content of steeped rice, and of starch at 96% relative humidity; amylose content, blue value, and starch–iodine blue values at 100°C; gelatinization temperature, alkali spreading, and clearing values and lintnerization loss of starch; protein content; and $S_{20,w}$ of amylopectin (Tables I and II).

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# Distribution of Phytate and Nutritionally Important Elements among the Morphological Components of Cereal Grains

Boyd L. O'Dell,* Ana R. de Boland, and Samuel R. Koirtyohann

Kernels of rice, wheat, and corn, a high lysine and a commercial hybrid variety, were dissected into the major components, including germ, endosperm, and pericarp. The whole kernels and fractions were analyzed for total P, phytate P, K, Mg, Ca, Fe, Cu, Zn, and Mn. Phytate phosphorus made up more than 80% of the total phosphorus and, in the case of corn, nearly 90% of phytate was in the germ. The concentration of phytate in rice and wheat germ was high but the major proportion in the total kernel existed in the outer layers. In general the highest concentrations and proportions of the mineral elements of corn were in the germ. There were no significant differences between the high lysine and control corn samples except that the high lysine corn contained a higher concentration of potassium. Although the highest concentrations of some trace elements were found in germ fraction of rice and wheat, the highest proportions were present in the pericarp and aleurone layers.

Cereal grains constitute a major source of human food and animal feeds. Although they contribute most significantly as a source of carbohydrate and to a less extent as protein, their potential contribution of minor nutrients, including the trace elements, is frequently overlooked. The degree and type of milling used in preparation of human food has an important effect upon the concentration of the minor nutrients available to the consumer.

In evaluating the nutritional value of a foodstuff one must consider not only the concentration of a particular nutrient but also its biological availability to the animal that consumes it. Inositol hexaphosphate, phytate, strongly binds several mineral elements and from the nutritional point of view is deleterious in that it renders zinc unavailable (O'Dell, 1969). There is also evidence that it decreases the availability of iron (Sharpe *et al.*, 1950), magnesium (Roberts and Yudkin, 1960), and calcium (Harrison and Mellanby, 1939). Phytate is found almost exclusively in plants and primarily in seeds. Cereal grains and oil seeds are particularly rich sources of phytate. For these reasons it is important to know the distribution of phytate and the nutritionally important mineral elements in plant seeds that serve as sources of human and animal food.

The purpose of this study was to determine the distribution of nutritionally significant substances among the dissectable components of the economically important cereal grains. Because of its potential significance in human nutrition, high lysine corn was compared to a commercially available hybrid variety of corn grown at the same location and during the same season.

Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201.

## MATERIALS AND METHODS

**Source and Dissection of Seeds.** The corn samples were produced by the Pioneer Hi-Bred Corn Co. near Johnston, Iowa. The high lysine corn contained 10.2% crude protein and 0.44% lysine, air dry basis; the commercial hybrid, which served as the control, contained 8.1% protein and 0.30% lysine. The wheat was a soft (Arthur) variety and was produced locally by the Agricultural Experiment Station. The

Table I. Analysis of Control Corn Kernels[a]

| Fraction, % Element | Whole 100, Concentration | Germ 12 | | Endosperm 82 | | Hull 6 | |
|---|---|---|---|---|---|---|---|
| | | Concentration | Distribution,[b] % | Concentration | Distribution, % | Concentration | Distribution, % |
| Total P, % | 0.30(2)[c] | 2.04(10) ± 0.13[d] | 80.0 | 0.05(4) ± 0.01 | 13.3 | 0.13(2) | 2.3 |
| Phytate P, % | 0.25(4) ± 0.03 | 1.80(4) ± 0.20 | 88.0 | 0.01(4) ± 0.003 | 3.20 | 0.02(3) ± 0.004 | 0.4 |
| Zn, ppm | 18.8(10) ± 2.7 | 106(6) ± 5.8 | 67.6 | 6.66(6) ± 1.65 | 29.1 | 20.3(3) ± 0.3 | 6.3 |
| Fe, ppm | 20.9(5) ± 1.9 | 145(5) ± 15 | 83.3 | 10.7(6) ± 1.4 | 42.1 | 31.8(4) ± 8.6 | 8.8 |
| Mn, ppm | 5.20 ± 0.40 | 34.6(5) ± 3.1 | 80.0 | 2.25(6) ± 0.40 | 35.6 | 15.8(4) ± 2.3 | 17.6 |
| Cu, ppm | 1.50(4) ± 0.18 | 7.28(5) ± 1.60 | 58.0 | 0.87(6) ± 0.31 | 48.0 | 7.25(2) | 28.0 |
| Ca, ppm | 44.0(4) ± 3.1 | 109(6) ± 17 | 25.0 | 29.5(6) ± 1.9 | 50.0 | 582(3) ± 110 | 75.0 |
| Mg, % | 0.11(3) ± 0.05 | 0.84(6) ± 0.01 | 90.0 | 0.02(6) ± 0.002 | 14.5 | 0.08(2) | 4.5 |
| K, % | 0.35(2) | 1.69(4) ± 0.06 | 71.4 | 0.07(4) ± 0.002 | 16.3 | 0.25(2) | 4.3 |

