weight less than 30,000 (the cut-off point in the ultrafiltration experiments of Knuckles et al., 1975) since it is absent in the diafiltered retentate. This same impurity is also noncoagulable from neutral or alkaline solutions since it is absent in the heat coagulated protein of Edwards et al. (1975) (see Table II).

Reducing agents, such as sodium bisulfite and 2-mercaptoethanol, are frequently used when attempting to isolate soluble active enzymes and undenatured protein fractions (Anderson and Rowan, 1967; Wolf, 1972). In attempting to improve the solubility of the protein precipitated at room temperature, several reducing agents were added to the CBJ (before precipitation) at the following levels: sodium bisulfite, 0.156 *M*; sodium dithionite, 0.078 *M*; and mercaptoethanol, 0.40 *M*. The samples were precipitated and washed at pH 3.5 at room temperature, and the protein reslurried in pH 8.5 borate buffer (0.1 *M*) for 1 hr. There was no increase in solubility when using the reducing agents compared to the untreated control. In related work, Betschart (1974) reported that mercaptoethanol was ineffective in increasing the solubility of freeze-dried, acid-precipitated alfalfa protein.

By acid precipitating the alfalfa protein at 2°, the native solubility is preserved. This protein concentrate, being free from the dark green color and most of the grassy flavor of the typical LPC, and still retaining its native solubility, should have many uses in the food industry. The protein can be further purified by membrane filtration if necessary.

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Changes in Concentrations and Interrelationships of Phytate, Phosphorus, Magnesium, Calcium, and Zinc in Wheat during Maturation

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Changes in concentrations and interrelationships of phytate, P, Mg, Ca, and Zn were studied in the heads of 11 varieties (Roshan, Jolgeh, Koohrang, Derakhshan, Ommid, Navid, Deihim, Siano, Penjamo, Tobari, and Akowa) of wheat (*Triticum aestivum* L.) during maturation. The maturation period was divided into prechlorophyll, chlorophyll, and postchlorophyll destruction stages. Concentrations of total P, Mg, and Zn showed an upward trend and that of Ca fluctuated during maturation. Phytate P concentration doubled mainly at the expense of nonphytate P during the chlorophyll destruction stage. Concentration of total P was related to nonphytate and phytate P during prechlorophyll and chlorophyll destruction stages, respectively. Phytate P, Mg, and Ca concentrations were interrelated during the postchlorophyll destruction stage. By the end of this period, phytate P constituted 75% of the total P of the wheat heads. The rate of phytin synthesis in wheat during maturation, together with the need of chlorophyll data in such studies, are discussed.

As early as 1934 it was suggested that cereal P was physiologically unavailable, because a major portion of it was present as inositol hexaphosphate or phytate (Bruce and Callow, 1934). Since then similar results have been reported by other investigators (Gillis et al., 1957; Nelson, 1967; Hintz et al., 1973). It was found that in wheat, phytate P varied from 49 to 80% of the total P of the grain (Knowles and Watkins, 1932; Booth et al., 1941; Asada et al., 1968; O'Dell et al., 1972; Abernethy et al., 1973). The mixed Mg, Ca, and K salts of phytate are termed phytin (Averill and King, 1926). Phytin is the principal storage form of P in most seeds (Earley and DeTurk, 1944).

A number of investigations have demonstrated that phytate might be responsible for decreased physiological availability of dietary Ca (Harrison and Mellanby, 1939; McCance and Widdowson, 1942; Krebs and Mellanby, 1943; Hoff-Jorgensen et al., 1946; Cullumbine et al., 1950; Nelson et al., 1968; Berlyne et al., 1973; Reinhold et al., 1973), Zn (O'Dell and Savage, 1960; Prasad et al., 1963; O'Dell, 1969; Reinhold, 1971; Halsted et al., 1972; Reinhold et al., 1973), and Mg (McCance and Widdowson, 1942; Roberts and Yudkin, 1960; Likuski and Forbes, 1965).

