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LIPIDS IN RAW AND GRANULATED POTATOES

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled LIPIDS IN RAW AND GRANULATED POTATOES

submitted by WING HONG PUN in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

Paul Zakk

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Sauer

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Date April 20 1979

ABSTRACT

Lipids and fatty acid composition were analyzed in raw potatoes, cultivar 'Netted Gem', and at various steps of a freeze-thaw granule processing. Potato lipids were isolated and fractionated by column and thin-layer chromatography. Fatty acid composition of the isolated lipid fractions was determined by gas-liquid chromatography. Phospholipids were 52% of the total lipids, followed by galactolipids (20%), steryl lipids (16%), and neutral lipids (12%). Phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol were the major constituents in the phospholipid fraction. The glycolipids contained mostly digalactosyl and monogalactosyl diglycerides. Steryl lipids were primarily free sterols, steryl esters, esterified steryl glucosides and steryl glucosides. Sterols found were cholesterol, stigmasterol, and β-sitosterol, which was about 80% of the total sterols. Neutral lipids were present as free fatty acids and triglycerides. Although the isolated lipid fractions varied significantly in their fatty acid composition, the major fatty acids present were linoleic, linolenic and palmitic acids. Lipid losses in steam-cooking, mashing, and dehydration steps were 14.7%, with the major portion being phospholipids. However, only a small decrease in the unsaturation ratio (sum of polyunsaturated over sum of major saturated fatty acids) was observed.

Lipid distribution within membranes and organelles of raw and cooked potato cells *in situ* was examined by transmission electron microscopy. Proximate lipid composition of membranes was achieved by

using five lipid solvents of various polarities. The least polar solvent, petroleum ether, removed the lipid bodies, lipids within cell walls, and the membranes around peroxisomes and starch grains. Acetone extraction did not affect tonoplast and mitochondrial membranes, whereas butanol saturated with water washed out all the membranes except tonoplast. Mixed solvent systems of chloroform--methanol (2:1 v/v) and methanol-ethanol (1:1 v/v) disorganized all cellular structures. They solubilized the lipo-protein components of membranes and denatured the hydrophilic globular proteins. Unlike the distribution of membranes in situ in raw potatoes, the processed potatoes showed the cell wall to be swollen or gelled in places, while the cytoplasmic structures were left as denatured masses of indefinite shape lying between the gelled starch and the cell wall. The gelling of starch and cell wall structures appeared to have sandwiched the denatured cytoplasmic material into a membrane-like boundary around the gelled starch. No imbedded lipid was detected within the gelled starch. All indications suggested alteration of such spatial organization of cellular structures and, probably, their constituents, although steam-cooking had not resulted in cell rupture.

Finally, membrane lipids were analyzed in isolated and purified cellular fractions. During isolation and purification treatments, nupercaine was added to the buffer medium of pH 7.8. Nupercaine not only helped preserve the structure and functions of the biological membranes but also aided the inhibition of potato tuber lipid acyl hydrolase and lipoxygenase activities. A lipid-containing membrane around starch grains was present only in freshly

harvested tubers or tubers stored at room temperature. It contained mainly neutral lipids. The cell wall and the peroxisomal fractions also contained mainly neutral lipids, predominantly as steryl esters. Plasmalemma was enriched in phospholipids, with phosphatidyl choline and phosphatidyl ethanolamine as major constituents. Galactolipids were only present in small amounts, and the major steryl lipid was esterified steryl glucoside. Mitochondria and endoplasmic reticulum had the highest contents of phospholipid and high unsaturation ratios. The highest degree of unsaturation in endoplasmic reticulum was found in its glycolipid fraction, especially in monogalactosyl diglyceride. The presence of cellular membranes containing significant amounts of unsaturated lipids would suggest that special treatment would be required during and/or after processing to avoid off-flavor development in granulated potatoes.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
DEAE-cellulose	Diethylaminoethyl cellulose
DGDG	Digalactosyl diglyceride
DPG	Diphosphatidyl glycerol
EDTA	Ethylenediamine tetraacetic acid
EGS	Ethylene glycol succinate
ESG	Esterified steryl glucoside
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FS	Free sterol
LAH	Lipolytic acyl hydrolase
LOX	Lipoxygenase
MGDG	Monogalactosyl diglyceride
OV-101	Methyl-silicone
PA	Phosphatidyl acid
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PG	Phosphatidyl glycerol
PI	Phosphatidyl inositol
PS	Phosphatidyl serine
QF-1	Trifluropropyl-methyl-silicone

SE	Esterified sterol
SG	Steryl glucoside
TEM	Transmission electron micrograph(s)
TG	Triglycerides
TLC	Thin-layer chromatography
Tris	Tris (hydroxymethyl) aminomethane

I. INTRODUCTION

The quality of lipid in a food is an important determinant of its acceptability. Although the lipid content of potato is relatively small, lipids are important as structural components of cellular membranes and, when hydrolyzed or oxidized, affect the organoleptic properties of the processed tuber. Factors affecting the extent of oxidation are: (1) fatty acid composition of the lipid, (2) presence of oxygen, light and other prooxidants and (3) temperature. This study was undertaken to determine the effects of granule processing steps on the constituents of potato lipids and their fatty acid composition. While this work was in progress, the effect of household cooking methods on the lipid composition of potatoes (Mondy and Mueller, 1977a) and the fatty acid composition of potatoes baked by microwave and conventional methods (Maga et al., 1977) were reported.

As part of the above objective, it was important to ascertain the nature and changes in the spatial distribution of lipids in raw and processed potatoes. To the best of the author's knowledge this aspect of potato lipid research has not been studied in any detail. For this purpose, in situ detection was performed in raw and processed potatoes using a novel technique in which transmission electron microscopy was applied in conjunction with specific solvent extraction of lipids. This provided information about the nature and distribution of cellular lipids in both raw and processed potatoes. Finally, the

in situ information on lipids of membranes was checked and confirmed by a detailed chemical analysis of isolated cellular fractions. These analytical data on potato lipids are discussed in relation to their contribution in the development of off-flavors in processed products.

II. REVIEW OF THE LITERATURE

A. Raw Potato

1. General Aspects

Potatoes (Solanum tuberosum, L.) are among the largest food crops per consumption, and are a nutritionally important part of human diets around the world. They have a high content of carbohydrates and proteins, and are a good source of vitamin C. Due to their importance in food processing, potatoes were and still are the object of many scientific and applied technological studies. These include chemical composition, biochemical and physiological studies during growth and post-harvest storage, and changes induced by processing: cooking, mashing, freezing, thawing, granulation and dehydration. Related to these are changes in color, flavor and texture.

Dehydrated instant mashed potato granules are widely used by consumer and institutional markets. The rapid growth of their production in Alberta over an extended processing season has spurred research into complex problems related to flavor quality, shipping and storage stability, vitamin C retention, rehydration rate, and textural qualities.

The flavor of dehydrated granules does not compare favorably with that of freshly mashed potatoes. Since this is partly due to an extensive pyrazine loss experienced in drying steps of processing, suggestions for flavor enhancement have been made (Marce and Hadziyev, 1977). In addition, the extensive destruction of vitamin C during add-back and freeze-thaw granule processing has necessitated a vitamin

C enrichment step (Jadhav et al., 1975).

Significant increases in granule production can be expected only after major improvements in shipping and storage stabilities are worked out. Such improvements are necessitated by the fact that longer storage can result in a gradual development of an objectionable rancid off-flavor associated with lipid oxidation. Since lipids are well-known precursors in the development of rancidity, it is necessary to elucidate the lipid content and its composition in raw tubers and to follow changes during the freeze-thaw granule process. In the subsequent sections, the author has tried to review the literature pertinent to (i) lipids in raw and processed potatoes, (ii) methodology of processing, (iii) microstructural aspects, (iv) lipid-degrading enzymes in tuber, and (v) lipids as precursors in rancidity development.

2. Potato Lipids

a. Total Lipids

In recent years considerable attention has been given to the study of the composition and metabolism of lipids in photosynthetic tissues and seeds, but relatively little work has been applied to plant storage tissues. Information on potato tuber tissues to date is primarily qualitative, and, in common with most studies on plant lipids, quantitative aspects are frequently confined to determination of fatty acid composition.

Konig (1920), an early potato research pioneer, gave the composition of potato lipids as 76.17% carbon, 11.85% hydrogen, and 11.98% oxygen, with 10.89% unsaponifiable matter.

The quantity of lipids in white potato may be too small to be

of nutritional significance. Surveys of the literature (Lampitt and Goldenberg, 1940; Kröner and Völksen, 1950) indicated that the fat content of ether-extractible matter of the potato ranged from 0.02 to 0.2% on a fresh weight basis, with an average of about 0.1%. Exhaustive extraction of potato granules with petroleum ether yielded about 0.15% fat (dry basis), but further extraction of the potato residue with ethanol gave an additional 0.20% of petroleum ether--soluble material (Talburt and Smith, 1967).

Völksen (1950) found that potatoes yielded 0.1% of a fat with an iodine number of 95.0 and a saponification number of 165.7. Fat from the outer portions of tubers had an iodine number of 101.6 and a saponification number of 161.7, whereas fat from uneven portions had an iodine number of 90.5 and a saponification number of 178.8.

Quantitative estimation of lipids from dried potatoes was carried out by Highlands et al. (1954) who reported about 0.17% potato fat (dry basis) that was extracted with petroleum ether from air-dried and vacuum-dried potatoes. The iodine number was between 158 to 160, and the stability, as measured by the increase in peroxide value during incubation at 64°C, was about the same as that of olive oil.

Total amounts of lipid extracted from Pontiac and Ontario potatoes did not change significantly with storage (Mondy et al., 1963). However, the concentration of lipid was greatest in the periderm and lowest in the vascular storage parenchyma and pith (Talburt and Smith, 1967). The fat from the outer layers of the tuber was a brown, viscous oil, while that from the inner part was light colored and butterlike in consistency (Schwimmer and Burr, 1967). Ohad et al. (1971) found the

relative amount of lipids decreased during growth of potato tubers, and they observed fluctuations in the relative lipid content during storage.

