selvendran and O'Neill, 1987) as follows:

Samples (120 mg) were dispersed in CDTA (10 mL; 50 mM; 6 h, room temperature) and centrifuged (12000*g*), and the supernatant was recovered. The pellet was washed twice with distilled water, and the washings were added to the supernatant. The residue was retained for further extraction. The supernatant was dialyzed and then freeze-dried to recover the CDTA-soluble

Table 1. Composition of Fiber Derived from Component Seed Tissues of Mung Bean Flour

		composition (mg/g)								mg/g	
sample	tissue wt (%)	deoxy	Ara	Xyl	Man	Gal	Glc	UA[b]	total[c]	whole tissue[d]	whole seed[e]
cotyledon	*93.0*										
insol		4.6	68.4	21.2	14.3	10.5	148.3 (58.4)	57.9	325.2	107.3	99.8
sol		0.6	5.7	1.9	98.8	20.2	33.1	18.3	178.5	4.9	4.6
hull	*5.9*										
insol		5.3	39.3	130.3	1.9	9.5	288.9 (30.3)	157.5	632.7	420.3	24.8
sol		2.4	28.8	5.6	21.5	19.3	27.4	40.7	145.7	10.2	0.6
embryo	*1.1*										
insol		3.7	61.1	46.1	3.2	19.8	107.8 (18.7)	104.5	346.2	263.6	2.9
sol		6.7	63.2	25.3	12.5	38.4	72.0	329.6	547.7	81.8	0.9

[a] Tissue wt = percent dry weight of whole seed. [b] UA = uronic acid. [c] Total = total sugars in destarched fraction. [d] Whole tissue = total NSP in sample tissue. [e] Whole seed = total NSP contribution to whole seed. Values in parentheses for 1 M sulfuric acid hydrolysis. Samples of fiber were analyzed in duplicate for NSP.

material. The CDTA-insoluble residue was dispersed in Na_2CO_3 (10 mL; 50 mM) at 1 °C for 16 h and then allowed to reach room temperature before centrifugation (12000*g*) and recovery of the supernatant as above. The supernatant pH was adjusted to 5.5 using acetic acid, before dialysis and recovery of Na_2CO_3-solubilized material by freeze-drying.

Analysis of Samples for NSP. Sugars were released from residues by acid hydrolysis. Water-insoluble samples were dispersed in 72% H_2SO_4 for 3 h followed by dilution to 1 M and hydrolyze at 100 °C for 2.5 h. A 1 M H_2SO_4 hydrolysis (100 °C for 2.5 h) was also included to estimate cellulose by difference. Water-soluble samples were hydrolyzed in 1 M H_2SO_4 as above. Neutral sugars released were derivatized as their alditol acetates before analysis by GLC (Selvendran et al., 1979). Uronic acids were estimated colorimetrically, as total uronic acid (Blumenkrantz and Asboe-Hansen, 1973), using a 1-h 1 M H_2SO_4 sample hydrolysate.

RESULTS AND DISCUSSION

Analysis of the mung bean flour gave a moisture content of 78 mg/g with free sugars 53 mg/g dry weight and starch 361 mg/g dry weight. For black gram flour, moisture content was 109 mg/g, free sugars 40 mg/g, and starch 338 mg/g dry weight. These values are comparable with those reported in *Food Composition Tables* (Holland et al., 1991).

Analysis of Component Seed Tissues in Mung Bean. Cotyledon tissue (93.0%) was the major contributor to the mung bean seed (Table 1), with seed hulls (5.9%) and embryo tissue (1.1%) being the other tissue types present. These tissue types were analyzed for soluble- and insoluble-fiber content, as NSP (Faulks & Timms, 1985). Determination of NSP was made in duplicate, with data reported as the mean.

