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# Enhancement Effect Study of Some Organic Acids on the Calcium Availability of Vegetables: Application of the Dynamic in Vitro Simulated Gastrointestinal Digestion Method with Continuous-Flow Dialysis

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The effect of added organic acids on the calcium availability of vegetables was investigated using the dialysis profiles obtained from an in vitro simulated gastrointestinal digestion with continuous-flow dialysis method. Citric acid was the most effective enhancer followed by tartaric, malic, and ascorbic acids. For amaranth, which has a low calcium availability (5.4%), a significant increase of availability was observed with increasing concentrations of all acids studied. With the continuous-flow dialysis approach, organic acids could be observed to promote the dialyzability even at an elevated intestinal pH. An enhancement effect from added organic acids was not clearly observed for Chinese kale, which itself contains a high amount of available calcium (52.9%).

**KEYWORDS: Calcium availability; vegetables; organic acid; in vitro method; continuous flow**

## INTRODUCTION

Vegetables, especially green leafy vegetables, are known as a rich source of dietary calcium. Unfortunately, some vegetables with high contents of calcium show very low availability. The low calcium availability in vegetables was derived from the presence of some substances (phytate, oxalate, and dietary fiber components) which bind calcium to form unabsorbable compounds (*1*, *2*).

The effect of some organic acids on calcium availability has been documented. An enhancement effect by ascorbic acid (*3*) and citric acid (*4*, *5*) was reported. Many literature data on the effect of an enhancer or an inhibitor on the mineral availability are available. These studies were often carried out by adding the enhancer or inhibitor directly to foods followed by an in vitro or an in vivo availability evaluation. The in vitro method was widely performed by the method or modified methods of Miller (*6*) using a simulated gastrointestinal digestion with an equilibrium dialysis procedure. The methods involve enzymatic digestion with pepsin at pH 2 followed by digestion−dialysis in the presence of pancreatin−bile extract (PBE) at a gradual pH change from 2 to 7.5. The earlier conventional dialysis procedure provides a single value of the dialyzable amount of the element of interest at the equilibrium condition. It is simple and has been well accepted. However, in the equilibrium method, the dialyzed components are not continuously removed, as occurred in the intraluminal digestive tract, and therefore, this approach does not mimic the dynamic absorption process in the body and does not give information of time-dependent changes of dialysis during the course of gastrointestinal digestion.

Therefore, in vitro gastrointestinal digestion with continuous-flow dialysis procedures have been proposed as a closer simulation of the in vivo physiological conditions as opposed to that based on equilibrium dialysis, because dialyzable components are continuously removed from the digestion mixture during dialysis (*7*−*10*). The continuous-flow procedures also are readily adaptable to automatic computer control (*9*) and on-line detection (*11*, *12*). The computer-controlled in vitro dynamic system was applied for many case studies of the bioavailability of both minerals (*13*, *14*) and food mutagens (*15*).

Continuous monitoring of dialyzed minerals and pH change during dialysis provides profiles which are believed to be useful for the interpretation of enhancing or inhibiting effects. The aim of this work was to apply the dynamic in vitro simulated gastrointestinal digestion with continuous-flow dialysis method for the first time to demonstrate the use of dialysis profiles to investigate the effect of some organic acids on the calcium dialyzability of vegetables by looking into the time-dependent profiles obtained. Amaranth and Chinese kale were selected in this study to represent vegetables of low and high calcium availability, respectively (*16*). Four common organic acids, i.e., ascorbic, citric, tartaric, and malic acids, were studied.

## EXPERIMENTAL DETAILS

**Equipment and Materials.** For measurement of calcium in dialysates, a Perkin-Elmer model 3100 flame atomic absorption/emission spectrometer (FAAS/FAES) was used. Calibration standards were

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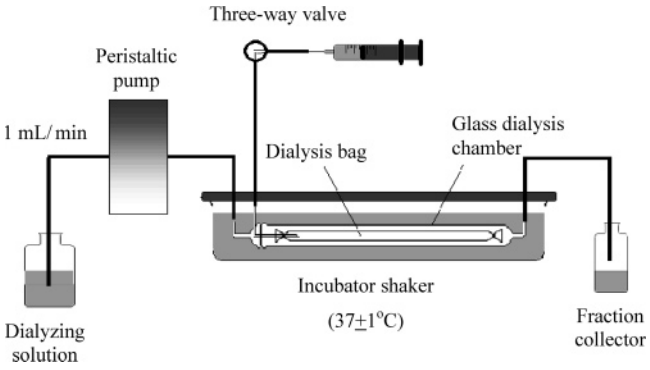


**Figure 1.** Diagram of the proposed continuous-flow in vitro dialysis system.

prepared in sodium bicarbonate of the same concentration as the dialyzing solution. The measurement was carried out with an air−acetylene flame. The calcium emission intensity was monitored at 422.7 nm with a 0.7 nm slit width.

