

## LIPIDS OF CURED CENTENNIAL SWEET POTATOES

**SUMMARY**—Lipids isolated from cured Centennial sweet potatoes were identified and quantitated by a combination of column and thin layer chromatography. These lipids were shown to consist of 42.1% neutral lipids, 30.8% glycolipids and 27.1% phospholipids. Triglycerides and steryl esters were the major lipids of the neutral fraction. Among the phospholipids, phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol were the most abundant. Galactolipids and the steryl glucosides were also present. The predominant fatty acids were stearic, palmitic, oleic, linoleic and linolenic.

### INTRODUCTION

OXIDATIVE deterioration of the lipids of dehydrated sweet potato flakes is a major obstacle to consumer acceptance of this food product. However, very little is known about the nature of lipids present in sweet potatoes. Boggess et al. (1967; 1970) studied lipid changes in a number of varieties of sweet potatoes including Centennials during curing and storage by separating lipids into "nonphospholipids," "cephalin" and "lecithin." These workers found the lipid content to be 0.44% (fresh weight) for cured Centennials stored for 180 days at 15.5°C.

On the other hand, the lipid make-up of white potato tubers has been studied exhaustively (Lepage, 1968; Galliard, 1968). These workers found that white potatoes contained approximately 0.10–0.13% lipids on a fresh weight basis. Phospholipids were present in the greatest amounts followed by glycolipids and neutral lipids.

It is the purpose of this paper to identify and quantitate the lipids of cured Centennial sweet potatoes.

### EXPERIMENTAL

#### Sweet potatoes

Centennial sweet potatoes used in this study were harvested at Benson, N.C. (1969) and cured at 85°F and 80% relative humidity (r.h.) for 2 wk. The cured roots were then stored at 61°F and 60% r.h. for 9 months.

#### Reagents and chemicals

Only reagent grade solvents, with the exception of 95% ethanol, were used. All solvents were carefully distilled and stored in the dark in brown-glass bottles. Silicic acid (–325 mesh) (Sigma Chemical Co., St. Louis, Mo.) and silica gel G and H (Brinkmann Instrument, Westbury, N.Y.) were used for chromatography. Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, N.J.) was used for lipid purification. Lipid

standards were obtained from Sigma Chemical Co. and Supelco (Bellefonte, Pa.).

#### Lipid extraction and purification

Cured Centennial sweet potatoes from the 1969 crop were peeled, diced and a 50g sample ground with boiling 95% ethanol (3.5:1 v/wt) for 2 min in a Waring Blendor. The slurry was allowed to settle and filtered. The filter pad was then exhaustively extracted with chloroform-methanol (2:1 v/v) until extracts were colorless. The extracts were combined and an aqueous sodium chloride solution (10%) added to give two layers. The bottom layer containing the lipids was removed and the upper layer was extracted with 3 equal portions of chloroform. The chloroform extracts were combined with the original lower layer and evaporated. The extracted aqueous layer was shown to be lipid free by TLC examination of a concentrated aliquot. The lipid slurry was freeze-dried, taken up in chloroform-methanol (2:1 v/v) and filtered.

The crude lipid extract was freed from non-lipid contaminants by passage through a Sephadex G-25 column (Wuthier, 1963) followed by freeze drying to remove water. The purified lipids were dissolved in chloroform-methanol (2:1 v/v), transferred to a tared vial and the solvent evaporated under nitrogen. The vial was then placed in a desiccator and evacuated to remove the last traces of solvent. The lipid was then weighed. So that the lipid could be expressed on a dry weight basis, a 5g sample of sweet potato was dried under vacuum at 80°C to constant weight and the moisture loss determined.

