

A STUDY ON THE CHANGES IN THE COMPOSITION
OF WHEAT LIPIDS DURING STORAGE

by

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INTRODUCTION

Understanding of the role which lipids play in the processing and the storage behavior of wheat and wheat products and the need for a detailed knowledge of the composition of lipids in wheat and flour has been emphasized by many authors.

In the wheat kernel, lipids form 1-2 percent of the endosperm, 8-15 percent of the germ and about 6 percent of the bran, with an average value of only 2-4 percent of the whole kernel (Sullivan, 1940). Nevertheless, wheat lipids have engaged the attention of cereal chemists, as research over many years has suggested that they are involved in processing, storage, and in complex biochemical transformations during plant development and germination.

Until quite recently, progress in plant lipid research has been slow because of the great number of compounds present and the lack of simple techniques for separation of the individual components. Recently, separation of lipid classes has been accomplished by a number of techniques, such as counter current distribution (CCD), silicic acid-column chromatography, gas-liquid chromatography (GLC) and thin-layer chromatography (TLC). By the use of these methods, it is now possible to make separations clearly, quickly and reproducibly. In particular, the variety and characteristics of the glycolipids and phospholipids present in wheat are now better known because of these techniques.

Modern combine-harvesting as well as unfavorable weather conditions often result in an increased moisture content, which together with high summer temperatures, create special problems in regard to storage of wheat. The need of storing surplus wheat for extended periods, particularly in

countries like the United States, has prompted questions regarding the effect of different factors including moisture, temperature and oxygen supply on the quality of wheat and wheat products in storage.

Numerous authors have studied the changes taking place during storage in different cereals under various conditions and have suggested a number of parameters for routine control. The proposed tests include viability, germ damage, fat acidity and glutamic acid decarboxylase determinations to predict storage behavior and the extent of actual damage to the stored grain. All these tests have their limitations and are much more useful in assessing actual damage than in predicting incipient deterioration.

Extensive work has been done on the role of fat acidity as an index to indicate deterioration in cereal grains. A literature review indicates, however, some contradictory data regarding the significance of free fatty acids in predicting storage behavior and the extent of actual damage by fatty acids to stored grain. Relatively little is known on transformations occurring in wheat lipids during storage. An understanding of the basic biochemical mechanisms involved in the breakdown of wheat lipids by new techniques would enable us to reduce losses due to spoilage as well as to develop better means of detecting deterioration at its early stages when the quality of the grain has not yet markedly decreased. The purpose of this study was to follow changes taking place in the composition of polar and nonpolar lipids of wheat stored at elevated moisture levels and temperatures. The methods used to fractionate the extracted lipids into different components included qualitative and quantitative thin-layer chromatography and fractionation on silicic-acid columns.

REVIEW OF LITERATURE

Earlier work on deterioration of cereal grains in storage has been reviewed by several authors including Kretovich (1945), Anderson and Alcock (1954) and Geddes (1958). However, the basic biochemistry of grain deterioration has become a subject of limited investigation only recently.

Biochemical effects of moisture in grain: The low respiratory activity in air-dry wheat (Linko and Milner, 1959) is localized almost entirely in the embryo. Exposure of embryos to water vapors increased carbon dioxide evolution approximately ten-fold. An increase in moisture content is generally followed by a slow, steady increase in respiration until a certain critical moisture is reached (Bailey, 1940; Bottomley *et al.*, 1952; Kretovich and Ushakova, 1940). Bailey and Gurjar (1918) explained the sharp increase in the respiratory rate after exceeding the critical moisture level by suggesting that when water uptake exceeds the binding capacity of biocolloids in the grain, free water becomes available for metabolic reactions.

Gilman and Baron (1930) observed that the sharp increase in respiratory rate occurred at the same moisture level required for the growth of certain saprophytic fungi. Since this discovery, the importance of molds in the relationship between moisture content and respiratory activity of cereal grain has become well-established (Carter and Young, 1950; Milner and Geddes, 1954; and Christensen, 1957).

Recent evidence indicates, however, that extensive deleterious biochemical changes may occur in grain before damaging fungal development. Studies with soybeans (Milner and Geddes, 1946 a, b) and wheat embryos (McDonald and Milner, 1954) showed that significant carbon dioxide evolution occurred immediately on wetting, resulting in highly elevated respiratory quotient values.

This excessive carbon dioxide evolution recedes several hours before germination of fungal spores affected the subsequent respiratory pattern (McDonald and Milner, 1954). That the metabolism of the seed itself is involved has been established (Milner and Geddes, 1946 a). The results indicate that enzymatic processes are stimulated simply by increasing the moisture content of air dry wheat embryos.

Germ damage and non-enzymatic browning: Damage to kernels is accelerated by high moisture content and elevated temperature, and seems to be associated with anaerobic conditions (Carter and Young, 1945). Milner *et al.* (1947 a,b) speculated on the possibility of a Maillard-type non-enzymatic browning reaction as a cause of germ damage in wheat. Pearce (1943) observed that an increase in the fluorescence of a wheat germ extract was related to the extent of damage, and concluded that deterioration of proteins would be mainly responsible for the spoilage. Milner and co-workers (Cole and Milner, 1953; McDonald and Milner, 1954) presented further evidence that increased fluorescence was characteristic of damaged embryos. That such a change can occur in the dry state has been demonstrated by Richards (1956).

Linko and Milner (1959) showed recently that considerable changes in the composition of free amino acids and the closely related keto acids might take place in wheat immediately following wetting. The most characteristic change was a rapid decrease of the quantity of free glutamic acid accompanied by a corresponding increase in free γ -aminobutyric acid. They concluded that the amino acids liberated from proteins by enzymes activated by the increased moisture content may react with reducing carbohydrates as a primary cause of the brown pigments in damaged embryos.

Sugars: Ramstad and Geddes (1942) found a marked increase in reducing

sugars in soybeans stored at more than 15% moisture content. This was followed by an equally marked decrease in non-reducing sugars. Many other workers have shown similar changes with different types of cereals (Glass et al., 1959; Houston et al., 1957; Bottomley et al., 1952). Linko et al. (1960) studied changes in the composition of soluble carbohydrates of wheat germ during storage at moisture levels and temperatures pertinent to conditions which produced germ-damaged wheat and they observed several unknown compounds believed to be intermediates in non-enzymatic browning. Glucose and fructose did not increase as much as might have been expected from the breakdown of sucrose and raffinose. The difference was attributed to the reaction of reducing sugars with free amino acids.

Phytase: Courtois and Perez (1948) found that hard wheat contained more phytase activity than soft wheat and that the activity increased on germination. Peers (1953) investigated the distribution of phytase in soft wheat and found that starchy endosperm and aleurone fractions contained 34.1 and 39.5% of total phytase respectively. Germ and scutellum contained 2.9 and 15.3% respectively. Hard wheats averaged about 20% more activity than soft wheats. Phytase activity of the soft wheat increased about six fold on germination.

Glass and Geddes (1959) observed that during wheat deterioration, inorganic phosphorus increased. They attributed this increase, which is accelerated by high moisture and elevated temperatures, to the action of phytase on phytic acid. Although they found a greater increase in fat acidity than in inorganic phosphorus, the latter seemed to increase more rapidly at the early stages of deterioration. Kyoko and Shoji (1962) reported that inorganic phosphorus increased, especially in endosperm fraction, when rice was stored for two and six months at 30° and 80% relative humidity. Inorganic

phosphorus in the brown rice increased to 130-160% when stored for a year in air-conditioned warehouses and increased to 230-270% when stored in ordinary warehouses. This was accompanied by a decrease of a certain colloidal substance in the water extract of brown rice meal. The authors suggested the increase in inorganic phosphorus as a parameter for measuring physiological changes occurring in rice kernel during storage.