This study was carried out in an attempt to provide data on changes in concentrations and interrelationships of phytate P, nonphytate P, total P, Mg, and Zn in wheat during maturation.

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MATERIALS AND METHODS

Seven Iranian (Roshan, Jolgeh, Koohrang, Derakhshan, Ommid, Navid, and Deihim) and four foreign (Siano, Penjamo, Tobari, and Akowa) varieties of wheat (*Triticum aes-*

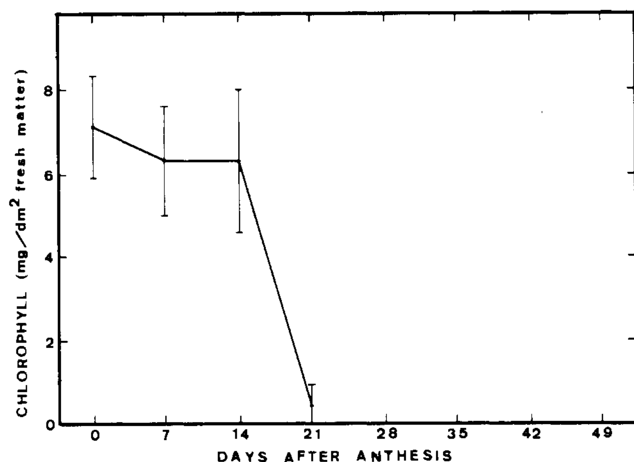

Figure 1. Average changes in concentration of chlorophyll in wheat (*Triticum aestivum* L.) leaves during maturation; vertical lines indicate standard deviation.

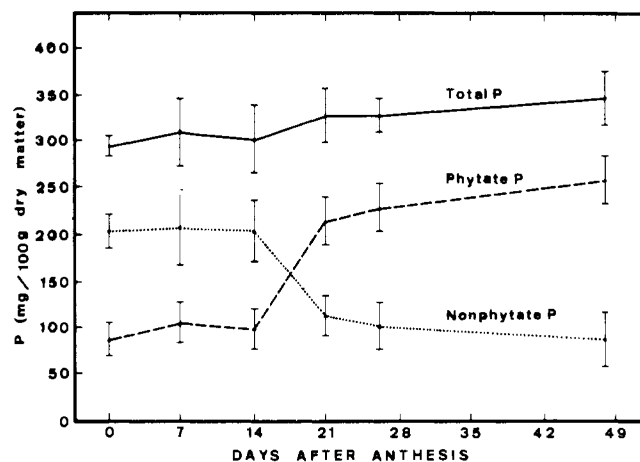

Figure 2. Average changes in concentrations of total P, phytate P, and nonphytate P in wheat (*Triticum aestivum* L.) heads during maturation; vertical lines indicate standard deviation.

tivum L.) were used. These varieties were planted on Dec 10, 1972, at the Pahlavi University, College of Agriculture Experimental Station, Shiraz, Iran. The experiment had a randomized complete block design with four replications. Each plot consisted of four 5 m rows at 50 cm distance. At 5 to 22 day intervals after flowering, heads of 20 plants of each variety were taken at random from each plot. The samples were dried at 100° for 24 hr, ground in a coffee mill, and stored in plastic bags until analysis. At the same time that the heads were sampled, fresh leaves of the plant were taken for chlorophyll determination.

