# PEARL MILLET LIPIDS: COMPOSITION AND CHANGES DURING STORAGE

by

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# TABLE OF CONTENTS

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# INTRODUCTION

Pearl millet, Pennisetum Americanum, is one of the most important food crops in tropical and subtropical regions of Asia and Africa. Its protein and oil content is equal or superior to wheat, corn, sorghum, and rice (Casey, et al., 1977; Burton, et al., 1972). It is a staple food which supplies up to 90% of the calories for many millions of poor people in the world, and therefore constitutes a major portion of the limited lipid they consume.

Pearl millet is a tall crop that usually reaches a height of six to ten feet when mature. However, within the last ten years high yielding dwarf varieties have been developed (Burton and Fortson, 1966; Casady, et al., 1976) which should reduce lodging, facilitate seed harvest, and make pearl millet a more desirable cereal crop.

The genotype and the environment in which a grain is grown will influence its chemical and nutritive value. Little is known about the nutritional content of pearl millet cultivars. The first objective of this study, therefore, was to characterize these important nutrients in pearl millet cultivars.

Ground millet is often consumed as a gruel or made into flatbread. However, the meal does not keep well; unpleasant odors and tastes often appear shortly after the grain has been ground. Thus, the second objective of this study was to determine the role of lipid may have in contributing to decreased storage stability of ground millet.

# REVIEW OF LITERATURE

The term millet is used for several genera of small seeded grasses. Rooney (1978) listed five widely used varieties of millet by their scientific and common names. Millet, however, in this report will refer to pearl millet unless otherwise specified.

## Millet Lipid Content

The percent lipid extracted from pearl millet ranges from 2.8% to 8.0% and averages 5.4% (Freeman and Bocan, 1973). This wide range of lipid content could be due to genetic differences, since high yileding hybrids have higher lipid contents than low yielding varieties (Sharma and Goswami, 1967; Freeman and Bocan, 1973). In addition, Jellum amd Powell (1971) have shown that extraction procedures are important in determining the amount and composition of oil from pearl millet grain. In any case, it appears that pearl millet grain is higher in lipid content than most other cereals (Burton, et al., 1972).

The oil of millet, as with other cereal grains, is concentracted in the germ, pericarp, and aleurone layer (Rooney, 1978). Belova, et al., (1970) reported that 24% of lipids from millet seed are concentrated in the germ.

The lipids in cereal grains are predominantly non-polar (triglyceride) in nature and are readily extracted by solvents such as hexane or petroleum ether. The percentage of lipid extracted from pearl millet as compared to other millets is shown in Table I. Finger millet showed the lowest yield of lipid compared to the other samples due probably to the chloroform-

Table I. Percentage of Lipid Extracted from Millets

| Millet Sample | Percent Extracted |
|---|---|
| Pearl Millet[a] | |
| African entries | 4.95-5.70 |
| American entries | 4.85-6.70 |
| Indian inbreds | 5.35-5.92 |
| Indian varieties | 4.22-6.77 |
| Hybrids | 4.65-7.40 |
| Proso millet[b] | 3.80-4.90 |
| Foxtail millet[b] | 4.00-5.00 |
| Finger millet[c] | 1.85-2.10 |
| Japanese millet[b] | 5.50-6.30 |

[a]Sharma and Goswami, 1969.

[b]Miller, 1958.

[c]Mahadevappa and Raina, 1978.

methanol solvent system that was used in the extraction procedure instead of petroleum ether.

Lipids associated with the starch of the endosperm of seeds (bound lipids) are generally of the polar class and must be extracted with polar-solvents, such as methanol, chloroform, and others. A bound lipid content of 0.5% has been reported for millet (Pruthi and Bhatia, 1970).

Polar and non-polar lipids have been separated by thin-layer chromatography. Two fractions from pearl millet were tentatively identified by Pruthi and Bhatia (1970). Triglycerides were reported to be the principal consitituents in the non-polar fractions whereas lecithin was found to be the major components of the polar fraction. Badi et al., (1976) compared the polar and nonpolar lipids in each fraction from wheat, sorghum, and pearl millet using thin-layer chromatography. The bound nonpolar lipid of all three grains contained similar components as did the free nonpolar and bound polar lipids. The pattern of polar-free lipids differed widely for millet and wheat.

A range of fatty acid composition of pearl millet oil has been reported (Rooney, 1978; Freeman and Bocan, 1973). In general, pearl millet oil tends to be higher in palmitic and stearic acids and lower in oleic acid than oil from sorghum and corn (TableII). Pearl millet oil contains significantly more saturated acid. However, most of the fatty acids present in millet are unsaturated.

Busareva et al., (1972) analyzed the composition of fatty acids in lipids of freshly harvested millet by gas chromatography.

Table II. Fatty Acid Composition of sorghum, Corn, and Pearl Millet Oils.

| Component | Corn[a] (%) | Sorghum[a] (%) | Pearl millet[a] (%) | Range for 65[b] selfed lines |
|---|---|---|---|---|
| Ether extracts | 4.5 | 3.4 | 5.6 | 4.1- 4.6 |
| Palmitic | 12.7 | 14.3 | 19.0 | 16.7-25.0 |
| Stearic | 2.6 | 2.1 | 5.0 | 1.8- 8.0 |
| Arachidic | 0.4 | 0.2 | 0.5 | 0.3- 1.0 |
| Oleic | 26.0 | 31.0 | 25.0 | 20.2-30.6 |
| Linoleic | 56.3 | 49.0 | 46.0 | 40.3-51.7 |
| Linolenic | 1.6 | 2.7 | 3.2 | 2.3- 5.1 |

[a] Rooney (1978).

[b] Jellum and Powell (1971).

Linoleic acid was the predominate acid of the free and bound lipids (69.6-72.4% and 44.5-53.4% respectively). On the other hand, palmitic acid was the predominate acid of the more "firmly bound" lipids (35.2-46.7%). Differences in composition for each type of lipid were thought to be due to differences among individual varieties. Nechaev et al., (1973) also showed that fatty acids in the free lipids of millet were principally palmitic, oleic, and linoleic acids.

Lipid Changes During Storage

Until recently, millet was ground by the African housewife just prior to consumption. Today milling technology has been used to produce millet flour (Thiam et al., 1976). The technology of hulling and milling, however, do not completely separete the germ from the rest of the kernel. Consequently, since the germ contains considerable quantities of lipid, these lipids are absorbed by the milled products resulting in problems related to quality changes during storage.

Work done on changes in the fatty acid composition of millet lipids during storage (Nechaev et al., 1973; Carnovale and Quaglia, 1973) suggests that rapid alterations in millet flour quality were mainly due to hydrolytic decomposition of the lipids, with lipase being the mediator in the reaction (Féron and Bouquet, 1948; Thiam et al., 1976). On the other hand, Thiam et al., (1976) found that lipoxygenase activity was practically non-existent in stored millet (30 C, 90-95% R.H.). UV spectra of lipid extracts from deteriorated flour showed no evidence of oxidation of unsaturated fatty acids. The author postulated that

phenolic compounds in the grain acted as antioxidants.

Carnovale and Quaglia (1973) studied lipids in milled products preserved for three months in a controlled chamber at 30 C and 95% relative humidity. The fatty acid composition of ether extracts were analyzed as methyl esters by gas chromatography. They noted that considerable losses occurred between the second and third month of preservation. Palmitic acid progressively decrease during atorage, and linolenic acid decteased to trace levels. On the other hand, stearic and oleic acid levels remained stable. An increase in linoleic acid was also observed in all milled products of millet. This agreed with the report of Nechaev et al., (1973) who showed an increase in linoleic acid content of stored millet seed of the Saratovstoe 853 and Veselopodoluanskoe 367 varieties having a moisture content of 12.8% and 13.3%, respectively. Carnovale and Quaglic (1973) also showed that, in contrast to wheat lipids (Daftary and Pomeranz, 1965), triglycerides were less stable during storage compared to polar lipids.

Lipid degradation during storage can occur via microbiological processes as well as by natural enzymes in millet flour. However, no microbial conditions were given by investigators other than Thiam et al., (1976). They also observed a significant increase in yeast and bacteria growth when millet flour was stored in a confined atmosphere at 30 C, 90 to 95% R.H. Large amounts of alcohols were identified in millet flour suggesting that fermentation played the major role in the deterioration process. When flours were stored under semi-aerobic conditions

at the same temperature and humidity, mold growth was also detected, accompanied by off-odors and tastes. Conversely, flour stored at 50% relatively humidity 30 C for 4 months or at 5 C, 50% R.H., for 10 months, did not show any deterioration in odor or taste.

