FACTORS CONTRIBUTING TO THE DEVELOPMENT OF RANCIDITY IN GROUND PEARL MILLET (*Pennisetum americanum* [L.] Leeke) DURING STORAGE

by

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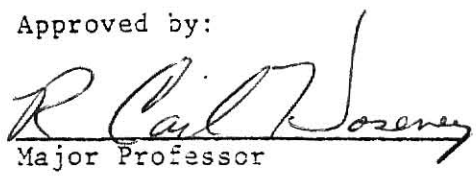

Major Professor

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INTRODUCTION

Pearl millet (Pennisetum americanum [L] Leeke) is the largest seeded and most widely grown of the millets (Hoseney et al, 1982). It is one of the most drought tolerant of man's food crops and is grown primarily in the dry Sahel Zone across Africa and in the semi-arid areas of India (Hoseney et al, 1982). In the areas where it is grown, the yield is often low (Hoseney et al, 1982), therefore, if the work put into producing the millet is to be rewarded with a supply of food throughout the year, proper storage is essential (Vogel and Graham, 1978).

Until recently, pearl millet was ground daily by the housewife and in amounts large enough for only immediate needs. Nowadays, more grinding is done in power mills (Thiam et al, 1976).

Millet flours have a serious drawback; they do not keep well. Off odors and taste are often apparent in a short time. Mechanical milling of millet would be more popular if its milled products had a longer preservation period (Carnovale and Quaglia, 1973). Millet meal stored at 19°C, 58% relative humidity (RH) had detectable changes in odor after only 4.5 days (Lai and Varriano-Marston, 1980b). Since millet is the nutritional basis of a large part of the world, especially in developing countries, more knowledge of the changes occurring in the lipid or fat is needed (Deyoe and Robinson, 1979).

The objective of this study is, therefore, to determine the storage stability of pearl millet and determine the factors contributing to the development of rancidity in millet meal during storage.

LITERATURE REVIEW

The storage of fat containing food almost inevitably brings about the development of off-flavors and off-odors. Those are referred to collectively as "rancid" and are a consequence of the decomposition of lipids.

Stability and Rancidity

In flour and other mill products, typical rancid odors, indicative of hydrolytic and/or oxidative deterioration of lipids, appear quickly under adverse storage conditions. To better understand what is happening during adverse storage that causes objectionable flavors, a review of the chemistry of rancidity is necessary.

Deteriorative changes in fats or oils may be either oxidative, resulting in typical rancid flavors and odors, or hydrolytic, resulting in the production of free fatty acids (FFA) (Pomeranz, 1974). Hydrolytic action of lipases on fats containing short carbon chain fatty acids may also bring about the formation of off-flavors even at very low levels. Hexanal, an oxidation product, has been reported to have a flavor threshold of 150 ppb (Labuza, 1971).

Hydrolytic rancidity. The action of lipases on triglycerides (TG) gives hydrolytic rancidity. Lipases are enzymes that hydrolyze ester linkages of emulsified gylcerides at an oil-water interface. Hydrolytic rancidity is particularly important in butter. It results from the release of short-chain fatty acids such as butyric, caproic, and caprylic by the action of lipases (Lundberg, 1962). It is also active in low moisture and non-aqueous environments (Morrison, 1978). Most lipases encountered hydrolyze triglycerides in a stepwise fashion as follows:

$$TG \xrightarrow{L} DG + FFA$$

$$DG \xrightarrow{L} MG + FFA$$

$$MG \xrightarrow{L} Glycerol + FFA$$

However, there are many exceptions depending on the enzyme and its specificity. There are four basic types of specificities associated with lipases: glyceride, positional, fatty acid, and stereospecificity.

Glyceride specificity is characterized by a preferential hydrolysis of low molecular weight TG over high molecular weight substrates. An example is the plant lipase like castor bean lipase which hydrolyses monoacid short-chain TG more rapidly than long ones (Jensen, 1971).

The best known example of positional specificity is that of pancreatic lipase, which hydrolyses only the primary ester of TG. No lipase that hydrolyses only the secondary ester from a TG molecule has been reported to date (Richardson, 1976).

Fatty acid specificity occurs when one fatty acid is more rapidly hydrolyzed than another, both being attached to the same position of similar triglyceride molecules. An example of fatty acid specificity is that of a lipase from Geotrichum candidum for oleic acid (Richardson, 1976). G. candidum elaborates an extra cellular lipase that is highly specific for fatty acid containing Cis 9 and Cis 9-Cis 12 unsaturation (Jensen, 1971).

Although no lipase has been reported to present stereospecificity, there are some indications that this enzymatic property among lipases exists (Jensen, 1971). Stereospecificity is the ability of an enzyme (lipase) to catalyse the production of fatty acids from triglycerides that have identical spatial conformation. Karnovsky and Wolff (1960) established that pancreatic, lipoprotein, and wheat germ lipases are not stereospecific.

Oxidative rancidity. Oxidative rancidity is often considered the major factor in the development of off-odors and flavors in fats and food containing fats (Lundberg, 1962; Buttery et al, 1961; Labuza, 1971; Mecham, 1978; and Frankel, 1980). It has been well established that among fatty acids, the unsaturated moieties cause the development of most rancid flavors in foods. The mechanism of the breakdown reaction has been extensively reviewed by Lundberg (1962) and more recently by Frankel (1980).

The overall autoxidation mechanism includes three stages: initiation, propagation, and termination.

Initiation: $RH \xrightarrow{\text{initiator}} R^{\cdot} + H^{\cdot}$

The production of free radicals may occur by thermolysis, hydroperoxide decomposition, metal catalysis, photolysis, or the result of enzyme action.

Propagation: $R^{\cdot} + O_2 \rightleftharpoons ROO^{\cdot}$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

With unsaturated fats, susceptibility to autoxidation is dependent on the availability of allylic hydrogens for reactions with peroxy radicals.

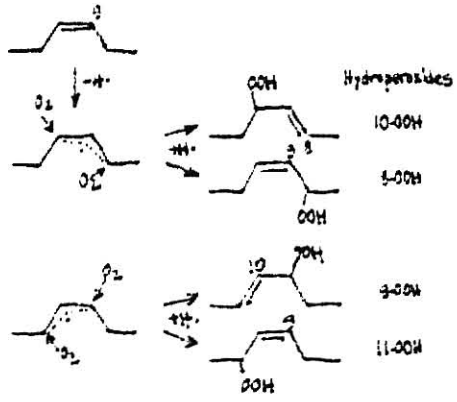
Termination: $R^{\cdot} + R^{\cdot} \longrightarrow R - R$

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow \text{nonradical product.}$$

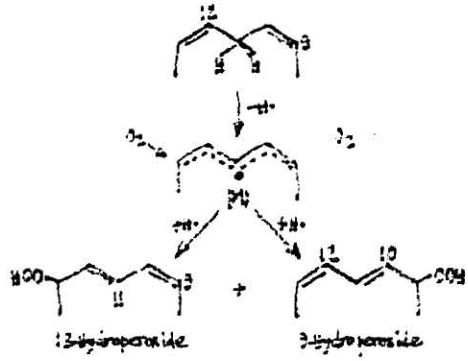
Unsaturated fatty acids can be oxidized by free radical autoxidation. The mechanism of autoxidation of oleate, linoleate and linolenate are shown in Fig. 1.

Fatty acids can also be oxidized by singlet oxygen. In the most stable triplet state, oxygen is not reactive with unsaturated compounds. The activation of oxygen by electronic excitation forms singlet oxygen, which reacts readily with unsaturated fatty acids. Singlet oxygen can be generated in a

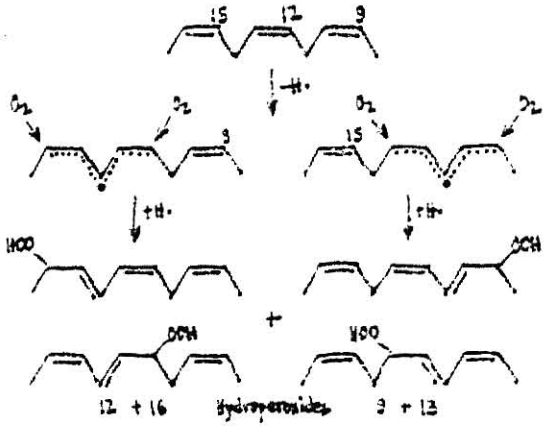
Fig. 1. Fatty acid oxidation by free radical formation (Frankel, 1980).



a-Mechanism of oleate autoxidation



b-Mechanism of linoleate autoxidation



c-Mechanism of linolenate autoxidation

wide variety of ways. Frankel (1980) cited a number of papers reporting several potential sources of singlet oxygen, the most important being exposure to light. All autoxidation mechanisms reported indicate the formation of peroxides before decomposition. The generally accepted scheme of decomposition of hydroperoxides is given in Fig. 2.

Lypoxygenase. Free radical formation can also be initiated by means of lypoxygenase. The reaction (Fig. 3) taking place in both the presence and absence of oxygen, is initiated after enzyme and substrate have bound and the abstration of a hydrogen from C-8 methylene group of the linoleic and substrate (Faubion and Hoseney, 1981).

Millet Rancidity

It is now well established that millet, after grinding, does not keep very well. However, there is no agreement upon what the causes for the instability might be. Carnovale and Quaglia (1973) found no change in the amino acid composition during storage. The total fatty acids, followed by G.L.C. during storage, showed significant quantitative changes in composition. They attribute those changes to hydrolytic decomposition of lipids and to a lesser extent to oxidative process.

Thiam et al (1976), on the other hand, found that the majority of the volatile compounds emitted were alcohols, indicating that fermentation processes were occurring. At the same time, hydrolytic action of lipases appeared to increase with increase in meal moisture. Ultraviolet (UV) spectrophotometry of lipid extracts produced no evidence of oxidation of the unsaturated fatty acids.

Lai and Varriano-Marston (1980b) found that 4.5 days were required after grinding millet before significant odor changes were noted. Their millet meal was stored at 19^{o}C and 58% RH.

Fig. 2. Some routes of hydroperoxide decomposition (Labuza, 1971; Gray, 1978).

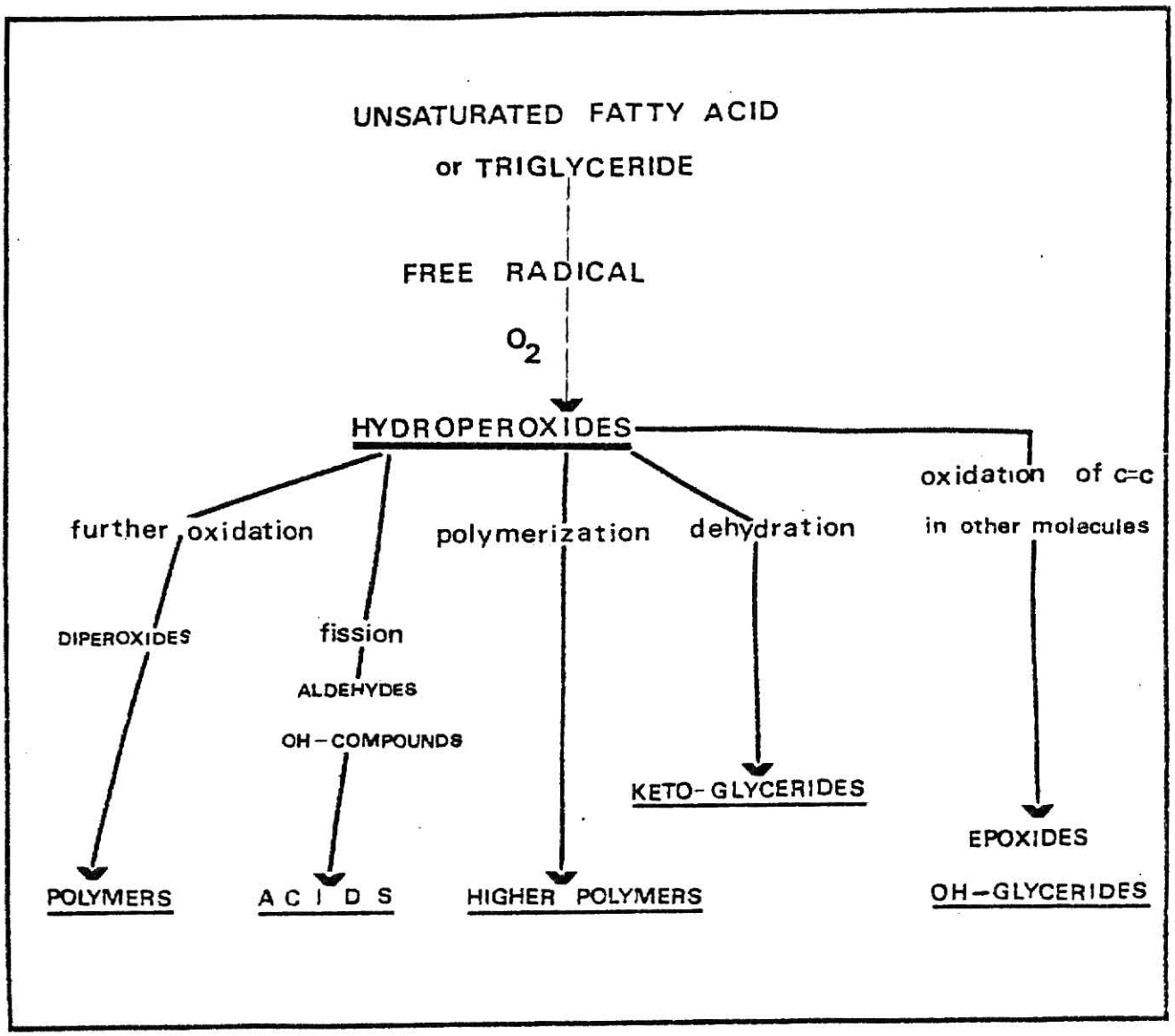
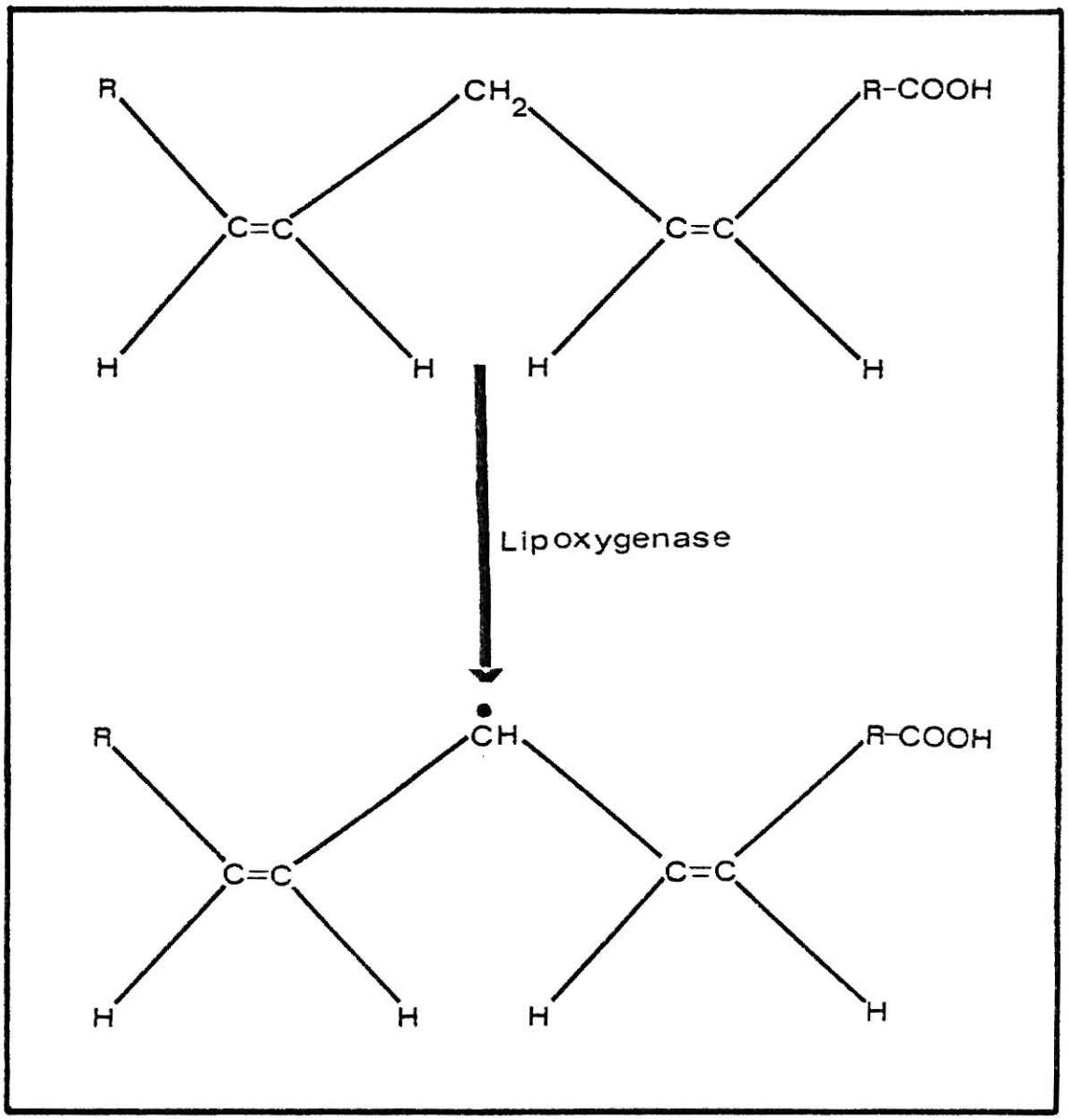

UNSATURATED FATTY ACID
or TRIGLYCERIDE
FREE RADICAL
O_2
HYDROPEROXIDES
further oxidation
polymerization
dehydration
oxidation of c=c
in other molecules
DIPEROXIDES
fission
ALDEHYDES
OH-COMPOUNDS
KETO-GLYCERIDES
EPOXIDES
POLYMERS
A C I D S
HIGHER POLYMERS
OH-GLYCERIDES

Fig. 3. Free radical formation by specific attack of lipoxigenase on fatty acids possessing Cis, Cis-1,4-pentadiene unsaturation (Faubion and Hoseney, 1981)


R
CH2
R-COOH
C=C
C=C
H
H
H
H
Lipoxygenase
R
CH
R-COOH
C=C
C=C
H
H
H
H

To judge the quality of fats and food containing fats, the consumer uses an organoleptic evaluation. However, sensory analysis is not practical for routine analyses and usually lacks reproducibility even though it is one of the most sensitive methods available.