[a] Analysis based on air dry weight. [b] Percentage of the element in the component part. [c] Number of analyses. [d] Mean plus or minus standard deviation.

Table II. Analysis of High Lysine Corn Kernels[a]

| Fraction, % Element | Whole 100, Concentration | Germ 15 | | Endosperm 79 | | Hull 6 | |
|---|---|---|---|---|---|---|---|
| | | Concentration | Distribution,[b] % | Concentration | Distribution, % | Concentration | Distribution, % |
| Total P, % | 0.31(2)[c] | 1.78(8) ± 0.63[d] | 87.1 | 0.03(4) ± 0.005 | 6.5 | 0.10(2) | 3.2 |
| Phytate P, % | 0.27(4) ± 0.03 | 1.61(4) ± 0.15 | 88.9 | 0.01(4) ± 0.001 | 3.0 | 0.07(3) ± 0.002 | 1.5 |
| Zn, ppm | 21.5(5) ± 0.99 | 93.6(6) ± 3.8 | 65.1 | 8.57(6) ± 2.12 | 31.4 | 33.5(2) | 9.4 |
| Fe, ppm | 2.22(6) ± 0.7 | 115(6) ± 8.5 | 77.6 | 7.96(5) ± 1.26 | 28.2 | 49.0(3) ± 1.4 | 13.0 |
| Mn, ppm | 6.78(6) ± 0.59 | 25.2(6) ± 1.9 | 56.0 | 2.24(6) ± 0.28 | 26.1 | 18.0(4) ± 0.22 | 16.1 |
| Cu, ppm | 1.50(4) ± 0.36 | 5.62(4) ± 0.88 | 56.0 | 0.69(4) ± 0.16 | 36.0 | 8.40(4) ± 3.20 | 34.0 |
| Ca, ppm | 52.0(8) ± 12.0 | 82.1(6) ± 12.3 | 20.0 | 30.9(6) ± 5.9 | 40.0 | 466(3) ± 14 | 60.0 |
| Mg, % | 0.12(2) | 0.74(6) ± 0.02 | 91.7 | 0.01(4) ± 0.001 | 6.7 | 0.05(4) ± 0.03 | 2.5 |
| K, % | 0.49(2) | 1.97(4) ± 0.10 | 65.3 | 0.11(4) ± 0.01 | 18.4 | 0.42(2) | 6.1 |

[a] Analysis based on air dry weight. [b] Percentage of the element in the component part. [c] Number of analyses. [d] Mean plus or minus standard deviation.

rice was a long grain brown rice supplied by the Arkansas Rice Growers Cooperative, Stuttgart, Arkansas.

Dissection was preceded by soaking the seeds in distilled water overnight in a refrigerator. The swollen seeds were frozen and aliquots removed for dissection as time permitted. Immediately after dissection the components were dried *in vacuo* and then allowed to reach equilibrium with the air before analysis. Stainless scalpels and tweezers were used for separating the components as completely as possible, but the physical separation was imperfect. The corn kernels were partitioned into hull, germ, and endosperm; wheat into hull (beeswing), germ, aleurone, and endosperm; rice into germ, endosperm, and pericarp (including aleurone). The morphology of the cereal grains as summarized by Matz (1969) was used as a guideline for the dissection.

**Analytical Methods.** Since only small quantities of some components, such as germ, were available, semimicro methods were utilized for analysis of these components. A sample, ranging from 5 to 25 mg, was wet-ashed in a test tube using 0.5 ml of distilled $HNO_3$ and two drops of $HClO_4$. The test tubes were placed in a graphite block which was heated on a hot plate. The temperature was raised slowly to about 140°C and most of the nitric acid allowed to evaporate. The heat was then increased until fumes of perchloric acid appeared (about 200°C) to complete the digestion. The ash was heated with water to assure complete dissolution and diluted to the calibration mark on the test tube (2 or 5 ml, depending on sample size).