A pH meter (Denver Instrument model 215, Colorado) with a glass combination electrode was used for all pH measurements. Commercial standard buffers (Merck, Darmstadt, Germany) of pH 4.00 ± 0.01 and 7.00 ± 0.01 were employed for the pH calibration. An incubator shaker from Grant Instrument, model SS40-D2 (Cambridge, England), was used to incubate the samples at 37 ± 1 °C.

**Chemicals and Samples.** The enzymes pepsin (P-7000, from porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas), and bile extract (B-6831, porcine) were from Sigma (St. Louis, MO). A pepsin solution was prepared by dissolving 0.16 g of pepsin in 1 mL of 0.1 M hydrochloric acid and a PBE mixture by dissolving 0.004 g of pancreatin and 0.025 g of bile extract in 5 mL of 0.001 M sodium bicarbonate (*6*). Calcium standard solution (1000 mg/L) was prepared by dissolving an appropriate amount of calcium carbonate (Carlo Erba, Italy) in 1% (v/v) hydrochloric acid. The dialyzing solution was prepared by dissolving an appropriate amount of sodium bicarbonate in 1 L of purified water. The optimum concentration of sodium bicarbonate was determined from the titratable acidity, which was determined by titrating a 2.5 mL aliquot of peptic-digested sample to which 625 μL of PBE mixture was added, using standard 0.01 M NaOH as a titrant, to a pH of 7.5 (*10*). Ascorbic, malic, citric, and tartaric acids were obtained from Fluka Chemicals (Switzerland) and were confirmed to contain an undetectable amount of calcium at the concentrations being used.

Fresh amaranth, Chinese kale, and other vegetables were purchased from local markets and were cleaned and rinsed with purified water. Only the edible parts were then dried at 65 °C to constant mass and ground to store in a desiccator for use throughout the study to ascertain that similar vegetable lots were used.

**Determination of the Total Calcium Content of Food Samples.** A 0.5 g amount of sample was accurately weighed in a TFM vessel, and 10 mL of a $HNO_3/H_2O_2$ (3:2 v/v) mixture was added. Then acid dissolution was performed in a microwave digestion system (Milestone, model MLS-1200 Mega, Connecticut) according to the manufacturer's instructions. The clear solution was diluted with purified water to obtain a volume of 50.0 mL. The solution was then transferred to a polyethylene bottle. The calcium content was determined by flame atomic emission spectrometry using standard addition calibration.

**Design and Setup of the Continuous-Flow Dialysis System (*10*).** A continuous-flow dialysis (CFD) system was designed to serve three objectives as follows: to facilitate a gradual pH change at the early stage of dialysis (30 min), with the pH being maintained at 7.5 at the later stage (after 60 min), to provide a convenient means of addition of enzymes at the time and amount required, and to enable continuous removal of dialyzable components during dialysis.

The proposed dialysis system is presented schematically in **Figure 1**. A dialysis chamber was designed to allow containment of the dialysis tubing, around which the dialyzing solution could flow during dialysis. The chamber (ca. 20 cm in length and 0.8 cm inner diameter) and its covers were constructed in-house from borosilicate glass. Dialysis tubing of MWCO 12000−14000 (Spectro/Por, Thomas Scientific) was used. To prepare the dialysis chamber, dialysis tubing of 10 mm flat width and ca. 17.5 cm length was tied at both ends, one end with a silicone tube (2 mm inner diameter and 5 cm long) inserted for the injection of a peptic digest sample and required enzymes. The other end of this silicone tube was pierced through an aperture in the chamber cover to allow convenient addition of a peptic digest aliquot and PBE mixture via a three-way valve by a syringe. The cover was tightly sealed onto the chamber with a silicone gasket and a rubber band. The dialysis chamber was placed in a shaking water bath at 37 ± 1 °C. The dialyzing solution ($NaHCO_3$) from the reservoir was pumped through the chamber using a peristaltic pump (Eyela, model MP-3N, Japan) with a flow rate of 1.0 mL/min. Dialyzable components in the peptic digest suspension could pass through the dialysis membrane and be collected in plastic collectors.

Although the proposed dialysis system can be connected to the pH meter and elemental detection instrument for on-line detection, in this study we chose to collect the dialysate fractionwise for subsequent calcium determination. In this way, dialysate samples are not totally consumed and can be kept for further analyses or later confirmation.

**Simulated Gastrointestinal Digestion Procedure with Continuous-Flow Dialysis.** Simulated gastrointestinal digestion of food samples was carried out starting with peptic digestion with pepsin in a batch system, followed by pancreatic digestion with PBE in the CFD system (see the flow chart in **Figure 2**).

