AN EVALUATION OF THE ANTIOXIDANT PROPERTIES OF GRAIN SORGHUM

by

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CHAPTER I

INTRODUCTION

Fats and oils in stored foods undergo autoxidation which result in flavor and other quality changes commonly known as rancidity. Rancidity in fats not only renders food unpalatable but is also responsible for the partial destruction of the essential fatty acids and other dietary nutrients such as vitamins A, E, and perhaps D, and certain of the B complex vitamins (Cummings, et al., 1931; Burr, et al., 1943; Quackenbush, 1945; Siedler, et al., 1954; Lea, 1962; Kummerow, 1962; Stuckey, 1962).

The lipids in grain sorghum are important to animal and human nutrition but may contribute to the development of off-flavors and rancidity in sorghum-based products (Wall, et al., 1970). In addition to naturally occurring lipids, it is customary to have fats and oils added to food for specific purposes such as color and flavor enhancement, or texture improvement (Bradshaw, 1959). Such exogenous sources of lipids also contribute toward the development of lipid deterioration which in turn is responsible for a decreased stability of lipids and hence a shorter shelf-life of the particular food products.

In the food industry, the most common approach to the control of oxidative rancidity is through the use of synthetic antioxidants such as butylated hydroxy anisole (BHA), and butylated

hydroxy toluene (BHT). However, two problems are encountered with the use of synthetic antioxidants.

First, there is the question of "carry through," that is, the ability of the antioxidant to survive a baking or cooking process to retain its effectiveness to protect the fat in the finished product. Second, there is the question of toxicity as the products of lipid oxidation can be toxic as well as unpleasant (Johnson, 1971). Of the synthetic inhibitors, BHA and BHT have long enjoyed the reputation of being safe food additives (World Health Organization Tech. Rept. Ser. No. 228, 1962). Results of toxicity studies have shown them to be completely excreted by man within 14 days with no accumulation (Johnson, 1971). Toxicity studies on NDGA (Nordihydroguaiaretic acid), another synthetic antioxidant, has been performed only with rats. Since enzyme pathways in animals are not necessarily the same as those in humans, the usage of NDGA has been banned in some countries such as the United States, but not in others (Johnson, 1971). Such a discord among the various countries concerning the usage of certain synthetic antioxidants has created problems. Therefore, it would be highly desirable, not only from the safety point of view, but also from an economic standpoint, to reduce the need for food additives whenever possible.

No published data related to the oxidative stability of grain sorghum are available. Watson (1970) observed an unusual stability of the germ fraction of the sorghum grain to oxidation

and hypothesized the presence of a powerful antioxidant. If grain sorghum has the ability to act as a natural antioxidant it can then serve a two-fold purpose: in sorghum-based food products by prolonging their keeping quality and by being part of the nutrient itself.

As a result, this study was undertaken: 1) to determine the antioxidant activity of various fractions of grain sorghum; 2) to evaluate the antioxidant potential of selected sorghum fractions in preserving a food product high in lipid content, namely pastry, from undergoing rapid oxidative deterioration; and 3) to correlate sensory evaluations of rancidity scores of pastry with their corresponding peroxide values obtained from chemical analysis.

Hypotheses

Three main themes concerning antioxidants and peroxidation of fats seem to warrant investigation:

1) The antioxidant potential of grain sorghum has not yet been investigated;
2) The rate of autoxidation of fats seems to increase with time and temperature;
3) Peroxidation of fats can be delayed or probably prevented through the use of suitable antioxidants, the rate of retardation of which is proportional to the amount of antioxidant used.

Therefore, to ascertain the antioxidant activity of grain sorghum, the following null hypotheses were tested:

1) There is no significant difference in the relationship of temperature with antioxidant activity;
2) There is no significant difference in the relationship of time with antioxidant activity; and
3) There is no significant difference in the relationship of amount of grain sorghum used in the pastry with antioxidant activity.

CHAPTER II

REVIEW OF LITERATURE

Grain Sorghum

Structure and Composition of Grain

The structure and chemical composition of grain sorghum are similar to those of corn (Watson, 1959; Wall, et £]^., 1969; Hubbard, et al., 1950; Ross, et ^., 1960; Kramer, 1959). However, the chemical composition varies widely among the sorghum grain varieties and hybrids (Quisenberry, e^ ^., 1970).

In structure, the sorghum kernel (Fig. 1) is a flattened sphere approximately 4.00 mm long by 3.5 mm wide by 2.5 mm thick (Rooney and Clark, 1968). It is composed of three main parts, the outer covering (pericarp or bran), the storage tissue (endosperm), and the germ.

In composition, sorghum generally has more protein than corn, less fat, about the same amount and proportion of carbohydrate components, more tannins and wax except for the yellow endosperm varieties, but no xanthophyll and carotenoid pigments (Kramer, 1959).

The percentages of germ, bran, and endosperm of sorghum grain, along with the proximate analysis of each portion, are shown in Table I. These are the mean values of five different varieties of sorghum which were hand-dissected.

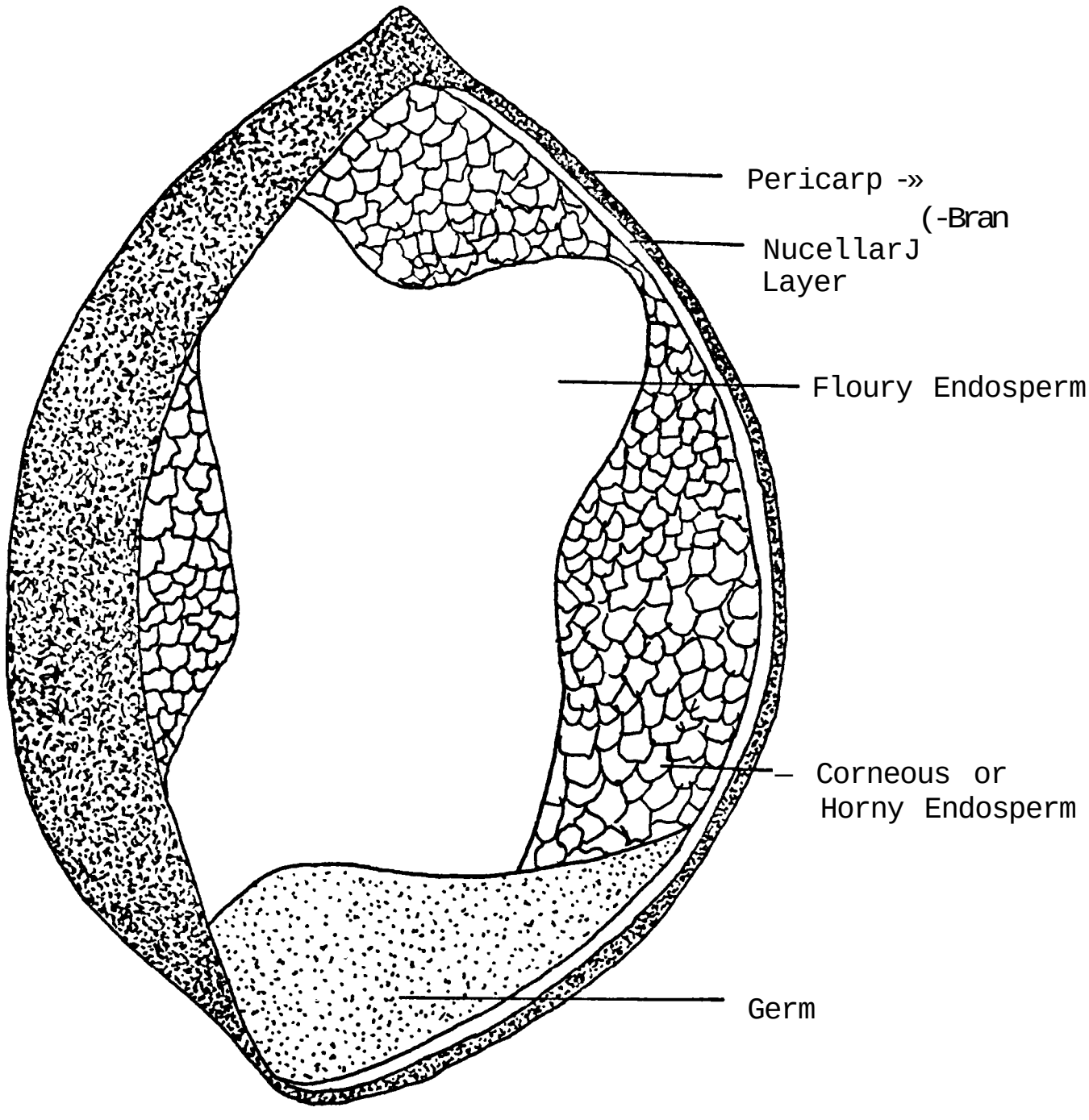


Fig. 1.—Structure of the Sorghum Kernel.

TABLE 1

COMPOSITION OF WHOLE SORGHUM GRAIN AND HAND-DISSECTED FRACTIONS^

Fraction	Kernel %	Protein %	Starch %	Oil %	Ash %
Whole Grain	100.0	12.3	73.8	3.6	1.65
Endosperm	82.3	12.3	82.5	0.6	0.37
Germ	9.8	18.9	13.4	28.1	10.36
Bran	7.9	6.7	34.6	4.9	2.62

Hubbard, et al., 1950

Lipids

Sorghum oil is extracted primarily from the wet-milled germ fraction (Wall and Blessin, 1969) and can be divided into two main types, nonpolar and polar. The nonpolar lipids contain triglycerides mainly, with lesser amounts of hydrocarbons, sterol esters, fatty acids, diglycerides, sterols, and phospholipids, one half of which is lecithin and the remainder cephalin (Wall and Blessin, 1969) The oils vary in color from light green to amber, depending upon the pigments in the sorghum variety (Rooney and Clark, 1968).

The fatty-acid composition of sorghum grain oil is nearly the same as that of com oil (Baldwin, et^ al• > 1951; Kummerow, 1946), with sorghum containing slightly less unsaturated fatty acids but some linolenic acid (Rooney and Clark, 1968). Major fatty acids in

a typical sorghum oil are linoleic, 52 per cent; oleic, 32 per cent; palmitic, 10 per cent; stearic, 4 per cent; and linolenic, 1 per cent (Baldwin, 1951).

Pigments

The pigments of sorghum can be divided into two broad categories, the carotenoids and phenolic compounds (Rooney and Clark, 1968). The common varieties of sorghum contain only a trace of carotenoids (Rooney and Clark, 1968). However, sorghum varieties found in Nigeria and India with a yellow endosperm contain appreciable amounts of carotenoids (Wall and Blessin, 1970).

The sorghum kernel contains varying amounts and types of pigments in the pericarp and nucellar layer of the bran. They are absent in the germ and endosperm (Dimler, 1965).

The phenolic pigments are mainly flavonoid in nature. Leucoanthocyanins or anthocyanogens, a group of colorless flavonoid pigment precursors (Blessin, et al. , 1963; Wall, et^ SLL. , 1970) undergo chemical change to produce anthocyanidins (Wall, et^ al., 1969) and apparently impart astringency to foods and beverages (Wall, et^ aJ[^., 1970). Pelargonidin and cyanidin, two anthocyanidins, have been reported to be found in the integument of sorghimi (Olifson, et al. , 1971). Generally, anthocyanogens are found in yellow milo and red kafir sorghum but not in white kafir, waxy, and yellow endosperm types (Blessin, et al., 1963).

Other flavonoid pigments, namely, apigenidin, quercetin and kaempferol, have been identified in the pericarp (Wall, et al., 1969; Olifson, 1971).

Tannins

Sorghum grain varieties with brown seed color are characteristically high in tannins (Wall, et al., 1970). Tannins are found chiefly in the pericarp and have been implicated as the cause of bitterness and unpalatability (Dimler, 1965; Blessin, e£ al., 1963; Chang, et al., 1964; Wall, et^ al•, 1970). White sorghum grain contains less tannins than the more pigmented varieties (Dimler, 1965).

Tannins have an inhibitory effect on various enzymes. Milles and Keen (1947) isolated an amylase inhibitor mainly found in the bran fraction of sorghum grain. The inhibitor was extremely resistant to heat and was soluble in ether. Enzymes whose activities are decreased by tannins are amylase, peroxidase, pectinase and cellulase (Bell, e^al., 1965; Bell, et_ al., 1962; Lyr, 1965).

Vitamins

The amount of individual vitamins varies considerably among varieties of sorghum (Hubbard, et^ aJ^., 1950). In determining the average distribution of B-complex vitamins, Hubbard and his associates hand-dissected fractions from five varieties of sorghum and found that the germ has two to five times the quantity of vitamins present in endosperm and bran. Ascorbic acid was reported to be

present in the whole grain (21 ug per gm), but not in the main fractions of germ, bran and endosperm (Wall and Blessin, 1970).

Minerals

According to Wall and Blessin (1970), phosphorus, magnesium, potassium and silicon are the major minerals in sorghum grain. From 40 to 75 per cent of the phosphorus present is in the form of phytate phosphorus. Other minerals include copper (3-10 ppm), iron (38-150 ppm) and manganese (16-30 ppm).

Acids

Of various grains analyzed, sorghum has the highest percentage of its phosphorus present in phytic acid (Wall and Blessin, 1970). In the whole grain phytate phosphorus ranged from 0.20-0.37 per cent; among the fractions, the germ has the highest content of phytate, ranging from 0.54-1.91 per cent; bran from 0.19-0.49 per cent; and grits, from 0.03-0.07 per cent (Wall and Blessin, 1970).

Enzymes

The most extensively studied enzymes of sorghum grain are those that degrade starch, namely the amylases (Wall and Blessin, 1970). Among the oxidative enzjmies, peroxidase, an iron-porphyrin enzyme that promotes oxidation of substrates by hydrogen peroxide, is found to be present in the sorghum grain (Wall, et_ al., 1970).

Lipid Oxidation

Lipid oxidation can be divided into two classes: enzymatically catalyzed oxidations, and autoxidation which are apparently autocatalytic (Johnson, 1971). The main difference between these two classes is that oxidative reactions that are catalyzed by enzymes cannot be stopped by antioxidants and generally the enzymes must be inactivated.

Lipid oxidation results in fat rancidity. Gardner and Inglett (1971) classified rancidity into two types: hydrolytic fat rancidity and oxidative fat rancidity. The former is most often caused by lipase activity and the latter can be caused by lipoxygenase or autoxidation or both.

When oxygen attacks the unsaturated, double-bond linkages of fatty acids contained in fats and oils, autoxidation is said to occur. The energy required for this reaction to proceed is obtained from sunlight, heat or from chemical components in the food (Bauernfeind, et al., 1970). Other factors affecting the velocity of such a reaction are moisture, the amount of air in contact with the fat, the presence or absence of pro-oxidants and antioxidants (Lundberg, 1954; Lundberg, 1962; Johnson, 1971).

Autocatalytic autoxidation, the most common type of lipid autoxidation, is characteristic of the fatty acid moieties of such products as lard, shortenings, cooking and salad oils, and food products in which they are used (Lundberg, 1962). Such an autoxidation can be divided into four periods. These periods

are induction period, peroxidation period, period of main autoxidation, and period of decline in autoxidation (Khan, 1954).

The initiation processes of autoxidation occur during the induction period and peroxidation period (Khan, 1954). During the induction period, the amount of oxygen absorbed is very small and is a measure of stability of a fat or oil (French, et^ al., 1935). However, once started, the autoxidation process is represented by a period of rapidly accelerating oxygen absorption, the rate increasing with the concentration of peroxidation (French, et al., 1935; Khan, 1954). The onset of rancidity usually coincides with or follows shortly after the end of the induction period (French, et. al., 1935).

Mechanism

The overall oxidation mechanism whereby molecular oxygen reacts with the unsaturated fatty acids of the lipids resulting in the typical production of volatile off-flavors is shown in Figure 2.

Based upon detailed studies of autoxidation of ethyl linoleate. Farmer (1946), and Bolland (1946) have worked out a mechanism for the autoxidation of fat. The mechanism was generalized by Lundberg (1962) and is represented in Figure 3.