Several chemical and physical methods for assessing lipid oxidation are reviewed by Gray (1978). There is no ideal chemical or physical method that correlates well with changes in organoleptic properties of oxidized lipids throughout the course of autoxidation.

Although the volatile carbonyl compounds are only minor side products during autoxidation of fatty acids, they are of great significance for the aroma of foods because their threshold concentrations for odor and taste are very low (Labuza, 1971). One of these compounds that seems to be widely distributed in deteriorated fats and food containing deteriorated fats is hexanal. The level of hexanal in autoxidized potato granules was reported to be 2.5 ppm as determined by GLC of a pentane extract of the steam distillate (Buttery et al, 1961). It amounted to four times the concentration of any other component. Hexanal was found to be among the major cleavage products of enzymic oxidation of linoleate (Chan et al, 1976).

Fritsh and Gale (1977) determined the concentration of hexanal in low fat dehydrated food in 10 minutes by GLC analysis of the vapors from a 15 g sample suspended in boiling water. They found that the onset of rancid odors occurred when the hexanal concentration increased to between 5 and 10 ppm. Besides with an oat cereal, they obtained good correlation between sensory evaluation and hexanal concentration between 0.3 and 5 ppm.

The identified volatile carbonyl compounds arising from the autoxidative breakdown of linoleic acid at low and moderate temperatures were hexanal (66%) as major, 2-octenal (18%), 2-heptenal (6%) and 2,4-decadienal (5%) as minor

components (Badings, 1970).

Among cereals, millet is probably the grain that develops most rapidly objectionable flavors and odors after grinding. There are at least three possible reasons: a) the fat content is high, b) the degree of unsaturation of fatty acids is higher than in other major cereals or c) the antioxidants occurring naturally in other cereal grains are lacking in millet.

Studies on fatty acid composition of pearl millet indicate that pearl millet lipids tend to be higher in saturated fatty acids and lower in unsaturated oleic and linoleic acids (Jellum and Powell, 1971; Rooney, 1978; Lai and Varriano-Marston, 1980a) than as other cereals. The measurement of lipoxygenasic activity and the UV spectrophotometry of the lipoidic extracts led Thiam et al (1976) to conclude that the enzymatic oxidation of lipids was practically nonexistant. The same authors attribute this to the presence of phenolic compounds acting as antioxidants. Thus, neither item b) or c) above appear to be true.

MATERIALS AND METHODS

All storage studies were conducted on replicate samples and analyses were done at least in duplicates. Different levels of relative humidities were used in this study. The moisture content of pearl millet meal was adjusted depending upon the experiment conducted.

Materials

Pearl millet samples used in this study were Serere 3A grown at Hays, Kansas in 1979, HMP 1700 grown at Hays, Kansas in 1980, and a bulk obtained from a blend of equal quantities of thirteen different entries grown at Minneola, Kansas in 1980. The Hard Red Winter Wheat used was a blend of varieties.

Three different makes of grinders used were: The Udy Cyclotech laboratory mill with a screen opening of 400 microns, a Ross experimental roller mill with 22 and 28 corrugated rolls, and an Alpine pin mill. After roller milling, the stock was sifted on a sieve equivalent to 375 microns screen opening. Both polyethylene bags and cotton bags were used.

Analytical Methods

Extractable fat was determined on a Goldfish apparatus by extraction with petroleum ether (bp 38-55^{o}C) for 4 hours.

Moisture content was determined by the approved AACC Method number 44-15A (1962).

Mold count: The quantitative determination of molds in millet meal was by the method of Christensen (1946).

Peroxide value was determined following the method of Takagi et al (1978). The average standard deviation was 0.33.

Fat acidity was determined by the approved AACC rapid method for corn, method number 0203.

Total fatty acids (TFA) from free lipids were analyzed by GLC using the method of Kates (1964) of transesterification of fats and oils.

Free fatty acids (FFA) from free lipids were quantitatively isolated by TLC (silica gel 60 F 254). The solvent system used was hexane/diethyl ether/acetic acid (60/40/1). FFA were localized on the TLC plates by subjecting the edges of the TLC plate to iodine vapor. The FFA spots had a neat contour and no trailing was apparent. The FFA band was then scraped off the plate and the FFA recovered by filtration through a scintered glass filter funnel with four fractions of 50 ml petroleum ether each. The internal standard used for TFA was trimargarin and for FFA, margaric acid.

Preparation of methyl esters of fatty acids. Fatty acids were analyzed by GLC as methyl esters. The preparation of methyl esters was by the transesterification method of Kates (1964). An aliquot of lipid extract was pipetted into a 50 ml combination hydrolysis and extraction flask. A known amount of trimargarin standard solution was added and the solvent evaporated under a stream of nitrogen. Four and a half milliliters methanolic-HCL (0.7N) were added to the lipid extract and refluxed for 2 hours. The volume was adjusted with methanol-water (9:1) to just fill the side tube. The fatty acid methyl esters (FAME) in the methanol-water phase were then extracted with four 15 ml fractions of petroleum ether and collected in a round flask. The excess solvent was removed in a rotavap and the FAME transferred into a small vial. The remainder of the solvent removed under a stream of nitrogen and the sample diluted to a known volume with Heptane.

Fatty acid methyl esters were determined with a Hewlett Packard 5750 gas chromatograph equipped with a flame ionization detector (FID). The column was 6 ft long, 1/8" inner diameter in stainless steel. It was packed with 10% SP 2330 chromosorb WAW 100/120 mesh (Supelco Inc.). The injection port temperature was 270°C, that of the FID was 260°C and the column was 210°C. Nitrogen carrier

gas flow was 25 ml/min. The sample volume injected of FAME extract was one microliter.

Relative peak areas were determined by multiplying the peak height by the width of the peak at half height. Weight percentage compositions were calculated by applying calibration factors obtained from chromatograms of known mixtures as indicated in the standard AOAC method. Peaks were tentatively identified by comparing the relative retention times to those from the standard reference mixture run under the same conditions. FFA weight percentages were determined on the basis of the quantity of free lipids spotted on the TLC plate.

Hexanal determination. The method used for hexanal determination in millet and wheat was that described by Fritsh and Gale (1977). Seven and a half grams of ground sample were weighed into a 250 ml erlenmeyer flask and 2 ml of the internal standard solution (4-heptanone solution, 25 ppm) added. Boiling distilled water was then added up to the 150 ml mark and the flask immediately capped with aluminum foil. The content of the flask was swirled vigorously for 10 seconds and then five milliliters of head-space gas was withdrawn with a gas-tight syringe and injected into the gas chromatograph injection port.

Hexanal was determined with a Hewlett Packard 5750 gas-liquid chromatograph equipped with a flame ionization detector (FID). The column was 10 ft long, ¼" inner diameter in aluminum. It was packed with 10% silicone OV-101 chromosorb WAW 60/80 mesh (Supelco Inc.). The column temperature was 100°C, the injection port temperature 200°C and the FID, 150°C. Nitrogen carrier gas flow was 25 ml/min and the sample size injected was 5 ml head-space.

Relative peak areas were determined by multiplying the peak height by the width of the peak at half height, and weight part per million (ppm) of hexanal in the sample was obtained by multiplying the peak area ratio of

hexanal to the internal standard heptanone by a calibration factor.

Experimental Procedures

Effect of grinding on the stability of millet meal during storage. Serere 3A and HMP 1700 millet samples were used in this study. Grinding was with a Udy laboratory mill and a Ross experimental laboratory roller mill. HMP 1700 was ground on the Ross roller mill and sifted to get a maximum stock thru 50GG. The overs of 50GG, representing 3% of the original sample, were reincorporated into the thrus in one sample (mixed for 30 min), and in another sample the 3% overs were not reincorporated.

In the first test, whole millet grain (Serere 3A) and whole millet meal, ground in a Udy mill, were stored in replicates at 25°C in plastic bags for 10 days. The increase in fat acidity and peroxide value were followed on both whole grain and whole meal.

In the second test, whole millet meal (HMP 1700) was ground with a Udy mill and on the experimental roller mill or a combination of the two mills and stored at 27°C, 64% RH in cotton bags. The fat acidity and peroxide value was followed for a 100 hr period.

Effects of varying container type on storability of millet meal. In the first test, HMP 1700 was used. It was ground in an experimental Ross roller mill and sifted to get a maximum stock thru 50GG.

Polyethylene bags and cotton bags were used as containers for storage of millet meal. Storage conditions were 27°C, 66% RH in a ventilated atmosphere (KSU, Entomology Department). Samples were stored in replicates and analyses of fat acidity and peroxide value were carried out every 12 hrs in duplicates.

In the second test of this study, the millet used was a bulk obtained by blending different entries from Minneola, Kansas. The bulk was cleaned in a scourer, to remove the glumes and dust on the grain surface, a dockage tester

was used to remove broken kernels and foreign grains using a sieve, and finally a Kice aspirator was used to eliminate lighter particles.

Grinding of the whole untempered grain was with an experimental Ross roller mill with 22 and 28 corrugation rolls. The stock was ground to pass thru a 50GG. The initial moisture content of that sample was 11.0%. Samples were stored in replicate plastic and cotton bags in an incubator at 35°C. The samples were analyzed for fat acidity, peroxide value, total fatty acids, and free fatty acids.

Effects of high humidity on the stability of millet meal during storage. HMP 1700 grown in Hays, Kansas was used for this study. The grinder used was a Udy laboratory mill with a 400 microns screen opening. The ground samples were mixed and stored in replicates in cotton bags at 30°C, 80% RH and analyses on extractable fat, moisture content, mold count, fat acidity, and peroxide value were carried out in duplicate.

Effect of moisture content storability of millet meal. A bulk millet (Minneola, 1980) was used in this study. The whole grain was ground in an experimental Ross roller mill and the ground material was adjusted to the following moisture contents: 13.8%, 11.0%, 9.7% and 3.8%.

One fourth of the ground millet stock was put to temper in an incubator at 90% RH for 12 hrs to bring the moisture content up to 13.8%. The second fraction, being at 11.0% moisture content level, was stored as is. The third fraction of millet meal was dried under the hood for 12 hrs to bring the moisture content down to 9.7%. Finally, the fourth fraction was dried in a lyophilizer for 15 hrs to bring the moisture content down to 3.8%. Whole millet meal samples were then separately put in replicate polyethylene bags and stored at 35°C for 15 days.

Samples were taken once every 5 days from each bag and analyzed for extractable fat, fat acidity, peroxide value (PV), total extractable fatty acids

(TFA), and free fatty acid (FFA).

Effect of decortication and degermination of millet meal on its stability during storage. The material used in this study was a bulk millet grown in Minneola in 1980. The clean bulk millet was decorticated in a Satake decorticator and passed through a Kice blower to eliminate lighter particles. A suitable amount of decorticated millet was ground as is and the second fraction of decorticated millet was tempered to 22% moisture for 6 hrs and milled on a Ross mill to produce low-fat grits (Abdelrahman et al, 1982).

Low-fat grits obtained from pearl millet were then dried under the hood to a moisture content of 10.8%. Ground decorticated millet, with a moisture content of 12.2%, and degermed millet (low-fat grits) were then stored in an incubator at 35°C in replicate polyethylene bags for 15 days. Sampling was once every 5 days. Extractable fat, fat acidity, peroxide value, total fatty acids, and free fatty acids were run in duplicate for each replicate.

Hexanal as a measure of oxidative rancidity. The materials used were bulk millet grown in Minneola in 1980 and HRWW previously cleaned in the cleaning house of the pilot mill. The bulk millet was cleaned as mentioned previously.

Both millet meal and whole wheat flour were ground in an Alpine pin mill and an equal amount of each ground material was extracted for 16 hrs in a Soxhlet apparatus. Extracted millet lipid and wheat lipid was dried in a vacuum oven at 40°C for 30 min to remove all the petroleum ether solvent. The extractable fat contents were 5.6% and 1.5% for pearl millet meal and whole wheat meal, respectively.

Reconstitution. Millet lipids (ML) were reincorporated into the defatted millet meal and defatted whole wheat meal so that the final fat content of reconstituted millet meal and reconstituted whole wheat meal was about that found in wheat. Similarly, wheat lipids (WL) were reincorporated into the defatted

whole wheat flour and defatted millet meal in the same proportions indicated above for millet lipids. The reconstitution of millet meal and whole wheat meal was done by mixing the defatted sample and the extracted lipid at high speed in a Stein mill.

Millet meal (M), defatted millet meal reconstituted with millet lipids (M + ML), defatted millet meal reconstituted with wheat lipids (M + WL), whole wheat meal (W), defatted whole wheat meal reconstituted with wheat lipids (W + WL) and defatted whole wheat meal reconstituted with millet lipids (W + ML), were stored separately in polyethylene bags in an incubator at 35°C for 1 month.

Effect of atmospheric oxygen on stability of millet meal. A bulk millet grown in Minneola in 1980 was used in this study. It was cleaned as in the previous experiments and ground in an experimental roller mill to pass through a 50GG.

Two quart size air-tight glass jars with wide mouths were used to store millet meal with 11.0% moisture content in an incubator at 35°C. Sample size (100 g) was such that the oxygen in the jar would not be a limiting factor during storage of millet meal under atmospheric oxygen.

A second jar was stoppered with a rubber stopper. The stopper was fitted with two glass tubes equipped with stopcocks to allow evacuation of oxygen and introduction of nitrogen into the jar. The oxygen trapped in the jar and that adsorbed by the sample was eliminated by successive use of a water pump, to evacuate air from the jar, and use of nitrogen to replace that air.

Fat acidity and peroxide value were determined in duplicates on each sample, the first day of storage and after 30 days. The values obtained for the millet meal stored under atmospheric oxygen were then compared to those obtained in the millet meal stored under nitrogen.

Effect of the quantity and quality of the lipid fraction on storability of millet meal. Hard red winter wheat and the bulk millet were ground in an Alpine pin mill and extracted with petroleum ether for 16 hrs in a Soxhlet apparatus.

The defatted residues were dried at room temperature in a hood until the solvent odors were no longer detected. The solvent in the fat extract was evaporated under a mild heat source almost to dryness. The flask containing the fat residue was then put in a vacuum oven at 40°C for 30 min to remove the remaining solvent.

The defatted residues were then reconstituted with appropriate amounts of lipids in a Stein mill for 1 min (Chung et al, 1981). Millet lipid was incorporated into defatted millet meal and defatted whole wheat flour and wheat lipid was incorporated into defatted whole wheat flour and defatted millet meal in the proportions indicated in Table 1.

A similar reconstitution was done with same samples of wheat and millet but the proportions in which lipid is incorporated is different. The incorporation of lipids into the defatted residues was as shown in Table 2. All the samples were stored at 30°C, 60% RH in cotton bags for 5 days. Samples were taken every day and fat acidity determined in duplicate.

Table 1. Reconstitution study on effect of fat content on fat acidity.

	M	M + ML	M + WL	W	W + WL	W + ML
Defatted sample wt (g)	-	32.4	32.4	-	32.9	32.9
Fat wt (g)	-	2.6	0.64	-	0.64	2.6
% fat of sample	5.55	7.43	1.94	1.82	1.97	7.42

Table 2. Reconstitution study on effect of fat quality on fat acidity.

	M	M + ML	M + WL	W	W + WL	W + ML
Defatted sample wt (g)	-	36.75	36.75	-	37.77	37.77
Fat wt incorporated (g)	-	2.14	2.14	-	0.65	0.65
% fat of sample	5.36	5.50	5.50	1.72	1.69	1.69

RESULTS AND DISCUSSION

Effects of grinding on the stability of millet meal. A study was conducted to show the effect of grinding to various particle size on the stability of millet meal during storage. The extent of oxidative decomposition was measured by peroxide value and the hydrolytic action of lipase was measured by fat acidity.