In the simulated peptic digestion step, a dried homogeneous vegetable sample (0.5 g) or cooked vegetable (equivalent to a 0.5 g dry mass) was suspended in 10 mL of purified water and adjusted to pH 2 with 6 M hydrochloric acid. The sample suspension volume was finally adjusted to 12.5 mL with purified water and spiked with 375 μL of pepsin solution. This digestion process was performed in an incubator shaker at 37 ± 1 °C for 2 h. The titratable acidity of the peptic digest was determined by titrating a 2.5 mL aliquot to which 625 μL of PBE mixture was added, using standard 0.01 M NaOH as a titrant, to a pH of 7.5. This titratable acidity was used for calculation of the optimal concentration of the dialyzing solution (*10*).

After the simulated peptic digestion, intestinal digestion with continuous-flow dialysis was carried out. The dialysis chamber was prepared as described earlier. Before use, the dialysis tubing was flattened to remove any air bubbles or liquid inside. Then, the 2.5 g peptic-digested sample was added via the three-way valve using a syringe connected to the silicone tube insert. The dialysis temperature was maintained at 37 ± 1 °C. The peristaltic pump was switched on to start the pancreatic digestion with a flow rate of 1 mL/min. The dialysate from the chamber was collected at 10 mL intervals in plastic vials for 60 min and then at 10 or 30 mL intervals for an additional 90 min. The freshly prepared PBE mixture (625 μL) was added into the dialysis bag by means of syringe injection via the three-way valve at 30 min. All dialysate fractions were subjected to pH measurement and calcium determination after completion of the pancreatic digestion.

**Simulated Gastrointestinal Digestion Procedure with Equilibrium Dialysis.** The peptic digestion was carried out similarly as mentioned above. Then a 2.5 mL portion of the peptic digest was added into the dialysis bag in the chamber of the dialysis system setup. However, instead of flowing the dialyzing solution, 3.0 mL of dialyzing solution containing an amount of $NaHCO_3$ equivalent to the titratable acidity of the peptic digest was injected into the dialysis chamber to fill the space in the chamber outside the dialysis bag. The sample was incubated in a shaking water bath at 37 ± 1 °C for 30 min before 625 μL of pancreatin−bile extract mixture was added and incubation continued for an additional 2 h. The dialysate was collected for subsequent determination of the calcium content.

**Estimation of Calcium Availability.** The amount of dialyzed calcium in a sample after the simulated gastrointestinal digestion was calculated from the summation of dialyzed calcium in all dialysate fractions and was expressed as a percentage of the total amount of calcium present in the sample:

$$\text{availability (\%)} = (D \times 100)/(WA)$$

where $D$ = total amount of dialyzed calcium (μg), $W$ = amount of sample used (g) as the dry mass of the original sample, and $A$ = concentration of calcium in the original dry sample (μg/g).

