A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content

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A continuous in vitro method for estimation of the bioavailability of minerals and trace elements is presented. This in vitro method is believed to be more representative of in vivo physiological conditions than in vitro methods based on equilibrium dialysis, because dialysable components are continuously removed from the pancreatic digestion mixture. The continuous in vitro method is compared with the equilibrium in vitro method with respect to the dialysability of Ca, Mg, Fe, Cu and Zn from eight different types of bread (varying in phytic acid content). The results show a pronounced effect of continuous removal of dialysable components from the pancreatic digestion mixture on the dialysability of minerals and trace elements. Furthermore, removal of dialysable components influences the effect of phytic acid on the bioavailability of minerals and trace elements. For these two reasons the importance of removal of dialysable components in vitro for the estimation of bioavailability in vivo needs further investigation. The bioavailability of minerals and trace elements from bread samples is not related to the phytic acid content only. Therefore, the effect of phytic acid on the bioavailability of minerals and trace elements cannot be studied separately from the effects of other components on bioavailability.

Continuous in vitro method: Bioavailability: Minerals and trace elements: Phytate

A good mineral balance is of importance to animals and man. Deficiency, overdose, or imbalances between inorganic nutrients have a negative effect on health (Mertz, 1981; Nielsen, 1988; Prasad, 1988). However, it is not the dose of a mineral that is important to maintain balance, but rather the amount that is bioavailable.

In the small intestine several components of our food form soluble or insoluble complexes with minerals and trace elements. These food components may influence the bioavailability of these minerals and trace elements by influencing their availability for absorption. Components that may have a positive effect on the bioavailability of minerals and trace elements are citric acid, ascorbic acid, lactose and some amino acids (Hallberg et al. 1986; Hazell & Johnson, 1987a, b; Sandström & Cederblad, 1987; Saxena & Seshadri, 1988; Schuette et al. 1989), while phytic acid, dietary fibre and polyphenolic compounds may have a negative effect (Brune et al. 1989; Lönnerdal et al. 1989; Spivey Fox & Tao, 1989; Torre et al. 1991).

In vivo experiments with experimental subjects are the best way to study the bioavailability of minerals and trace elements to man. In vivo experiments, however, are
time-consuming and very expensive, and often quite variable results are obtained which are
difficult to interpret. As an alternative, the rat is often used as a model for man (Schricker
1989). Those experiments, however, are limited by uncertainties with regard to differences
in metabolism between rat and man. Reddy & Cook (1991) recently reported that rat
studies cannot be used to assess the quantitative importance of dietary factors in human Fe
nutrition.

In the past few years in vitro methods to assess the bioavailability of minerals and trace
elements have gained popularity because of their accuracy, speed of analysis and relatively
low costs.

The solubility of minerals and trace elements under simulated conditions of the stomach
(pH 1–2, 37°C) as an index of bioavailability has been studied by Narasinga Rao &
Prabhavathi (1978), Lock & Bender (1980) and Forbes et al. (1989). In general, correlations
with in vivo bioavailability were poor. Narasinga Rao & Prabhavathi (1978), Wien &
Schwartz (1983), Hunt et al. (1987), Sandberg et al. (1989), Schwartz & Nevins (1989) and
Turnlund et al. (1990) have investigated the solubility of minerals and trace elements after
simulation of the conditions in the stomach (pH 1–2, 37°C) and small intestine (pH 6.5–8,
37°C) as a measure of in vivo bioavailability. The authors report contradictory results with
respect to the correlation between in vitro and in vivo bioavailability. Miller et al. (1981)
used the dialysability of Fe under simulated conditions of the stomach and the small
intestine as an index for its bioavailability. This method has been the basis for several in vitro
methods for estimation of the bioavailability of Fe and Zn. Promising correlations
between in vitro dialysability and in vivo bioavailability were reported (Schricker et al. 1981;
Hazell & Johnson, 1987b; Hurrell et al. 1988; Forbes et al. 1989; Sandström & Almgren,
1989). Miller et al. (1981), however, conclude that the prediction of bioavailability with in
vitro methods is relative rather than absolute because not all important physiological
factors can be simulated in vitro. Thus, in vitro methods may be very useful for ranking
purposes.

