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Effects of Extrusion Cooking on Starch and Dietary Fibre in Barley

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ABSTRACT

Wholemeal barley flour was extruded in a twin-screw extruder under different process conditions and the effects of extrusion on degree of gelatinization, content of starch and dietary fibre as well as the enzymic availability of starch were examined.

Although in general starch in extruded samples was highly susceptible to enzymic digestion in vitro, *a decrease in starch recovery during enzymic analysis was observed in some samples. The decrease was accompanied by an increase in the fibre fraction. However, this increase did not account for the total starch loss, probably due to formation of undigestible starch fragments too small to precipitate in the 80% ethanol used in the fibre assay.*

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INTRODUCTION

Extrusion cooking (E-C) is increasingly used for production of food for human consumption as well as for animal feed. The elevated pressure and high temperature applied during the process result in altered physical and chemical properties of the extruded products. Nutritional effects of processing depend on numerous factors, including type of extruder, process parameters and screw combination (Björck & Asp, 1983; Asp & Björck, 1989).

At present much interest is being focused on the nutritional properties of starch following food processing. Both the rate and extent of starch uptake are of nutritional concern (Jenkins *et al.*, 1987). Cereals are a common ingredient in extruded products. As cereals are an important source of dietary carbohydrate, it is essential to evaluate effects on these compounds during E-C. Björck *et al.* (1984*a*) found a higher *in vitro* susceptibility of starch to α-amylase in wheat flour extruded in a twin-screw extruder compared to the corresponding boiled flour. However, when including a pretreatment with pepsin prior to incubation with α-amylase the differences between boiled and extruded wheat products were abolished (Holm *et al.*, 1989). These data suggest that protein–starch interactions may affect results obtained *in vitro*. This is of methodological importance, as the susceptibility of starch to α-amylase is often used to predict rate of starch digestion *in vivo*. A high degree of gelatinization (DG) and high availability for α-amylolysis has been confirmed, also, in other studies with wheat products processed in a twin-screw extruder (Holm *et al.*, 1988; Lingström *et al.*, 1989). Although DG of extruded products normally is high, Chiang and Johnson (1977*b*) observed important variations in degree of gelatinization when processing wheat at different conditions in a single screw extruder. Further, Gomez and Aguilera (1983) found an increase in DG of corn extrudates when feed moisture content was reduced. Thus, different process conditions may affect the susceptibility of extruded products to α-amylase.

Several authors have observed a decrease in starch content after E-C (Schweizer & Riemann, 1986; Theander & Westerlund, 1987; Fornal *et al.*, 1987). With wheat extruded in a single screw extruder, Chiang & Johnson (1977*b*) found significant increases in contents of glucose, maltose, maltotriose and maltotetraose. These results indicate breakdown of α(1 → 4)glucosidic bonds of malto-oligosaccharides and starch during extrusion. Similar results were obtained with wheat starch extruded in a twin-screw extruder by Theander and Westerlund (1987). With increasing extrusion temperatures, they found increasing amounts of starch fragments soluble in 80% ethanol. However, part of the soluble fraction was 1,6-anhydrosaccharides. Hence, highly reactive anhydro-compounds produced

in E-C may react with starch or fragmented starch through trans-glycosidation reactions to form new branched glucans which are partly resistant to amylolytic enzymes. Depending on the molecular weight, these undigestible starch fragments may or may not be analyzed as dietary fibre (DF). Further, Theander and Westerlund (1987) found 5-hydroxymethyl-2-furaldehyde in extracts of extruded wheat starch. This indicated thermal degradation of glucose derived from fragmented starch. A decrease in starch content in heat-treated products can also be caused by retrogradation whereby starch molecules may become resistant to amylases unless solubilized in 2M KOH or dimethyl sulphoxide (Englyst *et al.*, 1983; Englyst & Cummings, 1984). Unless removed, this resistant starch will appear in the DF-residue (Björck *et al.*, 1986). However, Siljeström *et al.* (1986) found no retrograded starch in extruded wheat flour.

