☒Calcium Phytate:

Effect of pH and Molar Ratio on in vitro Solubility

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ABSTRACT

Calcium and phytate phosphorus were highly soluble below pH 4 at all molar ratios of calcium (Ca) to phytic acid (PA) studied (0.5-12.67). As pH is increased above 4, there is a drop in solubility, the magnitude of which depends on the Ca:PA molar ratio. Above pH 6, the greatest calcium precipitation occurred at molar ratios between 4 and 6.5; both lower and higher Ca:PA molar ratios showed higher calcium solubility. In contrast, phytate phosphorus solubility decreased in proportion to the Ca:PA molar ratio, showing essentially complete precipitation above the Ca:PA ratio of 5. The pentacalcium phytate salt probably predominates when calcium is not limiting. Under calcium-limiting conditions, the complexes resolubilize as the pH is increased above 7.

INTRODUCTION

Phytic acid has been implicated as a major factor affecting mineral bioavailability by complexing minerals and rendering them insoluble, and by general implication of biological unavailability (1). Thus, it is important to know the solubility characteristics of mineral-phytate complexes under the variety of conditions that might be encountered during processing or consumption of the food. One of the first studies on the solubility of mineral-phytate complexes as a function of pH was carried out by Jackman and Black (2). Calcium phytate showed a rapid drop in solubility as the pH was raised above 6, an observation confirmed by Saio et al. (3) and in accordance with that of McKinney et al. (4). Hoff-Jorgensen (5) and Crean and Haisman (6) observed formation of precipitates at even lower pH. These authors, and Evans and Pierce (7), also studied the composition of the precipitated calcium phytate. Recently, Oberleas and Moody (8) studied precipitate formation by calcium phytate complexes using a reaction mixture containing a calcium (Ca) to phytic acid (PA) molar ratio of one. They also demonstrated the synergistic effect of calcium with other minerals. Tangkongchitr et al. (9) reported solubility of phytate phosphorus using a molar ratio of 0.80 at pH 5-9, and Rendelman (10) reported solubility at pH 5.3-6.5 for a 7.8 Ca:PA molar ratio.

This paper reports on a study of solubility characteristics of calcium-phytate complexes over a wide range of pH and Ca:PA molar ratios. Although it is recognized that other components in a food system, such as fiber and protein, may complex calcium or phytic acid and affect their solubility, it would be difficult to ascertain the true mechanism of the interactions of phytic acid and minerals in a system as complex as a real food product due to the multiplicity of simultaneous reactions. Thus, to avoid confounding interpretation of the data, these initial studies were done with pure systems which could serve as a reference for future studies with real systems.

MATERIALS AND METHODS

Sodium phytate (> 98% pure, Sigma Chemical Co., St. Louis, MO) and reagent grade calcium chloride were used. Stock solutions of 73.35 mM or 7.738 mM calcium chloride (depending on the final molar ratio required) and 15.80 mM phytic acid were prepared using deionized water. Reaction mixtures were made by adding 10 mL of the sodium phytate solution to various amounts of $CaCl_2$ solutions to

provide the Ca:PA molar ratio sought in the reaction mixture. Ten mL of 1 M NaCl was added to minimize variations in ionic strength. The pH was then adjusted to the desired values using 1 N NaOH or 1 N HCl. Deionized water was added to bring the total reaction volume to 100 mL, thus resulting in an effective phytic acid concentration of ~ 1.6 $\times$ 10^{-3} M and Ca^{2+} concentration of 8.0 $\times$ 10^{-4} to 2.0 $\times$ 10^{-2} M in the reaction mixture. Ionic strengths of final reaction mixtures were calculated to be 0.11-0.17, which apparently is the range of ionic strengths needed to maintain an activity coefficient for calcium similar to that in the human gut (10). The reaction mixture was flushed with nitrogen and incubated overnight at 30 C. Since no buffers were used to avoid confounding interpretation of the data due to possible interaction of calcium and/or phytic acid with buffer components, the pH would sometimes drop during incubation; the magnitude of change depended on pH and the molar ratio of Ca:PA. The pH then was readjusted to the initial value if necessary. After incubation, the mixtures were centrifuged at 12,000 $\times$ G for 15 min at room temperature, and an aliquot of the supernatant carefully removed for analysis of calcium and phosphorus. A minimum of two replications of this procedure were made for all molar ratios and all pH values. Controls were also run to test for insolubility of $CaCl_2$ and sodium phytate solutions themselves. No precipitates were observed in these controls under our experimental conditions.