As the absorption of minerals and trace elements is taking place in the complex
environment of the small intestine, simulation of the conditions prevailing in the small
intestine is probably the most critical step for in vitro methods aiming at prediction of the
bioavailability of minerals and trace elements. In vitro methods based on the method of
Miller et al. (1981) use equilibrium dialysis of minerals and trace elements across a
semipermeable membrane as a model for the passage across the intestinal wall. It is
assumed that the minerals and trace elements that are dialysable are available for
absorption in the small intestine. In contrast with the situation in vivo, however,
components that pass the membrane are not removed. As we expect these components to
influence the equilibrium dialysis of minerals and trace elements, we hypothesize that a
dynamic in vitro method taking removal of dialysable components into account leads to a
better estimate of bioavailability in vivo. Therefore, we developed an in vitro method for
continuous dialysis of minerals and trace elements based on a hollow-fibre system. In this
way it is possible to remove dialysable components continuously from the pancreatic
digestion mixture.

In the present paper the continuous in vitro method is described in detail. Possible
interactions between the hollow-fibre membrane and minerals and trace elements were
investigated. As we expected an influence of pH on dialysability, the influence of pH during
pancreatic digestion was studied for both the continuous in vitro method presented here
and the equilibrium in vitro method described by Miller et al. (1981). The results of the
continuous in vitro method described here were compared with the results of the
equilibrium in vitro method with respect to the bioavailability of Ca, Mg, Fe, Cu and Zn
from different types of bread. The bread samples were chosen so as to contain variable phytic acid contents. The influence of phytic acid on the dialysability of Ca, Mg, Fe, Cu and Zn was investigated with both \textit{in vitro} methods.

**MATERIALS AND METHODS**

**Bread samples**

White bread, brown bread, wholemeal wheat bread, rye bread, brown bread with sunflower seeds, white bread with hazelnuts, sour-dough fermented brown bread and sour-dough fermented brown bread with sunflower seeds were bought in local stores. The breads were chosen so as to have varying contents of phytic acid (between 0.1 and 8.2 g/kg dry matter). The breads were dried at 60$^\circ$C for 24 h and milled on a 0.5 mm sieve.

Pepsin suspension: 8 g pepsin (\textit{EC} 3.4.23.1) powder (from porcine stomach mucosa; Sigma Chemical Co., Poole, Dorset) was suspended in 50 ml 0.1M HCl.

Pancreatin–bile extract mixture: 1 g pancreatin (from porcine pancreas; Sigma) and 6.25 g porcine bile extract (Sigma) were dispersed in 250 ml 0.1M NaHCO$_3$. In the \textit{in vitro} method with continuous dialysis the pancreatin–bile extract mixture was used at twice this concentration.

**In vitro method with equilibrium dialysis**

The \textit{in vitro} method with equilibrium dialysis was performed according to Miller \textit{et al.} (1981) and Hazell \& Johnson (1987b) with slight modifications. The method consists of three parts: peptic digestion, pH adjustment, and pancreatic digestion with equilibrium dialysis.

\textit{Peptic digestion.} Dry food sample (25 g) was suspended in 200 ml Milli Q water (Millipore Co., Etten-Leur, The Netherlands) in a plastic bottle. After adjusting the pH to 2.1 with HCl, 7.5 ml pepsin suspension was added. The pH was adjusted to 2.00 $\pm$ 0.03, the weight of the sample was brought to 250 g with Milli Q water and the sample was incubated in a shaking water-bath at 37$^\circ$C for 2 h. The pH was adjusted to 2.00 every 30 min.