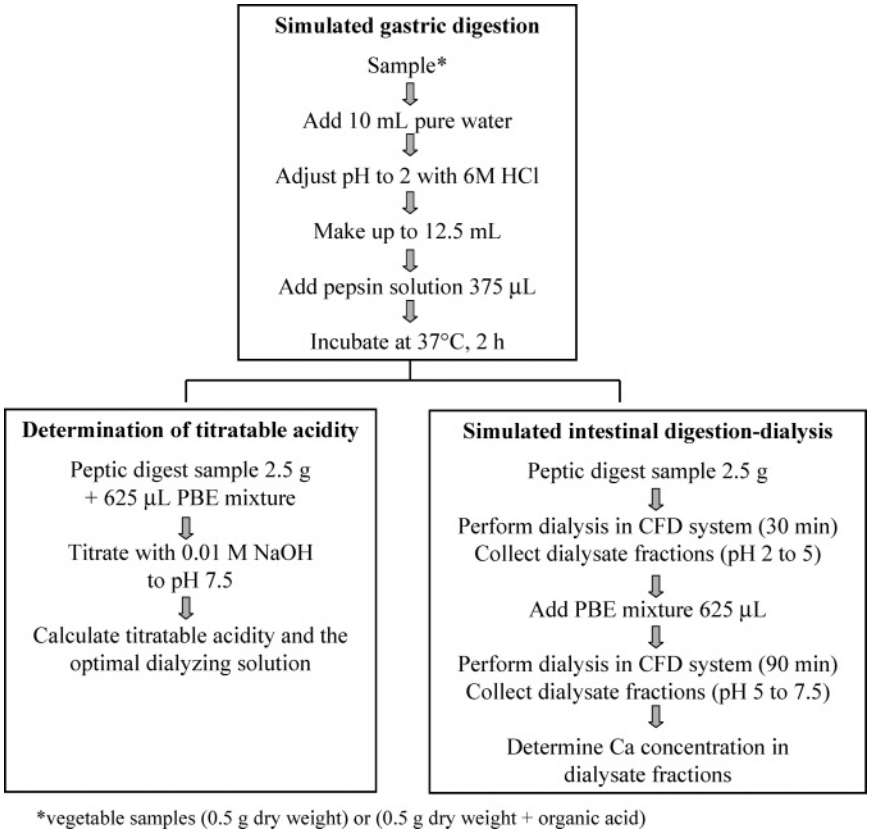


**Figure 2.** Flow chart of the simulated gastrointestinal digestion with continuous-flow intestinal digestion–dialysis.

## RESULTS AND DISCUSSION

**In Vitro Simulated Gastrointestinal Digestion: Equilibrium vs Dynamic Dialysis Approaches.** The differences between the in vitro gastrointestinal dialysis with traditional equilibrium and the continuous-flow dialysis approaches are important to consider. Only a single value of the dialyzable amount of minerals at the equilibrium condition is obtained from the equilibrium dialysis procedure, whereas a time-dependent change of the dialyzed amount of mineral during the course of gastrointestinal digestion is obtained from the continuous-flow approach. A dynamic in vitro method with continuous removal of dialyzed components should be a better estimation of availability than the equilibrium in vitro method. Naturally, the results obtained from the two approaches are different, owing to the fact that the equilibrium method is based on the dialysis equilibrium of minerals between both sides of the dialysis membrane. Dialysis ceases when the concentrations of dialyzable components on both sides are equal. On the other hand, in the dynamic continuous-flow in vitro method, all the dialyzable components could possibly permeate through the membrane because fresh dialyzing solution was fed to the system continuously. To demonstrate this fact, standard 100 $\mu$g/mL calcium carbonate in 1% (v/v) HCl was subjected to the two dialysis procedures using the procedure described. The calcium availability as determined by the continuous-flow in vitro method was higher than that obtained from the equilibrium dialysis method (**Table 1**). The dialyzed amounts obtained from the equilibrium approach were found to be dependent on the volume ratio of the peptic digest and the dialyzing solution as summarized in **Table 1**. For a given case in which the volumes of the peptic digest and the dialyzing solution are equal (10 mL each), the dialyzed amount from the continuous-flow in vitro method was approximately 2 times that of the equilibrium in vitro method. In other words, the dialyzed amount accounted for only half of the dialyzable amount in the equilibrium dialysis. Since the continuous-flow approach provides the total dialyzable amount through continuous removal of dialyzable minerals, the results from the equilibrium dialysis should be corrected to match the value obtained from the continuous-flow dialysis procedure using the following equation:

$$D_{\text{corrected}} = D_{\text{uncorrected}}(V_s + V_d)/V_d$$

where $D_{\text{corrected}}$ (%) = the corrected $D$ (%), $D_{\text{uncorrected}}$ (%) = the $D$ (%) obtained from the amount dialyzed in the equilibrium dialysis, $V_s$ = sample volume (mL), and $V_d$ = dialyzing solution volume (mL).

This rationale was confirmed by examining the effects of the sample volume and dialyzing solution volume on the dialyz-

**Table 1.** Effect of the Sample Volume to Dialyzing Solution Volume Ratio (S:D) on Dialyzability in the Equilibrium Dialysis Method[a]

| dialysis method | sample vol (mL) | dialyzing solution vol (mL) | S:D | $D$[b] (%) $D_{\text{uncorrected}}$ (%) | $D$[b] (%) $D_{\text{corrected}}$ (%) |
|---|---|---|---|---|---|
| equilibrium | 10.0 | 20.0 | 0.5 | 28.1 ± 1.0 | 42.2 ± 1.5 |
| | 10.0 | 10.0 | 1.0 | 22.0 ± 1.1 | 44.0 ± 2.2 |
| | 10.0 | 5.0 | 2.0 | 12.6 ± 0.8 | 37.8 ± 2.4 |
| continuous flow | 2.5 | flowing | | 41.2 ± 1.4 | |

[a] The sample was a 100 $\mu$g/mL (2.5 mL) standard calcium carbonate solution ($n = 3$). [b] $D$ for the equilibrium method is provided as uncorrected values ($D_{\text{uncorrected}}$) and corrected values ($D_{\text{corrected}}$), while that of the continuous-flow method needs no correction. Each $D_{\text{corrected}}$ value shows no significant difference with the $D$ obtained from the continuous-flow method as evaluated by the $t$ test at $P = 0.05$.