#### Separation of lipids into classes

The silicic acid column procedure of Lepage (1968) was used to separate the purified lipid extract into neutral lipid, glycolipid and phospholipid fractions. A 1 × 15 cm silicic acid column was washed with 3 column volumes each of methanol, chloroform and hexane. Samples (ca. 100 mg) were transferred onto the column in hexane, the columns were eluted with 30 ml each of 2%, 5% and 50% ether in hexane, then with 30 ml each of 5%, 10%, 30% and 50% methanol in chloroform and finally with 50 ml of methanol. After testing each fraction by TLC, similar fractions were combined to give neutral lipid, glycolipid and phospholipid fractions. After evaporation of the solvent, the percent distribution of the three lipid classes was determined from the weights of the major fractions.

#### Analysis of neutral lipids

Neutral lipids were identified by comparison

on TLC with authentic standards. The TLC system used was that of Nagy and Nordby (1970). Lipids in hexane were spotted onto activated silica gel H-coated plates and developed in ethyl ether-benzene-ethanol-acetic acid (40:50:2:0.2 v/v) to a distance 10 cm above the point of sample application. After this development the plate was dried for 30 min in vacuo. The plate was then developed in hexane-benzene-acetic acid (80:25:1 v/v). The solvent was allowed to travel 16 cm above the application point. The plate was removed, dried, sprayed with 50% sulfuric acid and charred in a muffle furnace at 190°C for 35 min.

The neutral lipids were quantitated after charring by densitometry on a Densicord 542 Densitometer (Photovolt Corp., N.Y.). The light source was fitted with a 1 × 6 mm sub-stage slit and a 0.3 × 6 mm collimating slit was placed over the phototube. Silica gel H plates (3 × 20 cm) were developed overnight in chloroform, dried and activated at 110°C for 1 hr. The silica gel layer was then scribed to give a lane 8 mm wide (Downing, 1968). The sample was applied within the lane and the plate developed and charred as described above. The plate was scanned on the densitometer and the area of each component obtained by triangulation. Three plates were run and the areas of each component averaged. Standards were run along with the unknown lipid samples. The percentage composition of each component was obtained from the areas after correction of sterol area for abnormally high carbon yields (Downing, 1968).

#### Analysis of glycolipids

Monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG) and cerebrosides (CER) were identified by comparison on TLC with known standards. The systems used were silica gel H layers containing magnesium acetate with solvent systems (A) chloroform-methanol-water (65:25:4 v/v/v) and (B) CHCl<sub>3</sub>-methanol-acetic acid-water (170:25:25:5 v/v/v/v). Visualization was by spraying with 50% H<sub>2</sub>SO<sub>4</sub> and heating at 110°C. Steryl glucoside (SG) and esterified steryl glucoside (ESG) were identified by a positive Liebermann-Burchard spot on TLC. In addition these materials were isolated by TLC and acid hydrolysed. Glucose and free sterol were obtained from SG while glucose, free sterol and free fatty acid were obtained from ESG (Lepage, 1964).

Glycolipids were quantitated by isolation of the individual lipids by TLC followed by hexose measurements in duplicate on acid-hydrolysed lipids (Svennerholm, 1956). ESG, SG and MGDG were separated on plates prepared from silica gel H (20g) containing magnesium acetate (0.5g) developed in solvent system (A). After development the area corresponding to each lipid was removed, extracted with chloroform-methanol-water (30:30:1) and assayed for hexose according to Svennerholm (1956). DGDG and CER were treated in the same manner except solvent system (B) was used for TLC separation. Standard solutions of glucose and ga-

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lactose were carried through all steps with the exception of TLC. The percent distribution of each component was obtained from the hexose values.

#### Analysis of phospholipids

Phospholipids were identified by separation with two dimensional TLC on silica gel H impregnated with magnesium acetate according to Rouser et al. (1970). Individual lipids were identified by spraying with one of the following reagents: ninhydrin reagent (0.4% in butanol containing 2% acetic acid) and heating at 110°C for phospholipids containing free amino groups; modified Dragendorff reagent for choline-containing phospholipids (Bregoff et al., 1953); the specific phospholipid spray of Dittmer and Lester (1964); or the sulfuric acid formaldehyde spray of Rouser et al. (1970) followed by charring at 180°C. Additionally identification was accomplished by comparing  $R_f$  values with those of reference compounds.