As shown in Figs. 4 and 5, fat acidity and peroxide value did not change significantly in the whole grain. But in the whole millet meal (Udy mill), dramatic changes occurred in both fat acidity and peroxide value as the sample was stored in a plastic bag at room temperature.

Fat acidity increased steadily over all the storage period. Peroxide value increased during the first 5 days and then decreased. After 10 days, it was near its original value.

Those results indicate that shortly after grinding, millet lipids undergo hydrolytic decomposition as well as oxidative degradation. Those results are in agreement with those of Carnovale and Quaglia (1973) and Lai and Varriano-Marston (1980b) but contradict those of Thiam et al (1976).

In a second series of storage studies ($27^{\circ}C$, 64% RH), samples were stored in cotton bags. The samples were ground either in a roller mill or a Udy laboratory mill or a combination of both. Fat acidity of the roller mill ground sample (RMGS), the Udy mill ground sample (UMGS) and the sample ground in roller mill and reground by Udy (URMGS) all increased during storage (Fig. 6). However, the rate of increase was much different for each of the samples. The fat acidity increased at a slower rate for the RMGS sample than for the URMGS sample which was, in turn, slower than the UMGS sample.

Because the Udy mill gives a much finer grind than did the roller mill, it appears that the particle size of the meal may affect the storability of

Fig. 4. Fat acidity of millet meal (Serere 3A) and whole millet grain (Serere 3A) stored at 25°C in polyethylene bags.

☆ Whole grain (WG)

● Ground grain (GG)

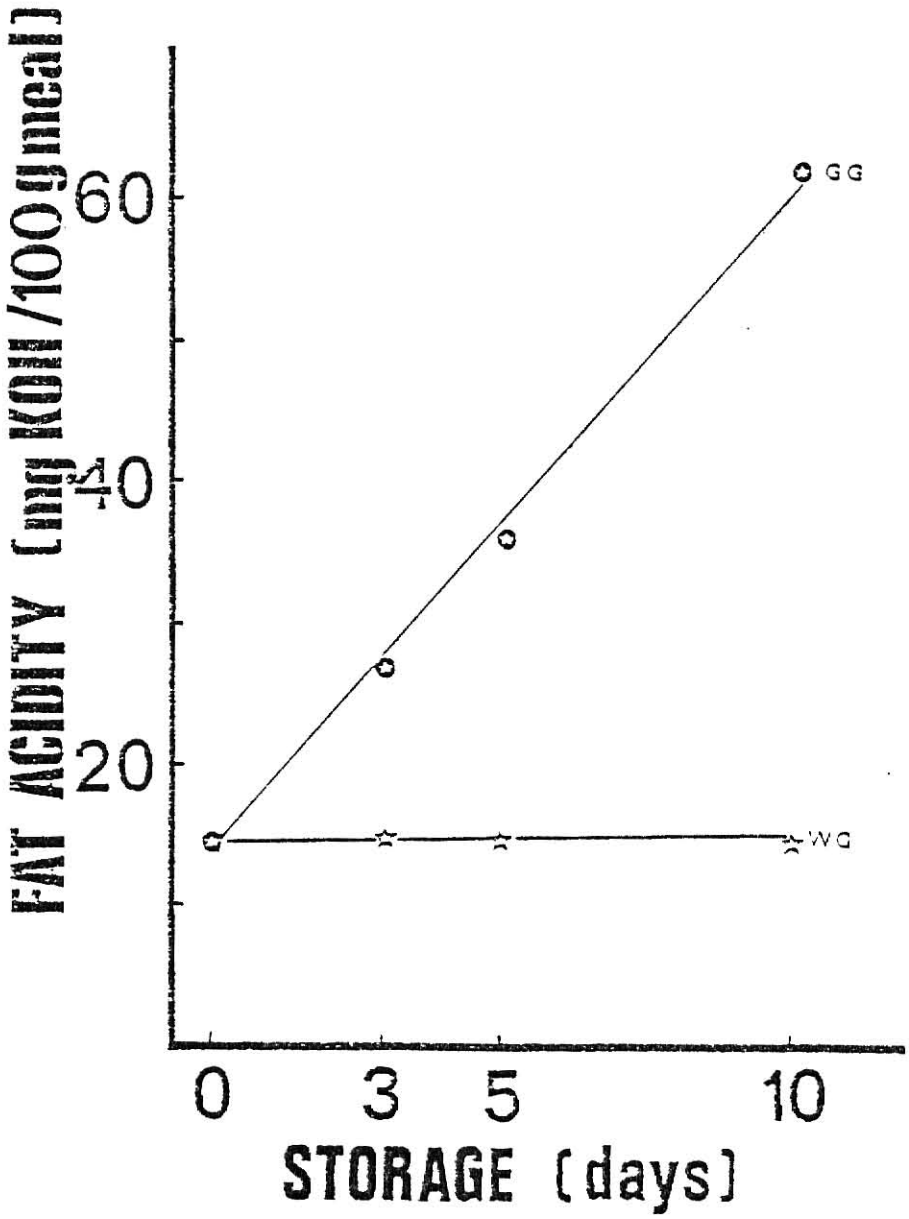

FAT ACIDITY [mg KOH/100g meal]
60
40
20
GG
WG
0
3
5
10
STORAGE [days]

Fig. 5. Peroxide values for millet meal (Serere 3A) and whole millet grain (Serere 3A) stored at 25°C in polyethylene bags.

✶ Whole grain (WG)

✡ Ground grain (GG)

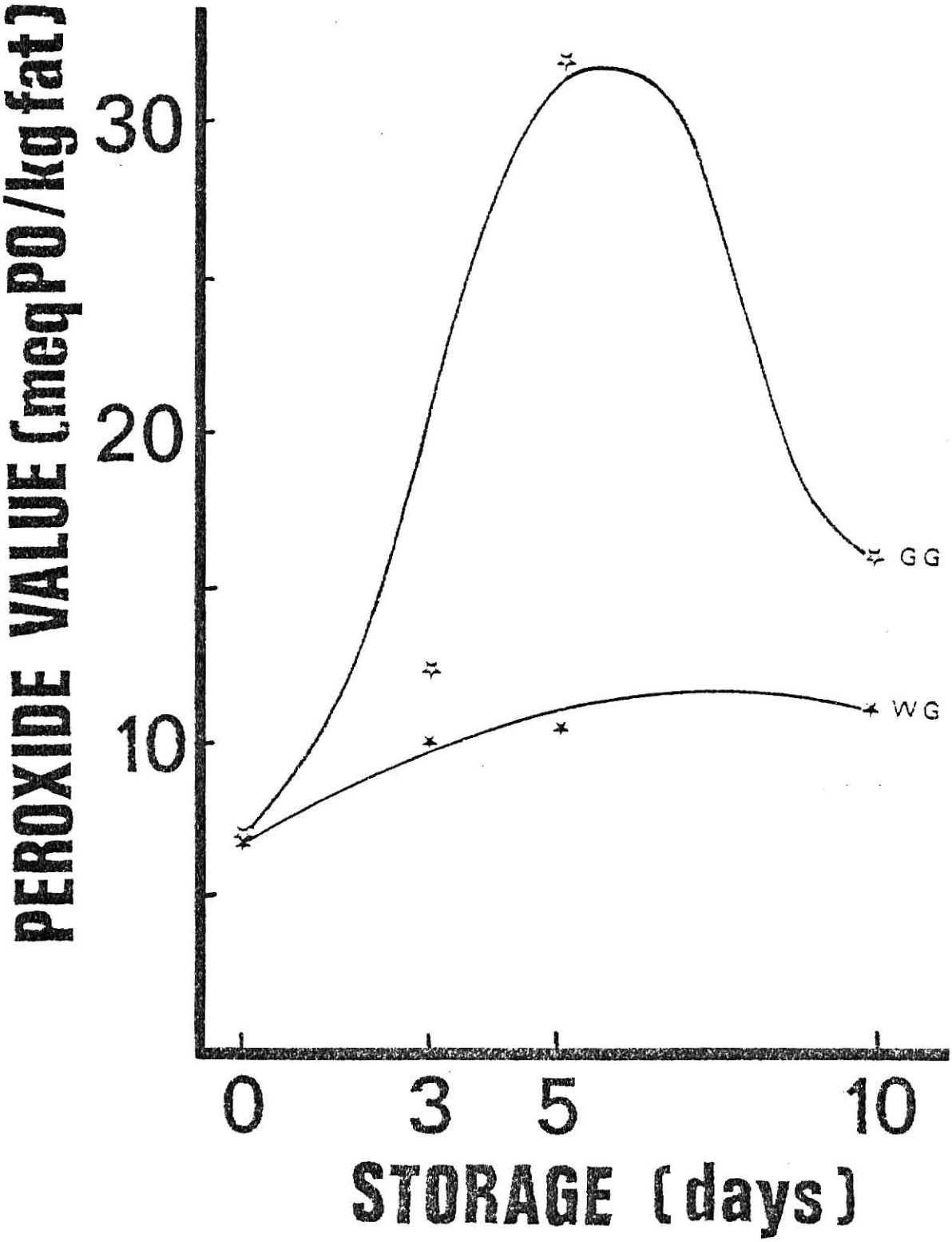
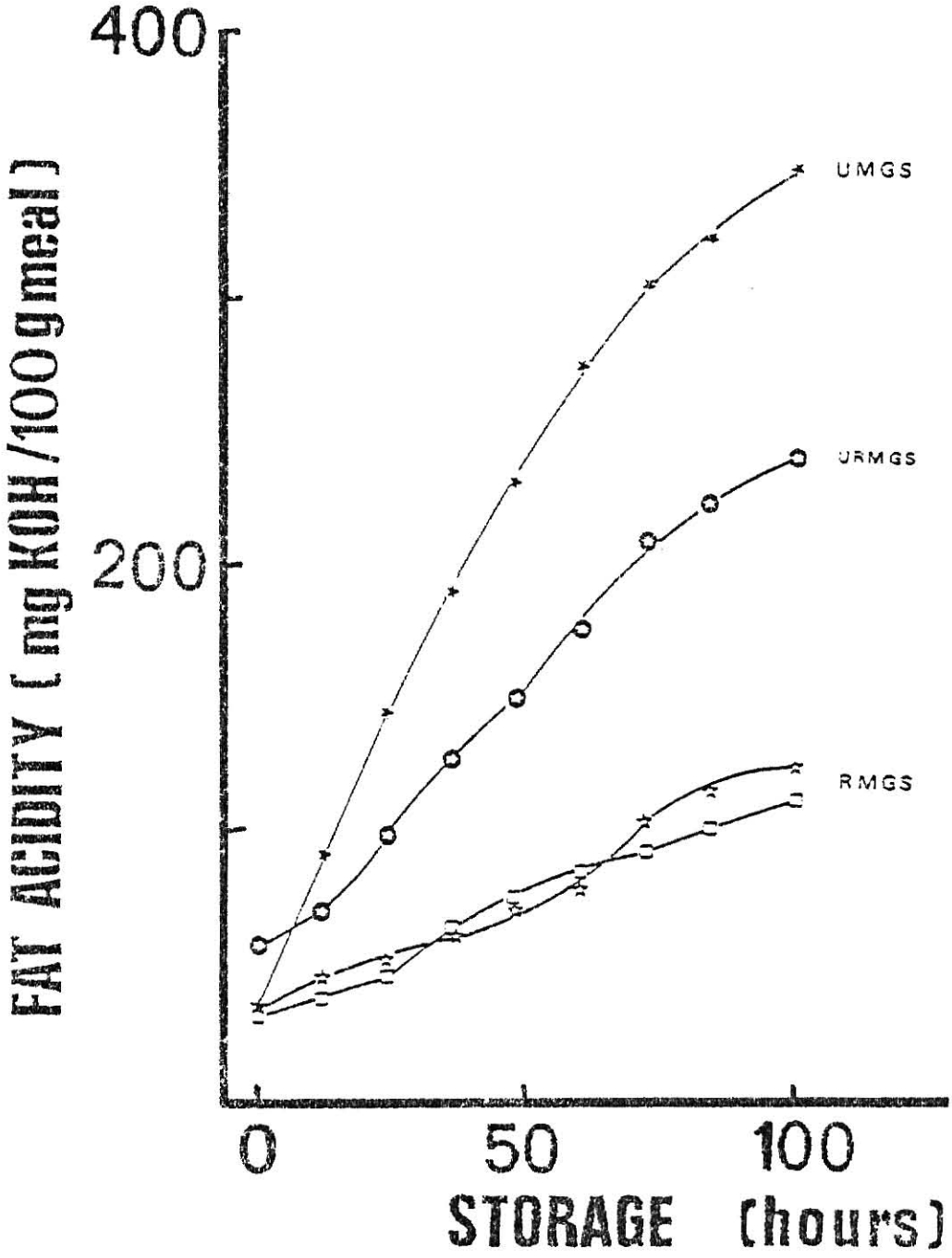

PEROXIDE VALUE (meq PO/kg fat)
30
20
10
GG
WG
0
3
5
10
STORAGE (days)

Fig. 6. Fat acidity of millet meal (HMP 1700) stored at 27°C, 64% RH in cotton bags.

- □ Roller mill ground millet excluding 3% overs of 50GG (RMGS)
- ✡ Roller mill ground millet including 3% overs of 50GG (RMGS)
- ✦ Udy mill ground millet (UMGS)
- ✪ Roller and Udy mill ground millet (URMGS)


400
200
0
50
100
FAT ACIDITY (mg KOH /100 g meal)
STORAGE (hours)
UMGS
URMGS
RMGS

millet. The finer the particle size, the lower the stability of millet meal during storage. It is also possible that the finer the particle size, the more fat is extracted by benzene (the solvent for fat acidity). If that was the case, the original values of fat acidity for the same samples ground at two different degrees of fineness would be different. This was not true, therefore, ground millet gives a higher fat acidity value than the coarsely ground millet over the storage period.

In contrast to the previous study (Fig. 5), peroxide values (Fig. 7) were much lower. We assume that the type of containers (cotton bags vs plastic bags) had an effect on the peroxide value.

Effects of varying container types on storability of millet meal. In the preceding study we showed different patterns of peroxide formation. The difference in the experimental conditions was the container type. In the first study, millet meal (Serere 3A) was stored at room temperature (25°C) in plastic bags (Fig. 5) and in the second part (HMP 1700) millet meal at 27°C, 66% RH in cotton bags (Fig. 7). In this study we varied the type of bag and measured peroxide value, fat acidity, total fatty acids, and free fatty acids.

Fat acidity increased for samples stored both in plastic and cotton bags but at different rates (Fig. 8). The initial moisture content of the millet meal was 9.5%; and after 112 hrs of storage, it was 9.8% in the sample stored in a plastic bag and 12.2% in the sample stored in the cotton bag. The fat acidity increased more rapidly in the sample stored in the cotton bag presumably because of the higher moisture level for that sample. The hydrolytic action of lipases, as measured by fat acidity, is known to be more rapid at higher moisture levels.

The peroxide value (Fig. 9) had a different pattern for samples stored in plastic bags as opposed to samples stored in cotton bags. In plastic bags, the peroxide value increased up to 5.1 meq PO/kg of fat after 62 hrs of

Fig. 7. Peroxide value of millet meal (HMP 1700) stored at 27°C, 64% RH in cotton bags.

