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FOOD PRODUCTS FROM CORN GERM: ENZYME ACTIVITY AND OIL STABILITY

SUMMARY—Full-fat corn germ was cooked by heated rolls at roll temperatures ranging from 99–204°C. The resulting flakes were examined for peroxidase, lipase, lipoxygenase (formerly lipoxidase) and linoleic acid-hydroperoxide-isomerase activity. Stability of the oil in germ flakes was also investigated. The amount of enzyme activity found in corn germ before roll-cooking was dependent on the post-harvest history of the corn. After picker-sheller harvest, drying the grain in heated air significantly inactivated enzymes. Cooking the germ by heated rolls at temperatures of 124°C and above completed inactivation of all the enzymes studied except peroxidase. Residual amounts of peroxidase activity remained in almost all samples. The oil in heated germ was resistant to hydrolytic rancidity when stored under nitrogen at a relative humidity as high as 75%. If the germ was stored in air at 75% relative humidity, mold developed on many of the samples. Oxidative rancidity, as measured by the peroxide value of the extracted oil, increased to about 20–40 meq peroxide/kg oil after 170 days storage only in the samples heated at or above 124°C. Low peroxide values (1.5–6 meq peroxide/kg oil) of oil from relatively unprocessed germ were postulated to result from the action of linoleic acid-hydroperoxide-isomerase. This enzyme catalyzes the conversion of hydroperoxide to products not measured by peroxide value.

INTRODUCTION

STABILITY of oil in full-fat oilseed products depends upon a complex set of variables. Oil decomposition can be caused by enzymic activity or by other catalysts present in the oil environment. Hydrolytic fat rancidity is most often caused by lipase activity. Oxidative rancidity can be caused by lipoxygenase or autoxidation, or both. Initially, either oxidation produces hydroperoxides (Privett et al., 1955; Farmer et al., 1943), which may initiate further oxidations (Lundberg, 1962) or decompose into secondary products (Keeney, 1962). Some catalysts of autoxidation promote the simultaneous formation and decomposition of hydroperoxides (Maier and Tappel, 1959). Autoxidation of oils in complex systems has been studied as functions of humidity (Tjhio et al., 1969), presence of transition metal ions (Maier and Tappel, 1959; Tjhio et al., 1969), protein (Labuza et al., 1969) and singlet oxygen (Rawls and Van Santen, 1970) to name a few of the more important variables.

Enzymes thought to influence oil stability—lipoxygenase and lipase—can be inactivated by heat in corn. According to Wagenknecht (1959), lipoxygenase activity in underblanched sweet corn induced off-flavors in fractions containing the germ. Inactivation of corn lipoxygenase by heat was also studied by Popov and Cheleev (1960). Corn lipase, located primarily in the germ, was inactivated at 65°C in water suspension by Torzhinskaya (1959).

Linoleic acid-hydroperoxide-isomerase is another enzyme which could affect the oxidative stability of corn germ lipid. Its activity in corn germ extracts converted linoleic acid hydroperoxides to hydroxy-oxooctadecenoic acids (Gardner, 1970). This enzyme has not been investigated as to its effect on oxidative stability of food products.

A study was initiated of roll-cooked corn germ to determine the conditions required to inactivate enzymes and to improve oil stability. We can now report conditions that are required to inactivate peroxidase and a number of lipid active enzymes. The effects of heat processing on hydrolytic and oxidative activity in the germ oil were also investigated.

MATERIALS & METHODS

Sources and preparation of germ

The corn germ used in roll-cooking studies was obtained from a commercial dry corn mill. The source of the corn germ and the method of roll-cooking it are described by Gardner et al. (1971).

Hand-dissected germ from a commercial hybrid dent corn grown at the University of Illinois was used only to study the effect of post-harvest drying conditions on enzyme activities. The corn, hybrid (B37TMS X H84) (Oh43RF X A619), was harvested at a 30% moisture content by a picker-sheller combine, and then dried to 15% moisture at various temperatures. After drying, the corn was stored 9–11 months at 1°C before it was used for enzyme studies. Another variety, hybrid X800 (Bear Hybrid Corn Co., Decatur, Ill.) was crib-dried.

Enzyme assays

Peroxidase activity was assayed by the method of Vetter et al. (1958b), as modified by Gardner et al. (1969). Activities were calculated as the mean of six or more replicates. The mean relative standard deviation of the values was 12% when the peroxidase activity was greater than a trace amount.