Lipids in wheat: Products of wheat milling vary in their lipid content. The germ and bran contain much higher concentrations of lipids than the starchy endosperm. In addition, certain relationships exist between alcohol-extracted and ether-extracted lipids. Sullivan and Near (1928) have shown that the lesser amount of lipid extracted by ether contained a smaller percentage of phosphorus than the lipid material extracted by ethanol. In dark flours the phosphorus content of ether-extracted lipids decreased markedly and of alcohol-extracted lipids slowly. Phospholipids were present in larger proportion in endosperm than in bran and germ. Nelson et al. (1963 a, b) observed by fractionation on silicic acid columns that bran and germ lipids contained more than 50% triglycerides, but endosperm lipids were composed of 30% triglycerides and more than 50% phospho- and glyco-lipids. McKillican and Sims (1964) observed that the removal of the germ before milling reduced the triglyceride content of the free lipid of the flours. According to them, bound lipids (extractable with water-saturated butanol) are mainly (80%) phospho-lipids, while free lipids (extractable with hexane) are mainly triglycerides.

The nitrogen content of lipid extracts have been found to follow generally the same trend as the phosphorus content. However, the nitrogen content consistently exceeded the amount that would be contributed by phospholipids; i, e., the molar N/P ratios were appreciably larger than unity. The presence of lipoprotein or polypeptide constituents might account for most of this ex-

cess of nitrogen (Ball et al., 1942; Sullivan, 1940). Fisher et al. (1964) found molar N/P ratio in Folch- washed flour lipids, to be between 1.25 and 1.56 and indicated the presence, in addition to lipids of N/P ratio 2:1, of non-lipid nitrogen either in the form of lipoprotein, lipoaminoacids or as nitrogeneous impurities solubilized by lipid.

Nelson et al. (1963, a,b) observed that a rather large amount of non-lipid material was extracted with water-saturated butanol from whole wheat, bran and germ but all of the material extracted from endosperm was soluble in petroleum ether. Wheat lipids extractable by water-saturated butanol but not by ethyl ether consisted almost entirely of polar material as distinguished from the predominantly non-polar nature of the ethyl ether extractable substances. The ether extract was smallest in patent flour, increased with decreasing refinement of flour and was greatest in bran and germ (Sullivan and Near, 1928). The same trend was observed by Bailey (1947) and by Horder et al. (1954).

Nelson et al. (1963 b) observed that total, as well as triglyceride fatty acids present in bran, germ and endosperm were generally similar. The main fatty acids in barley, oat and rye lipids were shown to be palmitic, oleic, linoleic and linoleinic by Aylward and Showler (1962). Distribution of fatty acids in lipids from various classes of wheat varied slightly (Fabriani, 1962).

Sullivan et al. (1936) found that ether-extractable endosperm lipids, but not germ lipids, were essential for bread making. Nelson (1962) found that baking quality was impaired in water-saturated butanol extracted flour.

Phospholipids: Tóópler (cited by Bailey, 1947) reported as early as 1861, the presence of 0.25 to 0.28% of phosphorus in flour oil. However, the difficulties of working with phospholipids largely prevented for many years the recognition of classes other than lecithins and cephalins.

Phosphatidic acids were reported to occur in wheat germ lipids (Channon and Foster, 1934); the proportions of phosphatidic acid; lecithin: cephalin were given as 4:4:1. Subsequently, Barton-Wright (1938 b) examined the petroleum ether extracts of germ, bran, and low-grade and patent flours, and reported that phosphatidic acids were the predominant type of phospholipids in both germ and flour, but not in bran. The occurrence of a large proportion of phosphatidic acids, in flour phospholipids, has been questioned recently, however, by Mason and Johnston (1958).

This discrepancy may be explained by the presence in cereals of phospholipases capable of hydrolyzing choline from lecithin to form phosphatidic acids (Acker, 1956; Acker and Ernst, 1954). Such a degradation of phospholipids was first demonstrated by Hanahan and Chaikoff (1947) and confirmed more recently (Hanahan, 1957, 1960).

The isolation of glycerophosphoinositophosphatic acids from wheat germ was reported by Faure and Morelec-Coulson (1953, 1957).

Fisher and co-workers (1956, 1957, 1958) reported that material precipitable by acetone from a dry carbon tetrachloride extract of dry flour could be separated by counter current distribution into plasmalogens, serine containing phospholipids, phosphatidyl ethanolamine and galactose-containing glycolipids. Two fractions, out of six, were essentially free of lecithin. Mason and Johnston (1958) reported the presence of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylcholine and glycolipids in lipids extracted with water-saturated butanol from two flours. The phospholipids contained only 0.07% inositol and the proportion of inositol-containing lipids appeared to be much lower in wheat than in corn or soybean phospholipids.

An equally complex picture of the phospholipids of wheat products has

been shown by chromatography on silicic acid. Daniels (1958) reported that the phosphorus content of five fractions ranged from 0.18 to 3.25% and the high phosphorus, lecithin-rich band contained little carbohydrates. Fisher and Broughton (1960) separated lipids by silicic acid column chromatography into 13 fractions. Coulson and Sommerville (1961) extracted lipids from gluten with chloroform-methanol and water-saturated butanol. Chromatography on silicic acid-impregnated paper indicated the presence of 13 phospholipid components. Fractionations of lipids from wheat flour by silicic acid column chromatography were reported by Houston (1961) and by Wren and Elliston (1961).

Recently, Fisher et al. (1964) separated and identified 1,2- and 1,3-diglycerides among the less polar lipids of wheat flour. Two-dimensional thin-layer chromatography revealed 23 components, including phosphatidylcholine and phosphatidylethanolamine.

Glycolipids: The presence of a carbohydrate residue in complex lipids of wheat was observed in 1908 by Winterstein and Hiestand and confirmed by Sullivan (1940) and Antener and Høgl (1947). The precise nature and structure of wheat glycolipids was unknown until the presence of two galactosyl-glycerol lipids, beta-D-galactopyranosyl-1-glycerol and alpha-D-galactopyranosyl-1,6-beta-D-galactopyranosyl-1-glycerol, accounting for a large proportion of the carbohydrate in the lipid, was demonstrated by Carter et al. (1956).

In the study by Mason and Johnston (1958) galactosylglycerol lipids were concentrated by counter current distribution of an acetone-insoluble lipid fraction. The digalactosyl-glycerol lipids, described by Carter et al. (1956) constituted up to 40% of the phospholipid fraction. Carter and co-workers later (1961 a,b) described the purification of monogalactosyl glycerol lipid by chromatography on silicic acid.

The presence, in acetone extracts of gluten, of components yielding galactose and glycerol upon hydrolysis, has been reported by Zentner (1960).

Extraction of lipids from wheat: Wheat, as is true of many other plant products, has a certain amount of its total lipids bound with the proteins and carbohydrates in such complexes that the normally employed fat solvents e.g., ether, acetone etc. do not give complete extraction.

The first problem we encounter in studies of wheat lipids is how to extract the lipid material. It is common knowledge that the amount of lipid material extracted depends on the solvent used. No completely satisfactory method for total lipid extraction is as yet available. The procedures that have been devised to approach complete extraction of lipids from cereal products generally rely on treatment with aqueous ethanol, when hydrolysis of the lipids is to be avoided, or with acid-alcohol mixtures when hydrolysis of fatty material is not objectionable. The problems involved in the development of suitable procedures were discussed for example by Hertwig (1923) and Meara (1955).

Although treatment with a mixture of polar organic solvent and water is necessary to render the lipid material in the cereal products readily available for ether extraction, more prolonged treatment with 95% or absolute ethanol probably has been an effective and most frequently used procedure. Alternatives to ethanol include water-saturated n-butyl alcohol, chloroform: methanol: water mixtures, and acetone: water mixtures. According to Mecham and Mohammad (1955), water-saturated n-butyl alcohol disrupts lipid-protein complexes.

Water-saturated n-butyl alcohol has the disadvantage of extracting large amounts of non-lipid material and of a high boiling point. On the other hand, Mecham and Mohammad (1955) observed that flour lipids occur in a form rela-

tively soluble in polar solvents, that is preserved in the n-butyl alcohol extractant but is altered in hot ethanol. Tsen et al. (1962) adapted a rapid method for extracting lipids from animal tissues to use on wheat products, employing a mixture of chloroform, methyl alcohol and water. Considerably more lipid was extracted than by petroleum ether, but slightly less than by water-saturated butanol.