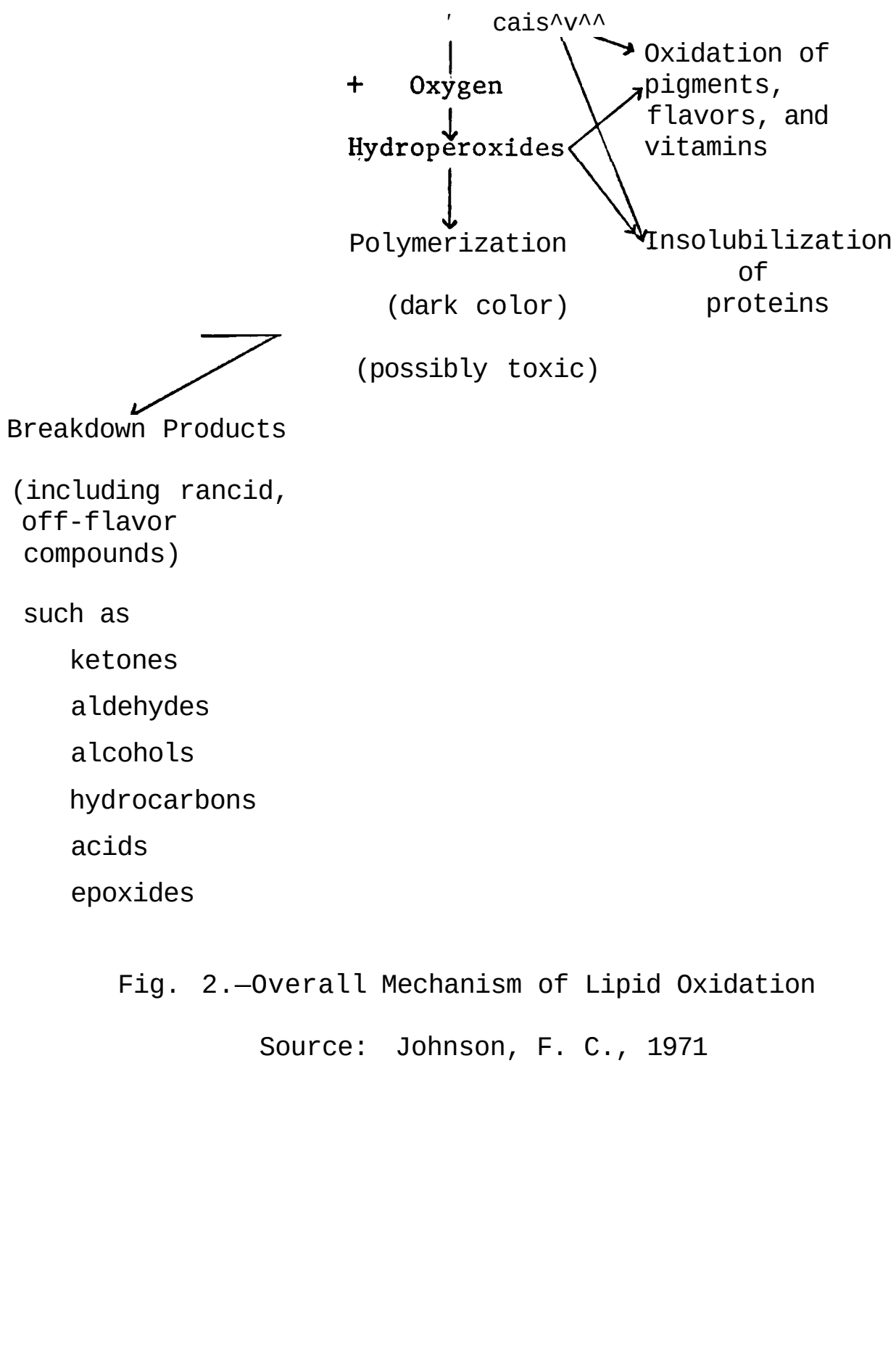


Fig. 2.—Overall Mechanism of Lipid Oxidation

Source: Johnson, F. C., 1971

Initiation:

RH + 0, -* free radicals

ROOH
(ROOH)^ J -> f ree radicals (£.^., R-, R0«, H0», etc.)

Propagation:

R« + O_2 ->R02.

R0«' + RH -> R' ROOH

Termination:

R« + R-

R« + RO_2 -> stable (non-radical) end products

R0«« H RO.

Fig. 3.—^Mechanism of Autocatalytic Autoxidation

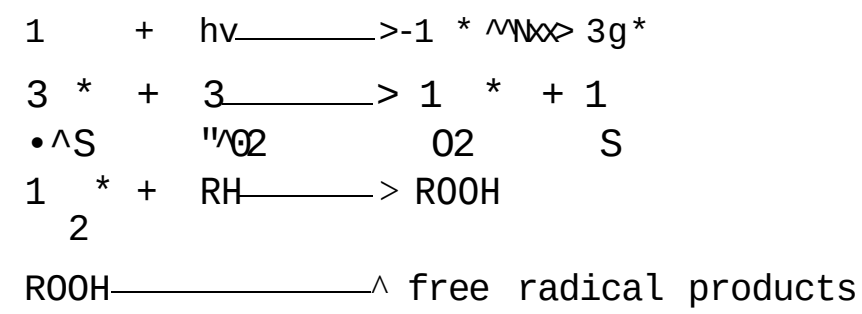
Where	RH	=	fatty acid	RO'	alkoxy radical
	ROOH	=	hydroperoxide	R»	alkyl radical
	RO^*	=	peroxy radical	HO'	hydroxyl radical

The mechanism as proposed by Lundberg (1962), and represented diagrammatically in Figure 3, is almost universally accepted and has been thoroughly studied (Gunstone, et al., 1945; Farmer, 1946; Bol-land, 1946; Gunstone, 1950). However, one factor remains unsatis-factorily explained: the origin of the initial free radicals necessary to begin the process in an oil completely free of hydro-peroxides (Gunstone, £t^ al., 1945; Khan, 1954; Farmer, 1946; Privett, et^ al. , 1962). How the first peroxides are formed is a major question to be asked since the principal chain initiation

reaction involves the decomposition of previously formed hydroxides by a second order process (Lundberg, 1954; Rawls, ej^ al., 1970).

Two recent studies (Ingold, 1961; Rawls, et al., 1970) have centered around the possible role of singlet oxygen in the primary initiation reaction. According to Rawls and his coworkers (1970), hydroxide formation requires a change in total electron spin since both the substrate and product are in singlet states, while oxygen is a triplet. Conservation of spin is violated making the reaction improbable. These researchers postulated if singlet oxygen was the species involved, then the reaction was possible for the formation of original hydroperoxides.

Pigments, commonly found in plants and animal sources of fatty acids, serve as sensitizers, and coupled with visible light, lead to the photosensitized production of oxygen in its singlet state,



^^^^1 = singlet sensitizer

1 * = excited singlet state sensitizer

3_* = excited triplet state sensitizer

3
0 = normal triplet oxygens

1 *
0^ = excited singlet state oxygen

hv = uv light energy in photons

Fig. 4.—Role of Singlet Oxygen in the Initiation of Fatty Acid Autoxidation.

Primary and Secondary Products

It is now widely accepted that hydroxides are the primary product of the reaction of oxygen with unsaturated lipids (Keeney, 1962).

With most fats and oils, autoxidation does not proceed very far before considerable proportions of secondary products develop (Lundberg, 1954). Even in the initial stages of autoxidation a small proportion of these is formed concurrently with the peroxides. For the most part, however, the secondary products are formed by a decomposition of the peroxides by interaction of the peroxides with other materials in autoxidizing fat, and by further oxidation of the peroxides (Lundberg, 1954). Lea (1962) outlined some of the routes by which the first-formed hydroperoxides can decompose (Fig. 5).

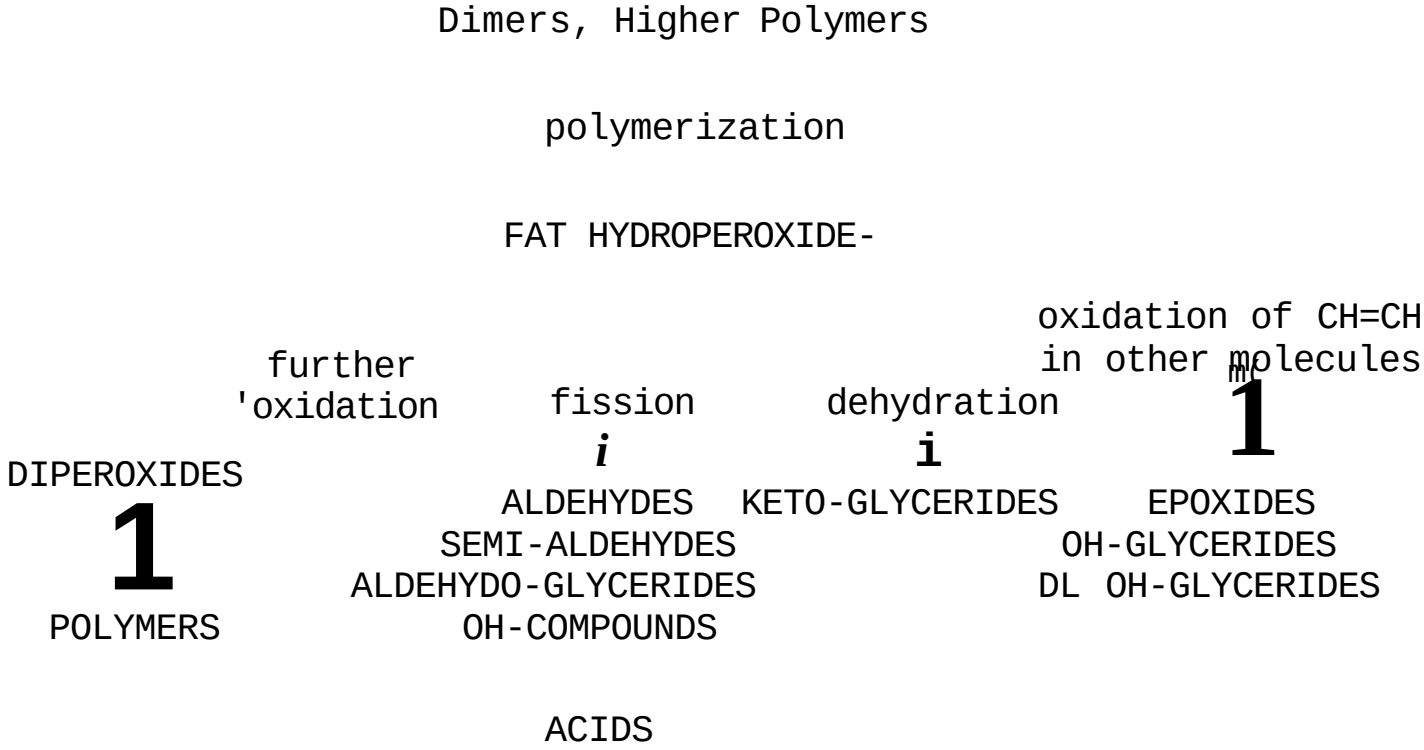


Fig. 5.—Some Routes of Decomposition of Fat Hydroperoxide.

Decomposition of hydroperoxides are catalyzed by metals, hemes, and oxidative enzymes and can be accelerated by heat (Aylward, et a]^., 1969).

According to Henick and his associates (1954), the hydroperoxides are all odorless and tasteless. It is some of the volatile cleavage products, namely the aldehydes, which are believed to be primarily responsible for the characteristic flavors of oxidized fats (Keeney, 1962). The best known oxidative off-flavors are rancidity and the reversion of flavor (Hoffman, et al., 1962).

The mechanism whereby peroxides decompose to form secondary degradation products has been investigated and and has been shown to be via free radical chain reactions (Keeney, 1962).

Accelerators of Lipid Oxidation

It has long been known that the autoxidation of fats may be markedly accelerated by various materials (Lundberg, 1954). A discussion of the factors affecting the development of rancidity in fats is necessarily an over-simplification in that each factor must be reviewed separately. In practice, however, each factor does not have an independent role, but exerts its influence in conjunction with many others (Bradshaw, 1959).

In addition to such factors as heat and light, various prooxidant substances, in small concentrations, markedly accelerate the rate of autoxidation in lipid materials. Among those that are important in relation to food fats are various metals, metal salts and

organic compounds of metals; oxidative enzymes, such as hemoglobin and other hematin compounds; and photochemical pigments which act as accelerators in the presence of light (Lundberg, 1962).

Temperature

According to Lundberg (1962), at ordinary temperature, the effect of increasing temperature on the rate of autocatalytic autoxidation is somewhat greater than in most chemical reactions. Increasing temperature accelerates not only the chain propagation reactions, but also the peroxide decomposition, thereby making a greater concentration of free radicals available for the initiation and propagation of reaction chains.

One situation whereby an increase in temperature results in an accelerated rate of fat oxidation can be demonstrated on a mix containing fat and flour (Bradshaw, 1959). As the temperature increases, not only does the rate of oxidation of the fat increase, but due to seepage and soaking up of the fat on the flour particles, the surface area of the fat is also increased. This in turn gives additional opportunity for the fat oxidation to take place. Swern (1961), in his review of autoxidation, quoted the temperature range of 0-40° C as being the lowest temperatures whereby many of the oxidation reactions involved in rancidification of fats occur.

In addition, it has been proposed that increased temperature can be responsible for the direct attack of oxygen on the

unsaturated fatty acid (Bolland, ^ al., 1947; Russel, 1956; Shelton, ^ al^. , 1963). Perhaps at the higher temperatures, the singlet-state oxygen formation increases (Labuza, 1971).

Metals

Among the more effective positive catalysts, or pro-oxidants, are various metals or metallic salts (Lundberg, 1954; Bradshaw, 1959). The transition (heavy) metals, particularly those possessing two or more valency states with a suitable oxidation-reduction potential between them (e^.^. , iron, cobalt, copper, manganese, etc.), both decrease the induction period and increase the rate of oxidative deterioration of food lipids (Ingold, 1961; Labuza, 1971).

Copper in particular is a very strong pro-oxidant, reducing the normal stability of lard at 208° F to half its normal value at a concentration of 0.05 ppm (Bailey, 1964). Copper is also a well-known catalyst for the oxidation of ascorbic acid and various poly-phenols (Tappel, 1955).

Osterberg and Sjoberg (1971) reported that of the copper ions present in food stuffs, those bound in the form of complexes to nitrogen, oxygen, and sulfur ligand atoms seem to catalyze the oxidation of lipids. However, no attempts have been made thus far to identify a certain copper complex as active.

Various mechanisms have been proposed for the effects of trace metals and several possible mechanisms of metal catalysts

have been described by Uri (1961 a). One of the more important of these is "reduction activation," involving the oxidation of a metal ion, as well as the production of hydroxyl ions and free radicals:

M"^"^ + ROOH_____> M^'^-'-^"^ + 0H~ + RO-

Since trace metals such as copper, manganese, and iron are to be found in cereal products, wrapping materials and enrichment ingredients, it is not surprising that the oxidation of fats is a common problem in the food industry (Bradshaw, 1959).

Biological Catalysts

Mechanisms involved in the peroxidation of fatty materials by biological catalysts, particularly lipoxidases and hematin compounds have been studied extensively by Tappel (1961, 1962). Lipoxidase, with no known prosthetic group or co-factor, is a highly specific catalyst for the peroxidation of unsaturated fatty **acids** that contain the cis - cis 1,4 - pentadiene system (Lundberg, **1962;** Tappel, 1961, 1962). Thus, lipoxidase will oxidize linoleic, linolenic and arachidonic acids, esters and triglycerides, but not those of oleic acid and its derivatives (Dillard, et al., 1961; Aylward, et al., 1969).

Lipoxidase, now known as lipoxygenase (Gardner and Inglett, **1971),** is widely distributed, particularly in legumes, potatoes, tomatoes, and various herbs (Aylward, et al., 1969). Tappel (1962)

cited the presence of lipoxidase in certain cereal grains and oil seeds. Most published data concern soya bean lipoxygenase, although recent studies (Wagenknecht, 1959; Gardner, 1970; Gardner and Inglett, 1971) on corn lipoxygenase, lipase, peroxidase and linoleic acid hydroperoxide-isomerase have been reported. Very little information is available on cereal enzymes in general (Bradshaw, 1959), and research on the enzymes of sorghum grain has been limited (Rooney and Clark, 1968). Wall and Blessin (1970) reported the presence of peroxidase, but there was no mention of lipoxidase. More work remains to be done in this field.

Inhibitors of Lipid Oxidation

Various substances known as antioxidants have a marked retarding effect on the autoxidation of fats and oils. Because of their importance to the prevention of rancidity in food fats, much effort has been devoted to searches for new and better antioxidants, and to studies of the mechanism whereby antioxidants exert their effect (Lundberg, 1954).

Observations from a number of studies on the inhibition of lipid oxidation have indicated that more than one type of action may occur, depending upon the conditions of the reaction and the type of system being studied (Stuckey, 1962). Four possible mechanisms by which an inhibitor may function as a chain stopper for the free radical chain mechanism of lipid oxidation have been proposed (Shelton, 1959). These are: 1) hydrogen donation by the

antioxidant; 2) electron donation by the antioxidant; 3) addition of R0«» to the aromatic ring of the antioxidant; and 4) formation of a complex between RO • and the aromatic ring of the antioxidant.

Types of Antioxidants

Lea (1958) classified antioxidants into two main types—primary antioxidants and synergists.

Primary Antioxidants

Primary antioxidants are usually ortho, para, di and poly phenolic compounds and aromatic amines (Lea, 1958; Matill, 1945). They function primarily in two ways, acting as chain terminators or peroxide decomposers.

The chain terminators function by removing alkyperoxy radicals (R00«) or alkyl radicals (R*), which are chain propagating radicals, by donating a hydrogen or an electron to the radical (Holdsworth, et al. , 1964; Labuza, 1971). A general scheme of the reaction is presented in Figure 6.

Chain reaction:

R* + ©2 ——— ^ ROO*

ROO* + RH ——— > ROOH + R*

Inhibition:

R« + AH ——— > RH + A.

ROO' + AH ——— > ROOH + A'

A* + X ——— ^ Non radical (inactive product)

Where AH = a phenol or amine antioxidant
X = some other moiety

Fig. 6.—General Scheme of Inhibition of Lipid Oxidation by Chain Terminator

A variety of sulfur-containing compounds has been found to possess the property of decomposing peroxides (Holdworth, £t al., 1964; Bateman, £t al., 1962; Bernard, et al., 1961). Their action involves the destruction of chain initiating hydroperoxides. A typical mechanism proposed by Holdworth (1964) is presented in Figure 7.

Chain initiation:

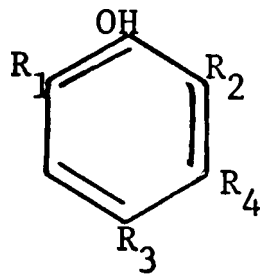
ROOH————————> RO* + -OH
2R00H————————> ROO- + HO + RO-

Inhibition:

ROOH + inhibitor————^ Non radical product

Fig. 7.—General Scheme of Inhibition of Lipid Oxidation by Peroxide Decomposers

Stuckey (1962) observed that all primary antioxidants are structurally similar in containing unsaturated benzene rings plus either hydroxy or amino groups. To be a good antioxidant, the compound must be able to have an effective delocalization of the unpaired electron produced in the reaction with the free radical (Labuza, 1971). The more effective the resonance forms, the better the antioxidant. Therefore, substitution of R- and R^ (ortho) and $R^{\wedge}$ (para) are much more effective and greatly enhances the potency of the antioxidant than at the meta R, position because of the greater number of resonance forms possible (Morawetz, 1949; Thompson, 1956; Labuza, 1971).