□ Roller mill ground millet excluding 3% overs of 50GG

✡ Roller mill ground millet including 3% overs of 50GG

★ Udy mill ground millet

✪ Roller and Udy mill ground millet

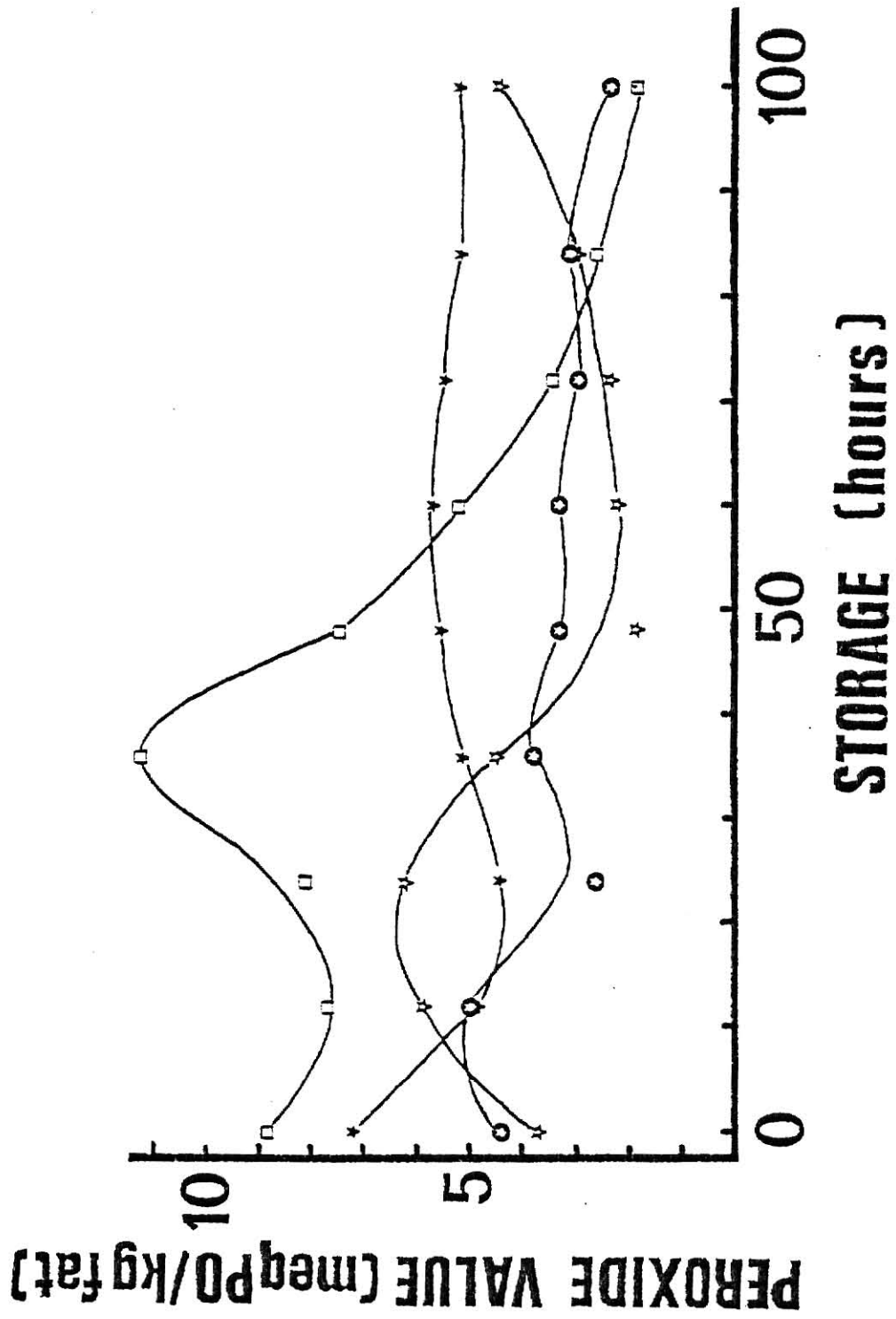

PEROXIDE VALUE (meqPO/kg fat)
10
5
0
50
100
STORAGE (hours)

Fig. 8. Fat acidity of millet meal (HMP 1700, ground in a Ross mill) stored at 27°C, 66% RH in cotton and polyethylene bags.

□ Cotton bags

✡ Polyethylene bags

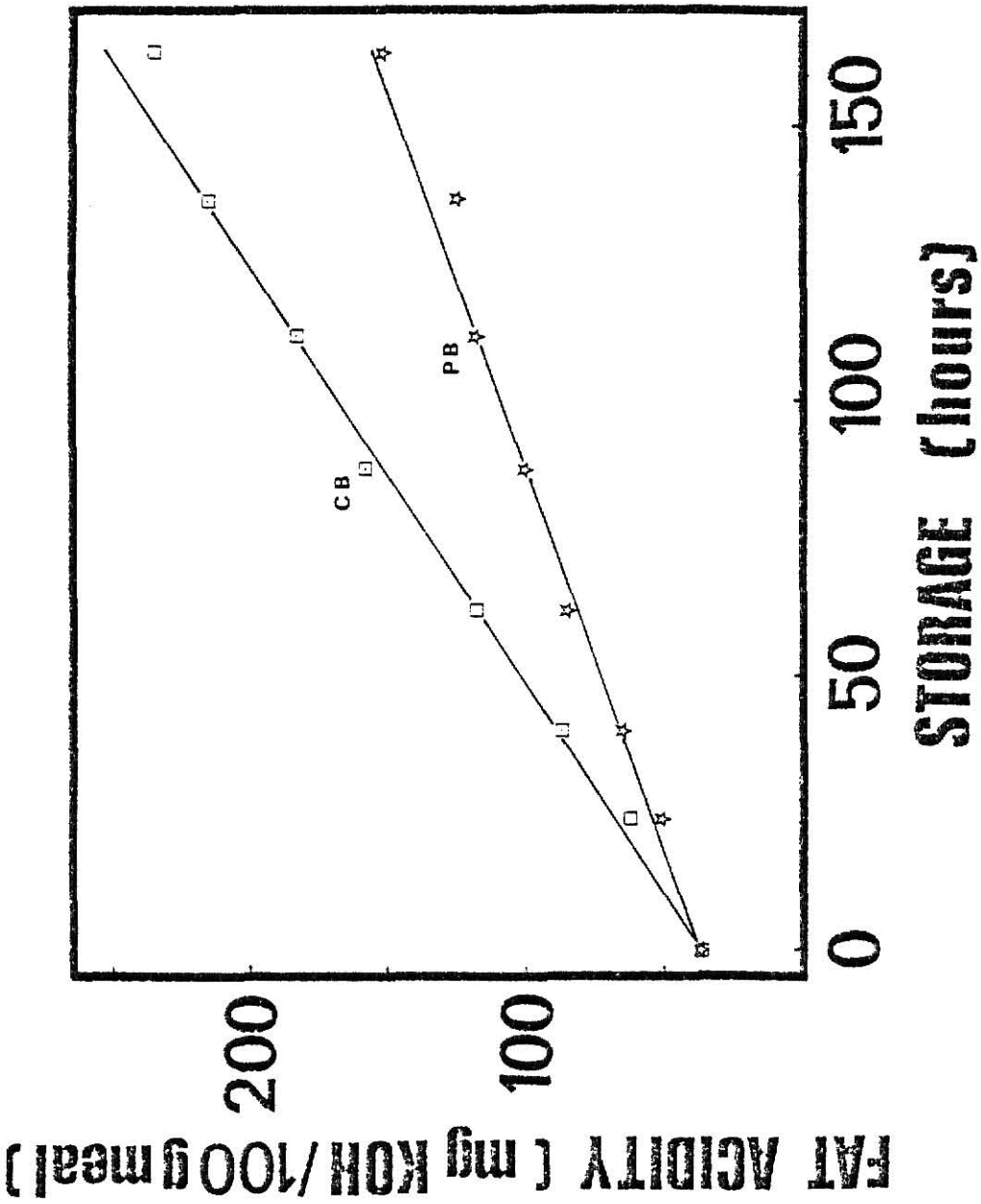
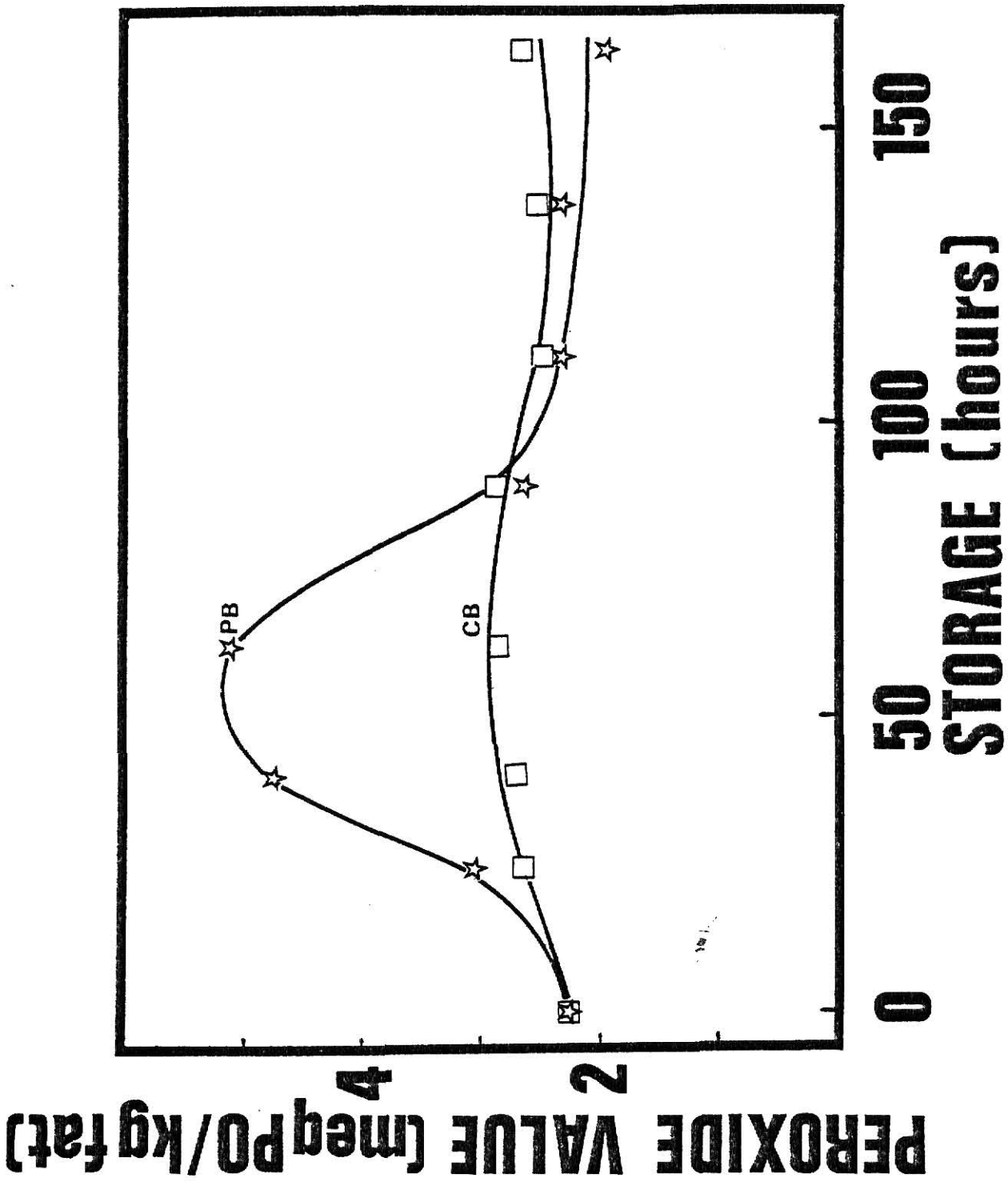

FAT ACIDITY (mg KOH/100 g meal)
200
100
0
50
100
150
STORAGE (hours)
CB
PB

Fig. 9. Peroxide value of millet meal (HMP 1700) stored at 27°C, 66% RH in polyethylene and cotton bags.

☆ Polyethylene bags

☐ Cotton bags


PEROXIDE VALUE (meqPO/kg fat)
4
2
0
50
100
150
STORAGE (hours)
PB
CB

storage and then decreased to near its original value. The end of the induction period (10 hrs) corresponded to a fat acidity of 45 mg KOH/100 g meal, somewhat higher than that reported by Lai and Varriano-Marston (1980b).

The peroxide value of millet meal stored in cotton bags remained constant throughout the storage period. Why does the peroxide value differ when the sample is stored in plastic rather than cotton bags? We think the moisture level might affect the level of peroxidation of lipids. In addition, both the plastic and cotton bags were stored in a ventilated atmosphere. The cotton bags allow air flow through the sample and accordingly sweeps away the volatile compound formed by oxidation of lipids. The plastic bags constitute a barrier to air flow but not to oxygen. In both containers, oxidation of lipids has undoubtedly occurred, however, for samples stored in cotton bags the peroxides are lost at a rate higher than the rate of formation. Thus, no peroxides are detected in samples stored in cotton bags.

The development of oxidative rancidity in flour was reported by Fine and Olsen (1928) to be promoted by low moisture levels. Thiam et al (1976) found that millet flour with moisture content of 25-30% did not produce evidence of oxidation of unsaturated fatty acids.

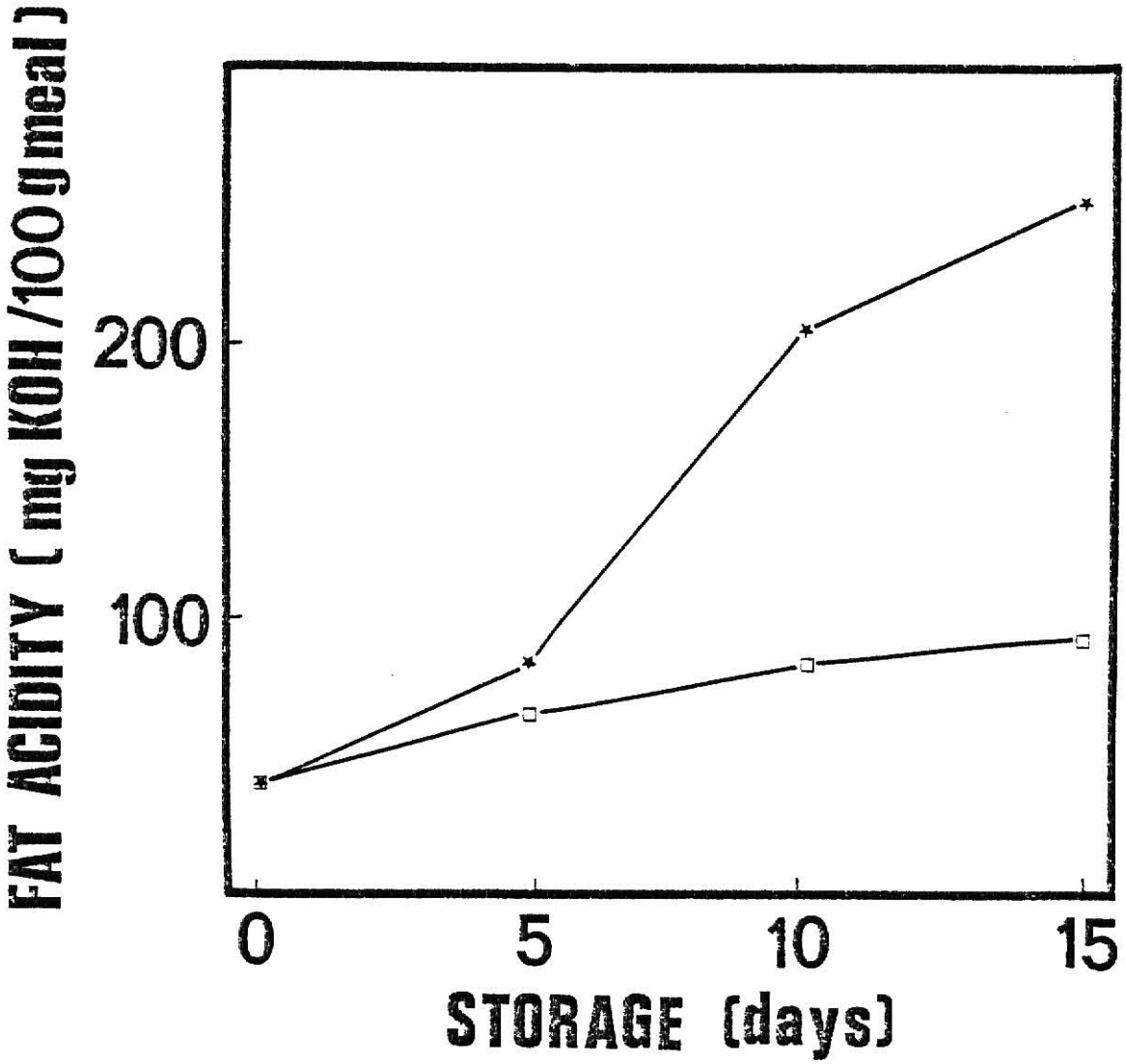
Total fatty acid and free fatty acid in stored millet meal.

Fat acidity. Fat acidity (Fig. 10) increased from 58.12 to 252.10 mg KOH/100 g meal in the sample stored in a plastic bag. The moisture content of that sample did not change significantly during storage. The fat acidity increased at a much slower rate for a sample stored in a cotton bag. In that sample the moisture content decreased from 11.0% to 5.8% during storage. Thus, the moisture content of whole millet meal stored in cotton bags decreased sharply during storage. Because the moisture content has a direct effect on the hydrolytic action of lipases, this explains why the fat acidity was lower in the millet meal stored in cotton bags.

Fig. 10. Fat acidity of millet meal (bulk) during storage at 35°C in polyethylene and cotton bags.

✱ Polyethylene bags

□ Cotton bags


FAT ACIDITY (mg KOH /100 g meal)
200
100
0
5
10
15
STORAGE (days)

Peroxide value. It was shown previously (Figs. 5 and 9) that peroxide value increases after grinding to a certain level and then decreases to near its original value. In this study, 4 days were required to grind all the millet samples and adjust their moisture contents. Obviously the sample underwent changes during this time. We assume that the decrease in peroxide value observed for millet samples stored in plastic and cotton bags (Fig. 11) is consistent with our previous results. The decrease in peroxide value observed in Fig. 11 corresponds to the decrease in peroxide value observed in both Figs. 5 and 9.

During 15 days storage, the peroxide value of the sample stored in a plastic bag decreased from 7.73 to 4.41 (Fig. 11). A similar decrease in peroxide value was found for the sample stored in a cotton bag. The peroxide values of the latter dropped from 6.35 to 3.67 which is slightly lower than those for the sample stored in polyethylene bags. This confirms our previous results showing that samples stored in plastic bags are higher in peroxide value than samples stored in cotton bags.