Since potato tubers normally accumulate polysaccharides rather than triglycerides (TG) as their major source of reserve energy, the lipid content of these tissues is generally low, and is mainly comprised of phospholipids and glycolipids. The distribution of lipids in potato peels and in potato flesh was reported by Fricker (1970). The peel was obtained by removing a 1 mm thick outer layer from the tuber, and the clean periderm or skin was readily peeled off after freeze-drying. The lipid content of the peel was 2% (dry weight basis), and that of the flesh 0.61%, while the whole tuber gave 0.77%. When the skin was removed as a clean periderm, the lipid contents were 0.58% for the flesh, 3.36% for the periderm and 0.70% for the entire tuber.

Galliard (1973) analyzed potato tubers from 23 cultivars obtained at harvest. It was shown that the total lipid content ranged from 0.08% to 0.13% fresh weight or about 0.5% dry weight. Results were similar for all cultivars and no significant differences in the individual lipid components were observed between cultivars. The author stressed that the majority of potato lipids were polar, phospho- and glycolipids, hence, polar solvents must be used for efficient lipid extraction. In addition, potato tissues should be treated in boiling isopropanol to inactivate the high levels of two lipid-degrading enzymes, lipolytic acyl hydrolase (LAH) and lipoxygenase (LOX).

The major lipids in potato tubers are those associated with the lipoprotein membrane structure, i.e., phospholipids and glycolipids.

It is unlikely that tuber lipids play a major role in energy storage; thus, the lipid composition of potatoes should reflect the biophysical properties of the membrane structures of the cells and subcellular organelles (amyloplasts, vacuoles, mitochondria, plastids, endoplasmic reticulum, etc.). It is well established that lipoprotein structures only function efficiently with respect to osmotic properties, ion transport, etc., when the "fluidity" of these membranes is strictly controlled. The major factor responsible for membrane "fluidity" at a given ambient temperature is the degree of unsaturation of fatty acids in the constituent lipids. The degree of unsaturation tends to increase with reduced temperature to maintain membrane viability.

Berkeley and Galliard (1974a) reported that the main potato lipid constituents were phospholipids and galactolipids, which were essential for maintaining the integrity of membrane structures. They were rich in polyunsaturated fatty acids. Subsequently in 1975, Galliard and his co-workers concluded from a series of studies that neither the choice of potato cultivar nor of storage temperature was likely to reduce the polyunsaturated fatty acid content of potato sufficiently to avoid the problems experienced commercially in the oxidative degradation of polyunsaturated fatty acids to produce off-flavors in potato products.

Quantitative data were obtained over three consecutive years for lipids in the bud and stem and regions of Katahdin tubers (Mondy and Mueller, 1977b). It was found the total crude lipid was richer in the bud than the stem end of the tuber. They also found that potatoes with greater crude lipid and phospholipid contents were less

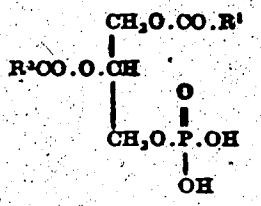
susceptible to enzymatic browning. They concluded that the lipid content of the tuber, although relatively low, was important in determining cellular integrity and resistance to bruising.

b. Phospholipids

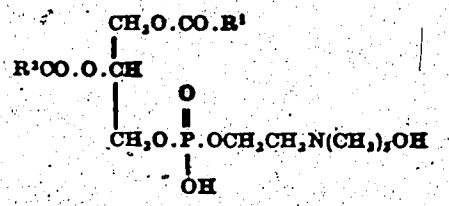
Phospholipids are glycerol phosphatides in which one of the primary hydroxyl groups of glycerol is esterified with phosphoric acid. In most phosphoglycerides the hydroxyl groups at carbon atoms 1 and 2 are esterified with two fatty acid residues. In addition most phosphoglycerides contain an alcohol component in which the hydroxyl group is esterified with phosphoric acid. The most abundant phosphoglycerides in plants are PC and PE (Figure 1). Other common phosphoglycerides in plants are PI, PG and PS.

Phospholipids are about 20% of the total lipids in many photosynthetic tissues (Kates and Marshall, 1975). Phospholipids in leaves and green algae consist mainly of PC, PG, PE and PI, with PC being the major component. However, the amount of phospholipids in nonphotosynthetic plant tissues is much higher than in photosynthetic tissues. Galliard (1968) found phospholipid to be the predominant component of total lipids, at about 45% by weight. PC was about 25.8%, PE 12.6%, and PI 6.3%, the latter being rich in palmitic acid. Similar results were obtained by Bolling (1973), who found that the three major phospholipids in potato tubers were PC, PE, and PI.

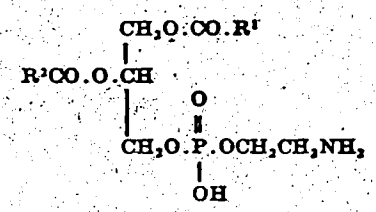
Spengler and Schormüller (1972) reported the separation by Sephadex G-25 and silicic acid column chromatography of polar lipids derived from vacuum freeze-dried raw potatoes. They fractionated the polar lipids on a DEAE-cellulose column and classified the individual fractions by TLC and by chemical analysis of hydrolyzed products.



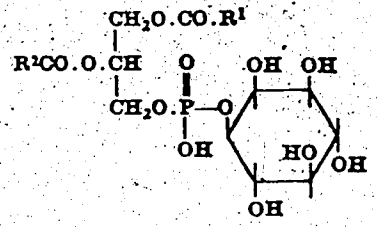
I. Phosphatidic acid



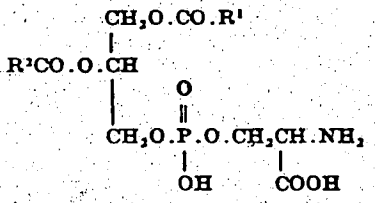
II. Phosphatidyl choline (lecithin)



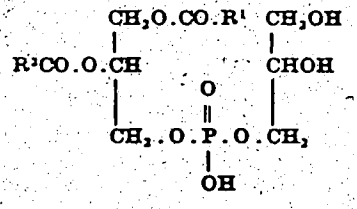
III. Phosphatidyl ethanolamine



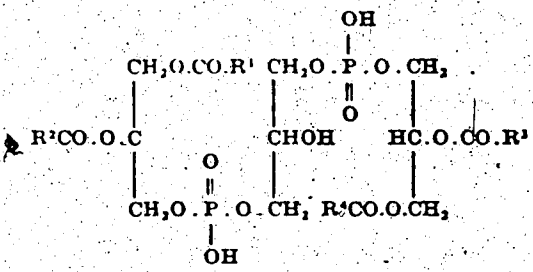
IV. Phosphatidyl-myo-inositol (monophosphoinositide)



V. Phosphatidyl serine



VI. Phosphatidyl glycerol



VII. Diphosphatidyl glycerol (cardiolipin)

Figure 1. Structures of the principal plant phosphoglycerides.

They found SE, FS, MGDG, cerebrosides, PC, PE, PI, PA and a sulfolipid in the polar lipid fraction.

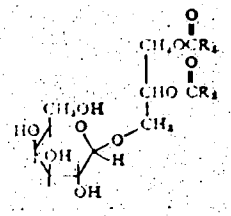
Phospholipids have been shown to be present in subcellular organelles of most plant tissues. PC and PE are the major phospholipids of purified mitochondria from non-photosynthetic tissues. They are the most abundant of the membrane phospholipids, and constitute more than 70% by weight of the total lipids in potato mitochondria (Meunier and Mazliak, 1972). The outer membrane has twice as much PC as PE, whilst the inner membrane has almost equal quantities of these compounds.

The lipid composition of plant mitochondria was investigated by Schwertner and Biale (1973). They reported that 17.6% by weight of potato mitochondria was lipids. Phospholipids, predominantly PC and PE, were 56% by weight of the total lipids. McCarty et al. (1973) also reported that highly purified potato mitochondria contained phospholipids exclusively. From in vivo studies, Mazliak et al. (1975) concluded that membrane phospholipids were synthesized mostly in the microsomes, and less so in mitochondria. This review makes it obvious that cellular organelles exchange their lipids. Moore et al. (1973) showed endoplasmic reticulum to be the site of phospholipid metabolism. Similarly, Beevers (1975) suggested that endoplasmic reticulum was intimately involved, specifically in the production of phospholipids destined for inclusion in the membranes of many organelles. Earlier, Ben Abdelkader and Mazliak (1970) found the microsomal membranes of potato tubers contained PC, PE, and PI as major components of the phospholipid fraction. Demandre (1975) reported that potato plasmalemma membranes were rich in phospholipids, PC and PE being predominant.