A bulky attachment helps to protect the antioxidant radical and gives more stability toward further reaction, but this also makes it more difficult to react with the peroxy radical (Labuza, **1971).**

Synergists

Synergists or metal deactivators are substances which delay or inhibit the autoxidation of fats (Mattill, 1945). These agents affect the initiation rate, that is, control the source of the production of free radicals before the propagation step (Labuza, 1971). They are usually inactive or feebly active when used alone, but in the presence of primary antioxidants they function by inhibiting the pro-oxidant activity of trace metals (Lea, 1958). In the latter function, they act as chelating agents which coordinate with trace metals making them less reactive (Stuckey, 1962; Labuza, 1971).

Since most foods contain natural antioxidants and many contain significant amounts of catalytically active metals (Lea, 1958), this is an important group of protective substances. Nevertheless, metals naturally present in a food may already be bound in stable

complexes and such complexes can be much less or much more catalytically active than the metal in the ionic form. In such cases, ordinary deactivators may be ineffective (Lea, 1958).

Synergists include acid type inhibitors, polyphosphates, certain phospholipid fractions and derivatives and various acids (Mattill, 1945; Lea, 1958). Mattill (1945) subdivided the acid type inhibitors into three groups. The first, and most commonly used, are the dibasic organic acids such as malonic, maleic, malic **and** citric acid. The second group are inorganic acids such as sulphuric acid and phosphoric acids. The third group of the acid inhibitors comprises di- and poly- phenolic acids; gallic acid is the best example and is effective in animal fat devoid of phenolic inhibitors by virtue of the fact that it is itself phenolic. In vegetable oil, it is also effective, because of its acid character. Numerous other nitrogen-, sulfur-, and phosphorus-containing chelating agents have also been shown to possess powerful metal-deactivating properties in fats and oils, and some of the less toxic of these substances may find application in foods (Lea, **1958).** Olcott, et^ al. (1963) have shown that the antioxidant properties of purified phospholipids are negligible, but they do have good sjniergistic effectiveness due to the nitrogenous moieties on lecithin and cephalin.

Several mechanisms have been proposed to explain the phenomenon of synergism. Stuckey (1962) suggested that synergism is probably due to hydrogen or electron donation and supported his

postulation by using a mixture of BHA and BHT in pastries prepared with lard as the shortening ingredient. Another investigator (Lundberg, 1962) has demonstrated that the chelation of pro-oxidants commonly present in food fats, notably copper and iron, is the principal mechanism involved.

A completely different theory of the mechanism involving synergism of mixed free radical acceptors has been advanced by Uri (1961 b). When a mixture of hydroquinone (AH) and ascorbic acid (BH) is added to a fat, the latter is prevented from being an effective acceptor of RO - because of a lower steric factor and a lower activation energy (due to a lower bond association energy). Hence, the following reaction sequence would then materialize:

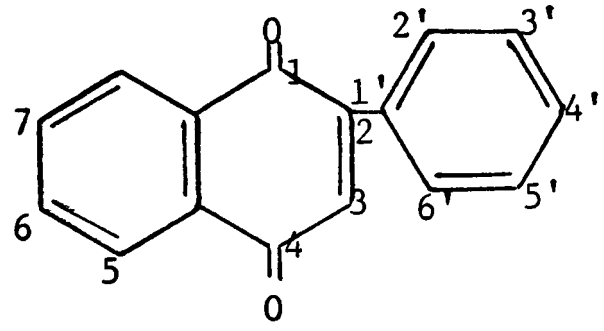
$$R02' + AH \longrightarrow ROOH + A-$$

$$A- + BH \longrightarrow AH + B-$$

Multifunctional Antioxidants

The line of demarcation between the different classes of antioxidants is not always sharp. Some substances combine in their molecules both chain-breaking and metal-deactivating properties (Lea, 1958). Such is the case, for example, with the flavonoid compounds which encompass the anthocyanidins, anthocyanins, anthocyanogens, flavones, flavanols, flavanones and other related 15-carbon substances (Gardner and Inglett, 1971; Bate-Smith, 1954). The last three named pigments are reported to be responsible for the

antioxidant properties in many plant tissues (Lewis and Watts, 1958; Pratt and Watts, 1964; Pratt, 1972). In fact, Watts (1962) cited plant flavonoids as among the most potent of phenolic antioxidants. They are generally present as glycosides (Mathew and Parpa, 1971), the sugar moiety being responsible for their water solubility (Watts, 1962). The antioxidant activities of flavonoid compounds are closely related to their chemical structures (Pratt, et al., 1964), and each of the functions, chain breaking and metal deactivating, of the flavonoids can be exercised at several points of the molecule (Lea, 1962). The antioxidant effects of flavones are increased by the synergistic action of citric or phosphoric acid (Janey, et aJ^., 1960).



FLAVONE SKELETON

Interest in flavonoids as antioxidants in food stuffs is increasing continually. One of the important factors contributing to the promise of these compounds for this purpose is their widespread occurrence in the plant kingdom and their presence in many foods now consumed (Mathew and Parpa, 1971).

Oxygen Uptake

The absorption of oxygen by artificial oil-in-water emulsion can be employed for the testing of antioxidant activities (Lea, 1958). Such a polarographic measurement of oxygen consumption has the advantage of measuring rapid reactions during early stages of initiation, and is one of the fastest methods for recording rates of lipid peroxidation. Rates of oxygen consumption in 10^{-3} M per second can be obtained with lipoxidase as a catalyst (Tappel, 1962).

According to Martin (1961), the first automatic recording oxygen absorption apparatus was devised for the determination of induction periods of fats and fatty materials. This apparatus has since been modified.

The basic principle lies in the detection of oxygen by a polarographic sensor (Beckman Instruments, Inc., 1968). The working elements of the sensor, cathode and anode, are separated from the sample by a selectively permeable membrane which permits oxygen and other gases to pass through, but excludes the oil in the sample (Beckman Instruments Inc., 1968; Spiehler, 1971). Thus, the sensor measures the partial pressure of oxygen, which is usually expressed in milliliters of mercury regardless of the medium (Spiehler, 1971).

Oxygen in the sample diffuses through the membrane and is reduced electrochemically at the cathode. This reaction causes a current flow, proportional to the partial pressure of the oxygen in the sample (Beckman Instruments, Inc., 1968; Spiehler, 1971).

The electrode reactions are:

$$O_2 + 2H_2O + 4e \longrightarrow 4OH \qquad \text{Cathode Reaction}$$

$$4Hg + 4Cl \longrightarrow 4HgCl + 4e^- \qquad \text{Anode Reaction}$$

Hydroperoxide Determination

Since hydroperoxides are widely accepted as the primary products of lipid autoxidation, quantitative determination of peroxides is the most commonly used analytical method for following the course of autoxidation reactions (Link and Formo, 1961; Swern, 1961).

Lea (1931) was probably the first researcher responsible for developing the peroxide test, in an attempt to devise a new test for rancidity, satisfying the following requirements:

(a) to have a quantitative knowledge of the state of oxidation in any specimen of fat;

(b) to be able to detect and follow the very earliest stages in the process of oxidation; and

(c) to possess the means of comparing the potential keeping qualities of any two or more fats.

Lea*s technique has been used in modified forms by other workers, including Wheeler (1932), for studying autoxidation. Generally, the iodometric procedures for peroxide determination are based on the ability of peroxides to oxidize iodide salts to free iodine, with subsequent measure of the latter giving indirectly the amount of peroxide originally present in the sample (Dahle and Holman, 1961).

The iodometric methods have been criticized by Banks, et al., (1961) on the ground of alleged low values, due to reabsorption by the unsaturated lipid of about 8 per cent of the iodine liberated. In Lea*s own work on linoleate autoxidizing at 37° C, he found by the iodometric method a peroxide value corresponding only to 91 per cent of the oxygen absorbed (Lea, 1952). These observations are in substantial agreement with those of Lundberg and Chipault (1947), who obtained a value of 94 per cent. These determinations would provide allowances for a possible error in the iodometric method (Lea, 1962).

However, in studies conducted to investigate the various methods used for estimating rancidity and their interrelationships, the iodometric method was found to be one of the most satisfactory procedures.

Lea (1962) found fairly close agreement between the iodometric method and the slower stannous chloride method. White (1941) examined a number of methods and reported that of the procedures employed, peroxide oxygen and Kreis test were most closely related at high and low levels of rancidity. Moreover, the peroxide oxygen test was found to be less affected by variations in the concentration of the fat, was simple to operate, and has general applicability. Pearce (1942) also found the peroxide method to be a convenient measure of oxidative rancidity in fats and correlated highly with other rancidity measurements. Grants and Lips (1946) reported that of the objective tests correlated with rancidity as assessed by odor

scores, the a-dicarboxyl values and peroxide oxygen determined by the iodometric procedure gave the highest associations.

The peroxide test is useful for comparison purposes (Lea, 1952; Wheeler, 1932), particularly when the absolute value obtained is of less importance than the adequate reproducibility, sensitivity and simplicity of technique (Lea, 1952). It is a useful method to employ in following the development of off-flavors in a given compound or formulation although its relative level may vary from product to product (Pohle, et^ a]^., 1964).

Olfactory Evaluation

The basic objectives of sensory testing are to determine whether the food products are acceptable to a consumer, whether this acceptability can be maintained in production and how long it can be maintained on the shelf (Dawson, ejt al., 1951; IFT Committee on Sensory Evaluation, 1964; Ellis, 1971).

In an organoleptic test involving the sense of olfaction, the basic procedure is the delivery of an odorous material to a subject and the measurement of his response to intensities, differences, or affective qualities (Stone, et_ al., 1965). Hence, it is a subjective evaluation based on human judgment which is individual and sometimes inconsistent (Amerine, et^ al^., 1965; Sawyer, 1971). According to Prince and his associate (1958), different persons show markedly different sensitivities to a given odor, and these differences vary widely with different odors. These differences in

sensitivity, moreover, cannot be estimated from the results obtained with a particular "reference" odor, but must be established from long-term averages for the material concerned.

In 1947, Moser, et^ al^. , commented that no substitute has been found for the human sense of taste and smell in the study of flavor problems. This is particularly true for the sense of smell when one is made aware of the fact that for the triggering of human olfactory neurons by a powerful odorant as few as eight molecules are required, and that as few as 40 molecules can produce an identifiable olfactory sensation (Teranishi, et_ al. , 1971).

In recent years, there has been an increasing effort to establish objective methods via chemical and physical tests for measuring properties of food that can be related to human odor and taste sensory properties (Sjostrom, 1968; Stone, et^ al., 1965). However, even the most elaborate device can reveal nothing about intensity nor quality of a smell (Foster, 1964). Among many analytical instruments investigated, the gas chromatograph coupled with mass spectrometer was reported to be the most sensitive (Sjostrom, 1968). Nevertheless Kendall and Neilson (1964) found that the gas chromatograph does not easily detect odorants in the concentrations at which they produce important changes in odor characteristics and does not tell when blends are occurring nor when new odors are produced. Hence, perhaps it is justifiable to state that as long as human beings are the final judges of flavor.

organoleptic evaluation will probably be required in flavor problems and that instrumental techniques such as gas-liquid chromatography will supplement, not substitute for olfactory examination (Stone, et_ al^., 1965).

Since human judgment is the backbone of an organoleptic test, careful selection and training of panel members is important (Prince, et^ al., 1958). Boggs and Hanson (1949) have stressed that the selection and criterion lies in the consistency and acuity exhibited by the individual in detecting differences in degree of rancidity. They recommend that this can be done by giving the panel members preliminary tests and only those who show a consistency in their acuity toward rancidity should be selected. Other criteria in panel selection are the consideration to be placed on panel attitudes, the amount of information which should be given to the panel, motivation, health, reliability, experience, fatigue, composition and size of panel, and changes, if any, in the panel members, availability, age and sex (Dawson, et al., 1951; Prince, et al., 1958).

Panel attitude is of importance to the success of an experiment. Hence, one should try to create and maintain the interest and confidence of the individuals on the panel (Boggs and Hanson, 1949). One way this can be done is to hold conferences where results are presented. According to Dawson, £t a_l. (1951), the judges should be kept informed of their performance at least in general terms if too much information could influence results and

as long as they cannot identify particular samples and do not become prejudiced. Motivation should also be emphasized, as suitable motivation could make the individual more selective in his response (Amerine, et_ _aa^., 1965). In addition, the subjects should be made aware of the importance of their contribution (ASTM Manual on Sensory Testing Methods, 1968).

Fatigue is an important criterion, as one should avoid not only the actual tiring of the nasal sense organs but also the possible mental or psychologic fatigue that results if panel members are presented with too many samples at one time or too often during a single day (Boggs, et ^1^., 1949). The recommended number of samples during one test is three or four (Boggs, £t al., 1949; ASTM Manual on Sensory Testing Methods, 1968).

The technique or methodology employed for odor testing also plays an important role in organoleptic research. There are numerous techniques available for odor measurement. Stone, *Q^ al_<-* (1965), cited the Sniff Method as being one of the most successful odor measurement techniques involving the presentation of samples in beakers, test tubes, or jars for evaluation. The subject removes the cover and inhales the vapor above the solution. No elaborate equipment is required, and success has been considerable. Also, this method is simple and economical, since in terms of cost it is by far the most inexpensive test available.

CHAPTER III

METHODOLOGY

Oxygen Uptake

Materials

Linoleic Acid

Linoleic acid, lot number K-2A obtained from the Hormel Institute, Austin, Minnesota, was used and had a stated purity greater than 99 per cent by gas-liquid chromatography and thin-layer chromatography.

Lipoxidase

Soybean lipoxidase, lot number Lt 3B-488, was obtained from Sigma Chemical Company, St. Louis, Missouri.

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Tween 20

This is polyoxyethlene (20) Sorbitan monolaurate, practical grade, purchased from J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Grain Sorghum Fractions

The grain sorghum used in this study consisted of samples of Funk G-766W hybrid supplied by Grain Product, Dodge City, Kansas.

The samples were dry-milled products derived from a 1968 crop grown in southwestern Kansas. Seven fractions, whole grain, pearled grain, ground pearled grain, germ, endosperm, mill-fines (impact milling), and mill-fines (cutting and cracking) were used. For an explanation of these various fractions of grain sorghum, see Appendix B,

Reagents

Phosphate Buffer Solution

One-tenth molar phosphate was used as the buffering agent. It was prepared from potassium monohydrogen phosphate (J. T. Baker Chemical Company), and potassium dihydrogen phosphate (J. T. Baker Chemical Company), and distilled water. The pH was adjusted to 6.5 at 25 ± 1° C.

Standard Unsaturated Fatty Acid Emulsion

The emulsions were always prepared immediately before use by a slight modification of Surrey's method (1964). One gm of linoleic acid was added drop by drop to 20 ml water in which 1 ml Tween 20 was dissolved. The contents were mixed thoroughly to disperse the acid into a fine emulsion. Then 6 ml of 1 N KOH (J. T. Baker Chemical Company) was added and the mixture once again agitated with a magnetic stirrer until a clear transparent solution was obtained. To this solution 180 ml of 0.1 M phosphate buffer was added. The final volume was made up to 400 ml with distilled water.

Lipoxidase Solution

Fifty mg soybean lipoxidase was weighed accurately and made up to 100 ml using pH 6.5 phosphate buffer.

Grain Sorghum Slurry

A given weight of grain sorghum fraction was blended with 100 ml pH 6.5 phosphate buffer for five minutes at 5000 R.P.M. with a Sorvall Omni-Mixer homogenizer equipped with an ice-water bath.

Instruments

A Beckman Fieldlab™ Oxygen Analyzer with a Beckman 39553 Oxygen Sensor was utilized for the determination of the velocity of fatty acid oxidation. An accessory linked the Oxygen Analyzer to a recorder (Speedomax XL Recorder Flatbed - 680 Series, Leeds and Northrup) which recorded graphically the rate of oxygen uptake by the test sample.

Calibration of Oxygen Analyzer

Prior to calibration, a clean, charged and stabilized sensor was ensured. An air-saturated pH 6.5 phosphate buffer was used as a liquid calibration medium. -i

The oxygen analyzer was calibrated at room temperature (26 ± 2° C) and at 142 mm Hg, the partial pressure of oxygen in air and in a liquid medium at 100 per cent saturation with oxygen. The adjustment in partial pressure from the normal value of 160 mm Hg was made

because of the area elevation and was calculated as follows:

Barometric Pressure in Lubbock = 26.6" = 675.64 mm Hg

Therefore $\frac{X}{675.6} \wedge \frac{160}{760}\wedge$

$X = 142$ mm Hg

Procedures

Twenty millimeters of the standard saturated fatty acid emulsion was pipetted into a 25 ml Erlenmeyer flask with a rubber capped arm. Two and one half ml of a test solution for antioxidant activity measurement was added to the flask. The sensor of the oxygen analyzer was inserted in such a manner that only the liquid was displaced and no air bubbles were entrapped. After starting the magnetic stirrer, 2.5 ml of lipoxidase solution was added through an injection syringe. Immediately after the addition of the catalytic solution, the oxygen tension change in the fatty acid emulsion was recorded. The apparatus for this experiment is shown in Figure 8.

Preparation of Pastry

Materials

Grain Sorghum Fractions

Two fractions were used, the germ and the mill-fines (impact-milling).