\textit{pH-adjustment for pancreatic digestion.} The titratable acidity was determined as described by Hazell \& Johnson (1987b). The suspension after peptic digestion was divided into five portions of 20 g each which were transferred into plastic bottles. Segments of dialysis tubing (molecular weight cut-off 12000–14000, diameter 28.6 mm; Spectra/Por, Spectrum, Houston, TX, USA) containing an amount of NaHCO$_3$ (60 g/l) equivalent to the titratable acidity filled up to 25 ml with Milli Q water were placed in each bottle. The bottles were incubated in a shaking water-bath for 30 min at 37$^\circ$C. For one bottle the incubation was stopped at this moment ($t_0$).

\textit{Pancreatic digestion with equilibrium dialysis.} To each of the four remaining bottles 5 ml pancreatin–bile extract mixture was added and the samples were incubated in a shaking water-bath at 37$^\circ$C for 0.5, 1, 2.5 or 4 h ($t_0$, $t_1$, $t_2$5 and $t_4$) respectively. Depending on the buffering capacity of the food samples, the resulting pH after dialysis against NaHCO$_3$ and addition of the pancreatin–bile extract mixture varied between 6.7 and 7.0. At the end of the pancreatic digestion the pH was measured; during pancreatic digestion the pH remained fairly constant.

In the dialysates the concentrations of Ca, Mg, Fe, Cu and Zn were determined. A blank was run in each experiment to correct for small amounts of dialysable minerals and trace elements from the reagents.

The method is based on the formation of an equilibrium across a semipermeable membrane. In general, an equilibrium was reached after 2.5 h. Consequently, the amount of dialysed Ca, Mg, Fe, Cu and Zn was calculated as the mean value of $t_2$5 and $t_4$. As the volumes at both sides of the membrane are equal, the amounts of dialysed Ca, Mg, Fe,
Cu and Zn represent only half the amounts dialysable. For this reason the amounts of
dialysable minerals were calculated as twice the amount dialysed. The dialysability was
expressed as a percentage of the amounts of Ca, Mg, Fe, Cu and Zn present in the food
sample. The dialysability was calculated according to the following equation:
\[
\text{dialysability (\%) = } \frac{2D}{WA} \times 100,
\]
where \(D\) is the amount of mineral dialysed, calculated as the mean of the values at \(t\) 2.5 and
\(t\) 4 (mg), \(W\) is the dry weight of the food sample used for pancreatic digestion (g), \(A\) is the
concentration of mineral present in the dry food sample (mg/g).

**In vitro method with continuous dialysis using a hollow-fibre**
The *in vitro* method with continuous dialysis was partly based on the *in vitro* methods
described by Miller *et al.* (1981) and Hazell & Johnson (1987b). The simulation of the small
intestine was different. Furthermore, samples and reagents were used at twice the
concentration of those described for the method with equilibrium dialysis because
otherwise the levels of minerals and trace elements in the (continuous) dialysate are
sometimes too low to allow accurate determination. The continuous *in vitro* method
consisted of three parts: peptic digestion, pH adjustment, and pancreatic digestion with
continuous dialysis.

**Peptic digestion.** A dry food sample (50 g) was suspended in 175 ml Milli Q water
(Millipore Co.) in a plastic bottle. After adjustment of the pH to 2.1 with HCl, 15 ml pepsin
suspension was added. The pH was adjusted to 2.00±0.03, the weight of the sample was
brought to 250 g with Milli Q water and the sample was incubated in a shaking water-bath
at 37°C for 2 h. The pH was adjusted to 2.00 every 30 min.

