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Extrusion cooking of a high-fibre cereal product

1. Effects on digestibility and absorption of protein, fat, starch, dietary fibre and phytate in the small intestine

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1. The effect of extrusion cooking of a high-fibre cereal product on digestibility of starch, fibre components and phytate in the stomach and small intestine was studied by in vivo digestion in ileostomy subjects, as well as its effect on ileostomy losses of fat, nitrogen, sodium and potassium.

2. Seven ileostomy subjects were studied during two periods (each of 4 d) while on a constant low-fibre diet supplemented with 54 g/d of a bran–gluten–starch mixture (period A) or the corresponding extruded product (period B).

3. Extrusion cooking, using mild conditions, did not change the content of starch, dietary fibre components or phytate of the bran product, but the phytase (*EC* 3.1.3.26) activity was lost. During the period using the extruded bran product, there was a significant increase in recovery of phytate-phosphorus (period A, 44% of intake; period B, 73% of intake). The amount of fibre components, fat, fatty acids, N, Na, K, water and the ash weight of the ileostomy contents did not differ between the two periods. Only 0·6 and 0·7% respectively of ingested starch was recovered in ileostomy contents in periods A and B, while the fibre components were almost completely recovered.

4. Extrusion cooking, using even mild conditions, may lead to a considerable impairment in the digestion of phytate, probably due to a qualitative change in phytate and a loss of phytase activity. Starch, before and after extrusion cooking, is almost completely digested in the stomach and small intestine while fibre components are digested to a very small extent.

Since extrusion cooking is used increasingly for the production of weaning food, breakfast cereals and bread products, a thorough knowledge of its effect on nutritional value is essential. Very little information is available in the literature on the effects of extrusion cooking on dietary fibre in cereals and other fibre-rich foods (Björck & Asp, 1983).

Under certain conditions a chemical modification of starch and fibre components can occur (Westerlund & Theander, 1984). Extrusion cooking of food also leads to the formation of amylose–lipid complexes (Mercier, 1980) which may affect its digestibility. Studies in rats have demonstrated a decrease in protein nutritional value of foods after extrusion cooking (Harper & Jansen 1981; Linko *et al.* 1981; Björck *et al.* 1983).

The significance of these effects on the digestion and absorption of nutrients in man is not known. However, the glucose and insulin responses (Björck, 1984) seem to increase after consumption of extruded bran as compared with unprocessed bran.

Cereals are rich in phytate and fibre components which have a mineral-binding capacity in vitro, suggesting that they might have an inhibitory effect on mineral absorption. Studies in man indicate that the high content of phytate rather than fibre is the main negative factor for mineral absorption (McCance & Widdowson, 1942*a*, *b*; Hallberg, 1984; Nävert *et al.* 1985).

The effect of extrusion cooking of cereals on phytate has not been considered in the literature. In the present investigation, we aimed to determine whether extrusion cooking of a high-fibre cereal product under mild conditions affects the digestibility of starch, dietary

fibre components and phytate in the stomach and small intestine of man and if it has any effect on digestion and absorption of fat and protein and excretion of sodium and potassium.

SUBJECTS AND METHODS

Subjects

Seven subjects, four men (mean age 50 years, range 39–58 years) and three women (mean age 31 years, range 23–39 years), volunteered to take part in the study.

All the subjects had previously been proctocolectomized for ulcerative colitis (three subjects) or Crohn's disease of the colon (four subjects), and had well-established ileostomies, with only a minor portion of the terminal ileum (30–50 mm) removed. The ileostomies functioned properly and the volumes of excreta were within the normal range without the use of drugs. No drugs were taken during the study.

Experimental model

The study on each subject extended over a period of 2 weeks. During four consecutive days of the first week, starting on Monday at breakfast and ending on Friday morning, the patients were given a constant low-fibre diet with the addition of either 54 g/d of a bran–gluten–starch mixture (period A) or the corresponding extruded product (period B) in random order. At the weekend the patients had no dietary restrictions. During the next week, a similar regimen was followed, using the reverse supplement.

Extrusion cooking and conditions.

The extrusion was performed in a Creusot-Loire BC extruder (Firminy, France) with co-rotating double screw with the following configuration: transport, low pressure, medium-pressure, high-pressure and reverse-screw elements. No external heat was transferred to the barrel or the screws during extrusion. Instead, the barrel was cooled with a specially-designed air-cooling device. Mass temperature and pressure were measured with a Dynisco probe in the compression chamber just before the dies and were determined as 120° and 5·9 MPa respectively. The screw speed was 150 rev./min. During extrusion of the mixture, which was composed of (g/kg) 300 bran, 600 starch and 100 gluten, water was added at 82 ml/kg.

