Factors Affecting Hydrolysis during Breadmaking of Phytic Acid in Wheat Protein Concentrate[1]

G. S. RANHOTRA, Nutrition Laboratory, American Institute of Baking, Chicago, Ill. 60611

ABSTRACT

Although phytic acid increases linearly when increasing concentrations of wheat protein concentrate (WPC) are added to hard winter wheat flour, the phytase activity does not increase linearly and the pH of the breads baked from these blends increases progressively above the optimum for phytase activity. However, the progressive decrease in the rate of phytic acid hydrolyzed during breadmaking as WPC increases in the blends, as was reported earlier, may be caused by increased inhibition of phytase activity or rephosphorylation (by excessive accumulation of inorganic phosphorus) of partially hydrolyzed phytic acid, or both.

Compared to hard red winter wheat flour, wheat protein concentrate (WPC, a low-fiber flour prepared from fibrous mill-fractions) is high in lysine and protein as well as in phosphorus and certain other dietary essentials (1–3) and thus may be potentially important in the human diet. Most of the phosphorus in WPC is, however, tied up in phytic acid complexes which generally render it physiologically unavailable and also interfere with the availability of certain other mineral elements. In blends of WPC and wheat flour, some of the phytic acid is hydrolyzed during breadmaking. However, with increasing concentrations of WPC, the amount of phytic acid hydrolyzed during breadmaking decreases until in an all-WPC bread virtually none of the phytic acid initially present is hydrolyzed (4), in spite of the increase in phytase activity (5). This paper presents results of studies on some of the factors affecting phytic acid hydrolysis during breadmaking.

---

[1]Presented at the 57th Annual Meeting, Miami Beach, October 1972.

MATERIALS AND METHODS

Hard red winter wheat flour and wheat protein concentrate (WPC) was provided by Dixie-Portland Mill, Arkansas City, Kansas. Pup loaves were made by a sponge-dough procedure (4) based on the formula: flour, 100 g., salt, 2 g., yeast, 3.0 g., yeast food, 0.5 g., lard, 3.0 g., nonfat dry milk, 4.0 g., sugar, 5 g., calcium propionate, 0.2 g., and monoglycerides, 0.5 g. Standard conditions were: sponge time, 210 min., floor time, 30 min., intermediate proofing, 20 min., pan proofing, 60 min., and baking, 20 min. at 450°F. (232°C.) Seventy percent of the flour, all of the yeast and yeast food, and 70% of the total water were used in the sponge. For analyses, loaves were air-dried and ground in a Waring Blendor to particle size 40 (U.S. sieve size). Doughs to be analyzed were frozen and freeze-dried at the end of sponge fermentation and ground similarly. The pH of bread crumbs and of dry loaf ingredients was determined by the AOAC methods (6). Total phosphorus was determined by the volumetric method of AOAC (6) and inorganic phosphorus by the method of Pons and Guthrie (7). Phytic acid was measured by the method of Makower (8), based on the content of iron precipitated as ferric phytate. Calculation of phosphorus content was based on a 4:6 iron:phosphorus molecular ratio. Residual phosphorus, representing nonphytic acid organic phosphorus and including that from lower phosphoric esters of inositol, was calculated using determined values for inorganic phosphorus, phytic aicd phosphorus, and total phosphorus. Phytase acitivity in flour, WPC, and their blends was measured by the method of Peers (5). Samples were incubated at 55° in 0.2M acetate buffer (pH 5.15) containing 0.004M $MgSO_4$ using 1.6×10^{-3}M phytic acid to obtain a final substrate concentration of about 0.3 mg./ml. (0.15 mg./ml. in the case of wheat flour). A 2-ml. aliquot of the suspension (total volume, 15 ml.) was removed at zero hour and after 1 hour of incubation. Inorganic phosphorus was determined immediately by the method of Fiske and Subbarow (9). This method was used because it permitted analyses of samples to be completed in less than 1 hr., which was required, but impossible with the method of Pons and Guthrie (7). One unit of phytase activity is defined as the amount of enzyme releasing 1 mg. of inorganic phosphorus from 1.6×10^{-3}M phytic acid at pH 5.15 in 1 hour. A different sample of WPC was used in present studies than was used earlier (4) and it was appreciably higher in total (840.6 mg.%), phytic acid (763.7 mg.%), and inorganic (75.6 mg.%) phosphorus.

