# Wheat Germ in Breadmaking. II. Improving Breadmaking Properties by Physical and Chemical Methods[1]

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**ABSTRACT**

Raw germ decreased loaf volume of white bread, whereas up to 15% glutathione-inactivated germ or equiprotein amounts of dialyzed sodium chloride extracts of germ had no deleterious effect. The addition of free polar wheat flour lipids increased loaf volume of bread nutritionally enriched with germ or germ proteins. High levels of sucrose monomyristate had deleterious effects on gassing power and on proof height and loaf volume in controls and in germ-enriched bread. Phosphatidyl choline (lecithin) had no effect on loaf volume of control bread, but increased substantially loaf volume of bread nutritionally enriched with up to 30% germ or 9% germ proteins. Results were best if the lecithin-to-germ ratio was between 1:10 and 1.5:10. Several phospholipids varied in their effects on loaf volume and crumb grain. Inositol phosphatide, synthetic DL alpha-lecithin, and phosphatidyl ethanolamine increased loaf volume most; phosphatidyl serine had little effect.

Rohrlich and Bruckner (1) reported that improving nutritional value of bread effectively requires the addition of 8 to 10% germ. According to Kent-Jones and Mitchell (2), germ bread should contain at least 10% of processed wheat germ, calculated on dry basis of the bread.

High levels of raw wheat germ have a deleterious effect in breadmaking. Several theories have been proposed to explain the action and mechanism by which germ components affect breadmaking. Means to counteract the detrimental effects of germ in breadmaking were known long before the identity of the components was established. The harmful effect is decreased by several treatments, such as heating, increasing fermentation time, and increasing oxidant levels, and certain of their combinations.

Investigations of Sullivan (3) have shown that wheat endosperm, but not wheat germ, contains lipids important in breadmaking. The deleterious effects of glutathione in germ were demonstrated by Sullivan et al. in 1936 (4). Most later investigations on the effects of germ in breadmaking have centered around assay of glutathione and the mechanism of its action and inactivation. Sullivan et al. (5) recommended heating wheat germ in a stoppered container at 80°C. to eliminate the undesirable effects of glutathione. Autoxidation of thiol groups in glutathione

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was believed to take place. Effects of oxidants, pH and composition of dough, and temperature on glutathione oxidation were studied by Ziegler (6). Hullet and Stern (7) reported that glutathione was eliminated enzymatically during fermentation. According to Stern (8), fermentation decreases soluble and protein-bound thiols in wheat germ. The oxidation of the thiols in fermenting dough was attributed to action of dehydrogenase. Smith and Geddes (9) found that increasing fermentation time or oxidant level, or both, decreased the harmful effects of 5 to 10% germ.

Several investigators reported beneficial effects of germ or germ components in breadmaking. According to Grewe and LeClerc (10), adding small amounts of steeped germ and potassium bromate has an improving effect. Greer et al. (11) identified in yeast-fermented germ two benzoquinone derivatives that acted as bread improvers.

Previous studies from our laboratories showed that synthetic sucroesters counteracted deleterious effects on loaf volume and crumb grain of high levels of soy flour (12); the improvement of wheat germ-enriched bread baked without shortening was small (13). The purpose of this study was to investigate the use of physical treatments and chemical additives in the baking of white flour doughs that were nutritionally enriched with wheat germ.

## MATERIALS AND METHODS

### Flour

The flour, designated as Regional Baking Standard (RBS), was milled from a composite of many wheat varieties that were harvested at numerous locations throughout the southern, central, and northern Great Plains of the United States in 1968. The flour (14% m.b.) had a protein content of 12.7% (N X 5.7), ash content of 0.42%, good loaf-volume potential, and medium mixing time.

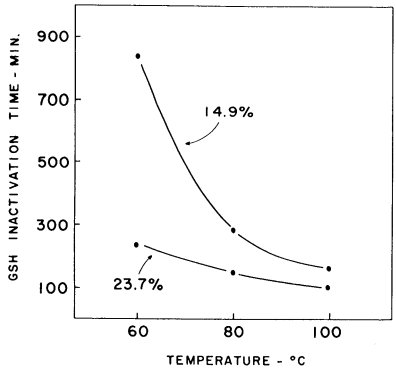
### Germ and Germ Extracts

For baking, unless stated otherwise, glutathione-inactivated germ was used. The heat-treated germ was dried to about 4% moisture, and most lipid was extracted with petroleum ether in 1-lb. batches in a Soxhlet. The low-lipid germ was ground on a Hobart mill and re-extracted with petroleum ether.