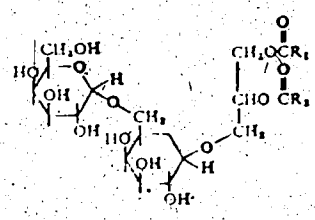
Mondy and Mueller (1977b) reported that potato discoloration was related to anatomy and lipid composition. They found the phospholipid content was higher in the bud end than the stem end. Also, they suggested that the variation in lipid content between the two ends could be explained by the histological development of the tuber. Reeve et al. (1969) observed that during tuber development the bud was more immature than the stem region, and that the former maintained a greater capacity for cellular division, particularly during the later stages of development. The fact that bud region cells were younger and smaller brought about an increase in lipoprotein membrane surfaces within a given volume, and consequently, gave a greater lipid content. Since lipids are a major component of cellular membranes, Mondy and Mueller (1977b) concluded that the membranes of potato tissue with a reduced lipid content were more susceptible to either chemical or mechanical disruption. This would result in greater susceptibility to discoloration by the action of freed enzymes on polyphenols that were initially separate from the enzymes within the mitochondria by lipid membranes.

c. Glycolipids

Plant tissues contain lipids in which 1,2-diacyl-sn-glycerols are joined to sugar moieties by a glycosidic linkage at position 3 (Hitchcock and Nicols, 1971; Kates, 1970). These lipids are major constituents of plants and microorganisms. The most abundant sugar in higher plants and algae is galactose, whereas mannose and glucose are more often found in bacteria. The principal glycolipid components in plants are mono- and digalactosyl diglycerides (Figure 2). Galactosyl diglycerides are formed by transfer of the galactose from uridine

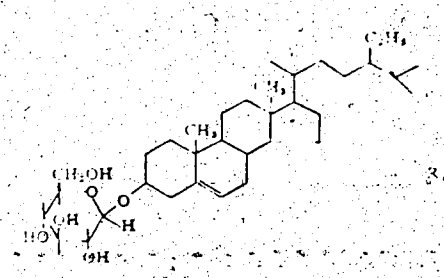


monogalactosyl diglyceride (MGDG)

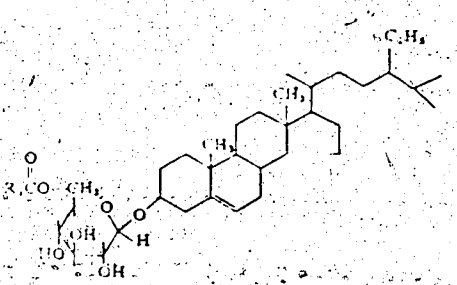


digalactosyl diglyceride (DGDG)

Figure 2. Structures of the principal plant glycolipids.



steryl glucoside (SG)



esterified steryl glucoside (ESG)

Figure 3. Structures of the principal plant steryl lipids.

diphosphate-galactose to a diglyceride. They are also major acyl lipids of the chloroplast.

Galactosyl diglycerides, when present in large quantities, may act as reserves of metabolic energy (Smith and Wolff, 1966; Rosenbert, 1967). Although the lipid contents of potato tissues are too insignificant to serve as energy reserves, galactolipids in potato tubers are present in surprisingly high concentration (Galliard, 1968; Bolling, 1973) as compared to their content in etiolated leaf tissue. In addition, the relative amounts of MGDG to DGDG in potato tissues are just the reverse of those reported for etiolated barley leaves (Gray et al., 1967).

Demandre (1975) reported the presence of MGDG and DGDG in potato plasmalemma membranes. Ben Abdelkader (1972) reported galactolipids amounted to 16% of total lipids in microsomes of potato tubers. McCarty et al. (1973) found that highly purified plant mitochondrial preparations from potato tubers contained no galactosyl diglycerides. However, Schwertner and Biale (1973) showed that their plant mitochondrial preparation contained galactolipids. They also reported that the galactolipid content was markedly higher in chloroplasts than in mitochondria.

In a recent study, Mannella and Bonner (1975) found that the outer mitochondrial membranes of potato contain about 40 µg sugar per mg of mitochondrial proteins. This would suggest the presence of galactolipids in potato mitochondria. However, this has not yet been proved, nor has the distribution of galactolipids in other cellular organelles been investigated.

d. Steryl Lipids

Gurr and James (1975) stated that sterols exist in a great many tissues as a mixture of the free alcohols and their long chain fatty esters. The major sterol in higher plants is β-sitosterol. Most of the sterols are combined with sugars as steryl glycosides, and in some cases the sugar moiety may be acylated with fatty acids. These compounds are known as esterified steryl glycosides (Figure 3). The ratio of steroids to phospholipids and glycolipids is low in the intracellular membranes like chloroplast lamella or mitochondria, but relatively high in the membranes which surround cells such as plasmalemma.

Völksen (1950) reported that non-saponifiable lipid fraction of potato contained cetyl alcohol and a non-nitrogenous steroid. Schreiber and Osske (1962) reported the presence of sitosterol and two unidentified sterols in potato leaves. Johnson et al. (1963) identified cholesterol in the sterol fraction of potato stem and leaves.

Fricker (1970) reported that the main constituent of steryl lipids in potato tubers was ESG, but significant amounts of SG, FS and SE were also found. In a detailed study, Lepage (1964a) showed β-sitosterol to be the major sterol component (85% of the total sterols) in SG and ESG fractions of steryl lipids.

Attention has also been given to the molar composition of sugar moiety in SG and ESG. Galliard (1968) reported a molar ratio near one-to-one of sterol to glucose in steryl lipids of potato tubers.

Dupéron et al. (1972) studied the distribution of steryl lipids in cellular organelles of potato tubers. They reported that

steryl lipids were present mostly in the microsomes, followed by mitochondria. The starch grains and the nuclei contained very little steryl lipids. The steryl lipids present in the starch grain fraction were mostly in the form of free sterols, whereas the steryl lipids in the microsomes and mitochondria were mostly ESG and SG. In a recent review on steryl lipids, Mudd and Garcia (1975) pointed out that the highest concentration of phytosterols occurred in the microsomal fraction, with the second highest concentration in the mitochondrial fraction. Nuclear and chloroplast fractions also contained phytosterols, but in smaller proportions. The presence of major phytosterols, β-sitosterol, stigmasterol, campesterol and cholesterol, in most subcellular organelles led Mudd and Garcia (1975) to suggest that they may have a physiological role associated with the structure and function of membranes. Moreover, stigmasterol and cholesterol are presumably intermediate and end products during the conversion of β-sitosterol to cholesterol (Figure 4), as proposed by Heftmann (1971), who also suggested that β-sitosterol may function as a reserve supply from which plants can produce other sterols.

e. Triglycerides and Other Neutral Lipids

Fatty acids often occur as esters of the trihydric alcohol, glycerol. The properties of TG depend on the fatty acids present, their relative amounts, and their positions in the molecule. In general, when unusual acids are accumulated in a plant, they are stored mostly as TG. Plants which accumulate fats as their major source of energy reserves (e.g., oil seeds) are rich in TG. It is

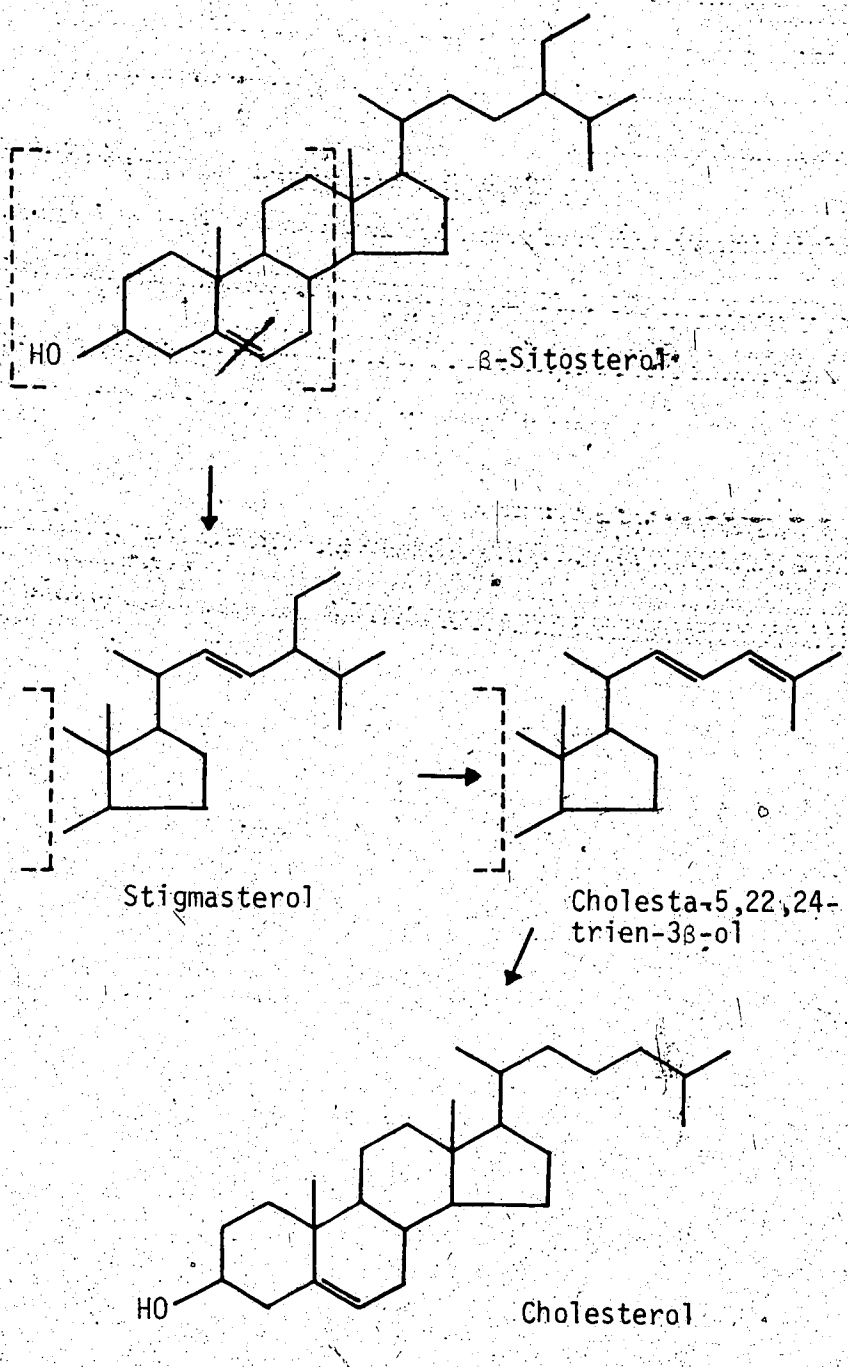


Figure 4.

Postulated Biochemical conversion of β-sitosterol to cholesterol

well-known that TG play only a small role in membrane structure, and are involved mainly as convenient and efficient channels of cellular fatty acid storage. Polysaccharides, rather than TG, are the major source of reserve energy in potato tubers, and their TG content is generally low. Galliard (1968) reported that, although TG content in potato tubers was low, it was the only major component in the neutral lipid fraction. Fricker (1969) discovered an appreciable amount of TG in potato lipids. Free fatty acids (FFA) were present in a lesser proportion.