Calcium and magnesium were determined on a dilution of the original solution using a Perkin-Elmer Model 303 atomic absorption spectrophotometer. Lanthanum was added (1000 ppm) to prevent interference from phosphorus. Potassium was determined on the same solution by use of flame emission photometry. Zinc, iron, manganese, and copper were determined on the original solution by use of atomic absorption spectrophotometry. Automatic background compensation (Perkin-Elmer Model 303-0295) was used for the copper determinations. In cases where the sample size limitation was extreme, copper was determined by a standard addition, atomic absorption method using an electrically heated graphite furnace (Perkin-Elmer HGA-70).

Total phosphorus was determined after wet-ashing of the total sample; phytate phosphorus was determined by wet-ashing ($HNO_3$ and $H_2SO_4$) the ferric chloride precipitated fraction of an acid extract (1.2% HCl containing 10% sodium sulfate) of the samples (Early and DeTurk, 1944). The final determination was by the AOAC (1965) colorimetric method.

At least two different samples of each cereal were dissected at different times and all samples were analyzed at least in duplicate. All values were averaged and the standard deviation calculated.

## RESULTS AND DISCUSSION

Analyses of the control corn are summarized in Table I. The concentrations of elements in the whole kernel and its components are presented as well as their distribution among the fractions calculated as a percentage of that present in the whole kernel.

Phytate phosphorus constituted more than 80% of the total phosphorus, and corn germ contained 88% of the phytate phosphorus in the kernel. A similar observation that most (85%) of the phytate of the corn kernel exists in the germ was made by Hamilton *et al.* (1951). It is significant that a large proportion (68%) of the zinc, an element strongly bound by phytate, is also present in the germ. In fact, except for calcium, which is present in an insignificant concentration, all of

Table III. Analysis of Wheat Kernels[a]

| Fraction, % Element | Whole 100, Concentration | Germ 3.5 Concentration | Germ 3.5 Distribution,[b] % | Endosperm 70.5 Concentration | Endosperm 70.5 Distribution, % | Aleurone 23 Concentration | Aleurone 23 Distribution, % | Hull 3 Concentration | Hull 3 Distribution, % |
|---|---|---|---|---|---|---|---|---|---|
| Total P, % | 0.42(4)[c] ± 0.01 | 1.66(5) ± 0.04[d] | 14.3 | 0.11(5) ± 006 | 19.0 | 1.39(2) | 76.2 | 0.08(5) ± 0.02 | 0.7 |
| Phytate P, % | 0.32(4) ± 0.01 | 1.10(4) ± 0.06 | 12.9 | 0.001(3) ± 0.0003 | 2.2 | 1.16(3) ± 0.02 | 87.1 | 0 (3) | 0 |
| Zn, ppm | 40.4(4) ± 3.7 | 222(5) ± 15.6 | 19.3 | 14.1(7) ± 3.0 | 24.6 | 119(4) ± 11.4 | 68.2 | 88.7(5) ± 11.5 | 7.0 |
| Fe, ppm | 54.6(4) ± 3.6 | 235(4) ± 12.8 | 15.1 | 21.5(4) ± 2.5 | 27.6 | 186(4) ± 9.4 | 78.6 | 110(4) ± 15.6 | 6.4 |
| Mn, ppm | 56.4(4) ± 2.4 | 402(5) ± 13.4 | 25.0 | 8.80(5) ± 1.54 | 10.9 | 130(4) ± 7.9 | 53.2 | 182(5) ± 9.2 | 10.3 |
| Cu, ppm | 4.25(4) ± 0.26 | 18.5(2) | 15.2 | 2.80(4) ± 0.58 | 45.8 | 12.4(4) ± 0.8 | 67.3 | 22.6(4) ± 1.34 | 16.9 |
| Ca, ppm | 335(4) ± 14 | 1760(5) ± 45 | 20.0 | 173(6) ± 38 | 46.7 | 730(4) ± 40 | 53.3 | 2570(5) ± 1061 | 26.7 |
| Mg, % | 0.15(4) ± 0.003 | 0.54(5) ± 0.02 | 13.3 | 0.02(5) ± 0.003 | 9.3 | 0.58(4) ± 0.03 | 86.7 | 0.13(5) ± 0.02 | 2.0 |
| K, % | 0.37(5) ± 0.005 | 0.91(5) ± 0.05 | 8.1 | 0.12(5) ± 0.003 | 21.6 | 1.10(4) ± 0.14 | 67.6 | 0.24(5) ± 0.07 | 2.2 |

[a] Analysis based on air dry weight. [b] Percentage of the element in the component part. [c] Number of analyses. [d] Mean plus or minus standard deviation.