**pH adjustment for pancreatic digestion.** Titratable acidity was determined as described by
Hazell & Johnson (1987b). After peptic digestion 20 g suspension was transferred into a
reaction vessel. A segment of dialysis tubing (molecular weight cut-off 12000–14000,
diameter 28.6 mm; Spectra/Por, Spectrum, Houston, TX, USA) containing NaHCO₃
(60 g/l) equivalent to the titratable acidity filled up to 5 ml with Milli Q water was placed
into the reaction vessel. The reaction vessel was incubated for 30 min at 37°C in a shaking
water-bath. After this stage the contents of the dialysis tubing were added to the reaction
vessel. The dialysis tubings were rinsed with 5 ml Milli Q water which was also added to
the reaction vessel.

**Pancreatic digestion with continuous dialysis.** Pancreatin–bile extract mixture (5 ml) was
added to the reaction vessel and the mixture was incubated for 4 h at 37°C. During this
pancreatic digestion the mixture was led through a hollow-fibre system. Every 30 min the
dialysate was collected. As in the *in vitro* method with equilibrium dialysis, the resulting pH
after addition of NaHCO₃ and pancreatin–bile extract mixture varied between 6.7 and 7.0.
During pancreatic digestion the pH in the reaction vessel decreased by 0.1–0.4 units
(depending on the type of sample).

The concentrations of Ca, Mg, Fe, Cu and Zn in the dialysates were determined. Every
four or five experiments a blank experiment was carried out to correct for small amounts
of dialysable minerals and trace elements from the reagents.

The hollow-fibre system is represented schematically in Fig. 1. The reaction vessel (1) was
placed in a water bath at 41°C (the temperature inside the reaction vessel was 37°C); in this
reaction vessel the pancreatic digestion takes place. The suspension was pumped via a
peristaltic pump (2) through the suction tube (3) into the hollow-fibre (4) (Amicon,
molecular weight cut-off 10000, Type H1P3-20; Amicon Division, W. R. Grace & Co.,
Components in the suspension that could pass the hollow-fibre membrane were dialysed and collected in a plastic bottle (5). The dialysis flow was 2 ml/min. That part of the suspension that could not pass the hollow-fibre membrane was pumped back into the reaction vessel via the recycle tube (6). In the reaction vessel these components could be digested further. The recycle flow was 50 ml/min. The volume in the reaction vessel was kept constant by a siphon (7). The siphon vessel was filled with Milli Q water (pH 7.0). The suction tube had a plastic cap on the end, this cap had some holes on the top to maintain a constant pressure inside and outside the cap. A fine filter cloth was stretched over the plastic cap to prevent large food particles from entering the hollow-fibre system. A magnetic stirrer was put inside the plastic cap to prevent clogging of the filter cloth. A filter was placed in front of the hollow-fibre membrane to prevent clogging of the membrane. The pressure on the hollow-fibre membrane could be measured by a manometer (8); this pressure may not exceed 170 kPa. After each experiment the hollow-fibre system was cleaned by pumping successively 0.1 M-HCl and water through the system.

The amount of dialysed material (dialysability) was expressed as a percentage of the total amount present in the food sample. The dialysability was calculated according to the following equation:

\[
\text{dialysability (\%)} = \frac{D}{WA} \times 100,
\]

where \(D\) is the total amount of mineral dialysed in 4 h (mg), \(W\) is the dry weight of food sample used for pancreatic digestion (g), \(A\) is the concentration of mineral present in dry food sample (mg/g).

**Investigation of interactions between the hollow-fibre membrane and Ca, Mg, Fe, Cu and Zn**

The possibility of interactions between the hollow-fibre membrane and Ca, Mg, Fe, Cu and Zn was investigated by dialysing pure salts. CaCl\(_2\) was dissolved in Milli Q water (pH 7.0), MgSO\(_4\) was dissolved in 0.1 M-phosphate buffer (pH 7.0) and FeSO\(_4\) was dissolved in 0.1 M-phosphate buffer (pH 7.0). In the last case a precipitate was formed immediately. The supernatant fraction was filtered and the Fe content determined. As the amounts of CuSO\(_4\) and ZnCl\(_2\) soluble in phosphate buffer of pH 7.0 were too small to be dialysed and analysed accurately, these salts were dissolved in 0.1 M-Tris buffer (pH 7.0).

