Phytochemistry, 1972, Vol. 11, pp. 1899 to 1903. Pergamon Press. Printed in England.

FATTY ACID COMPOSITION OF IMMATURE POTATO TUBERS

T. GALLIARD

Agricultural Research Council, Food Research Institute, Colney Lane, Norwich NOR 70F

(*Received* 6 *December* 1971)

Abstract—The fatty acid composition of the tubers from four varieties of potato was determined at different stages of maturity. Contrary to published data, no fatty acids with conjugated double bonds were found in the endogenous lipids. Conjugated unsaturation was present, however, in the fatty acids of aqueous homogenates of potato and was due to artefacts produced by enzymic breakdown of endogenous lipids during aqueous extraction methods.

INTRODUCTION

WITH the exception of some oil seeds,[1,2] the occurrence in plants of fatty acids containing conjugated double bonds is rare, although trace amounts of this type of fatty acid have been reported in some bulbs.[3] Recently, the presence of relatively large amounts of fatty acids with conjugated unsaturation (up to 35% of the total fatty acids) were reported in immature potato tubers.[4,5] Studies in our laboratory on the lipid composition of potato tubers during maturation have failed to detect conjugated unsaturation in endogenous lipids. This paper demonstrates that conjugation of double bonds and formation of artefact fatty acids occurs by enzymic reactions during certain aqueous extraction procedures.

RESULTS

When tissue from immature potato tubers was 'killed' with refluxing aqueous methanol before extraction of the lipids, the qualitative lipid composition observed by TLC analysis was similar to that previously described in mature tubers.[6,7] However, homogenization of the tissue in aqueous media resulted in a marked loss of glycolipids and phospholipids and a corresponding appearance of lipid breakdown products. A rapid breakdown of lipids in homogenates of mature tubers has also been described.[7,8]

The UV spectra of lipids extracted from intact tissue or from homogenates of the immature tubers used in the present work showed marked differences. The homogenate lipids had a complex spectrum (Fig. 1) with a major peak at 230–235 nm (conjugated diene) and absorption at higher wavelengths. The lipids extracted directly from the tissue showed no significant peaks above 200 nm (Fig. 1). Similar spectra to those shown for the King

[1] C. Y. HOPKINS and M. J. CHISHOLM, *J. Am. Oil Chem. Soc.* **45**, 176 (1968).
[2] C. R. SMITH, *Prog. Chem. Fats Lipids* **11**, 139 (1970).
[3] J. B. NICHOLS and A. T. JAMES, *Fette, Seifen, Anstrichm.* **66**, 1003 (1964).
[4] J. H. SCHWARTZ, R. E. LADE and W. L. PORTER, *J. Food Sci.* **33**, 115 (1968).
[5] J. H. SCHWARTZ and J. S. ARD, *J. Agric. Food Chem.* **18**, 952 (1970).
[6] T. GALLIARD, *Phytochem.* **7**, 1907 (1968).
[7] T. GALLIARD, *Phytochem.* **9**, 1725 (1970).
[8] T. GALLIARD, *Biochem. J.* **121**, 379 (1971).

Edward potatoes in Fig. 1 were obtained for the three other varieties. Table 1 compares the conjugated diene content of tissue and homogenate lipids for the four varieties.

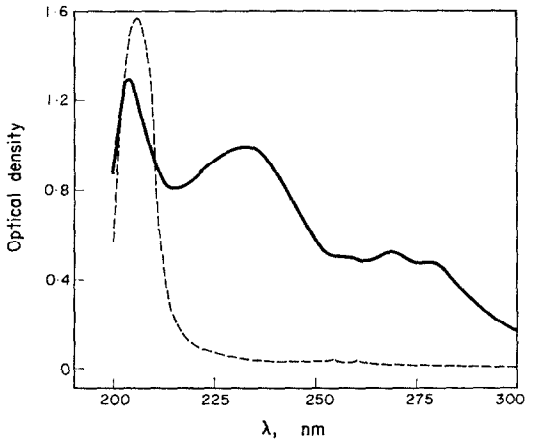

FIG. 1. UV SPECTRA OF THE LIPIDS EXTRACTED FROM INTACT TISSUE AND AQUEOUS HOMOGENATES OF POTATO TUBER.

Absorptivity measurements were made on lipids extracted from intact tissue (- - - - -) or from a water homogenate (——) of King Edward potatoes (av. fr. wt—25 g), sampled 76 days after planting. Ethanolic solutions (5 ml) contained the lipid representing 0·5 g fr. wt of original tissue.

Analysis by TLC of the lipid breakdown products in water homogenates showed a complex mixture containing free fatty acids, conjugated hydroperoxy-, hydroxy- and keto-diene derivatives of fatty acids as well as other, unidentified, carbonyl compounds. When 2 mM sodium metabisulphite, pH 5·5, was used as the homogenizing medium (to prevent phenolic oxidation reactions), the high 233 nm absorption of the lipid extract was retained, but a less complex pattern of lipid breakdown products was seen; the major product (see later) being a conjugated dienol fatty acid derivative.

