Titration Studies of Phytic Acid

W.J. EVANS, E.J. McCOURTNEY and **R.I. SHRAGER**, Southern Regional Research Center, PO Box 19687, New Orleans, LA 70179

ABSTRACT

The 12 pK_a values for phytic acid have been obtained from pH titration data and nonlinear regression analysis. The pK_a values ranged from 1.9 to 9.5. The results are discussed in terms of previously determined pK_a values. Possible reasons are given for the differences between previously determined values and present values.

INTRODUCTION

Oilseeds have high levels of phytic acid and, although various structural formulas for phytic acid have been proposed, the 2 which have attracted the most attention are those of Anderson and Neuberg (1,2). The Anderson structure implies 12 titratable hydrogen ions, the Neuberg 18. Current evidence based on ^{31}P nuclear magnetic resonance (NMR) data and X-ray crystallography (3-5) seem to leave little doubt that the structure proposed by Anderson is the proper choice.

Although there is some question as to whether 5 of the phosphates lie in an axial or in an equatorial plane, in either case, the structure suggests tremendous chelating potential. Thus, phytate binds to various metal ions, forming insoluble complexes in the physiological range of pH. This results in an impairment of the absorption of these metal ions. Erdman (6) has recently summarized the nutritional implications of phytates.

We are currently studying the binding of cations to phytic acid. In several aspects of this work, it is necessary to know the various ionization constants of phytic acid. Because earlier investigations (4,7,8) indicate that the pK_a values of phytic acid are probably sensitive to such factors as the nature of the counter cation used for titrations and the ionic strength, it seemed necessary to determine the pK_a values under the same conditions that are being used in the study of the metal-phytate complexes.

MATERIALS AND METHODS

Phytic acid was prepared by ion exchange procedures as previously described (9). Stock solutions, 0.01 M, of both phytic acid and potassium phytate were prepared by appropriately diluting phytic acid solutions with deionized, distilled water and potassium hydroxide. The concentrations of the phytic acid and potassium phytate were determined by measuring the phosphorus content (10).

The titration experiments were done in the following manner. Ten mL of either 0.01 M phytic acid or potassium phytate were placed into a 100-mL volumetric flask along with 5 mL of 4 M KCl and varying amounts of either 0.1 N KOH or 0.1 N HCl. The flask was then filled to the mark with deionized, distilled water and ca. 30 mL of each of these solutions was taken for the pH measurements. All pH measurements were taken with a Radiometer PHM 64 research pH meter with GK2401C electrode. The temperature of all measurements was at 25.0 ± 0.005 C.

RESULTS AND DISCUSSION

Figure 1 shows a plot of the data obtained from the titration experiments. The pH values represent the average of 3 readings differing by ± 0.01 pH unit at most. The term $\bar{n}$ represents the average number of hydrogen ions bound at the pH in question. This quantity is calculated from:

$$\bar{n} = \frac{C_{HCl} - (H^+) + (OH^-)}{C_S}, \qquad [I]$$

where C_S is the concentration of potassium phytate and (OH^-) was calculated from $pK_{W_c} = 13.75$ in 0.2 M KCl (11). In the instance where phytic acid was titrated, the term $\bar{h}$ representing the average number of titratable hydrogen atoms ionized, is applicable. This quantity is calculated from:

$$\bar{h} = \frac{C_{K^+} + (H^+) - (OH^-)}{C_A}, \qquad [II]$$

where C_A is the concentration of phytic acid. In Equation II, C_{K^+} is equal to the concentration of KOH added and does not inlcude the contribution of K^+ from the KCl. It follows that if N is equal to the total number of titratable hydrogen atoms, then:

$$N - \bar{n} = \bar{h}. \qquad [III]$$

Analysis of the titration data was done as follows. The general n-site Adair-type binding model is:

$$\bar{n} = [\sum_{i=1}^{N} i\, b_i\, h^i] / [1 + \sum_{i=1}^{N} b_i\, h^i], \qquad [IV]$$

where b_i is derived by curve-fitting, and h is the hydrogen ion concentration. If the binding process were simply random, with no site-site interaction, the b_i would be products of site-specific association constants. As it is, the b_i are best viewed as parameters to be found and used in Equation V below.