Temperature and time required to inactivate glutathione depended on moisture content (Fig. 1). Unless stated otherwise, germ with a moisture content of 14.9% was heat-treated at 80°C. for 8 hr. The heat-treated germ was dried to 3.6% moisture, and contained (on as-is basis) 33.7% protein (N X 5.45), 5.6% ash, 0.5% crude fat, and 2.3% crude fiber. Germ extracts used were obtained as described in Part I of this investigation (14).

### Lipids

Sucrose monomyristate, mono-oleate, and monotallowate were purified products from Colonial Sugars, Gramercy, La. Phospholipids were from Nutritional Biochemicals Corp., Cleveland, Ohio. According to the manufacturer's data, the refined soy lecithin was 90% pure; the synthetic beta-gamma dipalmitoyl DL alpha cephalin and the synthetic beta-gamma dipalmitoyl DL alpha lecithin were each over 99% pure. The phosphatidyl ethanolamine had an amino-N of 1.55%, total N of 1.70%, and alpha amino-N below 0.02%. Inositol phosphatide was 92% pure, and



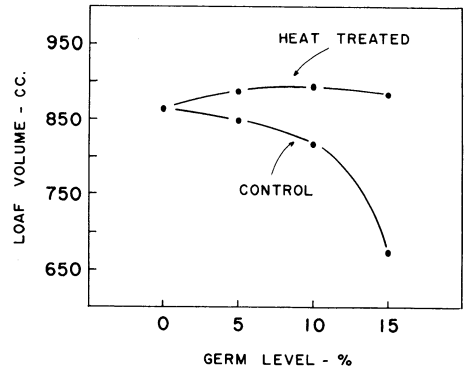




**Fig. 1 (left). Effects of germ moisture (14.9 or 23.7%) and temperature on time required for glutathione inactivation.**

**Fig. 2 (right). Loaf volume of bread baked with 100 g. flour and various amounts (% on flour basis) of raw and heat-treated germ.**

alpha amino-N was less than 1.5%; choline, cholesterol, and carbohydrates were each less than 0.1%. Elemental analysis of phosphatidyl serine gave 55% C and 7% P; the alpha amino-N was less than 0.1%, inorganic-P below 0.05%, and the material was choline-free. Polar wheat flour lipids were prepared from the petroleum-ether (b.p. 35° to 60°C.) extract of flour, by fractionation, on a silicic acid column as described previously (15).

#### Analytical Procedure

Moisture, ash, protein, crude fat, and crude fiber were determined as described in AACC Approved Methods (16). Inactivation of glutathione in germ was determined by the nitroprusside method[5]: 2 g. of germ was shaken with 12 ml. of a saturated ammonium sulfate solution, allowed to stand 10 min., and filtered. To the filtrate, 5 to 6 drops of a 2% sodium nitroprusside solution was added, followed by 2 ml. of concentrated ammonium hydroxide. The presence of glutathione was indicated by a clear purple color.

Pressuremeters were used for gassing-power determinations. Gassing power was determined on doughs prepared from 10 g. of flour and all ingredients (except shortening) used in the baking formula. The water-absorption of 100% was used. The doughs were fermented for 5 hr. and the pressure was determined at 1-hr. intervals.

The baking procedures of Finney and Barmore (17,18) for 100 g. flour, and the adaptation of Shogren et al. (19) for 10 g. flour were used. In samples containing wheat germ, nonfat dry milk was omitted. All baking tests were made in duplicate; average-of-replicate differences of 25 and 1.75 cc. were significant at the 0.05% level in loaves baked by the 100-g. and 10-g. methods, respectively.

[5]Private communication from E. Levin, Viobin Corp., Monticello, Ill.