In a previous study of extruded white and wholemeal wheat flour, a slight increase in total DF content was observed after extrusion (Björck *et al.*, 1984*b*). Moreover, a redistribution of insoluble to soluble DF was seen in extruded white wheat flour. Balance experiments in rats revealed that the increase in soluble components increased the hind-gut fermentability of wheat fibre. An increase in DF content was also noted by Theander and Westerlund (1987). The DF content in extruded wheat flour increased with increasing extrusion temperatures, mostly due to formation of lignin-like substances and chemically modified starch. In contrast, Schweizer and Reimann (1986) and Siljeström *et al.* (1986) found no changes in DF content when extruding wheat flour. Furthermore, Fornal *et al.* (1987) found decreased contents of cellulose and lignin in extruded mixtures of buckwheat and barley. Thus, the effects on total DF content during E-C appear somewhat contradictory.

Due to its high content of DF and high proportion of soluble fibre, barley is an interesting cereal from a nutritional point of view. The purpose of the present investigation was to study effects of different extrusion conditions on contents of starch and dietary fibre in wholemeal barley flour. In addition, effects on *in vitro* availability of starch to α-amylase were studied as well as the degree of gelatinization.

MATERIALS AND METHODS

Materials

Danish whole grain barley was used in the experiments. The barley was ground in a hammer mill to pass a 1·3 mm sieve and extruded in a Clextral BC 45 twin screw extruder under different process conditions (Table 1). The

TABLE 1
Process Conditions in the Clextral BC 45 Twin Screw Extruder

	Sample number							
	3	*5*	*9*	*15*	*21*	*23*	*25*	*37*
Mass temperature (°C)	120	180	120	180	150	150	105	150
Feed moisture content (% w/w)	18	15	27	27	21	21	21	21
Feed rate (g/min)	557	265	265	535	200	600	400	400
Screw speed (r/min)	110	110	110	110	150	150	150	150

screw consisted of three co-rotating elements (c), one reverse element (r) and one co-rotating element (Feed-cccrc-die). Total length was 50 cm. The screw combination and die geometry were kept constant (die diameter 7 mm).

Extruded materials were air-dried at room temperature and ground in a hammer mill to pass a 0·5 mm sieve. Raw barley flour was ground in the same way. Wholemeal wheat flour was included as a reference in some experiments. In some cases, boiled flours were studied. Suspensions (1·7% w/v) of the raw flours were gelatinized by heat-treatment in a boiling water-bath for 15 min.

Methods

All analyses were done at least in duplicate and results are given on dry weight basis (dwb).

Starch was determined according to Holm *et al.* (1986). The method includes boiling with a thermostable α-amylase (Termamyl) and a subsequent degradation to glucose with amyloglucosidase. Liberated glucose was measured with a glucose oxidase-peroxidase reagent and results were expressed on polysaccharide basis (glucose × 0·9). Percentage of starch gelatinized during E-C was determined as described by Chiang and Johnson (1977*a*). However, a glucose oxidase-peroxidase reagent was used for determination of liberated glucose as described for starch determination.

Dietary fibre was measured using the enzymic gravimetric method of Asp *et al.* (1983). The monomeric composition of the fibre fraction was further characterized by GLC-analysis according to Theander and Westerlund (1986). The fibre fraction was pretreated with 12M H_2SO_4 (30°C, 60 min), and further hydrolyzed by boiling with 1M H_2SO_4 for 2 h. The hydrolyzed sample was filtered and the insoluble residue was quantified as Klason lignin. The released monosaccharides in the filtrate were reduced to alcohols with KBH_4 and acetylated using 1-methyl-imidazole as a catalyst. The alditol acetates were determined by GLC with allose as internal standard.

Uronic acids were determined using a decarboxylation method (Theander & Westerlund, 1986). The DF constituents were expressed in polymer weight.

The amount of starch remaining in the fibre residue obtained with the enzymic gravimetric method was determined by dissolving the residue in 2M KOH for 30 min, followed by neutralization with HCl and incubation with amyloglucosidase. Released glucose was then determined as described above. Residual starch in the fibre residue was determined by omitting the KOH step. Resistant starch was calculated as the difference between the value obtained with KOH solubilization and the value for residual starch (Björck *et al.*, 1986).