Diets

The intake was kept constant on an individually-selected energy level to maintain energy balance; the subjects were weighed at the start and end of each period. The study was preceeded by a test day in which breakfast was composed of white bread, margarine, cheese and milk, coffee or tea and was served in the metabolic ward. The subjects took the lunch, dinner and supper to their homes for consumption. For lunch the subjects could choose either plaice or chicken, both dishes with rice and sauce. The dinner consisted of fillet of pork, rice, sauce and grated cheese and the supper contained white bread, margarine, cheese and a sponge cake. The subjects could choose either tea or coffee for breakfast and supper, and beer, mineral water or milk for lunch and dinner. For each patient the same menu was repeated daily throughout the study.

In period A, the ingredients of the product (32·4 g wheat starch, 5·4 g gluten and 16·2 g wheat bran) were mixed in the boiled rice, half the mixture taken at lunch and the other half at dinner. Gluten and starch were heated with the rice for 5 min to permit gelatinization of the starch. The bran was added just before serving. In period B, 54 g of the extruded bran product, which resembled a crispbread, was taken daily, half of it with lunch and the

other half with dinner. All the food for the subjects was prepared in advance in the metabolic-ward kitchen. The same batches of fish and meat were used for all patients. Meat and fish portions were stored at −18° after cooking, then thawed and heated in an oven, either by the subjects themselves, when they had the meals at home, or by the staff in the metabolic-ward kitchen.

No other food consumption was allowed and the subjects were instructed not to leave any food.

Duplicate portions of the diet were collected on days 2 and 4 in each period, homogenized and freeze-dried to constant weight as previously described (Sandberg *et al.* 1981) and then analysed.

Collection of ileostomy contents

Ileostomy contents were collected during four consecutive days in each period (from Monday morning to Friday morning). Ileostomy bags were changed every 2 h during the day, commencing at 07.00 hours and ceasing on retiring at night. The bags were immediately frozen on dry ice in a Dewar vessel which the subjects kept at home. The following day they left their frozen ileostomy bags at the metabolic ward, where the bags were weighed, stored at −20° and then freeze-dried to constant weight. The freeze-dried ileostomy contents from each day were pooled and homogenized and portions taken for analysis.

Analytical methods

Determinations of the wet weight, dry weight, ash weight and nitrogen content of the diets and ileostomy contents were performed as previously described (Sandberg *et al.* 1981). Neutral polysaccharide constituents of diets, of the extruded bran product and the raw bran, gluten and starch were determined according to the method of Theander & Westerlund (1986). Freeze-dried samples (1 g) were extracted with ethanol (800 ml/l; 75 ml, 45 min) and hexane (50 ml, 30 min). The extracted residue was dried at 50° in a water-bath. Starch was digested with a thermo-stable amylase (Termamyl, Novo) in acetate buffer in a boiling water-bath for 30 min and then with amylo-glucosidase (*EC* 3.2.1.3) at 60° for 16 h. Absolute ethanol (80 ml) was added and stirred and the mixture cooled (+4°, 30 min), centrifuged and decanted. The precipitate was centrifuged and washed twice with 80 ml ethanol and then with 50 ml acetone. Starch was analysed by spectrophotometric determination of glucose in a portion of the combined supernatant fractions.

The starch-free residue was stirred with acetone, centrifuged and decanted. The residue was air-dried overnight at room temperature. Neutral polysaccharide constituents of the starch-free residue were determined, after sulphuric acid hydrolysis and gas–liquid chromatography of their alditol acetates. β-D-Allose was used as an internal standard. Klason lignin was determined gravimetrically.

Determination of starch and neutral polysaccharide constituents of ileostomy contents was performed according to the method of Theander & Åman (1979) as previously described (Sandberg *et al.* 1981), except that β-D-Allose instead of *myo*-inositol was used as the internal standard.