Phospholipids were quantitated by phosphorous analysis of the individual components in duplicate after two dimensional TLC as described above. The results were converted to percent of total lipid by multiplying phosphorous values by the appropriate factor (Rouser et al., 1966).

#### Fatty acid analysis

The major fatty acid content of the total lipid extract and that of each of the three fractions was determined by converting an aliquot of each fraction into methyl esters (FAME). FAME were prepared by transesterification. About 5 mg of lipid was mixed with 1 ml anhydrous methanolic hydrogen chloride (5%) and sealed under nitrogen in an ampoule. The mixture was then heated at 80–85°C for 3 hr. The FAME were extracted with hexane, washed with 1N sodium carbonate solution, dried and purified by TLC.

Purified FAME were separated on an F and M model 810 gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) equipped with a flame ionization detector. Dual 6 ft × 1/8 in. aluminum columns maintained at 180°C packed with EGSSX' (10%) on chromosorb P (100/120 mesh, Applied Science, State College, Pa.) were employed. Carrier gas (nitrogen) flow was 38 ml/min. Assignment of identity was based on comparison of retention times with those of authentic reference samples.

The relative quantities of fatty acids of Centennial sweet potatoes were obtained by comparing the peak areas of FAME with those of a known mixture. The percentage composition of the known mixture (Applied Science) was confirmed with better than ± 1% precision and accuracy. Gas chromatographic detector response was linear over the range studied.

## RESULTS & DISCUSSION

THE LIPID content of cured Centennial sweet potatoes from this study, was found to be 2.7% (dry weight). Boggess et al. (1967; 1970) found 1.6% lipids for Centennials. This discrepancy could easily be due to differences in curing and storage or methodology. Further comparison of the make-up of Centennial lipids as found by Boggess et al. (1967; 1970) and this study is not possible because of the differences in methodology.

In contrast, white potatoes were found

(Galliard, 1968; Lepage, 1968) to contain about 0.5% lipid (dry weight). Although white potatoes and sweet potatoes are not closely related botanically, they are similar in that both are high in carbohydrates and low in lipids. Moreover, both are processed and sold as dehydrated food products. Possibly the relatively high lipid content of Centennials is one of the factors responsible for the greater storage stability of white potato flakes as compared to that of sweet potato flakes. Lepage (1968) found 16.5% neutral lipids, 45.5% phospholipids and 38.0% glycolipids for Netted Germ white potato tubers. We have found 42.1% neutral lipids, 27.1% phospholipids and 30.8% glycolipids for Centennial sweet potatoes.

For the purpose of this study neutral lipids are defined as those which contain neither phosphorous nor sugar in their molecules while glycolipids contain sugar and phospholipids contain phosphorous. The identity and amount of each lipid class and each component is given in Table 1.

#### Neutral lipids

Neutral lipids make up 42.1% of the total lipid of Centennial sweet potatoes. Of these lipids triglycerides are the most abundant followed by steryl esters, diglycerides, hydrocarbons and free sterols. No attempt was made to further study the components of the neutral fraction with the exception of the pigments. An investigation of the pigments of Centennials has been conducted previously (Purcell and Walter, 1968). Carotenoids, responsible for the orange color of sweet potatoes, were shown to consist of mostly  $\beta$ -carotene (86.4%) and small amounts of a large number of other carotenoids. In this study, most of the pigment was isolated in the neutral lipids and was found to amount to 2.3% of the total lipids.