Analytical Methods

Total phosphorus was measured by the method of Bartlett (11). Standard curves for phosphorus (P) were run for each batch of samples due to day-to-day variation. Calcium (Ca) was analyzed by atomic absorption and by the calcein/back titration method of Ntailianas and Whitney (12); both methods gave essentially identical results. All assays for P and Ca were done in duplicate for each sample.

Data are plotted in the figures as means of replicate observations. A bracket with a data point represents two standard deviations with at least 2 degrees of freedom (df). No brackets with a point indicates either the bracket is too small to be visible, or that df < 2, and thus no standard deviation was calculated.

RESULTS AND DISCUSSION

The Ca:PA molar ratios studied ranged from 0.5 to 12.67 and the pH range was from 2 to 9; in some cases, up to pH 11 was studied. Further, the solubility characteristics of both phytic acid (in terms of phosphorus) and calcium were measured in this study since the degree of insolubility of phytic acid is not necessarily an indication of the degree of insolubility of calcium and vice versa.

Figures 1 and 2 show typical solubility profiles at two different Ca:PA molar ratios. As shown in these and subsequent figures, calcium and phosphorus show a high degree of solubility below pH 4, regardless of Ca:PA molar ratio. Titration studies (13) indicated that phytic acid showed four major inflection points at pH 4.4, 6.0, 7.0 and 10.0, corresponding to NaOH:PA molar ratios of 6, 7, 8 and 12, respectively. Since six of the strongly dissociated protons in the phytic acid molecule have pK < 3, and two have pK of 5.3 and 6.5, the appearance of insoluble precipitates above
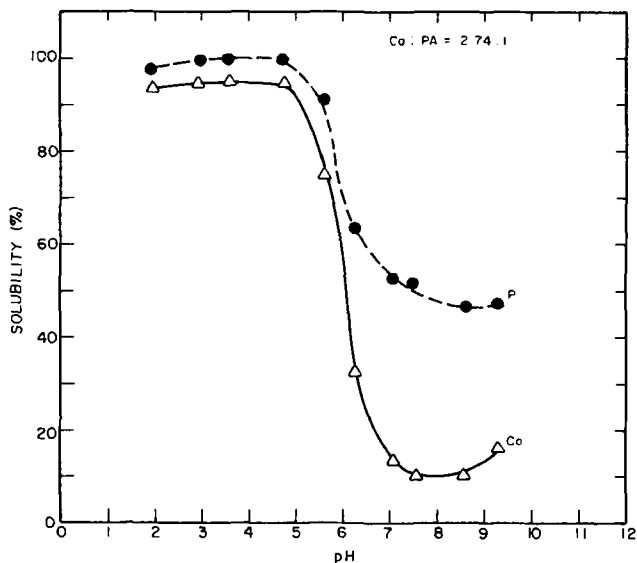
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FIG. 1. Solubility of calcium (Ca) and phytate phosphorus (P) in calcium phytate at Ca:PA molar ratio of 2.74.
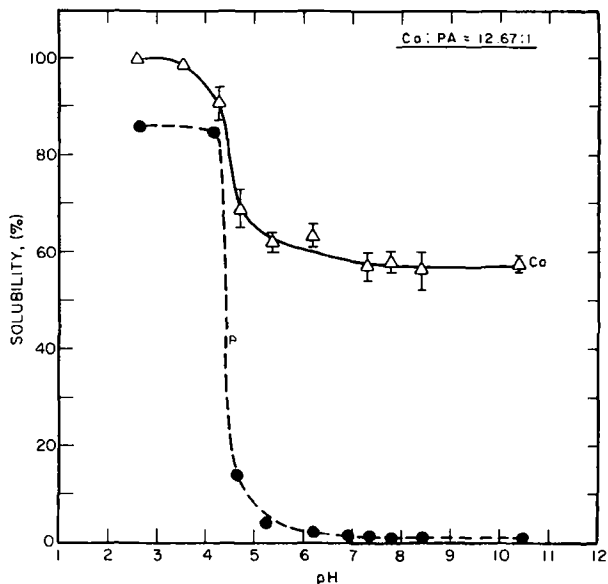