Cotyledon. Cotyledon tissue had an NSP content of 112.2 mg/g, of which 95.6% was insoluble and 4.4% was soluble. The soluble fraction had a high content of mannose (55%), the bulk of which can be inferred to arise from intracellular glycoproteins, including lectins (Selvendran and O'Neill, 1982). Pectic polysaccharides, on the basis of the contribution of arabinose, galactose, and uronic acid (galacturonic), accounted for only 25% of the soluble NSP. In contrast, pectic polysaccharides rich in arabinose were the major noncellulosic polysaccharides in the insoluble-fiber fraction, accounting for 42% of the NSP. The glucose released from the insoluble fiber by 1 M H_2SO_4 hydrolysis (noncellulosic glucose) was unexpectedly high. In most soft tissues, e.g. apple, potato, and runner bean parenchyma, the noncellulosic glucose from NSP is low and derived mainly from xyloglucan. The xyloglucan: cellulose ratio is about 1:7 (Ryden and Selvendran, 1990a,b), whereas in mung bean cotyledon cell wall material it was 1:2 (Gooneratne et al., 1992) and would account for the relatively large proportion of glucose released by 1 M H_2SO_4 hydrolysis of the fiber.

Embryo. In embryo tissue the fiber content was 345.4 mg/g of embryo, of which 75% was insoluble and 25% was soluble. The bulk of the soluble fiber contained pectic polysaccharides. Polysaccharides containing xylose and glucose were also present in a relatively high proportion. Pectic polysaccharides and cellulose were the major components of the insoluble fiber. The insoluble fraction also included an appreciable amount of xylose-containing noncellulosic polysaccharides, the bulk of which can be inferred to be xyloglucans. From the low proportion of embryo tissue in the seed, the contribution of the embryo to seed fiber content will be small.

Hulls. Hull tissue had a fiber content of 430.5 mg/g, of which 98% was insoluble and 2% was soluble. The bulk of the soluble fiber arose from the pectic polysaccharides. Cellulose was the major component of the insoluble fraction (45%); the low proportion of glucose in hull fiber susceptible to hydrolysis using 1 M H_2SO_4 being in marked contrast to the high proportion released from cotyledon and embryo tissues. The insoluble fiber from the hull was also enriched in pectic polysaccharides (33%) and xylose-containing polysaccharides (20%). Since relatively little glucose was hydrolyzed using 1 M H_2SO_4, and from the knowledge that xylans have also been isolated from soya bean hull (Aspinall et al., 1967) and pea hull (Ralet et al., 1993a), the bulk of the xylose was inferred to arise from xylans rather than xyloglucans. Although seed hulls accounted for only 5.9% of the mung bean seed, its high fiber content does make it a significant contributor to the overall fiber content in mung bean, particularly the contribution of xylans and cellulose.

On the basis of tissue analysis, the contribution of different tissues to the fiber content of the mung bean seed was as follows: embryo, 0.4%, hull, 2.5%; and cotyledon, 10.4%, to give a total fiber content of 13.3%, as NSP. This compares well to the value of 12.2% ± 0.4% derived from direct analysis of the flour (Table 2).

The results of the analysis of dietary fiber from mung bean flour, extruded mung bean and black gram flour using the method of Faulks and Timms, are shown in Table 2. Results shown are the mean of determinations made in duplicate from mung bean flour and black gram flour and in triplicate for the extruded mung bean flour samples. The fiber content of mung bean flour was 121.8 mg/g, of which 4.4% was soluble and 95.6% was insoluble. The composition of the soluble fiber was comparable to that from the cotyledon whereas the composition of the

Table 2. Composition of NSP Derived from Mung Bean Flour and Extrusion Cooked Product and Black Gram Flour Using the Procedure of Faulks and Timms

sample	% material recovered[a]	composition (mg/g) deoxy	Ara	Xyl	Man	Gal	Glc	UA	total sugars	original sample
mung bean flour										
insol[b]	31.9	4.0	71.2	59.1	7.9	10.2	161.0 (48.3)	51.6	365.0	116.4
sol[c]	2.9	0.6	5.9	12.5	114.7	16.8	13.2	19.9	183.6	5.3
total[d]	*34.8*	*3.7*	*65.8*	*55.2*	*16.8*	*10.8*	*148.7*	*48.9*	*349.9*	*121.8 ± 4.1*
mung bean extrud										
insol	33.0	2.4	42.4	39.5	7.8	8.7	167.9 (101.2)	39.5	308.2	101.7
sol	3.7	1.3	67.1	6.5	79.2	12.8	216.2	27.7	410.8	15.2
total	*36.7*	*2.3*	*44.8*	*36.2*	*15.0*	*11.1*	*172.6*	*38.3*	*320.3*	*116.9 ± 4.5*
black gram										
insol	35.3	2.8	68.4	32.0	10.1	28.1	100.6 (12.6)	71.0	313.0	110.5
sol	10.1	6.3	122.2	2.8	34.6	68.2	6.8	68.3	309.2	31.2
total	*45.4*	*3.6*	*80.4*	*25.5*	*15.6*	*37.0*	*79.7*	*70.4*	*312.2*	*141.7 ± 3.3*

