Factors Affecting Rancidity in Ground Pearl Millet (*Pennisetum americanum* L. Leeke)[1]

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ABSTRACT

Increases in fat acidity and peroxide value show that pearl millet rapidly becomes rancid after it is ground. Fat acidity and peroxide value of stored whole pearl millet grain did not vary significantly over the same time period, showing that deterioration occurs more rapidly in 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. However, millet meal stored in cotton bags showed no peroxide accumulation. This may suggest that the procedure measures unknown volatiles and not peroxides. Free fatty acids and fat-acidity data indicated that all the acidity produced during storage was the result of free fatty acids. The proportions in which free fatty acids were released were similar to those found in total fatty acids. Therefore, the hydrolytic action of lipase appeared to be random. 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 of storage and then leveled off. No explanation is offered for the leveling off. Hexanal is a major product of oxidative degradation of lipids. No hexanal was produced in millet meal stored for 15 days in polyethylene bags. As in other cereals, hexanal increased after 21 days of storage. 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 may not be the result of the classical oxidative degradation of lipids. Reconstitution studies of millet meal and whole wheat meal and their lipids showed that 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 samples containing wheat lipids than for those containing millet lipids. Lipase appeared to be more active in defatted millet than in defatted wheat.

Pearl millet (*Pennisetum americanum* L. Leeke) has the largest seeds and is the most widely grown millet (Hoseney et al 1982). Millet does not store well after grinding, but the cause of the instability is not known. Carnovale and Quaglia (1973) found no change in the amino acid composition during storage, but the total fatty acids showed significant quantitative changes in composition. They attribute those changes to hydrolytic decomposition of lipids and, to a lesser extent, to oxidation.

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

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

Millet is probably the cereal grain that most rapidly develops objectionable odors and flavors after grinding. There are at least four possible reasons: the fat content is high; the degree of unsaturation of fatty acids is higher than in other major cereals; the antioxidants occurring naturally in other cereal grains are lacking in millet; or enzymatic activity is greater.

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 fatty acids than other cereals (Jellum and Powell 1971, Rooney 1978, Lai and Varriano-Marston 1980a). The measurement of lipoxygenase activity and the UV spectrophotometry of the lipoidal extracts led Thiam et al (1976) to conclude that the enzymatic oxidation of lipids was practically nonexistent. The same authors attributed this to the presence of phenolic compounds acting as antioxidants. Thus, the second, third, and fourth reasons mentioned above did not appear true.

The objective of this study was to determine what factors contribute to the development of rancidity in millet meal during storage.

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MATERIALS AND METHODS

Materials

Pearl millet samples used in this study were Serere 3A grown at Hays, KS, in 1979, HMP 1700 grown at Hays, KS, in 1980, and a sample obtained from a blend of equal quantities of 13 different entries grown at Minneola, KS, in 1980. The hard red winter wheat used was a blend of varieties.

Three different grinders were used: the UDY laboratory mill with a screen opening of 400 μm, 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 having the equivalent of a 375-μm screen opening. Both polyethylene bags and cotton bags were used for storage.

Methods

Extractable fat was determined on a Goldfisch apparatus by extraction with petroleum ether (bp 38–55°C) for 4 hr. Moisture content was determined by approved AACC method 44-15A (1962). The quantitative determination of molds in millet meal was as described by Christensen (1946). The method of Takagi et al (1978) was used to determine peroxide value. The average standard deviation was 0.33. Fat acidity was determined by AACC method 0203, the rapid method for corn.

Total fatty acids (TFA) from free lipids were analyzed by gas-liquid chromatography (GLC), using the method of Kates (1964) of transesterification of fats and oils. Free fatty acids (FFA) from free lipids were quantitatively isolated by thin-layer chromatography (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 plates 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 acids recovered by filtration through a sintered-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.