The single rational expression IV can be regarded as a sum of simpler rational terms as in Table I. The validity of such a sum can be verified by noting that algebraic addition of the terms in Table I yields a result in the form of Equation IV. It is to be further noted that any sum of rationals

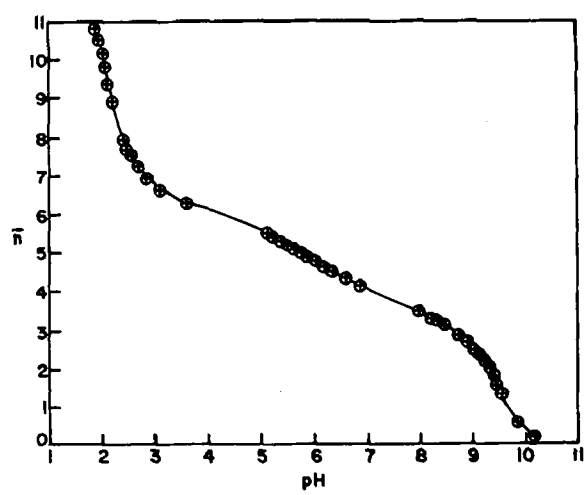

FIG. 1. Comparison of experimental (0) and theoretical (+) points in titration of phytic acid or potassium phytate. The term $\bar{n}$ represents the average number of hydrogen ions bound at the pH in question. Because experimental and theoretical values coincide, data points appear as circled (+).

of the type in Table I will do likewise provided the n_i sum to 12. After exploring various such forms, it became evident that the form in Table I was a simple, adequate description of the data. For this phase of the work, the MLAB computerized, mathematical modeling program was used (12). Curve fitting in this program is accomplished by a constrained Levenberg-Marquardt procedure (13). The program also provides asymptotic standard errors as given in Table I.

TABLE I

Nernstian Formula, $\bar{n} = \sum_{i=1}^{8} \frac{n_i(a_i h)^{n_i}}{1+(a_i h)^{n_i}}$, with Corresponding a_i and n_i Used to Simulate the Observed Saturation Curve

i	a_i	$\log(a_i)$[a]	n_i[b]
1	3.39343E9	9.53 ± 0.02[c]	2
2	1.55452E9	9.19 ± 0.07	1
3	9.48045E7	7.98 ± 0.06	1
4	1.77105E6	6.25 ± 0.04	1
5	1.58674E5	5.20 ± 0.05	1
6	1.44858E3	3.16 ± 0.06	1
7	2.38832E2	2.38 ± 0.01	2
8	8.33579E1	1.92 ± 0.01	3

[a]Log a_i indicates the central pH value at which the ith binding process occurs, analogous to midpoint redox potential in electrochemistry. For each binding process, n_i is 1; log a_i is the pK_a.

[b]n_i indicates the number of protons bound at the ith stage, analogous to the number of electrons passed in a redox transition due to potentiometric titration.

[c]Asymptotic standard error provided by the curve-fitting computer program.

The simplest way to compute partial saturation terms, that is, the fraction of total with i protons bound, is by the formula:

$$f_i(pH) = b_i h^i / [1 + \sum_{j=1}^{12} b_j h^j], \qquad [V]$$

where the denominator in V is the product of all the denominators in Table I. The coefficients b_j in V proved too large to express in the DEC-10 computer floating point number system, so V was reexpressed as shown in Table II to avoid various overflow problems. Manipulations to derive the numbers in Table II from those in Table I were done on the REDUCE symbolic processing system (14). The results were checked by comparing the curve:

$$\bar{n} = [\sum_{i=1}^{12} i\, c_i\, Z^{i-6}] / [Z^{-6} + \sum_{i=1}^{12} c_i\, Z^{i-6}], \qquad [VI]$$

to the curve in Table I. The 2 curves are identical within round-off error. (The c and z in Eq. VI are from Table II.) A comparison of the experimental and theoretical points are shown in Figure 1. The model and the data agreed well enough so that the + signs and circles appear to graphically coincide, although there are errors. Thus, the logarithms of the a_i in Table I represent the 12 individual pK_a of phytic acid according to the present titration study.