[a] % material recovered = percent sample recovered after gelatinization procedures. [b] Insol = buffer-insoluble fraction recovered after gelatinization procedures. [c] Sol = buffer-soluble/ethanol-insoluble fraction recovered after gelatinization procedures. [d] Total = total fiber contributed from insoluble and soluble fractions, with total fiber g of original sample the mean ± 1 SD. Values in parentheses from 1 M sulfuric acid hydrolysis. Samples were analyzed in duplicate for mung bean flour and black gram and in triplicate for the extruded material.

Table 3. Composition of NSP Derived from Mung Bean Flour and Its Extrusion Cooked Product Using the Procedure of Englyst

sample	% material recovered[a]	composition (mg/g) deoxy	Ara	Xyl	Man	Gal	Glc	UA	total	original sample
flour										
insol[b]	16	4.7	63.4	124.3	3.9	9.6	294.5 (49.1)	41.2	541.6	86.7
sol										
ppt[c]	9	7.1	81.9	20.1	16.0	30.2	92.7	116.0	364.0	32.8
snt[d]	8	0.9	3.9	0.9	7.4	3.9	39.2	22.6	79.0	6.2
total[e]	*33*	*4.4*	*54.0*	*66.0*	*8.0*	*13.8*	*177.6*	*57.1*	*380.9*	*125.5*
extruded										
insol	8	4.0	36.5	75.7	5.1	9.3	205.4 (40.4)	20.8	356.8	28.5
sol										
ppt	15	7.0	121.9	21.9	12.8	35.1	83.6	82.8	365.1	54.8
snt	8	2.2	22.2	1.5	10.8	6.4	89.7	17.6	150.4	12.1
total	*31*	*5.1*	*74.1*	*30.5*	*10.3*	*21.0*	*116.6*	*50.0*	*307.6*	*95.4*

[a] % material recovered = percent of material recovered after gelatinization procedures. [b] Insol = buffer-insoluble fraction recovered after gelatinization procedures. [c] ppt = soluble material precipitated in 80% ethanol (v/v) and dialyzed. [d] snt = material soluble in 80% ethanol (v/v) recovered by dialysis. [e] Total = total fiber contributed from the insoluble and the soluble fractions. Results shown are for determinations made in duplicate for flour and in triplicate for the extruded material. Values in parentheses are from 1 M sulfuric acid hydrolysis.

insoluble fiber was enriched in xylose and glucose, i.e. cellulose and xylans from the hull. The proportion of glucose hydrolyzable using 1 M H_2SO_4 (30%) in the insoluble fiber reflected the high xyloglucan content of mung bean cotyledon tissue.

The fiber content of extruded mung bean flour was 116.9 mg/g, of which 13.0% was soluble and 87.0% was insoluble. The increased soluble fiber content relative to the raw flour was due to mainly an increased solubility of arabinose-containing pectic polysaccharides resulting from a slight degradation of pectic polysaccharides. The notable increase in glucose-containing soluble NSP was probably due to the presence of some "resistant starch" formed during the extrusion process; this starch was resistant to amylolytic digestion. The mannose content of the fiber from extruded mung bean flour was similar to the raw flour, with also a similar distribution between soluble and insoluble fractions. The insoluble-fiber content of the extruded flour showed a depletion in pectic polysaccharides relative to the raw flour, consistent with the degradation and solubilization of pectic polysaccharides during extrusion.