# MATERIALS AND METHODS

## Materials

Pearl millet samples were grown in Hays, Kansas. Seventeen HMP550 $S_1$ lines (Tift $23db_1$/82PI185642) that were harvested in 1977 were studied. In addition, four bulk population were studied: HMP550, a combination of 110 $S_1$ lines; HMP1700 (PI263540/ Tift $239DB_2$/2*Serere 3A); RMPI(S)CI (parentage from Serere 3A, Serere 17 and Tift $239DB_2$); and Serere 3A, developed by Serere Exp. Stn., Uganda, Africa. The latter cultivar was harvested in 1975.

For the storage studies, an HMP550 bulk pearl millet population was used. The grain was ground in an experimental roller mill to pass a 50GG screen. The meal was stored in replicates in cotton bags under the following storage condition: 19 C, 58% R.H.; 27 C, 64% R.H.; 42 C, 75% R.H.. After designated storage periods, samples were taken using a hollow tube as a probe.

## Methods

Histology. Whole kernels of millet were soaked in distilled water overnight at 4 C. Kernels were then hand sectioned and stained with Oil Red O or Nile Blue method (Bancroft, 1975) for localization of lipids. Lipase location was identified by the Tween method (Bancroft, 1975) and the $\beta$-naphthyllaurate method according to Sastry et al., (1977). Transmission micrographs were taken on a Reichert (Austria) light microscope; a Wild Herrbrugg microscope was used to record images of stained half-kernels.

Lipid extraction. Millet was ground by a Wiley mill using a

40 mesh screen. Free lipids were extracted with petroleum ether (bp 38 to 55 C) in a Goldfish apparatus for 14 hours. Bound lipids were then extracted from the residue two times with water-saturated n-butanol in a Stein mill. In the first extraction, 50ml of solvent was added and the residue plus solvent were mixed for 4, 2 and 2 min., with 1-min. intervals between extractions. The second extraction was done three times, 2 min. each at 1-min. intervals. The rest of drying and re-extraction procedure was as described by Daftary and Pomeranz (1965a).

Thin-layer chromatography. The free and bound lipid fractions were characterized by thin-layer chromatography on silica gel using chloroform:methanol:water (65:25:4, v/v) to separate polar lipids and chloroform to separate nonpolar lipids. Spots were visualized by spraying the plates with a chromic $H_2SO_4$ reagent; a ninhydrin reagent for detection of free amino groups; a modified Dragendroff reagent for choline phosphatides and galactolipids; $\alpha$-naphol containing reagent specific for sugar-containing compounds (Stahl, 1969); or with a molybdenum blue spray specific for any phosphorous containing compounds (Dittmer and Lester, 1964). Lipids were tentatively identified by co-chromatography with known lipid compounds, by comparision of relative Rf values, and by reactions of the specific sprays.

Gas chromatography. Esterification of fatty acids was carried out by placing an aliquot of lipid extract in teflon-stoppered culture tubes and evaporating to dryness under a stream of $N_2$. A measured amount of tricosanoic acid (internal standard) and 2ml of 0.5N methanolic NaOH were added. The vial was sealed

and the contents were heated in a boiling water bath for about 2 min. followed by addition of 5ml $BF_3$-methanol and heating again for another 2 min. Tube contents were then transfered to a 50ml separatory funnel using 20ml of water. Fatty acid methyl esters were extracted with hexane and analyzed on the gas chromatograph.

Fatty acid methyl esters were identified on a Hewlett Packard 5750 Gas Chromatograph with a flame ionization detector. The column was 6 ft.X1/8in. packed with 10% Sp-2330 in 100/120 chromosorb W AW (Supelco Inc.). Injection temperature port was 250 C; column temperature was 195 C and $N_2$ carrier gas flow was 40 ml/min. Relative peak areas were determined by multi-plying the peak height by the width of the peak at half height. Weight percentage compositions were calculated by applying correction factors obtained from chromatograms of known mixtures. Peaks were tentatively identified by comparing the relative retention times with those from the standard regerence mixtures run on the same column under the same conditions.

Moisture content and mold count. Standard AACC methods (1962) were used for moisture determinations and mold count.

Sniff test. The sniff test was conducted using seven selected panelists (3 females, 4 males) who had previous sensory evaluation experience. Panelists were first acquainted with the aroma characteristics of the fresh and aged millet meal samples in order to define a frame of reference for future comparisions.

Samples from the three storage conditions which had been taken at the same time period were presented to the panel in odor-free glass jars. Six samples were evaluated at each sitt-

Fig. 1. Score sheet used for sniff test.

Name:_____ Date:_____

Please sniff these samples very carefully and compare to the standard, or reference samples. Mark an 'X' on the appropriate place of the line given that best describes your feeling about each sample.

Fresh Aged

Sample _____

Comments:

Sample _____

Comments:

Sample _____

Comments:

ing. All samples of stored meal were coded and presented in a random order. Panelists were asked to sniff the samples one at a time and compare them with the reference samples. Results and comments were recorded on a score sheet (Fig. 1). The sniff test value was derived by measuring the distance in centimeters from the fresh reference point to the point marked by the panelist.

Fat acidity. Fat acidity was determined by the AOAC rapid method (1960).

Peroside value. For peroxide value determinations, a 5 g sample was mixed with 50ml chloroform in a Stein mill for 3 min. The peroxide value of thefiltrate was determined using the method of Takagi et al. (1978).

Statistical analysis. An analysis of variance was run on the data (Snedecor and Cochran, 1967) and Duncan's (1955) multiple range test was applied to determine significant differences among means. Simple linear correlations between variables were calculated.

# RESULTS AND DISCUSSION

## Composition of Lipids

Lipid location. Lipid location may be an important factor contributing to storage stability of products milled from pearl millet. Histochemical studies on half-kernels showed that most lipids were concentrated in the germ and covering layers of the grain (Fig.2). Further observations on thin sections indicated that although the endosperm bound small quantities of lipid stain, most of the lipids were concentrated in the aleurone layer.

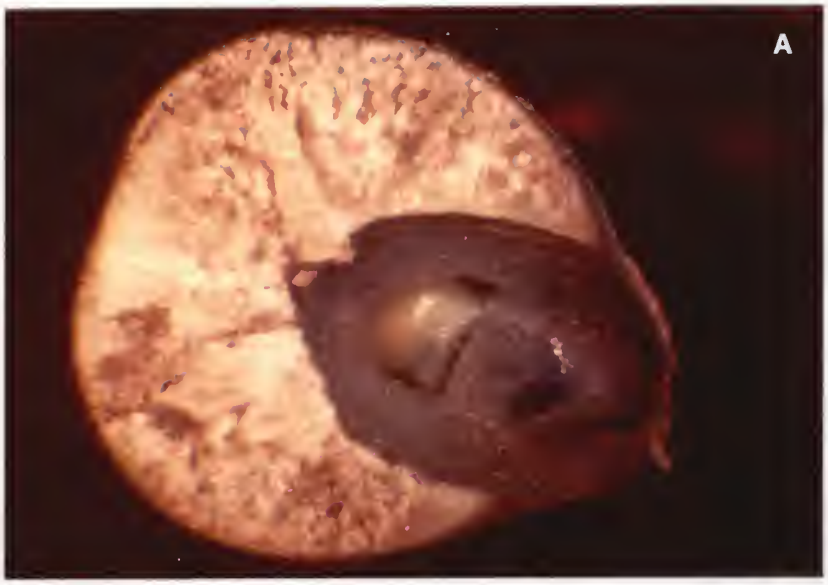
Locations of bound lipids were studied by extracting free lipids from half-kernels in petroleum ether overnight with a mechanical mixer. The kernels were washed and stained with the Oil Red O (Bancroft, 1975) and compared to the unextracted half-kernels. The results suggested that most of the bound lipids were located in the germ and covering layers.

Storage stability should be markedly improved if the major lipid containing structures are removed by milling. However, if the milling process could completely separate the endosperm from the germ and outer layers, which is nearly impossible by today's technology, rancidity of lipids in the endosperm might still occur in the stored milled product.

Free and bound lipids. Percentage of free and bound lipids in pearl millet cultivars are given in Table III. Free lipid content ranged from 5.66% to 7.08%; values which are in agreement with those reported in the literature (Rooney, 1978).