Phytate in ileostomy contents, diets, the extruded bran product and the raw ingredients was determined as phytate-phosphorus by a modification (Sandberg *et al.* 1982*b*) of the iron-precipitation method of Ellis *et al.* (1977). Samples of 0·5 g freeze-dried diet or ileostomy contents were extracted with 20 ml 0·5 M-hydrochloric acid containing sodium sulphate (50 g/l), filtered through a Munktell OOH filter, frozen overnight and filtered through an MF-Millipore filter (0·10 μm pore size) under pressure. Each sample was then precipitated with ferric ions by adding 1 ml ferric chloride (4 g/l) in 0·5 M-HCl containing Na_2SO_4 (50 g/l) to 50 ml of the filtrate. The mixture was kept in a 95° water-bath for 20 min,

cooled and centrifuged at 2300 rev/min for 20 min at 20°. The ferric precipitate was washed three times with 5 ml 0·5M-HCl and then dissolved in 1 ml concentrated H_2SO_4. From each tube 0·75 ml was withdrawn for wet-ashing at 295° for 15 min; 0·25 ml hydrogen peroxide (300 ml/l) was added, and then the wet-ashing was continued for another 15 min. The digest was quantitatively transferred to a 10 ml flask, diluted with approximately 8 ml demineralized water, kept in a boiling water-bath for 15 min and then cooled and made up to volume. The flasks were allowed to stand at room temperature overnight and then analysed for P according to Fiske & Subbarow (1925). Addition of calcium hydrogen phosphate to samples from the diets and ileostomy contents verified that no co-precipitation of inorganic phosphate occurred, when phytate was precipitated with Fe^{3+} as described previously.

Phytase (*EC* 3.1.3.26) activity in the extruded-bran product was determined by analysis of phytate before and after incubation at optimal conditions for phytase, i.e. at 55° with ten times its weight of water for 17 h at pH 4·5. After incubation, the product was freeze-dried before analysis of phytate.

Total fat and fatty acids were analysed according to Van de Kaamer *et al.* (1949).

Statistical methods

For statistical comparison of the results from the two periods, Student's paired *t*-test was used.

Ethical considerations

The Ethical Committee of Sahlgren Hospital approved the study.

RESULTS

Diets

The composition of the diets is summarized in Table 1. The extruded product and its raw ingredients were analysed separately and their composition is given in Table 2. There was no change after extrusion cooking except in phytase activity as measured indirectly. The raw bran contained 0·321 mmol phytate-P/g before incubation at optimal conditions for phytase and, after incubation, 0·085 mmol phytate-P/g. The extruded product contained equal amounts before and after incubation.

Ileostomy contents

The wet weight, dry weight, water, ash, N, fat and electrolyte concentrations of ileostomy contents are summarized in Table 3. The weight of ileostomy contents varied considerably from individual to individual and was greatest in patient no. 5, but the day-to-day variation in each period was small.

The excretion of N, fat and fatty acids did not differ between the two periods.

There was no significant difference in excretion of Na and K between the two periods. The Na excretion correlated well with the wet weight of ileostomy contents (*r* 0·99).

Polysaccharides and lignin excretion

Values for the different components of the polysaccharides in the ileostomy contents from periods A and B are given in Table 4. There was no significant difference between periods A and B in the excretion of any of the polysaccharide components.

Starch was almost completely digested in the stomach and small intestine. Only a mean of 0·6 (SE 0·09) % of the intake in period A and 0·7 (SE 0·12) % in period B was recovered in ileostomy contents.

Table 1. *Composition (g/d) of low-fibre diet*

	Mean	SE	Range
Energy intake			
MJ	9.0	0.61	6·3–10·8
kcal	2140	—	—
Calculated values*			
Water	896	87	540–1140
Ash	11·9	0·9	9·1–16·4
Fat	102	7·2	77–127
Carbohydrates	152	13·1	102–186
Analytical values			
Protein (nitrogen × 6·25)	98	6·7	79–132
Starch	107	7·5	83–131
NSP	6·7	0.98	3·6–10·6
Sodium (mmol)	126	14·3	69–171
Potassium (mmol)	37·3	2·3	29–48
Phytate-phosphorus (mmol)	2·11	0·18	1·32–2·76

NSP, non-starch-polysaccharides.
* Calculated from food tables (Swedish National Food Administration, 1978).

Table 2. *Chemical composition of raw ingredients and extruded product (g/kg fresh weight)*

	Bran	Starch	Gluten	Extruded product (g/kg: 300 bran, 600 starch, 100 gluten)
Moisture	86	96	51	81
Nitrogen	22	0	133	20
Starch	92	830	70	530
NSP	355	15	25	116
Klason lignin	73	1·0	18	17
Phytate-phosphorus (mmol)	415	0	3·6	125

NSP, non-starch-polysaccharides.

The mean excretion of Klason lignin during period A and period B was 1·2 (SE 0·19) g/24 h and 1·5 (SE 0·39) g/24 h respectively. There was no significant change.

Excretion and recovery of phytate

The mean excretion of phytate-P during period A was 3·87 (SE 0·49) mmol/24 h and, during period B, was 6·39 (SE 0·64) mmol/24 h, i.e. a significant increase when the extruded bran product was consumed ($P < 0·001$).