Fatty acid methyl esters were determined with a Hewlett Packard 5750 gas chromatograph equipped with a flame-ionization detector (FID). The stainless steel column was 6 ft long and had an o.d. of ⅛ in. It was packed with 10% SP 2330 chromosorb WAW 100/120 mesh (Supelco Inc.). The temperature of the injection port was 270°C, that of the FID was 260°C, and that of the column was 210°C. Nitrogen carrier gas-flow rate was 25 ml/min. The sample volume injected was 1 μl.

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. 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 Fritsch and Gale (1977).

Hexanal was determined with a Hewlett Packard 5750 gas-liquid chromatograph equipped with an FID. The aluminum column was 10 ft long and had an o.d. of ¼ in. It was packed with 10% silicone OV-101 chromosorb WAW 60/80 mesh (Supelco Inc.). The temperature of the column was 100°C, the injection port temperature 200°C, and the FID 150°C. Nitrogen carrier gas-flow rate 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. Weight (ppm) of hexanal in the sample was determined by multiplying the peak area ratio of hexanal to the internal standard heptanone by a calibration factor.

Defatting Millet and Wheat Meals

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 hr in a Soxhlet apparatus. Extracted millet lipid and wheat lipid were 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% for pearl millet meal and 1.5% for whole wheat meal.

Reconstitution

Millet lipids (ML) were reincorporated into the defatted millet meal and defatted whole wheat meal so that the final fat content of the reconstituted meals was comparable to that found in whole wheat meal. 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 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 (DM + ML), defatted millet meal reconstituted with wheat lipids (DM + WL), whole wheat meal (W), defatted whole wheat meal reconstituted with wheat lipids (DW + WL), and defatted whole wheat meal reconstituted with millet lipids (DW + ML), were stored separately in polyethylene bags in an incubator at 35°C for one month.

A second reconstitution experiment with samples of wheat and millet but with lipids incorporated into the defatted residues, as shown in Table I, was performed. All the samples were stored at 30°C, 60% rh, in cotton bags for five days. Samples were taken every day, and fat acidity determined in duplicate.

TABLE I
Reconstitution Study on Effect of Fat Content on Fat Acidity[a]

	M	DM + ML	DM + WL	W	DW + WL	DW + ML
Experiment 1						
Defatted sample wt (g)	···	32.4	32.4	···	32.9	32.9
Fat wt (g)	···	2.6	0.64	···	0.64	2.6
Percent fat of sample	5.55	7.43	1.94	1.82	1.97	7.42
Experiment 2						
Defatted sample wt (g)	···	36.75	36.75	···	37.77	37.77
Fat wt incorporated (g)	···	2.14	2.14	···	0.65	0.65
Percent fat of sample	5.36	5.50	5.50	1.72	1.69	1.69

[a] M = millet meal, ML = millet lipids, W = wheat meal, WL = wheat lipids, DM = defatted millet meal, and DW = defatted wheat meal.