Thus for complete and rapid extraction of the more polar lipid compounds, the presence of water is clearly necessary. It was reported by Herd (1927) and Anonymous (1929) that less fat was extracted by ether from completely dried flour and milling stocks than moist or partly dried stocks. Thus with non-polar solvents the level of moisture present during extraction is also a factor. This is recognized by the AOAC and AACC procedures which limit the moisture content of samples prior to lipid extraction.

Fat acidity: Deterioration of grain and of milled grain products in storage is accompanied by an increase in acidity. Besley and Boston suggested as early as 1914 acidity as a factor in determining the degree of soundness of corn. This acidity consists of (a) free fatty acids produced by the action of lipases on fats, (b) acid phosphates produced by the action of phytase on phytins and phosphatases on phosphatidic acids, and (c) amino acids produced by the action of proteolytic enzymes on proteins. At the very early stages of deterioration, fat acidity increased at a much greater rate than did either of the other two types or all types of acidities combined (Zeleny and Coleman, 1938). The work of Kelly et al. (1942) suggested that hydrolysis of fats seemed to be more pronounced than protein or carbohydrate hydrolysis. A number of authors including Zeleny and Coleman (1939), Swanson (1934) and Bolling (1961) suggested the determination of fat acidity as one of the best measures of grain damage.

Sullivan and Near (1933) reported that the alcohol-ether extract of wheat and its milled products dropped considerably in samples stored at or near their original moisture content of 13 to 14%. Several other workers have demonstrated that fats in cereals are hydrolytically broken down during storage (Fenton and Swanson, 1930; Carter and Young, 1945; Milner et al., 1947 and Hunter, 1951).

Barton-Wright (1938) observed that in a normal flour the acid value increased on storage to a maximum and then dropped off. Leustsen (1954) attributed abnormal acidity of flours to the presence of lactic acid with the commonly recognized acid hydrolytic products as contributory factors.

Fungal lipases: Fungi in seeds are commonly classified as field fungi and storage fungi. The major field fungi, *Alternaria* sp., *Helminthosporium* sp. and *Fusarium* sp., seldom cause storage deterioration with the exception of the last one, whereas the storage fungi *Aspergillus* sp. and *Penicillium* sp. are principally associated with decrease in quality during storage.

Although deterioration of stored grain by fungi was described more than 50 years ago (Duvel, 1909; Shanahan, 1910), only recently has the importance of the problem been recognized. Free fatty acids may result from the action of seed lipases, but it seems that in deteriorating stored wheat, they are primarily the product of action of fungal lipases. Sufficient evidence that the increase in fat acidity, that occurs when various kinds of grains are stored at higher moistures, is due mainly to the action of mold lipases rather than to the activation of seed lipases has been obtained by many investigators. Barton-Wright (1938) found that in flour with greater than 16% moisture, the oil content fell rapidly during storage and this was found to coincide with increase in fungal numbers. Kretovich (1940) observed a direct correlation between the lipase activity and moisture content of wheats.

Under normal conditions of storage, mold deterioration, such as occurs at unsafe moisture levels, would mask completely the more slowly developing deterioration occurring within the grain.

Some workers have reported that autoclaved grain inoculated with various storage fungi exhibited increases in fat acidity, similar to those formed in stored viable grain. There was often a fairly close correlation between increases in fat acidity and increases in mold count of stored grain. Dirks et al. (1955) differentiated mold and wheat lipases by using certain SH-blocking reagents and attributed the appearance of fat acidity in stored grain primarily to mold lipase activity. The hydrolysis of fats in stored grain is accelerated by the presence of fungi because of their high lipolytic activity (Goodman and Christensen, 1952; Loeb and Mayne, 1952). Fungi would thus seem to play a more dominant role in acidity increase than seed lipases. Golubchuk et al. (1960) found a good correlation between fat acidity, chitin content and mold spore count. Dirks (1954), Nagel and Semeniuk (1947) and Golubchuk et al. (1956) attributed the appearance of fat acidity in stored grain chiefly to the activity of fungal lipase. Nagel and Semeniuk (1947) found that various mold species differed in their ability to increase fat acidity of autoclaved corn and suggested that part of the measured fat acidity might be due to metabolic acids produced by the fungi from the carbohydrates. They reported that the greatest increase in free fatty acids were produced by P.chrysogenum I, P.chrysogenum II, A.niger, A.flavus, Mucor racemosus and A.amstelodomi. Bottomley et al. (1952) observed that the growth of A.flavus, A.candidus, Penicillium sp. and Fusarium sp. was often associated with a marked increase in fat acidity. They also suggested that decreases in fat acidity (after reaching peak) were possibly due to the metabolism of acids by the fungi. Thus evaluation of soundness of wheat, on the basis of fat

acidity alone, may be misleading as a more deteriorated sample may actually have a lower fat acidity. Fall in fat acidity might also be due to the utilization of the fatty acids by the fungi for their own further growth. This was confirmed in studies in which all of the fungi tested were able to utilize to some extent either corn oil, a monoglyceride, or fatty acid. Nagel and Semeniuk (1950) reported that different species of molds were known to differ greatly in the amounts of lipase and other enzymes they produced and in their ability to metabolize such products as fatty acids. In certain cases low fat acidity accompanied by high mold counts might have been due to the low lipase production by the species of molds which were prevalent or to the utilization of the fatty acids by molds. They reported that highest fat acidity coincided with the highest count of A.flavus at 40°, although there was a higher total mold count at 25°. In 1947, Milner et al. observed that fat acidity increased most in 18% moisture wheat, with *Aspergillus* sp., provided an oxygen atmosphere was maintained. Loeb and Mayne (1952) found that growth of A.chevalieri produced free fatty acids in rice bran sterilized by autoclaving, and demonstrated the relationship between micro-organisms and production of free fatty acids. The rate of production was slower than in untreated bran. Kartha (1963) demonstrated the broad specificity of certain isolated plant lipases.

Kretovich (1944) found that flour, milled from wheatbug-infested kernels, had increased titratable acidity. Pingale et al. (1954) observed an appreciable increase in fat acidity in insect-infested soft wheat.

Seed lipases: Green (1890) first showed the presence of lipase activity in germinating seeds. He observed that lipase of the resting Ricinus seed was present in a non-active form and was quickly activated by the addition of water. In 1920, Meharque reported that when corn spoiled, fat acidity increased threefold in the whole corn, slightly in the endosperm and more than

sixfold in the embryo. Pomeranz and Shellenberger (1961) indicated that scutellum was the main site of lipase activity. They reported that major sites of high lipid concentration and generally free fatty acids in a dormant sound wheat kernel were the germ and the aleurone, but as the germination period increased, zones farther from the scutellum tissue gradually increased in fatty acids.

Pett (1935) reported that germ lipase activity was considerably less than the scutellum lipase activity. Lipase activity of the endosperm was much lower than that of the scutellum. Bran contained a significant amount of lipase. Rothe (1958) observed that the lipase activity of the bran was nearly twice and that of the germ thrice as much as that of the whole kernel. The outermost surface layers showed extremely high activities apparently caused by the presence of microorganisms.

Regarding distribution of fatty acids in sound and "sick" wheat kernels, Milner et al. (1947) reported that fat acidity paralleled maximum lipase activity in bran fractions and that the enzymatic activity decreased with flour refinement in the order of the total fat decrease of the various fractions milled from sound wheat. The milled fractions of "sick" wheat, on the other hand, showed the highest fat acidity in the low grade flour. The high lipase activity of the aleurone layer and scutellum relative to that of the germ was apparently responsible for this effect.