TABLE 1. CONJUGATED DIENE CONTENT OF THE LIPIDS FROM INTACT TISSUE AND WATER HOMOGENATES OF IMMATURE POTATO TUBERS

Potato variety	Av. tuber fr. wt g	E_{233} of lipid extract	
		Tissue	Homogenate
Arran Pilot	37	0·06	0·53
King Edward	25	0·05	0·99
Duke of York	38	0·04	0·84
Maris Peer	41	0·04	0·73

Method as in Fig. 1.

Homogenates in water were approx. pH 6. The high 233 nm absorption was found in lipids extracted from homogenates buffered between pH 4 and 6. At higher pH values (7–8), the 233 nm peak decreased and a peak was observed at 250 nm, due to the presence of a fatty acid derivative, the structure of which has not yet been determined.

The fatty acid composition of the endogenous lipids from four varieties of potato was determined at different stages of development of the growing tubers. The results for one

variety are presented in Table 2; results for the other varieties were similar and no significant changes in fatty acid composition were observed throughout the maturation period. No conjugated fatty acid derivatives were observed at any stage.

TABLE 2. FATTY ACID COMPOSITION OF POTATO TUBERS DURING MATURATION

Days after planting	68	75	82	89	96	103	117
Average fr. wt of tubers (g)	—	37	37	44	66	70	113
Fatty acid composition (%)							
Palmitic	19·4	15·1	16·4	21·5	17·3	16·9	15·4
Stearic	3·7	3·7	3·9	5·5	4·7	4·5	4·3
Oleic	4·5	4·9	6·4	1·7	2·1	2·1	6·5
Linoleic	56·6	59·9	56·9	58·2	59·7	57·0	62·9
Linolenic	15·8	16·4	16·4	13·1	16·2	19·3	10·9

Fatty acid methyl esters prepared from lipid extracts of potato (Arran Pilot) tubers were analysed by GLC. Minor components (less than 1% total) are omitted. Values are expressed as % of total fatty acid content based on peak area measurements.

Evidence that the destruction of endogenous polyunsaturated fatty acids was responsible for the observed artefacts in homogenate lipid extracts was provided by a comparison of the fatty acid contents of the lipids from both intact and homogenized tissue from immature tubers (Table 3). The saturated and monoenoic fatty acids showed similar concentrations (on a fresh weight basis) in both extracts, whereas a marked fall was observed in linoleic acid (64% loss) and linolenic acid (84% loss) in the homogenate lipid.

TABLE 3. COMPARISON OF FATTY ACID CONTENTS OF INTACT TISSUE AND WATER HOMOGENATES OF IMMATURE POTATO TUBERS

Fatty acid	Concn (μg/g fr. wt) Tissue	Homogenate
Palmitic	96	103
Stearic	24	20
Oleic	31	36
Linoleic	380	136
Linolenic	105	17

Method as Table 2. Results are given for Arran Pilot potatoes (av. wt 37 g) sampled 75 days after planting. Concentrations were calculated relative to an internal standard of heptadecanoic acid.

When ^{14}C-1-linoleic acid was added to the tissue before homogenization, the distribution of radioactivity in the lipid breakdown products of the homogenate was similar to the observed distribution of products on TLC analysis. In the presence of sodium metabisulphite, most of the radioactivity was recovered in the conjugated dienol derivatives.

To identify the major artefact causing the high 233 nm absorption, the lipids from a large-scale homogenate preparation were separated on a silicic acid column. Using methods similar to those previously applied to the identification of the products formed from

linoleic acid by potato lipoxygenase,[9] the material in the major 233-absorbing peak from the silicic acid column was identified as a mixture of conjugated dienol fatty acid derivatives. After methylation and reduction, analysis of the products by mass spectrometry showed a mixture of methyl-9-hydroxystearate (70%) and methyl-13-hydroxystearate (30%). IR analysis of the dienol methyl esters indicated mainly *cis–trans* diene conjugation together with some *trans–trans* contribution.

DISCUSSION

Immature potato tubers at all stages of development contained the same major fatty acids and a similar distribution of these fatty acids as mature tubers previously analysed in this[6] and other laboratories.[10–14] Linoleic and linolenic acid together represented 72–76% of the total fatty acids; palmitic acid was the major saturated component (15–21%).