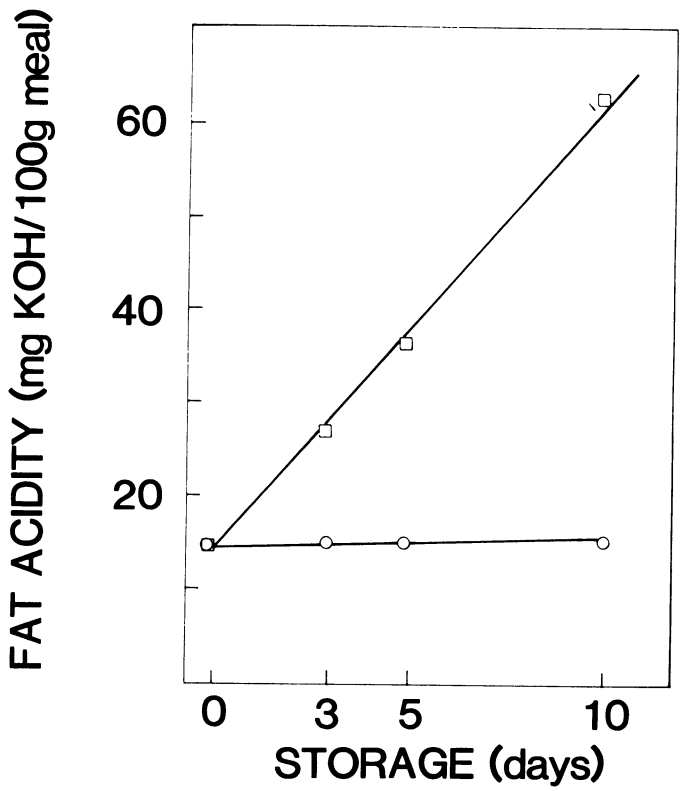

Fig. 1. Fat acidity of millet meal (Serere 3A) and whole millet grain (Serere 3A) stored at 25°C in polyethylene bags; O = whole grain, □ = ground grain.

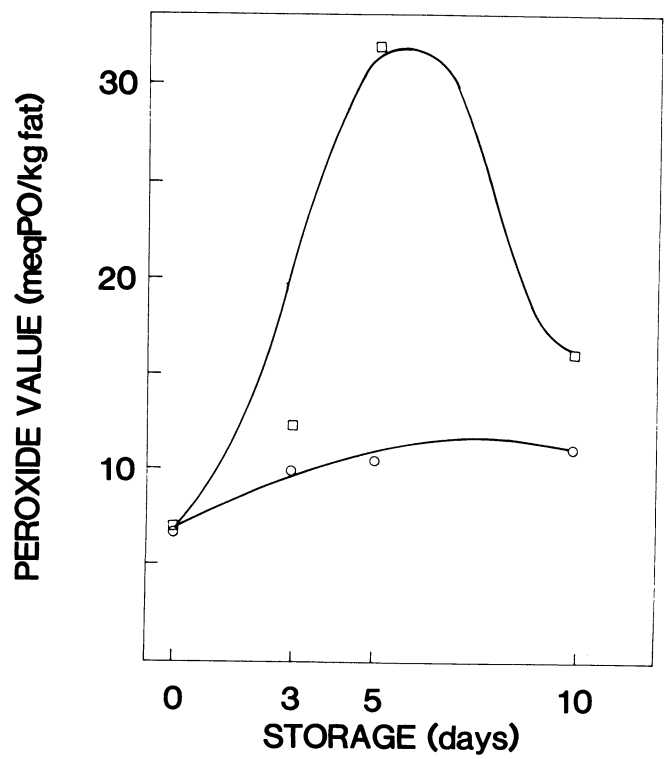

Fig. 2. Peroxide values for millet meal (Serere 3A) and whole millet grain (Serere 3A) stored at 25°C in polyethylene bags; O = whole grain, □ = ground grain.

Effect of Atmospheric Oxygen on Stability of Millet Meal

Two-quart, air-tight glass jars with wide mouths were used to store millet meal with 11.0% moisture content at 30° C. The 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 absorbed by the sample was eliminated when the air was evacuated from the jar and replaced with nitrogen.

Fat acidity and peroxide value were determined, in duplicate, for each sample on 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.

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 sizes on the stability of millet meal during storage. As shown in Figs. 1 and 2, fat acidity and peroxide value did not change significantly in the whole grain. But in the ground 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 during the storage period. Peroxide value increased during the first five 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).