Bound lipids ranged from 0.57% to 0.09%. Pruthi and Bhatia (1970) reported bound lipid contents of 0.47% to 0.55% for two

Fig. 2. (A) Cross-section of kernel stained with Nile Blue, showing coloration of the germ and covering layers. (84X)
(B) Cross-section of millet stained with Nile Blue, showing high concentration of lipid in aleurone and slight coloration of endosperm. (1260X)


A

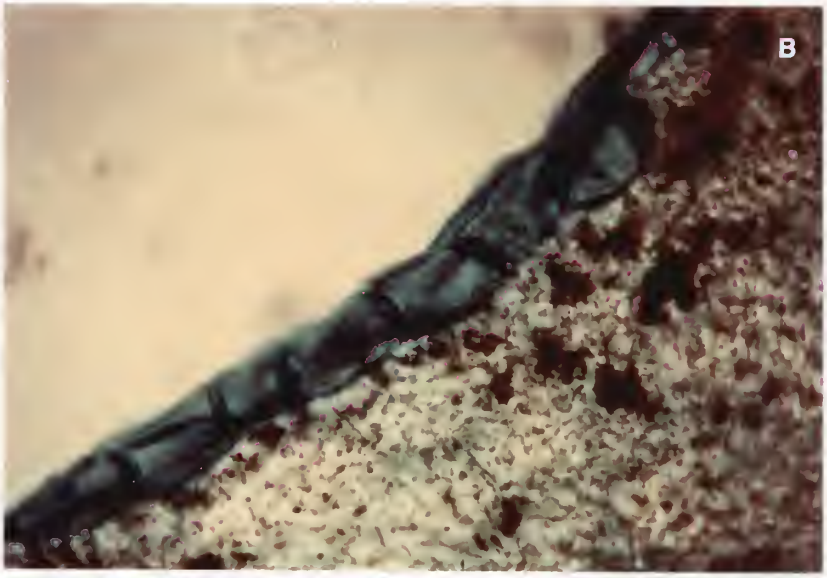

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Table III. Free and Bound Lipids of Pearl Millet Cultivars.

| Sample | Free lipid (%) | Bound lipid (%) |
|---|---|---|
| *Serere 3A | 6.20 | 0.59 |
| *RMPI(S)CI | 6.80 | 0.90 |
| *HMP1700 | 6.92 | 0.74 |
| *HMP550 | 7.08 | 0.82 |
| HMP550S$_1$ LINES | | |
| 114 | 6.33 | 0.88 |
| 120 | 6.21 | 0.77 |
| 134 | 6.45 | 0.65 |
| 149 | 6.40 | 0.77 |
| 155 | 6.46 | 0.73 |
| 160 | 6.14 | 0.75 |
| 179 | 6.50 | 0.75 |
| 180 | 6.32 | 0.74 |
| 189 | 5.87 | 0.76 |
| 191 | 6.19 | 0.78 |
| 193 | 6.07 | 0.64 |
| 196 | 5.66 | 0.57 |
| 200 | 5.88 | 0.70 |
| 201 | 5.95 | 0.73 |
| 218 | 6.03 | 0.68 |
| 220 | 7.03 | 0.64 |
| 240 | 6.56 | 0.67 |

*Bulk populations

Indian cultivars of pearl millet. High correlations were found between free and bound lipids for both line and bulk samples ($r = 0.99$ and $0.98$ respectively).

Lipid contents varied among cultivars. For example, Serere 3A contained significantly ($P < 0.05$) less free and bound lipids than the other cultivars.

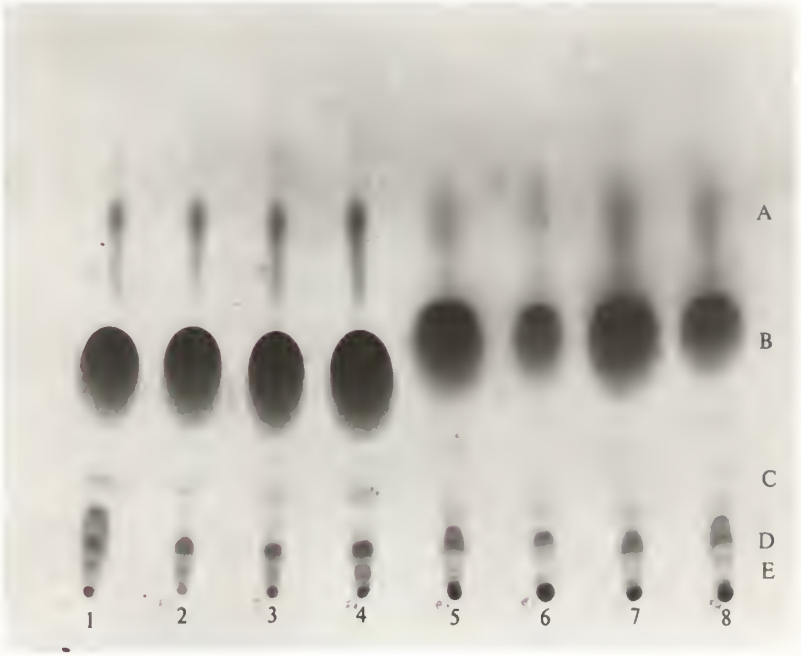
Thin-layer chromatography. Lipid components of free and bound fractions were separeted by thin-layer chromatography; results are shown in Figure 3. Nonpolar lipid components, both free and bound, were tentatively identified as : hydrocarbons and sterol esters, triglycerides, diglycerides, free fatty acids, and sterols. Triglycerides were the major nonpolar components. The $S_1$ lines were similar to the bulk samples in levels of both free and bound nonpolar lipids. In addition, the nonpolar lipid components of the bound and free lipid fractions were similar, which agrees with the work of Badi et al. (1976).

The polar bound lipids were tentatively identified as monogalactosyl glycerides, phosphatidyl ethanolamine, phosphatidyl choline and lysolecithin (Fig. 3). On the other hand, neutral lipids were the only components found in the free polar lipids. Like data reported by other authors (Badi et al., (1976), our data showed that phosphatidyl ethanolamine, digalatosyl diglycerides and phosphatidyl choline were not present in the free polar lipids of cultivars grown in Kansas.

Gas chromatography. Percent fatty acid compositions of free and bound lipids are similar for all pearl millet cultivars (Table IV and V). Pearl millets were high in unsaturated acids.

Fig. 3. TLC of free (1-4) and bound (5-8) nonpolar and polar lipids of pearl millet samples. Tentatively identified as: (A) hydrocarbons and steral esters, (B) triglycerides, (C) diglycerides, (D) free fatty acids, (E) sterols, (F) neutral lipids, (G) mono-galactosyl glycerides, (H) phophatidyl ethanolamine, (I) Phosphatidyl choline, (J) lysolecithin.

NONPOLAR




POLAR

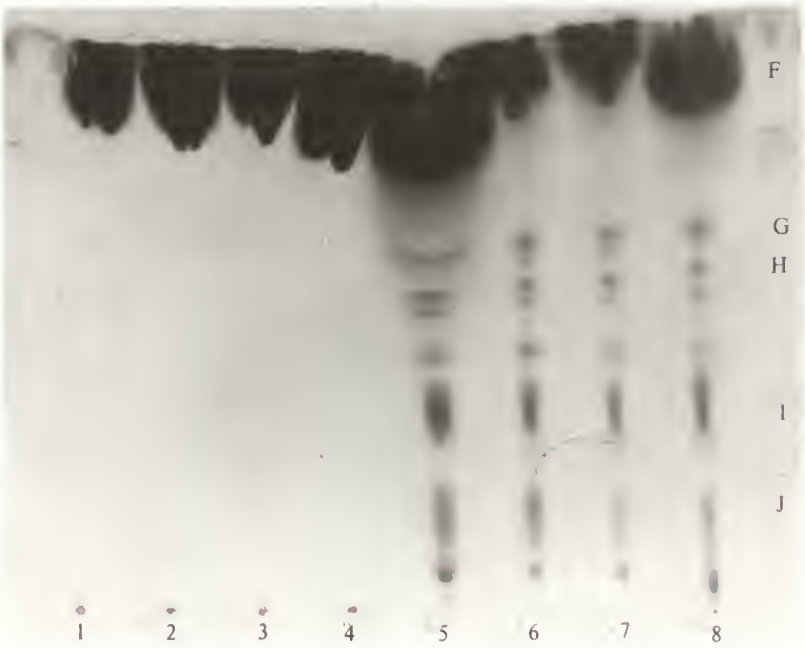


Table IV. Fatty Acid Composition (%) of Free Lipid from Pearl Millet.

