

The effect of vegetables and beet fibre on the absorption of zinc in humans from composite meals

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1. The absorption of zinc in humans from composite meals, was determined by extrinsic labelling of the meals with ⁶⁵Zn and measurement of the whole-body retention of the radioisotope.
2. Low-Zn (mean 25 μmol) chicken meals with 150 g white bread or 225 g potatoes, carrots, turnips, cabbage or green peas were studied. The effect of a beet-pulp-fibre preparation used as a breakfast cereal, in bread and as a meat extender on Zn absorption was also studied.
3. The mean percentage absorption from the chicken meals with white bread, carrots and cabbage was significantly different from the meals with potatoes, turnips and green peas. When the amount of Zn in the meals was taken into account a slightly higher absorption was observed from the white-bread meal compared with the meals with potatoes and cabbage, while no differences were seen between the vegetable meals.
4. The beet-pulp-fibre preparation did not affect the extent of Zn absorption when used as a meat extender. The absorption of Zn was higher when the beet fibre was included in bread than when used as muesli.
5. The results obtained suggest that, besides the low-Zn content in vegetables, a large intake of vegetables or a pure-vegetable-fibre preparation has no significant effect on Zn availability from animal-protein-based meals.

The adequacy of zinc in vegetarian diets has been questioned (Harland & Peterson, 1978; Freeland-Graves *et al.* 1980) and negative balances of Zn have been reported when diets high in fibre from fruits and vegetables were consumed (Kelsay *et al.* 1979; Kelsay & Prather, 1983). Many plant products contain phytate, which is a potent inhibitor of Zn absorption (Sandström *et al.* 1980; Turnlund *et al.* 1984; Nävert *et al.* 1985). The fibre components of vegetables, especially those rich in uronic acids, show a strong cation-exchange and mineral-binding capacity *in vitro* (McConnel *et al.* 1974; Davies, 1978). However, *in vitro* characteristics of single food components are probably of limited value for predicting Zn availability *in vivo* (Davies, 1978).

For evaluation of the absorption and utilization of a mineral for humans, growth rate or tissue retention can hardly be used as in animal experimental models. The conventional metabolic-balance technique has serious methodological limitations for mineral absorption studies. A special problem for Zn is the large endogenous losses, often of the same magnitude as the amount absorbed, which makes the possibilities of revealing effects on absorption limited. Studies with isotopes of the element of interest are usually more accurate and sensitive and better suited to identify factors that affect absorption. If extrinsic labelling with tracer doses of a radionuclide is used, it is assumed that isotope exchange takes place between the stable Zn in the meal and the added tracer. The lack of two suitable radionuclides of Zn to be used simultaneously in absorption studies makes validation in humans of the extrinsic-labelling technique difficult. Flanagan *et al.* (1985) compared the absorption of Zn from turkey meat labelled extrinsically and intrinsically, and found no difference in absorption. The virtually identical distribution of Zn and added ⁶⁵Zn in human milk and infant formulas also suggest rapid and complete exchange of an extrinsic label to food (Sandström *et al.* 1983). Using stable Zn isotopes, Janghorbani *et al.* (1982) found a consistently lower absorption from an extrinsic label than from an intrinsically labelled chicken. However, to

achieve sufficient enrichment with a stable isotope, tracer doses cannot be used and extrinsic labelling of a composite meal would in that case increase the total Zn content of the meal. The validity of the extrinsic label used in trace amounts thus still remains to be proved in man for foods other than chicken and turkey, especially as intrinsic labelling with sufficient enrichment is hardly feasible for more than a limited number of food components and the analyses are expensive.

In the present study composite meals were extrinsically labelled with ^{65}Zn and the absorption was determined from the whole-body retention of the isotope. The effect of vegetable fibres on Zn absorption was studied by comparing meals with different vegetables added to a low-Zn chicken meal and meals where the fibre content was increased by a beet-pulp-fibre preparation. The results indicate that vegetable fibre has no effect on Zn absorption.

MATERIAL AND METHODS

Subjects

Fifty-three men and non-pregnant women, aged 20–34 years and without known disease, volunteered for the study. Their serum Zn values were 11.4–16.1 $\mu\text{mol/l}$ and fell within reference values for our laboratory. The subjects were given written and verbal information about the aim and procedure of the study. Thirty-nine of the subjects participated on two occasions.