RESULTS AND DISCUSSION

In bread baked with blend A flour, which is a mixture of 70% wheat flour and 30% WPC (a flour donated by USDA for use overseas), substantial cleavage of phytic acid occurred (Table I), supposedly caused by phytase present. The concurrent increase in the levels of inorganic and residual phosphorus suggests that some of the phytic acid that underwent cleavage was only partially dephosphorylated. When 100 mg. of inorganic phosphorus was added, rephosphorylation to both the phytic acid and residual phosphorus levels occurred. With the addition of 200 mg. of inorganic phosphorus, some of this residual phosphorus was phosphorylated to completion, yielding additional phytic acid, so that its amount in the pup loaf now exceeded that initially present in the dry ingredients. No appreciable amount of phytic acid was formed with higher additions of inorganic phosphorus. Appreciable hydrolysis of phytic acid in all-WPC

TABLE I. EFFECT OF INORGANIC PHOSPHORUS ON THE PHYTIC ACID HYDROLYZED DURING BREADMAKING[a]

	Inorganic Phosphorus Added, mg.	Phosphorus mg./Pup Loaf			
		Total	Phytic acid	Inorganic	Residual
Dry loaf ingredients	0	403.4	280.8	45.5	77.1
Baked pup loaf	0	407.5	198.5	101.7	107.3
Baked pup loaf	100	502.6	230.6	149.0	123.0
Baked pup loaf	200	605.0	302.6	255.4	47.0
Baked pup loaf	300	698.4	304.6	374.4	19.4
Baked pup loaf	600	1006.2	299.5	705.3	1.4

[a]All breads were baked using blend A flour (70% wheat flour, 30% WPC).

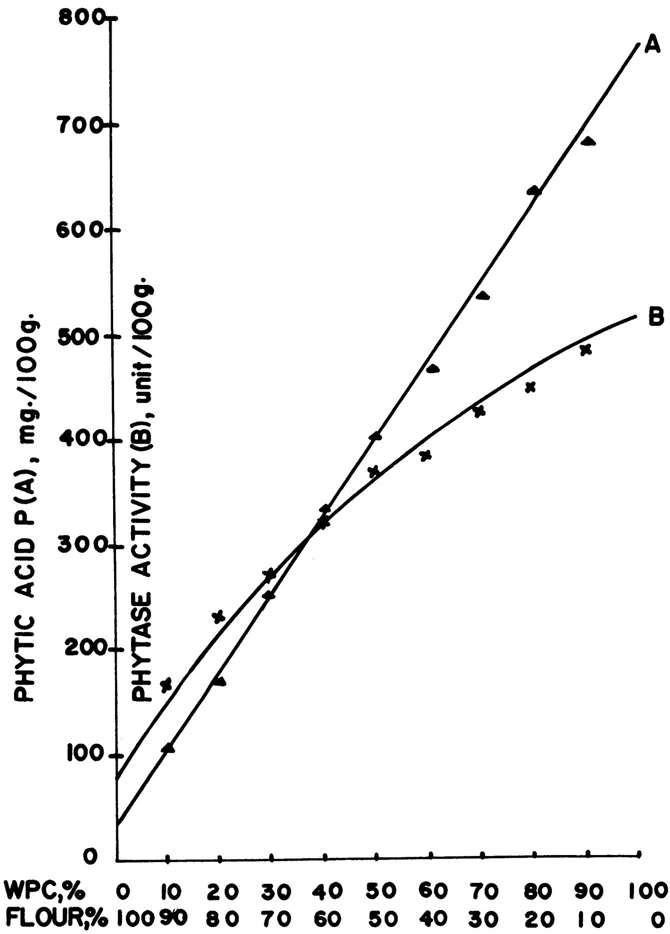

Fig. 1. Effect of increasing concentration of WPC in blends with hard winter wheat flour on phytic acid content and phytase activity.

bread occurs during steps early in breadmaking (4). Therefore, the lack of change observed in the overall phytic acid content of all-WPC bread might have, at least partially, resulted from rephosphorylation, triggered by excessive accumulation of inorganic phosphorus late in the breadmaking process, of partially hydrolyzed phytic acid. Addition of inorganic phosphorus was reported by Bianchetti and Sartirana (10) to inhibit phytase synthesis in germinating wheat embryo.

Figure 1 shows the phytic acid content and phytase activity of flour and WPC blends. Hard wheat flour is reported to contain higher phytase activity than soft wheat flour (5). The wheat flour used in present studies contained 73.6 units of phytase activity per 100 g. while WPC has an activity of 515.5 units per 100 g. (Fig. 1). Although phytic acid content increased linearly with increased concentration of WPC, the phytase activity increased at a decreasing rate as the WPC increased in the blend. Still sufficient phytase activity was present to have caused sizable phytic acid hydrolysis in all-WPC bread. A near lack of such hydrolysis can be attributed either to conditions such as excessive accumulation of inorganic phosphorus which favored phytic acid formation, or to those which inhibited phytase activity when WPC was mixed with ingredients used in bread formula, or both. Breads using various blends were made under conditions which involved no change in the standard baking conditions above. Thus, it seems likely that only the changes that occurred in pH, ionic concentration, etc., would have inhibited phytase activity in addition to inhibition caused by excessive accumulation of inorganic phosphorus.