We attribute this difference to the rate at which hydroperoxides decompose. In samples stored in cotton bags peroxides are less detectable because their rate of formation is less than or equal to their rate of decomposition. The air current prevailing in the incubator sweeps away volatile compounds from the decomposition of hydroperoxide and displaces the reaction toward increasing decomposition of hydroperoxides in pearl millet samples stored in cotton bags.

On the other hand, whole millet meal stored in polyethylene bags is not affected by air currents and, therefore, hydroperoxides build up. The rate of decomposition of hydroperoxide is lower than the rate of formation.

Total fatty acids (TFA). The TFA results are shown in Tables 3 and 4. The data indicate that there is not a significant change in total fatty acid

Fig. 11. Peroxide value of millet meal (bulk) during storage at 35°C in polyethylene and cotton bags.

☆ Polyethylene bags

✶ Cotton bags

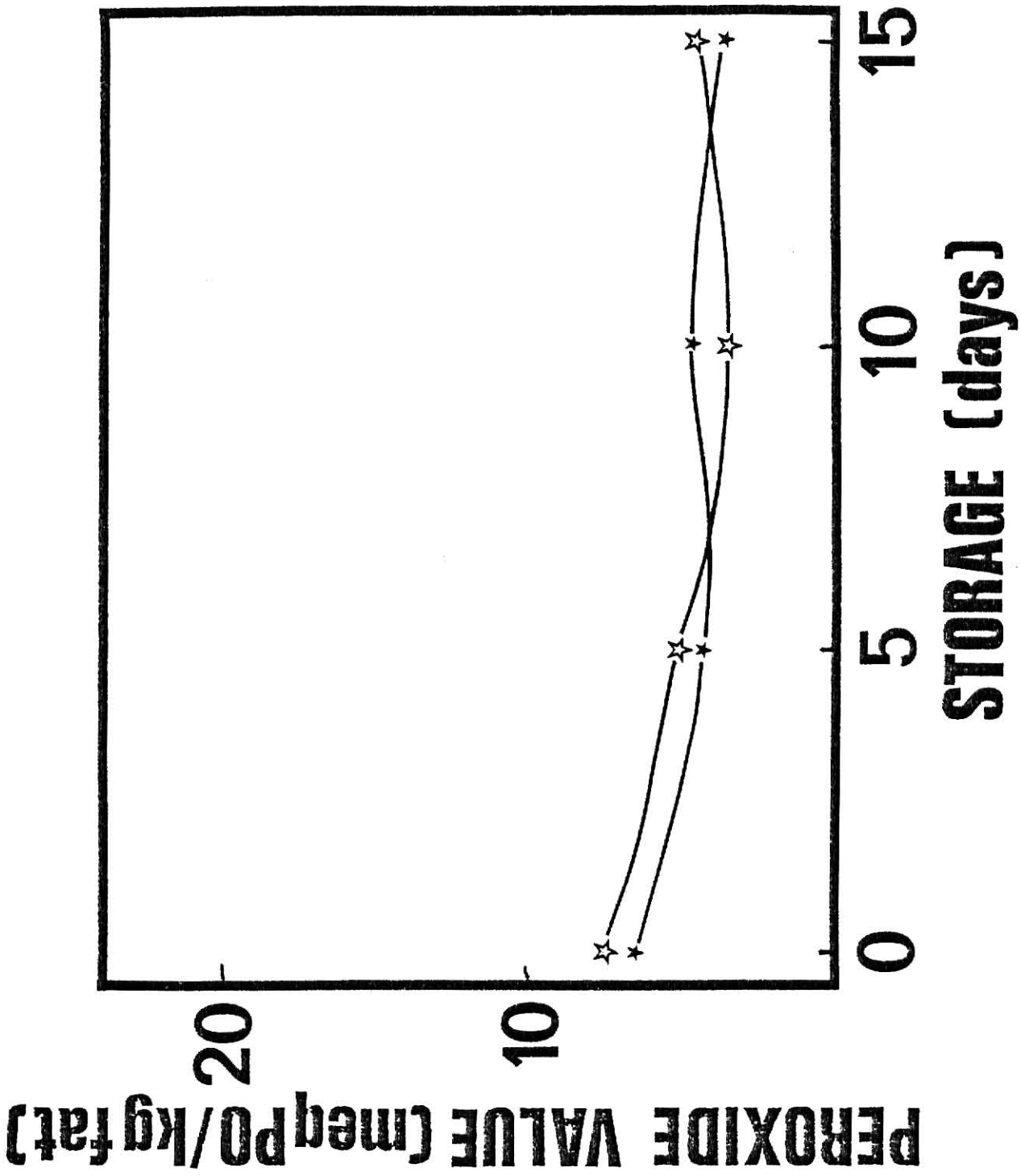

PEROXIDE VALUE (meqPO/kg fat)
20
10
0
5
10
15
STORAGE (days)

Table 3. Total Fatty Acids of Whole Millet Meal Stored at 10.96% Moisture in a Polyethylene Bag at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	3.15	(18.94)	3.81	(17.08)	1.96	(16.63)	0.94	(19.70)	0.56
18:0	1.04	(6.25)	1.44	(6.45)	0.71	(6.02)	0.30	(6.28)	0.35
18:1	4.70	(28.26)	7.00	(31.37)	3.47	(29.45)	1.26	(26.41)	4.03
18:2	7.36	(44.25)	9.56	(42.85)	5.36	(45.50)	2.12	(44.44)	2.39
18:3	0.38	(2.28)	0.50	(2.24)	0.28	(2.38)	0.15	(3.14)	0.37
Total	16.63		22.31		11.78		4.77		
Recovery[a/] (%)	93		93		97		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from chromatograms.

Table 4. Total Fatty Acids of Whole Millet Meal Stored at 10.96% Moisture in a Cotton Bag at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	3.15	(18.94)	3.89	(17.15)	2.28	(18.82)	0.88	(18.25)	0.37
18:0	1.04	(6.25)	1.38	(6.08)	0.72	(5.96)	0.26	(5.39)	0.33
18:1	4.70	(28.26)	6.65	(29.32)	3.29	(27.13)	1.35	(28.00)	3.87
18:2	7.36	(44.25)	10.21	(45.01)	5.52	(45.63)	2.21	(45.85)	1.45
18:3	0.38	(2.28)	0.55	(2.42)	0.29	(2.46)	0.12	(2.49)	0.25
Total	16.63		22.68		12.10		4.82		
Recovery[a/] (%)	94		93		98		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

under either storage condition.

Free fatty acids (FFA). The results for free fatty acid (FFA) are shown in Tables 5 and 6. The overall trend of FFA is an increase throughout storage of samples stored either in plastic or cotton bags. Because the TFA for samples did not change and the FFA increased, it appears that hydrolytic action of lipases is predominant. Had significant amounts of oxidative rancidity occurred, the level of total unsaturated fatty acid should have been lower.

Effects of high humidity on the stability of millet meal

Although millet is grown mostly in the semi-arid tropics, storage conditions where humidity was as high as 95% have been reported (Carnovale and Quaglia, 1973; Thiam et al, 1976). Therefore the storage of millet meal under high humidity conditions was studied.

When the meal was stored at 30°C and 80% RH, the moisture content increased the first 60 hrs and then levelled off at 14.5% (Fig. 12). The value agrees with the sorption isotherm for pearl millet (Kossou Kohounko, 1981).

The peroxide value was low and did not vary over the storage period. It appears that high humidity conditions decreases the amount of oxidative deterioration. This agrees with the findings of Carnovale and Quaglia (1973) and Lai and Varriano-Marston (1980b).

Fat acidity increased the first 90 hrs of storage and then levelled off at 450 mg KOH/100 g meal (Fig. 13). Why the fat acidity levels off is not clear. However, Goodman and Christensen (1952) reported that several fungi would metabolize appreciable amounts of fatty acids. Under similar storage conditions (30°C, 90-95% RH), Thiam et al (1976) reported that microflora growth brought about not only the consumption of sugars and alcohol-soluble oligosaccharides, but also lipids and particularly free fatty acids.

This levelling off of fat acidity at 450 mg KOH/100 g meal (dry basis) could be explained by the consumption of free fatty acids by the microflora.

Table 5. Free Fatty Acids of Whole Millet Meal Stored at 10.96% Moisture in a Polyethylene Bag at 35°C [a].

FFA	0 day		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	14.91	0.275	24.475	0.445	28.056	0.501	29.267	0.518
16:0	29.31	0.539	38.940	0.708	54.768	0.978	53.563	0.948
18:0	15.48	0.283	20.460	0.372	39.144	0.699	44.748	0.792

[a] Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Table 6. Free Fatty Acids of Whole Millet Meal Stored at 10.96% Moisture in a Cotton Bag at 35°C [a].

FFA	0 day		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	14.91	0.275	24.803	0.439	32.132	0.554	36.34	0.632
16:0	29.31	0.539	44.013	0.779	53.766	0.927	81.65	1.42
18:0	15.48	0.283	18.136	0.321	36.656	0.632	47.38	0.824

(a) Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Fig. 12. Moisture content of millet meal (HMP 1700) during storage at 30°C, 80% RH in cotton bags.

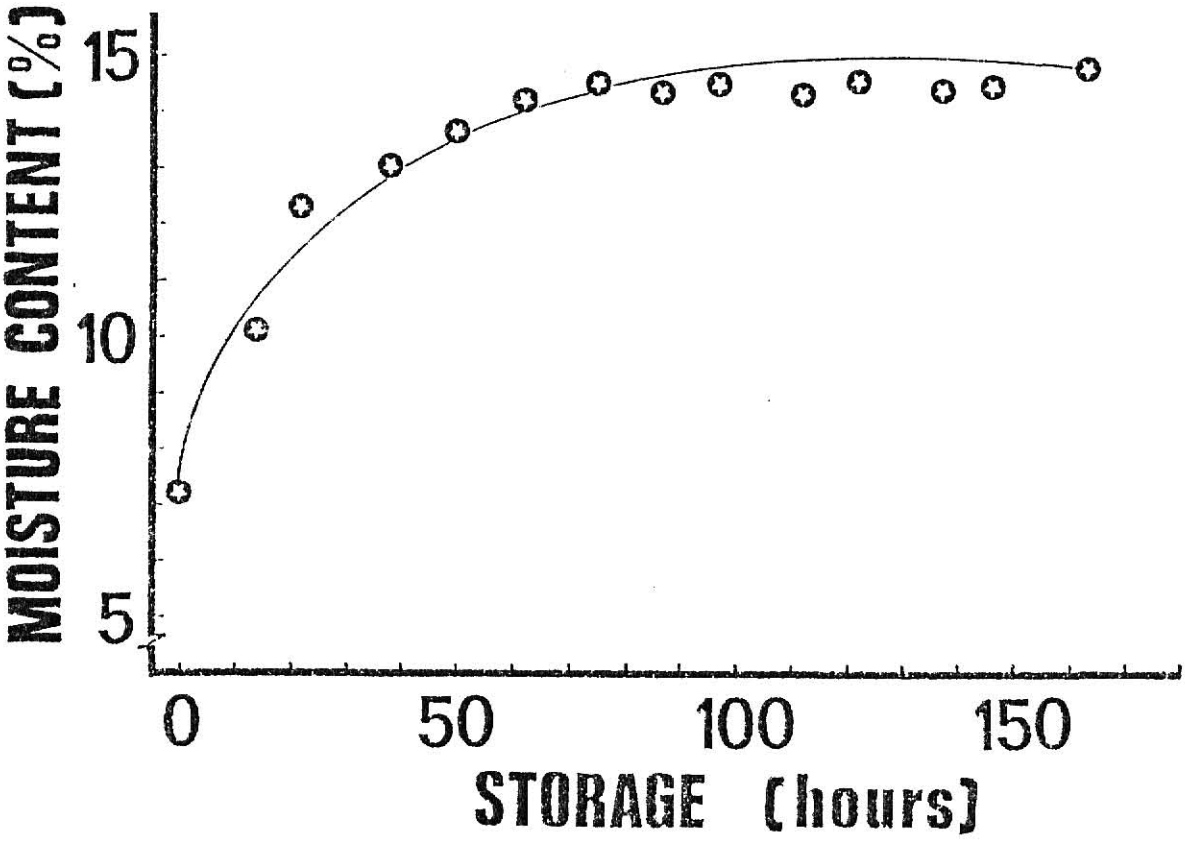
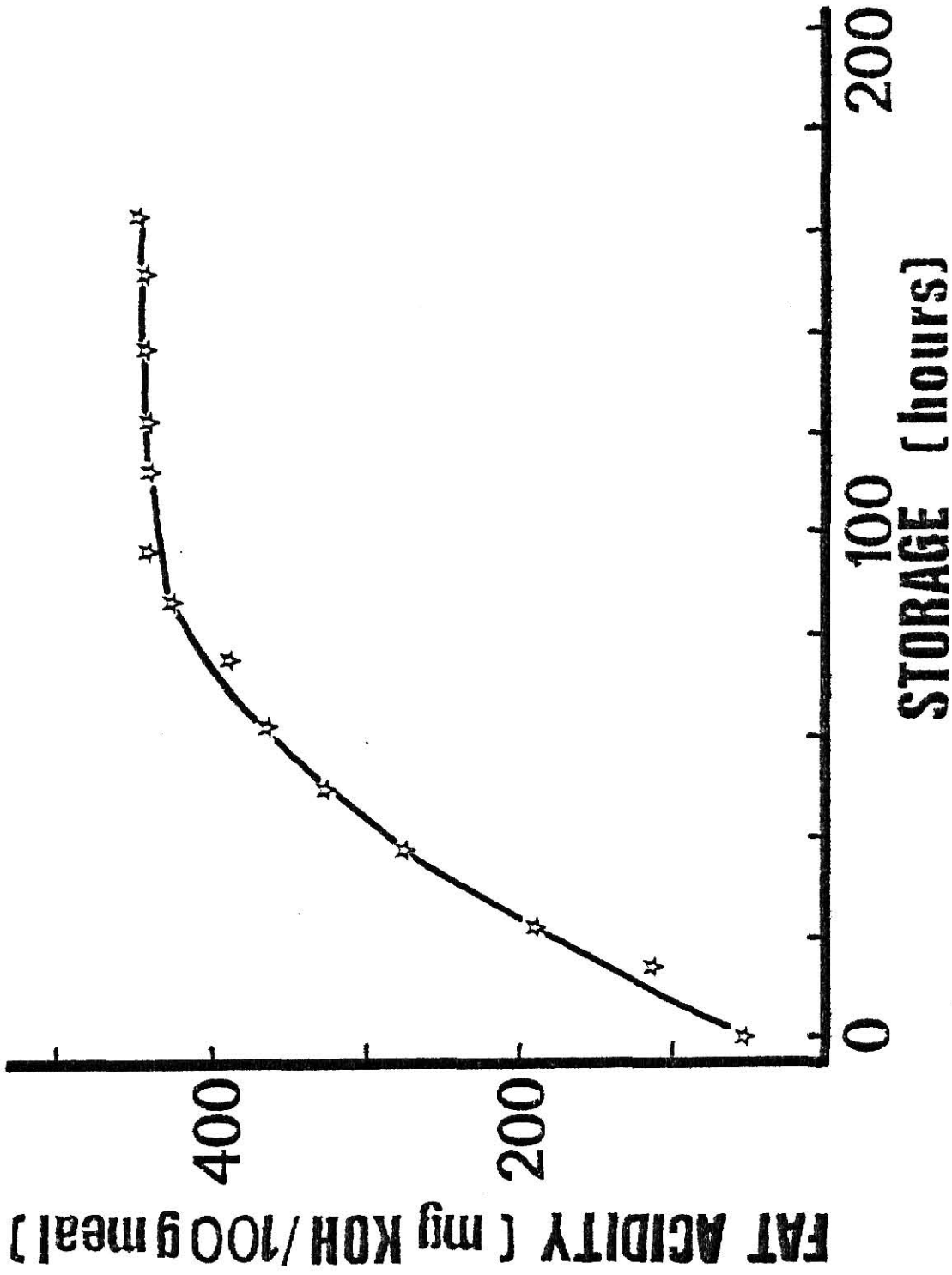

MOISTURE CONTENT (%)
15
10
5
0
50
100
150
STORAGE (hours)

Fig. 13. Fat acidity of millet meal (HMP 1700) during storage at 30°C, 80% RH in cotton bags.


FAT ACIDITY (mg KOH /100 g meal)
400
200
0
100
200
STORAGE (hours)

However, mold counts on the fresh sample and after 1 week of storage does not indicate a significant change during the storage period. The fresh sample gave a mold count of 2600 colonies per gram millet meal and after 7 days, the value was 2300 colonies per gram millet meal. Therefore, another explanation for the levelling off of fat acidity must be sought.

Effect of moisture content on storability of millet meal

The moisture content of the millet meal was adjusted to the following levels: 13.8, 11.0, 9.7, and 3.8. The meal was then stored in plastic bags at 35°C for 15 days. The time required to grind and adjust moisture contents prior to storing the millet samples in an incubator at 35°C was 96 hrs.