The project was approved by the Research Ethical Committee and the Isotopic Committee at Sahlgrenska Hospital.

Test meals

The effect of vegetable fibre on Zn absorption was studied in three types of meal: (1) a low-Zn meal of lunch- or dinner-type based on chicken served with bread alone (meal 1) or chicken served with bread and potatoes, carrots, turnips, cabbage or green peas (meals 2–6), (2) breakfast-type meal in which a beet-pulp fibre preparation was added as muesli (meal 7) or incorporated in bread (meal 8), (3) a lunch- or dinner-type meal based on beef and rice (meal 9) was compared with a similar meal in which the beet-pulp fibre preparation was used as a meat extender as recommended by the manufacturer (meal 10). The amounts of raw ingredients in the meals are given in Table 1. The beet fibre was a commercially available fibre preparation made from beet pulp (Fibrex 620^R; Fibrex AB, a subsidiary to the Swedish Sugar Company, Arlöv). The amount of beet fibre used in meals 7 and 8 was chosen to give a fibre content corresponding to 16 g wheat bran. To ensure uniformity the chicken meat was minced and meat patties were prepared and a meat sauce was prepared from the beef. The vegetables were minced after cooking in distilled water.

Analyses

After freeze-drying the test meals were analysed in duplicate for their contents of nitrogen, Zn, iron, calcium and phosphorus. All glassware was washed in 2.5 M-hydrochloric acid and rinsed in deionized water before use. Zn and Fe were assayed by atomic absorption spectrophotometry (Perkin Elmer Model 360, Norwalk, CT 06856, USA), after dry-ashing in Pyrex beakers overnight (450°). Three drops of nitric acid (10 M) were added and the ashing continued until a white residue was obtained. The ash was dissolved in 5 ml 5 M-HCl and the beakers covered with Parafilm (American Can Company, Greenwich, CT 06830, USA) and left overnight. The contents were then transferred quantitatively to 100-ml flasks, diluted to volume and left for at least 4 h before analysis. Ca and magnesium were assayed by atomic absorption spectrophotometry after wet-ashing (290–300°, 15 min) of 0.1 g freeze-dried samples in 1 ml concentrated sulphuric acid with the addition of 3 ml hydrogen peroxide (300 ml/l, 10 M). If necessary, another 2 ml H_2O_2 were added and

Table 1. Composition of test meals (g)

Meal no.	Chicken	White bread	Vegetables	Beet fibre*	Milk	Water	Butter	Beef	Rice†
1	75	150	—	—	—	150	25	—	—
2-6‡	75	50	225	—	—	150	25	—	—
7-8§	—	40	—	9.2	200	—	10	—	—
9	—	—	—	—	—	200	—	85	40
10	—	—	—	14.0	—	200	—	75	40

* Fibrex 620^R; Fibrex AB, a subsidiary to the Swedish Sugar Co., Arlöv.

† Dry weight.

‡ 225 g potato, carrot, turnip, cabbage or green peas.

§ Beet-pulp fibre added as müsli (meal 7) or incorporated into bread (meal 8).

the tube heated again until the digest was clear and colourless. Deionized water was added to volume and the samples analysed after addition of 1 ml 0.2 M-lanthanum oxide to 10 ml digest. The same digest was used to assay P according to Fiske & Subbarow (1925). Reference standards for Zn, Fe, Ca and Mg were prepared from Titrisol^R (Merck, Darmstadt, West Germany). Reference standard materials for Zn and Fe, with concentrations representative of those found in the diet samples, were run simultaneously and fell within the certified range (Orchard Leaves SRM 1571 and Bovine Liver SRM 1577 (a); National Bureau of Standards, USA). A freeze-dried reference diet from our laboratory was used for control of Ca and P analyses. The coefficients of variation for control materials of Zn, Fe, Ca, Mg and P were 3.1, 4.4, 4.1, 3.7 and 5.4% respectively. N analysis was performed by a micro-Kjeldahl technique (Technicon AutoAnalyzer, Tecator, Höganäs, Sweden). Starch and dietary fibre components of the diets were assayed according to the method of Theander & Westerlund (1986). Freeze-dried samples were extracted with ethanol and hexane. Insoluble and soluble fibre of the residue were isolated together by centrifugation after enzymatic removal of starch (with a thermostable α -amylase, Type III from *Bacillus subtilis*; Sigma, St Louis, MO 63178, USA, and amyloglucosidase, *Aspergillus niger* (EC 3.2.1.3.); Boeringer Mannheim GmbH Biochemica, Mannheim, West Germany) and precipitation of soluble fibre in ethanol (800 ml/l). Neutral polysaccharides in the dietary fibre fraction were analysed as alditol acetates by gas-liquid chromatography, and uronic acid constituents by a decarboxylation method (Theander & Åman, 1979). Phytate was assayed by a method suggested by Davies & Reid (1979). Cation-binding capacity was determined according to Croke (1969). Freeze-dried food samples were acid-washed with 0.01 M-HCl followed by titration to pH 7.0 using 0.01 M-potassium hydroxide. The cation-binding capacity was expressed as mmol KOH used per total dry weight.