#### Glycolipid composition

Glycolipids comprise 30.8% of the lipid fraction. This fraction contains MGDG, DGDG, cerebrosides, ESG, SG and an unidentified lipid. The lipid designated as MGDG contained a contaminating lipid. This was noted when the devel-

Table 1—Lipid composition of cured Centennial sweet potatoes

Lipid	% Weight total lipid
Neutral Lipids <sup>a</sup>	42.1
Triglycerides	26.9
Steryl esters	6.1
Diglycerides	3.8
Hydrocarbons	2.8
Sterol (free)	2.5
Glycolipids <sup>b</sup>	30.8
Monogalactosyl diglyceride <sup>c</sup>	13.6
Digalactosyl diglyceride	6.3
Cerebroside	4.7
Esterified steryl glucoside	3.5
Unknown	2.1
Steryl glucoside	0.6
Phospholipids <sup>d</sup>	27.1
Phosphatidyl ethanolamine	7.8
Phosphatidyl choline	7.0
Phosphatidyl inositol	5.1
Unknown <sup>e</sup>	3.0
Cardiolipid	1.6
Phosphatidyl glyceride	1.2
Phosphatidyl serine	1.1
Phosphatidic acid	0.4

<sup>a</sup>Individual components determined by duplicate densitometric analyses.

<sup>b</sup>Individual components determined by duplicate hexose analyses.

<sup>c</sup>Possibly contains a contaminating glycolipid.

<sup>d</sup>Individual components determined by duplicate phosphorous analyses.

<sup>e</sup>Positive Dragendorff Test for choline; negative ninhydrin test for free amine;  $R_f$  on TLC similar to lyso-phosphatidyl choline.

oped TLC plate was sprayed with 50% sulfuric acid and heated at 110°C. Under these conditions a yellow area was noted in the brown galactosyl diglyceride. If the plate is charred, the contaminating substance is not distinguishable from the galactolipid. Repeated attempts with numerous solvent systems did not resolve the two spots.

#### Phospholipid composition

As is usual, for nonphotosynthetic plant tissue, phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl inositol (PI) were found to

Table 2—Fatty acid composition of cured Centennial sweet potatoes<sup>a</sup> (Relative weight percent)

Lipid Fraction	% of Individual fatty acids in each fraction										
	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3'	19:0	20:0
Total Lipids	1.7	1.3	29.3	2.0	1.5	6.8	2.0	44.7	8.8		2.4
Neutral Lipids	2.3	1.7	32.2	1.8	1.7	7.8	2.0	39.0	9.6		2.2
Phospholipids	1.7	1.4	24.7	1.7	1.6	4.6	2.1	49.0	8.0		*d
Glycolipids #1 <sup>b</sup>	4.4	1.8	21.9	3.8	2.0	7.1	3.9	43.9	7.5		1.7
Glycolipids #2 <sup>c</sup>	2.9	2.8	17.5	2.2	3.4	5.9	2.1	49.4	9.7	3.5	*d

<sup>a</sup>Results based on the average of two gas chromatographic analyses.

<sup>b</sup>Diagalactosyl diglyceride, cerebrosides, and unknown glycolipids.

<sup>c</sup>Monogalactosyl diglycerides, steryl glucoside, and esterified steryl glucoside.

<sup>d</sup>Asterisk (\*) indicates trace amounts.

be the major components of the phospholipid fraction. An unknown lipid which gave a positive Dragendorff test and a negative ninhydrin test was tentatively identified as lyso-phosphatidyl choline on the basis of similar  $R_f$  values on two dimensional TLC. Phosphatidyl glyceride (PG) and cardiolipid (DPG) were tentatively identified by their chromatographic behavior on TLC.

#### Fatty acid composition

The major fatty acid composition of each of the lipid fractions as well as that of the total lipid is given in Table 2. Palmitic and linoleic acids are the most abundant fatty acids in all fractions. The neutral lipids contain 52.4% unsaturated acids while phospholipid and glycolipid fractions contain greater than 60% unsaturation. Our results for the total lipid fraction are in qualitative agreement with those of Boggess et al. (1970) who found palmitic and linoleic to be the most abundant fatty acids present. These work-

ers also found stearic and linolenic acid but in significantly smaller amounts.

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