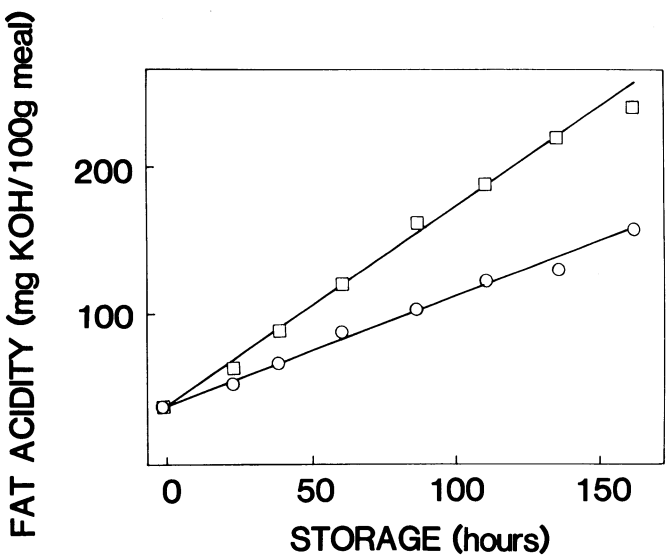

Fig. 3. Fat acidity of millet meal (HMP 1700, ground in a Ross mill) stored at 27° C, 66% rh, in cotton and polyethylene bags; □ = cotton bags, O = polyethylene bags.

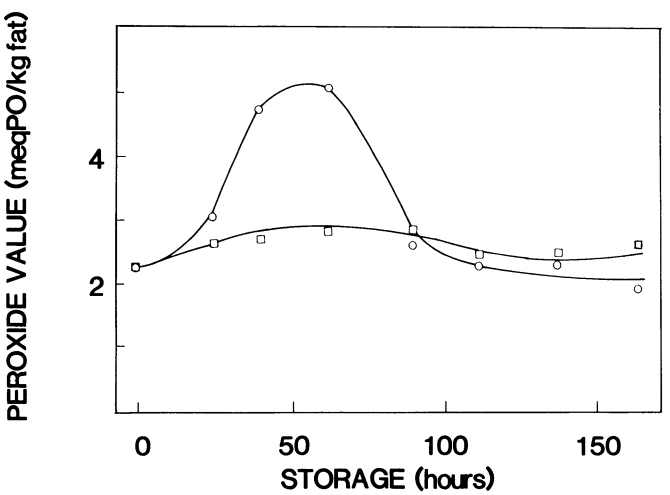

Fig. 4. Peroxide value of pearl millet (HMP 1700) stored at 27° C, 66% rh, in polyethylene bags (O) and cotton bags (□).

Effects of Varying Container Types on Storability of Millet Meal

Millet meal (HMP 1700) was stored at 27° C, 66% rh, in cotton and plastic bags. Fat acidity increased at different rates for samples stored in both plastic and in cotton bags (Fig. 3). The initial moisture content of the millet meal was 9.5%. After 112 hr of storage, it was 9.8% for the samples stored in plastic bags and 12.2% for the samples stored in the cotton bags. The fat acidity increased more rapidly in the sample stored in the cotton bags, presumably because of the higher moisture level for those samples. The hydrolytic action of lipases, as measured by fat acidity, is known to be more rapid at higher moisture levels.

The peroxide values (Fig. 4) for samples stored in plastic bags showed a different pattern than values for samples stored in cotton bags. In plastic bags, the peroxide value increased up to 5.1 meq peroxide value per kilogram of fat after 62 hr of storage and then decreased to near its original value. The peroxide value of millet meal stored in cotton bags remained constant throughout the storage period.

The reason for the difference in the two containers is not clear. Both the plastic and cotton bags were stored in a ventilated atmosphere. The cotton bags allowed air to flow through the samples, and this swept away volatile compounds. Peroxides are not volatile and thus would not be lost. There appears to be no logical explanation for why peroxides would form in the plastic bag and not in the cotton one. Perhaps the most logical explanation of the data is that the procedure measures some volatile material but not peroxides.