The recovery of phytate-P as a percentage of intake is shown in Fig 1. As the intake of phytate was equal in both periods the recovery increased significantly during the period with the extruded product ($P < 0·001$). Between 33 and 72% (mean 43·9%) of phytate-P from the diet was found in ileostomy contents during period A and between 55 and 104% (mean 72·9) during period B.

Table 3. *Wet weight, dry weight, water content, nitrogen, ash (g/24 h) and electrolytes, total fat and fatty acids (mmol/24 h) in ileostomy contents of seven subjects fed on a low-fibre diet with addition of 54 g bran–gluten–starch mixture (period A) or the corresponding extruded product (period B)*

(Mean values with their standard errors)

	Period A Low-fibre diet + bran–gluten–starch mixture			Period B Low-fibre diet + extruded bran product			Difference between periods A and B*
	Mean	SE	Range	Mean	SE	Range	
Wet wt	791	145	420–1525	779	158	381–1629	12
Dry wt	56·5	8·6	37·4–103·8	61·2	10·6	37·0–120·4	16·7
Water content	734	137	379–1421	718	148	344–1509	16
N	2·5	0·51	1·6–5·4	2·8	0·52	1·8–5·7	0·3
Ash	0·20	0·006	0·17–0·21	0·19	0·007	0·17–0·21	0·01
Sodium	91·9	15·1	54–172	87·4	16·0	42–174	4·5
Potassium	9·1	3·2	3·4–27	12·1	4·7	3·3–36	3·0
Total fat	16·1	5·8	5·5–49·6	20·2	8·6	7·0–70·4	4·1
Fatty acids	10·4	3·4	3·9–29·1	12·5	5·1	4·6–42·3	2·1

* Differences were not significant.

Table 4. *Polysaccharides (g/24 h) in ileostomy contents of seven subjects on a low-fibre diet with addition of 54 g bran–gluten–starch mixture (period A) or the corresponding extruded product (period B)*

(Mean values with their standard errors)

	Period A Low-fibre diet + bran–gluten–starch mixture			Period B Low-fibre diet + extruded bran product			Difference between periods A and B*
	Mean	SE	Range	Mean	SE	Range	
Starch	0·84	0·13	0·3–1·4	0·98	0·12	0·5–1·4	0·14
Neutral polysaccharide constituents:†							
Rhamnose	Trace	—	—	Trace	—	—	—
Fucose	0·46	0·06	0·21–0·68	0·50	0·08	0·25–0·88	0·04
Arabinose	2·02	0·16	1·45–2·60	2·12	0·17	1·55–2·54	0·10
Xylose	3·39	0·23	3·10–4·12	3·67	0·28	3·01–4·54	0·28
Mannose	0·65	0·15	0·23–1·36	0·69	0·15	0·32–1·45	0·04
Galactose	1·70	0·34	0·78–2·82	1·75	0·32	0·98–3·02	0·05
Glucose‡	5·70	0·82	3·37–9·89	5·81	1·07	3·54–11·81	0·11

* Differences were not significant.
† Anhydro sugar units (g/24 h).
‡ Corrected for starch.

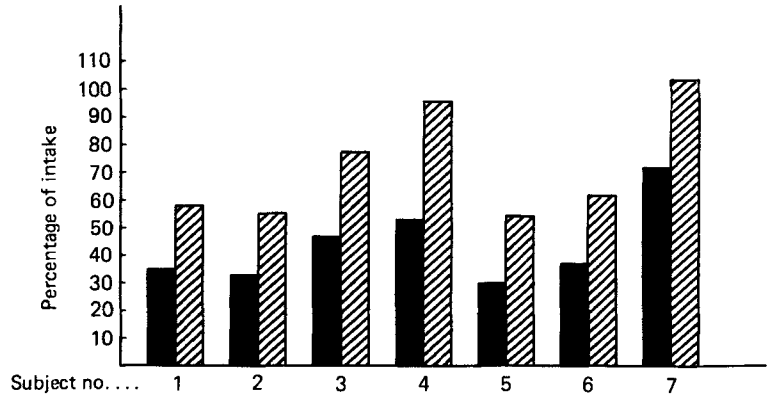

Fig. 1. Recovery of phytate in ileostomy contents of seven subjects studied during two periods on a constant low-fibre diet. In period A (■) the diet was supplemented with 54 g/d of a bran–gluten–starch mixture and in period B (▨) with the corresponding extruded product.