Phytate P concentration was determined by a modification of the method described by Oberleas (1971). Two grams of the ground sample was extracted with 40 ml of 1.2% HCl containing 10% Na_2SO_4 for 2 hr on a shaker with circular movement. The solution was allowed to stand overnight and then shaken again for 1 hr the following day. The extract was centrifuged at about 650*g* for 15 min. Five milliliters of cleared extract was added to 5 ml of distilled water and after the addition of 6 ml of 0.4% $FeCl_3{\cdot}6H_2O$ in 0.07 *N* HCl solution it was mixed and heated in a boiling water bath for 15 min. The precipitated ferric salt of phytate was isolated by centrifugation at 4000*g* for 15 min. The precipitate was washed with 5 ml of 4% Na_2SO_4 in 0.07 *N* HCl solution using a test tube shaker. After centrifugation at 4000*g* for 15 min, the precipitate was dissolved in 3 ml of concentrated H_2SO_4. One milliliter of the solution with 1 ml of 65% HNO_3 and 1 ml of distilled water were transferred into a Kjeldahl flask of 30 ml capacity and, after addition of three glass beads, the solution was allowed to sit overnight at room temperature. Wet digestion was carried out the following day. Five milliliters of distilled water was added to the digest with care while the digest was still warm and it was heated in a boiling water bath for 15 min to destroy pyrophosphate. The solution was diluted to 50 ml and inorganic P was determined by reduction of phosphomolybdic acid using ascorbic acid. To 2 ml of diluted digest, 3 ml of distilled water, 3 ml of 1.5 *N* H_2SO_4, 0.4 ml of 10% $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 0.4 ml of 2% ascorbic acid were added and mixed. The solution was allowed to stand for 20 min. Absorbance was measured at 660 mμ by a Bauch and Lomb Spectronic 20 spectrophotometer. Two milliliters of KH_2PO_4 solution containing 3.2×10^{-3} mg of P was used as the working standard for the determination.

For determination of P, Ca, Mg, and Zn concentrations, dried samples were subjected to wet digestion by a modification of the method described by O'Dell et al. (1972). One gram of the sample was soaked in 10 ml of 65% HNO_3 in a tall 200-ml beaker covered with a watch glass. The mixture was allowed to stand overnight and, after addition of 5 ml of 70% $HClO_4$, it was heated gently on a hot plate until digested as evidenced by fumes of $HClO_4$. The digest was then cooled and after addition of 25 ml of distilled water, it was heated and filtered through a Whatman No. 40 paper. The filtrate was diluted to 100 ml with distilled water and was used for subsequent analyses. Concentrations of Ca, Mg, and Zn were determined on a Zeiss Model PMQ II atomic absorption spectrophotometer, using the method of O'Dell et al. (1972). Lanthanum oxide was included to avoid interference by P. The concentration of P was determined as described earlier. Nonphytate P was calculated by subtracting the concentration of phytate P from that of total P of each individual sample. Chlorophyll was determined by the method of Arnon (1949).

RESULTS

Changes in chlorophyll concentration in wheat leaves are shown in Figure 1. The maturation period was divided into three stages: (a) a period of stable chlorophyll concentration (prechlorophyll destruction stage; 0 to 7 days after anthesis); (b) chlorophyll destruction stage (14 to 21 days after anthesis); and (c) postchlorophyll destruction stage (28 to 48 days after anthesis).

Changes in concentrations of phytate P, nonphytate P, and total P in wheat heads are shown in Figure 2. The concentration of total P showed a significant upward trend during the entire period of maturation ($r = 0.45$ for total P vs. time) (Table I), although this change was not statistically significant during the individual stages (Tables I and II). There was an increase in phytate P ($r = 0.83$ for phytate P vs. time) and a decrease in nonphytate P ($r = -0.75$ for nonphytate P vs. time) concentrations in wheat heads during maturation (Table I). The changes observed during this period occurred mainly during the chlorophyll destruction stage. Phytate and nonphytate P concentrations remained unchanged during the first stage of maturation. However, there was a significant increase in phytate P ($r = 0.94$ for phytate P vs. time) and a significant decrease in nonphytate P ($r = -0.88$ for nonphytate P vs. time) during the chlorophyll destruction stage (Table II). The concentration of phytate P showed a significant upward trend ($r = 0.53$ for phytate P vs. time) during the final stage of maturation, while that of nonphytate P remained constant (Table II).