**Table 2.** Total Calcium Content and Availability of Calcium from Various Vegetables As Determined by in Vitro Simulated Gastrointestinal Digestion with Continuous-Flow and Equilibrium Dialysis

| | | availability (%) ($n = 3$) | | | |
|---|---|---|---|---|---|
| | | | equilibrium in vitro method | | |
| vegetable | total calcium concn (mg/g dried sample) ($n = 3$) | continuous-flow in vitro method[a] | uncorrected | corrected[b] | availability (%) (other researchers) |
| amaranth, leaves | 26.7 ± 0.4 | 5.4 ± 0.4 | 3.0 ± 0.5 | 6.0 ± 1.0 | 4.1[c] (Kamchan et al. (*16*)) |
| awltree, leaves | 38.7 ± 0.6 | 10.1 ± 1.2 | 5.1 ± 1.0 | 10.2 ± 2.0 | |
| cabbage, edible parts | 4.5 ± 0.4 | 48.2 ± 1.3 | 21.6 ± 0.5 | 43.2 ± 1.0 | 64.9[d] (Weaver et al. (*20*)) |
| Chinese kale, edible parts | 16.1 ± 0.3 | 52.9 ± 1.1 | 22.2 ± 1.7 | 44.4 ± 3.4 | 58.8[d] (Weaver et al. (*20*)) |
| cumin, leaves | 23.6 ± 0.6 | 13.2 ± 0.6 | 5.5 ± 0.2 | 11.0 ± 0.4 | |
| hairy basil, leaves | 20.4 ± 0.5 | 31.2 ± 0.3 | 13.3 ± 0.9 | 26.6 ± 1.8 | |
| Indian penny wort, leaves | 15.3 ± 0.5 | 39.6 ± 0.7 | 17.1 ± 0.3 | 34.2 ± 0.6 | |
| ivy gourd, leaves and tips | 9.3 ± 0.6 | 38.4 ± 0.8 | 16.1 ± 0.6 | 32.2 ± 1.2 | |
| kitchen mint, leaves | 18.5 ± 0.4 | 33.9 ± 1.7 | 16.5 ± 1.6 | 33.0 ± 3.8 | |
| betel, leaves | 22.8 ± 0.5 | 2.25 ± 0.1 | 1.3 ± 0.2 | 2.6 ± 0.4 | 2.5[c] (Kamchan et al. (*16*)) |
| sesbania, tender tips | 10.1 ± 0.4 | 24.8 ± 0.2 | 10.1 ± 0.7 | 20.2 ± 1.4 | |
| spinach, edible parts | 10.3 ± 0.5 | 4.6 ± 0.5 | 1.9 ± 0.2 | 3.8 ± 0.4 | 4.6[d] (Peterson et al. (*19*)) |

[a,b] The correlation plot of data from the continuous-flow (*a*) and equilibrium (*b*) methods shows that $b = 0.853a + 0.638$, $r^2 = 0.991$. [c] Values from the in vitro method. [d] Values from the in vivo method.