Table IV. Analysis of Rice Kernels[a]

| Fraction, % Element | Whole 100, Concentration | Germ 2 Concentration | Germ 2 Distribution,[b] % | Endosperm 77 Concentration | Endosperm 77 Distribution, % | Pericarp 21 Concentration | Pericarp 21 Distribution, % |
|---|---|---|---|---|---|---|---|
| Total P, % | 0.31(4)[c] ± 0.008 | 1.30(4) ± 0.23[d] | 8.1 | 0.11(4) ± 0.005 | 27.4 | 1.04(4) ± 0.15 | 69.8 |
| Phytate P, % | 0.25(4) ± 0.003 | 0.98(3) ± 0.01 | 7.6 | 0.004(3) ± 0.001 | 1.20 | 0.95(2) | 80.0 |
| Zn, ppm | 19.5(4) ± 1.3 | 147(4) ± 8.2 | 14.3 | 10.6(4) ± 2.1 | 42.1 | 56.7(4) ± 6.8 | 60.0 |
| Fe, ppm | 24.3(4) ± 0.25 | 321(6) ± 9.7 | 14.4 | 13.0(3) ± 1.9 | 41.6 | 51.2(3) ± 5.8 | 43.2 |
| Mn, ppm | 42.4(4) ± 2.0 | 110(4) ± 8.4 | 8.6 | 6.80(4) ± 1.01 | 12.5 | 139(4) ± 12 | 67.6 |
| Cu, ppm | 4.8(4) ± 0.3 | 18.5(2) | 7.3 | 5.33(4) ± 0.47 | 85.8 | 10.3(2) | 44.2 |
| Ca, ppm | 154(4) ± 34 | 1144(6) ± 264 | 10.0 | 41.0(4) ± 12.0 | 7.5 | 721(3) ± 121 | 70.0 |
| Mg, % | 0.12(4) ± 0.007 | 0.56(4) ± 0.07 | 8.3 | 0.02(4) ± 0.001 | 12.5 | 0.51(4) ± 0.08 | 91.2 |
| K, % | 0.20(4) ± 0.003 | 0.61(4) ± 0.04 | 5.5 | 0.08(4) ± 0.01 | 30.0 | 0.45(3) ± 0.02 | 45.0 |

[a] Analysis based on air dry weight. [b] Percentage of the element in the component part. [c] Number of analyses. [d] Mean plus or minus standard deviation.

the cations determined were concentrated in the germ, *i.e.*, 60% or more. The unusually high recovery of calcium in the hull fraction may be due at least in part to contamination of the grain during harvesting and processing, in view of the occurrence of high concentration of calcium in the environment.

Grossly, high lysine corn differed from the control in having a higher proportion of germ, 15 *vs.* 12% (Table II). The concentration of elements in the total kernel did not differ significantly from that in the control corn. However, the concentrations in the fractions were such that the distribution of elements among the fractions was not appreciably different from the control corn except for manganese. As for the whole kernel, high lysine corn contained a higher percentage of potassium than the control corn but the germ contained a slightly lower proportion of the total potassium.

The wheat kernels contained a higher concentration of zinc, iron, maganese, copper, and calcium than the corn (Table III). The germ made up a relatively small portion (3.5%) of the kernel and, although the concentrations of elements were generally higher than in corn germ, the proportions of the total present in wheat germ were much less, 10–20%. The concentrations of zinc, iron, and manganese were extremely high in wheat germ. Nevertheless the major proportion (50–80%) of the elements in wheat was found in the aleurone (bran and middlings fraction). This fraction made up 23% of the total and the concentrations were higher than those of the total kernel. It is significant that 87% of the phytate phosphorus was also found in this fraction. The calculated recoveries of iron and copper were greater than 100% in this and other analyses. This may be explained in part by contamination during dissection and processing and, in the case of copper, by analytical errors resulting from the extremely low concentration and small sample size.

A summary of the rice analyses is presented in Table IV. Rice differed from wheat in that it contained lower concentrations of phytate, zinc, iron, and potassium concentrations in both the total kernel and, for the most part, in the germ. As in the case of wheat, the major proportions of all elements were found in the outer layers, which included the pericarp and aleurone.

There are few reports in the literature that show the distribution of mineral elements in cereal grains, but attention should be drawn to the work of Czerniejewski *et al.* (1964), who determined P, K, Mg, Ca, Zn, Fe, Mn, Cu, Mo, and Co in various wheat blends and in flour milled from these wheats. The flours contained less than 50% and for the most part less than 30% of the minerals present in the original wheat. McCall *et al.* (1953) analyzed eight varieties of rice grown under different conditions. They found that phytate phosphorus made up 40% of the total phosphorus in white rice and 90% of that in rice bran. The bran and white rice were analyzed for K, Ca, Mg, and Na but no trace element analyses were reported.