The distribution of TG, FFA and other neutral lipids in sub-cellular organelles of potato tubers has not been studied in any great detail. Schwertner and Biale (1973) reported that potato mitochondria contained neutral lipids, amounting to about 14% by weight of total mitochondrial lipids. Demandre (1975) also reported that potato plasmalemma were rich in neutral lipids, mainly steryl lipids.

f. Fatty Acid Composition

Völksen (1950) isolated and characterized linoleic, linolenic and palmitic acids in the saponifiable fraction of potato fat, but gave no quantitative data. Using spectrophotometric methods, Highlands et al. (1954) found 41% linoleic acid and 28% linolenic acid in the fat of Katahdin potatoes. In a more detailed study, Buttery et al. (1961) isolated linoleic and linolenic acids from Russet Burbank potatoes by use of gas-liquid chromatography, and identified them by their infrared spectra. They reported 53% linoleic acid and 20% linolenic acid.

A detailed study of lipids and their fatty acid composition using column, thin-layer, and gas-liquid chromatography was provided by Lepage (1968) for potatoes grown in Canada: Netted Gem (Russet Burbank) and Kennebec as representative of excellent processing and storage qualities, and Irish Cobbler and Sebago as fair-to-good storage quality. He found that the predominant fatty acids in Kennebec cultivars of potatoes were palmitic (19.5%), linoleic (44.8%), and linolenic (30.4%). The results showed only a few cultivar differences in content of some lipid classes, and no difference in the fatty acid composition of the total lipids. However, marked differences were observed in the fatty acid composition of some individual constituents of polar lipids. Galliard (1968) also analyzed the fatty acid composition of potato tuber lipids, and found that, although linoleic acid was the predominant fatty acid in most acyl lipids, ESG and PI were relatively rich in palmitic acid. Earlier, Lepage (1964b) found a similar high content of saturated acids in ESG, whereas MGDG and DGDG had higher contents of linoleic acid than other lipids. As in photosynthetic tissues and storage tissues (e.g., turnip, apple and bulbs), PC and PE had similar fatty acid contents; PI and PG were relatively rich in palmitic acid. Later analysis (Fricker, 1972) of fatty acid composition in potato lipids by gas chromatography and mass spectrometry also revealed that the major fatty acids present were linoleic, linolenic, and palmitic acids.

Mudd and Garcia (1975) reported that fatty acid analysis of galactosyl diglycerides of higher plants showed particularly

notable features: (a) galactolipids were highly unsaturated, (b) MGDG appeared to be more unsaturated than DGDG, and (c) palmitic acid was found predominantly in DGDG. These features were observed in potato galactolipids (Galliard, 1968). In addition, he reported that potato galactolipids were rich in linoleic acid. Earlier, Fricker et al. (1972) determined that MGDG and DGDG of potato tuber lipids were very rich in unsaturated fatty acids (linoleic and linolenic acids, 75.7% and 63.8%, respectively).

Galliard (1973) systematically analyzed numerous European potato cultivars and reported that linoleic and linolenic acids were 51 to 60, and 13 to 24%, respectively, of the total fatty acids. He also observed an inverse relationship between linoleic and linolenic acids which tended to maintain the amount of polyunsaturated fatty acids in all cultivars (70 to 76% of the total fatty acids). He concluded that at least 90% of the total fatty acids were represented by one saturated acid (palmitic) and two polyunsaturated fatty acids (linoleic and linolenic). Stearic and oleic acids were normally present in small amounts, and other fatty acids occurred only in trace amounts. Studies by Cherif and Ben Abdelkader (1970) revealed some compositional differences in the fatty acids obtained from the flesh and skin regions of Bintje potatoes, although the major fatty acids present in both regions were the same: linoleic, linolenic and palmitic acids.

Fatty acid compositions have also been reported for cellular organelles such as mitochondria, microsomes and plasmalemma.

Linoleic, palmitic and linolenic acids were the major fatty acids present in potato mitochondria (Ben Abdelkader et al., 1969). Meunier and Mazliak (1972) found that the outer membranes of potato mitochondria contained more saturated fatty acids than the inner membranes. Schwertner and Biale (1973) studied the fatty acid composition of total, neutral, phospho- and galactolipids of potato mitochondria. With the exception of neutral lipids, all the mitochondrial lipids contained mostly linoleic, palmitic and linolenic acids as major fatty acids. Their relative composition varied significantly between lipid fractions. Galactolipids contained relatively more linolenic acid than the other lipid fractions. Oleic acid was a major fatty acid in the neutral lipid fraction.

The microsomal membranes also contained the same fatty acids, but in a different relative composition (Ben Abdelkader and Mazliak, 1970). Cytoplasmic membranes (plasmalemma) had very similar fatty acid compositions (Demandre, 1975). It has also been found that, within the same membrane, PI contained generally more saturated acyl moieties than PC or PE, while DPG contained more unsaturated fatty acids than other lipids (Meunier and Mazliak, 1972). Mannella and Bonner (1975) analyzed the fatty acid composition of the outer mitochondrial and light microsomal membranes of potato tubers and found them to be almost identical.

B. Processed Potatoes

1. General Considerations

Dehydrated potato granules are one of the most popular potato products, and production is still increasing in volume and efficiency. They were first developed as a World War II military item in England, and were introduced into the United States for home use in 1947. Heisler et al. (1953) used solvent extraction for dehydration to produce potato granules. However, the process involved many stages of extraction and distillation, and the final product inevitably contained traces of the solvents employed. Moreover, the loss of some soluble materials during processing resulted in a product with inferior organoleptic quality.

Technological details of potato granule production were adequately reviewed by Olson and Harrington (1955), Feustel et al. (1964), and Gutterson (1971). At present, commercial processing of potato granules is primarily done by so-called add-back processes. Technological details of these and some other pilot plant processes, such as freeze-thaw, will be reviewed in subsequent sections.

2. Add-Back Process

As stated by Boyle (1967), the add-back process is the most accepted method of potato granule production. In general, this process is characterized by longer air exposure and heat treatments of the granules in mash-mixing, conditioning, predrying and drying steps. The simplified production steps are: (1) steam-peeling, (2) trimming, (3) slicing, (4) washing, (5) precooking-water blanching, (6) water cooling, (7) steam-cooking, (8) mash-mixing, (9) conditioning, (10) remixing, (11) air-lift drying, (12) cyclone granule collecting, (13) fluidized-bed drying, (14) fluidized-bed cooling and (15) sifting.

The most common steps in starch manipulation used in the process are precooking (Reeve, 1954b; Harrington, et al., 1959; and Potter et al., 1959), and conditioning or tempering of the moist mix at low temperature (Olson and Harrington, 1955). All these steps were aimed at inducing retrogradation of starch in cooked potatoes so that the starch gel would become less sticky and less soluble. Reeve (1954a & b; 1972), and Bartolome and Hoff (1972) reported that precooking or partial cooking prior to complete cooking tended to increase mealiness of the dehydrated products and to firm the potato tissue. Potter (1954) reported that granulation of the moist mix was easier to accomplish after such tempering. It also improved the texture of the product.

The moisture content of the cooked and mashed potatoes has to be reduced to less than 40% before the mash can be successfully

granulated to a fine powder without excessive cell damage (Olson and Harrington, 1955). To reduce the moisture content of mashed potatoes to the desirable level, recirculation of the dried granules to mix with the freshly mashed potatoes was developed as an important step of the add-back process.

The partially dried potatoes are then subjected to cooling and conditioning at low temperatures, followed by remixing and two stages of drying. The process of drying the potato granules is accomplished with air-lift and fluidized-bed dryers (Olson and Harrington, 1955; Harrington et al., 1959). In most cases granulated potatoes are dried to about 12% moisture in an air-lift dryer, and then to about 6% moisture in a fluidized-bed dryer. The advantages of using a fluidized-bed dryer are: (i) high heat-transter rates and (ii) greater uniformity of temperature (Scott et al., 1967). Finally, the product is cooled to approximately room temperature. The cooled dehydrated granules can be sieved to the desired mesh size to produce a quality product.

3. Freeze-Thaw Process

This process is a pilot plant production process and is characterized by shorter and milder heat treatments of the granules. The development of processing techniques for potato granules with freezing and thawing as an integral step was initiated by Greene et al. (1948), and later described by Harrington et al. (1951), Hall (1953), and Reeve (1967, 1969). Greene et al. (1948) found that freezing cooked potatoes caused a remarkable toughening of the cell wall, and formation of about 50% free moisture which could easily be expressed

from the potatoes after thawing without damaging the cells. Hall (1953) found that a longer freezing time resulted in more water being expelled from thawed potatoes. Harrington et al. (1951) reported that slow-freezing, or quick-freezing followed by slow-thawing of cooked potatoes caused a freezing-out of water from the solubilized starch, leaving a firm structure that would maintain its physical properties throughout subsequent dehydration. Reeve (1967, 1969) observed that freezing and thawing of cooked potatoes reduced the swelling capacity of the gelled starch and influenced its textural properties. This had advantageous effects in the manufacture of granules by rendering them more friable after thawing so that granulation was readily accomplished. A freeze-thaw process involving the steps shown in Figure 5 has been introduced by Ooraikul (1973).

