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Fatty Acid Composition and Oxidation of Cowpea (*Vigna unguiculata*) Flour Lipid

Mark E. Ukhun

Chemistry Department, University of Benin,
Benin City, Nigeria

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ABSTRACT

The lipids extracted from cowpea flour before and after storage at water activities (a_w) *of 0·11, 0·33 and 0·75 and at 5, 25 and 40 °C for 6 months were examined for their fatty acid composition and oxidation.*

Linoleic, linolenic and oleic acids, in decreasing order, were the unsaturated fatty acids recorded. The saturated fatty acids were palmitic, stearic and arachidic in decreasing order. The total unsaturated fatty acids concentration was higher than the total saturated fatty acids, with palmitic acid being the single dominant fatty acid.

Saturated/unsaturated fatty acid ratios (s/u ratio) and lipid conjugated diene absorbance at 233 nm indicated that the a_w *of 0·33 and the storage temperature of 5 °C were the most effective in mitigating the oxidation of the cowpea lipid.*

Oxidation rates of the unsaturated fatty acids were related to their levels of unsaturation.

INTRODUCTION

Cowpeas (*Vigna unguiculata*) have always constituted an important part of the diets of the peoples of West Africa (Lewicki, 1974).

Legumes as a group—of which cowpea is one—represent a valuable source of protein for the world's ever-increasing population, especially in

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the Third World countries. Bressani *et al.* (1963) have estimated that about 20–30% of the protein intake of the poor in Latin American countries is derived from legumes. About 8·5 million tons of protein are obtained from legumes annually.

In recent times, attempts have been made to prepare ready-to-eat foods from cowpeas by various dehydration techniques (Onayemi & Potter, 1976). The great versatility of cowpeas as a base material for many food products such as 'moin-moin' and 'akara balls', soups, gravies and stews, calls for cowpea processing into instant, or semi-instant, forms that will be easier to process into the various cowpea-based foods. The milling operation carried out in the present studies was designed to meet, if only partially, this objective.

The optimum utilization of cowpea, either as grown or in its processed or semi-processed forms, requires a knowledge of the various chemical phenomena which can either enhance or adversely affect its quality. In an otherwise high protein food like the cowpea legume, lipid oxidation would be one of the most important chemical phenomena that could affect quality, as has been reported by many workers for other foods (Lea *et al.*, 1960; Carpenter *et al.*, 1962; Ponting *et al.*, 1964; Braddock & Dugan, 1973; Jarenback & Liljemark, 1975).

In recognition of this, lipid oxidation in milled cowpea flour in relation to water activity (Rockland, 1969; Labuza *et al.*, 1972), temperature and length of storage was investigated.

EXPERIMENTAL

Cowpea seeds (*Vigna unguiculata*) were obtained from the National Seed Co., New Orleans, USA. Raw cowpea powder was prepared from these seeds using a Hobart Manufacturing Co. Mill Model No. 3430. Powder able to pass through a No. 1 Standard sieve with a Tyler Equivalent of 16 mesh was thus obtained.

Four hundred grams of the milled cowpea flour were weighed, in duplicate, into two 500-ml glass beakers and kept in a desiccator. The same procedure was followed for each of the two other desiccators. The three desiccators containing the cowpea flour samples were kept on a laboratory bench at an ambient temperature of 25 °C. Water activities of 0·11, 0·33 and 0·75 were then established, respectively, in the stored samples in accordance with the method described by Rockland (1960).

Similarly, samples were stored at 5, 25 and 40 °C, respectively, at a uniform a_w of 0·75 in three separate desiccators. The duration of storage was 6 months.

The method of Folch *et al.* (1957) was used to extract the lipid from the samples, on a monthly basis. The extent of oxidation in the extracted lipid was assessed by measuring diene conjugation absorbance at 233 nm and by s/u measurements obtained from the results of gas–liquid chromatographic analyses of the extracted cowpea lipid.

For the diene conjugation absorbance measurements, 10 mg of the extracted lipid was placed in a 30-ml test tube into which 10 ml of *iso*-octane (2,2,4-trimethylpentane) was poured, and mixed thoroughly in a Fisher mini shaker. The mixture was then filtered through a Whatman No. 1 filter paper and absorbance values were taken in duplicate on a Beckman DU spectrophotometer at 233 nm using *iso*-octane as the blank.