Insofar as we have been able to determine, there have been 2 previous potentiometric titration studies of phytic acid wherein pK_a values were reported. The first of these was the investigation of Hoff-Jørgensen (7), the other that of Barré et al. (8). Our data are perhaps more directly comparable to those of Hoff-Jørgensen. Thus, Hoff-Jørgensen, in one case, titrated solutions of sodium phytate in 0.2 M NaCl. Final pK_a values, ranging from 2.20 to 9.83, were computed from a set of preliminary values by successive approximation. It is important to note that these values represent only 8 of the possible twelve ionization constants. Hoff-Jørgensen was unable to determine the 4 most acidic pK_a values. A knowledge of these acidic pK_a values becomes important in low pH studies of phytic acid and in its combination with certain metal ions such as the ferric ion.

TABLE II

Computation of Fractions of Partially Protonated Species

$$z = 10^6 \cdot h = 10^{6-pH}[a]$$

$$f_i(pH) = c_i z^{i-6} / [z^{-6} + \sum_{j=1}^{12} c_j z^{j-6}][b]$$

i	c_i[c,d]
1	1.651256E + 03
2	1.166593E + 07
3	1.901510E + 10
4	1.733764E + 12
5	3.282750E + 12
6	4.816653E + 11
7	6.910346E + 08
8	2.747643E + 04
9	3.968542E + 01
10	4.002581E − 04
11	1.591368E − 08
12	2.282482E − 11

[a]Z is a scaled (H^+) to keep all numbers within computable bounds.

[b]$f_i(pH)$ is the fraction of total phytic acid with i protons bound.

[c]C_i are parameters used for the computation of $f_i(pH)$. They were computed from Equation V and checked as indicated by Equation VI.

[d]The precision of these numbers are shown as they were generated by the computer. The number of significant figures is not intended to represent experimental accuracy.

Although Hoff-Jørgensen used a standard procedure for the preparation of crystalline sodium phytate, a possible reason as to why he was unable to titrate the 4 most acidic protons may have been due to a slight impurity. For example, the crystalline sodium phytate used in our investigation was stated by the supplier as 98% pure. Analysis by an independent commercial laboratory indicated the proper sodium:phosphorus ratio. However, attempts to use this material as received resulted in a number of instances in which pH measurements in the most acid region gave anomalous $\bar{n}$ values. More important, the $\bar{n}$ and $\bar{h}$ values, calculated from the titrations of the sodium phytate as received and the phytic prepared from it, did not follow the relation given in Equation III. From these observations, it would appear that the impurity which initially influenced our results was removed by ion exchange.

Barré et al. (8) titrated solutions of sodium phytate and phytic acid. The acid was prepared from the sodium phytate by ion exchange. They analyzed their data in terms of the Henderson equation and interpreted it as 6 strongly ionized protons with pK of 1.84, 2 weakly ionized protons with pK of 6.3, and 4 very weakly ionized protons with pK of 9.7. Analysis of our data in a similar manner gave 6 functions with pK_a values of 2.18, 2 with pK_a values of 5.73, and 4 with pK_a values of 9.21. However, the error curve of our data by this method of analysis was not random.

The differences observed between our data and those of Barré et al. could possibly be due to differences in starting materials used and the absence of supporting electrolyte in their titrations. Regarding the latter factor, in preliminary titrations of phytic acid without the added KCl, we obtained significantly different $\bar{h}$ vs pH values as compared to those obtained in the presence of KCl.