Zn absorption measurement

The test meals were labelled with 0.02 MBq ⁶⁵Zn Cl₂ (Amersham International plc, Amersham, Bucks) by addition to the meat patties and minced vegetables, the meat sauce or to the bread dough during preparation of the meals. The activity of each individual meal was measured in the whole-body counter. Owing to the size of the test meals different protocols were used; meals 7 and 8 were served to the subjects after 12 h of fasting while the other eight test meals, which had a larger volume and a higher energy content, were served at 12.00 hours and preceded at 08.00 hours by a standardized small breakfast consisting of white bread and milk. No other food was allowed for at least 3 h after intake of the labelled meal. The retention of the radionuclide was measured 14 d after intake of the labelled meal,

when it can be assumed that the unabsorbed fraction has left the body. Allowance was made for endogenous excretion of ^{65}Zn in the period between intake and retention measurement, based on the mean retention function obtained after intravenous administration in another group of subjects (Arvidsson *et al.* 1978), to obtain the absorption value. This excretion in 14 d is approximately 14% of the retention value. When the subjects participated a second time, allowance was also made for residual activity from the first meal using the same mean retention function (Arvidsson *et al.* 1978) to estimate the rate of excretion of retained ^{65}Zn . The whole-body counter used consisted of four large plastic scintillators in a floor–roof configuration connected to a multi-channel analyser system (Nuclear data 660, Schaumburg, Illinois, USA). The total effective counting time was 100 s.

In vitro digestion

The isotope exchange between stable Zn and added ^{65}Zn was studied after *in vitro* digestion as described by Hallberg & Björn-Rasmussen (1981). A weighed amount, 3% of the total dry weight, of freeze-dried vegetables or chicken was transferred to an Erlenmeyer flask. To this was added 75 ml 'gastric juice' (0.16 g pepsin (porcine stomach mucosa, no. P-7012; Sigma), 2 g sodium chloride, 10 ml 8M-HCl diluted to 500 ml) and 0.01 MBq ^{65}Zn . The flasks were shaken in a water-bath, 37° for 2 h. The pH was adjusted to 8 by adding drops of ammonium hydroxide (13 M). Trypsin (30 mg) (bovine pancreas, no. T-8253; Sigma) was added and the flasks incubated at 37° for another 4 h. The pH was reduced to 4 with HCl, the samples centrifuged for 15 min (2041 g) and the concentrations of Zn and ^{65}Zn determined in the supernatant fraction.

Statistics

For statistical evaluation of absorption data, analysis of variance and Duncan's new multiple-range test was used for the chicken meals, and Student's *t* test for paired observations for the beet-fibre meals. The statistical analysis was performed by a computer package (Mulreg 800; Idatron, Linköping, Sweden)

RESULTS

Nutrient content of meals

The contents of N, Zn, Ca, Mg, P, phytic acid, neutral polysaccharides adjusted for starch content and uronic acid in the meals and the cation-binding capacity of the vegetables and the fibre preparation are given in Table 2. The chicken meals and the meals with meat sauce had an N content corresponding to 20–30 g protein (14–23% of the energy content of the meals). The Zn content of the six chicken meals (nos. 1–6) was 15–27 μmol , with the chicken meat accounting for 6 μmol . The beet fibre had a low content of Zn (0.3 $\mu\text{mol/g}$) which made a minor contribution to the Zn content of meals 7 and 8 and resulted in a lower Zn content when the fibre was used as a meat extender (meal 10 v meal 9). The Fe content of the beet fibre was 2–8 $\mu\text{mol/g}$ and provided about half the Fe content of meal 7 and 30% of the Fe content of the meal with beef and beet fibre (meal 10). The phytic acid content of the meals was relatively low with the highest amount (220–230 μmol) in the meal containing green peas and the meal with 14 g beet fibre (meals 6 and 10 respectively). The content of neutral polysaccharides varied from 4 to 15 g and the uronic acids from 0 to 3.5 g, in bread and carrots respectively. The green peas showed the largest cation-exchange capacity. The phytic acid and neutral polysaccharide contents were correlated (r 0.80). No correlation was seen between the cation-binding capacity and the neutral polysaccharide contents or the uronic acid contents.