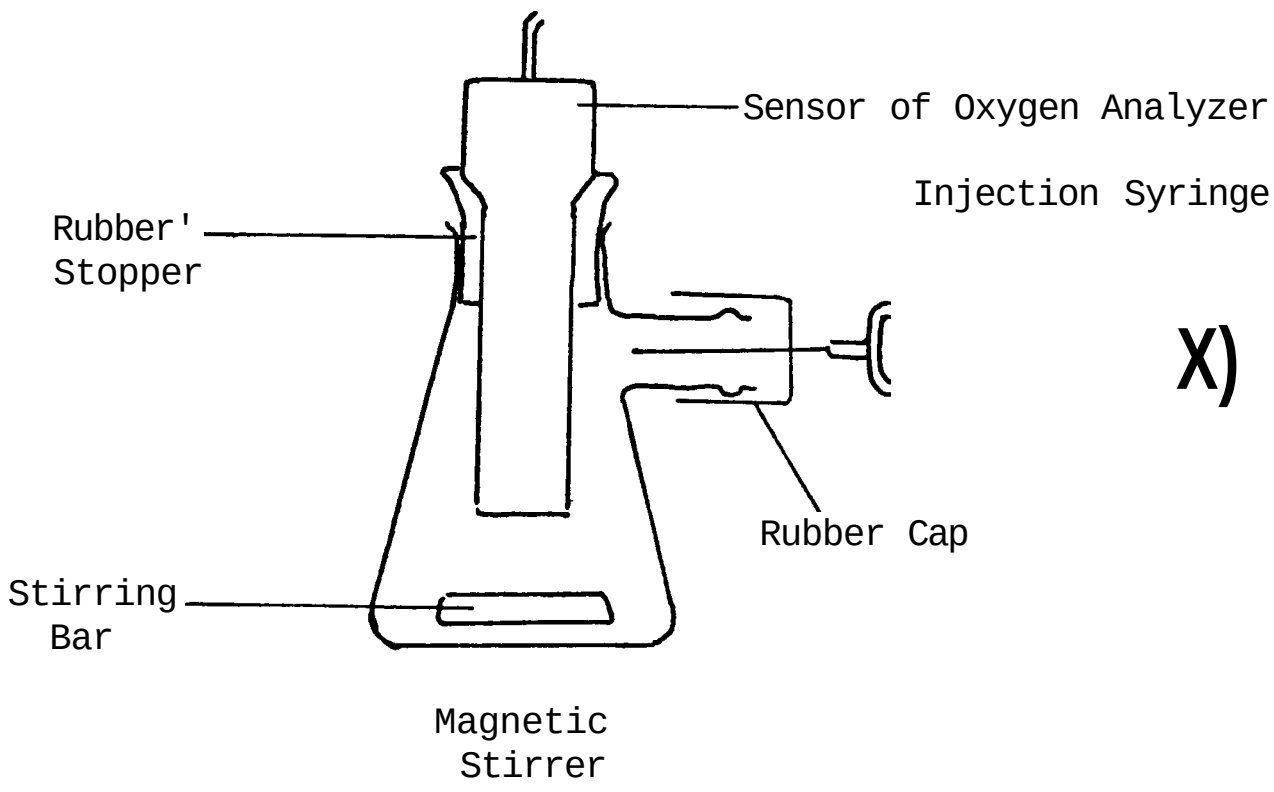


Fig. 8.—Apparatus for Measurement of Oxygen Consumption by Linoleic Acid.

Wheat Flour

"Gold Medal," all-purpose, bleached, enriched flour was used. It was purchased directly from a local supermarket.

Lard

This was obtained, by special arrangement, from Farm Pac Kitchens, Lubbock, Texas, before any synthetic antioxidants had been added. The lard was the "refined" product of pork which was steamed and filtered to eliminate impurities.

Salt

"Food Club" brand salt containing 0.01 per cent KI was used

Water

Distilled water was used.

Basic Recipe

The recipe developed for the sorghum pastry sample is shown in Table 2.

Procedures

All ingredients were weighed separately. The wheat flour, grain sorghum fraction and salt were mixed and seived into the mixing bowl of an electric mixer. One-half of the lard was added to the flour mixture and beaten for three minutes at low speed. The remaining lard was added and beaten for another minute and a half at the

TABLE 2

COMPOSITION OF GRAIN SORGHUM PASTRY SAMPLES

	Percentage of Grain Sorghum Fraction			
Ingredients	0%	7.5%	15%	30%
Wheat flour (gm)	100.0	92.5	85.0	70.0
Grain sorghum (gm)	0.0	7.5	15.0	30.0
Lard (gm)	54.0	54.0	54.0	54.0
Salt (gm)	3.0	3.0	3.0	3.0
Water (ml)	15.0	15.0	15.0	20.0

same speed. Fifteen ml of chilled distilled water was added to the mixture gradually in such a manner that uniform distribution was insured. The mixture was then beaten at low speed for one-half a minute.

Using a wooden rolling pin, the dough was rolled to one-eighth inch thickness and cut into rectangles, one inch by four inches in size. One-half of the pastry samples was stored raw, the other half was baked until golden brown, for 11 minutes at a temperature of 425° F before being stored. The storage temperatures were 37° C, 6° C, and -6° C for the pastry samples made from the germ fraction (designated as Batch I), and 37° C and 6° C for those made from the mill-fines, impact-milling (designated as Batch II).

Peroxide numbers were determined on the fat extracted from the pastry samples immediately after baking, and thereafter, at bi-weekly intervals. Olfactory evaluations on the pastry samples were run concurrently with the peroxide determinations.

Chemical Analyses

Materials and Reagents

Potassium Iodide (KI)

Seventy gm KI (J. T. Baker Chemical Company) was dissolved in 50 ml distilled water and stored in a brown bottle at 1-4° C.

Starch Indicator

A 0.5 per cent starch solution (W. H. Curtain and Company) preserved with salicylic acid was used.

Standard Potassium Dichromate

A 0.1 N standard potassium dichromate (J. T. Baker Chemical Company) was prepared by an approved A.O.A.C. method (1971) as follows:

Potassium dichromate (K2Cr20^) was dried at 100° C for two hours; 4.9032 gm was dissolved in one liter of distilled water.

Stock Sodium Thiosulfate Solution

A stock solution of sodium thiosulfate (J. T. Baker Chemical Company) was prepared in accordance with the A.O.A.C. methodology

as follows:

About 25 gm sodium thiosulfate (Na2S 0^-5H 0) was weighed and dissolved in one liter distilled water. The solution was boiled gently for five minutes and transferred while hot to a brown storage bottle.

When not being used, the stock Na2S202-5H20 was stored in a dark, cool place at 1-4° c. For peroxide determinations a 0.01 N solution was prepared by diluting the stock solution with distilled water. (Solutions less concentrated than 0.1 N are less stable and should be prepared just before use.)

Standardization of Sodium Thiosulfate

The stock sodium thiosulfate (approximately 0.1 N) was standardized against standard potassium dichromate as follows:

Twenty-five ml 0.1 N K.Cr 0 was pipetted into a 250 ml Erlenmeyer flask. An equal volume of distilled water was added, followed by 2 ml saturated KI and 5 ml concentrated HCl. This mixture was titrated against sodium thiosulfate solution until the red color, due to the free iodine, liberated from KI by K Cr 0 , had changed to a pale yellow. Ten ml starch solution (0.5 per cent) was then added and titration continued until the blue color changed to sea green. The reactions involved were:

K Cr 0 + 6KI + 14HC1 = 8KC1 + 2CrCl2 + 7H2O + 3I2
(green) (red)

3I2 + 6S2O2" = 61" + 3820^

Extraction of Fat

Each of the pastry samples was pulverized by a mortar and pestle, transferred into previously weighed beakers, and then weighed. To each pastry sample 20 gm anhydrous Na SO was added. The mixture was blended with 60 ml carbon tetrachloride (CCl,) for five minutes at 5000 R.P.M. using a Sorvall Omni-Mixer homogenizer equipped with an ice-bath. At the end of five minutes, the slurry was decanted into an Erlemeyer flask, covered with aluminum foil, and left in a refrigerator for fifteen minutes. It was then filtered through white ribbon filter paper (Schleicher and Schuell, No. 589).

Five milliliters of the clear filtrate was measured into a weighed moisture dish and evaporated over a hot water bath. The remaining fat was dried in an oven for two hours, cooled in a dessicator and weighed. From the weight of fat contained in the 5 ml filtrate, the amount of filtrate required to give approximately 0.5 gm fat was calcualted. This amount was measured into a 250 ml Erlenmeyer flask and CCl, added to bring the total volume to 20 ml.

Peroxide Determination

Peroxide values (P.V.) were determined by a modified Wheeler's method (1932) and were expressed in milliequivalents of sodium thiosulfate per kilogram of fat.

Thirty ml glacial acetic acid was added to the dissolved sample of fat followed by one ml saturated KI. The Erlemeyer flask

was covered with aluminum foil, shaken for one-half minute and left in the dark for exactly five minutes. Ninety ml distilled water was added and the mixture titrated against 0.01 N sodium thiosulfate to a light straw color. Ten ml 0.5 per cent starch indicator solution was added and titration continued until the blue color disappeared.

The peroxide value was calculated using the formula;

$$\text{P.V.} = \frac{\text{ml thiosulfate} \times \text{Normality thiosulfate} \times 1000}{\text{Weight of fat in grams}}$$

For Batch I (Germ Fraction) two titrations were performed on the lipid extract of the same pastry sample. For Batch II (millfines, impact-milling), four titrations were performed, two from each of the lipid extracts of two identically treated pastry samples. This variation in procedure was introduced on the assumption that an increased nimiber in the total number of extractions and titrations should decrease the percentage of experimental errors. However, the analysis of percentage deviations from the average results of Batch II peroxide values did not differ significantly from those of Batch I peroxide values.

Olfactory Evaluation

Following a common procedure, a screening trial was held initially to select potential panel members from available personnel. This also served the purpose of familiarizing the panel

members with the procedures, the score sheet, and the characteristic odor produced by rancid pastry samples.

As recommended by Amerine, et _a]^. (1965), members were given the comparison test and only those who could distinguish between the highly rancid and fresh samples were asked to participate as panel members. Ten judges, in accordance with the guidelines set out in the ASTM Manual on Sensory Testing Methods (1968), were selected. However, as the storage test proceeded, most of the panel members were found to be unable to distinguish between samples with small differences in rancidity. A second session was held in order to insure that it was the rancid odor which they were looking for, and not a host of other odors such as oiliness, meatiness, and the "baked" odor. Slight improvement in the panel's ability to detect odor differences was noted as the experiment progressed. Nevertheless, the performance of the majority of the panel members was found to be of low calibre. As a result, it was decided that new panel members should be recruited for subsequent storage tests using a different grain sorghum fraction. Only those who were capable of discriminating rancid odors in the testing of Batch I pastry samples were asked to remain as panel members. A total of fifteen members were selected as judges for the olfactory evaluation of Batch II pastry samples.

Method of Scoring

Since all of the panel members could not conveniently be present at the same time and because there was a limited number

of pastry samples, a procedure was followed that would enable the panel members to come in whenever they wished during the day of the testing.

The method of silent, individual scoring was used. To avoid mutual distraction among test subjects and to provide a testing area as free from odors as possible (ASTM Manual on Sensory Testing Methods, 1968), two individual booths were set up in an aircondi-tioned room. Samples of pastry contained in labeled 50 ml beakers and covered with aluminum foil were arranged on individual trays. A maximum number of six samples (ASTM Manual on Sensory Testing Methods, 1968) were presented to the judges at any one time. For both the baked and unbaked samples, a fresh and a rancid sample were given as standards.

Score Sheets

The method of rank-order coupled with a rating scale was employed (Amerine, et^ al•, 1965; ASTM Manual on Sensory Testing Methods, 1968). Descriptive terms, valued numerically from 4 (highest) to 0 (lowest) for calculation purposes, were used for rating the samples (see Appendix A). The panel members were encouraged to write comments in the spaces provided.

Statistical Methods

Analysis of Variance of Peroxide Values

To ascertain the main effects of temperatures, storage time and levels of grain sorghum and their interactions in the development of rancidity, a factorial design of the analysis of variance (ANOVA) was performed. As there were two different types of pastry samples, baked and unbaked, and two different batches, germ fraction and mill-fines, impact-milling, the three main effects were tested according to type within each batch. To ascertain whether there was a significant difference in the development of rancidity between the two different sorghum fractions, an analysis of variance was also performed resulting in a total of four ANOVA tests. These are:

(1) ANOVA on the three main effects of levels, storage time and temperatures and their interactions on the development of rancidity in the baked pastry samples containing the germ fraction;

(2) ANOVA on the same three named main effects and their interactions on the development of rancidity in the baked pastry samples containing the mill-fines, impact-milling fraction;

(3) ANOVA on the effect of baking on the development of rancidity; and

(4) ANOVA on the differences of the effect the two different sorghum fractions, germ and mill-fines, impact-milling, have on the development of rancidity.

The Newman-Keuls Multiple Comparison Test (After ANOVA Test) was performed on the main effects and interactions found to have significant F ratios. The difference of the means was compared with a critical value which was derived from the standard error and the distribution of Studentized Range Statistics (Winer, 1962) where the standard error was based upon the following formula: / Error Variance Estimate/n where the error variance estimate was in this instance the error within (Winer, 1962).

Analysis of Olfactory Evaluation

To obtain measures of the discriminative ability of the panel to distinguish between differences in rancid odors, the Wilcoxon Matched-Pairs Signed-Ranks test (Siegal, 1956) was used. All possible comparisons between the different levels of baked and unbaked pastry samples stored at 37° C and 6° C for both the batches (germ and mill-fines, impact-milling) were made.

Correlation of Olfactory Scores with Peroxide Values

Correlation coefficients between the olfactory scores and their corresponding peroxide values of both batches of pastry samples were performed (Siegal, 1956).

CHAPTER IV

RESULTS AND DISCUSSIONS

Oxygen Uptake

The average results of five experiments on the same preparation of linoleic acid substrate are shown in Tables 3, 4 and 5. There was no significant variation in the degree of oxygen tension reduction between the five experiments in either the control or test substrates. Findings of the antioxidant activities of seven different fractions of grain sorghum inhibited the linoleic acid oxidation catalyzed by lipoxidase in varying degrees proportional to concentration. Then using two levels of concentration of a particular sorghum fraction, the higher level consistently exhibited a higher antioxidant activity than the lower level of concentration in the same fraction.

TABLE 3

ANTIOXIDANT ACTIVITY OF WHOLE GRAIN FRACTION AND ENDOSPERM FRACTION

Antioxidant	Antioxidant Activity* Oxygen Tension Reduction in the Initial First Minute (mmHg)
None (Control)	71
Whole Grain (2.5%)	23
Whole Grain (5.0%)	10
Endosperm (2.5%)	83
Endosperm (5.0%)	44

*The substrate was linoleic acid; the catalyst was lipoxidase

TABLE 4

ANTIOXIDANT ACTIVITY OF PEARLED GRAIN AND GROUND PEARLED GRAIN FRACTIONS

Antioxidant	Antioxidant Activity* Oxygen Tension Reduction in the Initial first minute (mmHg)
None (Control)	72
Pearled Grain (2.5%)	79
Pearled Grain (5.0%)	17
Ground Pearled Grain (2.5%)	83
Ground Pearled Grain (5.0%)	21

*The substrate was linoleic acid; the catalyst was lipoxidase

TABLE 5

ANTIOXIDANT ACTIVITY OF GERM, MILL-FINES (IMPACT-MILLING) AND MILL-FINES (CUTTING AND CRACKING) FRACTIONS

Antioxidant	Antioxidant Activity* Oxygen Tension Reduction in the Initial first minute (mmHg)
None (Control)	70
Germ (2.5%)	2
Germ (5.0%)	-1
Mill-Fines (Impact-Milling) (2.5%)	9
Mill-Fines (Impact-Milling) (5.0%)	6
Mill-Fines (Cutting and Cracking) (2.5%)	9
Mill-Fines (Cutting and Cracking) (5.0%)	4

*The substrate was linoleic acid; the catalyst was lipoxidase

Second, the whole grain fraction had a significantly higher antioxidant activity than the endosperm fraction which did not exhibit any antioxidant activity at the 2.5 per cent level of concentration (Table 3 and Figure 9).

Third, the pearled grain fraction had almost identical antioxidant activities to those of ground pearled grain fraction at the 5.0 per cent level of concentration. At the 2.5 per cent level, both these fractions exhibited no antioxidant activities as was evident from data presented in Table 4 and Figure 10. The almost identical antioxidant activity of these two fractions indicated that grinding of the pearled grain did not impair the antioxidant properties of this particular fraction.

Fourth, initially or for about one minute, both of the mill-fines fractions had almost identical antioxidant activities (Table 5). However, as time elapsed, the antioxidant activity decreased at a slower rate in the impact-milled fraction of mill-fines than that in the cutting and cracking fraction (Figure 11).

Fifth, for both levels of concentration, the germ fraction had a higher antioxidant activity than the corresponding levels of either of the mill-fines fractions (Table 5 and Figure 11).

Finally, the germ fraction possessed the highest anti-oxidant activity followed closely by both fractions of the mill-fines. Taking into account the non-significant differences in the oxygen tension reduction in the initial minute of the controls.