The fiber content of the black gram was 141.7 mg/g, of which 22.0% was soluble and 78.0% was insoluble. While this was significantly higher than the soluble fiber of mung bean flour and was due to the increased amount of pectic polysaccharides rich in arabinose and also galactose, it is consistent for a nonendospermous legume (Selvendran et al., 1987). In red gram (*Cajanus cajan*), pectic polysaccharides rich in arabinose and galactose have also been identified from milling fractions (Swamy et al., 1991). The much lower mannose content of the soluble fiber from black gram indicated black gram to be relatively poor in intracellular glycoproteins. The insoluble fiber from black gram was rich in pectic polysaccharides and cellulosic glucose, and as in mung bean flour, a significant proportion of xylose can be inferred to arise from the hull tissue xylans.

Fiber content of mung bean products measured using the procedure of Englyst is shown in Table 3. Results shown are the mean of determinations made in duplicate for flour and in triplicate for the extruded product. The total recovery of fiber, as NSP, from the raw flour was similar to that found using the Faulks and Timms procedure. For the extruded flour, recovery of NSP was lower than that from the raw flour and was much lower than the value for extruded flour by the method of Faulks and Timms. The lower recovery persisted even after the inclusion of the NSP recovered in the ethanol-soluble fraction (6.2 mg/g for flour; 12.1 mg/g for extruded) and would indicate there was also a loss of degraded NSP due to dialysis. (A small amount of ethanol-soluble fiber may also arise by the procedure of Faulks and Timms, but this was not measured.) It was also notable that the proportion of soluble fiber was increased using the Englyst procedure

Table 4. Solubilization of NSP Derived from Mung Bean Using Differential Extraction Conditions[a]

	treatment	material recovered[b] (%)	composition of fiber extract (mg/g) deoxy	Ara	Xyl	Man	Gal	Glc	UA	total	NSP (mg/g of original sample)
flour											
saline soluble	(pH 7)	9.2	0.2	3.0	0.3	23.8	4.8	3.9	39.8	75.8	7.0
saline insoluble	(pH 7)	27.3									
	sol	3.0	1.7	12.7	3.4	105.0	13.5	18.8	46.0	201.1	6.0
	CDTA	4.5	2.7	21.6	11.4	7.5	4.7	6.7	143.2	197.8	8.9
	Na_2CO_3	0.6	3.0	65.2	14.7	10.3	12.2	10.1	81.9	197.4	1.2
	residue	15.5	6.0	92.7	92.1	4.5	14.5	243.3	102.3	555.4	86.1
extruded											
saline soluble	(pH 7)	3.9	1.3	102.4	12.8	26.5	33.8	76.0	24.6	277.4	10.8
saline insoluble	(pH 7)	32.0									
	sol	2.1	0.9	113.8	14.2	109.8	29.3	21.4	100.2	419.4	8.8
	CDTA	4.6	1.3	22.5	3.3	6.0	4.1	3.1	134.1	174.4	8.0
	Na_2CO_3	1.1	3.4	98.6	33.7	19.7	15.7	16.5	120.3	307.9	3.4
	residue	23.3	2.0	21.3	44.8	6.3	5.3	136.0	30.8	246.5	57.5

[a] Original samples were extracted in saline at pH 7, 37 °C, and dialyzed. For flour 12.9% of the sample was recovered as saline soluble and 75.0% as saline insoluble. For extruded product, 35.8% was recovered as saline soluble and 52.1% as saline insoluble. Saline-soluble and -insoluble residues recovered were destarched by the procedure of Faulks and Timms to yield material recovered (footnote *b*). Destarching the insoluble residue yielded a further soluble fraction (buffer sol). The destarched insoluble residue was then sequentially extracted using CDTA (CDTA sol) and Na_2CO_3. The residue is the fraction resistant to solubilization in Na_2CO_3. Total fiber recovery, as NSP, for flour was 109.3 mg/g of original sample and for the extruded product was 85.7 mg/g of original sample.

from 4.4% to 27.5% of the total fiber. The increased proportion of soluble fiber was due to enhanced levels of arabinose and uronic acid and to a lesser extent galactose. Thus degradation of pectic polysaccharides in the insoluble fiber matrix had occurred. That the enhanced solubility of fiber occurred after exposure to hot phosphate buffer at pH 7 would support β-eliminative degradation promoting solubilization of pectic polysaccharides (Barrett and Northcote, 1965). When phosphate buffer is used in other methods of fiber analysis (Prosky et al., 1984; Asp et al., 1983), it is used at 100 °C but at pH 6 and with only a 15-min exposure to the fiber compared to 30 min in the Englyst procedure. The Faulks and Timms procedure avoids the use of phosphate buffer.