## RESULTS AND DISCUSSION

The deleterious effects of raw germ on loaf volume are shown by the data summarized in Fig. 2. The addition of raw germ lowered loaf volume; the deleterious effect increased with increase in level of raw germ. Loaf volume of germ bread was consistently higher with glutathione-inactivated germ than with untreated germ. In bread baked with up to 15% heat-treated germ, no decrease of loaf volume was observed. Water-absorption increased at a rate of approximately 1% water per 1% heat-treated wheat germ. Mixing time decreased in germ-enriched doughs; the decrease depended on the germ level and was about 0.5 min. in doughs containing 15% heat-treated germ. Bromate requirement increased from 10 p.p.m. in controls (flour with no nonfat dry milk) to 70 p.p.m. in doughs with 15% heat-treated germ. The bromate requirement increased even more in doughs with raw germ; yet the additional oxidant eliminated only in part the deleterious effects of raw germ.

Previous studies (20) have shown that free polar flour lipids increased loaf volume. That effect is shown in Table I for the flour used in this investigation and baked without nonfat dry milk. A similar increase was determined when free polar flour lipids were added to bread baked with up to 15% heat-treated germ (Table II), or 4.5% of a sodium chloride germ extract (Table III). On an equiprotein basis, loaf volume of bread baked with a sodium chloride extract of germ was higher than that of bread baked with heat-treated germ. The higher loaf volume of bread baked with a germ-protein extract was obtained with or without added free polar flour lipids.

Calcium chloride extracts of germ were comparable, in their effects on loaf volume, to sodium chloride extracts of germ. In the case of calcium chloride extracts, dialysis to remove most of the salt was essential. Sodium chloride also was

TABLE I. EFFECTS OF POLAR WHEAT FLOUR LIPIDS ON LOAF VOLUME OF BREAD BAKED WITHOUT GERM

| Polar Lipids % | Loaf Volume cc. |
|---|---|
| 0 | 864 |
| 0.2 | 875 |
| 0.4 | 913 |
| 0.8 | 915 |
| 2.0 | 915 |

TABLE II. EFFECTS OF POLAR WHEAT FLOUR LIPIDS ON LOAF VOLUME OF BREAD BAKED WITH HEAT-TREATED GERM

| Germ Level % | Polar Lipids % | Loaf Volume cc. | Germ Level % | Polar Lipids % | Loaf Volume cc. |
|---|---|---|---|---|---|
| 0 | 0 | 864 | 15 | 0 | 885 |
| 5 | 0 | 890 | 15 | 0.8 | 925 |
| 5 | 0.2 | 900 | 15 | 1.0 | 945 |
| 10 | 0 | 895 | 15 | 2.0 | 955 |
| 10 | 0.4 | 930 | | | |

TABLE III. EFFECTS OF POLAR WHEAT FLOUR LIPIDS ON LOAF VOLUME OF BREAD BAKED WITH A SODIUM CHLORIDE GERM EXTRACT

| Germ Extract % | Polar Lipids % | Loaf Volume cc. | Germ Extract % | Polar Lipids % | Loaf Volume cc. |
|---|---|---|---|---|---|
| 0 | 0 | 864 | 4.5 | 0 | 942 |
| 1.5 | 0 | 913 | 4.5 | 0.2 | 960 |
| 1.5 | 0.2 | 903 | 4.5 | 0.4 | 985 |
| 3.0 | 0 | 958 | 4.5 | 0.8 | 1,005 |
| 3.0 | 0.4 | 956 | | | |

TABLE IV. EFFECTS OF SUCROSE MONOMYRISTATE ON LOAF VOLUME OF BREAD BAKED WITH GERM OR GERM EXTRACT

| | Germ or Germ Extract % | Sucrose Monomyristate % | Loaf Volume cc. |
|---|---|---|---|
| Wheat flour only | 0 | 0 | 864 |
| | 0 | 0.5 | 810 |
| | 0 | 1.0 | 800 |
| | 0 | 1.5 | 730 |
| | 0 | 2.0 | 710 |
| With petroleum ether-extracted germ | 5 | 0 | 850 |
| | 5 | 0.5 | 805 |
| | 5 | 1.0 | 660 |
| | 10 | 0 | 820 |
| | 10 | 1.0 | 745 |
| | 15 | 0 | 675 |
| | 15 | 1.5 | 660 |
| With petroleum ether-extracted and heat-treated germ | 15 | 0 | 885 |
| | 15 | 0.5 | 895 |
| | 15 | 1.0 | 840 |
| | 15 | 2.0 | 725 |
| | 30 | 0 | 813 |
| | 30 | 1.5 | 745 |
| With NaCl germ extract | 1.5 | 0 | 913 |
| | 1.5 | 1.0 | 715 |
| | 3.0 | 0 | 958 |
| | 3.0 | 1.0 | 905 |
| | 4.5 | 0 | 942 |
| | 4.5 | 0.8 | 860 |
| | 4.5 | 1.5 | 825 |