FIG. 2. Solubility of calcium (Ca) and phytate phosphorus (P) in calcium phytate at Ca:PA molar ratio of 12.67. Bracket represents two standard deviations.
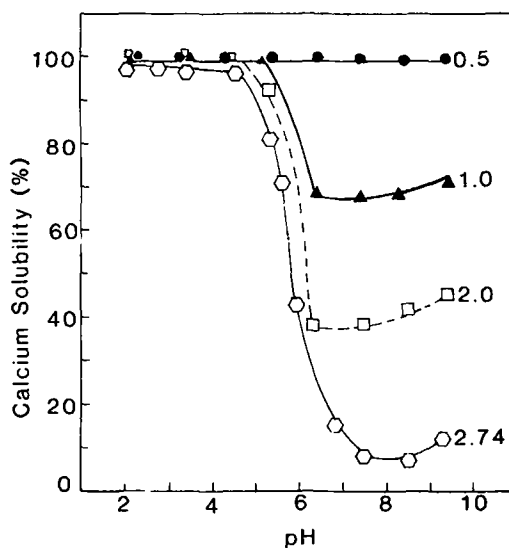

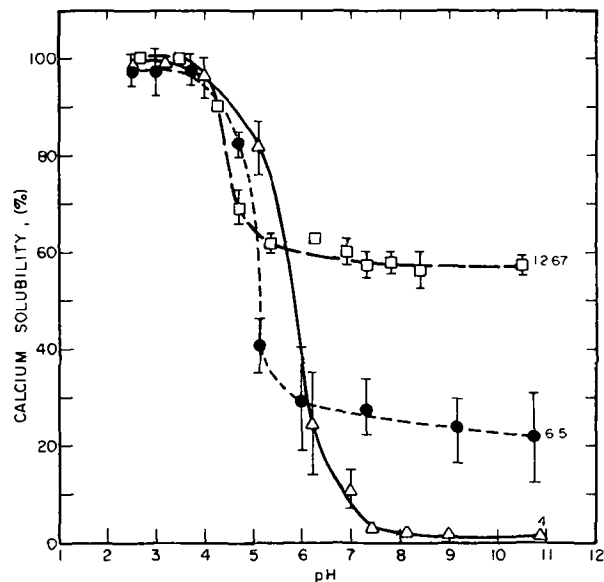FIG. 3. Effect of pH and Ca:PA molar ratio on solubility of calcium at low molar ratios of 0.5-2.74.

FIG. 4. Effect of pH and Ca:PA molar ratio on solubility of calcium at high molar ratios of 4-12.67.
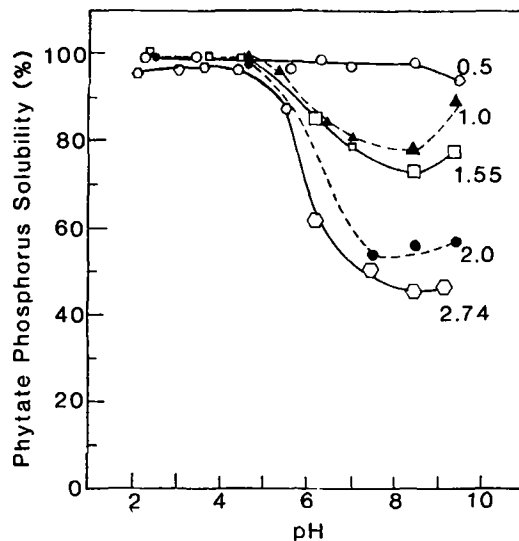

FIG. 5. Effect of pH and Ca:PA molar ratio on solubility of phytate phosphorus at low molar ratios of 0.5-2.74.

pH 4 indicates the appearance of the hexahydrogenated form of phytic acid followed by other negative species. Notice also that at the low Ca:PA molar ratio, phosphorus is more soluble than the calcium (Fig. 1), whereas at high Ca:PA molar ratio, the calcium is more soluble than phosphorus, indicating calcium is in excess (Fig. 2).