Our results are not compatible with the conclusions of Schwartz *et al.*[4,5] that immature tubers of potato contained a high proportion of fatty acids with conjugated unsaturation. On the contrary, the present results clearly show that conjugated unsaturation is not present in the endogenous lipids of immature tubers, but is found in artefacts derived from endogenous polyunsaturated fatty acids. The reported occurrence of conjugated fatty acids in immature tubers was based on methods using aqueous extractions without prior enzyme inactivation.[5]

In the present work, a major component of the conjugated fatty acids in the aqueous homogenates was 9-hydroxy-octadeca-10,12-dienoic acid. This is the same derivative as that proposed by Schwartz and Ard[5] to be a major component in the fatty acids from immature potatoes. In our extracts, this was a mixture of *cis–trans* and *trans–trans* isomers, whereas the previous workers reported only the *trans–trans*- isomer. It is possible that some isomerization takes place during extraction and analysis, because, as shown below, the probable precursor is the *cis–trans*-hydroperoxy-diene analogue.

The enzymic breakdown of polar lipids in homogenates of mature potato tuber was described previously.[6] More recent work[7–9] has shown that the breakdown proceeds from membrane-bound phospholipids and galactolipids, by the action of a lipolytic acyl hydrolase, to free fatty acids. Free linoleic and linolenic acids, which together represent the major proportion of fatty acids in the original lipids, are immediately attacked by a lipoxygenase enzyme that introduces a hydroperoxide group, predominantly at the 9-position on the fatty acids, to form a conjugated hydroperoxy-diene system. The further breakdown mechanisms are dependent upon pH, endogenous reducing agents and oxygen tension. Although the acyl hydrolase and lipoxygenase activities are present in tubers at all stages of development and subsequent storage, preliminary studies (unpublished) have shown a marked peak in lipoxygenase activity during the maturation phase and a subsequent decrease to a relatively low level at full maturity. This may explain the previous observations[5] that the content of conjugated unsaturated fatty acids in extracts of potato was highest for immature tuber and decreased with further maturation.

The dangers of enzymic hydrolysis of lipids during their isolation from plant tissues are well known. To avoid such processes, inactivation of the endogenous enzymes before dis-

[9] T. GALLIARD and D. R. PHILLIPS, *Biochem. J.* **124,** 431 (1971).
[10] N. J. MONDY, L. R. MATTICK and E. OWENS, *J. Agric. Food Chem.* **11,** 328 (1963).
[11] C. COTRUFO and P. LUNSETTER, *Am. Potato J.* **41,** 18 (1963).
[12] M. LEPAGE, *Lipids* **3,** 477 (1968).
[13] A. FRICKER, *Z. Lebensm. Forsch.* **142,** 24 (1970).
[14] A. CHERIF and A. BEN ABDELKADER, *Potato Res.* **13,** 284 (1970).

ruption of tissues is commonly used, e.g. steam or hot water blanching[15] and the use of hot solvents.[16] The present work has shown that, not only hydrolytic activity, but also oxidative enzymes in the tissues must be considered in the extraction and analysis of lipids and their component fatty acids. This is particularly important in a tissue like potato in which both hydrolase and lipoxygenase enzymes are active. Although the heat inactivation before homogenization is suitable for analysis of intact tissues, greater problems arise when analysis of subcellular fractions is required. Since both hydrolase and lipoxygenase are found in the particle-free supernatant fraction,[6] they can attack lipids in other cell fractions. Although both activities are reduced at high pH,[5–7] elimination of their potential activity will depend upon finding suitable inhibitors.

EXPERIMENTAL

Materials. Four varieties of potato *Solanum tuberosum* L. (Arran Pilot, King Edward, Duke of York and Maris Peer) were planted locally on 15 April 1971. Tubers were sampled at intervals from 68 to 117 days after planting.

Lipid extractions. Homogenates of tuber tissue were prepared as previously described,[7] using as homogenizing medium, either H_2O or a solution containing 2 mM $Na_2S_2O_5$ and 0·1 M NaOAc, pH 5·5. The crude homogenate was incubated aerobically by shaking 15 min at 25° then added to refluxing MeOH–H_2O (4:1, v/v) as before.[7]

Endogenous lipids were extracted as previously[7] from diced tuber tissue which had been heated in refluxing aqueous MeOH for 5 min.

Analytical methods. Procedures for TLC,[7] silicic acid column chromatography[9] and GLC of fatty acid methyl esters[6] were described previously. For quantitative determination of the fatty acid content of tubers and homogenates, heptadecanoic acid was added as internal standard prior to lipid extraction. UV spectra were recorded on EtOH solutions of the lipid extracts. Fatty acid breakdown products were identified using methods previously applied to the analysis of the products formed from fatty acids by potato lipoxygenase;[9] these involved TLC and UV analysis of the free acids, silicic acid column chromatography and IR spectra of the methyl esters and MS of the products obtained by catalytic reduction of the methyl esters.

Acknowledgements—I am indebted to Mrs. J. A. Matthew for experimental assistance.

[15] F. HAVERKATE and L. L. M. VAN DEENEN, *Biochem. Biophys. Acta* **106**, 78 (1965).
[16] M. KATES and F. M. EBERHARDT, *Can. J. Bot.* **35**, 895 (1957).

Key Word Index—Solanum tuberosum; Solanaceae; potato; fatty acids; unsaturation; lipoxygenase.