Lipoxygenase activity was determined by the rate of linoleic acid oxidation obtained in extracts of hexane-defatted germ. The oxidation procedure was described by Gardner (1970). The amount of linoleic acid oxidized at time intervals was determined by analyzing extracted portions for linoleic acid and oxidation products. The mixture of fatty acids was separated by thin-layer chromatography (TLC) as described previously (Gardner, 1970), and the weight per cent linoleic acid oxidized was determined by densitometry (Downing, 1968). Samples were analyzed in replicates of three to four. The mean relative standard deviation of the method was 10% when active samples were analyzed.

Linoleic acid hydroperoxide isomerase activity was assayed spectrophotometrically as described by Gardner (1970). Replicates were three or more determinations resulting in a mean relative standard deviation of 12%.

Table 1—Enzyme activities of germ dissected from corn subjected to various post-harvest conditions

Germ source[a]	Drying conditions (°C)	Enzyme activities[b]: Linoleic acid-hydroperoxide-isomerase (μmoles/min/g)	Lipoxygenase (μmoles/min/g)	Peroxidase (Δ Absorbance/min/g × 10^2)	Lipase[c] (mg fatty acid/day/g): Reconstituted	Full-fat
A	ca. 25	366 ±51	12 ±1	304 ±27	0.70	2.9
	88	216 ±17	10 ±1	275 ±27	0.48	1.6
	116	95 ± 0	7.3±0.7	197 ±16	0.26	0.8
	283	0.6± 0.1	0	9.9±1.0	0.06±0.01	0.4±0.2
B	Crib-dried	234	...	308	...	...
Dry mill	...	41± 4	0.8±0.4	50 ±8	0.19±0.01	0.6±0.2

[a]All germ was hand-dissected except for the dry-milled germ. Commercial hybrid dent corn: (A) hybrid (B37TMS X H84) (Oh43RF X A619); (B) hybrid X800.
[b]All activities were based on grams germ (dry weight). Standard deviation is shown where available.
[c]Lipase activity determined at 75% relative humidity (RH) under nitrogen.

Lipase activity was initiated by storage of the germ at controlled humidities according to the method of Acker and Beutler (1965). Germ samples were stored in humidity chambers as a full-fat meal (completely pulverized by mortar and pestle) or as a hexane-extracted meal reconstituted with 3.5% (by weight) crude corn oil. Lipase activity determined in the full-fat germ was generally greater than activity in the reconstituted germ; however, activity measurements with reconstituted germ were more accurate when lipase activities were low. At time intervals a sample of germ was removed and extracted with chloroform-methanol (2:1). The lipid from the extract was separated by TLC with isooctane-anhydrous ethyl ether-acetic acid (70:30:1) on Silica Gel G plates (250μ thick). The percentage of free-fatty acids separated by TLC was determined by densitometry (Downing, 1968). When activities were low, five to seven samples were analyzed; otherwise duplicates were determined. From the range between duplicates it was estimated that the variation of the data was about ±5% from the mean for reconstituted flakes and ±8% for full-fat flakes.

Analytical methods

The water absorption index (WAI) used to measure the degree of gelatinization was described by Gardner et al. (1971). Peroxide values of the oil (extracted at room temperature with hexane) were determined by the AOCS (1961) method. Moisture was analyzed by an AOAC (1960) method.

"Bound" lipid was extracted from hexane-defatted germ flakes with chloroform-methanol (2:1) and then washed according to Folch et al. (1957). The extract was taken to dryness and the residue extracted with chloroform to remove the lipid from methanol-soluble protein. The bound lipid was separated on Silica Gel G plates with chloroform-methanol-water (65:25:4) and acetone-acetic-acid-water (100:2:1).

RESULTS & DISCUSSION

Enzyme inactivation by post-harvest conditions

Enzyme activities in samples of corn germ varied considerably, often depending on the post-harvest conditions to which the grain was subjected. In modern agricultural practice corn is harvested at high moisture and dried to acceptable levels with heated air. Enzyme activities in germ decreased significantly upon heated air drying (Table 1).