Fat acidity. Fat acidity increased during the storage period for all moisture contents (Fig. 14). Higher moisture contents gave greater increases in fat acidity. This confirms the observation of Thiam et al (1976) that the lipid hydrolysis is higher with higher moisture levels.

The difference in fat acidity between samples the first day of storage was due to prior adjustments in moisture content. Since it took 96 hrs to grind and adjust millet samples' moisture content, the fat acidity increased during that period. The difference in fat acidity between samples the first day of storage is, therefore, consistent with prior increase in fat acidity.

Peroxide value. The peroxide value of whole millet meal with 13.8% initial moisture increased slowly from 3.8 to a maximum of 6 after 10 days and then decreased (Fig. 15). The peroxide value at other moisture levels does not appear to give a significant trend. The large variation in peroxide values at 0 days storage are assumed to be because of the time required to adjust moisture content of the samples.

Extractable fat. The ether extractable fat of millet meal did not show any significant changes during storage at the four moisture contents.

Fig. 14. Fat acidity of millet meal (bulk) during storage at 35°C in polyethylene bags. Effect of moisture.

□ 13.8% moisture content

✱ 11.0% moisture content

☆ 9.7% moisture content

✪ 3.8% moisture content

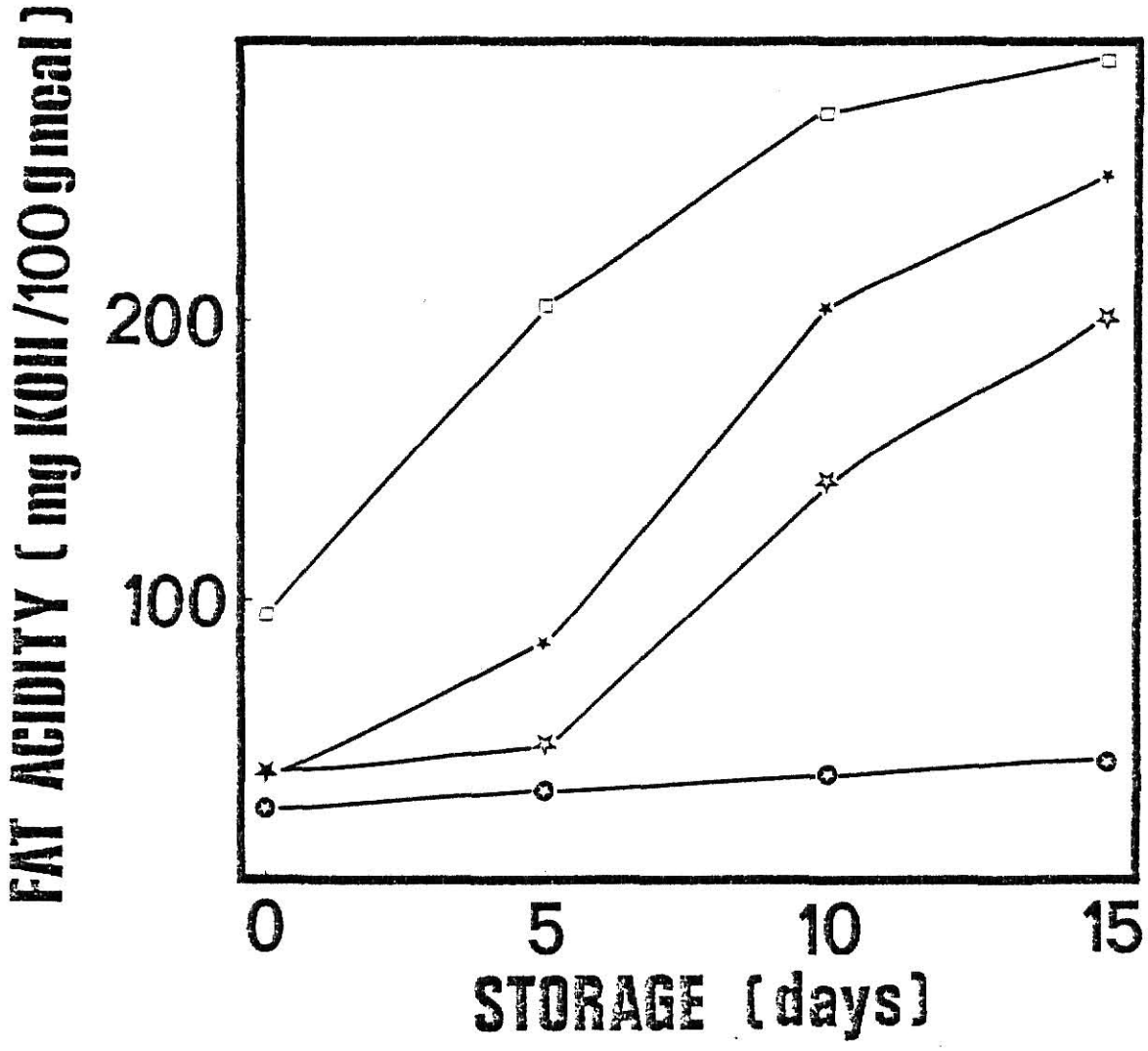

FAT ACIDITY (mg KOH /100 g meal)
200
100
0
5
10
15
STORAGE (days)

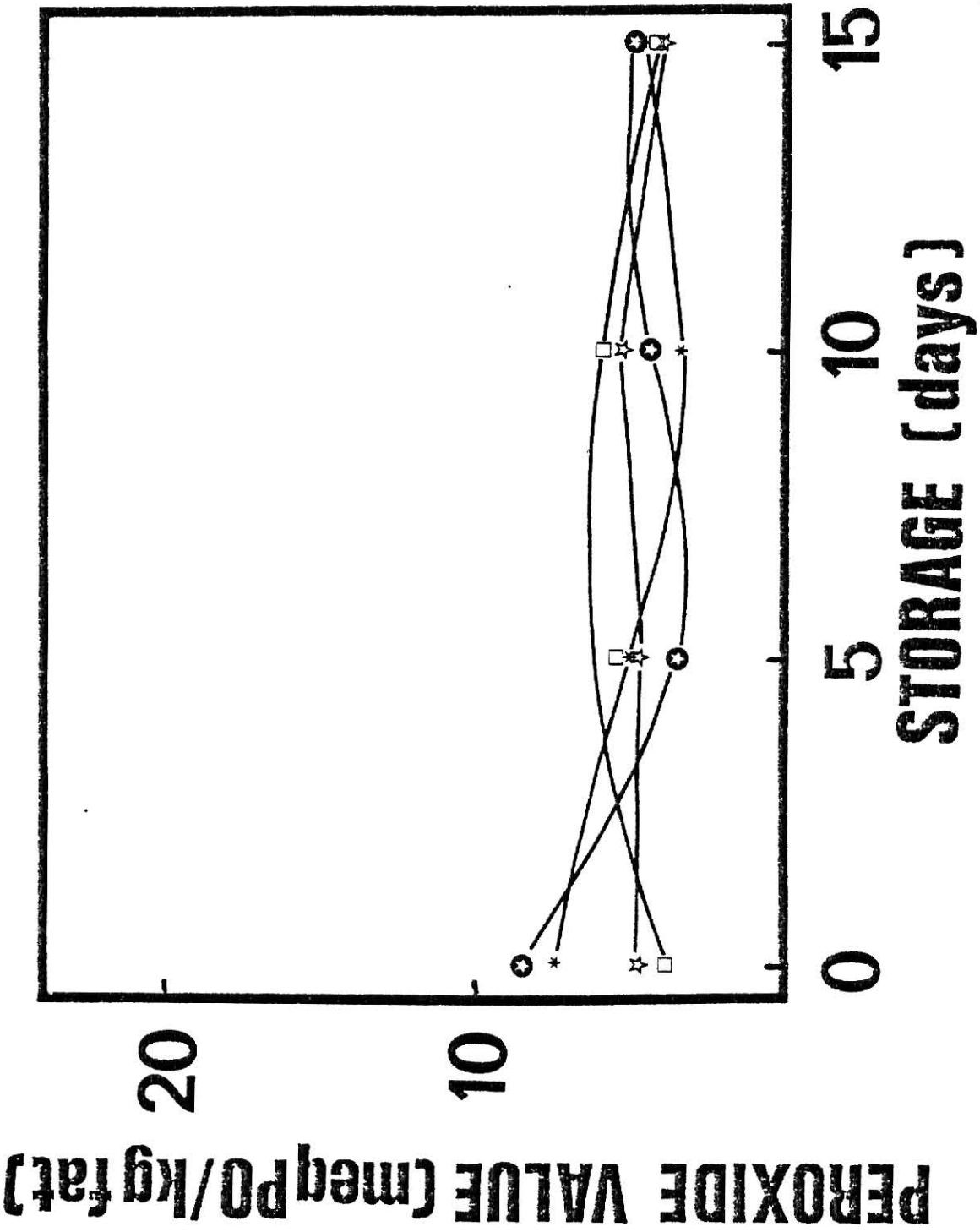
Fig. 15. Peroxide value of millet meal (bulk) during storage at 35°C in polyethylene bags. Effect of moisture.

⊡ 13.8% moisture content

* 11.0% moisture content

☆ 9.7% moisture content

✪ 3.8% moisture content


PEROXIDE VALUE (meqPO/kg fat)
20
10
0
5
10
15
STORAGE (days)

Total fatty acids (TFA). The levels of total fatty acids did not change significantly as a result of storing whole millet meal at various moisture content (Tables 3, 7, 8 and 9).

Free fatty acids (FFA). A steady increase in FFA was found for all four moisture contents (Tables 5, 10, 11 and 12).

Effect of decorticating and degerming of millet meal on its stability

What causes millet meal to produce objectionable rancid odors during storage is not known, but it is widely accepted that the lipid fraction is involved. The extractable fat content of pearl millet reported in the literature (Jellum et al, 1971; Rooney, 1978; Lai and Varriano-Marston, 1980a) exceeds the fat content of most other cereal grains.

According to Rooney (1978), the lipids in pearl millet are concentrated in the germ. More recently, histochemical studies on half-kernels showed that most lipids in pearl millet were concentrated in the germ and pericarp of the grain (Lai and Varriano-Marston, 1980a).

If we assume that the instability of millet meal during storage is caused by its high fat content, it will be of interest to study its storability when the pericarp and germs have been removed by milling. Until recently the milling techniques used for this cereal grain did not give complete separation of the germ from the rest of the kernel (Carnovale and Quaglia, 1973). They believed this to be the major cause for the alteration found during storage of millet products.

Abdelrahman et al (1982) developed a simple milling system using roller mills to produce low-fat grits (degermed meal) from pearl millet. In this part of our present study, we observed the effect of decorticating and degerming on the storage stability of millet products. The samples were stored in polyethylene bags at 35°C.

Table 7. Total Fatty Acids of Whole Pearl Millet Meal Stored at 13.81% Moisture in Polyethylene Bags at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	4.02	(18.55)	3.80	(17.20)	1.84	(16.70)	0.90	(19.56)	0.43
18:0	1.23	(5.67)	1.31	(5.93)	0.57	(5.17)	0.20	(4.34)	0.39
18:1	6.12	(28.24)	6.45	(29.19)	3.25	(29.49)	1.16	(25.22)	3.78
18:2	9.68	(44.67)	10.01	(45.31)	5.06	(45.91)	2.22	(48.26)	3.03
18:3	0.62	(2.86)	0.52	(2.35)	0.30	(2.72)	0.12	(2.61)	0.42
Total	21.67		22.09		11.02		4.6		
Recovery[a/] (%)	93		93		96		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

Table 8. Total Fatty Acids of Whole Pearl Millet Meal Stored at 9.67% Moisture in Polyethylene Bags at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	1.96	(17.80)	3.66	(16.31)	2.00	(16.93)	0.82	(17.01)	0.47
18:0	0.60	(5.44)	1.13	(5.03)	0.73	(6.18)	0.26	(5.39)	0.41
18:1	3.43	(31.15)	6.31	(28.11)	3.45	(29.21)	1.40	(29.0)	3.27
18:2	4.74	(43.05)	10.87	(48.44)	5.34	(45.21)	2.22	(46.06)	4.53
18:3	0.28	(2.54)	0.47	(2.09)	0.20	(2.45)	0.12	(2.48)	
Total	11.01		22.44		11.81		4.82		
Recovery[a/] (%)	93		93		97		97		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

Table 9. Total Fatty Acids of Whole Pearl Millet Meal Stored at 3.85% Moisture in Polyethylene Bags at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	4.44	(19.22)	3.75	(17.20)	1.98	(16.79)	0.93	(18.82)	0.63
18:0	1.37	(5.93)	1.33	(6.10)	0.71	(6.02)	0.28	(5.66)	0.42
18:1	5.83	(25.23)	6.36	(29.17)	3.42	(29.00)	1.37	(27.73)	4.07
18:2	10.76	(46.58)	9.87	(45.27)	5.33	(45.20)	2.23	(45.14)	2.41
18:3	0.70	(3.03)	0.49	(2.24)	0.35	(2.96)	0.13	(2.63)	0.39
Total	23.10		21.80		11.79		4.94		
Recovery[a/] (%)	94		93		97		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

Table 10. Free Fatty Acids of Whole Millet Meal Stored at 13.81% Moisture in Polyethylene Bags at 35°C.

FFA	0 days[b]		5 days[a]		10 days[a]		15 days[b]	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	1,296	0.024	8.736	0.156	38.787	0.725	14.465	0.263
16:0	7,884	0.146	31.136	0.556	58.315	1.090	334.510	6.082
18:0	1,890	0.035	8.400	0.150	45.314	0.847	70.620	1.284
18:1	13.176	0.244	---	---	---	---	520.245	9.459
18:2	13.662	0.253	---	---	---	---	501.270	9.114
18:3	1.026	0.019	---	---	---	---	44.330	0.806

[a] Values for FFA 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation. FFA were extracted from the FFA band, scraped off the TLC plate, using four fractions of 50 ml petroleum ether each (old method).

[b] FFA were directly esterified after scraping off FFA band from TLC plate. There was no prior extraction of FFA from the silica gel (new method).

Table 11. Free Fatty Acids of Whole Millet Meal Stored in 9.67% Moisture in Polyethylene Bags at 35°C [a].

FFA	0 days		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	17.489	0.318	20.832	0.372	21.505	0.391	34.998	0.614
16:0	26.014	0.473	44.295	0.791	47.025	0.855	99.75	1.750
18:0	23.209	0.422	25.927	0.463	26.070	0.474	30.951	0.543

(a) Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Table 12. Free Fatty Acids of Whole Millet Meal Stored at 3.85% Mositure in Polyethylene Bags at 35°C [a].

FFA	0 days		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	19.096	0.341	43.680	0.780	32.596	0.562	98.210	1.708
16:0	39.256	0.701	46.928	0.838	56.318	0.971	131.042	2.279
18:0	15.904	0.284	13.552	0.242	47.154	0.813	52.152	0.907

(a) Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Fat acidity. Fat acidity (Fig. 16) increased during the storage period for both decorticated and degermed millet samples. Degermed millet gave more hydrolytic decomposition than did the decorticated millet. Fat acidity of degermed millet was higher than that of decorticated millet but its rate of increase was lower.

The initial difference in fat acidity day 0 between degermed and decorticated samples was larger than that obtained after 15 days' storage. The large difference in fat acidity the first day of storage may have occurred because the degermed sample was tempered to 22% moisture for 6 hrs, ground, and then dried. During the 15 days of storage, the fat acidity of decorticated millet increased at a faster rate than for the degermed sample.

Peroxide value. The peroxide value (Fig. 17) of decorticated millet was relatively low and it did not significantly change during storage. The degermed sample was much higher and it varied during storage. The peroxide value was drastically higher after 10 days of storage and then decreased. Obviously oxidative rancidity was more important in degermed millet than it was in the decorticated samples.

Why degermed millet is more sensitive to autoxidation during storage is not known. Perhaps the germ lipids contain antioxidants that help protect the fatty acids from oxidation.

Extractable fat. The extractable fat of control, decorticated and degermed samples did not change significantly during storage. The average values were 5.75%, 5.40% and 2.43% for control, decorticated and degermed millet, respectively.

Total fatty acids. Total fatty acids did not vary significantly over the storage period for both decorticated and degermed samples (Tables 13 and 14).

Free fatty acids. Both decorticated and degermed millet showed increases

Fig. 16. Effect of decorticating and degerming of millet (bulk) on the stability of millet meal during storage at 35°C in polyethylene bags as measured by fat acidity.

- ✱ Whole millet meal (11.0% m.c.)
- ✪ Decorticated millet (12.2% m.c.)
- □ Degermed millet (10.8% m.c.)