| Fatty Acid | Sample | | | |
|---|---|---|---|---|
| | Serere3A | RMPI(S)CI | HMP1700 | HMP550 |
| C14:0 | 0.13 | trace | 0.21 | trace |
| C16:0 | 20.01 | 20.71 | 21.62 | 21.56 |
| C16:1 | 1.10 | 1.10 | 0.93 | 0.96 |
| C18:0 | 8.83 | 10.10 | 6.09 | 7.32 |
| C18:1 | 27.23 | 28.04 | 28.21 | 28.20 |
| C18:2 | 37.83 | 36.72 | 38.02 | 39.78 |
| C18:3 | 0.75 | 1.19 | 0.75 | 1.04 |
| C20:0 | 4.14 | 2.17 | 4.19 | 2.36 |
| C22:0 | trace | trace | trace | trace |

Table V. Fatty Acid Composition (%) of Bound Lipid from Pearl Millet Cultivars.

| Fatty Acid | Sample | | | |
|---|---|---|---|---|
| | Serere3A | RMPI(S)CI | HMP1700 | HMP550 |
| C10:0 | 3.41 | 0.73 | 4.0 | 1.18 |
| Unknown | 0.56 | 1.32 | 0.91 | 0.85 |
| C12:0 | 1.90 | 0.71 | 3.16 | 1.05 |
| C13:0 | 0.33 | 0.51 | 0.52 | 0.62 |
| C14:0 | 0.53 | 0.79 | 0.54 | 0.42 |
| C15:0 | 0.98 | 0.32 | 1.31 | 0.73 |
| C16:0 | 22.12 | 23.21 | 20.80 | 21.11 |
| C16:1 | 0.91 | 1.36 | 0.78 | 0.96 |
| C17:0 | 0.33 | 0.51 | 0.33 | 0.23 |
| C18:0 | 6.47 | 7.91 | 4.79 | 5.12 |
| C18:2 | 26.62 | 29.02 | 32.29 | 29.37 |
| C18:3 | 3.25 | 2.98 | 1.89 | 3.16 |
| C20:0 | 5.12 | 5.09 | 7.10 | 7.81 |
| C24:0 | 7.96 | 7.68 | 5.00 | 7.87 |

Averaging 67.89% of the total fatty acids of the free lipid fraction and 51.70% of the fatty acids of the bound lipid fraction.

Linoleic, oleic and palmitic acids were the principal fatty acids in both free and bound lipids (Figs. 4 and 5) which agrees with the results reported by others (Jellum and Powell, 1971; Rooney, 1978)for the fatty acid composition of millet free lipids. However, Agarwal and Sinha (1964) found that oleic acid (53.84%) was the major acid in their millet samples. Linoleic acid in our Kansas cultivars was slightly lower than that reported in the literature (Rooney, 1978; Jellum and Powell, 1971). Differences in extraction procedure as well as genetic variability may have contributed to the different fatty acid values reported by various authors.

Jellum and Powell (1971) reported that other unidentified fatty acids represented less than 1/2 of 1% of the total fatty acids in pearl millet. We found minute concentrations of minor fatty acids, including acids with odd numbered carbon atoms, in the bound lipid fraction. Short chain fatty acids have also been reported in wheat flour (Coppock et al., 1958), and in lipids of sweet potato (Alexandridis and Lopez, 1979).

In general, when pearl millet are compared with the seven cereal grains reported by Price and Parsons (1975), the millets have the typical fatty acids composition of most seeds, although our millet cultivars had higher stearic acid contents than in other grains.

Changes In The Lipids Of Pearl Millet Meal During Storage

Mycology. Microflora affects the quality of cereal grains

Fig. 4. Gas chromatography curve of fatty acid methyl ester of free lipids from pearl millet meal.

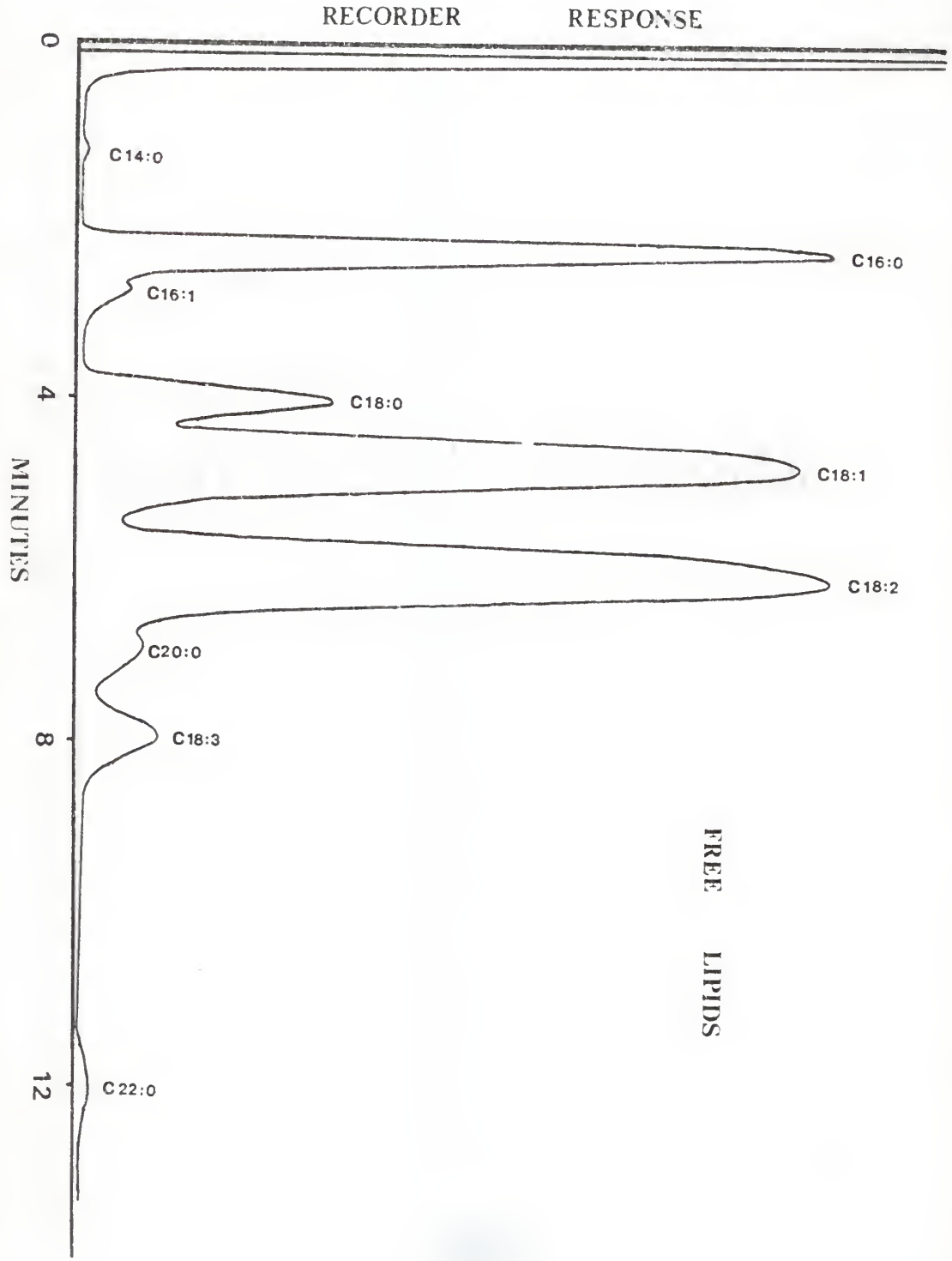
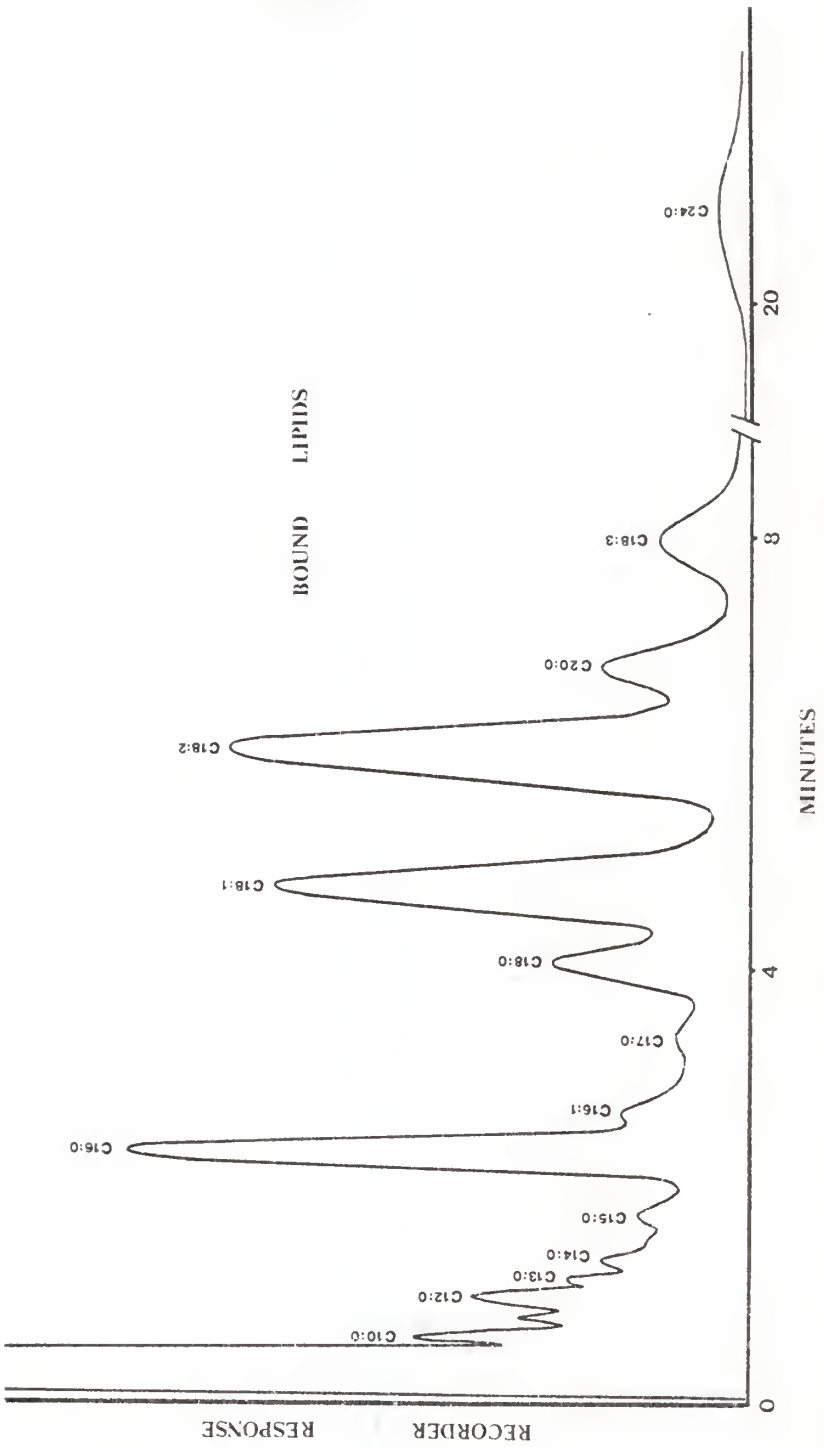