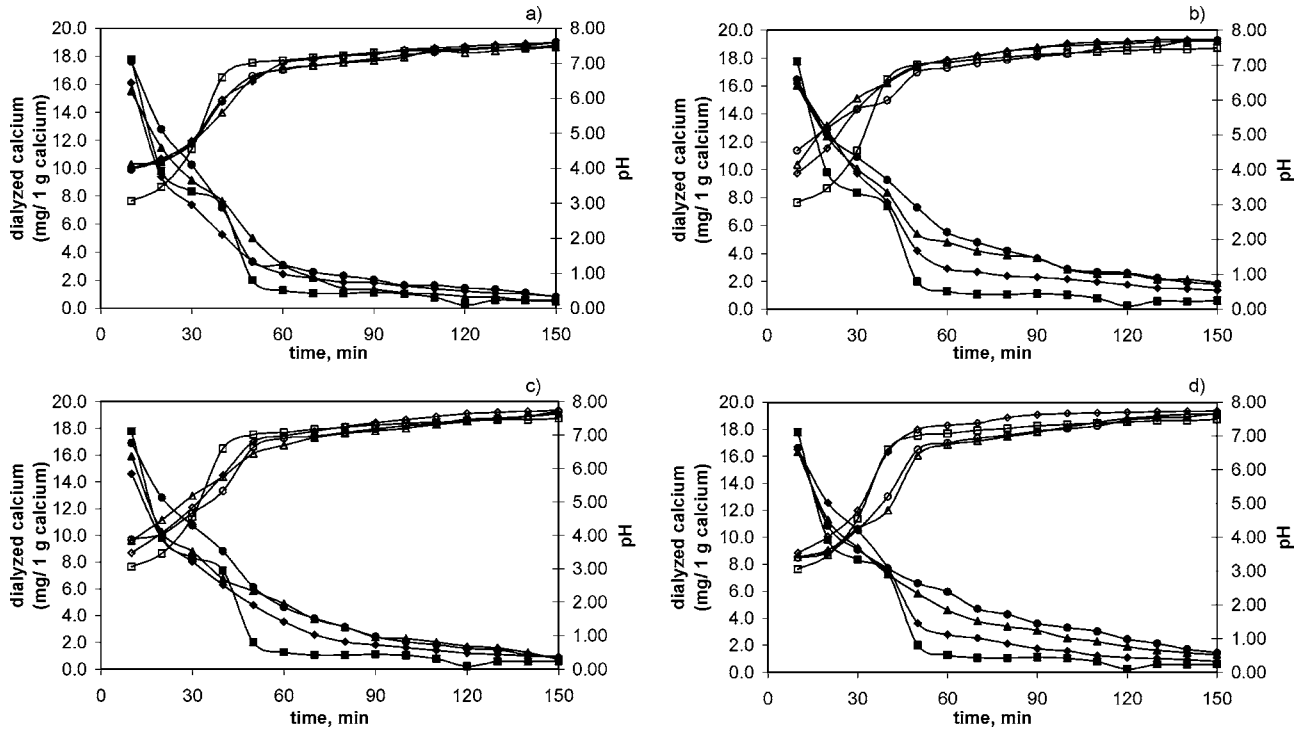


**Figure 3.** Dialyzed calcium and pH change during simulated intestinal digestion for amaranth with addition of varying concentrations of ascorbic acid (**a**), citric acid (**b**), malic acid (**c**), and tartaric acid (**d**), where ■, ◆, ▲, and ● represent amaranth with 0%, 1.0%, 2.5%, and 5% (w/w) acid, respectively. The corresponding pH profiles are presented with open symbols of the same type.

ability of a calcium standard as presented in **Table 1**, which shows a lower $D_{\text{uncorrected}}$ when the dialyzing solution volume is increased. $D_{\text{corrected}}$ gave results comparable with those of continuous-flow dialysis. This correction method should always be applied to get $D_{\text{corrected}}$, which is an accurate value expressing the dialyzable fraction for the equilibrium method.

**In Vitro Availability of Calcium for Various Local Vegetables.** The calcium availability of some vegetables as determined by the in vivo method has been reported (*16*−*20*). Total calcium and its availability of various local vegetables by an in vitro method based on continuous-flow dialysis and equilibrium approaches were determined, and the results are shown in **Table 2**. Some reported availability data are given in the last column for comparison. The results show that the corrected availability values of the equilibrium method were approximately 85% of the values obtained from the continuous-flow dialysis as calculated from the correlation plot ($b = 0.853a + 0.638$, $r^2 = 0.991$). The slightly lower availability values (%) from the equilibrium method could be due to systematic error from the correction factors obtained from the sample and dialyzing solution volumes used for correction. The accurate volumes were difficult to measure since the dialysis membrane may swell during use. This observation can also suggest that the results from the continuous-flow method should be more reliable than those obtained from the equilibrium method.

The in vitro availability of calcium for cabbage, Chinese kale, and spinach by the continuous-flow method was found to be 48.2%, 52.9%, and 4.6%, respectively. These results are close

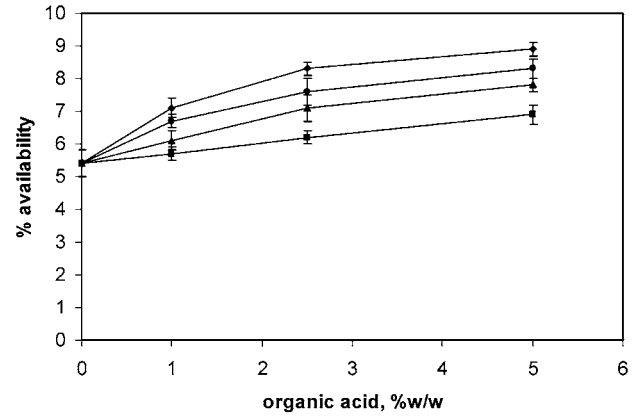


**Figure 4.** Relationship between the concentration (%) of organic acids added and the calcium availability (%) for amaranth: ascorbic acid (■), citric acid (◆), malic acid (▲), and tartaric acid (●).

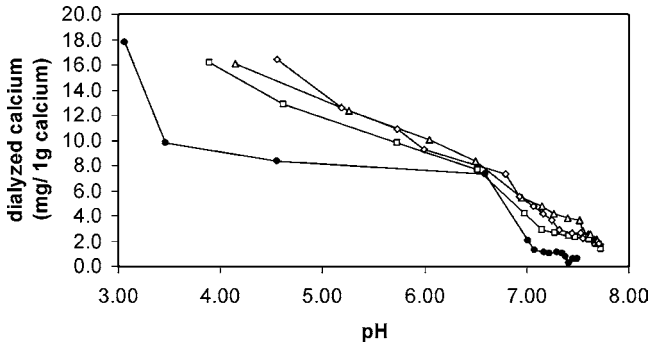


**Figure 5.** Dialysis profile as a graphical plot of dialyzed calcium against the pH value of the dialysate for amaranth with addition of varying concentrations of citric acid: 0% (●), 1% (□), 2.5% (△), and 5% (◇). Data were obtained from **Figure 3b**.

to the values of in vivo study reports of 64.9%, 58.8% (*20*), and 4.6% (*19*), respectively.