Figures 3 and 4 summarize effects of Ca:PA molar ratio on the solubility profile of calcium, and Figures 5 and 6 show effects of Ca:PA molar ratio on the solubility profile of phytate phosphorus. Our data generally show fairly good agreement with the data of Jackman and Black (2), Tangkongchitr et al. (9), Rendleman (10) and Oberleas and Moody (8) over the comparatively narrower ranges of the variables they studied.

At the low molar ratios, very little insoluble complex is formed throughout the pH range. As the molar ratio of Ca:PA increases, the pH region of the sharpest drop in calcium solubility (Fig. 3 and 4) and phosphorus solubility (Figs. 5 and 6) decreases. The phytate solubility phenome-
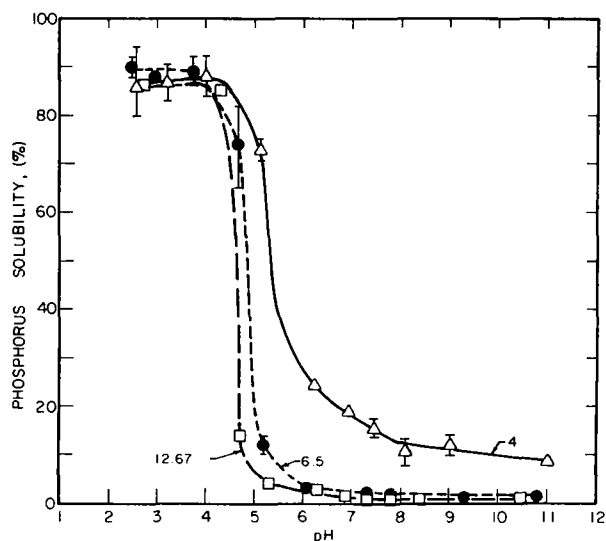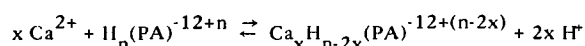
FIG. 6. Effect of pH and Ca:PA molar ratio on solubility of phytate phosphorus at high molar ratios of 4-12.67.

non could be explained by the law of mass action which can be stated as:

$$x\,Ca^{2+} + H_n(PA)^{-12+n} \rightleftharpoons Ca_xH_{n-2x}(PA)^{-12+(n-2x)} + 2x\,H^+$$

Higher Ca concentrations will drive the reaction to the right, competing with the hydrogens for the negative charges in the PA molecule. The displacement of the hydrogen ions will cause a "pH-drop," and also result in precipitation of the complex at a lower pH. In addition, the higher calcium concentration relative to phytic acid will result in greater complex formation and correspondingly relatively greater insolubility of the complex. Low pH, on the other hand, will drive the reaction to the left, resulting in relatively higher solubility of the calcium and the phosphorus.

Phosphorus Solubility

Maximum precipitation ($\sim$ 100%) of phytic acid phosphorus was achieved at pH 8 for the 6.5 and 12.7 molar ratios (Fig. 6) but only 90% precipitated at the 4 molar ratio, and even less precipitated at the lower ratios of 0.5-2.74 (Fig. 5). This is, of course, due to the limiting calcium concentration, i.e., not all possible sites on the PA molecule were saturated with calcium at the lower Ca:PA molar ratios. Other investigators reported generally similar results. Jackman and Black (2) reported the beginning of precipitation occurred only at pH 6 in a Ca:PA molar ratio of 6:1, and Hoff-Jorgensen (5) reported that it began at as low as pH 2.6. The data of Tongkongchitr et al. (9) show a drop in "phytate" solubility starting at pH 5.0 and leveling off to ca. 72% solubility at pH 9 for a Ca:PA molar ratio of 0.8, while Oberleas and Moody (8), using Ca:PA of 1:1, showed a sharp increase in precipitate formation at pH 6-7. The slight differences in results could be due to many factors, among them the source of phytic acid, purity of the reagents, method of separating the insoluble complex (e.g., insoluble phytate exists in such fine colloidal state that normal "G" forces in laboratory centrifuges may not be able to sediment all of the complex, thus leading to high values of "soluble" phytate [1]), the temperature used (higher temperatures decrease solubility of calcium phytate sharply [1]) and the total concentration of the reagents (too high a concentration may exceed the solubility of the reagents). Nevertheless, the general trends in solubility behavior of calcium phytate are clear, with indications that