RECORDER RESPONSE
0
4
8
12
MINUTES
C14:0
C16:0
C16:1
C18:0
C18:1
C18:2
C20:0
C18:3
C22:0
FREE LIPIDS

Fig. 5. Gas chromatography curve of fatty acid methyl esters of bound lipids from pearl millet meal.


BOUND LIPIDS
RECORDER RESPONSE
MINUTES
0
4
8
20
C10:0
C12:0
C13:0
C14:0
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C16:1
C17:0
C18:0
C18:1
C18:2
C20:0
C18:3
C24:0

during storage. Fungal growth is often related to increases in fat acidity of stored grains (Baker et al., 1959), and fungal lipases have been shown to be responsible for increased free fatty acids (Goodman and Christensen, 1952; Dinks et al., 1955). Therefore, it is essential that fungal growth be determined in all storage studies involving grains.

Microbiological studies were conducted on our stored millet meals. Samples were found to contain normal levels of field fungi typically found in cereal grains grown in Kansas. Alternaria, Fusarium, and Cladosporium were the major fungi present. Also identified, in small numbers , were the genera Mucor, Phoma, Aspergillus, and Penicillium. These latter storage fungi were present only in small numbers in some samples.

Mold counts declined drastically the first 12 weeks of storage of all samples except those stored at 42 C, 72% R.H.. In the latter storage condition counts increased after 9 weeks, with Penicillium being the predominant species present (Table VI). Thus, in the early weeks of storage, fungal growth probably had little affect on quality deterioration in millet meal.

All field fungi require high moisture conditions for growth, and therefore, they die off rapidly in grains that are held at moisture contents in equilibrium with relative humidities of 70 to 75% (Lutey and Christensen, 1963). On the other hand, storage fungi, such as Penicillium, are capable of growing at equilibrium relative humidities of 70 to 90%.

Sniff test. Sensory tests were conducted to determine if changes in quality could be detected during storage. During

Table VI. Effect of Different Storage Conditions on Mold Counts[1] in Stored Pearl Millet Meal.

| Time (Weeks) | Storage Condition | | |
|---|---|---|---|
| | 19 C 58% R.H. | 27 C 64% R.H. | 42 C 75% R.H. |
| Fresh | 14,800 | 14,800 | 14,800 |
| 3 | 10,800 | 11,250 | 400 |
| 6 | 10,200 | 6,100 | 200 |
| 9 | 10,750 | 4,100 | 1,250 |
| 12 | 5,150 | 3,000 | 31,800 |

[1]Mold counts reported as number of mold colonies per gram of sample.

the taste panel orientation, freshly ground millet (Fresh) and millet meal that had been stored at 42 C for over three months (Aged) were studied by the panel. The aroma of the FRESH sample was describes by the panelists as being fresh, green, and sweet, resembling creamed corn. On the other hand, a completely different aroma profile was given for the AGED sample; panelists described it as sour or acidic in odor, as well as musty, and dry and dusty.

A marked disappearance of sweet odors paralleled the increased appearance of dusty and oxidized characters in stored samples. In addition, the panel noticed that at high temperatures and humidities the time period necessary for detection of AGED characters was shortened. The sniff test data confirmed that there were significant ($P < .01$) changes in the odor response of the panelists after 12 hr. for samoles stored at 42 C, 75% R.H. On the other hand, periods of 60 and 108 hr. were required before significant odor changes were noted by panelists for millet meals stored at 27 C, 64% R.H. and 19 C, 58% R.H., respectively. A series of experiments were conducted to determine if the quality deterioration in millet meal that was noted by panelists was related to changes in lipid composition.

Gas chromatography. Changes in fatty acid composition of free and bound lipids of pearl millet during storage are presented in Table VII and VIII, respectively. There were no qualitative changes in the fatty acid composition of the lipids; however, some changes did occur in the quantities of fatty acids in the free and bound lipid fractions.

Table VII. Changes in Fatty Acid Composition (%) of Free Lipids of Pearl Millet Stored at 42 C, 75% R.H.

| Fatty Acid | Storage Time, Hours | | |
|---|---|---|---|
| | Fresh | 60 | 108 |
| C14:0 | trace | 0.09 | 0.08 |
| C16:0 | 21.56 | 20.83 | 19.19** |
| C16:1 | 0.96 | 1.19 | 1.19 |
| C18:0 | 7.32 | 7.38 | 7.82 |
| C18:1 | 28.20 | 28.10 | 29.85 |
| C18:2 | 39.78 | 38.21 | 37.53 |
| C18:3 | 1.04 | 3.45** | 3.75 |
| C21:0 | 2.36 | 0.78** | 0.61 |
| C22:0 | trace | trace | trace |

** Statistically significant at 0.01 level.

Table VIII. Changes in Fatty Acids Composition (%) of Bound Lipids of Pearl Millet Stored at 42 C, 75% R.H.

| Fatty Acid | Storage Time, Hours | | |
|---|---|---|---|
| | Fresh | 60 | 108 |
| C10:0 | 1.18 | 7.44** | 6.41 |
| Unknown | 0.85 | 0.34 | 0.68 |
| C12:0 | 1.05 | 4.28 | 7.08* |
| C13:0 | 0.62 | 0.65 | 2.20* |
| C14:0 | 0.42 | 0.18 | 0.30 |
| C15:0 | 0.73 | 2.60** | 2.72 |
| C16:0 | 21.11 | 21.65 | 21.19 |
| C16:1 | 0.96 | 0.59* | 0.51 |
| C17:0 | 0.23 | 0.52** | 0.90** |
| C18:0 | 5.12 | 3.27 | 3.23 |
| C18:1 | 19.49 | 17.65 | 15.22** |
| C18:2 | 29.37 | 28.08 | 26.52** |
| C18:3 | 3.16 | 2.13 | 1.32* |
| C20:0 | 7.81 | 1.19* | 0.89 |
| C24:0 | 7.87 | 9.38 | 10.67 |

* Statistically significant at 0.05 level.