Some vegetables such as amaranth, awltree, cumin, betel leaves, and spinach were found to contain high amounts of calcium but a very low in vitro availability (%). The reasons for the low availability were reported to be related to the inhibitors (phytate, oxalate, and dietary fiber components) which bind calcium to form unabsorbable compounds (*1*, *2*).

Amaranth and Chinese kale were selected as representative vegetables for low and high calcium availability, respectively, for the study of the effect of organic acids on availability.

**Effect of Organic Acids on the Calcium Availability of Amaranth.** Chemical bindings of calcium with food components are major factors affecting availability. Some food components favorably promote mineral absorption. The influence of various organic acids on calcium availability has been investigated. Some authors reported an enhancing effect by organic acids (*4*, *5*). Therefore, the effect of organic acids, commonly found in foods, on the calcium availability of vegetables was systematically studied. Ascorbic, citric, tartaric, and malic acids were directly added into the chosen vegetables at concentrations of 1% (w/w), 2.5% (w/w), and 5% (w/w) of the vegetable (dry mass), and the calcium availability was determined in the same manner. The results are depicted in **Figure 3**.

The dialysis profiles obtained from the dynamic continuous-flow system, showing the effect of the concentration of added organic acids, are illustrated in **Figure 3**. The changes in the dialyzed amount could be observed from the dialysis profiles. The profiles show an enhancing effect on calcium dialyzability at increased concentrations of organic acids. For example, the dialysis profiles of amaranth without citric acid dropped to the baseline when the pH of dialysis approached 7. On the contrary, dialysis went on even at pH higher than 7 when 1−5% citric acid was added. Other organic acids gave similar results. From

