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# Isolation and Composition of Glycolipids from Corn Flour

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*ABSTRACT*

*Total lipids extracted according to De Stefanis and Ponte* [Biochim. Biophys. Acta, ***176*** *(1969) 198–201] from corn flour (from Cameroon, variety white) amounted to 0·5% of the dry flour weight. The total lipids consisted of 90% neutral lipids, 5% glycolipids and 3% phospholipids. The glycolipids contained glucolipids as major components (4·5%) and minor amounts of a glycerolipidic fraction (0·6%). Glycolipid fatty acids were identified as linoleic, oleic and palmitic acids.*

## INTRODUCTION

The production of corn has been increasing in East Africa over the past thirty years in response to human food requirements (FAO, 1983). The corn flour is a major agricultural product used for making mixed flours.

Already an extensive study has been made of the composition of wheat flour lipids (Carter *et al.*, 1956, 1961*a*,*b*). It appears that wheat flour contains



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two galactosylglycerol lipids and a complex mixture of gluco- and mannocerebrosides (Carter *et al.*, 1961*a*; De Stefanis & Ponte, 1969).

Although the lipidic moiety represents a minor part of the corn flour, the composition of these lipids, and especially the nature of the fatty acids, is of interest as the corn flour is largely used in food products in East Africa.

The present study reports the results of the analysis of glycolipids of corn flour from Cameroon, variety white.

## MATERIALS AND METHODS

### Chemicals

All chemicals and solvents were purchased from E. Merck (FRG) or Prolabo (France), silicic acid Bio-Sil HA (325 mesh) from BioRad Laboratories (USA) and HPTLC glass plates of silica gel 60 F254 from E. Merck (FRG).

### Plant materials

Corn was obtained from the S.O.D.E.B.L.E. (Yaoundé, Cameroon). The kernels were pulverized in a IKA Universalmühle M 20 grinder. The powder was passed through a sieve.

### Extraction and fractionation of lipids

Lipids were extracted with methanol and hexane according to De Stefanis and Ponte (1969). The hexane-soluble extract was suspended in ethyl ether and mixed with silica gel. After 5 h the suspension was centrifuged. The supernatant contained neutral lipids; complex lipids were eluted from the silica gel with the following solvents: benzene–acetone 85:15 and 70:30, by vol. acetone–methanol: 97:3, by vol. methanol–water 95:5, by vol.

The procedure is summarized in Fig. 1.

The lipid extracts were fractionated on a silicic acid Bio-Sil HA column with elution by chloroform and chloroform containing increasing concentrations of methanol. Glycolipid fractions were eluted by chloroform–methanol in the ratios 95:5 or 90:10, by vol.

### Thin-layer chromatography

Thin-layer chromatography of lipids was performed on HPTLC plates with the following solvents: chloroform–methanol–water (65:25:4, by vol.) (Gray, 1967); methyl acetate–*n*-propanol–chloroform–methanol-0·25%