Other enzymes: Although certain phosphatases have been known to be present in cereals, little has appeared in the literature until recently concerning these enzymes in wheat. Berliner and Ruter reported in 1928 that wheat flour extracts contained a phosphatase capable of phosphorylating soybean phosphatide. A phosphomonoesterase and a phosphodiesterase were found in rice and bran by Uzawa (1932). The distribution of phosphomonoesterase in wheat before

and during germination was measured by Ignatieff and Wasteneys (1936). The leaves were found to have a higher concentration of phosphatase than any other part of the plant. The phosphatase activity in leaf blades of young sprouts of wheat was investigated by Sisakyan and Kobyakova (1940). They reported that the synthesizing activity of the phosphatases was decreased and the hydrolyzing activity increased when 30 to 40% of water was lost from the leaves. When 40 to 50% of the water was lost, both the synthesizing and hydrolyzing activity of the phosphatase was reported to increase.

Sarma and Giri (1942) suggested that phosphomonoesterase of rice and ragi may exist in both 'free' and 'bound' conditions; the 'free' form being water soluble, while the 'bound' form was not extracted by water. The significant increase in the total phosphatase content of rice during germination was attributed to freeing of the 'bound' phosphatase.

Booth (1944) clarified and extended the preliminary observations on the phosphatases of wheat. Phosphomonoesterase preparations containing hexose-diphosphatase and pyrophosphatase activities, prepared from wheat flour, hydrolyzed alpha- and beta-glycerophosphates. Alpha-glycerophosphate was hydrolyzed more rapidly than the beta- compound. Evidence presented by Booth indicated that there were at least two phosphomonoesterases in wheat, one of which hydrolyzes alpha-glycerophosphate more rapidly than beta-glycerophosphate. Booth's results also indicated the presence in wheat of a hexosediphosphatase, pyrophosphatase and a phosphodiesterase. Adenosinetriphosphatase, glycerophosphatase and thiaminepyrophosphatase have been demonstrated histochemically in wheat grains and sprouts (Glick and Fisher, 1945 a,b).

Factors affecting fat acidity increase: Pomeranz et al. (1956) showed that increase in mold growth was generally accompanied by a decrease in fat content and an increase in fat acidity. Different workers have attributed

increase in fat acidity to fungal growth, which is influenced by a number of factors including moisture, temperature, oxygen concentration, condition of the grain and previous history of the grain. (Christensen and Gordon, 1948; Tuite and Christensen, 1955; Karon and Altschul, 1944).

The molds due to their respiration cause heating of the stored grain. Johnson (1948) demonstrated that heating of the grain was due to the respiration of fungi rather than of the grain itself. Similarly, other workers (Isachenko et al., 1934; Gilman and Baron, 1930; Carter and Young, 1950) attributed heating of moist grains to the development of microorganisms.

Narayan Rao et al. (1954) observed during their study on husked, under-milled and milled rice in jute bags that there was a maximum increase in acidity of the fat present in the husked rice. Jarvi (1958) observed that the enzymic and bacteriological hydrolysis increased with damage and moisture content above 12%. Loeb and Mayne (1952) reported that in relatively dry (11.5% moisture) unautoclaved rice bran free fatty acids were formed without any detectable increase in microorganisms. There was even a slow formation of free fatty acids in bran with moisture content as low as 4.9%. Thus at moisture levels equivalent to less than 75% relative humidity, the increase in fat acidity is more likely attributable to causes other than microorganisms. Sharp (1924) observed that moisture from 4.8 to 14.8% at 22° or 35° had little effect on increasing acidity in normally ripened wheat and caused only slight increases in acidity in frosted, immature wheat.

Zeleny (1940) showed that at any given moisture level, the rate of heating tended to increase with fat acidity increase. He suggested fat acidity as a useful index of partly unidentified chemical, physical or biological transformations that appear to stimulate the respiration rate during deterioration.

Sorger-Domenigg et al. (1955) found that high levels of mold infestation markedly increased viability losses, development of germ damage, and increases in fat acidity during short-term storage at high moisture levels. On subsequent storage at 13-14% moisture, the mold count of most samples decreased but germ damage and fat acidity continued to develop, especially in the heavily infested moldy samples. Kelly et al. (1942) observed that drop in germination was associated with total damage and increased fat acidity. Fat acidity increased and viability decreased long before the appearance of external physical evidence of deterioration. For this reason, they considered the free fatty acid content of grain as a sensitive index of incipient deterioration.

Linko and Sogn (1960) reported that germ damage in wheat was followed by high fat acidity and fat acidity showed a high negative correlation with germination percentage. However, there seemed to be no correlation between fat acidity and viability with ten wheat crops of high viability. Holman et al. (1949) found that drop in germination was associated with increases in fat acidity of corn. The same type of relationship was found in case of soybeans (Holman and Carter, 1952).

Baker et al. (1957, 1959) found that, in general, storage damage of the type caused by molds and heating showed high positive correlation, while field damage showed low correlation, with fat acidity. In corn, high correlations between fat acidity and degree of damage were obtained for different types of damages. A low correlation was obtained for weevil damage during storage. Frost damage and immaturity, types of field damage, showed low correlations with fat acidity.

Lynch et al. (1962) found that, by most of the criteria used, samples stored under anaerobic conditions and hence without mold growth, deteriorated

almost as rapidly and equally in degree as did samples stored in aerobic conditions. The exception was fat acidity, which remained constant under anaerobic conditions while increasing tenfold under aerobic conditions.

Bottomley et al. (1950) reported that variations in relative humidity had a greater effect on fat acidity than differences in temperature or oxygen concentration. Fat acidity was uninfluenced unless oxygen was present. Decreasing the oxygen content from 21 to 5% produced no significant changes in fat acidity values but with 0.1% oxygen there was a highly significant reduction in fat acidity. Fat acidities were lower in samples at 100% than at 95% relative humidity despite the higher mold count and general deterioration at higher humidity. The correlation between mold count and fat acidity was only +0.20. Fat acidity did not exceed - under practically anaerobic conditions - a value of 40 even in advanced stages of fungal damage. In a later study (1952) it was found that in non-aerated samples there was relatively little change in fat acidity but a substantial decrease in viability, indicating fat acidity as a poor measure of grain storage under anaerobic conditions. Under partly anaerobic conditions, mold growth decreased at a much faster rate than fat acidity. Witt and Burdick (1963) observed that barley maintained under sound storage conditions for 1-1.5 years after harvest showed an increase of nearly 100% in fat acidity.

Baker (1962) investigated the fatty acid composition of oil from damaged corn and wheat. Chromatograms were similar in damaged and sound grain oil for fatty acids of chain length from lauric to linolenic. Lower molecular weight material appeared more prevalent in the free fatty acids from corn damaged by artificial drying, cob rot, or blue-eyed mold and in wheat damaged by heat. Linoleic acid appeared to be the predominant fatty acid and was followed by oleic and palmitic acids. Damaged wheat usually showed larger

amounts of lauric acid in the free fatty acids. Morrison (1963 c) reported that the free fatty acid content of flours varied according to the length of time the flour had been stored, but the free fatty acid composition remained remarkably constant. This is in agreement with the finding that there is no significant compositional difference between the free fatty acids of sound and damaged wheat and maize (Baker, 1962).

Sorger-Domenigg et al. (1955) observed that flour milled from wheat with high fat acidity levels had a high ash content, poor color, poor baking strength and impaired baking quality. Witzel (1957) suggested fat acidity as a good index of bread baking quality of flour. Morrison (1963 a,b) observed that dough quality was influenced by the free fatty acid content and by oxygen absorbed during the mixing process.

Determination of fat acidity: Various methods for fat acidity determination have been discussed in detail by Bailey (1944). Baker et al. (1957) found a reversible curvilinear relationship between extractable fatty acids and the moisture content of the grain. The oil extracted from the damp grain differed in appearance and composition from the oil extracted from dry grain. The higher percentage of nitrogen and phosphorus in the oil extracted from the damp grain indicated the presence of phospholipids. Lipids extracted from damp grain had lower iodine values than lipids from dry kernels. The authors emphasized that to secure comparable results, samples should be analyzed at a moisture content at or below 10%.