a more effective protein extractant than calcium chloride. Consequently, most baking experiments with germ extracts were made with sodium chloride-extracted proteins.

Adding high levels of sucrose-monomyristate lowered loaf volume substantially (Table IV). The decrease was determined in bread baked without germ, with

defatted germ, with defatted and heat-treated germ, and with a sodium chloride extract of germ. The deleterious effects of high levels of sucrose monomyristate resulted, in part at least, from impaired fermentation. Proof heights of doughs containing 1.5 to 2.0% sucrose monomyristate were substantially lower than those of controls containing no sucroesters.

Sucrose monotallowate added at levels of 1.5 to 2.5 g. to doughs made from 100 g. flour and 15 g. wheat germ was much less deleterious to proof height and loaf volume than sucrose monomyristate; sucrose mono-oleate had a slight improving effect. Bread baked without added wheat germ and 2.0% sucroesters showed a similar pattern. However, in bread baked with 8% soy flour, both sucrose monomyristate and sucrose mono-oleate were excellent improvers. Gassing-power determinations confirmed determinations of proof height. Sucrose monomyristate lowered gas production, except in doughs containing soy flour. Lecithin and sucrose mono-oleate had no effect on gassing power. The results indicate that in soy-enriched bread, the deleterious effects of sucrose monomyristate on fermentation and loaf volume are eliminated, and the monomyristate interacts effectively to improve bread quality.

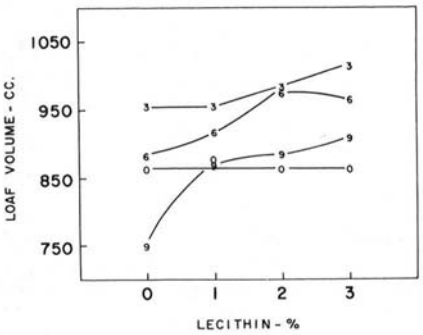
Free polar flour lipids contain phospholipids in addition to glycolipids (21). The main phospholipid in wheat flour is phosphatidyl choline (lecithin). The beneficial effects of a commercial purified soybean lecithin on loaf volume of bread nutritionally enriched with up to 9% of a sodium chloride extract of germ are summarized in Fig. 3. Adding lecithin, however, had no significant effect on loaf volume of bread baked without germ extract.

Similarly, loaf volume of bread baked with up to 30% heat-treated wheat germ was significantly increased by adding lecithin (Table V). The amount of lecithin required for maximum loaf volume increased with increase in germ level, especially in bread baked with up to 15% germ. In bread containing 10 to 30% wheat germ, loaf volume was highest if the lecithin-to-germ ratio was between 1:10 and 1.5:10. Both higher and lower lecithin levels were less effective.

Increase in loaf volume from adding lecithin to germ-enriched bread was accompanied by a slight opening of crumb grain. Adding small amounts of sucrose monomyristate counteracted somewhat the crumb-opening effect of lecithin. In soy-enriched bread an opposite effect was noted (12), however; sucroesters

TABLE V. EFFECTS OF LECITHIN ON LOAF VOLUME OF BREAD BAKED WITH HEAT-TREATED GERM

| | Loaf Volume of Bread Baked with Following Lecithin Levels | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Germ % | 0.0% cc. | 0.5% cc. | 1.0% cc. | 1.5% cc. | 2.0% cc. | 2.5% cc. | 3.0% cc. | 4.0% cc. | 6.0% cc. |
| 0 | 864 | 855 | 878 | 870 | 865 | 855 | 865 | ... | ... |
| 5 | 890 | 925 | 945 | 935 | ... | ... | ... | ... | ... |
| 10 | 895 | 940 | 943 | 1,010 | 973 | ... | 985 | ... | ... |
| 15 | 885 | 920 | 918 | 940 | 975 | 940 | ... | ... | ... |
| 20 | 865 | ... | 915 | ... | 920 | ... | 915 | 900 | ... |
| 30 | 818 | ... | 845 | ... | 840 | ... | ... | 855 | 820 |