The methods for peeling, slicing, washing, and sulfiting can be any standard methods used in pilot-scale work or industry. Abrasive peeling has been found to result in excessive loss of potato tissue with the peels. Steam-peeling or lye-peeling reduce the loss considerably. The peeled potatoes are longitudinally sliced into strips of about 1.2 x 1.2 cm to facilitate uniform and rapid heat transfer during cooking. Thorough washing of free starch grains from potato slices is necessary to avoid stickiness on the surfaces of the cooked potato slices, since gelatinization of the exposed starch grains would contribute to the pastiness of the final product on reconstitution. Sulfiting by soaking the potatoes in 0.5% sodium bisulfite solution for about 5 minutes has been found to be adequate for arresting enzymatic browning of the potatoes, and it also inhibits microbial

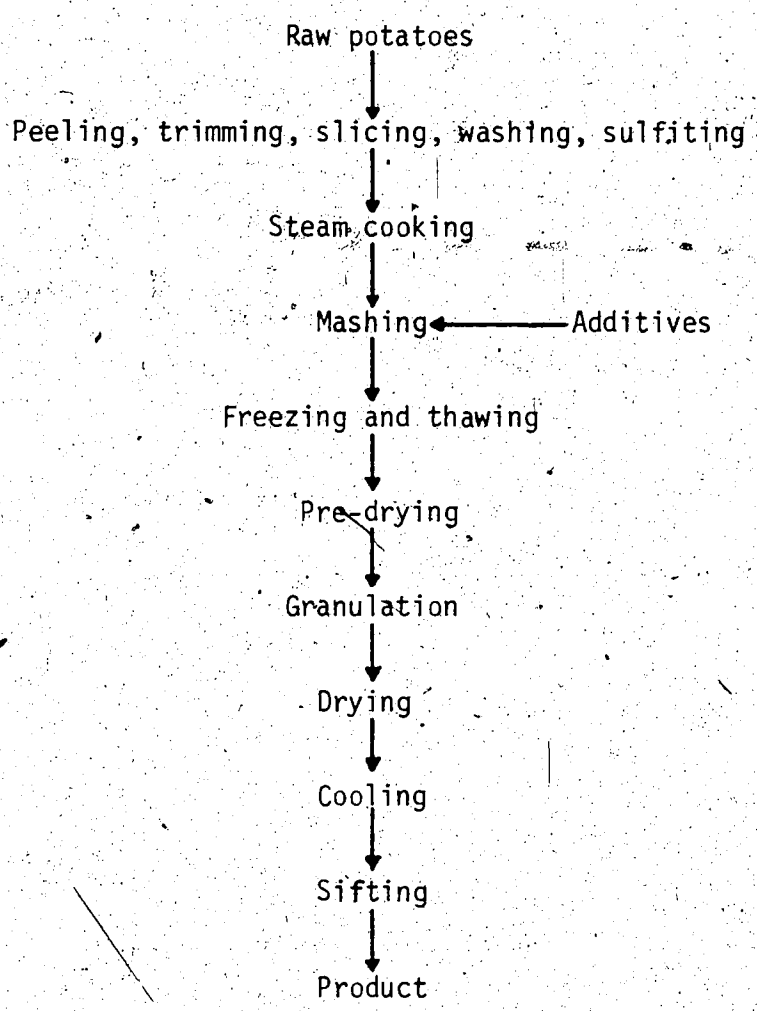


Figure 5. Flow chart of a freeze-thaw process.

growth.

The cooking step serves to: gelatinize starch grains contained in the storage cells, solubilize pectic substances in the middle lamella, and render the potato tissue more suitable for mashing and drying operations. Hence, in cooking potatoes for processing it is desirable to maintain cell wall strength and elasticity, to weaken intercellular bonds, and to render the soluble or amylose starch less soluble (Kintner and Tweedy, 1967). Overcooking causes sloughing or excessive tissue softening, therefore producing more damaged cells, while undercooking results in unmashed lumps and, consequently, higher amounts of broken cells (Severson et al., 1955, Harrington et al., 1959).

Either steam or boiling water can be used as a cooking medium for potatoes. In boiling water, loss of some soluble solids (sugars, proteins, ascorbic acid, solubilized starch, and metal ions) occurs through leaching. This may be beneficial, since excessive extracellular amylose leads to stickiness in mashed potatoes, reaction of reducing sugars and amino acids can produce non-enzymatic browning, and iron can lead to after-cooking darkening of the potatoes. However, the loss of these soluble solids results in the reduction of organoleptic qualities of the product as well as direct economic loss. Jericevic and Ooraikul (1977) and Fedec et al. (1977) reported that microscopic examination of steam-cooked potato tissue showed that large, swollen starch grains completely filled the cells, and also that almost all of the cell walls remained intact after cooking. Hence, steam-cooking is considered to be the most satisfactory method.

When potatoes are cooked, two important components of the potatoes, from the granule processing point of view, are affected, namely, starch and pectic substances. Upon cooking of potatoes at temperatures of 88 to 100°C, all starch grains are rapidly gelatinized. As the process continues, some solubilized starch diffuses out of the cell through primary wall pits in the cellulose matrix (Reeve, 1954a). The tissue cells become distended by the swollen gel and tend to separate, particularly with "mealy" tubers, due to the degradation of pectic substances between and in the cell walls (Reeve, 1967). It was observed that potato tissue at this stage was relatively soft and flexible, permitting cell separation by mashing without excessive damage. On the other hand, Bretzloff (1970) examined the cooked potato texture and appearance under a microscope and reported that no swelling of the cells was observed during cooking of tuber tissue in water. Hence, the hypothesis that starch gelatinization caused distention of potato cell walls during cooking was not supported by his results. In fact, Hoff (1972) disputed the existence of the "swelling pressure" which has been assumed to cause rupture of the cell wall or a rounding-off of the cell surface with resulting cell separation. He also presented an alternate interpretation of cell separation and rupture taking into account functions of calcium, organic acids, cell size, starch content, age and storage time, starch retrogradation and diffusion of amylose.

Personius and Sharp (1939) found that the decrease in cell adhesion after cooking was caused by the weakening of intercellular cementing material of potato tissue. Warren and Woodman (1974)

indicated that composition of the cell wall matrix was the major determinant of cell wall thickness and the degree of intercellular adhesion which ultimately determined the extent of cell separation during cooking. Again, Warren et al. (1975) suggested the possibility that breakdown of cooked potato tissues was due to excessive dehydration of cell wall material. Also, high levels of polyuronides and phytate and low levels of polyvalent metals in the cell wall material favored this breakdown. Moreover, intracellular gases which expanded during heating were forced from the tissue and contributed to the destruction of cell structure during cooking. Linehan and Hughes (1969b & c) suggested that amylose chains might act as a cement between potato tuber cells by formation of hydrogen bonds with polysaccharides of the cell wall. However, Keijbets et al. (1976) found no indication of hydrogen bonding between starch and cell wall polysaccharides which would lead to lower solubilization of pectic galacturonan.

When potatoes are cooked, heat energy is thought to disrupt or weaken some of the bonds in protopectin molecules, resulting in an increase in the water-soluble fraction of the pectic substances (Bettelheim and Sterling, 1955; Jaswal, 1969). Keijbets et al. (1974) reported that solubilization of pectic galacturonan during boiling of tubers (believed to induce loss of cell cohesion) was stimulated by all non-starch constituents except calcium, which inhibited solubilization with increased concentration, and concluded that calcium complexed more strongly with pectin than potato starch (Keijbets et al., 1976). Hughes et al. (1975) reported that the effect of calcium in increasing

the compressive strength of potato dices was small compared to the effect of reduced pH. This increased compressive strength could be overcome by extending the length of cooking.

Mashing determines the extent to which the cooked potato cells are separated and, consequently, the extent of damage sustained by the separated cells. The literature pertaining to mashing has been adequately reviewed by Reeve (1954a, 1959, 1970), Schwimmer and Burr (1967), and Linehan and Hughes (1969a). Ooraikul et al. (1974) found that potatoes mashed at temperatures close to that of cooking gave rather good cell separation with little damage of cell walls. On the other hand, mashing at low temperatures resulted in an increased percentage of broken cells. The effects of food additives such as surfactants on the quality of gelatinized potato starch and pectic substances have been described by Ooraikul and Hadziyev (1974).

The next and the most prominent step of this process involves freezing and thawing of the mashed product. It has long been recognized that freezing and thawing enhance the quality of granules. Rendle (1945), in his patent of the add-back process, introduced an optional step of freezing and thawing of cooked potatoes prior to admixing. He found that, when the mashed potato was frozen and immediately allowed to thaw, the resulting product was more granular and less gelatinous in texture than when freezing was omitted. Greene (1948) reported that freezing cooked potatoes resulted in a remarkable toughening of the cell wall. Consequently, approximately 50% of the moisture in the potatoes could be removed by pressing or centrifuging. He found that, although the macrostructure of the cooked potatoes was

greatly altered by freezing and thawing, the cells appeared unchanged and no ruptures were encountered.

Though some workers have shown that the rate of freezing is not of great importance to the effectiveness of the treatment, a moderate rate should be preferred in order to allow sufficient water to diffuse out of the cells into the intercellular voids while being frozen. After thawing to room temperature, predrying is applied to reduce the moisture content of the cooked mash to about 40% so that it can be granulated to a fine powder without excessive cell damage. The granulated potatoes are dried to about 7% moisture, then cooled and sieved to the desired mesh size. Predrying steps, granulation, drying and cooling steps are performed in a fluidized-bed dryer (Ooraikul, 1973).

4. Effect of Processing on Lipids

During processing into granules, potato lipids may deteriorate chemically by such means as autoxidation, hydrolytic decomposition, or browning reaction. Physical rendering of fat from potato tissue during cooking is also possible. The effects of steam-cooking and drying of potatoes on lipids have not been studied in any detail. However, such effects have been reported for some other food products and vegetables. Fricker et al. (1975) studied the influence of heat treatment of spinach at temperatures up to 100°C on total lipids and glycolipids content. They found that the amount of extractible lipids was dependent on temperature and duration of heat treatment applied.

Mondy and Mueller (1977a) reported the effects of household cooking methods on the lipid composition of potatoes. However, changes in

only the crude lipid and phospholipid content of cortex and pith tissues from Sebago potatoes and their fatty acid composition were dealt with. They suggested that histological changes during cooking, which involved cell rupture and a change in tissue structure, would permit lipids, without being hydrolyzed, to diffuse from the cell. Moreover, the lipids which functioned as structural components of cellular membranes, e.g., phospholipids and galactolipids, would not be free to escape from the tissue, except when fragmented from the membrane as a result of cell rupturing. High cooking temperature would render the lipid components more fluid and susceptible to damage. They found that cooking did not significantly alter the fatty acid composition of tubers. The loss of volatiles during cooking may account for higher phospholipid content in boiled tubers than in tubers cooked in either a conventional or microwave oven. Prolonged heating of tubers may result in membrane damage, particularly in the cortex area, and, hence, greater loss in phospholipids.