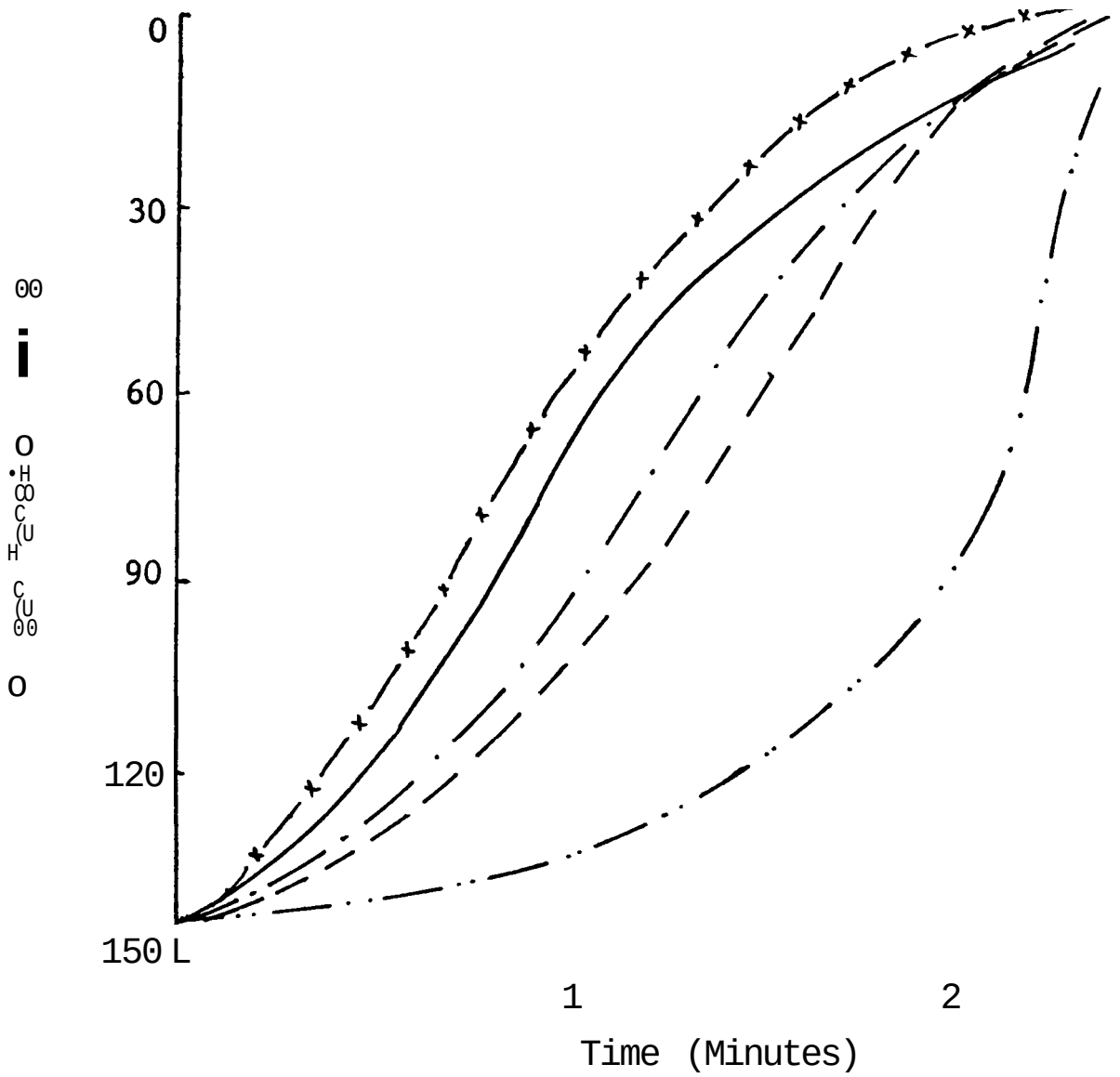


Fig. 9-—Comparison of the Antioxidant Activity of the Whole Grain Fraction and the Endosperm Fraction.

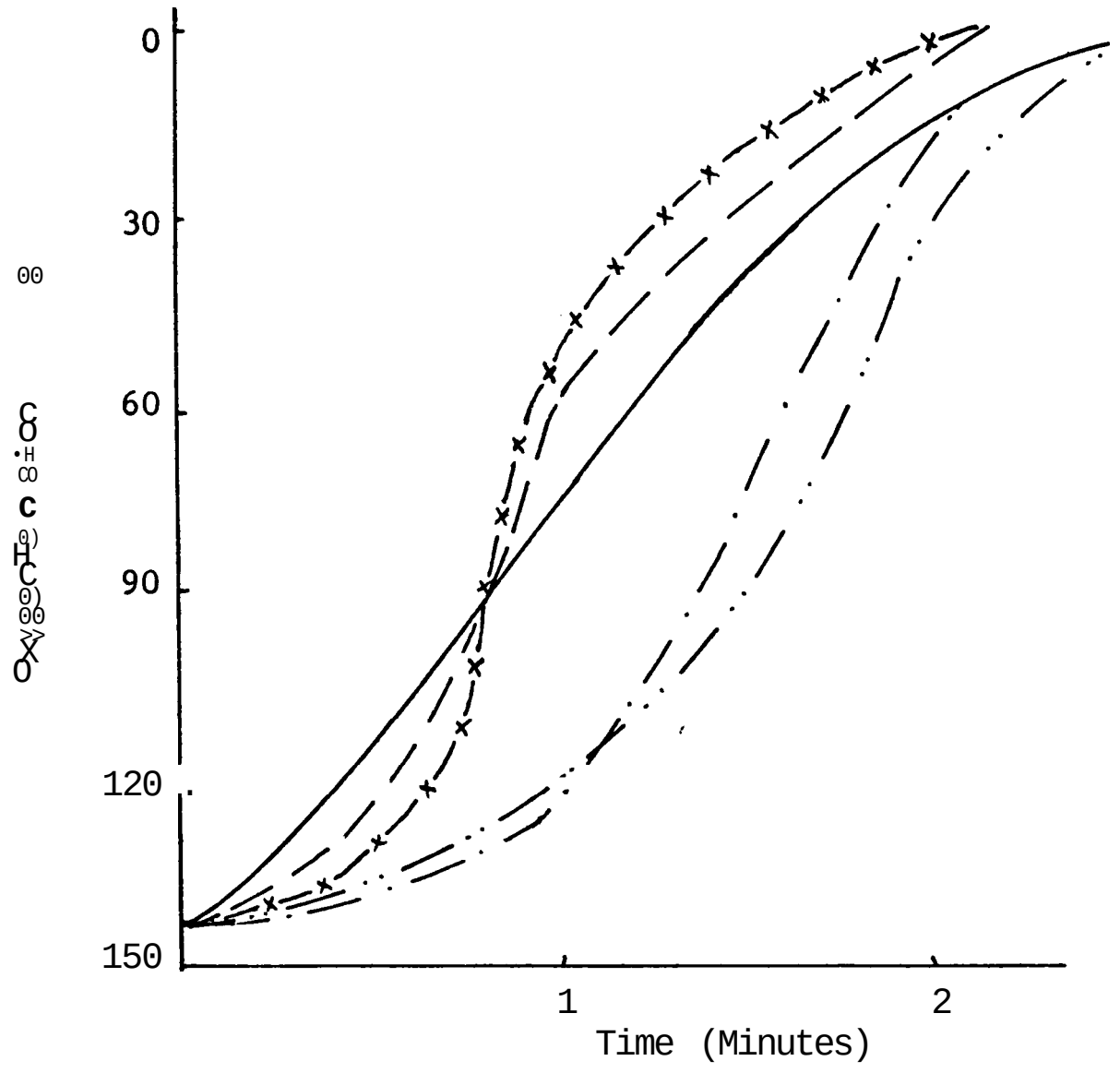


Fig. 10.—Comparison of the Antioxidant Activity of the Pearled Grain Fraction and the Ground Pearled Grain Fraction.

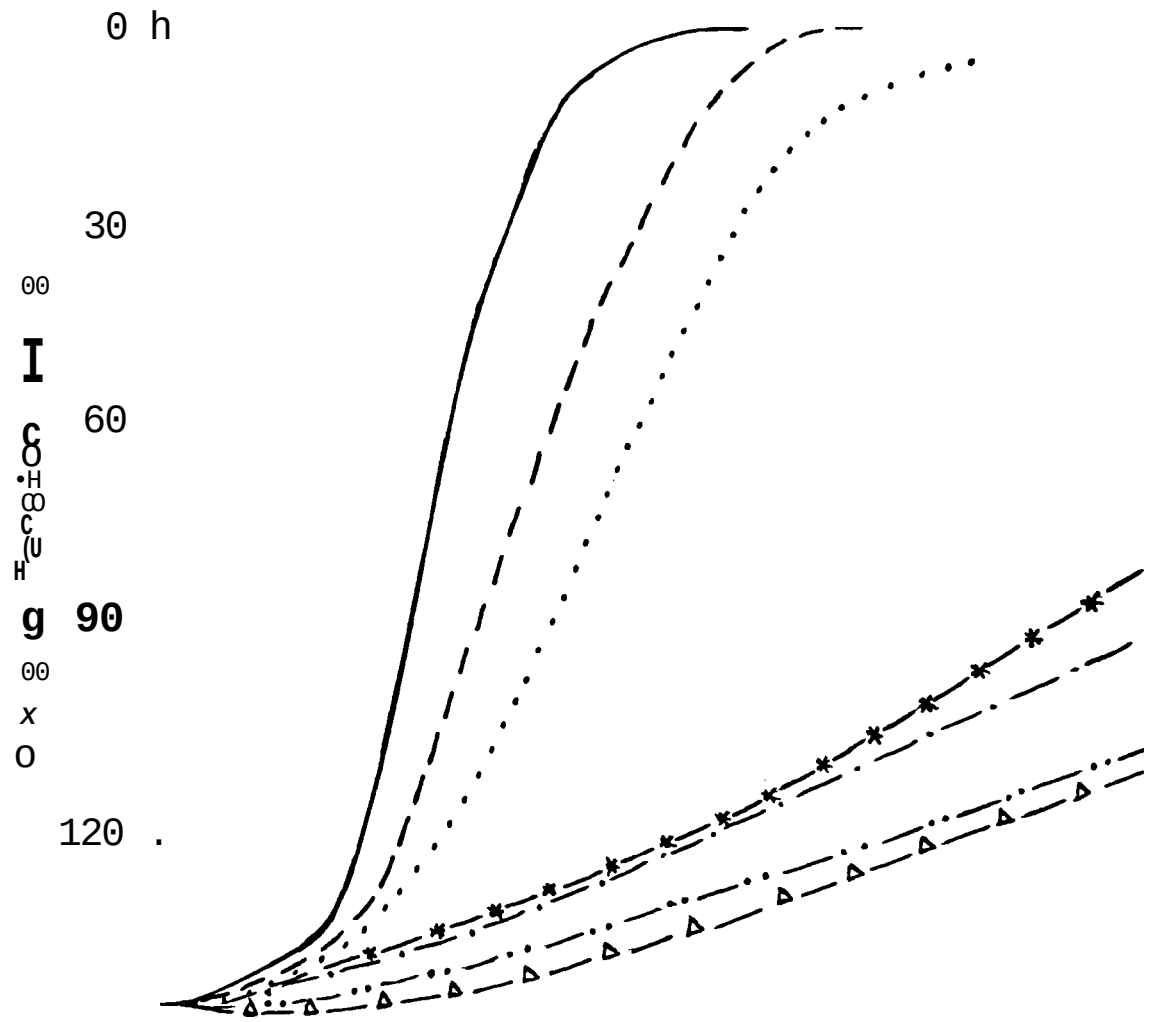


Fig. 11.—Comparison of Antioxidant Activity of the Germ Fraction, Mill-Fines (Impact-Milling), and Mill-Fines (Cutting and Cracking).

the antioxidant activity of various sorghum fractions were:

2.5 per cent	germ > mill-fines, impact-milling = mill-fines, cutting and cracking > whole grain
5.0 per cent	germ > mill-fines, impact-milling > mill-fines, cutting and cracking > whole grain > pearled grain > ground pearled grain > endosperm

At the 2.5 per cent level, pearled grain, ground pearled grain and endosperm were found not to exert any antioxidant activity.

Analysis of Peroxide Values

Actual data of peroxide values obtained by chemical analysis are presented in Tables 6, 7, 8, and 9. Corresponding data for the pastry samples containing the germ fraction and subjected to a storage temperature of -6° C were not presented as the peroxide values were found to be zero for all time periods tested (zero week to eighth week).

TABLE 7

PEROXIDE VALUES OF BAKED AND UNBAKED PASTRY SAMPLES WITH FOUR LEVELS OF GERM FRA.CTION STORED AT 6° C

Pastry Sample	Peroxide Value 0 week	5th week	8th week	15th week
Baked				
0.0 %	0.00	5.30	4.70	6.40
7.5	0.00	2.90	2.00	0.69
15.0	0.00	2.50	1.00	0.49
30.0	0.00	1.30	0.40	0.40
Unbaked				
0.0 %	0.00	0.00	0.00	0.00
7.5	0.00	0.00	0.00	0.00
15.0	0.00	0.00	0.00	0.00
30.0	0.00	0.00	0.00	0.00

^Each peroxide value is the average of two titrations using the lipid extract of the same pastry sample.

TABLE 8

PEROXIDE VALUES OF BAKED AND UNBAKED PASTRY SAMPLES WITH THREE LEVELS OF MILL-FINES (IMPACT-MILLING) STORED AT 37° C

Pastry Sample	Peroxide Value 0 week	2nd week	4th week	6th week
Baked				
0.0 %	2.05	26.95	322.53	764.45
7.5	0.73	1.45	14.88	82.70
15.0	0.50	1.63	6.38	13.40
Unbaked				
0.0 %	0.00	0.63	0.78	1.95
7.5	0.00	0.55	0.83	0.90
15.0	0.00	0.65	0.80	0.93

^Each peroxide value is the average of four titrations for two different pastry samples.

TABLE 9

PEROXIDE VALUES OF BAKED PASTRY SAMPLES WITH THREE LEVELS OF MILL-FINES (IMPACT-MILLING) STORED AT 6° C

Pastry Sample	Peroxide Value			
	0 week	2nd week	4th week	6th week
0.0 %	2.05	12.45	17.13	21.83
7.5	0.73	3.68	3.50	1.88
15.0	0.50	1.23	1.20	1.30

[a]Each peroxide value is the average of four titrations for two different pastry samples.

I) Effect of Levels, Storage Time and Temperature on the Development of Rancidity in Baked Samples Containing Germ Fraction

The analysis of variance performed for baked pastry samples containing four levels of germ fraction (Factor A) and stored (Factor B) at two different temperatures (Factor C) indicated that all main effects and their interactions were found significant beyond 0.005 level (Table 10).

The results of the Newman-Keuls Multiple Comparison and Multiple t-tests can be summarized as follows: first, significant differences beyond the 0.01 level were found between every pair of comparison of the four levels of germ fraction (A Effect); for example, between 0 and 7.5 per cent; 0 and 15 per cent and so on (Table 11). These results correlate with those shown in Figures 12 and 13 and indicate that the antioxidant activity in the germ fraction increased proportionally as the level or amount of this particular sorghum fraction increased in the pastry samples in the order of 0 < 7.5 < 15 < 30 per cent.

Second, significant differences beyond the 0.01 level were also found between all three pairs of comparison in terms of storage time (B Effect). The results were tabulated in Table 12. It can be seen that they corresponded well with those as shown in Figures 12 and 13. In Figure 12, the increase in peroxide values was directly proportional to the increase in storage time. This relationship

TABLE 11

NEWMAN-KEULS MULTIPLE COMPARISON TEST OF RANCIDITY OF BAKED PASTRY WITH FOUR LEVELS OF GERM FRACTION

Critical Value		Level of Grain Sorghum 0%	7.5%	15%	30%	
	X	299.12	190.64	164.58	121.31	X
14.03			125.74**	134.55**	177.99**	299.12
16.05				26.07**	69.52**	190.64
17.26					43.45**	164.58

** P < 0.01

applied to all pastry samples containing the germ fraction (7.5, 15, and 30 per cent). For the pastry samples containing no germ fraction (0 per cent), the peroxide values increased directly with time until the sixth week when a maximum was reached. After the sixth week, the peroxide value decreased with time. This is known as the phenomenon of maximum rate which is exhibited by lipids undergoing an advanced stage of lipid oxidation (Tobolsky, et al., 1950; Mesrobian, et^ al. , 1961; Lundberg, 1962) and appears to be due to thermal decomposition of the peroxide formed during the oxidation (Filer, et^ al. , 1945).