Fatty acid methyl esters of the cowpea lipid extract were prepared by the rapid method of Metcalfe *et al.* (1966) and thereafter subjected to gas–liquid chromatographic analysis of a 5830A Gas Chromatograph (Hewlett-Packard) equipped with a computerized integrator for peak area and percentage fatty acid quantifications. The glass column (6 ft × $\frac{1}{4}$ in outside diameter) was packed with 15 % DEGS on 80/100 mesh Chromosorb W. The operating conditions used were:

Injection temperature	220 °C
FID temperature	250 °C
Oven temperature	190 °C
Chart speed	1·00 cm/min

Nitrogen carrier gas flow rate was 27 ml/min. Peak identification was by comparison with known fatty acid methyl esters.

The rate of loss of each unsaturated fatty acid was obtained by using the formula:

$$R = \frac{X}{T}$$

where R = rate of loss, X = total change in per cent fatty acid over the 6-month storage period and T = length of storage (6 months).

The same formula was used to determine oxidation rate but with

X = total increase in lipid conjugated diene absorbance at 233 nm over the 6-month storage period.

RESULTS AND DISCUSSION

Table 1 indicates that the dominant fatty acid in cowpea lipid was palmitic acid ($C_{16:0}$). Of the unsaturated fatty acids, linoleic acid ($C_{18:2}$) was dominant. The total unsaturated fatty acid content was 54·6% compared with 45·4% for the saturated fatty acids. This gave an s/u ratio of 0·831. Although the high level of unsaturated fatty acids vis-a-vis the saturated ones is of nutritional significance (Scheig, 1968) it also means that the cowpea lipid would be prone to oxidation, with consequent adverse effects on its flavour and on food products based on undefatted cowpeas (Dugan, 1976). Traces of both behenic and lignoceric acids reported by Oyenuga (1968) were not detected in the cowpea lipid used in the present studies. This could be because Oyenuga analysed samples cultivated in Nigeria whereas those used in this study were cultivated in the USA where climatic and agronomic practices are not exactly the same as in Nigeria. In addition, varietal differences may have accounted for the different results (Ologhobo & Fetuga, 1983).

Table 2 shows an increasing s/u ratio of the lipid with increasing length of storage over the 6-month period at all water activities investigated. This implies loss of unsaturation. The decrease in the s/u ratio when the a_w was increased from 0·11 to 0·33 implies that the loss of unsaturation at the a_w of 0·11 was higher than at the a_w of 0·33. Correspondingly, the higher s/u ratio at the a_w of 0·75 than at the a_w of 0·33 implies that the loss of unsaturation at the latter a_w was lower than that at the former. A multistage trend in the relationship of lipid oxidation to changes in the a_w of the system is therefore discernible. Similar effects of a_w on lipid oxidation have been reported by Salwin (1962), Ayerst (1965) and Loncin

TABLE 1
Fatty Acid Composition of Cowpea Flour Lipid

Fatty acid	%
Oleic acid	13·2
Linoleic acid	27·8
Linolenic acid	13·6
Palmitic acid	35·1
Stearic acid	7·44
Arachidic acid	2·78

TABLE 2
Effect of Water Activity and Temperature on the s/u Ratio of Cowpea Flour Lipid During a 6-Month Storage Period

Month	a_w			*Temperature (°C)*		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	0·831	0·831	0·831	0·831	0·831	0·831
1	0·933	0·860	0·933	0·830	0·933	1·45
3	1·17	1·02	1·19	0·845	1·19	2·15
6	1·62	1·20	1·62	0·865	1·62	3·15

et al. (1968), among others. The lower s/u ratio at the a_w of 0·33 than at the a_w of 0·11, implying reduced oxidation at the a_w of 0·33, may have been due to free radical destruction and reduced rate of hydroperoxide decomposition; increased catalyst mobility and catalyst surface exposure may have accounted for the increased s/u ratio, implying increased oxidation, at the a_w of 0·75, compared with that at the a_w of 0·33 (Labuza, 1971). The a_w of 0·33 appears to be the critical a_w for cowpea lipid oxidation.