Concerning pH, Mollgaard et al. (11), Peers (5), and Gibbins and Norris (12) have quoted values of 5.15, 5.1 to 5.3, and 5.2 for the pH optimum of rye, wheat, and bean phytase, respectively. With increasing concentration of WPC in the blends, the pH of the resultant breads (also of dry ingredients) increased progressively away from the optimum (Fig. 2) for phytase activity; the values changed from 5.20 (all-wheat flour bread) to 5.75 (all-WPC bread). Peers (5) has reported a very rapid decrease in phytase activity on either side of the optimum. Addition of magnesium, which is required in phytase activation, had no apparent stimulatory effect

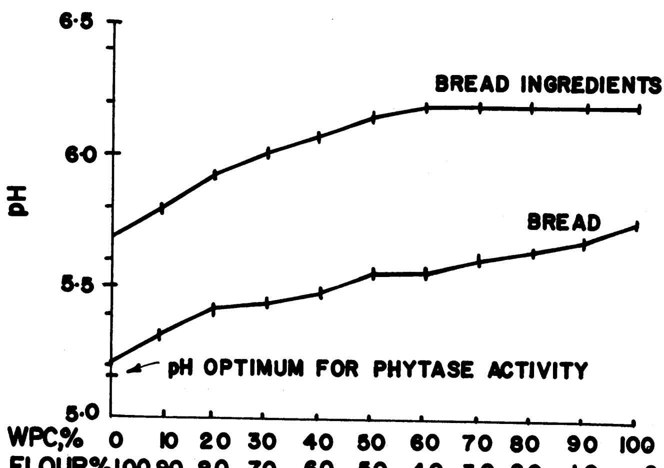

Fig. 2. Effect of increasing concentration of WPC in blends with hard winter wheat flour on pH of the resultant breads.

probably because WPC is quite high in magnesium (2). The effect of other ions was not studied but calcium used in conjunction with nonfat dry milk was reported earlier (4) to inhibit phytic acid hydrolysis in bread made with blend A flour.

In preliminary studies, it was observed that dough fermentation (blend A flour used) caused sizable hydrolysis of phytic acid which increased with the time of fermentation and that phytic acid hydrolysis also increased with baking temperature and time. Preliminary studies also revealed that while sizable phytic acid hydrolysis also occurred without the addition of yeast, yeast (3%) stimulated the hydrolysis still further. Some stimulatory effect was also observed when mineral yeast food was added (0.5%) simultaneously, but excessive addition of either the yeast or the yeast food had an inhibitory effect.

Acknowledgments

Thanks are due to Thomas Lehmann, Baking Technologist, and to Robert Loewe and L. Puyat, Chemical Technicians.

Literature Cited

1. WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., and DEYOE, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chem. 44: 48 (1967).
2. FELLERS, D. A., SHEPHERD, A. D., BELLARD, N. J., MOSSMAN, A. P., JOHNSTON, P. H., and WASSERMAN, T. Protein concentrates by dry milling of wheat millfeeds. II. Compositional aspects. Cereal Chem. 45: 520 (1968).
3. RANHOTRA, G. S., HEPBURN, F. N., and BRADLEY, W. B. Supplemental effect of wheat protein concentrate on the protein quality of white wheat flour. Cereal Chem. 48: 699 (1971).
4. RANHOTRA, G. S. Hydrolysis during breadmaking of phytic acid in wheat protein concentrate. J. Food Sci. 37: 12 (1972).
5. PEERS, F. G. The phytase of wheat. Biochem. J. 53: 102 (1953).
6. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
7. PONS, W. A., and GUTHRIE, J. D. Determination of inorganic phosphorus in plant materials. Ind. Eng. Chem. Anal. Ed. 18: 184 (1946).
8. MAKOWER, R. U. Extraction and determination of phytic acid in beans (*Phaseolus vulgaris*). Cereal Chem. 47: 288 (1970).
9. FISKE, C. H., and SUBBAROW, Y. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375 (1925).
10. BIANCHETTI, R., and SARTIRANA, M. L. The mechanism of the repression by inorganic phosphate of phytase synthesis in the germinating wheat embryo. Biochim. Biophys. Acta 145: 485 (1967).
11. MOLLGAARD, H., LORENZEN, K., HANSEN, I. G., and CHRISTENSEN, P. E. On phytic acid, its importance in metabolism and its enzymatic cleavage in bread supplemented with calcium. Biochem. J. 40: 589 (1946).
12. GIBBINS, L. N., and NORRIS, F. W. Phytase and acid phosphatase in the dwarf bean, Phaseolus vulgaris. Biochem. J. 86: 67 (1963).

[Received November 30, 1972. Accepted February 9, 1973]