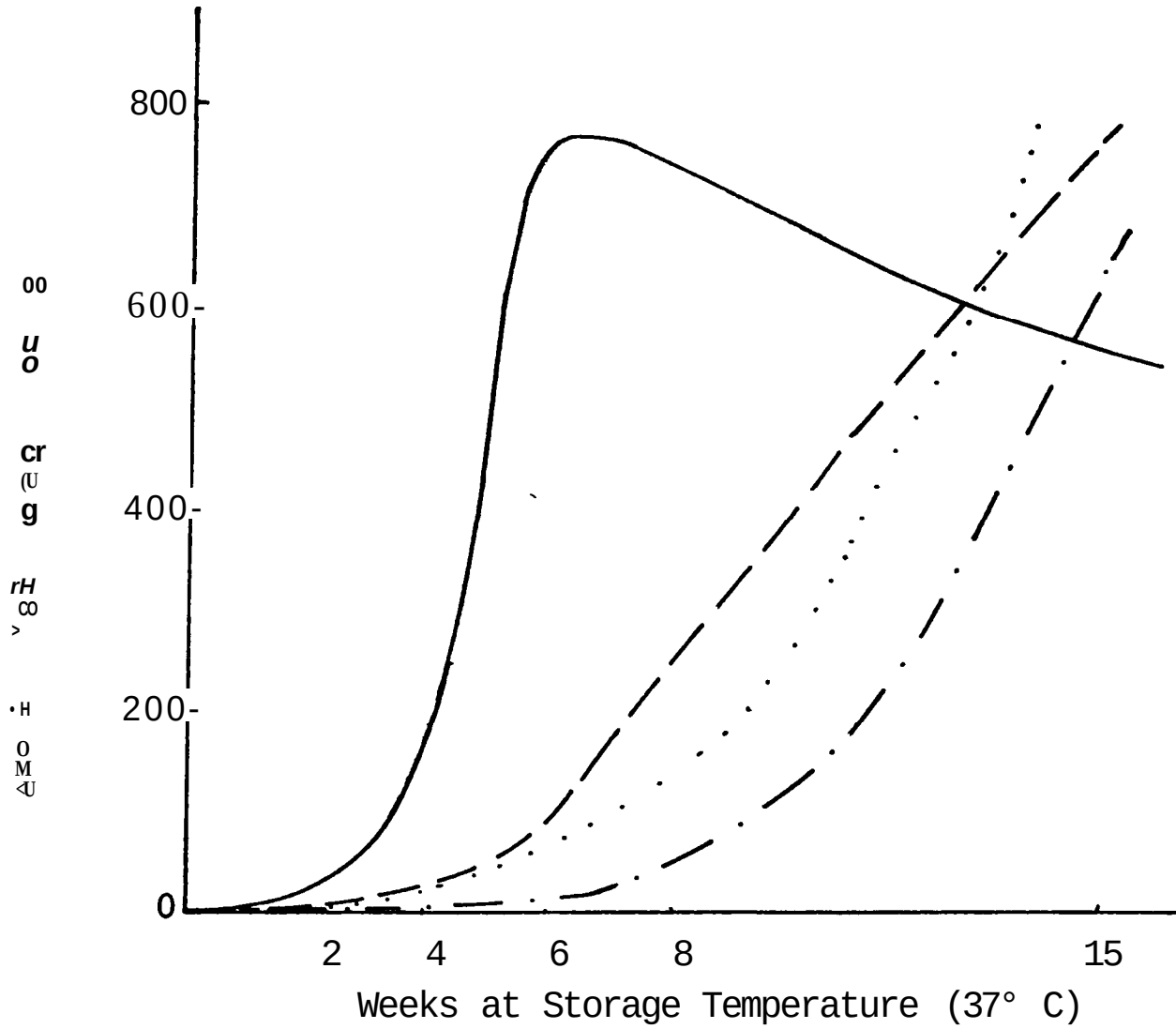


Fig. 12.—Rate of Peroxide Formation in Baked Pastry Samples Containing Four Levels of Germ Fraction Stored at 37"

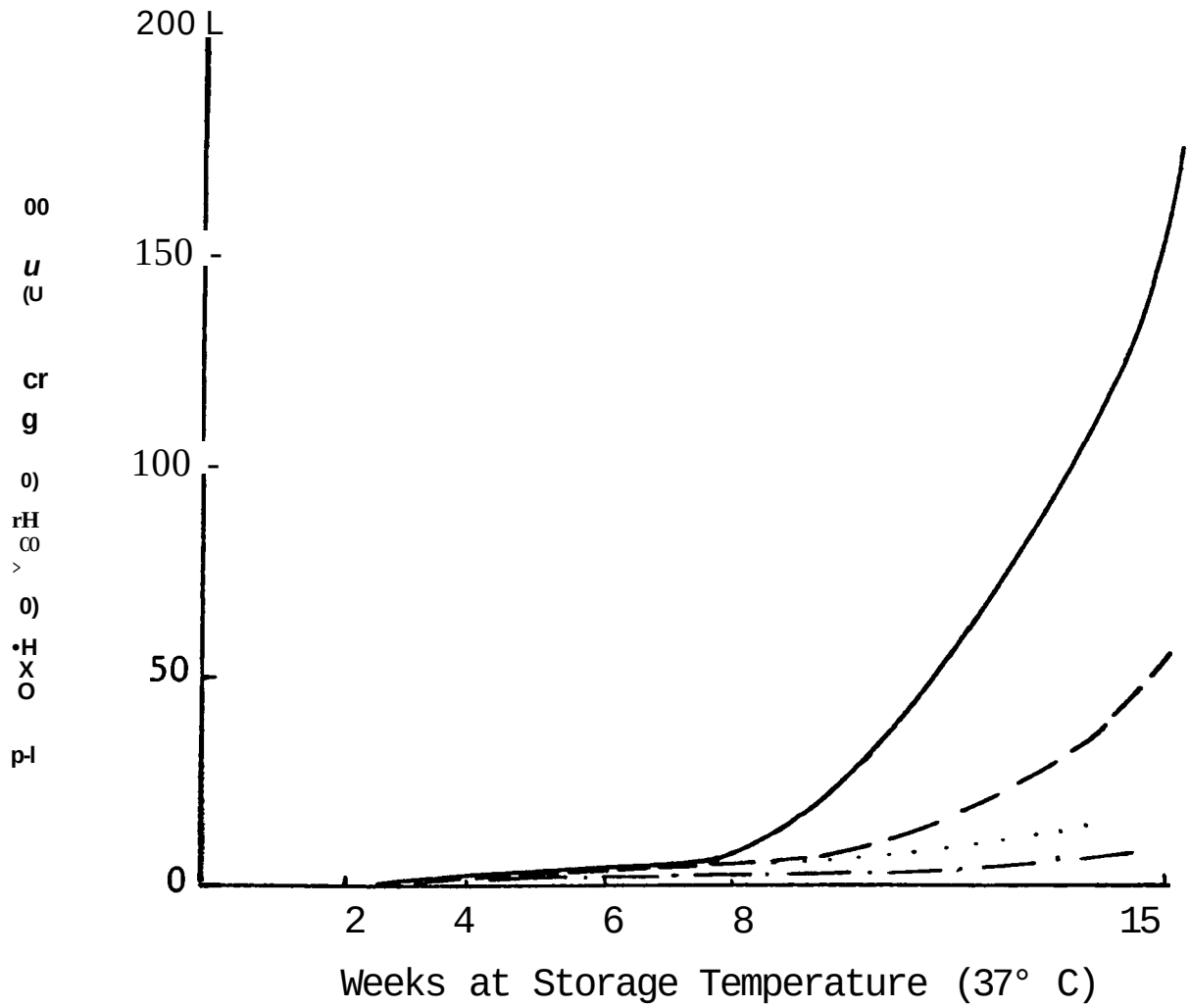


Fig. 13.—Rate of Peroxide Formation in Unbaked Pastry Samples containing Four Levels of Germ Fraction Stored at 37° C.

For the baked pastry stored at the lower temperature (6°C), peroxide values also increased with time, reaching a maximum at or around the fifth week (Figure 14). However, the phenomenon of maximum rate was not significant when compared with that exhibited by the 0 per cent baked pastry samples stored at 37° C (Figure 12). As the storage temperature was low (6° C), thermal decomposition could not have been a contributing factor in the breakdown of the hydroperoxides. Other factors such as trace metals or oxidative enzymes could be responsible in catalyzing hydroperoxide decomposition.

Third, in testing the interaction between the levels of germ fraction with storage time (AB Interaction), every comparison of the cell means was found to be significant at the 0.01 level.

The main effect of storage temperature (37° C and 6° C), that is, the C Effect with one degree of freedom, on the development of rancidity was in no need of further analysis as it is obvious from the data (Tables 6, 7, and 10 and Figures 12 and 14) that pastry samples stored at 6° C underwent lipid deterioration at a much slower rate than those kept at 37° C.

Every comparison of the cell means in the interaction between the levels of germ fraction with temperatures (AC Interaction) and that between storage time ,and temperatures (BC Interaction) were also found to be significant beyond the 0.01 level.

Data for the Newman-Keuls Multiple Comparison Test for AB, AC, BC, and ABC interactions are not tabulated nor presented since all comparisons had been found significant.

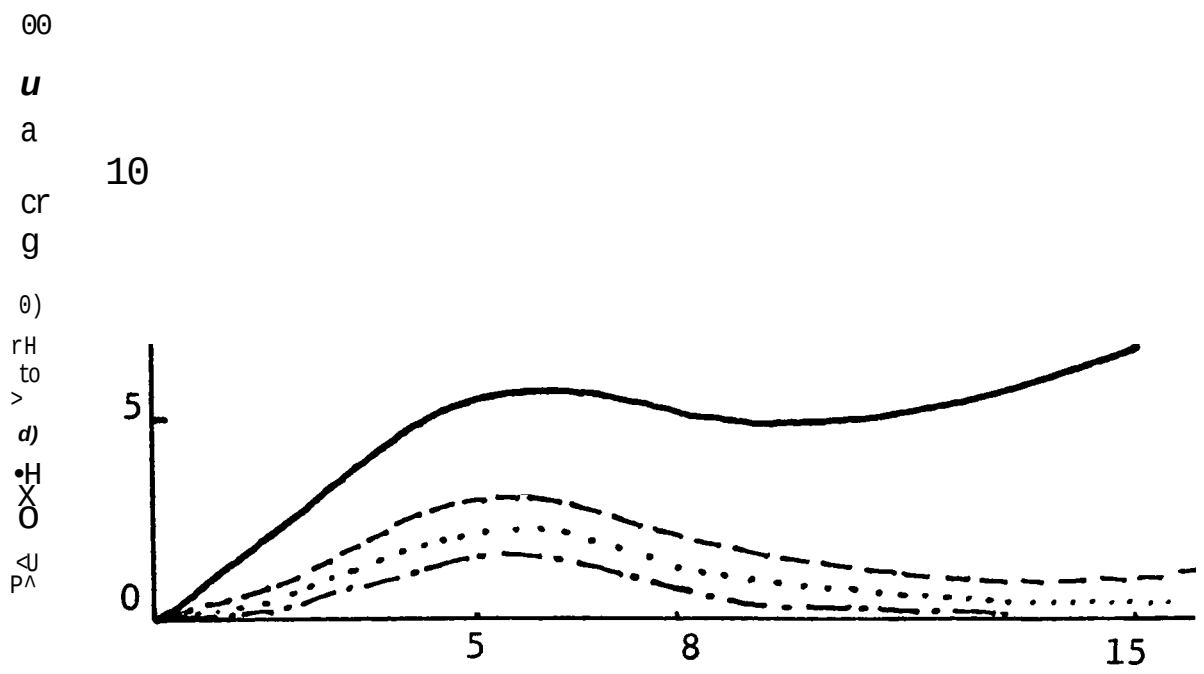


Fig. 14.—Rate of Peroxide Formation in Baked Pastry Samples Containing Four Levels of Germ Fraction Stored at 6° C.

TABLE 12

NEWMAN-KEULS MULTIPLE COMPARISON TEST OF RANCIDITY OF BAKED PASTRY CONTAINING FOUR LEVELS OF GERM AND STORED FOR VARIOUS PERIODS OF TIME

Critical Value		Storage Time (Weeks)			
		5 weeks	8 weeks	15 weeks	
	X	77.03	147.86	356.70	
14.03			70.84**	279.68**	77.03
16.05				208.84**	147.86

** P < 0.01

II) Effect of Levels, Storage Time and Temperatures on the Development of Rancidity in Baked Samples containing Mill-Fines, Impact-Milling

As shown in the analysis of variance on baked pastry samples containing three levels of mill-fines and stored at two different temperatures, all of the main effects and their interactions were found significant at the 0.005 level (Table 13). Subsequent Newman-Keuls Comparison Tests indicated the following findings:

First, on testing the inhibitory effect of levels of mill-fines (impact-milling) on lipid oxidation (A Effect), significant differences beyond the 0.01 level were found between 0 per cent and 7.5 per cent and between 0 per cent and 15 per cent (Table 14). These results suggested that the presence of 7.5 per cent or 15 per cent mill-fines (impact-milling) fraction in the pastry exerted an inhibitory effect on lipid oxidation as compared to 100 per cent wheat flour without mill-fines. No significant difference was found for the pair-wise comparison between 7.5 per cent and 15 per cent indicating that the inhibitory effect was not proportionately increased when the amount of the sorghum fraction was doubled. It is highly possible that the maximum amount of inhibition was reached somewhere between 7.5 per cent and 15 per cent.

Second, in examining the effect of storage time on development of rancidity (B Effect), significant differences beyond

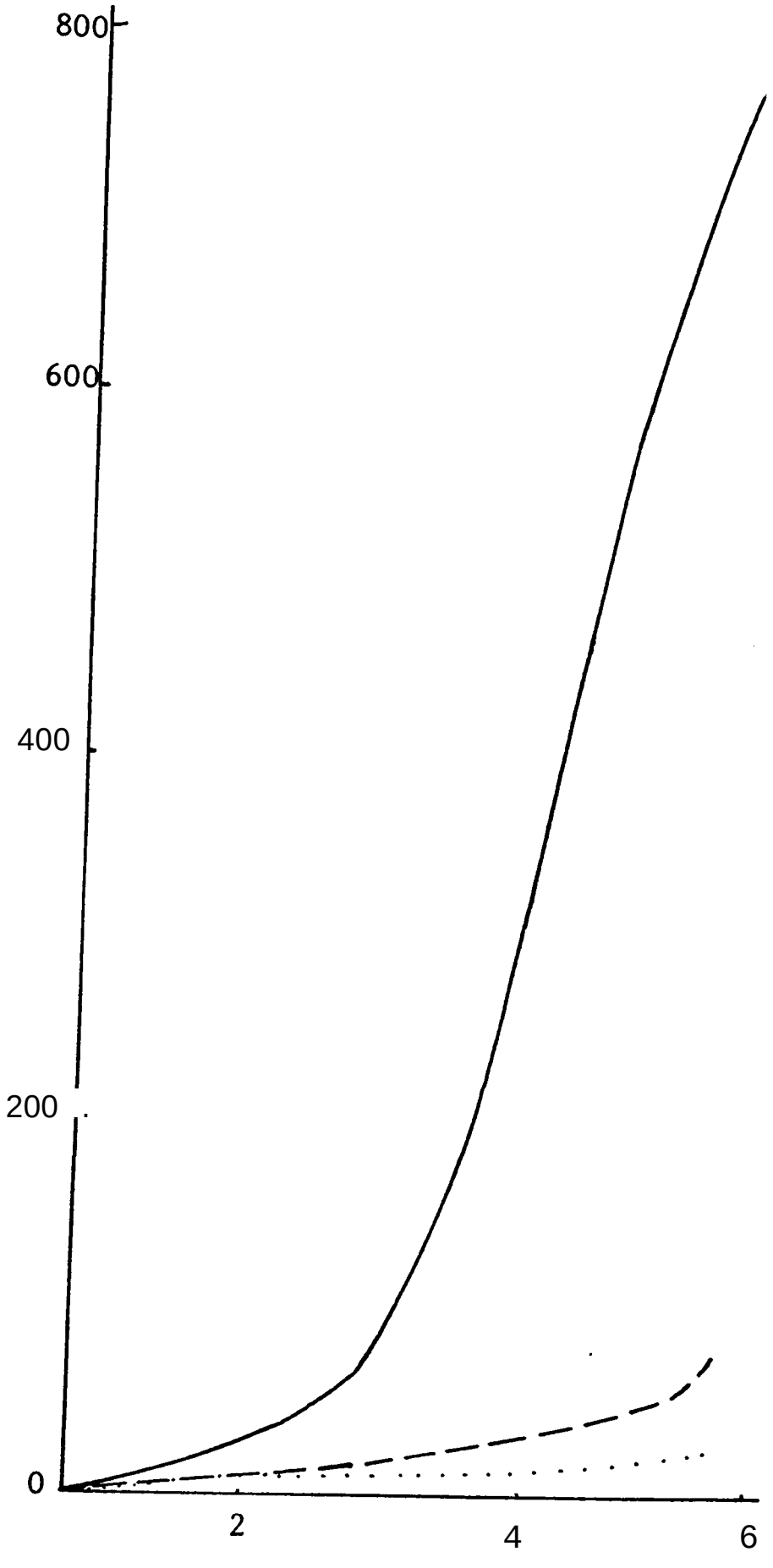
the 0.01 level were found for all possible pair-wise comparisons excepting that between zero week and the second week (Table 15). These results indicate that lipid deterioration increased with time, but the increase was more rapid after the second week. Figures 15 and 16 illustrate graphically the positive relationship of rancidity with storage time and that the rate of lipid oxidation was much greater in the zero per cent pastry samples as compared with the 7,5 per cent or 15 per cent pastry samples for both the storage temperatures (37° C and 6° C) and for the same time periods examined.

Third, following the previous procedure, the main effect of storage temperatures (C Effect) with one degree of freedom was not analyzed further as it is obvious from data (Tables 8, 9, and 13, and Figures 15 and 16) that the pastry samples stored at the higher temperature (37° C) underwent lipid oxidation more rapidly than those stored at the lower temperature (6° C).

Ill) Effect of Baking on the Development of Rancidity

In the pastry samples containing different levels of germ fraction and stored at 37° C, it is obvious from the data (Table 6 and Figures 12 and 13) that the baked pastry samples underwent lipid oxidation more rapidly than the unbaked samples. Therefore no analysis of variance was performed on this set of data.