The clear solutions were put in the reaction vessel of the hollow-fibre system and dialysed for 4 h. Other reagents were not added. The siphon vessel contained Milli Q water during
the experiment with Ca, 0·1 M-phosphate buffer (pH 7·0) during the experiments with Mg and Fe, and 0·1 M-Tris buffer (pH 7·0) during the experiments with Cu and Zn. Ca, Mg, Fe, Cu and Zn were determined in the dialysate and in the reaction vessel after dialysis.

Analytical methods
Phytic acid was determined after extraction with dilute HCl by ion-exchange chromatography with post-column derivatization and u.v. detection as described by Bos et al. (1991). Ca, Mg and Zn were determined with flame atomic absorption spectroscopy. Fe and Cu were determined with graphite-furnace atomic absorption spectroscopy.

RESULTS AND DISCUSSION
To estimate the bioavailability of minerals and trace elements a continuous in vitro method was developed as an alternative to the equilibrium in vitro method developed by Miller et al. (1981). In the equilibrium in vitro method components that pass the membrane are not removed, in contrast with the situation in vivo. As we expected these dialysable components to influence the equilibrium dialysis of minerals and trace elements, we studied the influence of the continuous removal of dialysable components from the pancreatic digestion mixture on the dialysability of Ca, Mg, Fe, Cu and Zn.

Investigation of interactions between the hollow-fibre membrane and Ca, Mg, Fe, Cu and Zn
The hollow-fibre membranes were made of polysulphone and should be inert. However, we investigated whether Ca, Mg, Fe, Cu or Zn bound to the hollow-fibre membrane, because this might lead to errors. Possible interactions were investigated by dialysis of pure salts. It was found that the Ca from the CaCl₂ solubilized in water (pH 7·0), the Mg from MgSO₄ solubilized in phosphate buffer (pH 7·0), the Cu from CuSO₄ and the Zn from ZnCl₂ solubilized in Tris buffer (pH 7·0) were completely recovered. Dialysis experiments with FeSO₄ were hampered because the phosphate buffer appeared to be largely contaminated with Fe. When only this phosphate buffer was dialysed in the hollow-fibre system the Fe from this buffer was completely recovered. No evidence was found for binding of Ca, Mg, Fe, Cu or Zn to the hollow-fibre membrane at pH 7. This, in combination with the inert character of the polysulphone membrane, led to the conclusion that dialysability measurements are not likely to be disturbed by binding of minerals or trace elements to the hollow-fibre membrane.

Repeatability of the continuous in vitro method
The repeatability of the continuous in vitro method was tested with a sample of wholewheat meal. The dialysability of Ca, Mg, Fe, Cu and Zn was tested in triplicate; the mean values obtained (%) were: Ca 35 (SD 4), Mg 57 (SD 3), Fe 21 (SD 3), Cu 76 (SD 5), Zn 24 (SD 6). It is concluded that the repeatability of the determination of the dialysability of Ca, Mg, Fe, Cu and Zn with the continuous in vitro method is good.

Influence of pH during pancreatic digestion on dialysability
The solubility of minerals and trace elements decreases and the binding of minerals and trace elements by dietary fibre and phytic acid increases with increasing pH (Fernandez & Phillips, 1982; Sri Kantha et al. 1986; Martin & Evans, 1986, 1987; Champagne, 1988; Champagne & Phillippy, 1989; Sandberg et al. 1989). Therefore, it is expected that pH influences the bioavailability of minerals and trace elements. We investigated the influence of pH during pancreatic digestion on the dialysability of Ca, Mg, Fe, Cu and Zn from a
Table 1. Influence of pH on dialysability (% of the amount present) of Ca, Mg, Fe, Cu and Zn from rye bread