While the bulk of the solubilized pectic polysaccharides could be precipitated by 80% ethanol, a significant amount was recovered by dialysis of the ethanolic solution and freeze-drying. The analysis of the ethanol-soluble NSP showed the presence of both pectic polysaccharides and some mannose-containing carbohydrate moieties. The mannose-containing moieties would have been released and lost from the intracellular glycoproteins by the activity of proteolytic enzymes present in Pancrex and indicate that the glycoproteins present in the NSP are susceptible to proteolysis and can be lost during dialysis. A glycopeptide with a carbohydrate moiety containing 10 sugar residues (mainly mannose) and 20 amino acid residues would have a MW around 5000, which would be lost through the 12000 MW cut-off dialysis membrane used in the present study.

The above observations on the solubilization of pectic polysaccharides were even more applicable to the extruded product. The fiber recovered from the extruded product (95.4 mg/g) was only 76% the amount recovered from the flour and was also lower than the value determined for the extruded product using the Faulks and Timms procedure. Thus, not only has there been a greater solubilization during the analysis but fiber, mainly of pectic polysaccharide origin, has been degraded to dialyzable fragments. This study has shown that differences in the analytical procedures used for fiber analysis can result in major modifications to the fiber matrix and the properties of the fiber recovered. However, through an understanding of the behavior of constituent fiber polysaccharides under the different analytical conditions, important information can be obtained on the properties of fiber as eaten in foods, i.e. how cooking and processing conditions might alter the fiber matrix and how this may influence formation and release of soluble fiber in foods.

To investigate more fully how fiber solubility may be affected under physiological conditions, the mung bean flour and extruded product were extracted, in duplicate, in physiological saline at 37 °C and, after starch depletion (Faulks and Timms, 1985), the saline-insoluble residues were sequentially extracted with CDTA followed by dilute Na_2CO_3, under conditions which caused minimum degradation of polysaccharides (Selvendran and O'Neill, 1987).

Following incubation at 37 °C and recovery of the dialyzed supernatant and insoluble residues, 12.9% of the flour and 35.8% of the extruded product were recovered in the soluble fraction. Amylolysis reduced the amount of soluble material recovered to 9.2% for the flour but only 3.9% for the extruded product, indicating that after extrusion much more of the solubilized material was starch (Table 4). The saline-solubilized fraction from flour contained 7.0 mg of fiber/g of original sample, compared to 5.3 mg/g of original sample measured by fiber analysis (Table 2), and comprised mainly mannose (from the glycoproteins) and uronic acid. The fraction solubilized from the extruded product using saline contained 10.8 mg of fiber/g of original sample, compared to 15.2 mg of fiber/g by fiber analysis, was relatively rich in pectic polysaccharides enriched in arabinose, and contained some mannose (glycoproteins) and an increased level of "xylan". The solubilization of pectic polysaccharides from extruded material using physiological saline clearly showed that pectic polysaccharides of the fiber matrix had been appreciably degraded during extrusion. Similarly, the increased level of xylose-containing polysaccharides suggests some solubilization of hemicelluloses. This would agree with the solubilization of pectic polysaccharides and hemicelluloses found from extrusion cooked pea hulls (Ralet et al., 1993b). Although pectic polysaccharides and hemicelluloses were solubilized during extrusion of pea hulls, there was no extensive degradation of polymeric structure. However, like the mung bean hulls, fiber frompea hulls is compositionally and structurally distinct from that in the cotyledons and there is also evidence that β-eliminative degradation of pectic polysaccharides can also give rise to low molecular weight oligomers (Kravtchenko et al., 1993). These may be lost during dialysis and hence overlooked during analysis.