Recently, research by Maga et al. (1977) showed only minor differences in overall relative fatty acid composition among four cultivars of potatoes baked by microwave and conventional methods. However, regardless of cultivar, the relative percent of total unsaturated fatty acids was consistently lower in the microwave products as compared to conventionally baked potatoes. In addition, the relative percentage of trans- fatty acids was 2.5- to 4-times higher in the microwave process compared to the same cultivars baked conventionally.

Fricker (1969) investigated the fatty acid composition of the

total lipids of freeze-dried potato by gas chromatography and showed that 90% of the fatty acids consisted of linoleic, linolenic, palmitic, and stearic acids. Differences in fatty acid composition of eight individual cultivars were not appreciable.

Changes in potato lipids during the granule dehydration process have not been explored. However, changes in coconut lipids during desiccation were studied by Krishnamurthy (1975) who found that the fatty acid composition of total lipids and the neutral lipid fractions was similar to that of coconut oil, and was not altered by desiccation. However, the phospholipid fraction, which was rich in unsaturated fatty acids, decreased considerably by desiccation. This indication of oxidation, even under mild conditions of desiccation, would explain the rancidity problem encountered with desiccated coconut and foods incorporating it.

Schwartz et al. (1968) investigated the fatty acid content of dehydrated potato dices and flakes. When dehydrated dices were prepared commercially by cooking diced potatoes, followed by drying in a forced-draft tray dryer, the polyunsaturated fatty acid content matched that of the original tubers. However, flakes made by a standard flake process contained somewhat less of these acids. In both products the degree of unsaturation of the fatty acids changed little, and no off-flavors developed when the samples were reconstituted.

C. Microstructure of Raw and Granulated Potatoes

As outlined in previous chapters, the major lipids in potato

tuber tissues are the polar, phospho- and glycolipids, all of which are associated with lipoprotein membranes of the cell or cell organelles. Electron microscopic examination of potato tuber (M.J.C. Rhodes, unpublished observations) has shown the presence of membranes around amyloplasts (starch grains), which are presumably of plastid origin. Since galactolipids have been shown to be concentrated in chloroplast membranes in leaves (Allen et al. 1966), it seems reasonable to suggest that these lipids in potato tubers may be components of membranes of plastid nature. The structural aspects in raw and processed potatoes are of paramount importance, because such studies provide basic information regarding the nature of principal cellular components. In this section some information on these cellular components will be reviewed. Since the objective of most workers in this area was primarily different from the present author, a correlation of microstructures is purely based on the assumption that, if a biological membrane was present around cellular components, it probably existed as a lipoprotein polymer.

Starch is present in the raw tuber as microscopic grains in the cell; lining the interior of the cell walls of the parenchyma tissue. The grains are ellipsoidal, with an average size of about 60 by 100 microns. Hall and Sayre (1970) used electron microscopy to examine potato starch grains and suggested that each starch grain was surrounded by a membrane.

In studying the amyloplast membranes during maturation and storage of potato tubers, Ohad et al. (1971) used transmission electron microscopy to follow the changes in the morphology of cells from

developing or mature tubers after storage at 4 or 25°C. They observed rows of parallel fibres in the heavy cell wall, and a thin layer of cytoplasm underlying the cell wall, which was intensely stained after osmium tetroxide treatment due to formation of osmic acid derivatives with the cell wall lipids. The vacuolar membrane occupied most of the cell volume. Ribosome-like particles and proplastids surrounded by a double membrane were observed within the cytoplasm. The amyloplast extruded into the vacuolar space, but was connected to cytoplasm by a "stalk". The cytoplasm contained several intact mitochondria, microsomes, and numerous ribosomes. After additional tuber growth, the stalk was broken and the vacuolar membrane was torn. The granule, still coated by the intact plastid membrane, and at least partially by tonoplast fragments, "fell" into the vacuole. However, the remnant of the cytoplasmic body of the cell along the cell wall did not spread or disperse into the vacuole. During all stages of tuber development the starch grains were surrounded by a membrane derived from the plastid envelope. Storage in the cold (about 4°C) resulted in disintegration and disappearance of this membrane; however, it remained intact during storage at 25°C. It was suggested that the cold-induced reduction in the starch content, and an accumulation of sugar during the storage of potato tubers probably involved damage to the membranes surrounding the starch grains. The disintegration of the membrane was thought to have some role in the preservation and maintenance of the starch grain, possibly by restricting contact between the enzymes involved in starch degradation and their respective substrates. However, at high temperature storage (10°C or

higher), this phenomenon was not observed. Moreover, cold storage did not affect other lipoprotein membranes of the cell or cell organelles, and starch membrane dissolution did not change the quantity or composition of total tuber lipids. Recently, Isherwood (1976) demonstrated that the starch membrane started to disintegrate in potatoes, cultivar Home Guard, when stored at 20°C.

Douce et al. (1972) used electron micrographs of purified potato mitochondria to show a homogeneous population of mitochondria with the inner and outer membranes clearly distinguishable. This was in agreement with Bonner (1965), who showed by electron micrograph that the mitochondria were bound by two membranes.

Microscopic examination of cooked potato tissues (Noraikul et al., 1974) showed that the large, swollen starch grains completely filled the cells, and that almost all of the cell walls remained intact after cooking. This implied that the cytoplasmic content of the cells was being pushed toward the cell wall.

Fedec et al. (1977) studied the ultrastructures of raw and processed potatoes by scanning electron microscopy. The technique provided very useful information for major cellular constituents, but could not reveal the nature or distribution of cellular lipids. However, the existence of membranous structures around these cellular components would suggest that the lipids were present as a structural component in these membranes.

D. Lipid Degrading Enzymes in Potato Tubers

1. Occurrence and Localization

Potato tubers have high activities of lipid-degrading enzymes. Galliard and co-workers (Galliard 1970; Galliard and Matthew, 1973; Galliard and Rayward-Smith, 1977) were among the first to demonstrate the presence of phospho- and galactolipases, and lipoxygenase (LOX) in tuber, and the rapidity with which lipids were hydrolysed and peroxidized in tuber homogenates, even at 0°C. MGDG was particularly susceptible to hydrolysis. The lipid-degrading enzymes, which had acidic pH optima, were present mainly in the particle-free supernatant fraction. Free lipids were hydrolysed more readily than those of lipoprotein membranes.

Wardale and Galliard (1975) reported the subcellular localization of lipolytic acyl hydrolase (LAH) and LOX. LOX was localized in discrete but fragile organs which were separate from mitochondria, microbodies and plastids. Microbody, mitochondrial, and microsomal fractions had little or no LOX activity. The authors concluded that, because of the high activity of LAH and LOX in potato tubers, attempts to prepare subcellular fractions would be made difficult by the degradative action of these enzymes on the membrane structures.

The distribution of LAH and LOX in potato tubers was reported by Galliard and Rayward-Smith, (1977). LAH's were 2- to 3-fold higher in activity at the bud end than at the stem end of tubers, whereas LOX was more concentrated in the cortical tissues outside the vascular ring.

Recently, in purified potato mitochondria, Bligny and Douce (1978) identified a membrane-bound LAH which was unmasked by $CaCl_2$

(5×10^{-3} M) and was responsible for rapid enzymatic hydrolysis of the membrane phospholipids, with the liberation of free fatty acids. They also indicated that the absence of detectable lysophospholipids at all Ca^{2+} concentrations and all pH-values ruled out the possibility that the phospholipid breakdown observed in intact mitochondria could be attributed to a phospholipase A_2 and a lysophospholipase operating in succession.

2. Isolation, Purification, and Enzyme Activities

A partially purified LOX from potato tubers was prepared by Galliard and Phillips, (1971). They found that it converted linoleic acid almost exclusively (95%) into 9-D-hydroperoxyoctadeca-trans-10,-cis-12-dienoic acid. The enzyme had a pH optimum at 5.5-6.0, and was inactive at pH 9.0.

The levels at harvest of two lipid-degrading enzymes, namely, LAH and LOX, in tubers from 23 cultivars of potato were measured by Galliard and Matthew, (1973). With the exception of the variety Desiree, all cultivars had very high levels of hydrolytic enzyme, ranging from 5 to 50 μmole of substrate/min/g fresh weight of tuber. In addition, all cultivars had high levels of LOX activity in the range of 1 to 25 μmole of O_2 consumed/min/g fresh weight of tuber. The combined action of these enzymes in the degradation of potato tuber lipids was also discussed.

Pinsky et al. (1973) separated two LOX isoenzymes from potato tubers by chromatography on Ectola cellulose. They further purified one fraction by chromatography on Sephadex G-200 column with phosphate buffer, pH 6.5, as eluent. Both enzymes had an optimum pH of 5.5.

The effects of growth and storage on enzyme activities were

reported by Berkeley and Galliard (1974b), who studied six European potato cultivars and investigated the activity of LAH and LOX during tuber growth in the field, and subsequent storage at 5°C. Both enzymes displayed relatively consistent levels of activity throughout the life cycle of the tuber. LAH showed an overall slight increase with time, and there was a slight decline in LOX activity during storage.

In a recent study, Shepard and Pitt (1976) separated and partially purified two lipolytic enzymes from potato tubers by column chromatography, and electrofocussing of the semi-purified extract. One enzyme, of higher isoelectric value, possessed LAH activity and some activity towards PC. The other enzyme possessed phospholipase and galactolipase activity, but showed a low LAH activity.

Hasson and Laties (1976a & b) claimed that the American potato cultivar Russet Burbank, once sliced, might lose as much as 20% of its galacto- and phospholipids in a matter of seconds, while up to 40% might be lost in just a minute. They isolated and characterized three distinct potato LAH's (Hasson and Laties, 1976a). Enzyme I was shown to be a neutral lipase which favored glyceryl triolein over di- and monolein, and showed no activity with phospho- and galactolipids. Enzyme II, while attacking glyceryl mono- and diolein, was basically a phospholipid and galactolipid acyl hydrolase. Enzyme III was an esterase, since it hydrolysed glyceryl monolein exclusively among the neutral lipids, and showed minimal activity on phospho- and galactolipids.

