NOTE

Effects of Phytase from Three Yeasts on Phytate Reduction in Norwegian Whole Wheat Flour

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Price control measures for agricultural products are an important tool in Norwegian nutrition policy. Farm subidies are directed toward influencing the consumption of nutritious foods by offering healthful foodstuffs to the consumer at a lower price. Thus, there is a somewhat lower price for whole grain cereals than for cereals or grains with low extraction rates.

Grain products of 100% extraction are rich sources of nutrient minerals. However some of the minerals may be bound by phytate, an organic compound normally present in greater amounts in whole grains than in refined products. The binding of minerals by phytate makes them unavailable for absorption into the body. The Norwegians, who do not fortify nor enrich their flour and cereals, are seeking ways to improve the mineral bioavailability of these products.

The most commonly consumed cereal product in Norway is whole grain yeast-leavened bread. During bread baking, much of the phytate is hydrolyzed due to the enzyme phytase, present both in the grain and yeast, thus improving the bioavailability of minerals in the bread.

In this in vitro study, the phytate content of five Swedish and Norwegian flours was compared with an American flour. Bakers' yeasts from Norway, the United States, and Sweden were combined with a Norwegian wheat flour of 100% extraction to determine which yeast during the fermentation process exhibited the greatest ability to reduce the phytate content of the flour.

Background

Phytate (myoinositol hexakisphosphate) is found throughout the plant kingdom as a natural constituent. Because phytate may bind certain minerals, making them unavailable for body utilization (deBoland et al 1975, Ellis et al 1987, Frølich and Asp 1985, Frølich et al 1984, Harland and Oberleas 1987, Harland et al 1988a, Oberleas and Harland 1981, Persson et al 1987, Schweizer et al 1984), it may be of nutritional concern. Not only does phytate bind minerals in the food in which it originates, but in a meal it can bind minerals from other foods that do not contain phytate. Moreover, it will bind endogenously secreted minerals that circulate in the gut as a part of the digestive process (Harland and Oberleas 1987). In extreme situations, phytate may cause mineral deficiencies. It is of importance to improve the bioavailability of minerals from plant sources for omnivores (Harland et al 1988b). A number of methods have been devised to lower phytate levels or to remove phytate from certain foods (Harland and Oberleas 1987, Cosgrove 1980).

As minerals, vitamins, and dietary fiber are found together with the phytate, a mechanical removal of phytate by removing the outer grain layers will lower the nutritive as well as the organoleptic qualities. However, to retain these qualities, the phytate may be removed enzymatically.

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Phytase is the enzyme that hydrolyzes phytate. When phytate is hydrolyzed, it loses its mineral binding capacity. Phytase is naturally present along with phytate in grain and can be increased reasonably through the addition of yeasts that also contain phytase. Therein lies the focus of these experiments.

MATERIALS AND METHODS

Foreign and domestic flours were purchased in the United States, Norway, and Sweden. All samples listed in Table I were analyzed by the AOAC-approved method to determine the current phytate content (Harland and Oberleas 1986).

Only the Norwegian flour of 100% extraction was used in the timed, temperature-controlled trials. Twenty grams of flour was shaken with 100 ml of 2.4% HCl in a shaker at room temperature for 1 hr. In order to reduce the analytical time for the timed, temperature-controlled experiments, the investigators extracted phytate from flour samples for only 1 hr instead of the 3 hr recommended. In previous experiments (*unpublished data*), we had learned that 95% of the phytate was solubilized within the first hour of shaking. After extraction, the suspension was vacuum filtered through Whatman No. 1 filter paper. The filtration process required approximately 20 min because with these proportions of flour and water, a paste tended to form. The filtrate was adjusted to a pH of 5.2 with concentrated NaOH, and 50 ml of the extract was placed into incubation flasks. Each of the yeasts was added in amounts of 5 g to each of the 50-ml suspensions, and the pH was again adjusted to 5.2. Incubation was performed in a shaking water bath at 50°C (Tecator 1024, shaking water bath). After the appropriate reaction time (0, 30, 60, and 120 min), 5-ml aliquots were removed and treated with 1 ml of 2.4% HCl to stop the phytase activity.

For these experiments we chose three strains of yeast: one each from Norway, Sweden, and the United States. For commercial and household use, only one strain is available in Norway. This is true of Sweden as well. The American Fleishman's cake yeast was purchased in 1-oz. packages at a local supermarket.

RESULTS AND DISCUSSION

Table I shows the results of the phytate analyses of the different flours. All of these values fell within the normal range for flours.

TABLE I
Phytate in Flours

Flour	Phytate (mg/g)
Swedish barley flour, 90% extraction	6.65 ± 0.15[a]
Swedish rye flour, 100% extraction	6.80 ± 0
Swedish whole wheat flour, 100% extraction, 10% moisture	7.70 ± 0.30
Norwegian whole wheat flour, 100% extraction	7.90 ± 0.20
Swedish whole wheat flour, 100% extraction, 9% moisture	8.15 ± 0.32
American whole wheat flour,[b] 100% extraction	9.60

[a]Each flour sample was analyzed in triplicate. Data are mean values ± standard error.
[b]Oberleas and Harland 1981.

TABLE II
Effect of Three Kinds of Yeast on Reduction of Phytate in Norwegian Flour (100% extraction)

Yeast	Amount (g)	Phytate (mg/g) Present After Incubation for 0 min	30 min	60 min	120 min
Norwegian	5	7.46 ± 0.01[a]	7.09 ± 0.13	6.94 ± 0.49	5.96 ± 0.09
American	5	7.63 ± 0.01	7.52 ± 0.12	7.30 ± 0.17	7.18 ± 0.04
Swedish	5	7.47 ± 0	7.77 ± 0.13	7.64 ± 0.17	7.72 ± 0

[a]Each value represetns a mean ± standard error of five determinations.

As would be expected, the higher the extraction rate of the flour, the higher the phytate content. Two varieties of Swedish flours were analyzed. Five experiments using yeast fermentation were performed in order to develop optimum reduction of the phytate over time. Prior tests of proportion of ingredients, pH of the extract, incubation time, and temperature led to the final experiment (which was repeated five times to confirm findings). The results appear in Table II.

At zero time and after 30 min of incubation, none of the yeasts affected the phytate level of Norwegian flour. The phytate level of 7+ mg/g for these various aliquots is similar to that presented in Table I (7.90 mg/g) for the Norwegian flour. However, after 60 min, there were 7 and 4% reductions of phytate in the Norwegian and American yeast mixtures, respectively, and after 120 min, a total of 20 and 6% reductions. There was essentially no change as a result of Swedish yeast phytase even after 120 min of incubation.

The Norwegian yeast appeared to exhibit the greatest activity and gave consistent results. The reduction of phytate due to yeast was, however, not very marked, suggesting that the phytase normally present in the grain is of greater importance for phytate reduction. Other investigators have pointed out (Harland and Harland 1980) that the endogenous phytase normally present in grains may play a larger role in phytate reduction than the addition of yeast phytase.

By using the Harland and Oberleas method for the phytate analyses (1986), an inherent error is introduced which should be clarified for the reader. The anion-exchange method is dependent upon the measurement of total phosphorus in the final eluate. In the step-wise hydrolysis of phytate by the enzyme phytase, lower inositol phosphates are formed (Frølich et al 1986), which are measured as phosphorus but do not have the mineral-binding capacity of the original phytate molecule. Thus, phytate values may be artificially inflated, and consequently the mineral-binding capacity normally associated with those phytate levels.

To have a more realistic explanation of the mineral binding phenomena, these experiments would profit from analyses of the aliquots by nuclear magnetic resonance spectroscopy (O'Neill et al 1980, Frølich et al 1986, Mazzola et al 1986, Sandberg and Ahderinne 1986). This method is able to detect the individual hydrolysis products that have different binding capacities.

As with any in vitro experiment, the next practical step is to test the system with breads to see whether the reduction in phytate can be produced in the more complex environment of a bread dough.

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