There was a significant negative correlation between changes in phytate and nonphytate P ($r = -0.90$) (Table I) during maturation. However, this relationship was not observed in all of the three stages. During the prechlorophyll destruction stage changes in concentration of phytate and

Table I. Correlation Coefficients among Concentrations of Phytate P, Nonphytate P, Total P, Mg, Ca, Zn, and Time during the Entire Period of Maturation (Lower Diagonal), and during Prechlorophyll Destruction Stage (Upper Diagonal) of Wheat (*Triticum aestivum* L.)

	Phytate P	Nonphytate P	Total P	Mg	Ca	Zn	Time
Phytate P		−0.45	0.35	−0.02	−0.14	0.39	0.37
Nonphytate P	−0.90[b]		0.77[b]	0.07	0.19	0.17	0.01
Total P	0.57[b]	−0.13		0.03	−0.01	0.46	0.28
Mg	0.42[b]	−0.27	0.46[b]		0.21	0.51[a]	−0.09
Ca	−0.32	−0.40[b]	−0.02	0.38[b]		0.01	−0.22
Zn	0.36[a]	−0.17	0.44[b]	0.27	0.05		0.22
Time	0.83[b]	−0.75[b]	0.45[b]	0.39[b]	−0.21	0.29[a]	

[a,b] Statistically significant at $P < 0.05$ and $P < 0.01$ level, respectively.

Table II. Correlation Coefficients among Concentrations of Phytate P, Nonphytate P, Total P, Mg, Ca, Zn, and Time during Chlorophyll Destruction (Lower Diagonal) and Postchlorophyll Destruction (Upper Diagonal) Stages of Wheat (*Triticum aestivum* L.)

	Phytate P	Nonphytate P	Total P	Mg	Ca	Zn	Time
Phytate P		−0.65[b]	0.49	0.61[a]	0.62[a]	0.35	0.53[a]
Nonphytate P	−0.85[b]		0.33	−0.17	−0.22	−0.33	−0.26
Total P	0.54[b]	−0.02		0.56[a]	0.53[a]	0.06	0.36
Mg	0.47	−0.19	0.67[b]		0.83[b]	0.00	0.36
Ca	−0.45	0.44	−0.06	0.05		0.07	0.69[b]
Zn	0.01	0.34	0.48	0.52[a]	0.43		0.00
Time	0.94[b]	−0.88[b]	0.38	0.38	−0.52[a]	−0.31	

[a,b] Statistically significant at $P < 0.05$ and $P < 0.01$ level, respectively.

Table III. Average Changes in Concentrations of Mg, Ca, and Zn in Wheat (*Triticum aestivum* L.) Heads during Maturation

Days after anthesis	Concentration in dry matter[a]		
	Mg, ppm	Ca, ppm	Zn, ppm
0	751 ± 170	67 ± 10	30 ± 3
7	728 ± 103	61 ± 13	31 ± 4
14	794 ± 177	62 ± 19	36 ± 4
21	874 ± 167	44 ± 8	32 ± 4
26	809 ± 215	37 ± 8	36 ± 6
48	1132 ± 659	63 ± 28	36 ± 7

[a] Mean plus or minus standard deviation.

nonphytate P were not significantly correlated (Table I). But there was a significant negative correlation between the two variables during chlorophyll destruction (r = −0.85) and postchlorophyll destruction (r = −0.65) stages (Table II).

There was a significant correlation between concentrations of total and phytate P (r = 0.57) (Table I) during maturation, although this relationship was not observed in all of the three stages. Changes in concentrations of phytate and total P were significantly correlated (r = 0.54) (Table II) only during the chlorophyll destruction stage. No significant relationship was found between total and nonphytate P, when the entire period of maturation was considered for statistical analysis, while they were significantly correlated (r = 0.77) (Table I) during the prechlorophyll destruction stage. The two variables were not related in the other two stages of maturation (Table II).