The s/u ratio is seen to increase with temperature increases from 5 °C to 25 °C to 40 °C. Chemical reactions, generally, are accelerated when temperature increases. This would be particularly true of the initiation process of lipid autoxidation in which the energy requirement for radical production by rupture of a carbon–hydrogen bond is about 80 kcal, which is regarded as excessive (Dugan, 1976). Accordingly, any input of thermal energy is likely to increase oxidation, as has been demonstrated in Table 2. The effectiveness of a storage temperature of 5 °C in retarding cowpea lipid oxidation is notable, as is the storage temperature of 40 °C in promoting cowpea lipid oxidation. Apart from supplying greater energy for the oxidation processes, the storage temperature of 40 °C may also have acted to increase the rate of oxygen diffusion into the system to promote faster lipid oxidation. This would have been particularly important in view of the milling operation which reduced the cowpeas to the flour form with more surface area and porosity for oxygen diffusion and oxidation reactions. This means that, given the generally warm Nigerian climate, a low temperature storage, say 5 °C, is advised for the milled cowpea if lipid oxidation and its attendant flavour problems are to be avoided.

The results shown in Table 3 indicate that, at all water activities and temperatures examined in this study, the rates of loss of the unsaturated

TABLE 3
Rates of Loss of Unsaturated Fatty Acids in Cowpea Flour Lipid Over a 6-Month Storage Period

Fatty acid	a_w			*Temperature (°C)*		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
Oleic acid	0·597	0·367	0·600	0·027	0·603	1·20
Linoleic acid	0·882	0·498	0·885	0·040	0·882	1·62
Linolenic acid	1·27	0·680	1·26	0·101	1·27	2·27

fatty acids were related to their levels of unsaturation. The magnitude of losses discernible in the Table may be the result of both autoxidation and lipoxygenase catalyzed oxidation. Lipoxygenase, which is principally a plant enzyme, is known to be distributed widely in legumes. Ericksson (1967) and Ericksson & Suensson (1970) demonstrated lipoxygenase activity in peas, and recommended blanching to inactivate these enzymes. Since no thermal treatment was given to the milled cowpea flour, their activity in the flour is predictable.

The complex nature of lipid oxidation calls for a combination of two or more methods in its monitoring in food systems. In the present studies, ultraviolet absorption of lipid conjugated dienes at 233 nm has been employed as an additional method of assessing oxidation of the cowpea lipid. The oxidation of polyunsaturated fatty acids produces peroxides

TABLE 4
Effects of Water Activity and Temperature on the Absorbance at 233 nm of Cowpea Flour Lipid During a 6-Month Storage Period

Month	a_w			*Temperature (°C)*		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	0·030	0·030	0·030	0·030	0·030	0·030
1	0·038	0·030	0·039	0·032	0·033	0·700
2	0·041	0·036	0·042	0·031	0·036	1·010
3	0·073	0·038	0·074	0·033	0·075	1·410
4	0·138	0·078	0·141	0·032	0·143	1·80
5	0·165	0·115	0·148	0·034	0·146	1·84
6	0·173	0·137	0·150	0·035	0·152	1·86
Lipid oxidation rate	0·024	0·018	0·020	0·001	0·020	0·305

and the position of the double bond shifts to a conjugated form. Conjugated linkages give rise to characteristic and intense absorption bands within the spectral range of 200–400 nm, while the absorption of isolated double bonds within the same region is very weak (Mehlenbacher, 1960). The results in Table 4, which show increasing absorbance with increasing length of storage and temperature and lower absorbance at the a_w of 0·33 compared with that at either the a_w of 0·11 or 0·75, are consistent with the s/u results already discussed. S/u ratio and a lipid diene absorbance of 233 nm appear, therefore, to be effective methods of following cowpea lipid oxidation under the storage conditions used in this study.

The differences in the extent of lipid oxidation at 5, 25 and 40 °C were statistically significant (α level 0·01, 12 degrees of freedom) using t test assessment of differences between means. At the same α level and degrees of freedom, differences in the extent of lipid oxidation at a_w's of 0·33 and 0·75 were also statistically significant. However, the obvious differences in lipid oxidation between the a_w 0·11 and a_w 0·33 samples and between the a_w 0·11 and a_w 0·75 samples were not statistically significant.

CONCLUSION

The unsaturated fatty acid content of the lipid of *Vigna unguiculata* examined in this study was higher than its saturated fatty acid content. This is nutritionally desirable, but it also increases the susceptibility of the lipid to oxidative deterioration.

S/u ratio and diene conjugation absorbance measurements at 233 nm gave agreeable results in the assessment of lipid oxidation in cowpea flour during a 6-month storage period.

An a_w of 0·33 and a temperature of 5 °C provided the greatest lipid stability of all storage conditions examined.

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