<table>
<thead>
<tr>
<th>Element</th>
<th>pH</th>
<th>Dialysability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vitro method with equilibrium dialysis</td>
<td>In vitro method with continuous dialysis</td>
</tr>
<tr>
<td>Ca</td>
<td>6.2</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>44</td>
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<tr>
<td></td>
<td>7.4</td>
<td>42</td>
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<tr>
<td>Mg</td>
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<td></td>
<td>6.6</td>
<td>69</td>
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<td></td>
<td>6.9</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>69</td>
</tr>
<tr>
<td>Fe</td>
<td>6.2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>18</td>
</tr>
<tr>
<td>Cu</td>
<td>6.2</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>41</td>
</tr>
<tr>
<td>Zn</td>
<td>6.2</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>49</td>
</tr>
</tbody>
</table>

sample of rye bread. Dialysability was measured with both the in vitro method with continuous dialysis and the in vitro method with equilibrium dialysis. The dialysability with the equilibrium in vitro method was studied at pH 6.2, 6.6, 6.9, 7.1 and 7.4, while the dialysability with the continuous in vitro method was studied at pH 6.6 and 7.1. The results are shown in Table 1. For the in vitro method with equilibrium dialysis there was a marked decrease in the dialysability of Ca and a slight decrease in dialysability of Fe with increasing pH. The dialysability of Mg, Cu and Zn was not affected by pH. For the in vitro method with continuous dialysis there was a decrease in the dialysability of Ca, Mg, Fe and Cu with increasing pH. The dialysability of Zn was not affected.

As the influence of pH may depend on the type of sample, we investigated the influence of pH on the dialysability of Ca, Mg, Fe, Cu and Zn from carrots for the in vitro method with equilibrium dialysis (values not shown). For carrots the dialysability of Ca, Mg, Fe and Zn was influenced by pH. Only the dialysability of Cu was not influenced. This demonstrates that the influence of pH is dependent on the type of food sample.

These results clearly show that there is a great influence of pH during pancreatic digestion on the dialysability of minerals and trace elements. The actual influence depends on the type of food sample and on the in vitro method used. Miller et al. (1981) found no or only a very small influence of pH during pancreatic digestion on the dialysability of Fe.
Dialysability of Ca, Mg, Fe, Cu and Zn from several types of bread: comparison between equilibrium dialysis and continuous dialysis

The dialysability of Ca, Mg, Fe, Cu and Zn from eight different types of bread was determined with both the equilibrium in vitro method and the continuous in vitro method reported in the present paper. Fig. 2 shows the results of the continuous dialysis of Ca, Mg, Fe, Cu and Zn from wholemeal wheat bread. The dialysis was almost complete after 3 h. The dialysability was calculated as the total amount of Ca, Mg, Fe, Cu or Zn dialysed in 4 h.

In the first hour the amount of dialysed minerals and trace elements was very high. This may have been due partly to the high initial concentration of minerals and trace elements and partly to the pH. At t 0, the pH varied between 5 and 5.5 and the pancreatin–bile extract mixture was added. In the next 30 min the pH rose to 7. As is shown in Table 1, a lower pH generally resulted in a higher dialysability. A similar pH course was found in the equilibrium in vitro method. The pH course observed in vitro agrees well with that in the in vivo situation: the pH in the duodenum is lower than in the jejunum where the pH is about 7 (Clemens et al. 1975). In the duodenum a large part of the minerals and trace elements is absorbed (Avioli, 1988; Fairbanks & Beutler, 1988; Shils, 1988; Solomons, 1988; Wilson & Greene, 1988), but it is not clear whether the higher initial concentration and the lower pH are the only important factors here.