In 1961, Baker reported a colorimetric method based on using aqueous cupric acetate to form soaps, which are soluble in benzene solution. The intensity of the resulting blue color was measured at 640 m μ . The relationship of fat acidity against percentage transmission, in case of corn and wheat was linear in the range of 20 to 100 fat acidity units. The method

was, therefore, of limited use for samples with below 20 (very viable) and above 100 (very deteriorated) units of fat acidity. Bautista (1962) found that the results correlated poorly with other standard techniques for fat acidity determination in wheat and corn.

Another colorimetric method based on using diphenyl carbazide has been suggested by Sedlacek and Rybin (1959). The reagent forms red color in a carbon tetrachloride solution and the color is measured at 550 m μ . No data are yet available on applicability of this method in testing of cereals.

MATERIALS AND METHODS

Sound, hard, red winter wheat of Comanche variety and soft red winter wheat of Seneca variety stored at 4° for about six months after harvest were used. The original moisture of the wheat samples was 12.8 and 13.4% respectively, and each was moistened to either 18 or 22% as described below. The wheat samples were placed in narrow-mouthed glass containers, the water was added and the contents mixed thoroughly. Then the containers were plugged with cotton and the wheat was allowed to attain the desired moisture levels during storage for 48 hours at 4°. The samples were stored at 49°, and were shaken daily. Sub-samples were removed for analyses as indicated. Part of each sub-sample was preserved in cold for mold counts.

Fat acidity: Fat acidity was determined by procedures 02-01 and 02-02 described in Cereal Laboratory Methods (AACC, 1962), using benzene and petroleum ether as extractants. Fat acidity determinations were made both on moistened samples and samples dried to 11-12% moisture in a forced draft oven below 60°. Part of the benzene extract was concentrated under vacuum and preserved at -20° for use in thin-layer chromatography.

Additionally, free fatty acids were determined in 35 samples of sound

wheat harvested in 1963 and stored for about six months at 4°.

Lipids were extracted within one hour after grinding on a Wiley laboratory mill to pass a 20 mesh sieve. For extraction with benzene, the grinder-extractor Stein Mill Model M was used. Fat acidity values were expressed as mgm. potassium hydroxide required to neutralize acids liberated from 100 gms. wheat (dry matter basis).

Moisture: Moisture was determined by drying the ground sample at 130° in an oven for one hour.

Mold counts: Mold counts were made by the procedure of Christensen (1946).

Column chromatography: Lipids from (a) original wheat, (b) wheat at 22% moisture and stored at 4° and (c) wheat at 22% moisture but stored at 49°, were separated into non-polar and polar fractions. Fifteen gram samples were extracted with 100, 50, and 50 ml. of water-saturated 1-butanol for 4, 2, and 2 minutes respectively, allowing 4 minutes between each extraction step. The combined extracts were decanted, filtered and evaporated almost to dryness under vacuum in a glass apparatus at about 45°. The extracts were kept under vacuum in a desiccator for 40 hours over phosphorus pentoxide at 4°, dissolved three times with Skellysolve B and the combined upper layers evaporated under vacuum. The lipids were dissolved in 80 ml. of a chloroform-methanol mixture (2:1), washed with 17.5 ml. of aqueous 0.04%, followed by two washings with 10 ml. each of aqueous 0.02% calcium chloride solutions, and the volume of the washed layer made to 50 ml. with chloroform. Total lipids were determined by drying to constant weight two 5 ml. portions, and the remaining 40 ml. were concentrated under vacuum to about 2 ml. for separation on silicic acid columns. Silicic acid columns were 15 cm. long and 2 cm. in diameter. Twenty gm. lots of silicic acid (Malincrodt, N.Y.) for chromatography of lipids were

washed with distilled water and dried at 120° for 4 hours. This was again washed twice with 60 ml. of a 7:1 and once with 60 ml. of a 15:1 chloroform-methanol mixture, and finally with 80 ml. chloroform. The slurry was transferred to columns and the neutral lipids were eluted with 120-150 ml. chloroform. The polar lipids were eluted with 120-150 ml. methanol. The completion of elution was checked in each case by heating of spots applied to thin-layer chromatography plates, sprayed with sulfuric acid. Each of the two fractions were concentrated to 100 ml. under vacuum and two 10 ml. aliquots were drawn from each for determination of neutral and polar lipid content, respectively. The remaining fractions were freed of solvent under reduced pressure, dissolved in a 2:1 mixture of chloroform-methanol and stored at -20° for thin-layer chromatography.

Preparation of lipid fractions for quantitative thin-layer chromatography:

For calibration purposes the lipid extract from wheat of Kaw variety prepared as described under column chromatography was separated into 10 fractions by elution from silicic acid (from Bio-Rad Lab., Richmond, Calif.), employing the apparatus described by Hirsh and Ahrens (1958). The neutral lipids were eluted by the solvents given by Barron and Hanahan (1958), followed by elution of polar lipids, according to Hanahan et al. (1957). The fractions contained mainly, (a) carbohydrates, (b) sterolesters and esters of higher alcohols, (c) triglycerides, (d) sterols and higher alcohols, (e) diglycerides, (f) monoglycerides, (g) polyglycerolphosphatides and glycolipids, (h) phosphatidyl ethanolamine and phosphatidyl serine, (i) inositol phosphatide, lecithin and lysophosphatidyl ethanolamine and (j) lysolecithin. The fractions were evaporated to almost dryness under reduced pressure and dissolved in 2:1 mixture of chloroform-methanol.

Thin-layer chromatography (TLC): Standard glass plates (20 cm. x 20 cm.)

were coated with a 250 micron layer of silica Gel G (E. Merck, A.G., Darmstadt, Germany), applied in a suspension of 30 gm. silica gel in 60 ml. distilled water. The plates were activated by drying at 130° for three hours and then stored in a desiccator.

The most useful solvent systems employed for the ascending development of lipid spots (20-40 microliters) were: (a) chloroform mixture i.e., chloroform-methanol-water (65:25:4) according to Wagner et al. (1961), (b) chloroform, and (c) ether mixture i.e., petroleum ether-ethyl ether-acetic acid (80:20:1).

All solvents were of analytical grade, redistilled from glass; ether was redistilled from above metallic sodium. The chromatographic jars were lined with filter paper to ensure saturation of the enclosed space with solvent vapors. The lipids were developed by ascending chromatography and when the solvent had travelled about 15 cm. from the original spot, the plates were taken out and dried at room temperature. Before spraying the chromatoplates, the separated components were visualized under ultraviolet light (long wave 3660 Å°). The other methods employed for visualization and detection included: general sprays, (a) exposure for 10-15 minutes to iodine vapor (Sims and Larose, 1962). The iodine was then removed by heating and the plates sprayed with sulfuric acid or specific reagents, (b) sulfuric acid; the chromatoplates, after preheating at 100° for 5 minutes, were sprayed with sulfuric acid and heated for 15 minutes at 130°. Most lipid components appeared as light black to intense black spots, depending upon the concentration and the nature of the lipid component. These charred spots were visualized under ultraviolet light and also used for quantitative thin-layer chromatography; specific sprays: (a) ninhydrin (Lepage, 1964); the chromatoplates were stained with 0.2% ninhydrin in 99% n-butanol and 1%

pyridine for phosphatidyl ethanolamine and phosphatidyl serine and free amino acids, (b) modified Dragendorff reagent (Mangold, 1961); choline containing phospholipids were orange and appeared immediately on spraying with this reagent, galactolipids gave orange spots that appeared more slowly, (c) molybdate reagent (Dittmer and Lester, 1964); on spraying with this reagent, phospholipids appeared as blue spots.

The lipid components separated by thin-layer chromatography were tentatively identified by comparing the Rf values with the data reported in literature (Mangold, 1961; Blank et al., 1964; Lepage, 1964, and Fisher et al., 1964), by using specific sprays and by comparing values with those of pure compounds. Among the neutral lipids, lauric acid, myristic acid, 1-monopalmitin, 1,2-dipalmitin, 1,3-dipalmitin, tripalmitin and tristearine (H.L. Mitchell, Biochemistry Department, Kansas State University) were used. Among the phospholipids (plant phosphoglyceride standards from Applied Science Laboratories Inc., State College, Pa.), lecithin phosphatidyl ethanolamine, and phosphatidyl serine were used as reference material.