Table 2. Content of nitrogen, zinc, calcium, phytic acid, fibre and uronic acids in the experimental meals and cation-binding capacity of vegetables or fibre components of the meals

Meal no.	Major components	N (g)	Zn (μ mol)	Fe (μ mol)	Ca (mmol)	P (mmol)	Mg (mmol)	Phytic acid (μ mol)	Neutral fibre polysaccharides* (g)	Uronic acids (g)	Cation-binding capacity (mmol)
1	Chicken, white bread	4.1	19	153	1.0	8.3	1.7	0	6.0	0	4.1
2	Chicken, potatoes	3.7	21	79	1.0	9.4	2.5	110	4.1	1.0	6.3
3	Chicken, carrots	3.4	19	72	2.8	8.2	1.7	60	5.7	3.5	6.0
4	Chicken, turnips	3.7	22	79	2.0	9.2	2.2	40	6.3	2.7	5.9
5	Chicken, cabbage	3.5	15	103	3.7	8.6	2.3	40	5.2	2.1	5.8
6	Chicken, green peas	4.5	27	127	1.7	11.8	2.8	230	11.1	1.6	11.3
7	Beet fibre (9.2 g) as muesli, white bread, milk	1.9	24	62	6.3	7.7	1.7	40	6.7†	2.3†	4.1
8	Beet fibre (9.2 g) in white bread, milk	1.8	24	55	8.0	7.3	1.7	65	6.7†	2.3†	4.1
9	Beef (85 g), rice	3.6	63	86	1.2	7.2	1.3	110	nd	nd	nd
10	Beef (75 g), beet fibre (14 g), rice	2.6	55	111	3.4	6.8	2.0	220	10.2†	3.5†	6.2

nd, not determined.

* Neutral polysaccharides corrected for starch content.

† Values from the manufacturer. Fibre contents of white bread or rice are not included.

Table 3. Zinc absorption from meals containing different vegetables and beet fibre (The meals were extrinsically labelled with ^{65}Zn and absorption was determined from measurement of the whole-body retention of the radionuclide)

Meal no.	Major components	No. of subjects	Zn absorption			
			%		μmol	
			Mean	SD	Mean	SD
1	Chicken, white bread	10	46.1 ^a	13.5	8.7 ^a	2.6
2	Chicken, potatoes	13	27.7 ^b	6.2	5.7 ^b	1.2
3	Chicken, carrots	13	41.2 ^a	8.8	7.8 ^{a,b}	1.7
4	Chicken, turnips	10	30.2 ^b	6.9	6.6 ^{a,b}	1.5
5	Chicken, cabbage	8	40.9 ^a	10.0	6.1 ^b	1.5
6	Chicken, green peas	6	27.2 ^b	5.2	7.4 ^{a,b}	1.4
7	Beet fibre (9.2 g) as müsli, white bread, milk	8	27.8	12.0	6.6	2.9
8	Beet fibre (9.2 g) in white bread, milk	8	39.5*	11.5	9.4*	2.7
9	Beef (85 g), rice	8	21.8	5.1	13.7	3.2
10	Beef (75 g), beet fibre (14 g), rice	8	25.7	7.5	14.1	4.1

a, b Values for meals 1–6 with different superscript letters within each column were significantly different ($P < 0.05$).

* Mean values were significantly different from those for meal 7 ($P < 0.05$).