The results of the present study clearly show that a major proportion of the nutritionally important mineral elements is lost to consumers by milling processes that involve degermination of corn or wheat, or removal of the pericarp and aleurone of rice and wheat. On the other hand these processes remove a large proportion of the phytate, which is deleterious in the utilization of at least some of the trace elements, notably zinc (O'Dell, 1969).

It noteworthy that the cereal grain fractions that are rich in phytate contain relatively little calcium. For this reason the

phytate in corn germ, for example, could not possibly exist there as phytin, $Ca_5Mg$ phytate, as is frequently stated. The chief cations associated with phytate in corn germ are potassium and magnesium. The analyses suggest that the ratios of phytate:Mg:K are approximately 1:3:5. From the point of view of human and livestock nutrition, it appears that high lysine corn is as valuable a source of nutrients, other than lysine, as a commercial hybrid. In fact it contains a higher concentration of potassium, a positive asset. Furthermore, the concentration of phytate was not different.

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# Rapid Method for Determining Aconitic Acid and Its Adaptability for Assaying Aconitate in Plant Extracts

Ralph B. Clark

Color formation in the acetic anhydride–pyridine method for aconitic acid was complete upon the addition of pyridine to the reaction and the absorbance could be measured immediately at 425 nm. Reaction mixtures were prepared and maintained at 0°C. The absorbance curve for aconitic acid concentration was linear to 80 μg. Absorbance losses were insignificant within 20 min after color formation and were 12% after 2 hr. Of the organic acids tested only *trans*- and *cis*-aconitic acids yielded significant absorbance under these conditions and the molar extinction coefficient of *cis*-aconitic acid was two-thirds that of *trans*-aconitic acid. Amino acids and sugars did not interfere with the assay. Crude extracts of plant material could be assayed directly.

Aconitic acid (1,2,3-prop-1-enetricarboxylic acid) accumulates at relatively high concentrations in some plants (Yoder, 1911; Nelson and Mottern, 1931; McCalip and Seibert, 1941; Buch, 1960; Beevers *et al.*, 1966; Stout *et al.*, 1967; Clark, 1969), especially in species from Gramineae and Ranunculaceae (Buch, 1960; Stout *et al.*, 1967). The importance of *cis*-aconitic acid in the tricarboxylic acid cycle is well documented; however, the importance of *trans*-aconitic acid is generally unknown. *Trans*-aconitic acid appears to be closely associated with the mineral status of plants (Coic *et al.*, 1961; Torri and Laties, 1966; Brown, 1968; Clark, 1968; Grunes *et al.*, 1970) and has been suggested as a causative factor to higher incidence of hypomagnesenia (grass tetany) in cattle (Grunes *et al.*, 1970). *Trans*-aconitic acid has also been known to inhibit aconitase (Dickman, 1961). Aconitic acid has been recognized as a complicating factor in the clarification and crystallization of refined sugar and molasses (McCalip and Seibert, 1941; Balch *et al.*, 1946; Ambler and Roberts, 1947). The presence of *trans*-aconitic acid in certain plant species has led scientists to seek more rapid and accurate methods for its determination.

U.S. Department of Agriculture, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

Several methods have been used for the determination of aconitate. Some of the disadvantages and problems involved in many of these methods are discussed by Poe and Barrentine (1968). These authors also described a colorimetric method for the determination of aconitate in sorgo juice (Poe and Barrentine, 1968) and in oats (Poe and Barrentine, 1970). In determining citrate, Spencer and Lowenstein (1967) noted that if the temperature was maintained at 0°C, aconitate yielded color while citrate yielded no color. No further description of the reaction was given. The object of this study was to determine if this difference in behavior of citrate and aconitate at 0°C could be used as a method for measuring the amount of aconitate in plant tissue.

## EXPERIMENTAL

**Materials.** The chemicals used in the procedures were of purified (ACS) grade and the organic acids, sugars, and amino acids were of high purity (obtained from Sigma Chemical Co., St. Louis). Standard solutions of compounds to be assayed were prepared in water and frozen until used for assay. Fully-expanded maize leaves about 3 weeks after emergence were the source of plant material used in this study.

**Methods.** PREPARATION OF PLANT MATERIAL FOR ACONITATE ASSAY. One-gram samples of dried leaf material were