Quantitative analysis of lipids by thin-layer chromatography: Lipid fractions separated from silicic acid columns and lipid components separated by thin-layer chromatography were measured quantitatively (Blank et al., 1964) by photodensitometry of spots charred by heating after spraying with sulfuric acid. Color intensity was determined with the help of densitometer, with automatic scanning stage and self-balancing potentiometer recorder.

RESULTS AND DISCUSSION

Fat acidity: Fat acidity of 35 samples of sound wheat was in the range of 10 to 17. The values were higher in benzene than in petroleum ether extracts (Table 1). TLC have shown that the difference could be attributed,

Table 1. Summary of fat acidity⁽¹⁾ values in lipids of 35 sound wheat samples extracted with benzene and petroleum ether.

	Benzene Extract	P. ether Extract
Maximum	17.6	16.4
Minimum	10.3	10.0
Average of 35 values	13.1	12.3

1. expressed on 'as is' basis; moisture between 11-13%.

in part at least, to different lipid components in the two extracts. Method 02-02 of Cereal Laboratory methods (AACC, 1962) using benzene as extractant was found to be very rapid and reliable when compared to method 02-01, using petroleum ether as extractant.

Free fatty acids in wheat moistened to about 18% and stored at 49° increased in the damp wheat, then decreased and finally increased when the moisture was below 10%. Barton-Wright (1938) and Bottomley et al. (1952) have reported the same trends in their studies.

From Table 2, it is clear that the benzene extracts contained consistently less titratable acidity, if dried prior to extraction to 11-12% moisture, then if titration was performed on moistened grain, thus confirming the observation of Baker et al. (1957). The moisture decreased as a result of partial loss of water of the wheat stored at 49° in loosely plugged containers for relatively long times. TLC of benzene extracts from damp wheat showed a number of unidentified compounds that were absent from extracts of dry wheat. Similarly, free fatty acids in wheat moistened to about 22% and stored at 49° increased at the beginning and then decreased. The moistures of the samples first decreased and increased in the later periods of storage (Table 3). The decrease in free fatty acids could be attributed to metabolism by molds as pointed out by Bottomley et al. (1952).

Table 3 shows a definite increase in fat acidity in the 22% moisture wheat samples stored at 4° for 132 days. This is also indicated in Table 4.

In wheats stored at 18 or 22% moisture, fat acidity values were around 50, despite intensive deterioration. This might be due to repression of mold growth by limited oxygen supply or could be attributed to higher temperature (49°) coupled with higher moistures. Bottomley et al. (1950) have reported in their study that as the temperature increased from 25° to 40°, there was

Table 2. Fat acidity in benzene extracts of 16% moisture wheat stored at 49°.

Length of Storage (Days)	Comanche						Seneca			
	Moistened Wheat			Redried Wheat			Moistened Wheat		Redried Wheat	
	% Moisture	Fat Acidity	% Moisture	% Moisture	Fat Acidity	% Moisture	% Moisture	Fat Acidity	% Moisture	Fat Acidity
0	18.1	18.9	12.1	12.1	13.9	17.6	17.6	18.9	12.2	12.7
2	18.2	18.9	13.4	13.4	13.8	17.6	17.6	18.9	13.7	14.7
5	17.9	20.9	12.4	12.4	15.1	17.1	17.1	20.9	12.4	15.1
8	17.7	21.7	11.7	11.7	15.6	17.0	17.0	21.9	11.9	15.6
15	17.1	43.1	11.6	11.6	34.9	16.4	16.4	27.5	11.6	21.3
31	16.7	53.3	11.7	11.7	45.5	15.8	15.8	38.1	12.0	34.5
42	14.6	47.1	-	-	-	13.8	13.8	41.0	-	-
56	12.0	39.0	-	-	-	11.8	11.8	39.8	-	-
70	9.7	47.1	-	-	-	9.6	9.6	48.7	-	-

Table 3. Fat acidity in benzene extracts and mold counts (colonies per gm.) of 22% moisture wheat stored at 49°.

Length of Storage (Days)	Comanche			Seneca		
	% Moisture	Fat Acidity	Mold Count ($\times 10^3$ /gm.)	% Moisture	Fat Acidity	Mold Count ($\times 10^3$ /gm.)
0	12.8	12.3	1	13.4	13.1	1
0	22.3	23.8	1	22.0	23.4	1
7	21.7	24.2	-	21.3	23.4	-
14	20.9	27.5	250	20.5	27.1	180
21	20.8	33.2	-	20.3	32.8	-
28	21.7	35.3	925	20.9	36.9	1125
42	21.6	30.8	1875	20.7	44.3	1475
56	21.9	27.1	50	21.0	43.1	2475
70	22.5	23.4	-	21.4	34.9	687
100	22.4	22.6	-	21.3	27.9	-
132	24.4	19.7	-	22.7	24.6	-
132	9.1 ⁽²⁾	10.3	-	9.1 ⁽²⁾	15.2	-
132 ⁽¹⁾	21.9	37.7	-	21.4	28.3	-
132 ⁽¹⁾	9.3 ⁽²⁾	26.7	-	9.6 ⁽²⁾	17.6	-

1. stored at 4°, after moistening to 22%.
2. redried in a forced draft oven below 60°.

a rise in fat acidity from 57.6 to 112.1, followed by a decrease to 51.1 at 45°. Withe and Onselius (1949) found that thermal inactivation of oat lipase was much greater when the heat treatment was carried out at 20% than at 12% moisture. Iinko (1960) has reported that during storage of moist wheat, enzymes themselves began to deteriorate rapidly due to the early increase in proteolytic activity. This enzyme inactivation was found temperature-dependent.

The increase in fat acidity, concomittant with increase in fungal growth is summarized in Table 3. Microbial counts of viable organisms in wheat stored beyond 70 days showed abundant bacterial growth; consequently mold counts of wheat stored longer are not included.

Thin-layer chromatography: TLC of benzene extracts from damp wheat (22% moisture) stored at 49° for 132 days (Fig. I and II) show a consistent decrease in triglycerides and an increase in free fatty acids. This is clearly shown by quantitative TLC (Table 4) where triglycerides decrease from 35.7 to 13.0% and free fatty acids increase from 2.0 to 11.2%.

Whereas the changes in the decomposition of triglycerides were relatively slow, the disappearance of certain components of the polar fraction proceeded at a more rapid rate (Fig. III and IV). Both the figures show higher extraction of phospholipids, especially phosphatidyl serine, at higher moisture (spots 1 and 2). This higher extraction of phospholipids at higher moisture is confirmed by quantitative TLC (Table 4), wherein the more rapid decrease of polar components than of triglycerides is shown. From these observations, it is now possible to suggest that early stages of grain deterioration might be detected by following the disappearance of certain phospholipids or by assays of enzymes involved in phospholipid metabolism, provided the enzymes are elaborated by fungi and are absent in sound grain. A decrease in phospho-

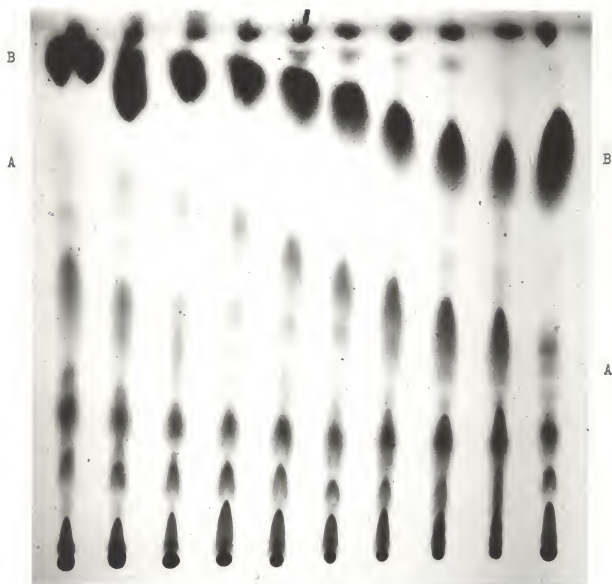


Fig. I. TLC of benzene extracts of 22% moisture Comanche wheat, (from left to right) stored for 0 (12.8% moisture), 0, 14, 28, 42, 56, 70, 100, 132 and 132 (at 40°) days at 49°. Developed with chloroform and visualized under ultraviolet after sulfuric acid spray. A = free fatty acids; B = triglycerides.