Hasson and Laties (1976b) purified and characterized a potato phospholipid acyl hydrolase, which in the pH range 7.5 to 8.5, was at least 10,000 times more effective with phospholipids than with galacto-

lipids. It was a soluble enzyme, readily distinguished from a neutral lipid lipase and a third LAH which, while acting on phospholipids, showed a decided preference for glyceryl monolein. The phospholipase had a pH optimum of 8.5. It was stimulated by Ca^{2+} at pH above 7.5, and inhibited by Ca^{2+} at lower pH; was not dependent on detergents, although stimulated by Triton X-100 to a moderate extent, and it remained very active at temperatures near 0°C.

In a subsequent study, Berkeley and Galliard (1976) reported the substrate specificity of potato LOX using a partially purified enzyme preparation from tubers of a potato cultivar with low LAH activity. LOX was fully active only on free linoleic acid or linolenic acid, and only acted directly on more complex glyceride moieties in the absence of any significant endogenous LAH activity.

Recently, separation and characterization of potato LOX was reported (Sekiya et al., 1977). They purified the enzyme by DEAE-Sephadex column chromatography and preparative polyacrylamide gel electrophoresis. The increase in absorbance at 234 nm showed that the purified enzyme caused the formation of hydroperoxide with a conjugated diene system. The enzyme had a optimal pH range of 6.0-6.3.

Nevertheless, the phospholipids of intact potato mitochondria were highly susceptible to degradation by potato phospholipase. It was suggested that this enzyme was involved in the extensive lipid breakdown which occurred in fresh potato slices following cutting, and in the deterioration of mitochondria during their preparation and aging. Since both LAH and LOX were found in the particle-free fraction of potato tissue homogenates after filtration, they could attack lipids in other cell fractions as well. Although both activities were

reduced at high pH, elimination of their potential activity would depend upon finding suitable inhibitors.

3. Control of Lipid-Degrading Enzyme Activities

LOX from potato tubers was found to be inactive at pH 9.0 (Galliard 1971); Farlier, Galliard (1970) reported that LOX had equal rates of oxidation with linoleic and linolenic acids, but no activity towards oleic acid. Also, they found that Ca^{2+}, CN^-, F^- and EDTA (all at 10^{-3} M) and p-chloromercuribenzoate at 2×10^{-4} M) had no effect on LOX activity. They used sodium metabisulfite (2×10^{-3} M) to inhibit lipoxygenase-mediated phenolic polymerization reactions.

Neither fraction of the two LOX isoenzymes separated from potato tubers was inhibited by CN^-, p-chloromercuribenzoate (pCMB), or EDTA at 10^{-3} M (Pinsky, 1973). However, they were inhibited by cysteine, and completely inhibited by 7 M urea.

Recently, Sekiya et al. (1977) found that LOX isolated from potato tuber was inhibited by saturated alcohols and SH-reducing reagents (cysteine and reduced glutathione).

Phospholipid acyl hydrolase, which was active on PC, was not affected by Ca^{2+} (10^{-3} M) and not inhibited by EDTA (10^{-3} M).

Nupercaine is a novel and potent LAH suppressor. Scarpa and Lindsay (1972) showed that it effectively suppressed phospholipase A_2 activity in rat-, and Moreau et al. (1974) in cauliflower bud mitochondria. Scarpa and Lindsay proved that nupercaine alone could effectively replace either EDTA or BSA, which were routinely used to improve the stability of isolated mitochondria. They also recommended the use of nupercaine in preserving the structure and functions of other biological membranes containing phospholipase A_2 during their

preparation and storage.

Moreau et al. (1974) found that phospholipase D was associated with a light membrane fraction which released PA. However, purification of cauliflower bud mitochondria by sucrose density gradient could eliminate the phospholipase D.

In lipid analysis, advantage was taken of the differential rates of breakdown of free and membrane-bound lipids (Galliard 1970). Nevertheless, pH control by buffering the isolation medium would suppress enzyme activities to a large extent. Thus, the control of lipid-degrading enzyme activities could be achieved by carefully controlling the pH during isolation of subcellular fractions, and adding potential enzyme inhibitors to the isolation medium.

The effect of pH on LOX activity was investigated in this study to determine the optimum pH for minimal LOX activity. The effects on LOX activity of some common inhibitors added to the extraction medium was also investigated.

The effect of nupercaine on coupled LAH-LOX activity was also studied, and its potential as a novel LAH suppressor was used to advantage to suppress the lipid-degrading enzyme activities.

E. Lipids as Precursors of Rancidity

Lipid oxidation and degradation are the major sources of off-flavors and rancidity in foods. In addition, oxidation of various unsaturated fats not only leads to loss of the essential fatty acids, but may also cause destruction of some vitamins and pigments, and a reduction in the biological values of proteins (Labuza, 1971). Since potato fat is relatively susceptible to oxidative deterioration, it could be a potential problem in processed potato products such as dehydrated granules, flakes, and similar combinations. In 1949 Burton suggested that off-flavor in mashed potato powder was due to oxidation of the endogenous "fat". Subsequently, lipid degradation has been demonstrated through autoxidation in dehydrated potatoes. Highlands et al. (1954) presented evidence indicating that certain off-flavors in dehydrated potato products were the result of unsaturated fatty acid oxidation.

The contribution of potato lipids to the gradual development of an oxidative off-flavor in stored potato granules was studied by Buttery et al. (1961). Russet Burbank potato granules were prepared by an add-back process. Sulfite (300 ppm as SO_2) was the only additive. Storage in air at room temperature caused an intensive autoxidation of linoleic and linolenic acids, resulting in a gradual loss of the polyunsaturated fatty acids. Granules stored in oxygen lost relatively higher amounts of linoleic and linolenic acids than those stored in air.

Since polyunsaturated fatty acids are responsible for off-flavor development, their content being expressed in a form of an unsaturation ratio was introduced to reflect a convenient index for the probability of autoxidation (the unsaturation ratio equals: a ratio of the sum of linoleic and linolenic acids to the sum of palmitic and stearic acids present in

the total lipids). Freshly dehydrated granules had an unsaturation ratio close to 3.0. This decreased to 1.2 after 4.5 months storage in air, and to 0.7 after 3 months storage in oxygen. The oxidation rates of linoleic and linolenic acids were similar. It was established that two moles of oxygen were taken up for each mole of linoleic and linolenic acids oxidized.

Off-flavor scores for the stored granules, as obtained by a sensory panel, increased with the decrease in unsaturation ratio. A plot of oxygen absorbed from the headspace versus storage time gave a curve typical of lipid oxidation. There was an induction period followed by rapid oxidation, and a tailing-off period.

Buttery et al. (1961) also analyzed the headspace vapor of the can or the vapor above hot reconstituted granules. They detected aldehydes (up to C_6), and hydrocarbons (up to C_5). Hexanal was the predominant volatile compound. Its concentration was about four times that of any other major component from granule autoxidation, and ten times that of the majority of other compounds.

The volatiles from autoxidized granules corresponded to theoretically expected degradation products of linoleic and linolenic acids. Evans et al. (1969) postulated thermal breakdown of hydroperoxides, yielding specific hydrocarbons. The oxidation of pure linoleic and linolenic acids produced mostly pentane and ethane, which were more than 90% of the hydrocarbons released (Arnaud and Wuhrmann, 1974). Measurement of the concentration of hydrocarbons (pentane in particular) was suggested for determination of the extent of oxidative deterioration of dehydrated mashed potatoes.

Potato granules stored in air do not show a high level of headspace volatiles, but show a large concentration upon steam distillation, or in the headspace of hot reconstituted granules. This suggests that the bulk of off-flavor constituents derived from oxidized lipids are produced through the breakdown of precursors after hot reconstitution.

The relation of hexanal in headspace to subjective flavor estimates was reported by Boggs et al.(1964). Commercially produced granules (7% moisture, 2.5 ppm BHT, and 250 ppm sulfite as SO_2) were sealed in cans under air, and stored at 22°C. A control sample was packed under nitrogen and stored at -34°C. Air-packed granules, when reconstituted, showed a hexanal increase proportional to storage time. The increase was slow during the first 2 months, suggesting an induction period. This was followed by a rapid change after 80 days, after which a regular hexanal increase occurred up to 4 months of storage. Their study showed that hexanal concentration was closely associated with flavor deterioration of dehydrated granules as judged subjectively.

Overseas shipment of granules in polyethylene-foil lined paper bags during summer may bring about extensive rancidification. Some shipments of add-back granules (7% moisture, 550 ppm sulfite as SO_2, and close to 10 ppm BHT) became rancid (Dornay Foods, 1976). Hexanal levels, determined by the procedure described by Buttery and Teranishi (1963) and Boggs et al. (1964) were four to five times higher than commercially permitted. The levels of SO_2 decreased at the same time, and tended to reflect the extent of rancidity. The samples with

minimal hexanal levels retained about 4 ppm of antioxidant, the slightly rancid ones only traces, while the highly rancid granules completely lost all antioxidant protection.

Walter and Purcell (1974) found that dehydrated sweet potato flakes underwent rapid oxidative deterioration unless stored in an atmosphere low in oxygen. Of relevance to rancidity problems of dehydrated mashed white potatoes was their suggestion that autoxidation of flakes occurred in a bimodal fashion, with surface lipids being oxidized at a faster rate than internally located, bound lipids. The loss of bound fatty acids was almost too slow to be detectable. Surface fatty acids showed an induction period of 18 days. The oxidation of unsaturated surface fatty acids was found to be independent of peroxide values. The fact that 76% of unsaturated lipids occurred in bound lipids (Walter et al., 1972), and that the bound lipids were oxidized at much lower rates than surface lipids, strongly suggested that environment was often more important for autoxidation than lipid composition.

A plausible explanation of bimodal autoxidation in flakes was that processing brought about a trapping of up to 90% of the lipids into gelled carbohydrates and protein matrices. This lipid protection might retard autoxidation. However, lipids on the flake surface were freely exposed to air and, consequently, were readily oxidized. Therefore, the rancidity responsible for short shelf life of dehydrated mashed potatoes might be attributed mostly to surface lipids.