TABLE II
Total Fatty Acids of Whole Millet Meal Stored at 11.0% Moisture in a Polyethylene Bag at 35°C

Fatty Acids	Control (%)	Millet Stored for 5 Days (%)	10 Days (%)	15 Days (%)	Average Standard Deviation (%)
16:0	18.94	17.08	16.63	19.70	0.56
18:0	6.25	6.45	6.02	6.28	0.35
18:1	28.26	31.37	29.45	26.41	4.03
18:2	44.25	42.85	45.50	44.44	2.39
18:3	2.28	2.24	2.38	3.14	0.37

TABLE III
Total Fatty Acids of Whole Millet Meal Stored at 11.0% Moisture in a Cotton Bag at 30°C[a]

Fatty Acids	Control (%)	Millet Stored for 5 Days (%)	10 Days (%)	15 Days (%)	Average Standard Deviation (%)
16:0	18.94	17.15	18.82	18.25	0.37
18:0	6.25	6.08	5.96	5.39	0.33
18:1	28.26	29.32	27.13	28.00	3.87
18:2	44.25	45.01	45.63	45.85	1.45
18:3	2.28	2.42	2.46	2.49	0.25

[a] Recovery was determined as the ratio of amount of fat esterified to the total fatty acid methyl esters calculated from the chromatograms, ranged from 93 to 98%.

TFA and FFA in Stored Millet Meal

TFA. The TFA results are shown in Tables II and III. The data indicate that there is no significant change in TFA under either storage condition. Had significant amounts of oxidative rancidity occurred, the level of total unsaturated fatty acid would have been lower.

FFA. The results for FFA are shown in Tables IV and V. 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. The proportion of free fatty acids released were similar to those found in the total fatty acids, so lipase action was probably random.

Effects of High Humidity on the Stability of Millet Meal

Although millet is grown mostly in the semiarid tropics, storage conditions with humidity as high as 95% have been reported (Carnovale and Quaglia 1973, Thiam et al 1976). As a comparison, we also studied the storage of millet meal under high humidity.

When the meal was stored at 30° C and 80% rh, the moisture content increased during the first 60 hr, then leveled off at 14.5%. This 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 decreases the amount of oxidative deterioration, which agrees with the findings of Carnovale and Quaglia (1973) and Lai and Varriano-Marston (1980b).

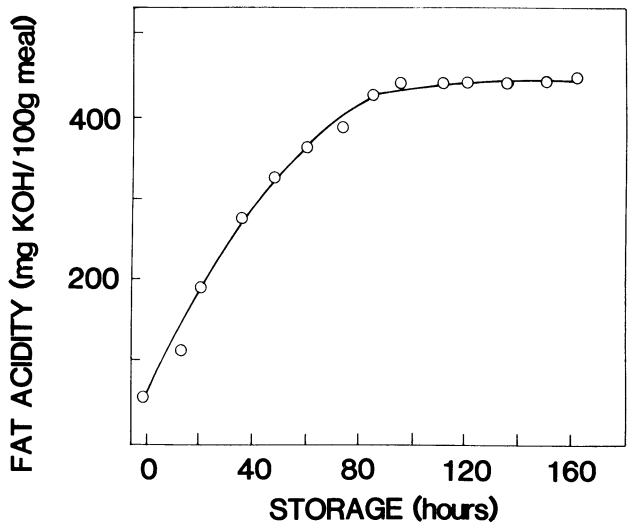

Fig. 5. Fat acidity of millet meal (HMP 1700) during storage at 30° C, 80% rh, in cotton bags.

Fat acidity increased during the first 90 hr of storage and then leveled off at 450 mg of KOH per 100 g of meal (Fig. 5). The reason the fat acidity levels off is not clear. Goodman and Christensen (1952) reported that several fungi could metabolize appreciable amounts of fatty acids. Under similar storage conditions (30° C, 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 FFA. This leveling off of fat acidity at 450 mg of KOH/100 g of meal (dry basis) could be explained by the consumption of FFA by the microflora. However, mold counts on the fresh sample and after one week of storage did not indicate a significant change during the storage period. The fresh sample gave a mold count of 2,600 colonies per gram of millet meal; after seven days, the value was 2,300 colonies per gram of millet meal. Therefore, another explanation for the leveling off of fat acidity must be sought.