In the pastry samples containing different levels of mill-fines (impact-milling) the results of analysis of variance showed


800
600
400
200
0
2
4
6
Weeks at Storage Temperature (37*^ c)
0% Mill-Fines
7.5% Mill-Fines
15% Mill-Fines

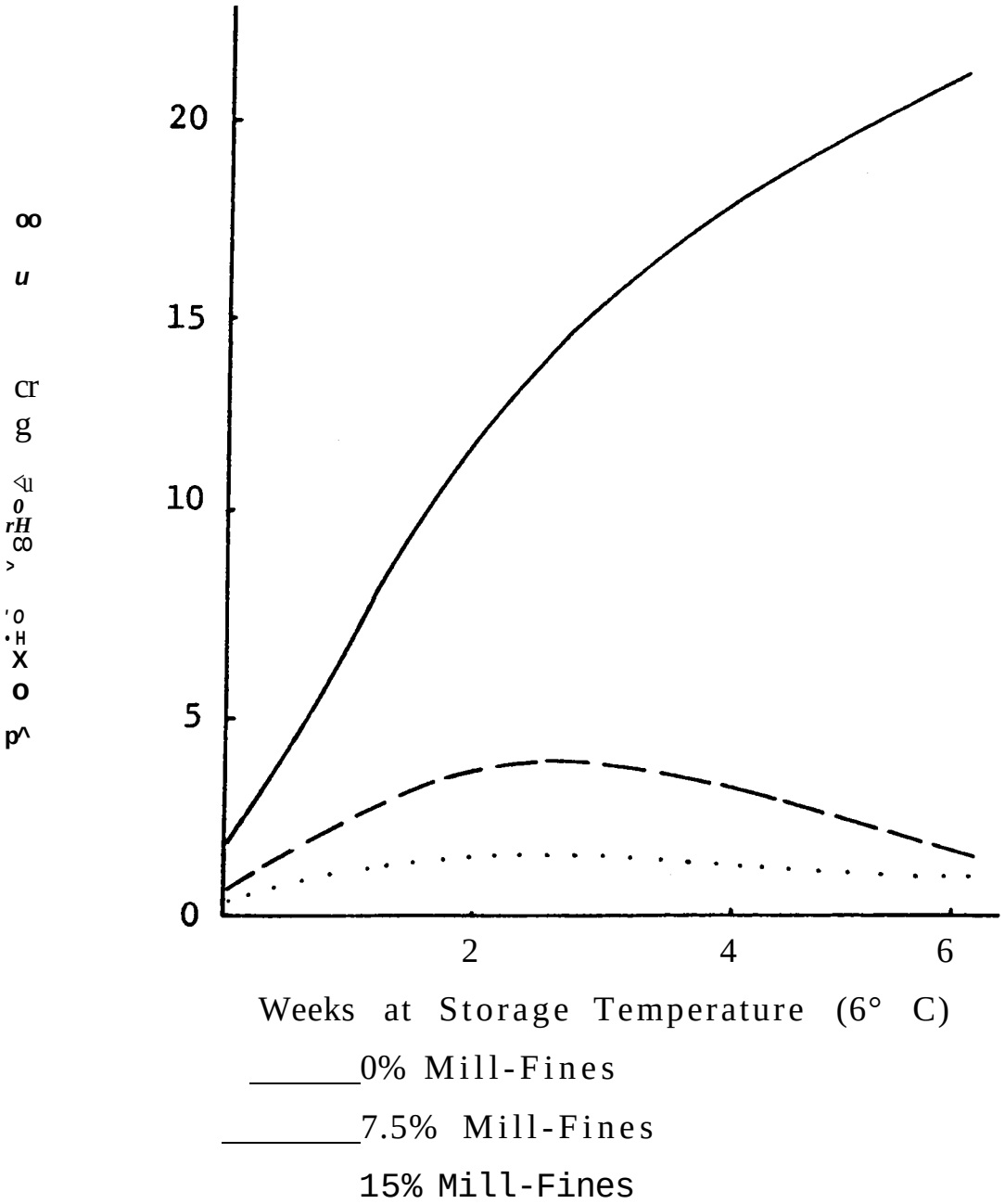


Fig. 16.—Rate of Peroxide Formation in Baked Pastry Samples containing three Levels of Mill-Fines (Impact-Milling) Stored at 6° C.

that there was a significant difference beyond the 0.005 level between the baked and unbaked samples (Table 16). Correlating this finding with the data presented in Figures 15 and 17, it is evident that the baked samples underwent lipid oxidation more rapidly than their unbaked counterparts. One possible explanation is the destruction of enzymes, biological pro-oxidants of lipid oxidation, in the baking process (425° F for 11 minutes). This explanation does not account for the fact that the unbaked samples with "intact" enzyme activities exhibited a lower rate of oxidation as compared to the baked samples at the same storage temperature. On the other hand, storage temperature of 37° C did not presumably fall within the optimal temperature range for the enzymes in question resulting in this pro-oxidant not being one of the factors involved in the peroxidation of lipids in the pastry samples under examination.

Another possible explanation is that one or more antioxidants present in the mill-fines fraction was heat liable and was destroyed during the baking process. Consequently, the unbaked samples exhibited a higher resistance to oxidation as opposed to their baked counterparts under identical conditions.

From data presented in Tables 6 and 8, it is clear that the effect of baked when compared to unbaked samples was similar for both the batches of pastry (germ versus mill-fines, impact-milling),

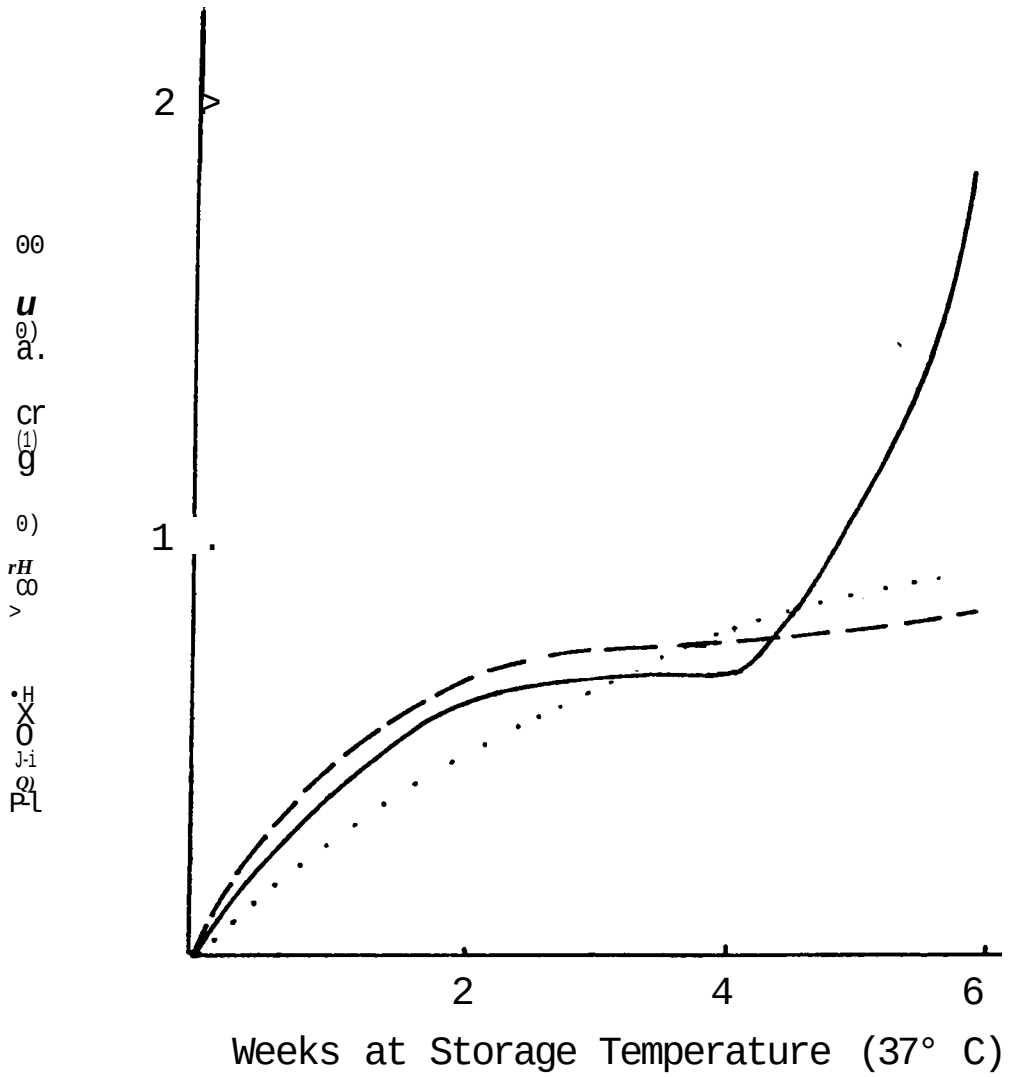


Fig. 17.—Rate of Peroxide Formation in Unbaked Pastry Samples containing Three Levels of Mill-Fines (Impact-Milling).

Hence, the postulations employed to explain the observed results for the batch containing mill-fines, impact-milling, also apply to the batch containing the germ fraction.

The main effects of levels of sorghimi fractions, germ or mill-fines, impact-milling (A Effect) and storate time (B Effect) were not analyzed further in this section as they were discussed in detail in the previous two sections.

IV) Effect of Type of Sorghum Fraction (Germ Versus Mill-Fines, Impact-Milling) on the Development of Rancidity

In the analysis of the antioxidant activity between two sorghum fractions (germ versus mill-fines, impact-milling) no significant difference was found (Table 17). This suggests that the two sorghum fractions investigated possessed approximately equivalent antioxidant activities.

In the cereal industry, particularly that of corn and wheat, and industries using these milled products, it has been the usual practice to utilize fractions other than the germ. This is due to the extremely high lipid content of the germ rendering this particular fraction more susceptible to lipid oxidation and a subsequent shorter shelf-life in both the raw product and germ-based products. The implication may have potential industrial and economic importance. Moreover, nutritionally, the germ as compared with other sorghum fractions is one of the richest sources of proteins, essential fatty acids and fat soluble vitamins.

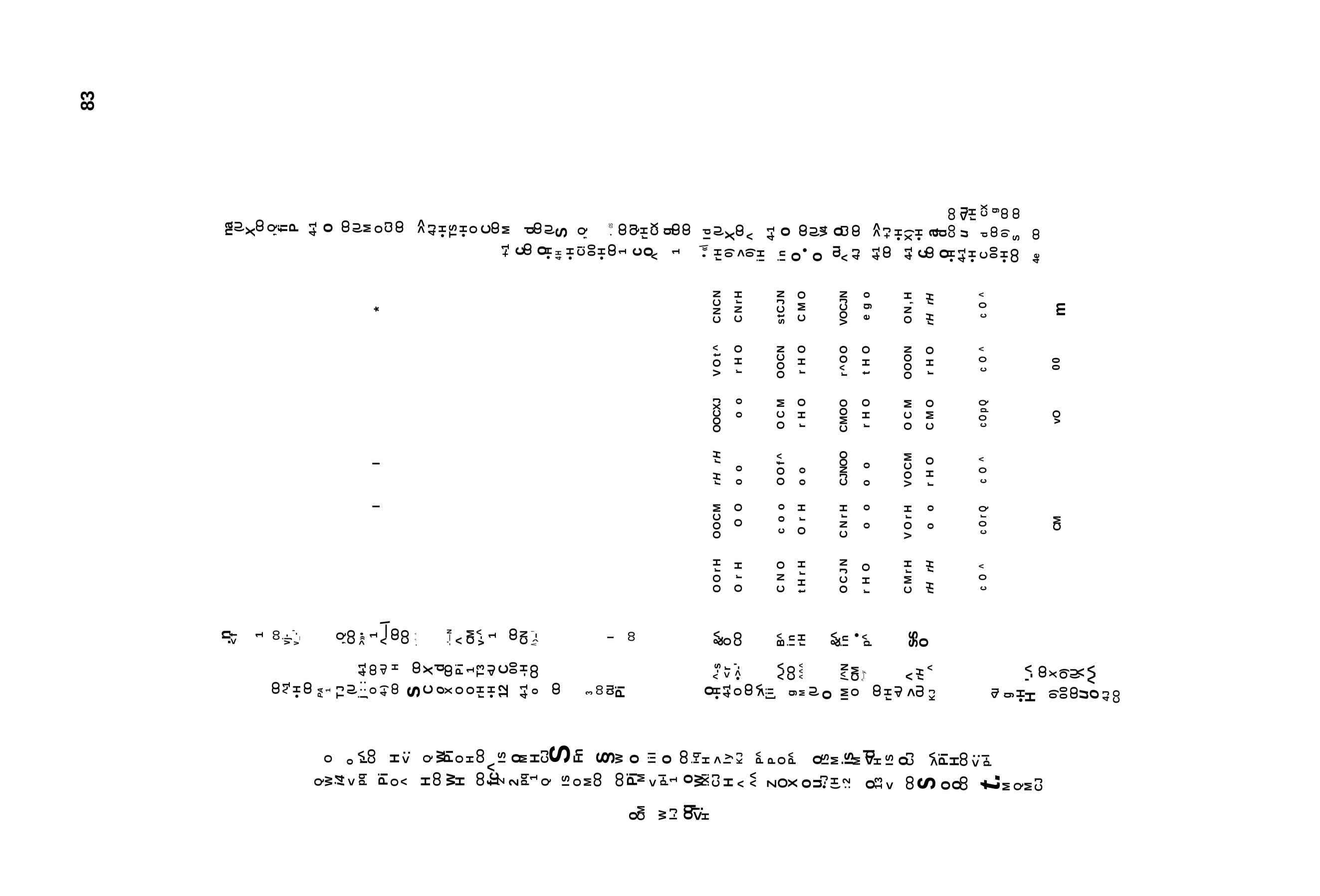
Olfactory Evaluation

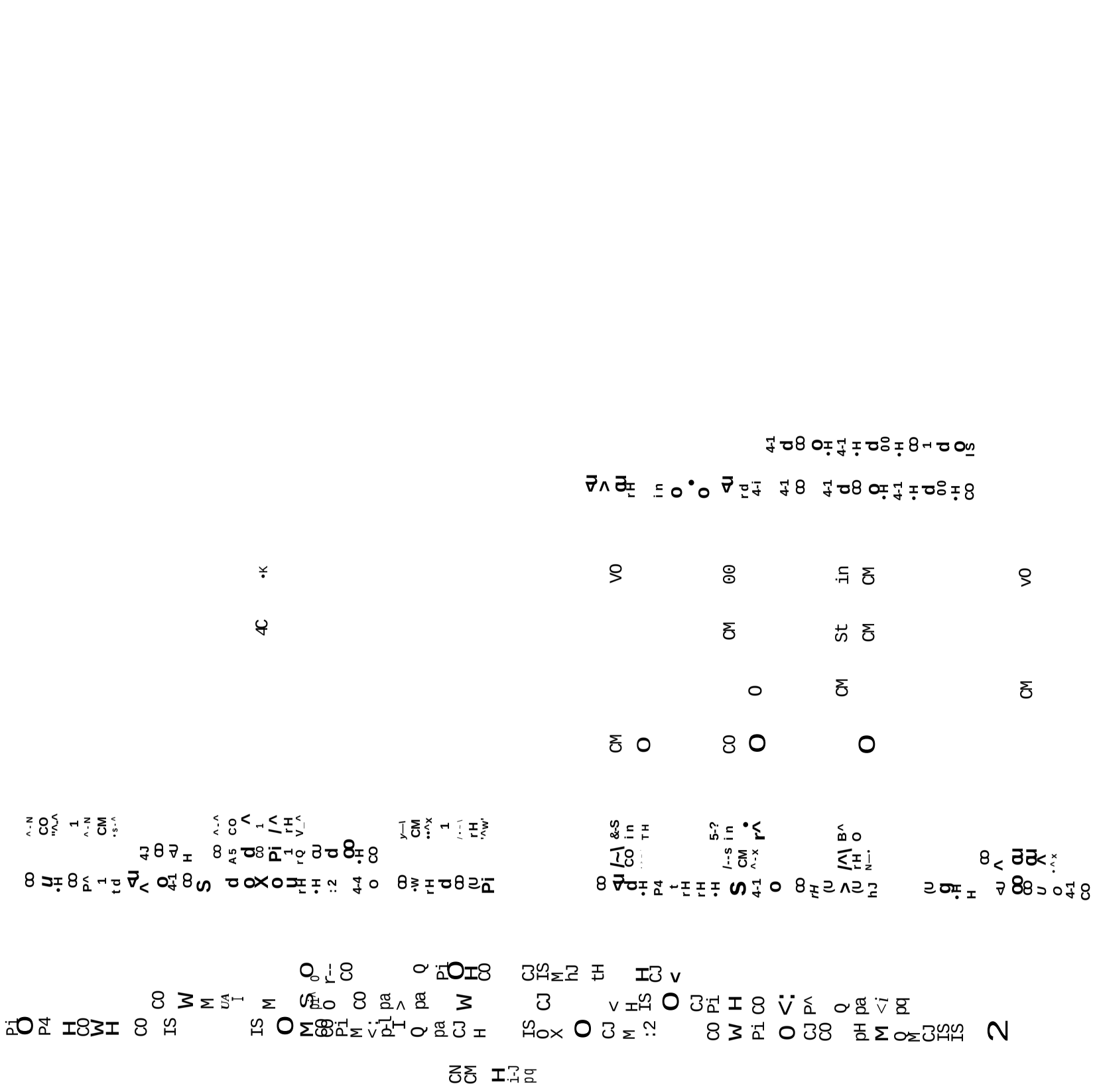
The rancidity scores used for performing the Wilcoxon Matched-Pairs Signed-Ranks Test were the average scores calculated for each sample at each level of the sorghum fraction. Tables 18, 19, 20, and 21 show the statistical evaluation by the same method for the germ fraction; Tables 22, 23, 24, and 25 show the statistical evaluation of the mill-fines, impact-milling.