** Statistically significant at 0.01 level.

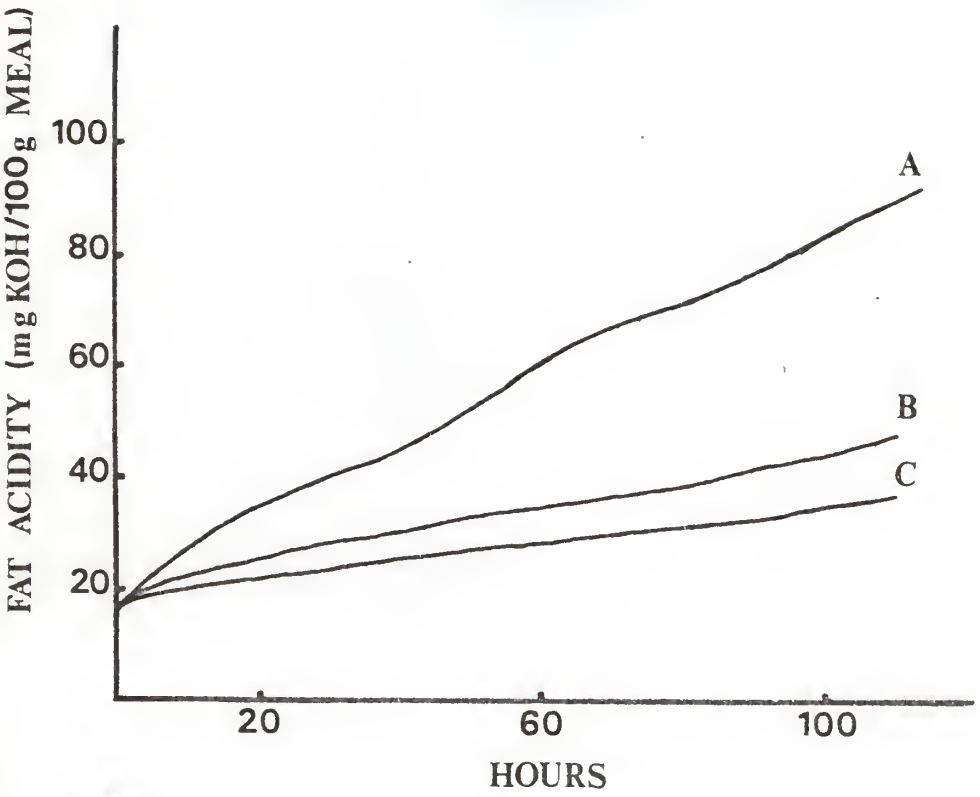
The free lipid fraction showed significant ($P < .01$) increases in linolenic acid after 60 hours storage. On the other hand, decreases in linoleic acid were observed in both free and bound lipids. These data contradict the reports of Nechaev et al. (1973) and Carnovale and Quaglia, (1973). However, those authors did not specify which type of millet they studied.

In contrast to wheat lipids (Daftary and Pomeranz, 1965b), triglycerides of millet appear to be less stable during storage than are the polar lipids (Carnovale and Quaglia, 1973). Triglycerides were reported to be the major nonpolar components of pearl millet lipids. Since more changes in fatty acid composition were observed in bound lipids than free lipids, one could reasonably expect that varieties with higher levels of bound lipids might be more susceptible to changes during storage. Further studies are needed in this area.

Fat acidity. Fat acidity and moisture content of millet meal stored at 42 C, 75% R.H. increased rapidly during the first 108 hours of storage compared to samples stored at 27 C, 63% R.H. or 19 C, 58% R.H. (Fig.6). It is interesting to note that the period for the detection of odor changes in samples from all storage conditions corresponded to a fat acidity value of 30 mg KOH/100g of meal. The high moisture content of the samples stored at 42 C may have accelerated the development of objectionable odors.

Peroxide value. Peroxide values of samples stored at all three conditions steadily increased in the early storage periods, reached a maximum, and then registered a gradual decline (Fig. 7).

Fig. 6. Changes in fat acidity of millet meal during storage.
(A) 42 C, 75% R.H.
(B) 27 C, 64% R.H.
(C) 19 C, 58% R.H.


FAT ACIDITY (mg KOH/100g MEAL)
100
80
60
40
20
A
B
C
20
60
100
HOURS

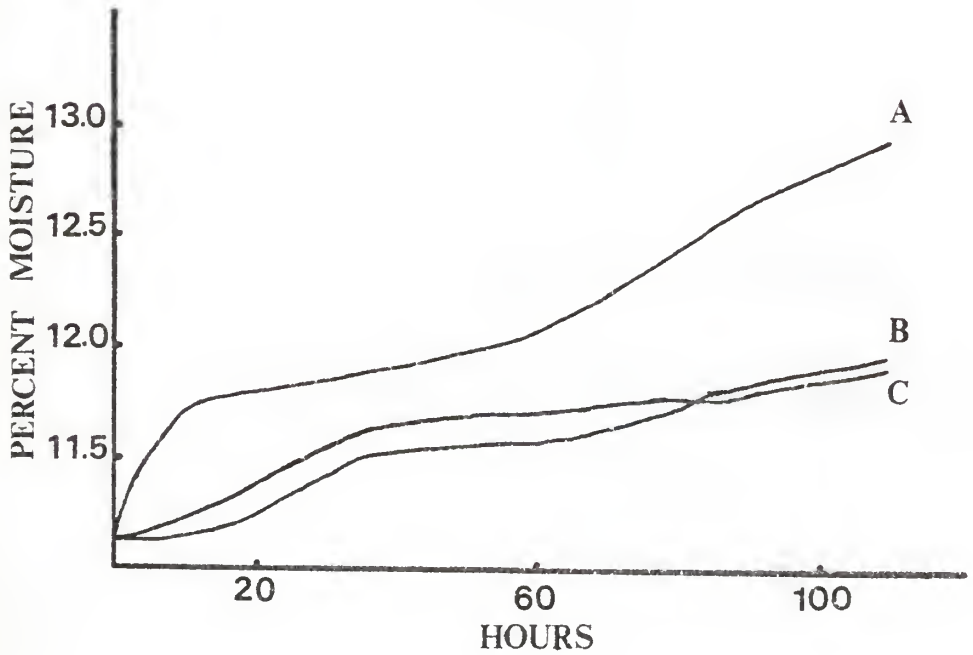
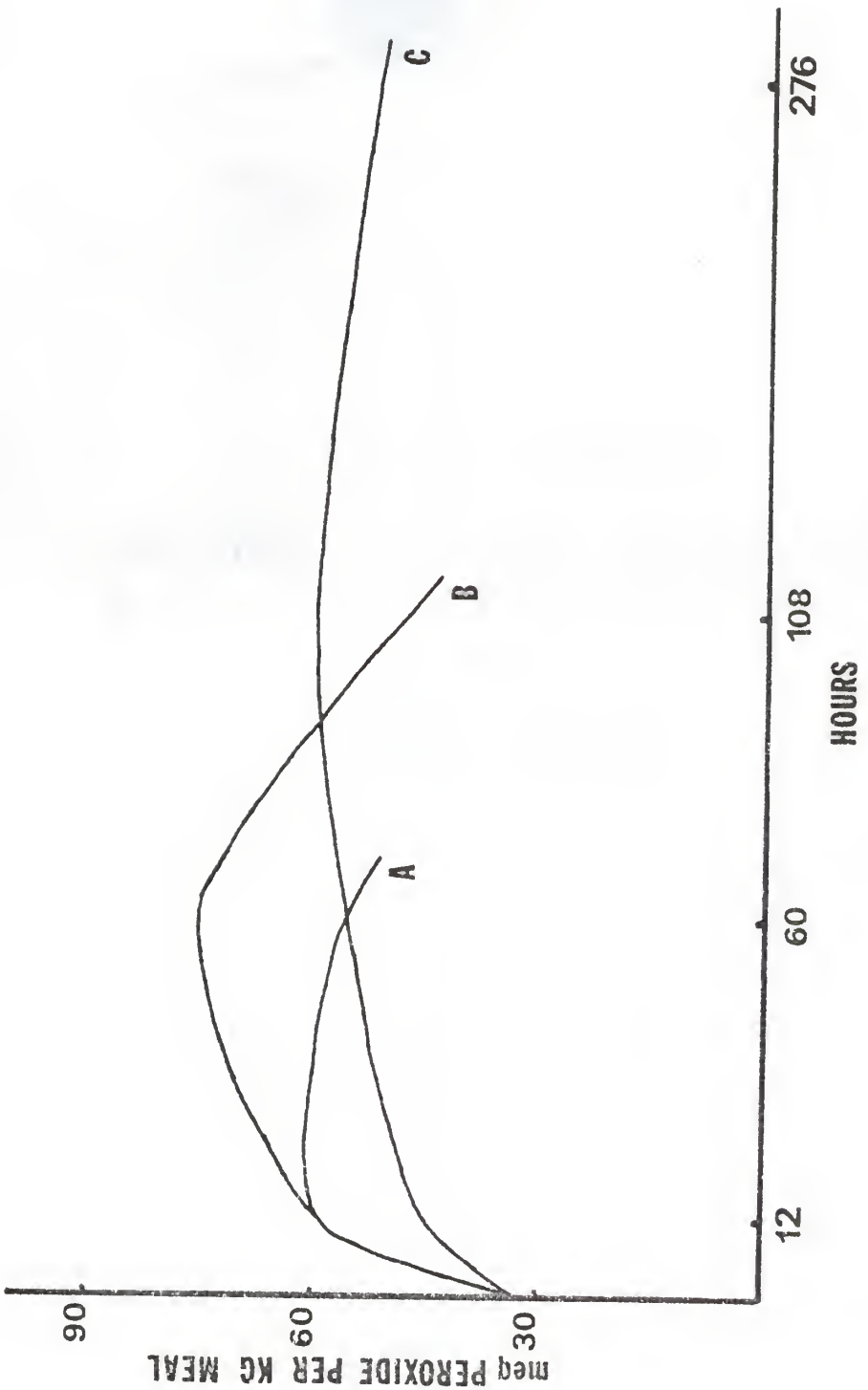