Costello et al. (4) have recently determined the 12 separate pK_a values of phytic acid by means of ^{31}P NMR spectroscopy. These determinations were done with tetrabutylammonium cation. They reported pK_a values ranging from 1.1 to 12. The differences between their data and those of the present investigation can be attributed to their use of the tetrabutylammonium cation. Thus, it is to be expected that the large tetrabutylammonium cation would bind to the phytate anion more weakly than a cation such as K^+. This weaker binding would undoubtedly be reflected in the pK_a values.

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Preparation of Low-Phytate Rapeseed Protein by Ultrafiltration: I. The Aqueous Extraction of Phytate from Deoiled Rapeseed Meals

R.D. SIY and **F.D.F. TALBOT**, Department of Chemical Engineering, University of Ottawa, Ottawa, Ontario, Canada K1N 9B4

ABSTRACT

The extraction of phytate and nitrogen- and phosphorus-containing compounds from Tower and Candle variety rapeseed meal and flour using aq sodium chloride solution has been investigated. Single-stage extraction experiments were made varying the v/n ratio from 10 to 500 mL of extract solution per g of sample and using an extract solution containing 0-10% (w/v) sodium chloride. The pH of the extract solution was varied from 3.0 to 10.0. It was found that phytate and phosphorus-containing compounds can be completely extracted whereas the extent of extraction of nitrogen-containing compounds ranged from 27 to 75% and depended on the extent of protein denaturation of the sample. The above observations were found to be independent of the pH of the extract solution. The extraction method is viewed as a first step in the membrane preparation of a low-phytate rapeseed protein.

INTRODUCTION

Rapeseed is an important source of vegetable oil. Associated with the production of rapeseed oil is rapeseed meal. Current interest in developing the meal as a new source of food quality protein is a direct consequence of the fact that it has a well-balanced amino acid composition. However, there are antinutrients associated with the edible protein, i.e., glucosinolates, erucic acid and phytates. Glucosinolates and erucic acid are the antinutritional factors which are themselves physiologically deleterious. Phytates are the antinutritional factors which induce a physiological deficiency, i.e., they affect the availability of minerals in the diet.

The methods proposed for inactivation or removal of glucosinolates from rapeseed proteins are numerous. Most detoxification methods consist of variations of heat inactivation and/or aqueous extraction. The most important development has been the breeding of low glucosinolate-low erucic acid varieties, e.g., Tower variety. However, using detoxified rapeseed protein (i.e., free from glucosinolates) Eklund (1) has shown that phytate has a detrimental effect on zinc metabolism. This conclusion has been reconfirmed (2,3).

Rapeseeds, like other oilseeds, are particularly rich in phytates. Nutritionally, phytate binds strongly with several essential minerals to form insoluble complexes. It is therefore capable of inducing a wide variety of physiological deficiencies, depending on the first limiting element in a specified diet.

The structure and configuration of phytic acid is shown in Figure 1. Phytate, the metallic salt of phytic acid, was first encountered as early as 1872 (4); however, its structure was not established until recently (5). This perhaps explains why no direct analytical method for phytate is available to date, and why so few studies have been published on its separation from plant protein.

The objectives of this project are (a) to determine the most favorable condition for aqueous extraction of phytate from deoiled rapeseed meal, and (b) to develop a suitable porous membrane process to effectively remove the extracted phytate from the dissolved proteins prior to their recovery as protein isolates. This paper deals with the aqueous extraction of phytate from deoiled rapeseed meals, a first step toward the preparation of low-phytate rapeseed protein by membrane separation.

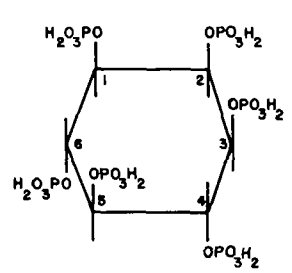

FIG. 1. Structure and configuration of phytic acid, myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate).