

Optimization of Temperature, Time, and Lactic Acid Concentration to Inactivate Lipoxigenase and Lipase and Preserve Phytase Activity in Barley (cv. Blenheim) During Soaking

A. RUTGERSSON,^{1,3} E.-L. BERGMAN,² H. LINGNERT,¹ and A.-S. SANDBERG²

ABSTRACT

Cereal Chem. 74(6):727–732

A barley-soaking process was studied to find conditions that inactivate the prooxidative enzyme lipoxigenase and the lipolytic enzyme lipase but preserve phytase activity to develop possible procedures for production of barley products with potentially high mineral bioavailability and good oxidative stability. Lactic acid concentration, temperature, and soaking time were studied. The study was done using a multivariate experimental design. Lactic acid concentration varied between 0 and 1%, temperature

varied between 45 and 70°C, and soaking time varied between 30 and 120 min. Although conditions under which lipoxigenase was inactivated were found, total inactivation of lipase was not obtained. Total lipoxigenase inactivation with <20% remaining lipase activity and >60% remaining phytase activity was reached after soaking in 1% lactic acid at suitable time-temperature combinations of 70–110 min and 53–58°C.

Barley has a high starch, dietary fiber, vitamin, and mineral content and, therefore, fits well with present dietary guidelines (Sundberg 1995). Human studies have shown that consuming barley products reduces postprandial glucose, insulin, and serum lipid levels. Due to its higher β -glucan content, barley contains as much total dietary fiber and soluble dietary fiber as oat (Ranhotra et al 1991). However, barley, like all other cereals, contains phytic acid (*myo*-inositol hexaphosphoric acid), which is an antinutrient that naturally occurs in foods and is present in large quantities in cereals (Lolas et al 1976). The antinutritive effect of phytic acid is caused by its ability to form chelates (phytates) with minerals such as zinc (Lönnerdal et al 1988, Sandberg 1991, Rossander et al 1992, Sandström and Sandberg 1992), calcium (Heaney et al 1991), and iron (Hallberg et al 1989, Brune et al 1992, Rossander et al 1992), making them unavailable for absorption in the human intestine.

Cereals are staple foods in a large part of the world and are a major source of minerals; therefore, it is important that the minerals from these products have a high bioavailability. The intake of large amounts of foods rich in phytate may cause mineral deficiency symptoms (Maga 1982, Torre et al 1991). However, the bioavailability of the minerals in cereals improves if phytate is degraded during food processing (Nävert et al 1985, Brune et al 1992). Dietary phytase can degrade phytate in the stomach and small intestine, which also improves mineral bioavailability (Sandberg et al 1987, Sandberg and Andersson 1988). By activating the naturally occurring enzyme phytase in cereals during food processing (soaking and malting), it is possible to degrade phytate to lower inositol phosphates, inorganic phosphate, and, in some cases, free *myo*-inositol (Cosgrove 1980). Phytase activity differs among cereals: rye has the highest activity, followed by wheat and barley, and oat has the lowest activity (Peers 1953, Bartnik and Szafranska 1987).

The prooxidative enzyme lipoxigenase and the lipolytic enzyme lipase, which occur in barley, also may be active during processing, which limits the shelf-life of the final cereal product. To improve the oxidative stability of cereal products, it is important to min-

imize the action of the lipoxigenase enzymes. Lipid oxidation during food processing may promote further oxidation during subsequent storage (Lingnert 1992). Yabuuchi (1976) hypothesized that lipoxigenase is the main factor in off-flavor production in malt. Lipoxigenase (EC 1.13.11.21) catalyzes the addition of molecular oxygen to the *cis*, *cis*-1,4-pentadiene system in polyunsaturated fatty acids, forming hydroperoxides. The hydroperoxides are decomposed or transformed into a variety of aldehydes and oxygenated fatty acids (Gardner 1988) that give rise to off-flavors. The undesirable flavors and hydroperoxides generated by lipoxigenase activity also are believed to interact with proteins, causing changes in food texture. Cereal lipids are rich in polyunsaturated fatty acids, making them good substrates for lipoxigenase action (Gardner 1988). Linoleic acid constitutes \approx 57% and linolenic acid \approx 5% of the fatty acid composition in barley (Morrison 1978). Lipase (EC 3.1.1.3) acts at the oil-water interface to release fatty acids from triglycerides (Brockenhoff and Jensen 1974), providing substrates for lipoxigenase enzymes that require the free acids as substrate. Degradation of fat, beginning with hydrolysis and followed by oxidation, leads to rancidification, which limits storage and handling of cereal products. Therefore, low lipase activity is desirable.

The aim of this study was to find soaking conditions for barley cv. Blenheim that inactivate lipoxigenase and lipase but preserve phytase activity to develop possible procedures for production of barley products with potentially high mineral bioavailability and good oxidative stability. The influence of lactic acid concentration, temperature, and time on inactivation of enzyme activities was studied to optimize a soaking process.

MATERIALS AND METHODS

Barley Seed

Whole barley kernels (cv. Blenheim) from the 1994 crop were provided by Skånska Lantmännen (Malmö, Sweden).

Experimental Design

A central composite face-centered design (CCF) was used with three variables and three replicates at the center point, for a total of 17 experiments. The three process variables studied included the lactic acid (*La*) concentration of the soaking agent (% v/v), the temperature during soaking (*T*), and the soaking duration (*t*, min). Experimental conditions at the center point were *La* = 0.5%, *T* = 57°C, and *t* = 75 min. The experimental number, scaled values, and real values are given in Table I. The scaled values were $x_1 = (La -$

¹SIK, The Swedish Institute for Food and Biotechnology-AB, P.O. Box 5401, S-402 29 Göteborg, Sweden.

²Chalmers University of Technology, Department of Food Science, P.O. Box 5401, S-402 29 Göteborg, Sweden.

³Corresponding author. Phone: +46 31 35 56 07. Fax: +46 31 83 37 82. E-mail: Annika.Rutgersson@sik.se.

0.5)/0.5, where L_a ranged from 0.0 to 1.0% (v/v); $x_2 = (T - 57)/12.5$, where T ranged from 45 to 70°C; and $x_3 = (t - 75)/45$, where t ranged from 30 to 120 min. A verification experiment was performed in the experimental domain (experiment 18).

Barley Kernel Soaking

The barley kernels (300 g) were soaked in 0.9 L of deionized water with a controlled lactic acid concentration at a specific temperature and time (described above). After soaking, the kernels were placed in deionized water at $\approx 4^\circ\text{C}$ with the same lactic acid concentration used during soaking. The kernels were cooled for 10 min and spread out between sheets of absorbent paper (Mölnlycke 11.51.15, Mölnlycke, Sweden). The paper was changed until it was no longer dampened by the kernels. Samples were frozen and stored at a temperature $< -20^\circ\text{C}$ until determination of enzyme activities. Duplicate samples were taken at the end of the soaking period for measurement of the water content and pH of the kernels. Approximately 30 g of kernels and 30 mL of soaking agent were removed from the soaking bath with a beaker. The beaker was cooled for 5 min in an ice bath, and the kernels were dried as described above.

Water Content

Approximately 5 g of barley kernels was weighed in an aluminum bowl. After drying in an oven at 105°C for 16 hr (Biltcliffe et al 1984), the water content was determined based on weight loss.

Measurement of pH

Approximately 5 g of barley kernels was ground for 15 sec in a coffee grinder (Braun AG type:4041, Kronberg/Ts., Germany). A sample (1.0-g wet weight) of ground barley was weighed in a sample tube, and 2.5 mL of distilled water was added. The tube was shaken for 1 min before the pH value was measured by a pH meter (Radiometer PHM 83, Copenhagen, Denmark).

Lipoxygenase Activity

Lipoxygenase extraction. Approximately 30 g of barley kernels was thawed and ground for 15 sec in a coffee grinder (Braun AG type:4041). A sample (1.0-g wet weight) of the ground barley was suspended in 10 mL of 0.1M potassium phosphate buffer containing 0.01M KCl, pH 6.7, in a beaker on an ice bath and magnetically stirred for 30 min. The suspension was centrifuged for 15 sec (Eppendorf 5415, Hamburg, Germany) at 14,000 rpm at room

temperature, and the supernatant was collected and stored on ice (for a maximum of 30 min) before analysis. The extractions were performed in duplicate.

Measurement of lipoxygenase activity. The lipoxygenase activity was measured at 25°C by a polarographic method described by Eriksson and Svensson (1970). The lipoxygenase activity was determined as the rate of O_2 consumption after addition of the barley extract to a linoleic acid substrate. This was done with an oxygen analyzer (Medelco AB, Hägersten, Sweden) equipped with a YSI 4004 Clark electrode (Yellow Springs Instrument Co., Yellow Springs, OH). The substrate was 10 mM linoleic acid (>99% Nu-Chek-Prep, Elysian, MN) in 0.1M potassium phosphate buffer, pH 6.7, and Tween 20 (0.3%) was used as the emulsifier. Supernatant (100 μL) was injected into a 1-mL reaction chamber containing the linoleic acid substrate. The maximum rate of oxygen consumption (shortly after the addition of the extract) was used as the measure of the enzymatic activity. The results are presented as a percentage of the activity in the raw material extract. The analyses were performed in duplicate.

Lipase Activity

Lipase extraction. Approximately 30 g of barley kernels was thawed and ground in a coffee grinder for 15 sec (Braun AG type:4041). A sample (1.0-g wet weight) of ground barley was suspended in 10 mL of 0.2M Tris-HCl buffer, pH 8.5, in a beaker on an ice bath and magnetically stirred for 30 min. The suspension was centrifuged for 15 sec (Eppendorf 5415) at 14,000 rpm at room temperature. The supernatant was stored on ice (maximum of 30 min) before analysis. The extractions were performed in duplicate.

Measurement of lipase activity. Lipase activity was measured by a fluorescence method first described by Heltved (1984) and later modified by Ekstrand et al (1992). Wheat germ lipase (Sigma Chemical Co., St. Louis, MO) was used as a reference to create a standard curve for lipase activity. The results are presented as a percentage of the activity in the raw material extract. The analyses were performed in duplicate.

Phytase Activity

Phytase extraction. Approximately 30 g of whole barley kernels was thawed, ground in a coffee grinder (Braun AG type:4041) for 30 sec, and 0.4 g was analyzed in duplicate for dry matter with a moisture balance (Precisa HA 300, Zurich, Switzerland). Phytase from the seeds (2-g wet weight) was extracted in duplicate by adding 20 mL of 0.1M sodium acetate buffer at 4°C , pH 5.0, according to Konietzny et al (1995). The samples were magnetically stirred for 2 hr at 4°C and centrifuged at $10,000 \times g$ at 4°C for 30 min. Because the samples contained various amounts of phosphate and inositol phosphates, the supernatants were treated with AG 1-X8 anion resin (Bio-Rad Laboratories, Richmond, CA) for 10 min at 4°C to remove the compounds. Finally, the samples were centrifuged again, and the supernatants were used as enzyme extracts.

Measurement of phytase activity. Phytase activity was measured by incubating the enzyme extract with sodium phytate at pH 5.2 and 47°C for 1 hr, which is optimal for phytase activity in barley extract (E.-L. Bergman and A.-S. Sandberg, unpublished data). The proportions of enzyme extract, sodium phytate, and buffer in the reaction mixture were equal to the proportion used by Lolos and Markakis (1977). The reaction mixture contained 2 mL of enzyme extract, 0.8 mL of 15 mM sodium phytate solution (previously adjusted to pH 5.2 with 2M HCl), and 9.2 mL of 0.13M citrate buffer (pH 5.2) in a final volume of 12 mL. The final concentration of buffer and phytate were 0.1M and 1 mM, respectively. After incubation, 4 mL of 2M HCl was added to stop enzymatic activity. The samples were evaporated to dryness and analyzed for inositol phosphates as described below. Phytase activity was calculated as degraded inositol hexaphosphate per minute and gram of dry matter and was related to the phytase activity in the raw material extract as percent remaining activity.

TABLE I
Soaking Conditions for Barley in Scaled and Real Values^a

Experiment	Scaled Value			Real Value		
	x_1	x_2	x_3	Lactic Acid (% v/v)	Temp. ($^\circ\text{C}$)	Time (min)
1	-1.0	1.0	1.0	0	70	120
2	1.0	1.0	1.0	1	70	120
3	-1.0	-1.0	1.0	0	45	120
4	1.0	-1.0	1.0	1	45	120
5	-1.0	1.0	-1.0	0	70	30
6	1.0	1.0	-1.0	1	70	30
7	-1.0	-1.0	-1.0	0	45	30
8	1.0	-1.0	-1.0	1	45	30
9	0.0	0.0	0.0	0.5	57	75
10	0.0	0.0	0.0	0.5	57	75
11	0.0	0.0	0.0	0.5	57	75
12	-1.0	0.0	0.0	0	57	75
13	1.0	0.0	0.0	1	57	75
14	0.0	-1.0	0.0	0.5	45	75
15	0.0	1.0	0.0	0.5	70	75
16	0.0	0.0	-1.0	0.5	57	30
17	0.0	0.0	1.0	0.5	57	120
18	0.0	0.2	0.6	0.5	60	100

^a $x_1 = (\text{Lactic acid} - 0.5)/0.5$, where lactic acid ranges from 0.0 to 1.0% (v/v); $x_2 = (\text{temperature} - 57)/12.5$, where temperature ranges from 45 to 70°C ; $x_3 = (\text{time} - 75)$, where time ranges from 30 to 120 min.

Determination of inositol phosphates. The evaporated samples were redissolved in 0.025M HCl (15 mL). The inositol phosphates were separated from the crude extract by ion-exchange chromatography (Sandberg and Ahderinne 1986). Inositol hexa-, penta-, tetra-, and triphosphates were determined by ion-pair C18 reverse-phase HPLC with formic acid-methanol and tetrabutylammoniumhydroxide as the mobile phase (Sandberg and Ahderinne 1986, Sandberg et al 1989). The HPLC comprised an HPLC pump (Waters Model 510, Waters Associates Inc., Milford, MA), a Chromasil ODS (5 µm, column length 150 mm, 2 mm i.d.), and a refractive index detector (ERC-7510 RI-detector, Erma Optical Works Ltd., Tokyo, Japan). The flow rate was 0.4 mL/min. Retention times and peak areas were measured by the laboratory data system, HP 3350 (Hewlett Packard Ltd., Palo Alto, CA). Injections were made with a 20-µL loop.

Data Analysis

Central composite designs make it possible to approximate the measured data (y_{obs}) using a response surface model (RSM, equation 1) expressed in scaled variables:

$$y_{obs} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1x_1 + b_{22}x_2x_2 + b_{33}x_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + e \quad (1)$$

$$e = y_{obs} - y_{calc} \quad (2)$$

where b_0 is a constant; b_1 , b_2 , and b_3 express the main effect of each process variable; b_{12} , b_{13} , and b_{23} show the interaction effects between the variables and the square coefficients; and b_{11} , b_{22} , and b_{33} reveal whether any of the variables give a maximum or minimum within the experimental domain. The difference between the experimental data (y_{obs}) and the model (y_{calc}) gives the residual (e) in Eq. 2 (Lindgren et al 1995). The results were examined by the computer program MODDE version 3.0 (Umetri AB, Umeå, Sweden). The RSM were estimated by multiple linear regression (MLR) for the 17 experiments in the CCF design. The replicates at the center point made it possible to estimate the pure error of the analyses, which was used to predict whether the models gave significant lack-of-fit (Carlsson 1992). The reliability of the models was evaluated by calculating the R^2 and Q^2 values for each model, where R^2 is the variation of the response explained by the model and Q^2 is the fraction of the variation of the response that can be predicted by the model (MODDE version 3.0). Q^2 should be >0.5 if conclusions are to be drawn from the model (Lindgren 1995). Generally, a model is considered excellent if R^2 and Q^2 exceed 0.9 (Lindgren et al 1995). A verification experiment was performed to estimate the predictive capacity of the models. Both the measured and predicted values are presented for each model.

RESULTS AND DISCUSSION

Water Content and pH

The water content and pH at the end of the 18 experiments are shown in Table II. Complete RSM, including the 17 experiments in the design, were estimated. The results from the measurements were evaluated statistically by MLR. Factors during the soaking that had a significant influence ($P < 0.05$) on the water content included temperature (x_2) and time (x_3). The model showed no significant lack-of-fit: $R^2 = 0.98$, and $Q^2 = 0.87$. The water content of the verification experiment agreed with the predicted value, falling within the confidence interval (Table III). The lactic acid concentration (x_1) and the square term of the lactic acid concentration (x_1x_1) during soaking significantly ($P < 0.05$) influenced the measured pH. The model showed no significant lack-of-fit: $R^2 = 0.98$, and $Q^2 = 0.82$. The pH of the verification experiment agreed with the predicted value, falling within the confidence interval (Table III).

Lipoxygenase Activity

The results of the 17 experiments and verification experiment 18 are shown in Table II. The results were evaluated statistically by MLR. A complete RSM, including the 17 experiments in the design, was estimated. Because the mathematical analysis (normal probability plot) showed that experiment 12 was an outlier, it was excluded. The achieved mathematical model, expressed in scaled variables, was:

$$y_{calc} (\% \text{ lipoxygenase activity compared to raw material}) = 8.0 - 3.8x_1 - 31.2x_2 - 10.3x_3 - 8.6x_1x_1 + 26.3x_2x_2 + 7.0x_3x_3 + 5.7x_1x_2 - 3.0x_1x_3 + 7.3x_2x_3 \quad (3)$$

The temperature (x_2), square terms of temperature (x_2x_2), time (x_3), and interaction term of temperature and time (x_2x_3) during the soaking significantly ($P < 0.05$) influenced lipoxygenase activity. The model showed no significant lack-of-fit (Table IV), and the estimation of the model gave $R^2 = 0.98$ and $Q^2 = 0.75$. The lipoxygenase activity of the verification experiment agreed with the predicted value, falling within the confidence interval (Table III).

Lipase Activity

The results of the 17 experiments and verification experiment 18 are shown in Table II. The results were evaluated statistically by MLR. A complete RSM, including the 17 experiments in the design, was estimated. The achieved mathematical model, expressed in scaled variables, was:

$$y_{calc} (\% \text{ lipase activity compared to raw material}) = 27.7 - 12.1x_1 - 27.3x_2 - 7.3x_3 + 4.1x_1x_1 + 19.7x_2x_2 + 0.6x_3x_3 + 9.7x_1x_2 - 2.5x_1x_3 + 5.2x_2x_3 \quad (4)$$

TABLE II
Water Content, pH, and Remaining Activity of Lipoxygenase, Lipase, and Phytase after Processing of Barley

Experiment	Water Content (%)	pH	Lipoxygenase Activity ^a (%)	Lipase Activity ^a (%)	Phytase Activity ^a (%)
1	35	5.7	-1	22	39
2	33	4.1	1	19	23
3	27	5.5	62	96	99
4	25	4.3	30	38	94
5	26	5.7	2	30	11
6	26	4.5	5	21	20
7	20	5.7	83	109	113
8	19	4.5	74	77	120
9	27	4.8	9	31	57
10	26	4.7	3	23	44
11	26	4.7	5	24	51
12	26	5.6	91	45	54
13	26	4.5	2	25	75
14	23	4.7	72	72	129
15	29	4.5	2	27	35
16	23	4.8	35	37	111
17	28	4.7	3	26	29
18	29	4.7	-1	26	29

^a Enzyme activities are given as percent activity in relation to activity in raw material extract.

TABLE III
Measured and Predicted Values^a for pH, Water Content, and Enzyme Activities for the Verification Experiment^b

	Measured Value	Predicted Value
pH	4.7	4.7 ± 0.1
Water content	29%	29 ± 1%
Lipoxygenase activity	-1%	0 ± 8%
Lipase activity	26%	20 ± 7%
Phytase activity	29%	36 ± 10%

^a The values were calculated by response surface modeling using the computer program MODDE 3.0 (Umetri AB, Umeå, Sweden).

^b Experiment 18: lactic acid conditions 0.5% (v/v), temperature 60°C, and time 100 min.

The temperature (x_2), lactic acid concentration (x_1), square terms of temperature (x_2x_2), interaction term of lactic acid concentration and time (x_1x_3), and time (x_3) during soaking significantly ($P < 0.05$) influenced lipase activity. The model showed no significant lack-

of-fit (Table IV), and the estimation of the model gave $R^2 = 0.98$ and $Q^2 = 0.69$. The lipase activity of the verification experiment agreed with the predicted value, falling within the confidence interval (Table III).

Phytase Activity

The results of the 17 experiments and verification experiment 18 are shown in Table II. The results were evaluated statistically by MLR. A complete RSM, including the 17 experiments in the design, was estimated. Experiment 16 was an outlier in the normal probability plot and was excluded from the evaluation. The achieved mathematical model, expressed in scaled variables, was:

$$y_{\text{calc}} (\% \text{ phytase activity compared to raw material}) = 50.9 + 1.6x_1 - 42.7x_2 - 0.7x_3 + 10.3x_1x_1 + 29.6x_2x_2 - 25.6x_3x_3 - 1.2x_1x_2 - 4.6x_1x_3 + 8.9x_2x_3 \quad (5)$$

The temperature (x_2), square terms of temperature and time (x_2x_2 and x_3x_3 , respectively), and interaction term of temperature and time (x_2x_3) during soaking significantly ($P < 0.05$) influenced phytase activity. The model showed no significant lack-of-fit (Table IV), and the estimation of the model gave $R^2 = 0.98$ and $Q^2 = 0.85$. The phytase activity of the verification experiment agreed with the predicted value, falling within the confidence interval (Table III).

Optimization of the Soaking Process

A high temperature during soaking inactivated all three enzymes: lipoxygenase, lipase, and phytase. The addition of lactic acid to the deionized water played a significant role in inactivating lipase. Lactic acid concentration did not significantly influence inactivation of either lipoxygenase or phytase. Hugues et al (1994) reported that at a lower pH, within a pH range of 4.65–7.0, the barley lipoxygenase from ungerminated barley was less stable against heat in the temperature interval of 50–65°C. It is unclear to what extent lactic acid concentration influenced pH within the kernels in the current experiments, but obviously, the lactic acid concentration affected lipase inactivation. Because lipase inactivation was facilitated by a high lactic acid concentration, 1% lactic acid was chosen for prediction of optimal conditions with respect to temperature and time.

Response surface plots for lipoxygenase, lipase, and phytase activities, in which the lactic acid concentration was set to 1.0%, are shown in Fig. 1A–C. Lipoxygenase was the most unstable enzyme of the three, followed by lipase. Phytase was the most stable enzyme during the soaking. As can be seen in Fig. 1A, complete inactivation of lipoxygenase was achieved within the experimental domain. Soaking for at least 50 min at 65°C was needed, according to the response surface plot, to inactivate lipoxygenase completely; 120 min was needed at 55°C. This agrees with the findings of Lulai and Baker (1976) who reported that lipoxygenase in aqueous extracts from ungerminated barley was rapidly inactivated at 65–70°C.

A response surface plot for lipase activity is shown in Fig. 1B. According to the response surface plot, complete inactivation of lipase could not be achieved within the experimental domain. The lowest lipase activity was achieved after soaking for 120 min at a temperature between 60 and 65°C. Soaking for at least 52 min at 65°C was needed to inactivate lipase to <20% of the original activity; at least 100 min was needed at 55°C.

A response surface plot for phytase activity is shown in Fig. 1C. According to the response surface plot, the highest phytase activity was achieved after soaking for about 70 min at the lowest temperature in the experimental domain. Throughout the temperature interval, there seemed to be optimum phytase activity after ≈60–80 min of soaking. The fact that the phytase activity in samples soaked at 45°C was higher than in the raw material when the soaking was shorter than 120 min (Table II) was unexpected. This might be due to an activation of phytase during soaking or pos-

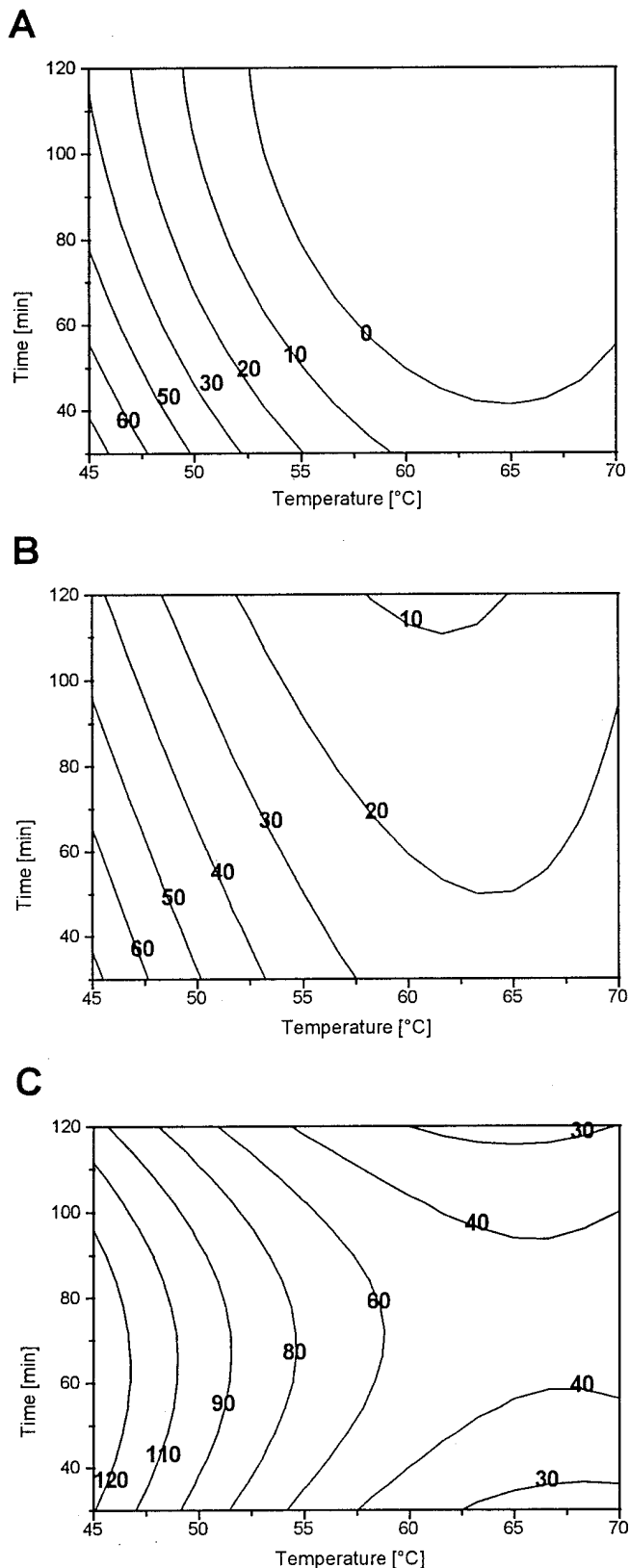


Fig. 1. Response surface plots, at 1% lactic acid concentration for **A**, lipoxygenase activity, **B**, lipase activity, and **C**, phytase activity in barley after soaking, based on equations 3, 4, and 5, respectively.

sibly to more efficient extraction of the enzyme from the barley after soaking than from the raw material. Activation of phytase during incubation of barley grains also was found by Gabard and Jones (1986).

There are some peculiarities in all three response surface plots (Fig. 1A–C), especially at higher temperatures. For instance, according to Fig. 1B, a longer time is needed at 70°C than at 65°C to achieve the same inactivation of lipase. However, the confidence intervals for the predicted enzyme activities were $\approx 10\%$ (Table III) and, for lipase, 7%. The confidence intervals differed within the experimental domain though, because a model established by an orthogonal central composite design yielded predictions with smaller variance for variables set near the center than for variables set toward the periphery of the explored domain (Carlsson 1992). Furthermore, as can be seen in Fig. 1B, the time-temperature dependence is much weaker at the higher temperatures. Taken altogether, this indicates that the difference in the amount of time needed to inactivate lipase at 65°C compared to 70°C, as shown in the figure, is not significant.

When Fig. 1A–C are superimposed (see Fig. 2), an area in the response surface plot meets the criteria of complete inactivation of lipoxygenase and $>60\%$ remaining phytase activity. Complete inactivation of lipase, however, could not be reached at the same time, but achievement $<20\%$ remaining lipase activity was possible. Experiment 13 was done on the border line of this area, at the limit for 20% remaining lipase activity. As shown in Table II, the remaining activities of lipoxygenase, lipase, and phytase were 2, 25, and 75%, respectively, for experiment 13, which agrees with the criteria described above.

Complete inactivation of lipase was not achieved, but complete inactivation of lipoxygenase is the more important factor with regard to flavor stability of cereal products. Further lipase inactivation possibly could be achieved during additional processing at a lower temperature, when the remaining phytase could degrade the phytate. A temperature of $\approx 57^\circ\text{C}$ is optimal for phytase activity in barley flour (E.-L. Bergman and A.-S. Sandberg, unpublished data), and a high lactic acid concentration probably would still be favorable for lipase inactivation. Fig. 1B shows that lipase is considerably inactivated at 57°C , but based on these experiments, it is difficult to tell what time would be needed for complete lipase inactivation in the second phase of processing. The

time needed in the additional processing also depends on how fast phytate degrades to a desirable level.

CONCLUSIONS

We conclude that almost complete inactivation of lipoxygenase and good preservation of phytase can be achieved by soaking whole barley kernels in a 1% lactic acid solution at suitable time-temperature combinations of 70–110 min and $53\text{--}58^\circ\text{C}$. Under such conditions, it also is possible to achieve 80% inactivation of lipase.

ACKNOWLEDGMENTS

This study was supported financially by the Nordic Industrial Foundation, Oy Lahden Polttimo Ab, Semper AB, SL Foundation and Nutek.

LITERATURE CITED

- Bartnik, M., and Szafranska, I. 1987. Changes in phytate content and phytase activity during the germination of some cereals. *J. Cereal Sci.* 5:23-28.
- Biltcliffe, D., Judd, H., and Wood, R. 1984. Convection oven determination of loss of mass on drying of quick frozen French fried potatoes. *J. AOAC* 67:635-636.
- Brockenhoff, H., and Jensen, R. G. 1974. Lipases. Pages 25-175 in: *Lipolytic Enzymes*. Academic Press: New York.
- Brune, M., Rossander-Hulthén, L., Hallberg, L., Glerup, A., and Sandberg, A.-S. 1992. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J. Nutr.* 122:442-449.
- Carlsson, R. 1992. Response surface methods. Pages 259-260 in: *Design and Optimization in Organic Synthesis*. Elsevier Science: Amsterdam.
- Cosgrove, D. J. 1980. Phytase. Pages 85-97 in: *Inositol Phosphates their Chemistry, Biochemistry, and Physiology*. D. J. Cosgrove, ed. Elsevier Scientific Publishing: Amsterdam.
- Ekstrand, B., Gangby, I., and Åkesson, G. 1992. Lipase activity in oats—Distribution, pH dependence, and heat inactivation. *Cereal Chem.* 69:379-381.
- Eriksson, C. E., and Svensson, S. G. 1970. Lipoxygenase from peas, purification and properties of the enzyme. *Biochim. Biophys. Acta* 198:449-459.
- Gabard, K. A., and Jones, R. L. 1986. Localization of phytase and acid phosphatase isoenzymes in aleurone layer of barley. *Physiol. Plant* 67:182-192.
- Gardner, H. W. 1988. Lipoxygenase pathway in cereals. Pages 161-215 in: *Advances in Cereal Science and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Hallberg, L., Brune, M., and Rossander, L. 1989. Iron absorption in man: Ascorbic acid and dose-dependent inhibition by phytate. *Am. J. Clin. Nutr.* 49:140-144.
- Heaney, R. P., Weaver, C. M., and Fitzsimmons, M. L. 1991. Soybean phytate content: Effect on calcium absorption. *J. Clin. Nutr.* 53:745-747.
- Heltved, F. 1984. Spectrophotometric assay for hydrolytic activity in germinating wheat. *J. Cereal Sci.* 2:179-185.
- Hugues, M., Bovin, P., Gauillard, F., Nicolas, J., Thiry, J.-M., and Richard-Forget, F. 1994. Two lipoxygenases from germinated barley—heat and kilning stability. *J. Food Sci.* 59:885-889.
- Konietzny, U., Greiner, R., and Jany, K.-D. 1995. Purification and characterization of a phytase from spelt. *J. Food Biochem.* 18:165-183.
- Lindgren, Å. 1995. Multivariate structure-property-relationships of non-ionic surfactants. PhD thesis. Umeå University: Umeå, Sweden.
- Lindgren, Å., Sjöström, M., Hellsten, M., and Lif, A. 1995. Modelling of detergency performance for some technical nonionic surfactants. *Tenside Surf. Det.* 32:300-309.
- Lingnert, H. 1992. Influence of food processing on lipid oxidation and flavor stability. Pages 292-301 in: *Lipid Oxidation in Food*. A. J. St. Angelo, ed. American Chemical Society: Washington, DC.
- Lolas, G. M., and Markakis, P. 1977. The phytase of navy beans (*Phaseolus vulgaris*). *J. Food Sci.* 42:1094-1106.
- Lolas, G. M., Palamidis, N., and Markakis, P. 1976. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. *Cereal Chem.* 53:867-871.
- Lönnerdal, B., Bell, J. G., Hendricks, A. G., Burns, R. A., and Keen, C. L. 1988. Effect of phytate removal on zinc absorption from soy formula. *Am. J. Clin. Nutr.* 48:1301-1306.
- Lulai, E. C., and Baker, C. W. 1976. Physicochemical characterization of

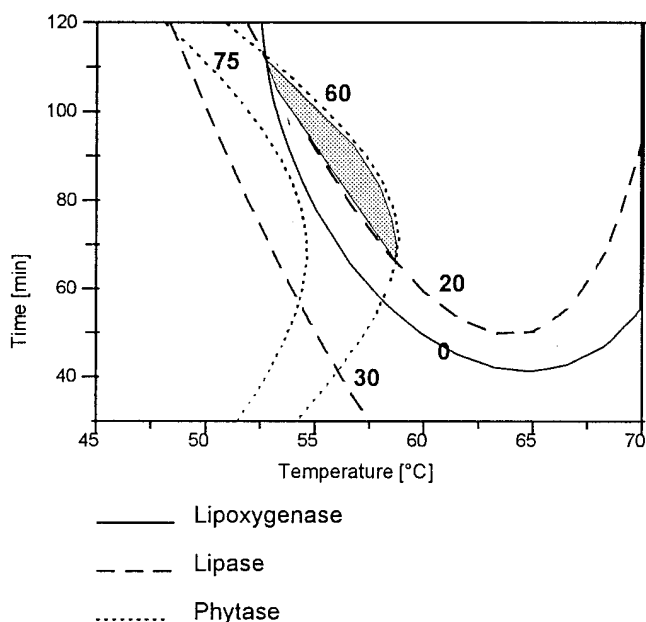


Fig. 2. The optimal area (shaded area) of the soaking process for barley at 1% lactic acid concentration, achieved by superimposing Fig. 1A–C.

- barley lipoxygenase. *Cereal Chem.* 53:777-786.
- Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *J. Agric. Food Chem.* 30:1-9.
- Morrison, W. R. 1978. Cereal lipids. Pages 205-348 in: *Advances in Cereal Science and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Nävert, B., Sandström, B., and Cederblad, Å. 1985. Reduction of the phytate content of bran by leavening in bread and its effect on absorption of zinc in man. *Br. J. Nutr.* 53:47-53.
- Peers, F. G. 1953. The phytase of wheat. *Biochem. J.* 53:102-110.
- Ranhotra, G. S., Gelroth, J. A., Astroth, K., and Bhatta, R. S. 1991. Relative lipidemic responses in rats fed barley and oat meals and their fractions. *Cereal Chem.* 68:548-551.
- Rossander, L., Sandberg, A.-S., and Sandström, B. 1992. The influence of dietary fibre on mineral absorption and utilisation. Pages 197-216 in: *Dietary Fibre—A Component of Food: Nutritional Function in Health and Disease*. T. Schweizer and C. A. Edwards, eds. Springer-Verlag: London.
- Sandberg, A.-S. 1991. The effect of food processing on phytate hydrolysis and availability of iron and zinc. Pages 499-508 in: *Nutritional and Toxicological Consequences of Food Processing*. M. Friedman, ed. Plenum Press: New York.
- Sandberg, A.-S., and Ahderinne, R. 1986. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.* 51:547-550.
- Sandberg, A.-S., and Andersson, H. 1988. The effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. *J. Nutr.* 118:469-473.
- Sandberg, A.-S., Andersson, H., Carlsson, N.-G., and Sandström, B. 1987. Degradation products of bran phytate formed during digestion in the human small intestine. Effect of extrusion cooking on digestibility. *J. Nutr.* 117:2061-2065.
- Sandberg, A.-S., Carlsson, N.-G., and Svanberg, U. 1989. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *J. Food Sci.* 54:159-161, 186.
- Sandström, B. M., and Sandberg, A.-S. 1992. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *J. Trace Elem. Electrolytes Health Dis.* 6:99-103.
- Sundberg, B. 1995. Barley for food. PhD thesis. Swedish University of Agricultural Sciences: Uppsala, Sweden.
- Torre, M., Rodriguez, A. R., and Saura-Calixto, F. 1991. Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* 1:1-22.
- Yabuuchi, S. 1976. Occurrence of a new lipoxygenase isoenzyme in germinating barley embryos. *Agric. Biol. Chem.* 10:1987-1992.

[Received December 18, 1996. Accepted July 12, 1997.]