Changes in concentrations of Mg, Ca, and Zn in wheat heads are shown in Table III. There was a significant upward trend for concentrations of Mg (r = 0.39 for Mg vs. time) and Zn (r = 0.29 for Zn vs. time) during the entire period of maturation (Table I), although these changes were not statistically significant during the individual stages (Tables I and II). The concentration of Ca did not show any change with time when the entire period of maturation was considered for statistical analysis, while it had a significant fall (r = −0.52) during the chlorophyll destruction stage followed by a rise to its original level during the next stage (r = 0.69) (Table II).

Changes in phytate P concentration were significantly correlated with those of Mg (r = 0.42) and Zn (r = 0.36) during maturation (Table I). There was a significant negative correlation between phytate P and Ca concentrations (r = −0.32) during this period. On the other hand, no significant correlation was found between changes in concentrations of Zn and phytate P during the individual stages of maturation (Tables I and II). Phytate P and Mg concentrations were not correlated during the first two stages of maturation. However, they were significantly associated (r = 0.61) (Table II) during the postchlorophyll destruction stage. Furthermore, changes in Ca concentration were significantly correlated with those of phytate P (r = 0.62) and Mg (r = 0.83) only during this period of maturation (Table II). There was a significant correlation between total P and Mg concentrations during chlorophyll (r = 0.67) and postchlorophyll (r = 0.56) destruction stages (Table II). Total P and Ca concentrations were significantly correlated (r = 0.53) (Table II) only during the postchlorophyll destruction stage.

DISCUSSION

At or immediately after anthesis, the major portion of the head is constituted of glumes, lemmas, paleas, rachis, and awns. The P data show that phytate P comprised an appreciable portion (30%) of total wheat head P during the prechlorophyll destruction stage (0 to 7 days after anthesis). In contrast, Abernethy et al. (1973) have reported a

very low content of phytate (almost nil) in wheat grain 7 days after anthesis. This discrepancy might be in part due to sampling differences, i.e., use of heads vs. grain. By the end of the maturation period, when the contribution of parts other than the grain to the head was minor, phytate P accounted for most of the wheat head P (75%) which agreed with results reported by others for wheat grain (Booth et al., 1941; Abernethy et al., 1973). Knowles and Watkins (1932), however, have reported lower values for phytate P (49% of total P) in the grain.

The high phytate concentration in wheat may explain the high incidence of Ca (Berlyne et al., 1973; Reinhold et al., 1973) and Zn deficiency (Prasad et al., 1963; Reinhold, 1971; Halsted et al., 1972; Reinhold et al., 1973) reported in the Middle Eastern villages, where unleavened bread made from whole wheat flour constitutes the major portion of the diet.

The major rise in phytate P concentration occurred during the chlorophyll destruction stage. In fact, phytate P concentration doubled by the end of this period (Figure 2). Earley and DeTurk (1944), in a study on phytate synthesis in developing corn grain, could not find a relationship between phytate and nonphytate P. They suggested that in the grain, P metabolism falls into two types: (a) formation of entire cellular structure of the fruit; (b) formation of phytate. Data in the present study indicate that both processes occurred simultaneously during the prechlorophyll destruction stage. But the latter process was the dominant, if not the only one, during the chlorophyll destruction stage. Phytate synthesis was almost complete by the end of this stage.

It has been suggested that soluble phytate may form a mixed precipitate with Ca and Mg (Earley and DeTurk, 1944; Roberts and Yudkin, 1960). Degradation of chlorophyll would be accompanied by release of Mg, which may contribute to additional precipitation of available phytate as phytin. This may be one of several factors driving phytin synthesis toward completion. The data on P together with the significant relationships found among phytate P, Mg, and Ca during the postchlorophyll destruction stage seem to support the proposed mechanism. Phytin precipitation would be further favored by the decrease in water content of the plants during maturation. Further work is under way to establish the mechanism of phytin synthesis in wheat.

The data indicated that in studies on developing wheat, determination of interrelationships among biochemical variables by consideration of only the period of maturation might result in erroneous conclusions. Since the availability or lack of chlorophyll will have a direct effect on metabolism of the plant, it is recommended to use chlorophyll data to study the relationships during the three stages of maturation.

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