the optimum molar ratio for maximum phosphorus precipitation is between 4 and 6.5.

Calcium Solubility

In contrast to the phosphorus behavior, maximum precipitation of calcium (ca. 100%) occurred only in the 4:1 molar ratio above pH 8 (Fig. 4). Our data closely match those of Rendleman (10) who observed the solubility of calcium drop from 72% at pH 5.3 to 23% at pH 6.5 at a molar ratio of 7.8. Increasing (Fig. 4) or decreasing (Fig. 3) the molar ratio appeared to result in higher calcium solubility. In the case of the higher calcium concentrations (Fig. 4), the higher solubility occurs because the calcium is in excess of that needed to saturate all available complexing sites. The higher calcium solubility at lower ratios, on the other hand (Fig. 3), has been explained before as being due to insufficient complexation of calcium by phytic acid, i.e., the phytic acid is in excess. It can also be seen by examining Figures 3-6 that, in the pH region of insoluble precipitate formation at all molar ratios of 4 and below, calcium is generally less soluble than phytic phosphorus, whereas at molar ratios of 6.5 and above, calcium is more soluble than phosphorus (also shown in Figs. 1 and 2).

These two sets of observations suggest that calcium would normally combine with PA to form neither the tetranor the hexacalcium salt, but probably the pentasubstituted calcium salt when calcium is not limiting. This was confirmed by an analysis of the precipitate as shown in Figure 7. For the 4:1 Ca:PA molar ratio solution, the precipitate assayed only 2:1 Ca:PA at pH 5, reaching a maximum of 4.6 at pH 7.5 and above. However, in the 6.5 and 12.67 Ca:PA reaction mixtures, the precipitate showed a maximum molar ratio of 5.1 and 5.5 at pH 8.0 and above.

The nonstoichiometric ratios showed by the precipitate could be due to several factors: (a) inaccuracies in the assay procedure, (b) impurities in the reagents, and (c) formation of calcium mixed salts (of various molar ratios). This was also observed by Hoff-Jorgensen (5) outside the
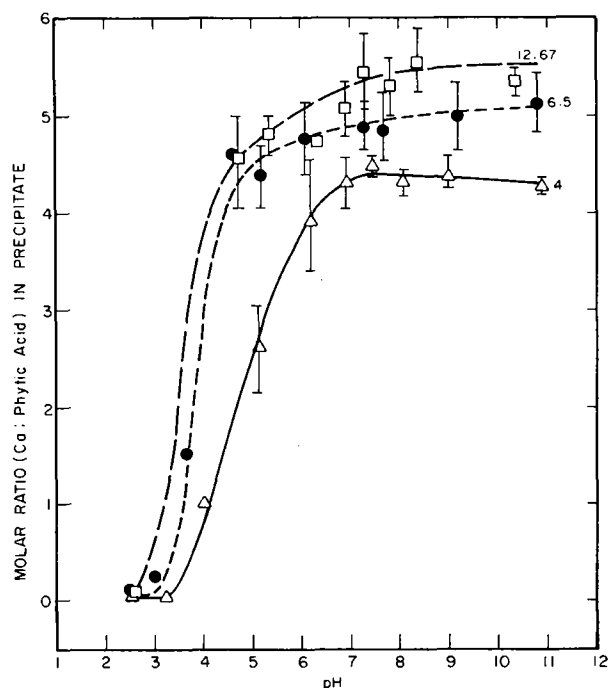

FIG. 7. Effect of pH and Ca:PA molar ratio in the reaction solution on the Ca:PA molar ratio in the precipitate. Bracket represents two standard deviations.

pH range of 4.6-6.9 and by Evans and Pierce (7), the latter group obtaining a molar ratio of 4.96 to 5.08 in the precipitate when using molar ratios of 6 to 24 at pH 5-6 in the reaction mixture. Tangkongchitr et al. (9), on the other hand, observed a precipitate molar ratio of 2.5 when the reaction mixture contained a 0.8 Ca:PA molar ratio at pH 5.5-9.0.