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. The content of hexanal, one of the major products of oxidative degradation of lipids, was run every day over a 20-day period. The results showed no increase of hexanal during the storage period.

In another study, we compared oxidation in pearl millet meal during storage with whole wheat meal stored under the same conditions. Whole wheat meal gave higher amounts of hexanal than did millet meal samples during the first three weeks of storage (0.2 versus 0.1 ppm). In both whole wheat and millet meal samples, the amount of hexanal produced was very low during three weeks of storage. The trend of hexanal formation observed here is similar to that reported in the literature (Labuza 1971).

Although the initial hexanal content of millet meal was half that of wheat, the amount of hexanal in millet meal after five weeks is very close to that of wheat. This indicates that, after the induction period, millet meal may produce hexanal more quickly than does wheat.

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 VI). The fat acidity values for the two samples were essentially identical, indicating that atmospheric oxygen has no effect on fat acidity values.

TABLE IV
Free Fatty Acids (FFA) of Whole Millet Meal Stored at 11.0% Moisture in a Polyethylene Bag at 35°C[a]

	0 Days		5 Days		10 Days		15 Days	
FFA	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 FFA 18:1, 18:2, and 18:3 are not given because significant loss occurred during separation on thin-layer chromatography plate due to oxidation.

TABLE V
Free Fatty Acids (FFA) of Whole Millet Meal Stored at 11.0% Moisture in a Cotton Bag at 35°C[a]

	0 Days		5 Days		10 Days		15 Days	
FFA	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 FFA 18:1, 18:2, and 18:3 are not given because significant loss occurred during separation on thin-layer chromatography plate due to oxidation.

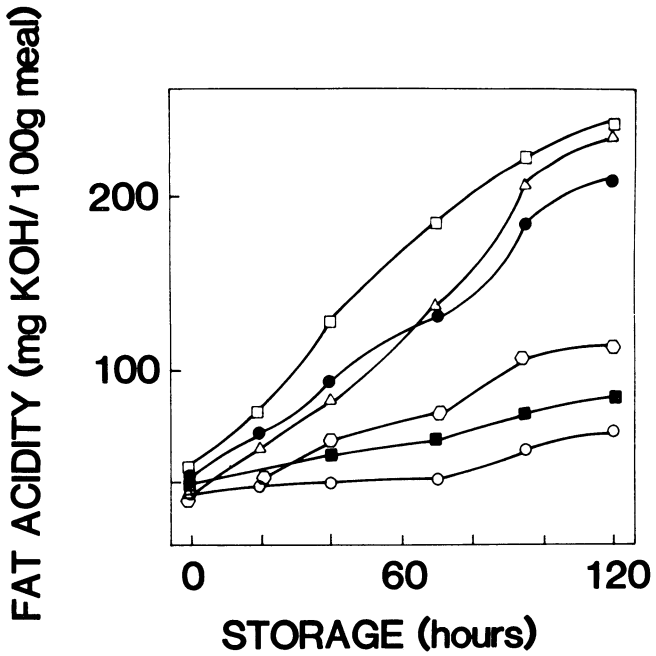

Fig. 6. Fat acidity during storage of reconstituted wheat and millet. Effect of lipid content: Δ = millet meal (M), 5.5% lipid; □ = defatted millet meal + 7.42% millet lipids (DM + ML); ⬡ = defatted millet meal + 1.97% wheat lipids (DM + WL); O = whole wheat flour (W), 1.82% lipid; ■ = defatted wheat + 1.97% wheat lipids (DW + WL); ● = defatted wheat + 7.42% millet lipids (DW + ML).