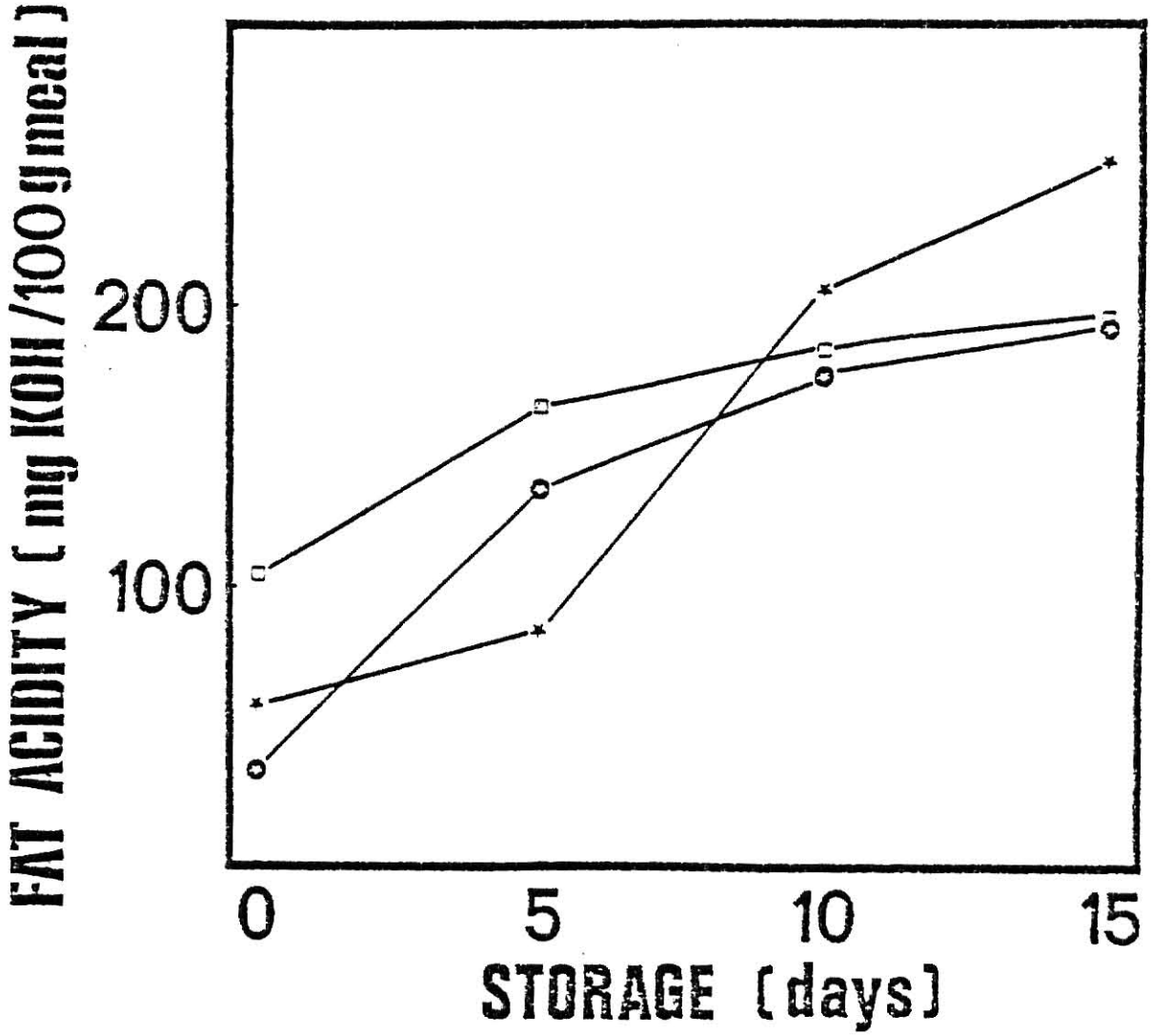

FAT ACIDITY (mg KOH /100 g meal)
200
100
0
5
10
15
STORAGE (days)

Fig. 17. Effect of decorticating and degerming of millet on the storability of millet meal during storage at 35°C in plastic bags as measured by peroxide value.

- Whole millet meal (11.0% m.c.)
- Decorticated millet (12.2% m.c.)
- Degermed millet (10.8% m.c.)

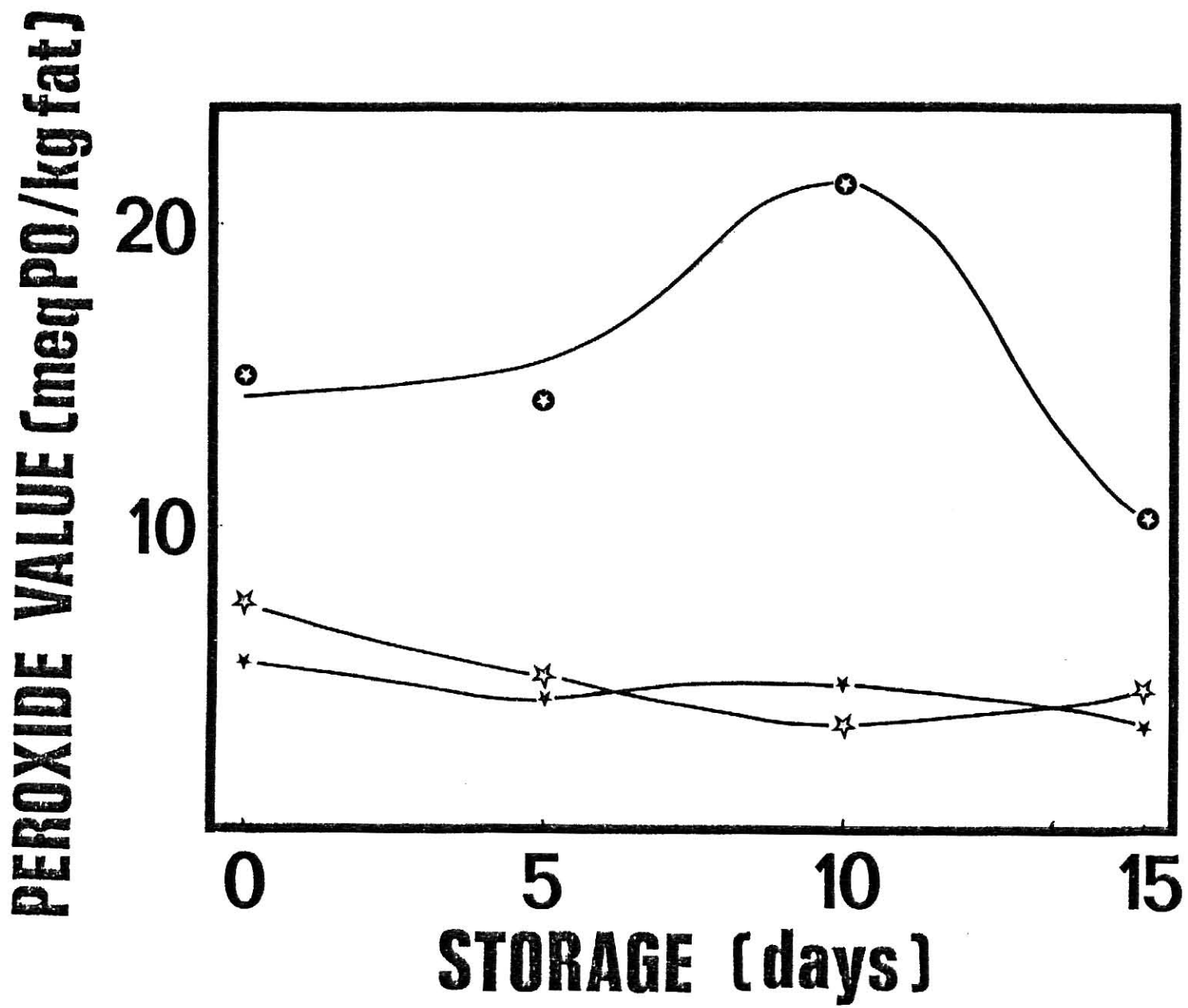

PEROXIDE VALUE (meqPO/kg fat)
20
10
0
5
10
15
STORAGE (days)

Table 13. Total Fatty Acids of Decorticated Millet Stored at 12.22% Moisture in Polyethylene Bags at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	1.69	(17.69)	1.69	(16.84)	1.88	(16.81)	0.86	(18.53)	0.67
18:0	0.52	(5.44)	0.60	(5.98)	0.66	(5.90)	0.25	(5.38)	0.32
18:1	2.97	(31.09)	2.93	(29.23)	3.25	(29.07)	1.30	(28.01)	3.37
18:2	4.12	(43.14)	4.60	(45.86)	5.09	(45.52)	2.15	(46.33)	3.78
18:3	0.25	(2.61)	0.21	(2.09)	0.30	(2.68)	0.08	(1.72)	0.45
Total	9.55		10.03		11.18		4.64		
Recovery[a/] (%)	83		87		97		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

Table 14. Total Fatty Acids of Degermed Millet Stored at 10.83% Moisture in Polyethylene Bags at 35°C.

Fatty Acids	Control		5 days		10 days		15 days		Average Standard Deviation
	μ g FAME	%	μ g FAME	%	μ g FAME	%	μ g FAME	%	
16:0	2.92	(17.33)	1.50	(17.46)	1.56	(16.14	0.69	(19.43)	0.75
18:0	0.98	(5.81)	0.46	(5.35)	0.56	(5.79)	0.20	(5.63)	0.28
18:1	4.97	(29.49)	2.64	(30.73)	2.88	(29.81)	0.91	(25.63)	4.21
18:2	7.58	(44.98)	3.78	(44.00)	4.34	(44.92)	1.67	(47.04)	3.77
18:3	0.40	(2.37)	0.21	(2.44)	0.32	(3.31)	0.08	(2.25)	0.79
Total	16.85		8.59		9.66		3.55		
Recovery[a/] (%)	93		95		97		98		

[a/] Recovery was determined as the ratio of amount of fat esterified to the total FAME calculated from the chromatograms.

in FFA during the storage period (Tables 15 and 16). This is consistent with the higher fat acidity values obtained.

Hexanal as a measure of oxidative rancidity

Millet meal (HMP 1700) was stored in glass jars, with loose lids so that oxygen was not a limiting factor. Hexanal content was run every day over 20 days period. The results (Fig. 18) show no increase of hexanal over all the storage period.

In another study we wanted to determine the extent of oxidation in pearl millet meal during storage and compare the results with those obtained for whole wheat meal stored under the same conditions.

The results show that whole wheat meal gave higher amounts of hexanal than did millet meal samples during the first 3 weeks of storage (Fig. 19). In both whole wheat and millet meal samples, the amount of hexanal produced was very low during the first 3 weeks of storage. The trend of hexanal formation obtained here is similar to that reported in the literature (Labuza, 1971).

Millet meal (M) had its hexanal content increase from 0.1 ppm to 0.22 after 5 weeks. When millet meal was reconstituted with millet lipids (M + ML), the hexanal content after 5 weeks increased to 0.25 ppm. When millet meal was reconstituted with wheat lipids (M + WL), its hexanal content decreased to a value of 0.21 ppm after 5 weeks of storage.

Besides, whole wheat meal (W) had a higher hexanal content (0.30 ppm) after 5 weeks' storage than did reconstituted whole wheat with wheat lipids (0.21 ppm) and reconstituted whole wheat flour with millet lipids (0.24 ppm).

Although the initial hexanal content of millet meal was half that of wheat, the amount of hexanal in millet meal after 5 weeks is very close to that of wheat. This indicates that past the induction period, millet meal

Table 15. Free Fatty Acids of Decorticated Millet Meal Stored at 12.22% Mositure in Polyethylene Bags at 35°C [a].

FFA	0 days		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	27.56	.530	30.888	0.572	30.564	0.566	30.250	0.550
16:0	13.468	.259	36.882	0.683	57.834	1.071	76.670	1.394
18:0	21.736	.418	22.842	0.423	23.976	0.444	27.830	0.506

[a] Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Table 16. Free Fatty Acids of Degermed Millet Meal Stored at 10.83% Moisture in Polyethylene Bags at 35°C [a].

FFA	0 days		5 days		10 days		15 days	
	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat	mg/100 g meal	g/100 g fat
14:0	1.554	0.074	2.562	0.122	6.462	0.275	4.837	0.225
16:0	9.072	0.432	34.23	1.630	52.405	2.230	56.760	2.64
18:0	10.143	0.483	11.46	0.546	13.230	0.563	11.889	0.553

(a) Values for free fatty acids 18:1, 18:2 and 18:3 are not given because significant loss occurred during separation on TLC plate due to oxidation.

Fig. 18. Hexanal values for ground millet (HMP 1700) stored at 35°C in glass jars.

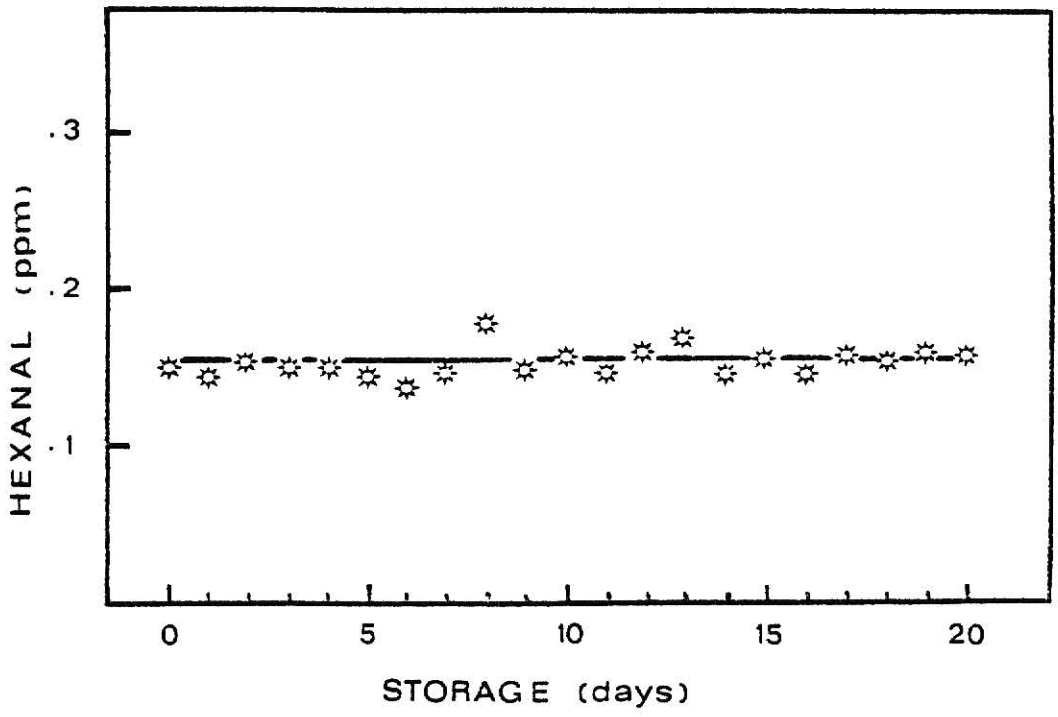
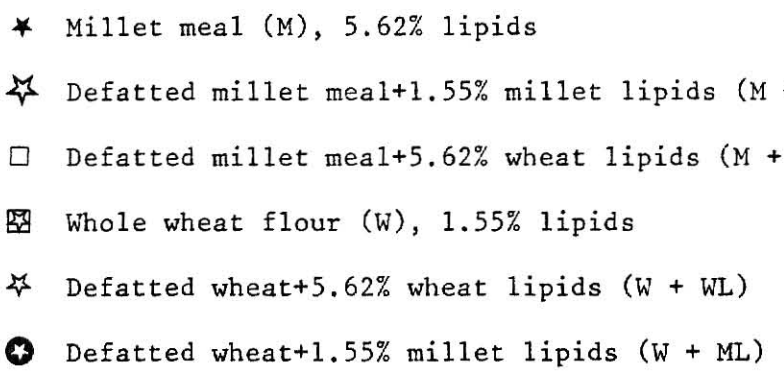
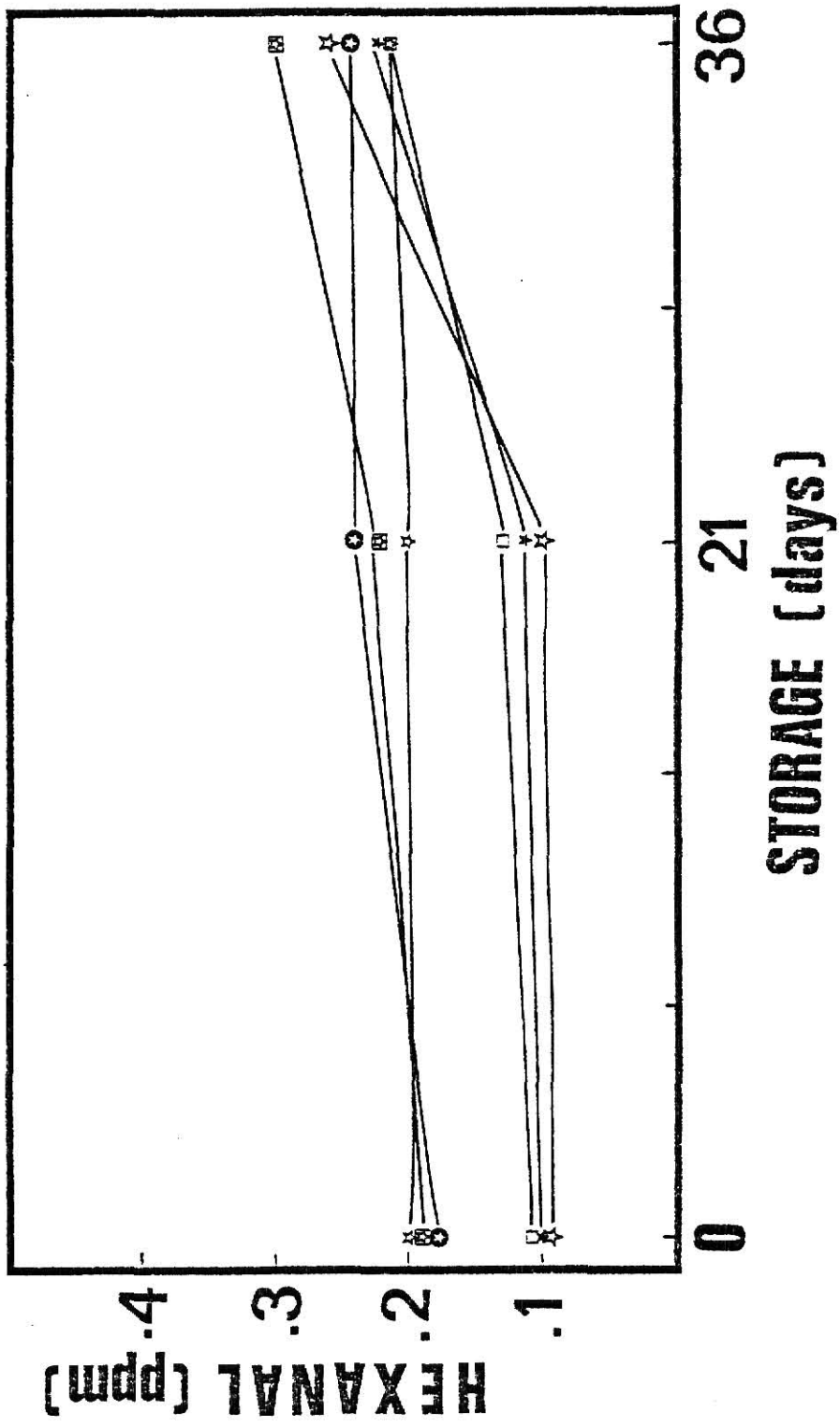

HEXANAL (ppm)
.3
.2
.1
0
5
10
15
20
STORAGE (days)

Fig. 19. Stability of ground millet (bulk) and wheat during storage at 35°C in polyethylene bags as measured by hexanal content.