In all probability, when the Ca:PA molar ratio is close to the optimum such as 5-6, the predominant species in the precipitate is the pentacalcium phytate salt as suggested by Evans and Pierce (7) and Hoff-Jorgensen (5). However, when the calcium is far in excess, it is possible that the excess calcium could displace the remaining hydrogens on the phytic acid molecule to produce the fully dissociated phytate anion (charge^{-12}), resulting in the formation of some hexacalcium salt. The mixed salts of penta- and hexacalcium phytate would result in Ca:PA molar ratios greater than 5 in the precipitate. (The coprecipitation of calcium hydroxide along with the phytate salt occurs only at very high pH and thus would not significantly affect our data.)

One interesting feature of the data observed at the low molar ratios (Figs. 3 and 5) is the minima in calcium and phosphorus solubility, i.e., the insoluble complex is apparently resolubilizing as the pH is increased above 7. This phenomenon can also be observed in the data of Oberleas and Moody (8); for a Ca:PA molar ratio of 1.0 the maximum amount of "precipitates" was formed at pH 7, and increasing the pH above 7 decreased the amount of precipitate formed. In our case, no resolubilization was observed at higher molar ratios, even when the pH was increased to pH 11 (Figs. 4 and 6).

This resolubilization phenomenon is probably related to the degree of ionization of the calcium-phytate complex. At lower Ca:PA molar ratios, it is probable that a major portion of the complex is in the form of the lower calcium-substituted phytates (mono-, di-, tricalcium phytates), i.e., they contain potentially ionizable hydrogens in their structure. As the pH is increased, the proportion of ionized hydrogens on these low-calcium phytate molecules increases, thus increasing the effective solubility of these complexes. The higher the Ca:PA molar ratio, i.e., the more saturated the PA molecule, the less the degree of ionization of the hydrogens at higher pH. This results in less resolubilization of the complex, as shown by our data in Figures 4 and 6, where even a pH of 11 did not resolubilize the high molar ratio complexes.

REFERENCES

1. Cheryan, M., Crit. Rev. Food Sci. Nutr. 13:297 (1980).
2. Jackman, R.H., and C.A. Black, Soil Sci. 72:179 (1951).
3. Saio, K., E. Koyama and T. Watanabe, Agric. Biol. Chem. 31:1195 (1967).
4. McKinney, L.L., W.F. Sollars and E.A. Setzkorn, J. Biol. Chem. 178:117 (1949).
5. Hoff-Jorgensen, E., Det. Kgl. Dan. Vid Sel. Mat.-Fys. Medd. 21(7):1 (1944).
6. Crean, D.E.C., and D.R. Haisman, J. Sci. Food Agric. 14:823 (1963).
7. Evans, W.J., and A.G. Pierce, JAOCS 58:850 (1981).
8. Oberleas, D., and N. Moody, in Trace Element Metabolism in Man and Animals, edited by J.M. Howell, J.M. Gawthorne and C.L. White, Aust. Acad. Sci., Canberra, Australia, (1981), p. 129.
9. Tangkongchitr, U., P.A. Seib and R.C. Hoseney, Cereal Chem. 59:216 (1982).
10. Rendleman, J.A., Cereal Chem. 59:302 (1982).
11. Bartlett, G.R., J. Biol. Chem. 234:466 (1959).
12. Ntailianas, H.A., and R.McL. Whitney, J. Dairy Sci. 47:19 (1964).
13. Grynspan, F., M.S. thesis, University of Illinois, Urbana, IL, (1982).