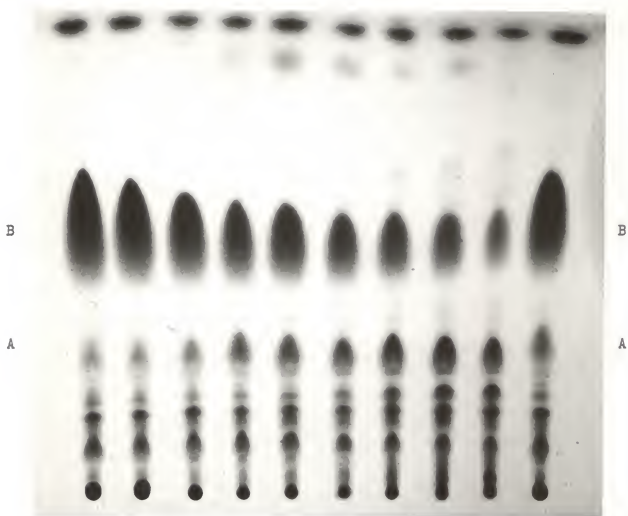


Fig. II. TLC of benzene extracts of 22% moisture Comanche wheat, (from left to right) stored for 0 (12.8% moisture), 0, 14, 28, 42, 56, 70, 100, 132 and 132 (at 49°C) days at 49°C. Developed with ether mixture and visualized under ultraviolet after sulfuric acid spray. A = free fatty acids; B = triglycerides.

Table 4. Changes in the composition of lipids (dry matter basis) of 22% moisture Seneca wheat stored at 49°, as determined by quantitative thin-layer chromatography (benzene extracts).

Length of Storage (Days)	Moisture %	Neutral Fractions		Polar Fractions (1)		
		Triglycerides % (3)	Free fatty acids % (3)	F % (3)	D % (3)	E % (3)
0	13.6	33.5	1.6	0.9	4.5	2.4
0	22.0	35.7	2.0	2.9	5.7	4.7
14	20.5	27.5	2.2	1.2	4.8	3.6
28	20.9	24.0	2.7	0.4	4.4	2.1
42	20.7	21.0	3.6	0.4	1.7	0.6
56	21.0	-	-	0.3	1.3	0.5
70	21.4	16.5	6.2	-	0.9	0.2
100	21.3	13.5	8.0	0.2	-	-
132	22.7	13.0	11.2	0.2	0.5	-
132 ⁽²⁾	21.7	26.5	2.2	1.3	4.9	3.6

1. tentatively identified as D = digalactosyl glyceride, E = monogalactosyl glyceride and F = phosphatidyl ethanolamine.
2. stored at 4°.
3. of the total lipids.

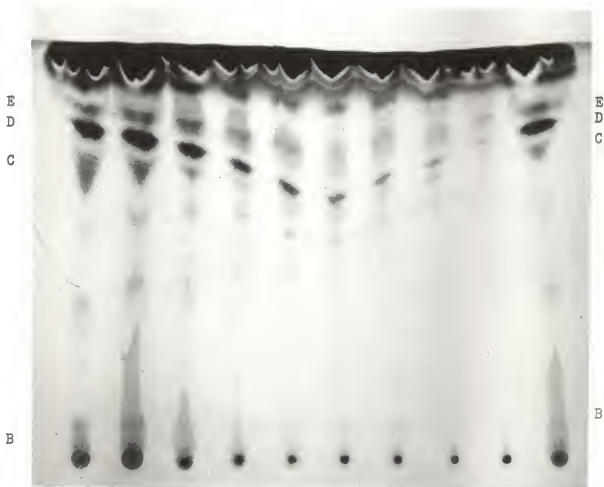


Fig. III. TLC of benzene extracts of 22% moisture Comanche wheat (from left to right) stored for 0 (12.8% moisture), 0, 14, 28, 42, 56, 70, 100, 132 and 132 (at 4°) days at 49°. Developed with chloroform mixture and visualized under ultraviolet after sulfuric acid ~~and~~ spray. B = Phosphatidyl serine; C = Phosphatidyl choline, D = digalactosyl glyceride, and E = monogalactosyl glyceride.

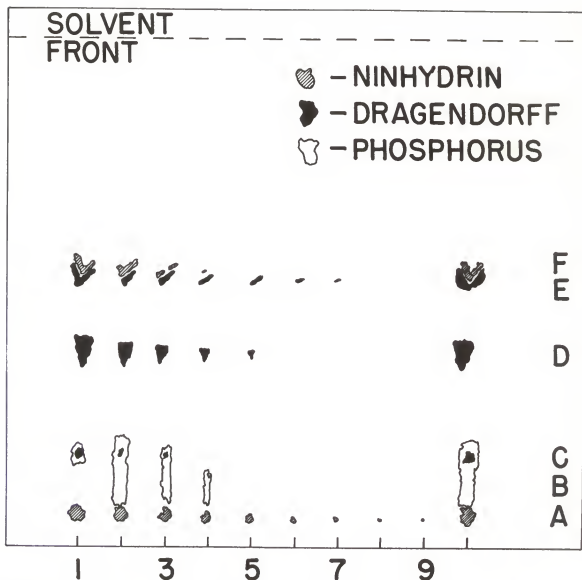


Fig. IV. TLC of benzene extracts of 22% moisture Comanche wheat (from left to right) stored for 0 (12.8% moisture), 0, 14, 28, 42, 56, 70, 100, 132, and 132 (at 4°) days at 49°. Developed with chloroform mixture and visualized after spraying with specific reagents. B = phosphatidyl serine; C = phosphatidyl choline; D = digalactosyl glyceride; E = monogalactosyl glyceride and F = phosphatidyl ethanolamine. The figure represents combined results from spraying one plate with ninhydrin followed by Dragendorff's reagent and one plate with ninhydrin, followed by phosphorous reagent. All spots shown, gave a phosphorous positive reaction. Only spots that did not give a ninhydrin or Dragendorff reaction are outlined as colored with the phosphorous reagent.

lipids in a polished rice during six months' storage at room temperature has been reported very recently by Katsuharu and Shintaro (1964).

To identify the phospholipids metabolized by the proliferating fungi, lipids separated on TLC by chloroform mixture were sprayed with reagents specific for free amino acids-, choline-, and phosphorus-containing lipids (Fig. IV). These compounds are tentatively identified as phosphatidyl serine (B), phosphatidyl choline (C), digalactosyl glyceride (D), monogalactosyl glyceride (E) and phosphatidyl ethanolamine (F).

Fungal attack was accompanied by elaboration of at least four compounds (at different storage periods) with varying Rf values, which could be seen under ultraviolet light without spraying the plate (Fig. V). As the formation of these auto-fluorescing compounds took place at a relatively early stage of damage, the sequence of their elaboration could be used to identify incipient deterioration, provided the metabolites are elaborated by all or most prevalent saprophytic fungi present in stored grain.

Column chromatography: The intensive disappearance of phospholipids in deteriorating grain is again illustrated in Table 5. Whereas both neutral and polar lipids decreased during storage, the neutral lipids decreased only slowly and actually constituted, at advanced stages of deterioration, a larger fraction of the residual lipid extract as phospholipids rapidly disappeared. The decrease in triglycerides and increase in free fatty acids in the non-polar fraction of water-saturated butanol extract is shown in Fig. VI.