Germ from a commercial dry-mill steam was greatly reduced in enzyme activities compared to germ from corn that was air dried at room temperature or crib dried (Table 1). Admixture of endosperm bits with dry-milled germ would cause some loss of enzyme activity by dilution. Endosperm, compared to germ, is relatively deficient in peroxidase (Gardner et al., 1969), lipoxygenase, linoleic acid-hydroperoxide-isomerase (Gardner, 1970) and lipase (Torzhinskaya, 1959). The dry-milled germ used in this study was about 60% germ, as judged from the 20% fat content of the sample (Gardner et al., 1971). Considering the magnitude of enzyme loss, the dilution of activity with endosperm could not be the primary cause of the low values. The corn probably was exposed to conditions of heat drying or storage detrimental to preservation of enzyme activity. The level of activity found in dry-milled germ may reflect the post-harvest history of an average sample of corn now used by dry millers in the Corn Belt.

Inactivation of enzymes with heated rolls

Commercial dry-milled germ was cooked by heated rolls as described by Gardner et al. (1971) to study enzyme inactivation. Roll temperatures of 124°C or higher inactivated all the lipid enzymes studied (Table 2). The amount of moisture used to temper the germ was also important for inactivation.

Denaturation of peroxidase by heat has often been used as an index of generalized enzyme inactivation in sweet corn (Vetter et al., 1958a, b). Almost all samples of heat-rolled germ had residual peroxidase activity (Table 2). Most unexpectedly, peroxidase activity persisted at some of the higher temperatures. In view of the short contact time on the rolls, activity may be preserved by insufficient heating time. In Figure 1, peroxidase activity is plotted against WAI. The WAI is a measure of the extent of heat treatment a sample of germ has received (Gardner et al., 1971). Most activity was gone at relatively low WAI values.

Lipoxygenase appeared to be inactivated at the lowest roll temperatures (Table 2). Due to the low lipoxygenase activity in unprocessed germ, detection of residual activity in the heated samples would be nearly impossible. Low lipoxygenase activity from corn germ was emphasized by comparison to linoleic acid-hydroperoxide-isomerase activity. In units of μmoles/min/g dry germ, the isomerase was about 50 times more active than lipoxygenase in unprocessed germ.

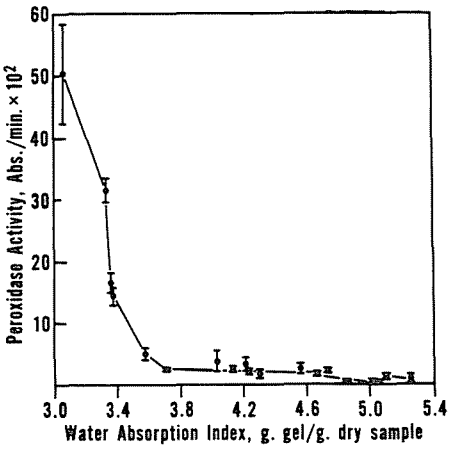

Fig. 1—Peroxidase activity of corn germ as affected by the degree of heat treatment measured as a function of the water absorption index. The vertical bars indicate standard deviation from the mean values.

Lipase activity in heat-rolled germ was assayed by determining free-fatty acid release under conditions similar to those encountered during storage of food products. The germ flakes after equilibrating with 50 or 75% relative humidity (RH) were stored at room temperature. At 50% RH the water content of full-fat germ flakes averaged about 9.2%; at 75% RH, 13.4%. The water adsorption at these two humidities was higher than for pure corn germ (Shelef and Mohsenin, 1966), pre-

Table 2—Inactivation of enzymes by heated rolls

Roll temp. (°C)	Temper moisture (%)	Enzyme activities[a]: Peroxidase (Δ Absorbance/min/g × 10²)	Linoleic acid-hydroperoxide-isomerase (μmoles/min/g)	Lipoxygenase (μmoles/min/g)	Lipase[b] (mg fatty acid/day/g)
Untreated	...	50 ±8	41 ±4	0.8±0.4	0.19±0.01
99	20	31 ±2	27 ±4	0	0.02±0.01
	25	17 ±2	8.7±1.5	0	0
	30	14 ±1	6.3±0.3	0	0
124	20	5.0±1.0	0	0	0
	25	2.4±0.3	0	0	0
	30	2.0±0.5	0	0	0
141	20	2.6±0.4	0	0	0
	25	1.7±0.3	0	0	0
	30	1.3±0.4	0	0	0
149	20	2.6±0.8	0	0	0
	25	2.5±1.7	0	0	0
	30	0.4±0.3	0	0	0
177	30	0.4±0.3	0	0	0
204	30	0.1±0.1	0	0	0

[a]All activities were based on grams germ (dry weight). Values represent the mean and standard deviation of replicate determinations.