DISCUSSION

Although the daily ileal contents emptying in the colon in healthy subjects is three times higher than that in ileostomy subjects (Philips & Giller, 1973), there appear to be no other differences as regards digestive or absorptive processes (Hill, 1976). The time taken for the head of a meal to reach the ileostomate's terminal ileum is identical to the mouth-to-caecum time of normal subjects (Holgate & Read, 1983). Fermentation by ileal bacteria does not seem to be a confounding factor in healthy ileostomates as breath hydrogen (Holgate &

Read, 1983) and volatile fatty acids (Chapman *et al.* 1985) in ileal effluent did not rise after a carbohydrate meal. In agreement with Chapman *et al.* (1985) we believe that healthy ileostomates are suitable for quantifying absorption by simple intake–output studies.

Formation of amylose–lipid complexes, during extrusion cooking, which are resistant to amylase digestion in vitro has been reported in the literature (Mercier, 1980). However, such complexes are completely digested and absorbed in the rat intestine (Holm *et al.* 1983). The present study also supports the idea that the amylose–lipid complex formed during extrusion cooking using mild conditions has no effect on starch digestion or excretion of fat and fatty acids.

The almost-complete digestion of starch in the stomach and small intestine in both periods of the present study is in agreement with our previous studies on the digestion of rice in ileostomy subjects (Sandberg *et al.* 1981, 1982*a*), but is in contrast with other recent studies which claim that up to 20% of the starch in a meal may be passed undigested to the colon as measured by the aspiration technique (Stephen *et al.* 1983) or by breath H_2 (Andersson *et al.* 1981). The discrepancy between our results and those of Stephen *et al.* (1983) may be due to the fact that in their study the sources of starch were not only rice but also bananas, potatoes and beans. Another possible explanation may be the difficulties in getting a representative sample of the intestinal contents when using the aspiration technique, or that intubation may affect intestinal transit and digestion.

No effect of extrusion cooking of the bran–gluten–starch mixture on the amount and composition of fibre components was found in the present study. This agrees with a collaborative study organized by Varo *et al.* (1983), in which the dietary fibre content of cooker-extruded wheat and whole-grain-wheat flour was compared with the raw materials. In the present study the digestibility of fibre components in the stomach and small intestine did not differ between the raw ingredients and extruded product. The fibre components seem to pass through the stomach and small intestine without digestion occurring, as we have shown earlier (Sandberg *et al.* 1981). The amount of polysaccharides derived from bacteria or endogenous material in ileostomy contents are included in the determination of fibre components and were supposed to be constant in the two periods when each patient served as his or her own control.

During the period of consumption of raw ingredients, a recovery of 44% of dietary phytate (mainly derived from wheat bran) was found, which corresponds well with our previous studies where 41% of the phytate in 16 g AACC wheat bran was recovered in ileostomy contents (Sandberg *et al.* 1982*b*). In both these studies, the microbial growth was minimized by the routine described previously and therefore the hydrolysis of phytate cannot be attributed to microbial phytase activity, but to hydrolysis by human phytase or alkaline phosphatase (*EC* 3.1.3.1). According to Bitar & Reinhold (1972), intestinal phytase activity is found in the mucosa of the small intestine of the rat, chicken, calf and man.

The amount of phytate analysed as phytate-P was not changed, or only slightly reduced, after extrusion cooking of the bran–starch–gluten mixture. However, in spite of the mild extrusion conditions, the phytate in the extruded product was much-less digestible in the stomach and small intestine than the phytate in the raw ingredients. There are two possible explanations for the reduced digestibility. First, there may have been loss of phytase activity during extrusion cooking and, second, there may have been a qualitative change in what we analysed as phytate-P. The first alternative is, however, contradicted by the results from our earlier study where the phytate in AACC wheat bran was largely digested (Sandberg *et al.* 1982*b*) although the enzymes, including phytase, were deactivated during preparation. The second explanation is the more likely. Precipitation with $FeCl_3$ for determination of phytate-P probably also measures both inositol hexaphosphate and inositols with four or

five phosphate groups. Inositol with four or five phosphate groups might be formed during extrusion cooking and might not be digested by the intestinal enzymes of man. Other indigestible phytate-complexes may also be formed. This will be further studied by qualitative analysis of phytate in food and ileostomy contents using high-pressure liquid chromatography.

In conclusion, mild extrusion cooking of a fibre-rich cereal product does not lead to important negative effects on the availability of protein, fat or starch. The possibility cannot be excluded, however, that this might occur with food products in the market which are extruded under higher pressure and temperature. Even under mild conditions, a possible impairment of mineral absorption, mediated by the resistance of phytate to digestion in the human gut, must be considered. The effect of extrusion cooking on apparent mineral absorption in the seven subjects studied will be reported separately.

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