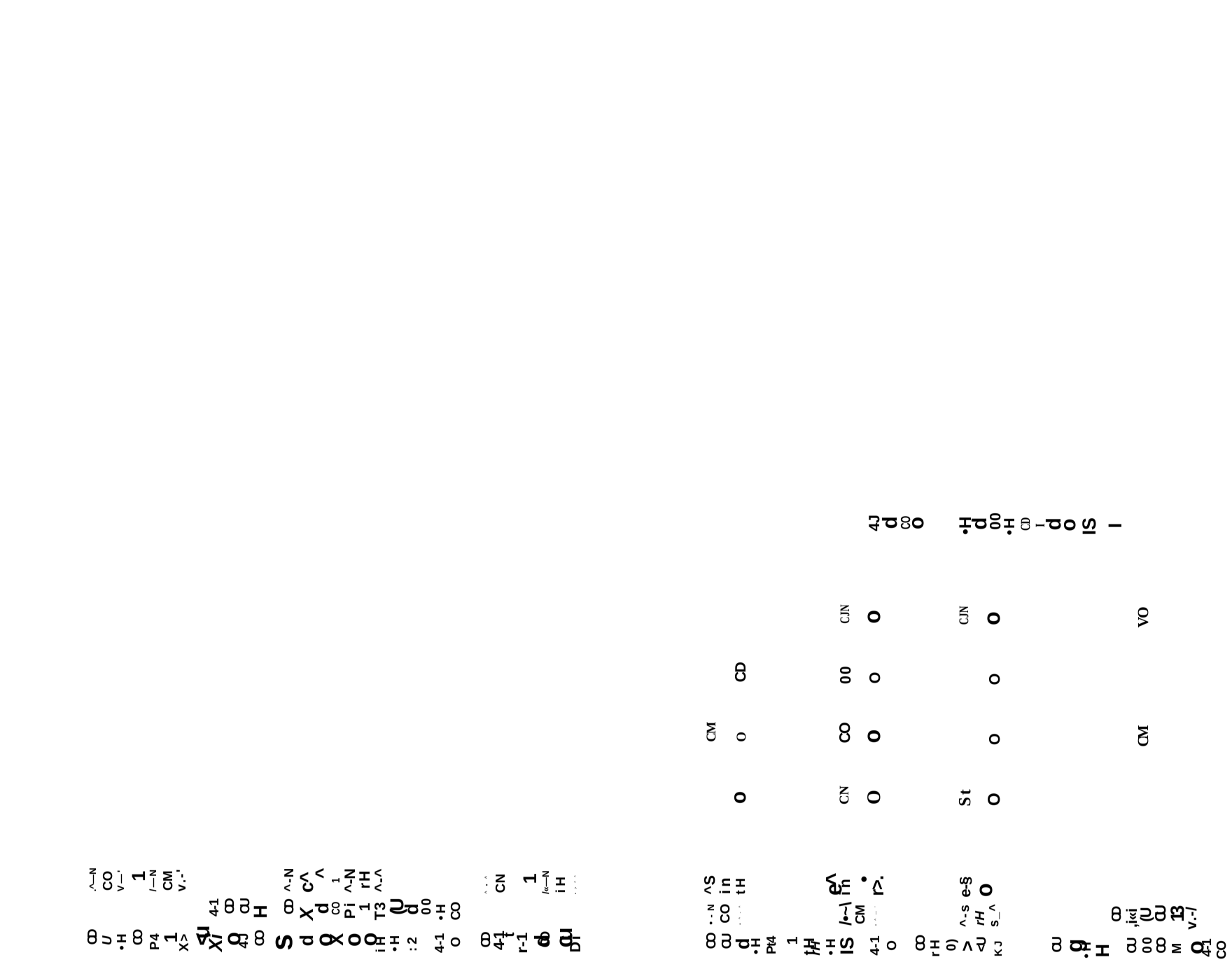
Germ

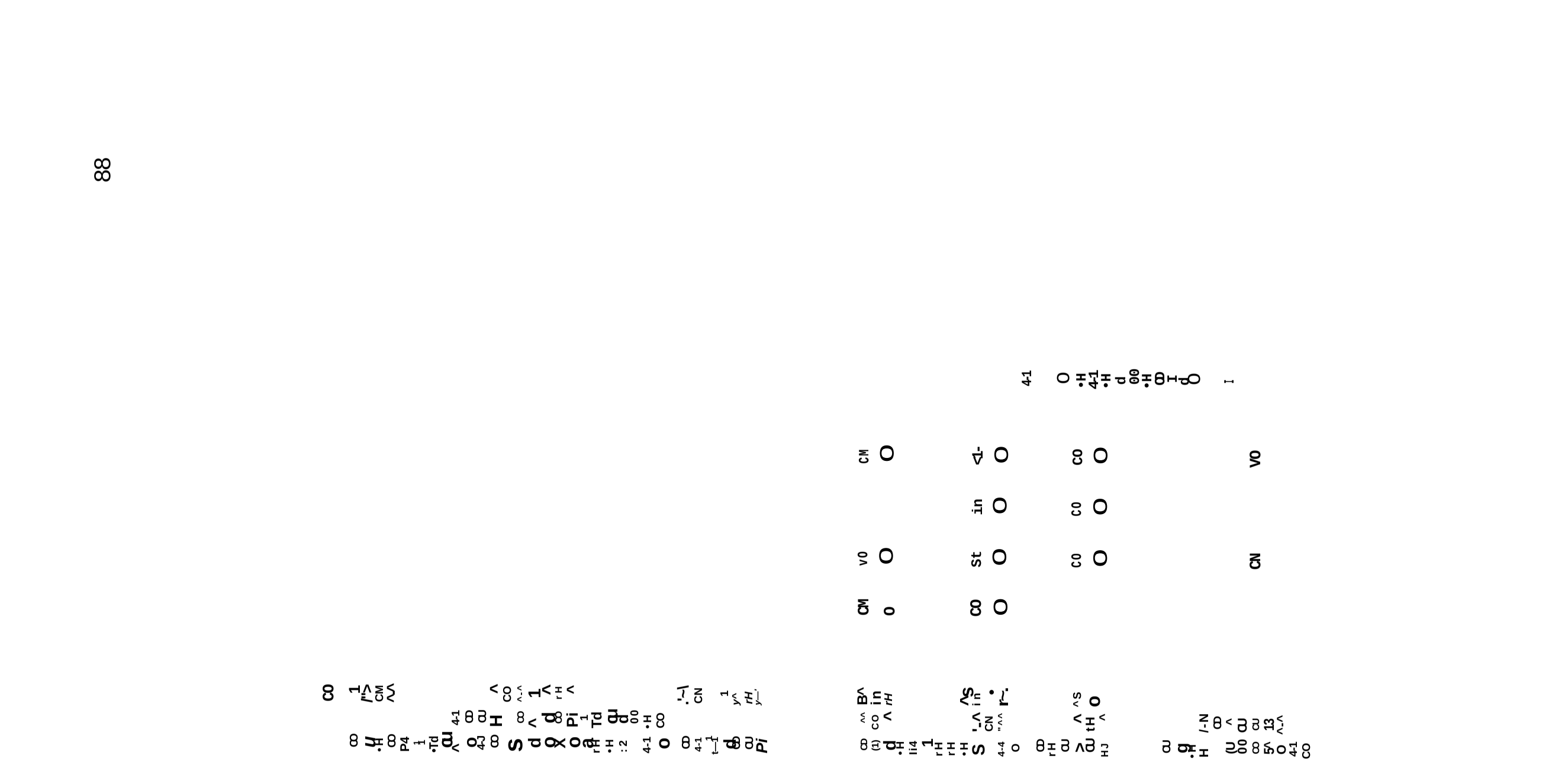
In Table 18, it is obvious that there was differentiation at the 0.05 level between the pair-wise comparisons of 0 per cent and 7.5 per cent, 0 per cent and 30 per cent, and between 7.5 per cent and 30 per cent germ for the storage period in the fourth week. For the storage period in the sixth week, significant differences were found between 0 per cent and 7.5 per cent, 0 per cent and 15 per cent, and 0 per cent and 30 per cent.

These findings suggest that the panel was able to distinguish only marked differences in rancid odors between the four different levels of grain sorghum, and only after the fourth and sixth week when rancidity, as indicated by peroxide values, had reached an advanced stage, particularly for the 0 per cent baked samples (see Table 6).









No significant differences were found for the unbaked pastry samples stored at 37° C (Table 19). Rancidity, as indicated by peroxide values (see Table 6), did not develop in all of the unbaked samples until the eighth week. For the storage period between zero week and the eighth week, most of the panel members assigned low scores to the pastry samples. Comparing these results with data in Table 6, it is evident that the panel was not able to distinguish non-rancidity (zero peroxide values) from low rancidity (peroxide values of approximately 10 and below). Of the fifteenth-week samples, when the differences in peroxide values between the four different levels of pastry samples were markedly increased, the panel was still unable to detect differences in all possible pair comparisons, indicating they were unable to differentiate between marked differences in rancid odors.

Comparisons between the baked and unbaked samples stored at 37° C (Table 20) indicate that differences were not significant until the fourth week. For both the fourth- and sixth-week time periods, with the exception of the pair-wise comparison between the baked and unbaked with 15 per cent germ, significant differences beyond the 0.05 level were found only for the pairs between baked and unbaked samples with 0 per cent germ. On the eighth week, all possible pair comparisons were found significant.

No significant differences, however, were found for all the comparisons between baked and unbaked samples at 6° C (Table 21).

Although low rancidity scores were assigned by panel judges to these samples (Table 21), no differentiation was made in the rancidity rating allocated to samples determined chemically to be non-rancid (zero peroxide values) or to have low peroxide values of approximately 10 or less (Table 7).

Mill-Fines (Impact-Milling)

Significant differences beyond the 0.05 level were found for the pair-wise comparison between 0 per cent and 7.5 per cent and between 0 per cent and 15 per cent for the storage periods of fourth and sixth week (Table 22). No significant differences were found for the pair comparisons of 7.5 per cent and 15 per cent for these storage periods. Comparing findings with data presented in Table 8 clearly only marked differences in rancidity were detected by judges. This trend is evident from examination of data presented in Tables 23, 24, and 25 and those of Tables 8 and 9 which present the peroxide values corresponding to the mean olfactory scores in the former set of named Tables.

Careful inspection of the rancidity scores given by the panel indicate that rancidity as determined chemically to have low peroxide values (peroxide value of approximately 10 and below) was not differentiated from non-rancidity (peroxide value of zero).

Correlation of Olfactory Scores with Peroxide Values^ ~

Results of correlation coefficients between the mean olfactory scores and the corresponding peroxide values of both batches of pastry samples were, first, in Batch I, there was no significant correlation ($r = 0.144$) between the panel olfactory scores and the peroxide values. Second, in Batch II, a correlation coefficient of 0.708 was obtained between the olfactory scores and their corresponding peroxide values (Table 26). This was significant at the 0.01 level (DuBois, P. H., 1965).

Using peroxide values as the reference standard of the intensity of rancidity these results would indicate that the panel members who participated in the olfactory rating of Batch I pastry samples (germ fraction) did not perform as well as those who participated in the olfactory evaluation of Batch II pastry samples (mill-fines, impact-milling).

TABLE 26

CORRELATION OF MEAN OLFACTORY SCORES WITH PEROXIDE VALUES

Pastry Samples	r	r^2
Germ	0.144	0.011
Mill-Fines (Impact-Milling)	0.708**	0.501

** P < 0.01 d.f. = 14

CHAPTER V

SUMMARY AND CONCLUSIONS

Synthetic antioxidants have been traditionally and presumably will continue to be utilized as additives in foods to increase their lipid stability and shelf life. Nevertheless, a natural source of antioxidant should prove an important asset to the food industry. The High Plains region of Texas is a leading grain sorghum producing area. Hence due to the abundant production the economic advantages afforded by this grain are readily noted. This study was initiated to evaluate the antioxidant potentials of grain sorghum.

Lipids undergo autoxidation leading to fat rancidity, a complex process resulting from the interaction of numerous factors. These factors can be divided into two categories, lipid accelerators and lipid inhibitors. Heat, light, metals, and biological catalysts such as enzymes are examples of lipid accelerators. Examples of lipid inhibitors are antioxidants which are subdivided into primary antioxidants, synergists and multifunctional antioxidants.

During the process of lipid oxidation, oxygen is absorbed and the rate of oxygen consumption by lipids is proportional to the amount of lipids present. An antioxidant suppresses the

uptake of oxygen by the lipids and the rate of inhibition is proportional to the amount of the antioxidant present. This affords a good method of assessing the antioxidant activities of a particular substance.

The development of rancidity is characterized by the formation of primary and secondary products. The former are mainly hydroperoxides which decompose to give secondary degradation products. Among the latter are volatile cleavage products, mainly aldehydes and ketones, which possess characteristic off-odors. The hydroperoxides can be determined by chemical analysis and the off-odors by olfactory means.

In this study the antioxidant activity of seven different fractions was evaluated objectively using a polarographic oxygen analyzer which measured the rate of oxygen uptake by an artificial lipid-emulsion containing linoleic acid in a dispersion of Tween 20 and water. The rate of oxygen consumption was accelerated by introducing into the emulsion an enzyme, soybean lipoxidase. Antioxidant activities of two different concentrations of each sorghum fraction (2.5 per cent and 5.0 per cent) was measured by the amount of suppression that particular sorghum fraction had on the accelerated rate of oxygen consumption as compared to a control containing no sorghum fractions.

Two fractions, germ and mill-fines, impact-milling were assessed by the oxygen uptake method to possess the highest

antioxidant activity. They were selected for the evaluation of their "inhibitory effect" on the development of rancidity in a food product high in lipid content, pastry. The pastry samples were prepared using a basic recipe with varying levels of the sorghum fraction (0 per cent, 7.5 per cent, 15 per cent, and 30 per cent) replacing wheat flour. One-half of the samples were baked at a temperature of 425** F for eleven minutes and the remaining half were unbaked. Both baked and unbaked samples were then stored at two different temperatures (37** C and 6" C) for periods of eight and fifteen weeks. At biweekly intervals, pastry samples were chemically analyzed for hydroperoxides, an index of rancidity. At the same time, a panel of semi-trained judges was asked to identify the host of rancid odors produced by degradation products of lipid oxidation.

Results of the study were:

1. At a concentration of 5 per cent, all of the sorghum fractions exhibited antioxidant activities. In decreasing order, the antioxidant activities were: germ > mill-fines, impact-milling > mill-fines, cutting and cracking > whole grain > pearled grain > ground pearled grain > endosperm.

2. At a concentration of 2.5 per cent, only four fractions were found to exert an antioxidant activity when compared with the control. In the decreasing order, they were: germ > mill-fines, impact-milling > mill-fines, cutting and cracking > whole grain.

3. The rate of suppression of lipid oxidation in the pastry samples containing germ or mill-fines, impact-milling was found to be proportional to temperature, storage time and amount of sorghum fraction contained in the pastry. That is:

i) lipid oxidation progressed more rapidly in pastry stored at a higher temperature (37** C) than in pastry stored at a lower temperature (6° C);

ii) lipid oxidation increased proportionately with storage time; and

iii) lipid oxidation decreased proportionately with an increased amount of sorghum fraction contained in the pastry samples. Generally, the results showed that lipid oxidation in the comparison of varying percentages of germ and mill-fines, impact-milling was as follows:

germ: 30 per cent < 15 per cent < 7.5 per cent < 0 per cent

mill-fines: 15 per cent < 7.5 per cent < 0 per cent

4. Baked pastry samples underwent lipid oxidation more rapidly than unbaked pastry samples.

5. The development of rancidity in pastry was suppressed by both the sorghum fractions (germ and mill-fines, impact-milling) when compared to controls containing no sorghum fractions. There was no significant difference between the antioxidant activities of these two fractions.

6. Human olfaction could prove to be a good detector of the development of rancidity, particularly with a well-trained panel. However, in this study, the panel members, with a few exceptions, were found not highly discriminative toward rancid odors. As a whole, the judges failed to distinguish non-rancidity (zero peroxide value) from low rancidity (peroxide value of approximately 10 and below).

7. A correlation (r = 0.708) was found between the olfactory scores and the corresponding peroxide values for the pastry samples containing varying levels of mill-fines, impact milling. No correlation (r = 0.144) was found for the samples containing different amounts of germ fraction.

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APPENDIX

A. Rancidity Odor Score Card for Grain Sorghum

B. Definition of Terms

APPENDIX A: RANCIDITY ODOR SCORE CARD FOR GRAIN SORGHUM

Name of Panel Member:

Date: Time:

Key:

Code Number	
0	Not Rancid
1	Just detectable
2	Moderately detectable
3	Strongly detectable
4	Very strongly detectable

DIRECTION:

Please lift the foil (DO NOT REMOVE IT) just before your test. Cover the container tightly again as soon as you have finished each test. Use the key to indicate the intensity of rancidity by placing the code numbers in the appropriate columns of the Rating Table.

Pause a minute or two between samples to allow recovery of your olfactory nerves. You may retest any of the samples again.

Please also indicate on the Comparison Scale the relative intensity in rancidity of the samples, using the appropriate sample numbers allocated to each sample.

RATING TABLE

SAMPLE NUMBER	RATING	COMMENT

COMPARISON SCALE

Worst Average Best

APPENDIX B: DEFINITION OF TERMS

Fal^ Stability; The stability of a fat is generally assessed as the length of time (induction period) that elapses before onset of rancidity, a condition denoted by a rapid rise in peroxide value and formation of rancid odors.

^^^^^- Sorghum: Grain sorghum is the term used to identify a group of plants in the grass family known by the scientific name Sorghum vulgare.

^^ain Sorghum Fractions: Grain sorghum fractions are the products of dry milling. The seven fractions used in this study are defined as follows: (Stickley, E. S., 1971)

1. Whole Grain: This is the whole kernel which was aspirated to remove stocks, glumes, undersize berries, cracked grains, and small foreign seeds.
2. Pearled Grain: Pearled grain is the fraction of the grain sorghum kernel from which the bran has been removed.
3. Ground Pearled Grain: This is the ground product of the pearled grain.
4. Germ: Germ describes the fraction of the sorghum grain kernel from which the bran and the endosperm have been removed.
5. Endosperm: This is the fraction of the sorghum grain kernel from which the bran and the germ have been removed.
6. Mill-Fines, Impact-Milling Process: These are the fines, through No. 30 stainless steel screen (diameter of opening = 0.0268 inch), from Impact-Milling which supplies the endosperm. Impact-Milling is a process whereby pearling and degermination are accomplished in a single step.
7. Mill-Fines, Pearling and Cracking Process: This is the minus 20-mesh from the cracking operation used in preparing the germ fraction.

Rancidity: Rancidity is a widely-known term covering many typical (mostly objectionable) off-flavors which are formed by autoxidation of all unsaturated fatty acids present. Various volatile components such as aldehydes and ketones are responsible for the off-flavors.