Starch susceptibility to pancreatic α-amylase *in vitro* was determined as described by Holm *et al.* (1985). An amount of sample corresponding to 500 mg starch (dry basis) was diluted to 55 ml with 0·05M sodium potassium phosphate buffer, pH 6·9, containing 0·04% (w/v) NaCl. A stock solution of α-amylase was prepared by adding porcine pancreatic α-amylase (40 μl, Sigma Chemical Co., St Louis, USA, containing 27 mg protein/ml and 1200 U/mg) to sodium potassium phosphate buffer (16 ml). The rate of starch hydrolysis was measured by incubating the substrate (55 ml) with this enzyme solution (1·25 ml) at 37°C. Samples (0·2 ml) were withdrawn at time intervals up to 1 h and mixed with a dinitrosalicylic acid reagent (DNS) for determination of reducing sugars. Maltose was used as a standard and the extent of hydrolysis (%) was expressed in maltose equivalents and calculated on a basis of starch content (100 × mg maltose equivalents × 0·95/mg starch).

In another series of experiments, samples were preincubated with pepsin prior to the incubation with α-amylase. The pH of the substrate solution was adjusted to 1·5 with 5M HCl. A pepsin solution (1 ml, 200 FID U) was added and incubation was carried out at 37°C for 1 h. The pH was adjusted to 6·9 with 5M NaOH and the sample was incubated as described above.

Statistical analysis

Data were examined by a one-way analysis of variance and differences between samples were calculated as the least significant difference (LSD) at the 5% level.

RESULTS

Starch content and degree of gelatinization

The contents of starch in extruded products and the corresponding raw barley flour are given in Table 2 together with the percentage of gelatinized

TABLE 2
Content of Starch (% dry basis) in Raw and Extruded Barley Flour (Mean Values of Four Determinations ± SD) and Starch Gelatinization (% total starch content)

Sample	*Starch (%)*	*Starch gelatinized (%)*
3	$61{\cdot}8^{a} \pm 1{\cdot}6$	100
5	$60{\cdot}4^{abc} \pm 0{\cdot}7$	90
9	$59{\cdot}8^{bcd} \pm 1{\cdot}8$	93
15	$60{\cdot}1^{abc} \pm 1{\cdot}8$	99
21	$58{\cdot}9^{cd} \pm 1{\cdot}0$	94
23	$56{\cdot}9^{e} \pm 1{\cdot}8$	96
25	$58{\cdot}1^{de} \pm 1{\cdot}7$	93
37	$59{\cdot}2^{bc} \pm 1{\cdot}6$	97
Raw Barley Flour	$61{\cdot}0^{ab} \pm 1{\cdot}1$	

Values with different superscript letters differ significantly ($p \leq 0{\cdot}05$).

starch. The starch content in some of the extruded products was significantly lower than that of the raw flour. The most prominent decrease was about 7% (sample No. 23). The decrease in analyzed starch occurred mainly in products extruded at the higher screw speed tested, 150 rpm.

The percentage of gelatinized starch varied from 90 to 100%. The least gelatinized starch was found in the product processed at the lowest water content (sample No. 5).

Enzymic availability of starch

The degrees of starch hydrolysis after incubating the samples with α-amylase alone or after a preincubation with pepsin are shown in Fig. 1. The *in vitro* susceptibility of starch in boiled or extruded barley flour was considerably higher than that of the corresponding raw flour. After 60 min of incubation the degree of hydrolysis was 22 and 76% for raw and boiled barley flour, respectively. For extruded products the corresponding figures varied from 76 to 81%, except for sample No. 5. In this sample the degree of hydrolysis was only 67% after 60 min of incubation, possibly due to the lower degree of gelatinization. Initially, the boiled flour was hydrolyzed faster than extruded products. Thus, after 5 min of incubation the degree of hydrolysis was 48% for boiled flour, but only from 26 to 36% for extruded products. Also after 15 min of incubation, boiled flour was hydrolyzed to a higher extent than extruded products. However, when incubation was extended to 30 min or more, most extruded products were hydrolyzed to a higher degree than boiled flour.