[b]Hexane-defatted germ reconstituted with 3.5% corn oil and stored at 75% RH under nitrogen

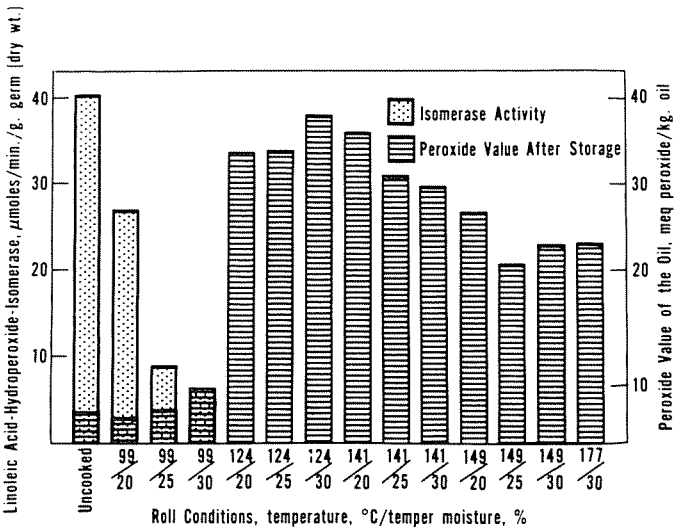

Fig. 2—Oxidation of the oil in cooked germ flakes during storage correlated with the inactivation of linoleic acid-hydroperoxide-isomerase. Storage was for 170 days at 5°C and 6.0 ± 0.9% moisture.

sumably owing to greater adsorption of water by endosperm fragments included in the dry-milled germ flakes. Samples of germ flakes stored at 50% RH did not increase much in free-fatty acid content. Low lipase activity even in unprocessed germ contrasted to the high lipase activity at 50% RH reported in oat flakes by Acker et al. (1965). It was necessary to increase lipase activity by using 75% RH so that more accurate measurements could be made. At 75% RH mold was a serious problem and its growth was evident in some samples within 30 days; mold growth was prevented indefinitely by storing the samples in a nitrogen atmosphere. Under nitrogen, lipase activity was not detected in most processed samples (Table 2). Residual activities would be extremely difficult to detect because of the low rate of lipase activity in the original unprocessed sample of corn germ obtained by dry-milling.

Oil stability in germ flakes

A study of oil stability in dry-milled germ must be considered in terms of the composition of the oil. Most of the oil is a neutral lipid fraction (20% of the germ flakes) composed primarily of triglycerides, but also subject to decomposition is the bound lipid, which is 0.9% by weight of the germ flakes. TLC of the bound lipid showed that it was composed of neutral lipids, phospholipids and glycolipids. Most changes in the lipid fractions were too subtle to be detected by TLC, except for changes caused by lipolytic activity.

Lipolysis (hydrolytic rancidity) is most often caused by lipase activity. As discussed previously, germ lipase was inactivated by heat treatment. However, rapid lipolysis took place in some air-stored samples in which the lipase was inactivated. This oil hydrolysis was probably due to infestation with mold. Fat acidity as a result of fungal growth is a phenomenon well known to workers knowledgeable with the problems of grain storage (Christensen and Kaufmann, 1969). As stated, storage at 75% RH resulted in conditions moist enough to cause mold in a few samples within a month. Spores were not always visible, but mycelia could often be detected microscopically or by matting of the flakes. Except for some of the most active samples reported in Table 1, native corn lipase (measured under nitrogen) was not very active when compared to lipolysis observed in samples infested with mold. Seed lipases are often comparatively inactive until activated or biosynthesized during germination, as was demonstrated in germinating corn (Torzhinskaya, 1959). It is doubtful that lipase would be a serious problem if storage moistures and RH are kept low, especially if the germ is heat processed.