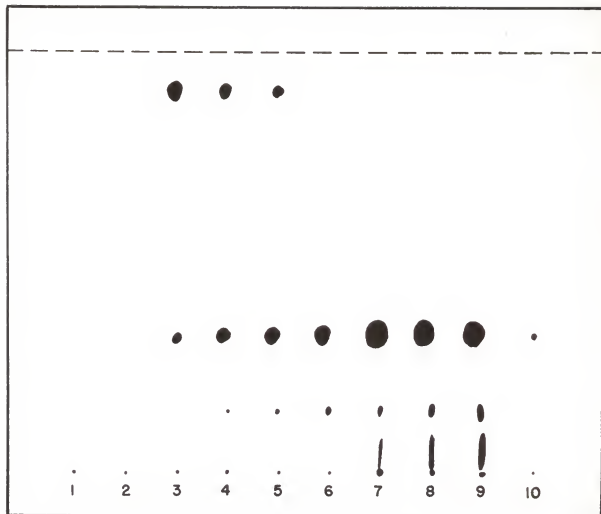


Fig. V. TLC of benzene extracts of 22% moisture Comanche wheat stored for 0 (at 12.8% moisture), 0, 14, 28, 42, 56, 70, 100, 132 and 132 (at 4^o) days at 49^o (from left to right). Developed with chloroform and visualized under ultraviolet without any spray.

Table 5. Total, neutral and polar lipids (on dry matter basis) extracted with water-saturated butanol from 22% moisture wheat stored at 49°.

Length of Storage (Days)	Comanche				Seneca			
	Total lipids %	Neutral lipids %	Polar lipids %	Recovery %	Total lipids %	Neutral lipids %	Polar lipids %	Recovery %
0(1)	1.97	1.26	0.63	96.0	2.27	1.47	0.77	98.7
140(2)	2.00	1.20	0.67	96.0	2.24	1.43	0.76	97.8
140	1.52	0.98	0.38	89.5	1.65	1.22	0.39	97.6
148	1.43	1.06	0.37	100.0	1.60	1.22	0.36	98.9
160	1.17	0.94	0.23	99.8	1.38	1.13	0.25	100.0

1. original control sample (12.8% moisture) stored at 4°.

2. 22% moisture wheat stored at 4°.

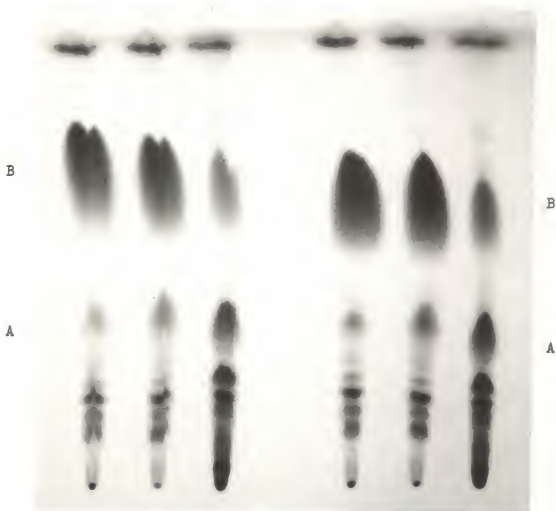


Fig. VI. TLC of neutral lipids from water saturated butanol extracts of 22% moisture Seneca wheat. From left to right, 0 days at 49°, 140 days at 4°, and 160 days at 49° samples. The right half is the replication of the left 3 spots. Developed with chloroform and visualized under ultraviolet after sulfuric acid spray. A = free fatty acids; B = triglycerides.

SUMMARY

Fat acidity of 35 sound wheat samples from the 1963 crop ranged between 10 and 17 (on 'as is' basis). The values were higher by 6 to 8 percent in benzene extracts than in petroleum ether extracts of wheat.

Benzene extracts of moistened wheat contained more lipids and gave higher fat acidity values than did extracts of wheat redried to 11-12% moisture. Fat acidity of wheat samples moistened to 18 and 22% moisture and stored at 49° for 70 and 132 days respectively, increased for some time and then decreased. Twenty-two % moisture wheat samples, stored at 4° for 132 days, showed increase in fat acidity, with decrease in triglycerides and a number of polar lipid fractions.

Changes in lipid composition during grain deterioration were followed by qualitative and quantitative thin-layer chromatography, and by fractionation on silicic acid columns. Deterioration of wheat was accompanied by formation of at least four unidentified compounds that showed auto-fluorescence under ultraviolet light. Grain deterioration was accompanied by lowering of non-polar and polar fractions of lipid. The breakdown of phospholipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides. Among the rapidly disappearing phospholipid components phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, digalactosylglyceride and monogalactosylglyceride have been tentatively identified.

The sequence of the formation of auto-fluorescent compounds during early stages of deterioration and the rapid disappearance of different phospholipid components have been suggested as possible indices to detect early deterioration in wheat.

SUGGESTIONS FOR FUTURE RESEARCH

The role of the lipases and particularly enzymes responsible for phospholipid breakdown has not been fully studied in connection with grain storage. This subject would constitute an interesting research problem. When such basic experimental data are secured, the effect of esterases on the storage of cereal grains and their milled products, and their relations to other aspects of the milling and baking industry, can be better evaluated.

Little is known about the physiological activities of viable seeds at very early stages of water imbibition. This is particularly true of moisture levels insufficient to produce germination, such as may occur during storage of grain. A need to understand the basic biochemical reactions taking place under these conditions is evident. It is hoped that the study of moisture levels required for the activation of various enzymes in wheat, would help evaluate the biochemical behavior of grain during storage at different moisture levels and temperatures.

Considerable uncertainty still exists regarding the reactions involved in fatty acid utilization by plants and molds, though in mammalian tissue, it is now fully established that fatty acids are degraded by β -oxidation and converted to acetyl-coenzyme A derivatives, which then enter the common metabolic pool of the cell. A better understanding of the phenomenon of increase followed by decrease in fat acidity during storage of grain, is highly desirable. The source of free fatty acids at different stages of deterioration, under different sets of storage conditions, should be investigated to establish the value of this determination in assessing changes in stored grain. Additional research is needed concerning the changes taking place in different phospholipid components and the formation of auto-fluorescent

compounds under normal storage conditions. When a clear picture is obtained of the primary reactions which eventually result in deterioration, means may be developed to slow down these processes and to extend the storage life of grain.

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A STUDY ON THE CHANGES IN THE COMPOSITION
OF WHEAT LIPIDS DURING STORAGE

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A number of parameters, including fat acidity, to predict incipient deterioration of wheat have been suggested. Literature review indicates, however, some contradictory data regarding the significance of free fatty acids in predicting storage behavior and the extent of actual damage to stored grain. Again, relatively little is known on transformations occurring in wheat lipids during storage.

The purpose of this study was to follow changes taking place in the polar and nonpolar lipids of wheat stored at elevated moisture levels (18% and 22%) and temperature (49°). The methods used to fractionate the extracted lipids into different components included qualitative and quantitative thin-layer chromatography (TLC) and fractionation on silicic acid columns. Tentative identification of some of the components was done with the help of standard compounds and Rf values reported in the literature.

Fat acidity of 35 sound wheat samples from the 1963 crop ranged between 10 and 17. The values were higher by 6 to 8 percent in benzene extracts than in petroleum ether extracts. Benzene extracts of moistened wheat contained more lipids and gave higher fat acidity values than did extracts of wheat redried to 11-12% moisture. Fat acidity of wheat samples moistened to 18 and 22% and stored at 49° for 70 and 132 days respectively, increased for some time and then decreased. Twenty-two percent moisture wheat samples, stored at 4° for 132 days showed an increase in fat acidity, with decrease in triglycerides and a number of polar lipid fractions.

Deterioration of wheat was accompanied by the formation of at least four unidentified compounds that showed auto-fluorescence under ultra violet light. As this took place at a relatively early stage of damage the sequence of elaboration of the fluorescing compounds has been suggested as a possible

index to detect early deterioration in wheat.

Grain deterioration was accompanied by lowering of nonpolar and polar fractions of lipid. The breakdown of phospholipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides. Among the rapidly disappearing phospholipid components, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, digalactosylglyceride and monogalactosylglyceride have been tentatively identified. This rapid disappearance of different phospholipid components has also been suggested as an index to detect incipient deterioration in wheat.