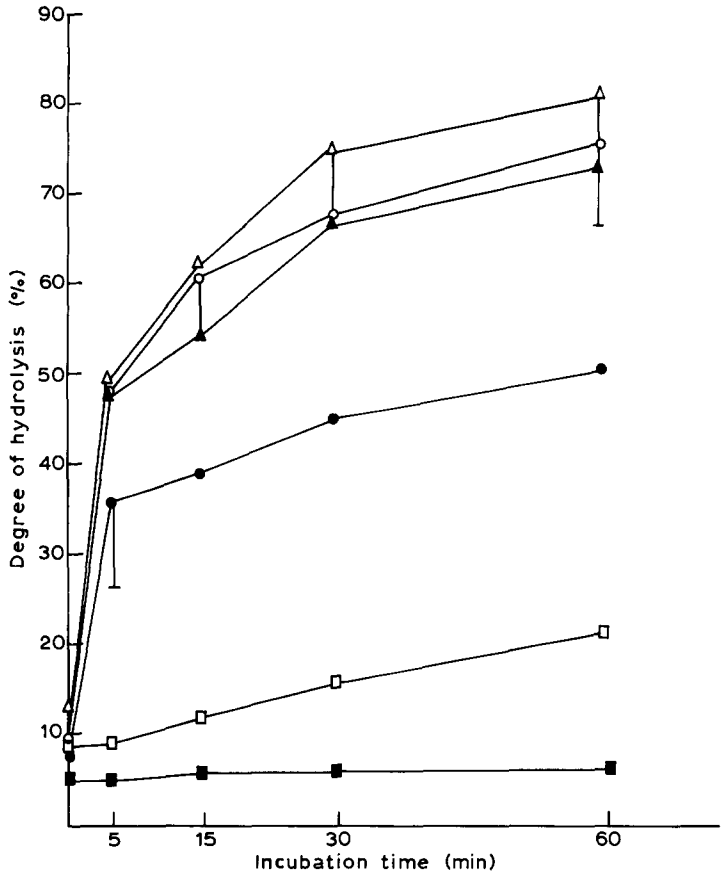

Fig. 1. Starch hydrolysis with pancreatic α-amylase *in vitro* (% of total starch content). Wholemeal barley; □ raw, ○ boiled, △ boiled + pepsin. Wholemeal wheat; ■ raw, ● boiled, ▲ boiled + pepsin. The bars (I) represent the intervals obtained with extruded barley.

A pre-incubation with pepsin slightly increased the susceptibility to α-amylase in the case of boiled barley flour whereas prominent protein–starch interactions were present in the boiled wheat flour reference. In contrast, pepsin had no effect on the availability of starch in either raw or extruded barley. When including pepsin, boiled barley flour was hydrolyzed to a higher degree than most extruded products at all incubation times tested. The difference was particularly noteworthy in the initial phase. However, the gap between extruded products and the boiled pre-incubated flour became smaller when incubation time was prolonged. Starch in both raw and boiled barley was hydrolyzed to a somewhat greater extent than starch in the corresponding wheat samples after 5 min as well as after 60 min of incubation.

TABLE 3
Content of Dietary Fibre (% dry basis) in Raw Barley Flour and Barley Flour Extruded under Different Conditions (Mean value of four determinations ± SD) Solubility determined at pH 4·5

Sample	*Dietary fibre (% dry basis)*		
	Insoluble	*Soluble*	*Total*
3	$17{\cdot}5^{cd} \pm 0{\cdot}1$	$3{\cdot}3^{a} \pm 0{\cdot}2$	$20{\cdot}8^{d} \pm 0{\cdot}2$
5	$17{\cdot}4^{cd} \pm 0{\cdot}2$	$3{\cdot}4^{a} \pm 0{\cdot}3$	$20{\cdot}8^{d} \pm 0{\cdot}3$
9	$17{\cdot}7^{c} \pm 0{\cdot}1$	$3{\cdot}7^{a} \pm 0{\cdot}2$	$21{\cdot}9^{bc} \pm 0{\cdot}9$
15	$19{\cdot}5^{a} \pm 0{\cdot}3$	$3{\cdot}4^{a} \pm 0{\cdot}4$	$22{\cdot}9^{a} \pm 0{\cdot}3$
23	$19{\cdot}1^{b} \pm 0{\cdot}1$	$3{\cdot}8^{a} \pm 0{\cdot}4$	$22{\cdot}9^{a} \pm 0{\cdot}4$
25	$17{\cdot}4^{cd} \pm 0{\cdot}2$	$3{\cdot}8^{a} \pm 0{\cdot}2$	$21{\cdot}2^{cd} \pm 0{\cdot}2$
37	$18{\cdot}9^{b} \pm 0{\cdot}2$	$3{\cdot}7^{a} \pm 0{\cdot}2$	$22{\cdot}5^{ab} \pm 0{\cdot}3$
Raw Barley Flour	$17{\cdot}3^{d} \pm 0{\cdot}1$	$2{\cdot}6^{b} \pm 0{\cdot}1$	$19{\cdot}8^{e} \pm 0{\cdot}1$

Values in the same row with different superscript letters differ significantly ($p \leq 0{\cdot}05$).