PERCENT MOISTURE
13.0
12.5
12.0
11.5
A
B
C
20
60
100
HOURS

Fig. 7. Changes in peroxide value of millet meal during storage.
(A) 42 C, 75% R.H.
(B) 27 C, 63% R.H.
(C) 19 C, 58% R.H.

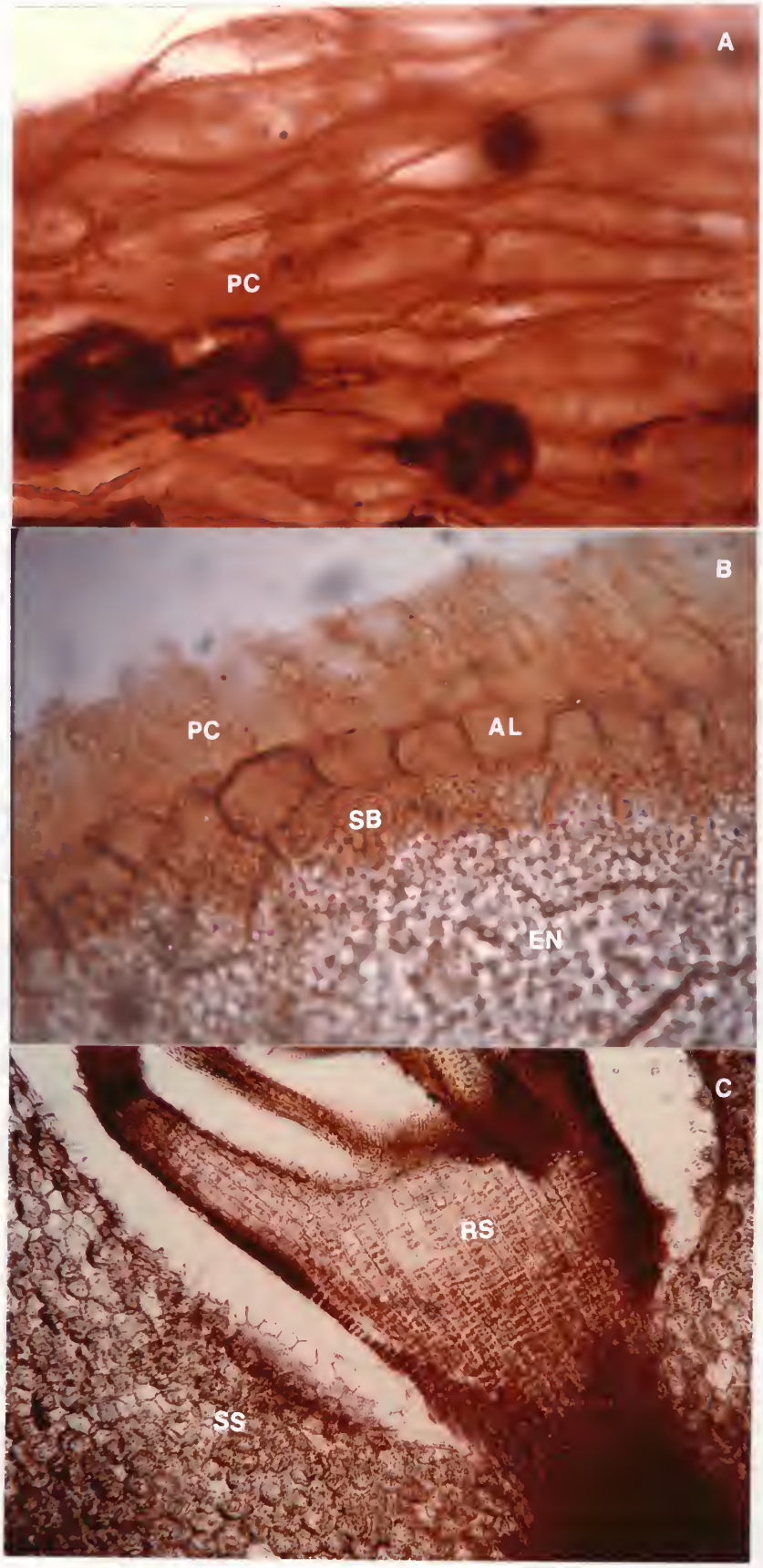

meq PEROXIDE PER KG MEAL
90
60
30
12
60
108
276
HOURS
A
B
C

However, the rate of change in peroxide value differed for each storage treatment. Samples stored at 42 C, 75% R.H. terminated the induction period earier than those stored at 27 C, 64% R.H. or at 19 C, 58% R.H. The end of the induction period coincided with the time after storage when panelists first detected an odor change.

Histology. Féron and Bouquet (1948) reported that lipase was present in millet seeds. Histochemical localization of lipase by Tween and β-naphthyllaurate method demonstrated that some millet lipase activity was present in all portions of the kernel. Further studies with thin sections indicated that the activity was located mainly in the germ, pericarp, aleurone, and sub-aleurone layers (Fig.8). Slight activities were observed in the areas below the sub-aleurone layer as well as in the other parts of the endosperm.

Although the β-naphthyllaurate method is for non-specific esterases, in this study it gave results identical to the Tween method for lipase activity.

Fig. 8. Photomicrographs of (A) Pericarp, (1,550X), (B) Outer layers, (1,260X), (C) Germ, (375X). PC = pericarp, AL = aleurone, SB = sub-aleurone, EN = endosperm, SS = sheath of shoot, RS = Rudimentary shoot.


A
PC
B
PC
AL
SB
EN
C
RS
SS

# SUMMARY AND CONCLUSIONS

Pearl millet grain appears to be higher in lipid content than most other cereals. The percent total lipid (free plus bound lipids) extracted from the grain ranges from 6.23% to 7.90%. Triglycerides are the major nonpolar components, and neutral lipids are the major polar components. In addition, millet lipids contain high levels of unsaturated fatty acids.

Studies on changes in millet lipids duting storage indicated that there were no qulaitative changes in the fatty acid composition of free and bound lipid fractions. However, some changes did occur in the fatty acid levels. More changes were observed in the bound than free lipids. Thus, one could reasonably expect that varieties with higher levels of bound lipids might be more susceptable to changes during storage.

Fat acidity and peroxide values suggested that both hydrolytic and oxidative changes occurred in lipids during storage. Undoubtedly, increases in fat acidity were due to lipase activity. Histochemical studies demonstrated that the bulk of the lipase activity was located in the germ and covering layers; most lipids were also located in those areas. If the milling process could completely separate the endosperm from the germ and outer layers, lipid degradation may still occur in the stored milled product, because low levels of lipase activity and lipid content were present in the endosperm.