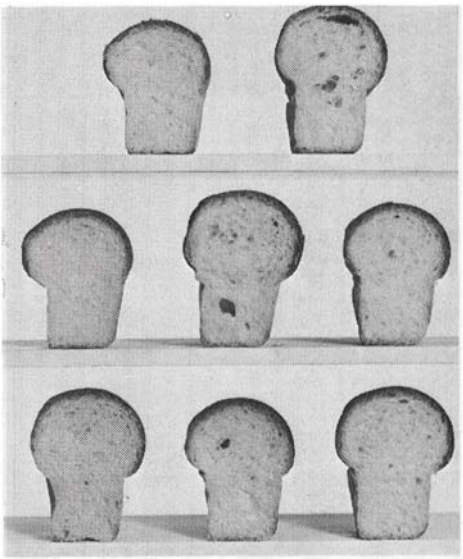



**Fig. 3 (left). Effects of partly purified lecithin on loaf volume of bread baked with 0, 3, 6, and 9% (on flour basis) of a sodium chloride extract of germ proteins.**

**Fig. 4 (right). Cut loaves of bread baked from 10 g. flour, 1 g. heat-treated wheat germ, and 0.2 g. various phospholipids. Left to right: top row, no phospholipids, polar wheat flour lipids; second row, soy lecithin, synthetic DL alpha lecithin, synthetic DL alpha cephalin; third row, phosphatidyl ethanolamine, phosphatidyl serine, inositol phosphatide.**

increased loaf volume and opened crumb grain; phospholipids had little effect on loaf volume but improved crumb grain.

Effects of various purified phospholipids on volume and crumb grain of loaves baked from 10 g. flour and 1 g. heat-treated germ are summarized in Table VI and Fig. 4. Bread baked with phosphatidyl ethanolamine was distinctly yellow, and with inositol phosphatide slightly yellow. The effects of phospholipids cannot be explained completely in terms of the charged groups, since synthetic DL alpha lecithin was a much more effective improver than soy lecithin; synthetic DL alpha cephalin (a phosphatidyl ethanolamine) had no effect, but phosphatidyl ethanolamine was an excellent improver. Variations in fatty-acid composition likely accounted for some of the observed differences. It is noteworthy, however, that whereas the negatively charged phosphatidyl serine had little effect on loaf volume of germ bread, the inositol phosphatide was an excellent improver. When the latter two phospholipids were used to replace polar wheat flour lipids in defatted flour baked with shortening, an opposite effect was recorded (22). That is, phosphatidyl serine effectively replaced wheat flour glycolipids, and inositol phosphatide had no effect. Those results, as well as the difference between the effects of sucroesters and lecithin in baking soy-enriched and germ-enriched bread, point to different modes of interaction. Those differences presumably result from variations in the size and

TABLE VI. EFFECTS OF PHOSPHOLIPIDS ON LOAF VOLUME OF BREAD BAKED FROM 10 g. FLOUR AND 1 g. GERM

| Lipid | Loaf Volume of Bread Baked with Following Phospholipid Levels | | | |
|---|---|---|---|---|
| | 0% cc. | 1.0% cc. | 1.5% cc. | 2.0% cc. |
| None (control) | 71 | | | |
| Polar wheat flour | | 81 | 78 | 79 |
| Soy lecithin | | 72 | 75 | 75 |
| DL Alpha lecithin | | 83 | 86 | 88 |
| DL Alpha cephalin | | 71 | 70 | 72 |
| Phosphatidyl ethanolamine | | 81 | 84 | 86 |
| Phosphatidyl serine | | 74 | 72 | 75 |
| Inositol phosphatide | | 86 | 86 | 90 |

composition of interacting proteins in various concentrates and their binding of various polar lipids. The relation of that binding to improvement in breadmaking is being investigated.

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