Dietary fibre

The contents of insoluble (IDF), soluble (SDF) and total (TDF) dietary fibre as measured with the enzymic gravimetric method are shown in Table 3. A significant increase in TDF ranging from 5 to 16% was observed in extruded products. The differences between extruded products were mainly due to differences in content of IDF which varied from 17·4% dwb (sample No. 25) to 19·5% dwb (sample No. 15). No significant differences in SDF were

TABLE 4
The Monosaccharide Composition and the Klason Lignin Content in the Dietary Fibre Fraction from Raw and Extruded Barley Flour (% dry weight basis)

Sample	*3*	*9*	*23*	*37*	*RBF*[a]
NSP	17·7	18·2	18·8	18·3	16·4
Arabinose	2·6	2·6	2·9	2·7	2·7
Xylose	5·9	5·9	5·9	6·0	5·8
Mannose	0·2	0·3	0·3	0·3	0·2
Galactose	0·3	0·3	0·3	0·3	0·3
Glucose	8·1	8·6	8·9	8·5	7·0
Uronic acid	0·6	0·5	0·5	0·5	0·4
Klason lignin	3·6	3·8	3·9	4·0	3·6
DF	21·3	22·0	23·4	22·3	20·0

[a] RBF = raw barley flour.

observed between extruded products. However, the fraction of SDF was significantly higher in all extruded products (15–18%) when compared to the corresponding raw barley flour (13%).

The fibre fraction of some samples was further characterized by their monosaccharide compositions (Table 4). These data indicate that the increased TDF content observed in extruded samples was mainly due to an increased content of glucans in the fibre fraction and hence to formation of in-vitro digestible starch. The greatest increase in indigestible glucans was 1·9% dwb and was observed in the sample which also showed the most prominent decrease in analyzed starch content (sample No. 23). The content of resistant starch in the fibre fraction was less than 0·05% dwb in all samples and the same was true for residual starch. Thus, the increased glucan content was due to alterations different from formation of retrograded resistant starch. A high correlation was seen between TDF values obtained with the gravimetric method and the method based on specific analysis of the monomeric composition of the fibre fraction ($r = 0{\cdot}973$).

DISCUSSION

The significant decrease in starch content seen in some extruded barley products is in agreement with observations made by Fornal *et al.* (1987) on mixtures of pure starches from buckwheat, barley and corn. They found that starch content was decreased by 8 to 10% during E-C compared with up to 7% in the present study. The considerable decrease in in-vitro starch digestibility in some samples is remarkable in view of the fact that only negligible effects were seen on the protein nutritional value in our products (Østergård & Björck, unpublished results). The decrease in starch content was most pronounced at the higher screw speed tested (150 rpm). In contrast, Björck *et al.* (1984*a*) and Siljeström *et al.* (1986) observed no changes in starch content in wheat processed in a twin screw extruder. The impact of different extrusion parameters on in-vitro availability of cereal starches needs further evaluation.

In the present study only negligible amounts of resistant starch were found. The loss in analyzed starch was therefore not a consequence of retrogradation but was rather due to thermally induced chemical modifications as described by Theander and Westerlund (1987). Thus, the increased glucan content in the fibre fraction of extruded samples indicates the presence of enzymically unavailable starch different from resistant starch. Possibly this modified starch consists of branched glucans formed by reactions of saccharides containing 1,6-anhydro-D-glucopyranose units with starch as previously described (Theander & Westerlund, 1987).