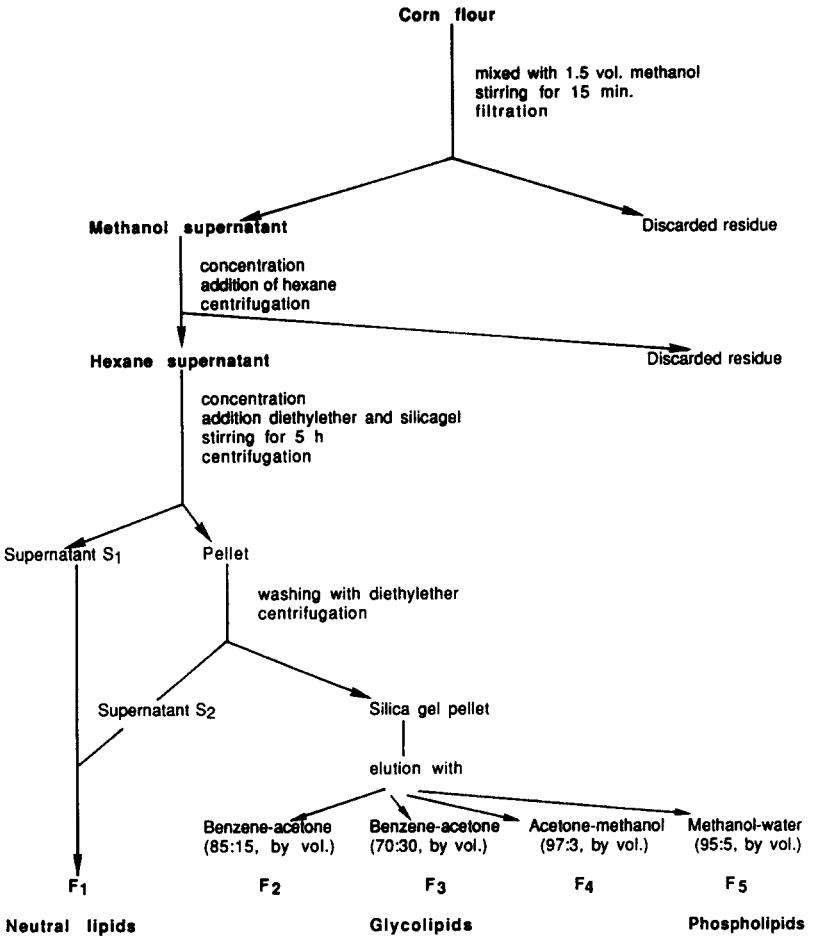


**Fig. 1.** Isolation of lipids from corn flour.

aqueous KCl (25:25:28:10:7, by vol) (Heape *et al.*, 1985). The detection of compounds was carried out by spraying the plates with Dittmer and Lester reagent modified by Vaskovsky and Kostetsky (1968) for phospholipids and with a 10% sulfuric acid/vanillin solution for lipids.

## Analytical methods

Hydrolysis of purified lipids was performed with 6M HCl for 48 h at 100°C. Fatty acids were recovered from the hydrolysate by chloroform and esterified with diazomethane; glycerol was acetylated with acetic anhydride/pyridine (1:1, by vol.) at 100°C for 15 min and analyzed by gas chromatography.

Neutral sugars analysis was performed after hydrolysis in 0·1M HCl for

48 h at 100°C, by gas chromatography after conversion of monosaccharides into alditol acetates according to Sawardeker *et al.* (1965).

### General methods of gas chromatography and mass spectrometry

Gas chromatography was carried out on an Intersmat IGC 120 F apparatus fitted with either ECNSS-M (3% on Gas Chrom Q, 80–100 mesh) column (0.6 × 150 cm, 110°C or 180°C) or with a capillary SP 2380 column (0·25 mm × 20 m, 210°C or 230°C) for glycerol and sugar derivatives. The same device, which was fitted with a capillary SP 2100 column (0·25 mm × 25 m, 140°C to 260°C) was used for the analysis of fatty acid methyl esters. Combined gas chromatography/mass spectrometry (GC/MS) was performed on a VG MM 305 apparatus (temperature 200°C, ionisation potential 70 eV and current intensity 200 $\mu$A), which was connected to a gas chromatograph equipped with a FFA1 capillary column (0·35 mm × 50 m, 60°C to 240°C).

## RESULTS AND DISCUSSION

### Lipid fractionation

The extraction of corn flour with methanol gave a crude lipid fraction: 0·5% of dry weight. The fractionation, as described above (Fig. 1), gave 90% of neutral lipids ($F_1$), 5% of glycolipids ($F_2$: 0·5%; $F_3$: 4%; $F_4$: 0·6%) and 3% of phospholipids ($F_5$): (Table 1).

The fractions $F_3$ and $F_4$ were purified by chromatography on Bio-Sil HA silicic acid with elution by solvent mixtures of chloroform–methanol (from

**TABLE 1**
Yields of Various Fractions after Solvent Fractionation of the Hexane-Soluble Extracts of Corn Flour Lipids

| *Fraction* | *Solvent system* | *Yield % Total hexane soluble lipids* |
|---|---|---|
| $F_1$ | Ethylether | 90 |
| $F_2$ | Benzene-acetone (85:15, by vol.) | 0·5 |
| $F_3$ | Benzene-acetone (30:70, by vol.) | 4 |
| $F_4$ | Acetone-methanol (95:5, by vol.) | 0·6 |
| $F_5$ | Methanol-water (95:5, by vol.) | 3 |

**TABLE 2**
Silicic-Acid Chromatography of $F_3$ and $F_4$ Lipids[a]

| *Solvent system chloroform:methanol by vol.* | $F_3$ | | $F_4$ | |
|---|---|---|---|---|
| | *Fraction* | *Yield* | *Fraction* | *Yield* |
| 100:0 | $F_3A$ | 9·7 | $F_4A$ | 16·1 |
| 95:5 | $F_3B$ | 68·9 | $F_4B$ | 8·3 |
| 90:10 | $F_3C$ | 10·2 | $F_4C$ | 26·9 |
| 85:15 | $F_3D$ | 3·1 | $F_4D$ | 11·2 |
| 80:20 | $F_3E$ | 2·5 | $F_4E$ | 13·6 |
| 75:25 | $F_3F$ | 2·3 | $F_4F$ | 6·6 |
| 50:50 | $F_3G$ | 1·4 | $F_4G$ | 0 |
| 0:100 | $F_3H$ | 1·9 | $F_4H$ | 17·4 |

[a] The yield values are expressed as a percentage of dry weight.