Sensory evaluation of stored millet meal indicated that at high temperatures and humidities the time period necessary for detection of changes in odor was shortened. It is interesting

to note that the time after storage when panelists first detected an odor change corresponded to the termination of the peroxide induction period, as well as to a fat acidity value of 30 mg KOH/100 g meal. Sensory evaluation appears to be a very sensitive method for assessment of millet meal deterioration during storage.

# REFERENCES

AACC. 1962. "Cereal laboratory methods", 7th ed. American Association of Cereal Chemists. St. Paul, Minnesota.

Agarwal, P.N. & Sinha, N.S. 1964. Chemical composition of the oil from the seeds of Pennisetum typhoideum (Bajra). Indian J. Agrom. 63:288.

Alexandrisdis, N. & Lopez, A. 1979. Lipid changes during processing and storage of sweet potato flakes. J. Food Science. 44:1186.

AOAC. 1960. "Official methods of analysis" 9th ed. Association of official Agrocultural Chemists. Washington, D.C.

Badi, S.M., Hoseney, R.C., & Casady, A.J. 1976. Pearl millet I. Characterization by SEM, amino acid analysis, lipid composition, and prolamine solubility. Cereal Chem. 53:478.

Baker, D., Neustdat, M.H. & Zeleny, L. 1959. Relationships between fat acidity values and types of damage in grain. Cereal Chem. 36:308.

Bancroft, J.D. 1975. "Histochemical Technique". 2nd ed. Butterworth & Co. Ltd. London.

Belova, Z.A., Nechaev, A.P., & Severineu, S.M. 1970. Content of fatty acids in the lipids of millet. Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol. 1:32.

Billek, G., Guhr, G. & Waibel, J. 1978. Quality assessment of used frying fats: A comparison of four methods. J. AOCS. 55:728.

Burton, G.W., & Fortson, J.C. 1966. Inheritance and utilization of five dwarfs in pearl millet (Pennisetum typhoides) breeding. Crop. Sci. 6:69.

Burton, G.W., Wallace, A.T. & Rachie, K.E. 1972. Chemical composition and nutritive value of pearl millet (Pennisetum typhoides (Burm.) Stapf and E.C. Hubbard) grain. Crop Sci. 12:187.

Busare, N.N., Denisenko, Ya. I., & Nechaev, A.P. 1974. Composition of fatty acids in free, bound and firmly bound lipids of millet. Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol. 2:132.

Carnovale, E., & Quaglis, G.B. 1973. Influence of temperature and humidity controlled storage on the chemical composition of milling products from millet. Ann. Technol. Agric. 22:371.

Casady, A.J., Paulsen, R.C., Hoseney, R.C., & Webster, O.J. 1976. Registration of pearl millet germ plasm. Crop. Sci. 16:745.

Casey, P., & Lorenz, K. 1977. Millet-functional and nutritional properties. The Baker Digest. 2:45.

Coppock, J.B.M., Fisher, N., & Ritchie, M.L. 1958. The role of lipid in baking. V. Chromatographic and other studies. J. Sci. Food Agric. 9:498.

Daftary, R.D., & Pomeranz, Y. 1965a. Changes in lipid composition in maturing wheat. J. Food Sci. 30:577.

Daftary, R.D., & Pomeranz, Y. 1965b. Changes in lipid composition in wheat during storage deterioration. J. Agr. Food Chem. 13:442.

Dirks, B.M., Boyer, P.D., & Geedes, W.F. 1955. Some properties of fungal lipase and their significance in stored grain. Cereal Chem. 32:356.

Dittmer, J.C., & Lester, R.L. 1964. A simple specific spray for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res. 5:126.

Duncan, D.B. 1955. New Multiple range and multiple F tests. Bimetrics. 11:1.

Féron, F., & Bonquet, F. 1948. The presence of lipase in millet flour. Annals de la Nutr. et de Lalimen. 2:537.

Freeman, J.E., & Bocan, B.J. 1973. Pearl millet : A potential crop for wet milling. Cereal Sci. Today. 3:69.

Goodman, J.J., & Christensen, C.M. 1952. Grain storage studies. XI. Lipolytic activity of fungi isolated from stored corn. Cereal Chem. 29:299.

Jellum, M.D., & Powell, J.B. 1971. Fatty acid composition of oil from pearl millet seed. Agro. J. 63:29.

Lutery, R.W. & Christensen, C.M. 1963. Influence of moisture content, temperature, and length of storage upon survival of fungi in barley kernels. Phytophth. 53:713.

Mahadevappa, V.G., & Raina, P.L. 1978. Lipid profile and fatty acid composition of finger millet (Eleusine coracana). J. Food Sci. Tech. 15:100.

Miller, D.F. 1958. "Composition of Cereal Grains and Forages" Publication 585, National academy of Science---National research council. Washington, D.C.

Nechaev, A.P., Busareva, N.N., Denisenko, Ya. I., & Kuznetsov, D.I. 1973. Changes in the fatty acid composition of millet lipids during storage. App. Biochem. Microbio. 9:617.

Price, P.B., & Parsons, J.C. 1975. Lipids of seven cereal grains. JAOCS. 52:490.

Pruthi, T.D., & Bhatia, I.S. 1970. Lipids in cereals. I. Pennisetum typhoideum. J. Sci. Food Agric. 21:419.

Rooney, L.W. 1978. Sorghum and pearl millet lipids. Cereal Chem. 55:484.

Sastry, B.S., Ramakrishna, M., & Raghavendra Rao, M.R. 1977. Histochemical localization of lipase in the rice grain. J. Food Sci. Tech. (Mysore) 14:273.

Sharma, K.P., & Goswami, A.K. 1969. Chemical constants of lipid content of high yielding varieties and hybrids of Bajra (Pennisetum Typhoideum) flour. J. Nutr. & Dietet. 6:316.

Snedecor, G.W., & Cochran, W.G. 1967. "Statistical Methods", 6th ed. The Iowa State University Press, Ames, IA.

Stahl, E. 1969. Thin-layer chromatography. SpringerVerlag. New York. 2nd ed.

Takagi, T., Mitsuno, Y., & Masumura, M. 1978. Determination of peroxide value by the colorimetric iodine method with protection of iodide as cadmium complex. Lipid. 13:147.

Thiam, A.A., Drapron, R., Richard-Molard, D. 1976. Cauale D'Alteration des Farines de Mil et de sorgh. Ann. Technol. Agri. 25:253.

# PEARL MILLET LIPIDS:COMPOSITION AND CHANGES DURING STORAGE

by

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The lipid composition of pearl millet cultivars was studied.. The percent free lipid extracted from pearl millet ranged from 5.66% to 7.08%; bound lipids ranged from 0.57% to 0.90%. Non-polar lipid components tentatively identified by thin-layer chromatography were: hydrocarbons, sterol esters, triglycerides, diglycerides, free fatty acids, and sterols. Triglycerides were the major nonpolar component. The polar bound lipids were tentatively identified as mongalactosyl glycerides, phosphatidyl ethanolamine, phosphatidyl choline, lysolecithin and neutral lipids.

Pearl millet cultivars were found to be high in unsaturated acids. Linoleic, oleic and palmitic acids were the main fatty acids in both free and bound lipids. Minute concentrations of minor fatty acids were present in only the bound lipid fraction.

The lipids of pearl millet were concentrated mainly in the germ and the covering layers. In addition, the endosperm portions bound small quantities of lipid stain.

Changes in millet lipids during storage were investigated. Tests were conducted, after designated storage periods, on pearl millet meal stored under the following conditions: 19C, 58%R.H.; 27C, 64% R.H.; and 42C, 75% R.H..

Gas chromatography studies showed that the free lipid fraction increased significantly ($P<.01$) in linolenic acid during storage. On the other hand, decreases in linoleic acid were observed in both free and bound lipids.

Fat acidity and peroxide value determinations indicated that deteriorative changes in ground millet during storage were

due, in part, to hydrolytic and oxidative changes in the lipid domponents. The mediator in hydrolytic rancidity is lipase which was found to be located mainly in the germ, pericarp, and sub-aleurone layers.

Fungal levels decreased in the 6 weeks storage period when fat acidity and peroxide values were determined. Therefore. fungal growth does not appear to be responsible for changes in lipid components.

A trained sensory panel detected a change in the odor of stored millet samples after 108 hr., 60 hr., and 12 hr., respectively, for 19C, 27C, and 42C storage conditions. The period of odor detection in each condition corresponded to a fat acidity of about 30 mg KOH/100 g meal. Therefore, sensory evaluation appears to be a good indicator of early deteriorative changes in millet meal.