HEXANAL (ppm)
.4
.3
.2
.1
0
21
36
STORAGE (days)

goes bad much more quickly than wheat does.

Effect of atmospheric oxygen on the stability of millet meal

The fat acidity of samples stored under atmospheric air and under nitrogen both increased dramatically (Table 17). The fat acidity values for the two samples were essentially identical which indicates that atmospheric oxygen has no effect on fat acidity values.

During the 30 days of storage, peroxide value decreased significantly from 7.73 to 4.11 for the sample stored under air and from 7.73 to 3.35 for the sample stored under nitrogen. Although the differences between the samples is small, the rate of decomposition of hydroperoxides appears to be more important for samples stored under nitrogen than for samples stored under air.

Effect of the quantity and quality of lipid fraction on storability of millet meal

Effect of fat content. All samples were stored at 30°C, 60% RH for 5 days and had increases in fat acidity (Fig. 20). The control wheat (W) had the lowest fat acidity. Defatted wheat meal reconstituted with wheat lipid (W + WL) had a higher fat acidity than the control presumably because the lipid is on the surface of the sample when it is reconstituted. When defatted wheat meal was reconstituted with millet lipid (M + ML), the fat acidity increased at a higher rate than it did for the control. When defatted millet meal was reconstituted with wheat lipid (M + WL), the fat acidity was much lower. It was obvious that the fat content influenced the fat acidity. Higher fat acidity values were obtained for those samples with higher fat contents. The same level of millet lipids (ML), (M + ML) and (W + ML) and the sample containing millet meal gave significantly higher fat acidity values. Also with the same level of wheat lipid (WL), the sample containing millet meal (M + WL) gave higher fat acidity than the sample containing wheat meal (W +

Table 17. Effect of atmospheric oxygen.

Millet meal stored under	0 days		30 days	
	Fat acidity (mg KOH/ 100 g meal)	Peroxide value (mg PO/ kg fat)	Fat acidity (mg KOH/ 100 g meal)	Peroxide value (mg PO/ kg fat)
Air	58.12	7.73	290.44	4.11
N_2	58.12	7.73	292.69	3.35

Fig. 20. Fat acidity during storage of reconstituted wheat and millet. Effect of lipid content.

■ Millet meal (M), 5.5% lipid

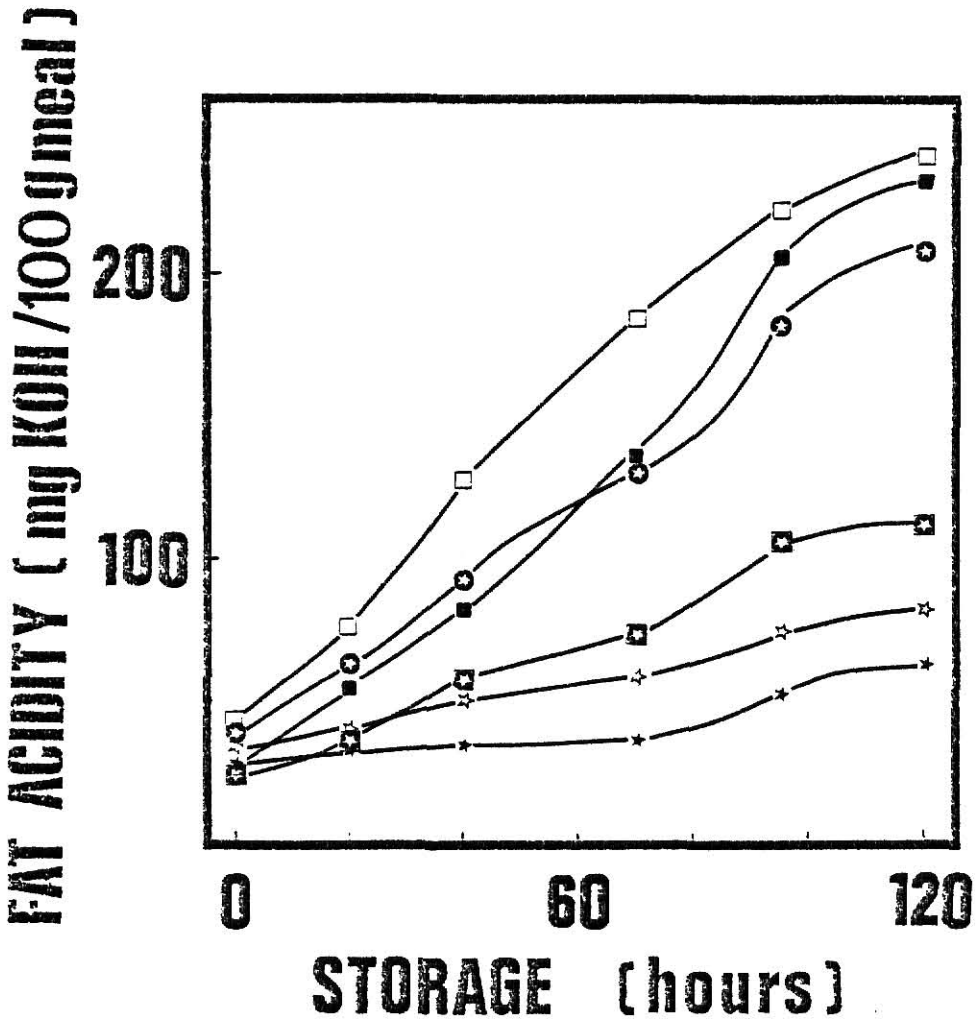
□ Defatted millet meal+7.42% millet lipids (M + ML)

Defatted millet meal+1.97% wheat lipids (M + WL)

Whole wheat flour (W), 1.82% lipid

Defatted wheat+1.97% wheat lipids (W +WL)

Defatted wheat+7.42% millet lipids (W + ML)


FAT ACIDITY (mg KOH /100 g meal)
200
100
0
60
120
STORAGE (hours)

WL). We assume the reason is that millet meal has a higher lipase activity.

Effect of fat origin. All wheat samples had lower fat acidity value than did millet samples (Fig. 21). When defatted wheat was reconstituted with wheat lipid (W + WL), the values of fat acidity were higher than that obtained with the control wheat (W). When defatted wheat was reconstituted with millet lipids (W + ML), the fat acidity values were lower than those obtained for the defatted wheat plus wheat lipids (W + WL) sample. This indicates that millet lipids are slightly less sensitive to hydrolytic decomposition than are wheat lipid.

Millet reconstituted with millet lipid (M + ML) also had higher fat acidity values than did the control millet (M). As found with the wheat samples, reconstitution of millet with wheat lipids gave a sharper increase in fat acidity than found for the other samples. Therefore, it appears that millet lipids are less sensitive to hydrolytic attack than are wheat lipids.

Fig. 21. Fat acidity during storage of reconstituted wheat and millet. Effect of lipid quality.

■ Millet meal (M), 5.36% lipid

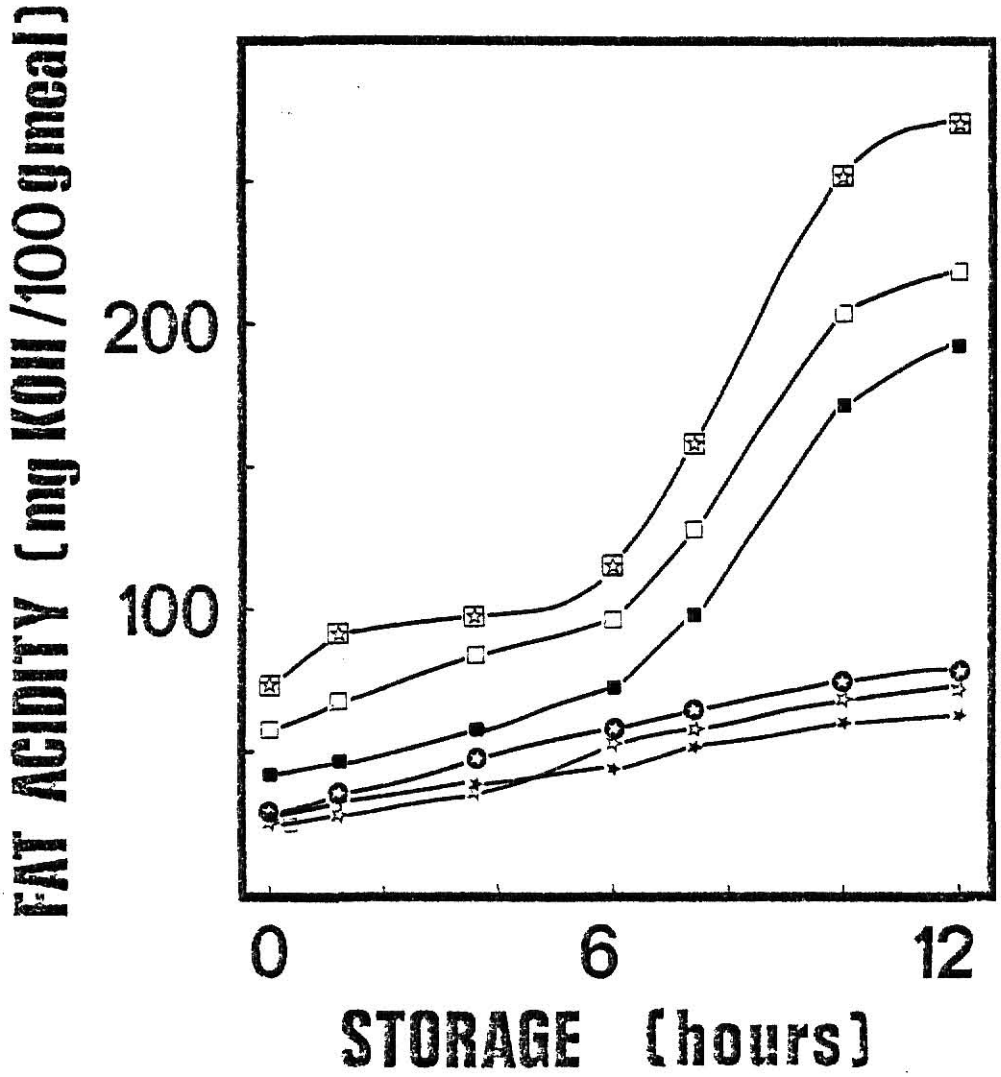
□ Defatted millet meal+5.50% millet lipids (M + ML)

Defatted millet meal+5.50% wheat lipids (M + WL)

Whole wheat flour (W), 1.72% lipid

Defatted wheat+1.69% wheat lipids (W + WL)

Defatted wheat+1.69% millet lipids (W + ML)


FAT ACIDITY (mg KOH/100 g meal)
200
100
0
6
12
STORAGE (hours)

CONCLUSIONS

Previous work (Lai and Varriano-Marston, 1980b) had shown that pearl millet goes rancid rapidly after it is ground. Increases in fat acidity and peroxide value accompanied millet meal going rancid. Fat acidity and peroxide value of stored whole pearl millet grain did not vary significantly over the same time period. This shows that deteriorations occurred more rapidly in the ground millet than in whole grain.

When millet meal was stored in polyethylene bags, the peroxide value increased rapidly and appeared to signal the start of rancidity. Millet meal stored in cotton bags showed no peroxide accumulation, suggesting that air either accelerates the decomposition of hydroperoxides or removed volatile components.

Free fatty acids and fat acidity data indicate that all the acidity produced during storage was the result of free fatty acids. The proportions in which free fatty acids were released are similar to those found in total fatty acids. Therefore, it appears that the hydrolytic action of lipase was of a random type.

Total fatty acids did not significantly change as a result of storage. Thus, no significant oxidative degradation of fatty acids occurred during the storage period.

Under high relative humidity (80%), fat acidity increased drastically during the first few days and then levelled off. This levelling of the fat acidity cannot be accounted for by the consumption of free fatty acids by the microorganisms present in millet during storage. The number of mold colonies found in millet was low the first day of storage and did not increase over that storage period. It also cannot be explained as a loss of fatty acid as a result of oxidation, because as mentioned above the total fatty acid

remained constant during the storage period. No explanation is offered for the levelling off.

Hexanal is the major product of oxidative degradation of lipids (Labuza, 1971). Hexanal determination on millet meal stored in polyethylene bags showed essentially no hexanal produced in 15 days. There was an increase in hexanal after 21 days of storage similar to that found with other cereals however, this does not correlate well with the rancid odors and flavors found earlier during storage of pearl millet. Thus, the peroxide value obtained after short storage is not the result of the classical oxidative degradation of lipids. Furthermore, peroxides may not be the result of lipid decomposition.

The reconstitution study of millet meal and whole wheat meal and their lipids showed that the fat content was the major factor contributing to the rapid increase of fat acidity in ground millet. When the same level of fat was used, the increase in fat acidity was significantly higher for sample containing wheat lipid than for those containing millet lipids. Lipase appeared to be more active in defatted millet than in defatted wheat.

Decorticated millet appeared more stable during storage than degermed millet because peroxide value was much lower for decorticated millet. We assume that endosperm lipids were more rapidly oxidized than were lipids from the covering layers or germ.

Fat acidity was directly related to the moisture content of millet meal. The increase in fat acidity was greater in samples with high moisture levels than it was for samples with low moisture levels.

Since the ultimate mean of detection of rancid odors is the sensory evaluation, decorticated millet and degermed millet should be stored and a sniff test conducted to determine if decortication and/or degermination improve the stability during storage. Additional work is also required to identify the

compounds giving the peroxide values after short storage.

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FACTORS CONTRIBUTING TO THE DEVELOPMENT OF RANCIDITY IN GROUND PEARL MILLET (Pennisetum americanum [L] Leeke) DURING STORAGE

by

Idir KACED

B.S., National Institute of Agronomy, Algiers, 1976

AN ABSTRACT OF A MASTER'S THESIS

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submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1982

ABSTRACT

A number of factors such as temperature, relative humidity, moisture content, type container, and oxygen were varied to study their effect on storability of ground millet meal. Titratable fat acidity, and free fatty acids were determined to measure the hydrolytic decomposition of lipids. Peroxide value, total fatty acids, and hexanal determinations was used to measure the oxidative deterioration of lipids.

Millet was decorticated and degermed to see if storage stability was improved. Decortication appeared to improve slightly the storability of millet meal. However, our data indicate that degermination did not improve millet meal stability. The highest values of fat acidity and peroxide value were obtained with the degermed millet.

Hexanal determination and total fatty acids did not produce any evidence of oxidative deterioration of lipids during short time storage. Objectionable flavors and odors are known to appear five days after grinding.

Peroxide values were more easily detected in millet meal stored in plastic bags than in cotton bags. Ventilation was found to be the major factor in the detection of hydroperoxides in millet meal.