Oxidative rancidity in a complex system, such as the full-fat flake, probably depends on many variables. Compounds that reportedly react with peroxides, such as proteins, are present in the flakes. The peroxide value (PV) measures the initial products of oxidation but does not always indicate the exact amount of oxidation that has taken place in an oil. Because of its limitation, the PV must be considered to be an empirical method in measurements of oxidative rancidity. Due to the presence of linoleic acid-hydroperoxide-isomerase in unprocessed corn germ, it was important in our study to measure the hydroperoxide concentration of the germ oil rather than the secondary products.

The PV of the oil from unprocessed germ was at a minimum, ranging from 1.5–6 meq peroxide/kg of oil regardless of storage conditions. Heat processing did not affect this low value (the PV of oil from cooked samples averaged 4.4 ± 0.8 immediately after processing), but after storage the samples heated at or above 124°C developed a higher PV. The reason for the low PV of oil from comparatively unprocessed germ may be due to a factor which was denatured by heat. Linoleic acid-hydroperoxide-isomerase could be the heat-labile factor preserving the oil from peroxide accumulation. This conclusion was supported by the data plotted in Figure 2. The PV of the oil increased during storage only in germ samples with no isomerase activity (and no lipoxygenase activity). Germ with isomerase activity yielded oil with low PV before and after storage. This observation was extended to a wider variety of storage conditions (Table 3). The conclusion was the same; i.e., the PV of oil from unprocessed germ remained low, whereas oil from heated germ underwent autoxidation that was typical of model systems of linoleate oxidation (Labuza et al., 1969). Heated samples stored at lower moistures had higher PV, except at the −20°C temperature where moisture has less influence on oxidation mechanisms. Our observation with corn germ was similar to a study by Lindemann (1953), who found that the oil in sorghum germ autoxidized within a few weeks after heat processing. Lindemann did not observe any significant changes occurring in the oil from heated corn germ within the same time period.

Table 3—Peroxide value of oil extracted from unprocessed and heated corn germ[a] after storage[b] at different moistures and temperatures

Storage temp. (°C)	Unprocessed (moisture, %) 3	Unprocessed (moisture, %) 7	Heated (moisture, %) 3	Heated (moisture, %) 7
−20	2.0	4.0	12.5	11.9
5	2.6	5.3	22.4	15.7
25	3.8	3.4	43.6	10.0

[a]The heated germ was slurried 1:2 (w/v) in water, heated to 100°C with stirring and dried as a thin layer in a 105°C oven. Both the heated and unprocessed germ were ground to −20 mesh.
[b]Storage time was 75 ± 5 days.

As pointed out earlier, we found that oil in heated corn germ will oxidize after a longer period of initiation than sorghum germ, possibly owing to a natural system of antioxidants in corn germ superior to that in sorghum germ.

The correlation of low PV in corn germ oil with linoleic acid-hydroperoxide-isomerase activity may be fortuitous, as other factors may be involved. It could be argued, for example, that heat destroys the integrity of the fat-containing bodies in the cell (liposomes) and that the

rendered oil is more exposed to the atmosphere. However, we have strengthened the hypothesis that isomerase is responsible for low PV. Samples of unprocessed germ assaying high activities of linoleic acid-hydroperoxide-isomerase were mixed and ground with samples of heat-processed germ containing oil with a high PV. The PV of the oil extracted from the mixture was the same as that of the unprocessed germ. The decrease in PV was so rapid that a study of the kinetics of the PV decrease was not practical. It does seem probable that low PV's are due to an enzyme, possibly linoleic acid-hydroperoxide-isomerase.

Considerable research effort has been conducted on lipoxygenase by other workers because it has been considered to be responsible for off-flavors and lipid oxidation in food products. Some of this effort has been on lipoxygenase inactivation studies. In corn, the products of lipoxygenase oxidation, fatty acid hydroperoxides, do not accumulate (Gardner, 1970), and this may explain why classical autoxidation of oils by hydroperoxidation does not occur in unprocessed samples. To avoid peroxidation of oils, heat processing to inactivate enzymes may be undesirable.

Alternatively, it is not known what effect the sequential oxidation of unsaturated fatty acids by lipoxygenase and linoleic acid-hydroperoxide-isomerase would have on off-flavor production. The products of this sequential reaction are hydroxyoxooctadecenoic acids, which conceivably could be susceptible to cleavage into short-chain aldehydes, ketones and acids. Perhaps many lipoxygenase-containing foods should be reexamined in view of the corn germ findings.

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Reference to a company or product name does not imply endorsement or recommendation by the U.S. Department of Agriculture.

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