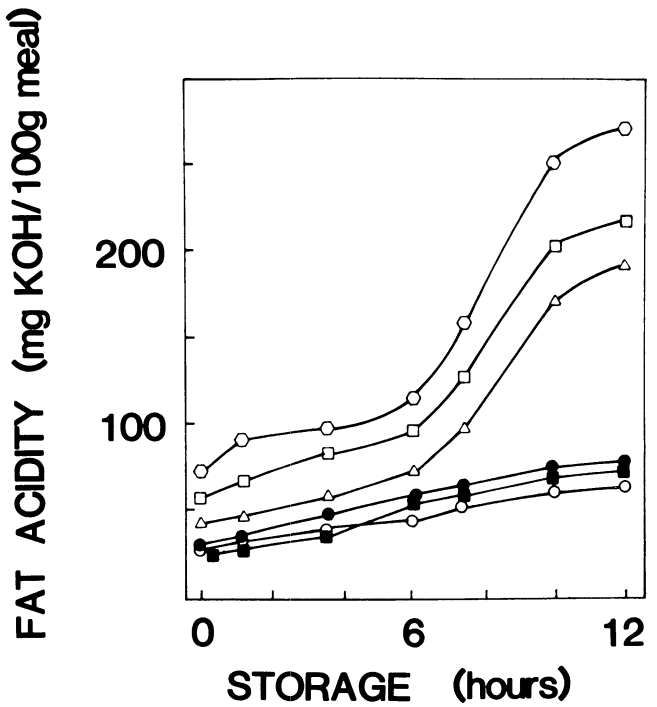

Fig. 7. Fat acidity during storage of reconstituted wheat and millet. Effect of lipid quality: Δ = millet meal (M), 5.36% lipid; □ = defatted millet meal + 5.50% millet lipids (DM + ML); ⬡ = defatted millet meal + 5.50% wheat lipids (DM + WL); O = whole wheat flour (W), 1.72% lipid; ● = defatted wheat + 1.69% wheat lipids (DW + WL); and ■ = defatted wheat + 1.69% millet lipids (DW + ML).

Effect of the Quantity and Quality of Lipid Fraction on Storability of Millet Meal

Both millet meal and wheat meal were defatted with petroleum ether. The lipids were recovered, and thus each defatted meal could be reconstituted with its own lipids or the lipids could be exchanged. This also gave the opportunity of increasing or decreasing the lipid content of the reconstituted meals.

Effect of fat content. All samples were stored at 30° C, 60% rh for five days. The control wheat (W) had the lowest fat acidity (Fig. 6). Defatted wheat meal reconstituted with wheat lipid (DW + WL) had a higher fat acidity than the control, presumably because the lipid was on the surface of the sample when it was reconstituted. When defatted wheat meal was reconstituted with millet lipid (DW + ML), the fat acidity increased at a higher rate than it did for the control. When defatted millet meal was reconstituted with wheat lipid (DM + 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. When the level of millet lipids (ML), (DM + ML), and (DW + ML) was held constant, the sample containing millet meal gave significantly higher fat acidity values than the wheat meal samples. Also, when the level of wheat lipid (WL) was held constant, the samples containing millet meal (DM + WL) gave higher fat acidity than did the sample containing wheat meal (DW + 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 values than did millet samples (Fig. 7). When defatted wheat was reconstituted with wheat lipid (DW + WL), fat acidity values were higher than those obtained with the control wheat (W). When defatted wheat was reconstituted with millet lipids (DW + ML), fat acidity values were lower than those obtained for the defatted wheat plus wheat lipids (DW + WL) sample. This indicates that millet lipids are slightly less sensitive to hydrolytic decomposition than are wheat lipids.

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

TABLE VI
Effect of Atmospheric Oxygen

	0 Days		30 Days	
Millet Meal Stored Under	Fat Acidity (mg KOH/ 100 g meal)	Peroxide Value (mg PO[a]/ kg fat)	Fat Acidity (mg KOH/ 100 g meal)	Peroxide Value (mg PO[a]/ kg fat)
Air	58.12	7.73	290.44	4.11
Nitrogen	58.12	7.73	292.69	3.35

[a] Peroxide value.

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