As a consequence of the continuous removal of dialysable components in the continuous in vitro method, the pH during the 4 h pancreatic digestion decreased slightly. This is in agreement with the situation found in in vivo experiments with pigs: the pH in the ileum is slightly lower than that in the jejunum (Clemens et al. 1975). In the equilibrium in vitro method, on the other hand, the pH remained rather constant during pancreatic digestion.
The dialysability of Ca, Mg, Fe, Cu and Zn from eight different types of bread as determined by the in vitro method with equilibrium dialysis and the in vitro method with continuous dialysis is presented in Table 2. In general, the dialysability of Ca, Mg, Fe and Cu determined by the continuous in vitro method is higher than that determined with the equilibrium in vitro method (up to a factor 3–4). For Zn both methods gave comparable dialysabilities. Linear regression analysis showed a positive linear correlation between the dialysabilities of Fe, Cu and Zn as determined by the two in vitro methods (r = 0.84, 0.89 and 0.96 respectively). For Mg and Ca no correlation was found.

These results show that removal of dialysable components from the pancreatic digestion mixture has a marked influence on the dialysability of minerals and trace elements. Whether removal of dialysable components leads to better estimates of bioavailability in vivo is currently being investigated.

The influence of phytic acid on the dialysability of Ca, Mg, Fe, Cu and Zn

The breads were chosen to contain variable amounts of phytic acid because phytic acid is known to have a strong negative influence on the bioavailability of some minerals and trace elements. The contents of phytic acid, Ca, Mg, Fe, Cu and Zn in the breads are presented in Table 3. Table 2 shows the dialysability of Ca, Mg, Fe, Cu and Zn from the breads.

The phytic acid content increased in the order white bread < brown bread < wholemeal wheat bread, whereas the dialysability of Fe and Zn as determined with the equilibrium in vitro method decreased dramatically. There was a smaller decrease in the dialysability of Cu. For the continuous in vitro method the dialysability of Ca, Fe and Zn decreased with increasing phytic acid content. There was a smaller decrease in dialysability of Mg. These results agree with other studies where in vitro and in vivo experiments have shown a strong negative influence of phytic acid on the bioavailability of Ca, Fe and Zn, and a less marked influence on the bioavailability of Mg and Cu (Spivey Fox & Tao, 1989; Torre et al. 1991).

Addition of sunflower seeds to brown bread led to a 3-fold increase in phytic acid content. This led to a decrease in the dialysability of Ca, Mg, Fe and Zn as determined by the equilibrium in vitro method. The dialysability of Cu increased. Apart from a small increase in Ca dialysability, the continuous in vitro method resulted in similar dialysabilities for brown bread and brown bread with sunflower seeds. This shows that phytic acid is not the only component influencing the bioavailability of minerals and trace elements from bread samples. Therefore, the effect of phytic acid cannot be studied separately from the effects of other components on the bioavailability of minerals and trace elements.

Addition of hazelnuts to white bread led to an increase in phytic acid content. At the same time the dialysability of Fe and Cu as determined with the equilibrium in vitro method decreased. When determined by the continuous in vitro method the dialysability of Ca, Mg and Fe decreased. Despite the rise in phytic acid content, both in vitro methods showed an increase in Zn dialysability.

Sour-dough fermentation of bread resulted in a significant reduction in phytic acid content. This has been reported previously by van Lonkhuijsen & van Gelderen (1985). Although the equilibrium in vitro method showed a small increase in Ca dialysability for sour-dough fermented brown bread and a small increase in Fe and Zn dialysability for sour-dough fermented brown bread with sunflower seeds, neither the equilibrium in vitro method nor the continuous in vitro method showed a clear positive influence of the decrease in phytic acid content on the dialysability of Ca, Mg, Fe, Cu and Zn. In general, reduction of the phytic acid content by sour-dough fermentation is believed to increase the bioavailability of minerals and trace elements because the phytic acid content is reduced. However, the phosphate produced from the phytic acid during sour-dough fermentation may also have a negative effect on the bioavailability of minerals and trace elements.
Table 2. Dialysability of Ca, Mg, Fe, Cu and Zn (% of the amount present) from eight different types of bread determined with the equilibrium in vitro method and the continuous in vitro method*  
(Means of duplicate analyses)