Zn absorption

The absorption of Zn is given in Table 3. From the chicken meals (nos. 1–6) the highest percentage absorption, 40–46%, was attained from the meals with white bread alone and with carrots or cabbage (nos. 1, 3 and 5). The fractional Zn absorption from these meals was significantly different ($P < 0.05$) from the other chicken meals. However, when the Zn content of the meals was taken into account the differences in amount of Zn absorbed were small. When the beet-fibre preparation was used to replace cereal fibre (meals 7 and 8) a higher absorption ($P < 0.05$) was observed when it was included in bread compared with its use as müsli. A partial replacement of meat with beet fibre had no effect on Zn absorption (meals 9 and 10).

The percentage absorption of Zn from the chicken meals correlated weakly and negatively to the content of phytic acid in the meal and to the in vitro cation-binding capacity ($r = 0.73$ and -0.67 respectively) but not to the content of uronic acids. No correlation was found between the absolute amount of Zn absorbed and any of the analysed components or in vitro characteristics.

In vitro digestion

After in vitro digestion all ^{65}Zn and virtually all native Zn was found to be soluble in the supernatant fraction after centrifugation of the acidified slurry of food and 'gastric juice' (Table 4). Only from the white bread was less of the native Zn than of the added ^{65}Zn recovered in the supernatant fraction after digestion.

DISCUSSION

In the present study the absorption of Zn from a composite meal was determined from measurement of the retained activity of a tracer-dose of ^{65}Zn 14 d post-absorption by the use of the mean rate of Zn excretion obtained earlier in another group of subjects (Arvidsson *et al.* 1978). The rate of Zn turnover in healthy subjects was, in that study (Arvidsson *et*

Table 4. *Effect of in vitro digestion on added ^{65}Zn and release of Zn from food to an aqueous solution*

(Food samples were digested with pepsin for 2 h and trypsin for 4 h at 37°. The samples were acidified and centrifuged and Zn and ^{65}Zn measured in the supernatant fraction. The values are means and ranges of four determinations expressed as percentage of total Zn content and added activity)

Food component	Zn in supernatant fraction (%)		^{65}Zn in supernatant fraction (%)	
	Mean	Range	Mean	Range
White bread	75	65–81	102	99–105
Potatoes	96	94–100	98	91–102
Carrots	92	89–98	101	100–103
Turnips	91	78–100	100	98–102
Cabbage	95	87–106	96	94–98
Green peas	93	86–100	97	95–98
Chicken	92	88–100	101	101–104

al. 1978), found to be relatively slow and the inter-individual differences were small, which indicates that Zn intake before or after administration of a labelled diet has little influence on the size of the fraction of the initially absorbed Zn that is re-excreted during the first 14 d in subjects with a 'normal' Zn status, as judged from dietary habits and serum Zn levels. As most of the subjects participated on two occasions it was also possible to make paired comparisons of the values for the chicken meals. However, this did not in any way change the conclusions and the same is the case if the results for the beet-fibre meals are treated as group values in the statistical analysis. The benefit of determination of each individual's turnover rate by extended measurements over a long period of time is therefore doubtful, especially as this does not improve the precision of the prediction of the early excretion rate before the non-absorbed fraction of the meal has left the body. The effect of a preceding meal on Zn absorption is not known; by serving a standardized breakfast to the subjects taking meals 1–6 and 9–10 it was hoped to be minimized.

Another potential source of error is the endogenous secretion of Zn in the intestine before absorption. Isotope exchange that takes place between the label of the meal and the Zn secreted in the gut would probably give an underestimate of the extent of absorption from the low-Zn meals in the present study. The values obtained are, however, similar to the absorption found from low-Zn diets by Wada *et al.* (1985) by the use of stable Zn isotopes. It is also reasonable to assume that the endogenous Zn secretion is similar after the meals, so that comparisons between the meals can be made.

The results from the *in vitro* digestion gives, due to the more or less complete recovery of native Zn in the acidified digest, no validation of the extrinsic-labelling technique. On the other hand the observation that Zn was in solution at low pH may suggest that isotope exchange may occur in the stomach, if this has not already taken place during the preparation of the meal. An incomplete isotope exchange would give an overestimate of the absorption since the *in vivo* percentage absorption increases when the content of Zn in meals decreases (Sandström *et al.* 1980; Sandström & Cederblad, 1980). If the *in vitro* digestion results are indicative of the isotope exchange *in vivo*, the lower *in vitro* extraction of Zn from the white bread would mean a falsely high absorption. However, for practical and radiation safety reasons it was not suitable to label the food samples for the *in vitro* study in the same way as the test meals, as this would have required a much higher amount of ^{65}Zn to achieve sufficient activity in the fraction of the meal used. The addition of the

radionuclide to the dough, as was done for the test bread, might give better conditions for isotope exchange than in the set up of the *in vitro* study.

Neither the result from the *in vitro* digestion or the cation-binding capacity could be used to predict the extent of Zn absorption from the meals. The *in vitro* digestion demonstrated a high solubility of Zn after acidification, a procedure that was found necessary in order to be able to centrifuge the otherwise highly-viscous samples. At a pH similar to that at the site of intestinal absorption, a cation-exchange capacity of the same magnitude as commercial cation exchangers was observed. Similar observations of the high pH-dependency for the solubility of Zn in food has been observed by Lyon (1984). Further development of *in vitro* techniques, which better mirror the chemical environment at the site of absorption, is necessary before such methods can be used to estimate the availability of Zn.

Some of the factors determining the extent of Zn absorption have been identified. We have recently shown a low absorption of Zn from wholemeal bread or bran rich in phytic acid and that the extent of Zn absorption is influenced by the Zn and the protein content of the meal (Sandström *et al.* 1980; Sandström & Cederblad, 1980; Nävert *et al.* 1985). Phytic acid has a high binding constant for Zn and a ratio of 15:1 for phytate: Zn has been found to impair growth rates in rats (Davies & Olpin, 1979). The amount of phytic acid in the meals in the present study is low compared with that found in whole-grain cereals (10 g bran contains about 0.5 mmol phytic acid) and despite the low Zn content of the meals the molar ratio phytic acid: Zn was less than 10 for all meals.

Dietary fibre content has also been suggested to affect Zn absorption. A negative Zn balance during 20 d on a high-fibre intake in the form of wholemeal bread or 10 g cellulose has been reported (Reinhold *et al.* 1976; Ismail-Beigi *et al.* 1977). Drews *et al.* (1979) observed an increased faecal Zn excretion after supplementing the diet with 14.2 g cellulose or hemicellulose during 4 d. As differences in fibre intake affect intestinal transit time (Cummings, 1982), methodological problems can be expected in short-term balances on high-fibre diets. In 30 d balances reported by Sandstead *et al.* (1979), no effect of 26 g of various types of fibre on Zn balance was observed. The amount of vegetables used in the present study, providing 5–12 g dietary fibre, can be assumed to represent a high intake for a single meal in an animal-protein-based diet. Chicken was used as the protein source because of its low Zn content and to increase the possibility of revealing nutritionally significant effects on Zn absorption. The results obtained indicate that vegetables *per se* do not impair Zn absorption from this type of meal. The low Zn content of most fibre-rich vegetables in comparison with cereal fibre sources should, however, be noted.

Zn absorption from the meals in which the beet-fibre preparation was used as muesli or included in bread, was higher than that observed in earlier studies using bran as the fibre source (Nävert *et al.* 1985). From meals with similar compositions and using identical test procedures, an absorption of Zn of 12% was reported for a bread containing 10 g bran as compared with 39.5% in the present study. The difference can probably be attributed to the low phytic acid content of the beet-fibre preparation. However, even after long-term fermentation of the bran-containing bread which reduced the phytic acid content to a level similar to that of the meals in the present study, the absorption of Zn was lower (19.8%) than that from the beet-fibre bread in the present study. It is therefore possible that factors other than phytic acid in fibre-rich cereals could influence Zn absorption. The difference between meals 7 and 8 in the present study could be due to physico-chemical changes of the fibre during the baking process. We have recently observed that extrusion cooking of a bran product reduces the apparent absorption of Zn (Kivistö *et al.* 1986). A milder heat treatment may have other effects on Zn availability.

The results from meals 9 and 10 also indicate that vegetable fibre is inert as regards Zn absorption. A vegetable-fibre preparation could therefore be used to partly replace meat

protein as a way to increase fibre intake when an increased vegetable intake is not possible. The beet-fibre preparation used in the present study had a relatively high mineral content; however it is possible that other ways of processing could result in a lower mineral content and also other effects on mineral absorption. Studies in pigs have shown that the extent of methoxylation of pectin greatly influences Zn absorption (Bagheri & Guéguen, 1985). The results obtained in the present study do not necessarily apply to other fibre preparations.

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