The high extent of gelatinization in the extruded barley products (DG > 90) confirms previous studies with wheat (Holm *et al.*, 1988). Gomez and Aguilera (1983) have proposed that the degree of gelatinization could be considered as an indicator of the extent of starch degradation during extrusion, as DG correlates very well with water solubility index which also expresses the degradation. From this point of view, the high DG observed in the present investigation indicates a high degree of degradation which is confirmed by the high enzymic susceptibility. However, when compared to a completely gelatinized boiled control, all extruded products displayed a somewhat slower initial rate of α-amylolysis, whether pepsin was included or not. Although the least gelatinized sample was the least susceptible to α-amylase, no correlation was observed between the extent of hydrolysis and DG within the narrow range tested (90–100). These results suggest that other factors might influence the initial phase of hydrolysis. Interactions between protein and starch are not likely to play a role as a pretreatment with pepsin had no significant effect on the subsequent susceptibility to α-amylase in either boiled or extruded barley. One possible explanation of the somewhat slower initial rate of amylolysis in the case of extruded barley could be the formation of various branched structures with 'non-starch' linkages due to transglycosidation as described above. Thus, in the case of chemically modified starches, it has been shown that physical hindrance caused by bulky chemical substituents reduces accessibility by α-amylase (Wotton & Chaundhry, 1979). However, the slower initial starch breakdown in extruded products is probably of no consequence for the rate of *in vivo* starch uptake.

It is interesting to note that, in contrast to boiled barley, prominent interactions between protein and starch were present in boiled wheat thus obstructing the availability to α-amylase. The surprisingly low susceptibility of starch in boiled wheat to α-amylase is in agreement with previous reports (Björck *et al.*, 1984*a*; Holm *et al.*, 1989). However, from a physiological point of view differences due to protein encapsulation are of limited importance as the material will be exposed to protein-degrading enzymes in the digestive tract. Therefore, a preincubation with pepsin is recommended when trying to predict *in vivo* susceptibility. A small difference persisted between the boiled barley and boiled wheat samples also after pepsin treatment, starch in boiled barley displaying a higher degree of hydrolysis in the late phase.

An increase in DF content was noted in extruded products. In some samples the reduction in starch content (i.e. in-vitro digestible starch) was greater than the concomitant increase in DF content. This can be interpreted as if part of the unavailable starch fragments were too small to precipitate in the 80% ethanol used in the DF assay. Schweizer and Reimann (1986) eluted two unidentified compounds together with sucrose and maltose during

HPTLC (high performance thin-layer chromatography) analysis of extruded wheat. According to Theander and Westerlund (1987) 1,6-anhydro-α-D-glucopyranose and low molecular weight saccharides with such end units can be identified in 80% ethanol extracts of heat-processed wheat flour by HPLC. Due to their solubility in ethanol they will not be analyzed as fibre. Similarly, *trans*-glycosidation reactions occurring during dry-heat treatment of pure starch resulted in a prominent decrease in starch content without a concomitant increase in analyzed DF content (Siljeström *et al.*, 1989). The availability of this type of 'chemically modified starch' to digestive enzymes or to microbial enzymes in the hind gut is not known.

Other suggested mechanisms to explain increased content of DF following heat-processing are the non-enzymatic browning reactions. Reducing sugars, formed during E-C, may react preferably with lysine to give Maillard products which are chemically stable and nutritionally unavailable (Nogushi *et al.*, 1982). Theander and Westerlund (1987) found that the increase in DF in extruded wheat was partly due to an increased Klason lignin content, probably caused by Maillard reactions. However, in the present study only minor increases in Klason lignin were observed, and the increase in DF content in extruded products did mainly result from formation of indigestible starch.

The solubilization of fibre during E-C of barley is in agreement with previous studies with wheat (Björck *et al.*, 1984*b*; Varo *et al.*, 1983; Siljeström *et al.*, 1986). It is possible that a higher proportion of soluble fibre may influence the fermentability characteristics of barley fibre in the hind gut as previously reported for extruded or popped wheat (Björck *et al.*, 1984*b*; Nyman *et al.*, 1987). Possible effects on, e.g. bulking capacity, are nutritionally important and should be evaluated.

CONCLUSION

From the present investigation it can be concluded that chemical and physical changes which occur in polysaccharides during E-C are dependent on the process conditions. Changes cannot be related to one single process parameter but are rather influenced by an interplay of parameters. However, it appears as if a higher screw speed increases formation of enzymically indigestible starch.

In general E-C renders the major proportion of the starch in barley highly susceptible to enzymic digestion *in vitro*. However, the recovery of starch during enzymic analysis decreased considerably in some extruded products. This could be due to formation of chemically altered glucan compounds as no retrograded 'resistant' starch was detected. An increase in DF content

was noted in extruded products. This increase could largely be accounted for as enzymically indigestible starch. In view of the current interest in starch malabsorption, further studies are needed to elucidate the digestibility and fermentability in vivo, of such in-vitro starch.

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