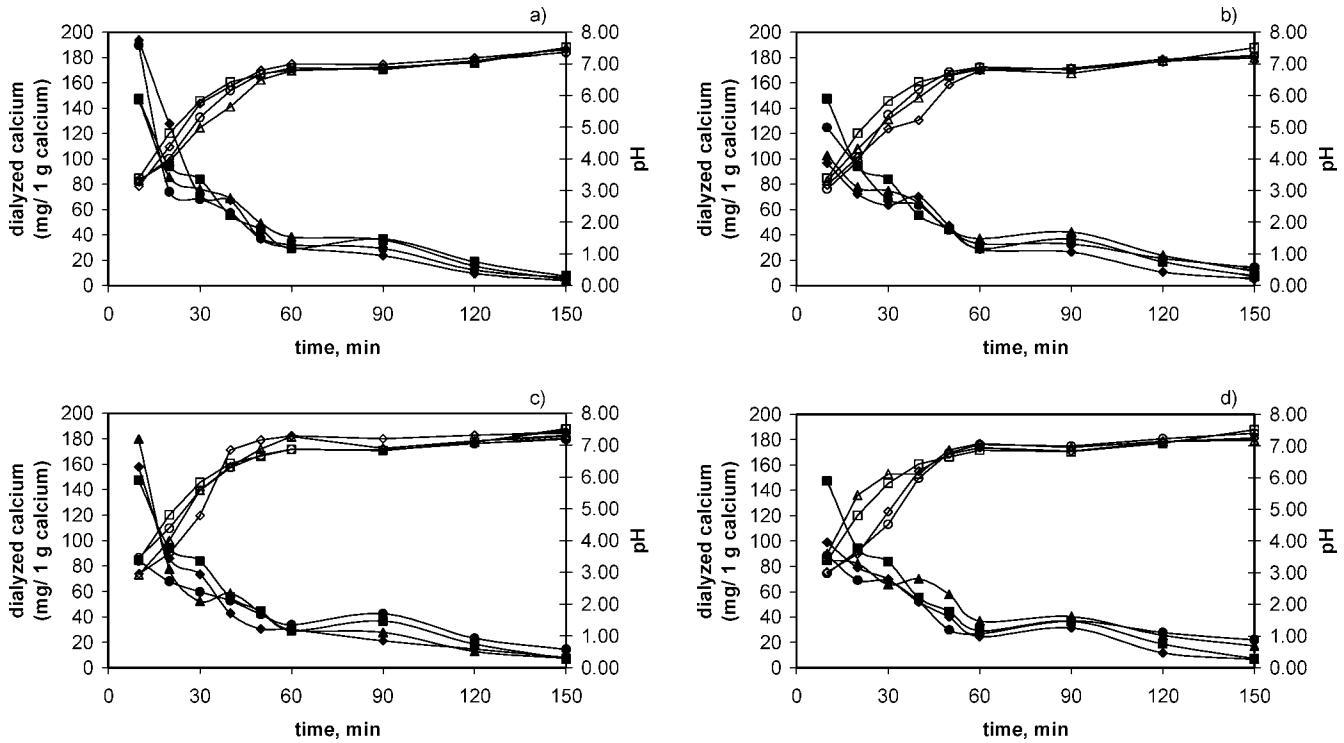


**Figure 6.** Dialyzed calcium and pH change during simulated intestinal digestion for Chinese kale at varying concentrations of ascorbic acid (**a**), citric acid (**b**), malic acid (**c**), and tartaric acid (**d**), where ■, ◆, ▲, and ● represent Chinese kale with 0%, 1.0%, 2.5%, and 5% (w/w) acid, respectively. The corresponding pH profiles are presented with open symbols of the same type.

the dialysis profiles (**Figure 3**), the enhancement effect was most pronounced when citric and tartaric acids of 5% were added.

Figure 4 illustrates the effect of the percentage of organic acid added on calcium availability, as calculated from the summation of dialyzed calcium in all dialysate fractions, for the amaranth sample. The effect on calcium dialyzability in a decreasing order is as follows: citric acid > tartaric acid > malic acid > ascorbic acid. This appears to be the same order as that of the first stability constants (log $K_1$) of calcium complexation with these organic ligands: citric acid (3.50), tartaric acid (1.80), malic acid (1.80), and ascorbic acid (0.19) (*21*).

From the observation of dialysis profiles, the following can be speculated. Most of the calcium in vegetables is bound with other dietary constituents. During the simulated gastrointestinal digestion, these constituents are broken down, and calcium is released as dialyzable forms. At low pH, the dialyzability is naturally higher than at high pH, owing to the favorable binding of anions with protons, leaving calcium as an ionic soluble form. When the pH increases, binding with various ligands occurs. Oxalate and phytate can bind with calcium to form precipitates which are not dialyzed. However, with the presence of organic acids, the organic acids favorably bind with calcium even at an elevated pH, resulting in the enhancement of calcium dialyzability. To demonstrate the dialysis of calcium at high pH in the presence of the organic acids studied, **Figure 3b** was replotted to obtain **Figure 5** showing the effect of citric acid on the dialyzed calcium at various pH values. This graph illustrates that the change in dialyzability was not solely contributed by the pH change, but also due to the organic acid added. This information cannot be obtained from the equilibrium dialysis approach.

**Effect of Organic Acids on the Calcium In Vitro Availability of Chinese Kale.** The dialysis profiles showing the effect of added organic acids in Chinese kale are presented in **Figure 6**. No enhancement of calcium dialyzability was observed when various organic acids were added at various concentrations. Because of the naturally existing promoters (citric and malic acids) in Chinese kale, most calcium was already present in readily dialyzable forms. The dialysis profiles (**Figure 6**) show continuing dialysis even after pH has reached 7.0. In relation to the high calcium availability of Chinese kale, some authors reported a low concentration of inhibitors (precipitators) such as phytate and oxalate in Chinese kale (*16*). Moreover, high concentrations of enhancers such as citric and malic acids were found as the major organic acids in Chinese kale at 22.13 and 1.51 mg/g, respectively (*22*). Lucarini et al. (*23*) presented *brassica* vegetables (e.g., broccoli, green cabbage, and kale) as a good source of dietary calcium and showed that dialyzable calcium in these vegetables was in both ionic and bound forms with a large fraction in the bound forms. Hence, the added organic acid did not show an observable effect on the calcium dialyzability in Chinese kale.

**Conclusion**. The calcium availability of various vegetables was determined by the in vitro simulated gastrointestinal digestion with continuous-flow dialysis method. The calcium in vitro availability in the vegetables studied was found to vary between 4.6% and 52.9%. The in vitro availability obtained as compared to the reported availability data from the in vivo measurement was very similar. This indicates the possibility of using the in vitro method as a rapid evaluation method for the availability study.

The study of the effect of organic acids on calcium dialysis showed a significant increase of availability at increasing concentrations of acid for amaranth. Citric acid was the most effective enhancer followed by tartaric, malic, and ascorbic acids as could be predicted from their stability constants of complexation with calcium. An enhancement effect was not clearly observed for Chinese kale, which was a representative vegetable of high calcium availability.

The dialysis profiles from continuous-flow dialysis, showing a time-dependent dialyzed amount and pH change, provide information which is not obtained from the equilibrium method. Thus, the enhancement effect of some organic acids on calcium availability was proved to be caused by calcium remaining dialyzable even at elevated pH conditions in the intestinal digestion stage. The dialysis profiles were proved to be useful for understanding the effect of food components on mineral dialyzability in the simulated gastrointestinal digestion model.

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