After saline extraction and dialysis, 75.0% of the flour and 52.1% of the extruded product were recovered as

saline-insoluble material. Amylolysis reduced the recovery of material to 27.3% for flour and 32.0% for the extruded product. During amylolysis the buffer-soluble fraction generated for the extruded product (2.1%) was much richer in NSP (277.4 mg/g) compared with the buffer-soluble fraction from the flour (3.0%; 201.1 mg/g). The solubility of the mannose-containing glycoproteins from both buffer–soluble samples was comparable. The fiber solubilized through saline extraction and amylolytic treatment of the extruded product was significantly enriched in arabinose compared to the flour, supporting the evidence from the soluble fractions that appreciable degradation of pectic polysaccharides had occurred during extrusion cooking. However, the extent of degradation was not sufficient to allow solubilization during treatment with saline.

The CDTA-soluble fractions from both the flour and extruded product were similar in fiber composition, being predominantly galacturonic acid, and represented around 0.8% of the fiber in each sample. However, the Na_2CO_3-soluble fraction from the extruded product was richer in pectic polysaccharides than that from the flour. These pectic polysaccharides are probably sugar esters cross-linked within the cell wall matrix and are released during mild saponification (Ryden and Selvendran, 1990a,b).

The solubilization of degraded pectic polysaccharides by the above solvents was reflected in the lower content of arabinose, galactose, and galacturonic acid in the final residue recovered after Na_2CO_3 extraction from the extruded product compared to that from the flour. From sequential extraction, up to and including Na_2CO_3 treatment, only 2.3% fiber was solubilized from flour and 3.1% from the extruded product. This compared to 0.5% (Faulks and Timms) and over 3.3% (Englyst) for flour by direct analysis and for extruded material, 1.5% (Faulks and Timms) and over 5.5% (Englyst). The much lower values for soluble NSP obtained by sequential extraction confirms that a substantial degradation of pectic polysaccharides can occur during fiber analysis, even beyond that due to cooking and processing procedures.

Distribution of pectic polysaccharides in each fraction generated through sequential extraction indicated that in flour the bulk of pectic polysaccharides remained associated with the insoluble residue. Using arabinose as a marker for the more readily degradable pectic polysaccharides regions, in the raw flour 87.7% of the pectic polysaccharides were associated with the Na_2CO_3-insoluble residue, with the Na_2CO_3 extract (2.4%), CDTA extract (5.9%), and other soluble fractions (4.0%) being minor contributors of pectic polysaccharides. Conversely, in the extruded product the Na_2CO_3-insoluble residue accounted for only 39.6% of the pectic polysaccharides, with the Na_2CO_3 extract (8.7%), CDTA extract (8.4%), and other soluble fractions (43.3%) being significant contributors of pectic polysaccharides. This is analogous to the situation found in the potato, where a large proportion of pectic polysaccharides in the cell wall material from the raw potato remains associated with cellulose (Ryden and Selvendran, 1990b), but in cooked potato the association is lost and pectic polysaccharides can be readily solubilized. In conjunction with the lower recovery of pectic polysaccharides from the extruded product and the potential for hemicelluloses to be solubilized during extrusion (Ralet et al., 1993b), this emphasizes the extent to which the cell wall matrix can be modified and how constituent polysaccharides can be degraded and become more predisposed to solubilization during cooking and processing.

Changes resulting from degradation and solubilization of pectic polysaccharides will influence the porosity of the fiber matrix and affect the accessibility of cell contents to digestive enzymes. Degradation of pectic polysaccharides during transit in the upper gut in the pig has also been shown to result in a reduction in particle size in vegetable material (Robertson et al., 1992), which will also influence the accessibility of cell contents to digestive enzymes. Since the glycemic index of a food will be strongly related to the accessibility of starch to digestion and absorption, it is reasonable to suppose that the insoluble pectic polysaccharide fraction of fiber will have an important influence in determining the glycemic index of starchy foods (Wolever, 1990) [i.e., the properties of the cell wall matrix will be important in determining nutritional response (Selvendran and Robertson, 1990)]. Thus, when considering the response to soluble or insoluble fiber in the diet it is important to consider how this is reflected in the food as eaten. Methods which minimize the degradation of fiber durings its estimation are therefore to be preferred for reliable and realistic estimates of the solubility properties in foods and to ensure that modification to the fiber matrix is minimized during the analysis.

CONCLUSION

In conclusion it is recommended that when the fiber content of foods is related to nutritional response, care should be taken to ensure that the fiber matrix is considered in the physiological state present in food as eaten.

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