100:0 to 0:100, by vol.). The results are reported in Table 2. The major components of $F_3$ ($F_3B$) and $F_4$($F_4C$) were eluted with, respectively, chloroform–methanol 95:5, by vol. ($F_3B$) and 90:10, by vol. ($F_4C$). On thin-layer chromatography $F_3B$ and $F_4C$ gave a major spot with $R_F = 0{\cdot}80$ ($F_3B$) and 0·60 ($F_4C$) in the solvent system of Gray (1967) and with $R_F = 0{\cdot}68$ ($F_3B$) and 0·62 ($F_4C$) in the solvent system of Heape *et al.* (1985).

## Sugar and glycerol analysis

Crude glycolipids and purified fractions $F_3B$ and $F_4C$ were analyzed after acid hydrolysis. The sugars were identified by gas chromatography as galactose and glucose (Table 3); glucose was the major sugar in $F_2$, $F_3$ and $F_3B$. Glycerol was only present in $F_4C$. These results showed that glucolipids were the major components of glycolipids of corn flour. By analogy with the results of Carter *et al.* (1961*a*) which had isolated

**TABLE 3**
Sugar Composition of Glycolipids from Corn Flour

| *Fraction* | *Galactose* | *Glucose* |
|---|---|---|
| $F_2$ | traces | + |
| $F_3$ | traces | + |
| $F_3B$ | 1 | 11·3 |
| $F_4$ | 1 | 1·9 |
| $F_4C$ | 1 | 0·75 |

The values are expressed as molar ratio to glucose.

**TABLE 4**
Fatty Acid Composition of $F_2$, $F_3$ and $F_4$ Lipids

| *Fatty acids* | $F_2$ | $F_3$ | $F_4$ |
|---|---|---|---|
| Hexadecanoic (16:0) | 35·3 | 20·7 | 23·6 |
| Octadecadienoic (18:2) | 27·0 | 36·9 | 38·4 |
| Octadecenoid (18:1) | 28·7 | 36·2 | 33·2 |
| Octadecanoic (18:0) | 9·0 | 7·6 | 4·7 |

The values are expressed as percentage of total peak areas.

glucocerebrosides in wheat flour we can assume that corn flour contains glucocerebrosides with smaller amounts of galactocerebrosides.

$F_4C$, which was the major component of the fraction $F_4$ eluted with the solvent acetone–methanol (97:3, by vol.), contained glycerol, glucose and galactose; it could be a lactosyl diglyceride or a mixture of monogalactosyl and digalactosyl diglyceride already reported by De Stefanis and Ponte (1969) in wheat flour, and of glucosyldiglycerides.

### Fatty acids analysis

Methyl esters of fatty acids were analyzed by gas chromatography combined with mass spectrometry. $F_2$, $F_3$ and $F_4$ contained the same fatty acids: hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$), octadecenoic ($C_{18:1}$) and octadecadienoic ($C_{18:2}$) acids (Table 4). However, the composition was different in glycolipids $F_2$, $F_3$ and $F_4$: glycolipids $F_3$ and $F_4$ contained a larger amount of linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids than glycolipid $F_2$, while palmitic acid ($C_{16:0}$) was predominant in $F_2$. Stearic ($C_{18:0}$) acid was found in a low level in the three fractions $F_2$, $F_3$ and $F_4$.

Similar results had been reported by Sosulski and Abdullahi (1988), who studied the fatty acid composition of endosperm and whole kernel corn samples. Carter (1961*a*) reported a higher percentage of linoleic acid in the galactolipids of wheat flour.

## CONCLUSION

The lipids of corn flour consist of 90% of neutral lipids, the polar lipids consist of 5% glycolipids and 3% phospholipids. The major fatty acids of glycolipids were palmitic, oleic and linoleic acids. Similar results had previously been reported for the fatty acid composition of germ, endosperm and whole kernel samples from other origins (Gupta *et al.*, 1979; Amed *et al.*, 1987).

Glycolipids represent a low amount of total lipids in agreement with the lipid composition of corn germ of Somali cultivars (Sosulski & Abdullahi, 1988). The major glycolipid fractions, $F_2$ and $F_3$ (90% of total glycolipids), contained a high proportion of glucose and no glycerol. This composition was quite different from that of major glycolipids isolated from various cereals, they are glyceroglycolipids (Carter *et al.*, 1956, 1961*a*,*b*; Fujino & Sakata, 1973; Obara & Kihara, 1973; Mahadevappa & Raina, 1984; Hemavathy & Prabhakar, 1988). In the corn flour, only the fraction $F_4$ (10% of total glycolipids) was found to be glyceroglycolipid.

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