<table>
<thead>
<tr>
<th>Type of bread</th>
<th>Dialysability</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>Equilibrium</td>
<td>12</td>
<td>52</td>
<td>36</td>
<td>50</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>53</td>
<td>76</td>
<td>46</td>
<td>64</td>
<td>27</td>
</tr>
<tr>
<td>Brown bread</td>
<td>Equilibrium</td>
<td>22</td>
<td>36</td>
<td>14</td>
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<td>7</td>
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<td>7</td>
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<tr>
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<td>50</td>
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<td>63</td>
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<td>with sunflower seeds</td>
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<td>25</td>
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<td>17</td>
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</table>

* For details of procedures, see pp. 851–854.

Table 3. Contents of phytic acid (g/kg dry matter (DM)), Ca, Mg, Fe, Cu and Zn (mg/kg DM) in various types of bread

<table>
<thead>
<tr>
<th>Type of bread</th>
<th>Phytic acid</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
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<tbody>
<tr>
<td>White bread</td>
<td>0.4</td>
<td>360</td>
<td>330</td>
<td>17</td>
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<td>Brown bread</td>
<td>2.9</td>
<td>470</td>
<td>890</td>
<td>32</td>
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<td>20</td>
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<td>4.3</td>
<td>880</td>
<td>1020</td>
<td>44</td>
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<td>430</td>
<td>1100</td>
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<td>38</td>
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<td>Brown bread with sunflower seeds</td>
<td>8.2</td>
<td>570</td>
<td>1670</td>
<td>47</td>
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<td>620</td>
<td>480</td>
<td>22</td>
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<td>360</td>
<td>760</td>
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<tr>
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<td>1380</td>
<td>40</td>
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</table>


It should be noted that although the relative dialysability of minerals and trace elements is lower for certain breads, the absolute bioavailability may be higher due to the higher absolute content of minerals and trace elements present in those breads (Table 2).

Our results show that for white bread, brown bread and wholemeal wheat bread there is a marked negative effect of phytic acid on the dialysability of Ca, Fe and Zn. Results for brown bread with sunflower seeds, white bread with hazelnuts and the two types of sour-dough fermented bread, however, show that the bioavailability of minerals and trace...
elements from bread samples is not related to the phytic acid content only. Therefore, it is concluded that the effect of phytic acid on the bioavailability of minerals and trace elements cannot be studied separately from the effects of other components on this bioavailability. Removal of dialysable components as performed in the continuous in vitro method appears to have a marked effect on the influence of phytic acid on the bioavailability of minerals and trace elements. This once more stresses the importance of a better understanding of the removal of dialysable components.

Conclusions

The in vitro method with continuous dialysis for the estimation of the bioavailability of minerals and trace elements presented here takes continuous removal of dialysable components into account. Experiments showed that there is no interaction between the hollow-fibre membrane used in the continuous in vitro method and Ca, Mg, Fe, Cu and Zn ions. Therefore, dialysability measurements are not likely to be disturbed by binding of minerals or trace elements to the hollow-fibre membrane. There is a large influence of pH during pancreatic digestion on the dialysability of Ca, Mg, Fe, Cu and Zn determined by both in vitro methods. The actual influence depends on the type of sample and on the in vitro method used.

For most types of bread the continuous in vitro method leads to higher dialysabilities of Ca, Mg, Fe and Cu than the equilibrium in vitro method. The dialysability of Zn is for most breads comparable for the two methods. It is concluded that removal of dialysable components has a marked influence on the dialysability of minerals and trace elements. However, the importance for the estimation of bioavailability in vivo needs further investigation.

Phytic acid has a negative effect on the bioavailability of Ca, Fe and Zn. However, the effect of phytic acid cannot be studied separately from effects of other components on the bioavailability of minerals and trace elements from breads. It has been shown that removal of dialysable components in vitro influences the effect of phytic acid on the bioavailability of minerals and trace elements.

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References