Potato flakes have a shelf life of only 6 months in air at 23°C, even when stabilized by incorporation of sulfite and antioxidants

(Sapers et al., 1972). Volatile components associated with storage changes can arise from reducing sugar - amino acid interaction, and from lipid oxidation. Storage for 6 months resulted in only small increases in low-boiling aldehydes, and in furfural. Phenylacetaldehyde, the major component of potato flake volatile concentrate, increased slowly during the first 3 months of storage, as did benzaldehyde. Differences between the level of furfural and the Strecker degradation aldehydes in air- and nitrogen-packed flakes were small and variable. These findings suggested that dehydrated potatoes, even flakes packed in nitrogen, might undergo further nonenzymic browning reactions during storage, yielding volatiles detrimental to flavor. However, the shelf life of flakes was not normally limited by flavor defects due to sugar - amino acid interaction. The major objectionable flavor defect was derived from lipid oxidation, which could be controlled to a certain extent by BHA or BHT, and by nitrogen-packing. Flakes stored in air at 23°C for up to 6 months showed a substantial increase in compounds clearly indicative of lipid oxidation (Sapers et al., 1972).

The foregoing literature survey clearly showed that lipids are primarily responsible for off-flavors in stored dehydrated potato products. Recently in our laboratory, Khan and Hadziyev (1978) studied accelerated autoxidation of potato lipids in dehydrated model systems and potato granules. Oxidation rates were highest with glycolipids, followed by phospholipids. Neutral lipids, which were mostly saturated, were oxidized very slowly. Lipid oxidation in dehydrated system was also influenced by the nature of the matrix on which they were spread,

being highest with starch, followed by pectic and cellulosic substances. Protein as a matrix had a protective effect on lipid oxidation. It was suggested (Khan and Hadziyev, 1978) that in processed potato products the most unsaturated lipids (galactolipids) would be most susceptible to oxidative degradation, and that this would be influenced by lipid orientation within or on the surface of the protein--carbohydrate matrices of the final products.

III. EXPERIMENTAL

Chemicals

Nupercaine hydrochloride was obtained from Ciba (Basel, Switz.). Fatty acid poor BSA was obtained from Calbiochem (Los Angeles, Ca.). Sucrose, galactose, EDTA, Tris, sodium dithionite and Celite were obtained from Fisher Scientific Co. (Fair Lawn, N.J.). Cysteine--HCl was from Matheson, Coleman and Bell (East Rutherford, N.J.), and sodium mercaptobenzothiazole from Eastman Kodak Co. (Rochester, N.Y.). Chromosorb P, 100/120 mesh, and EGS were from Chromatographic Specialties (Brockville, Ont.); MN-Kieselgel N from Macherey Nagel & Co. (Düren, Germany). Gas Chrom Q (80/100 mesh), trifluropropyl-methyl--silicone (QF-1), and FAME standards were obtained from Applied Science Laboratories, Inc. (State College, Penn.). Phospholipid standards were purchased from P.L. Biochemicals, Inc. (Milwaukee, Wis.). Silicic acid, chromatography grade, 100 mesh, was from Mallinckrodt (Montreal, Quebec), and methyl-silicone (OV-101) from Analabs (North Haven Conn.). Boron--trifluoride-methanol (14% w/v), BHT, p-chloromercuribenzoate, mono-, di- and triglycerides, fatty acids standards were from Sigma Chem. Co. (St. Louis, Mo.). Standard sterols were from Supelco (Bellefonte, Pa.). For electron microscopy work, propylene oxide was obtained from Polysciences Inc. (Warrington, Pa.). Glutaraldehyde and osmium tetroxide were from Steven's Metallurgical Corp. (New York, N.Y.). Araldite resin 502, dodecenyl succinic anhydride, DMP-30 (Dimethyl amino-methyl phenol), uranyl acetate and lead citrate, were obtained from Ladd Research Industries, Inc. (Burlington, Vermont).

Equipment

Centrifuges used were Janetzki T5 (Leipzig, Germany), Beckman J-21B preparative centrifuge and Beckman L2-65B preparative ultracentrifuge, with rotors SW-27 and a fixed angle type 30, Beckman Instr. Inc. Spinco Div. (Palo Alto, Ca.). The gas chromatograph was a Bendix Model 2500, Bendix Instruments Div. (Ronceverte, W. Va.). For visible and UV spectra a Pye Unicam SP 1800 UV spectrophotometer (Pye Unicam Ltd., Cambridge, England) and a Spectronic 20, Bausch & Lomb Inc. (Rochester, N Y.) were used. For transmission electron microscopy work, a Phillips EM-200 transmission electron microscope from Phillips Electronics Ltd. (Scarborough, Ontario) was applied. For sectioning, a Sorvall type MT2-B "Porter-Blum" ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn., U,S.A.) was used. A measuring magnifier was obtained from Bausch & Lomb Inc. (Rochester, N.Y.). For processing, a Kitchen Aid Mixer from Hobart Mfg. Co. Ltd. (Troy, Ohio) and a Manesty Petrie-Fluid Bed dryer, Model MP 10E Manesty Machines Ltd. (Speke, Liverpool, England), were employed. The densitometer used was a Chromoscan Model MK II, Joyce Loebel & Co. Ltd. (Gatehead, U.K.). A Cahn gram electrobalance, Cahn Div., Ventron Instr. Co. (Paramount, Ca.) used for gravimetric determinations of lipids. A Model 53 Biological Oxygen Monitor equipped with a Clark electrode was from Yellow Springs Instr. (Yellow Springs, Oh.). Other general equipment used was: Fisher Isotemp gravity convection oven and Fisher Accumet Model 230 pH meter from Fisher Scientific Co. (Fair Lawn, N.J.), Virtis homogenizer manufactured by Virtis Co. Inc. (Gardiner, N.Y.), a rotary evaporator, Buchi Rotavapor R, from Buchi Glasapparate Fabrik

(Flawil, Switz.), a Burrell wrist-action shaker, Burrell (Pittsburgh, Pa.), and a Sonicator Model S-75 (Branson, Danbury, CT.).

Methods

A. Production of Dehydrated Granules by a Freeze-Thaw Process

A semi-pilot scale freeze-thaw process introduced by Ooraikul (1973) was applied. Raw potatoes used were the Alberta grown cultivar 'Netted Gem' (Russet Burbank) of specific gravity 1.098. The process was as follows:

Cooking Potato tubers (10 kg batch) were peeled, trimmed, washed and sliced, then steam-cooked at atmospheric pressure for 35 minutes.

Mashing The cooked slices were immediately mashed for 2 minutes at a speed setting of 6 in a Kitchen Aid mixer equipped with a flat beater.

Freeze-thawing The hot mashed potatoes were spread on stainless steel trays, frozen in an air-blast freezer at -20°C for 3 hours, and then thawed at room temperature.

Predrying, granulation, drying and cooling steps These steps were performed in a fluidized-bed dryer, modified to have a rotary stirrer immediately above the porous plate. The thawed potatoes were charged into the bowl and predried with an air temperature setting of about 90°C and a stirrer speed of 20 rpm. When the moisture content of the potatoes dropped to 35-42% after about 16 minutes, the granulation step followed, using a lower temperature setting and minimum air velocity (about 30 cu ft per minute) and a stirrer speed of 400 rpm. After 10 minutes, the temperature and the air flow were increased to begin the drying step. After about 10 minutes of drying, the temperature and air flow were again gradually reduced to avoid cell damage due to abrasion of the granules in the air stream. The heaters were then turned off and the air flow maintained at a low rate to

reduce the granules' temperature to 20°C. In the dehydration steps, the highest temperatures of the drying air, as recorded at the base of the dryer and at the exhaust pipe, were 86°C and 60°C, respectively. The mid-point of the bowl with floating granules had an average temperature of 35°C, rising as high as 76°C but never reaching or exceeding 85°C.

B. Lipid Analysis

1. Sampling and Analysis

Triplicate samples were analyzed for lipids at the following stages: (I) Close to 0.5 kg of peeled and sliced raw tubers were taken from a 10 kg batch for processing. After dicing and mixing, 100 g were taken for analysis; (II) A 100 g sample was withdrawn from the hot mash; (III) 40 g of dehydrated granules were taken from the bowl of the dryer.

Moisture content was determined from weight lost after heating 10 g of sample at 55°C for 5 hours and then at 105°C for 2 hours in a gravity convection oven.

2. Lipid Extraction

Whole tubers were peeled, sliced and refluxed for 5 minutes in 200 ml boiling isopropanol, and then filtered. The residue was homogenized in a Waring blender with 300 ml chloroform-methanol (2:1 v/v), filtered, and re-extracted several times with small volumes of the same solvent. Combined filtrates were evaporated at 40°C in a vacuum flash rotary evaporator. The crude lipid residue was redissolved in 150 ml of the chloroform-methanol solvent, and non-lipid

contaminants were removed by shaking the solution in a separatory funnel with one-fifth its volume of 0.58% aqueous sodium chloride, and leaving it to equilibrate at 4°C (Folch et al., 1957). The upper phase was removed by a Pasteur pipet, and the remaining interface impurities were rinsed twice with small amounts of pure upper phase solvent. The lower phase containing the purified lipids was taken to dryness under vacuum as above. The resulting residue was then dissolved in anhydrous, ethanol-free chloroform, prepared by refluxing chloroform over calcium chloride for 2-3 hours. About 0.5 mg of BHT antioxidant was added, and the volume was made up to 10 ml. The solution was stored under nitrogen in the dark at -20°C.

Lipid from cooked and mashed potato samples and from dehydrated granules was extracted in a similar manner. However, the granules were first rehydrated to the moisture content of raw tuber.

The lipid yields were determined from 20 µl aliquots of lipid solutions that were dried to a constant weight in a stream of nitrogen at room temperature in aluminium-foil cups. Weighing was done with a Cahn gram electrobalance.

3. Lipid Fractionation by Silicic Acid-Celite Column Chromatography

Column chromatography was used to separate the total lipids into neutral lipid, glycolipid, and phospholipid fractions. Silicic acid was washed with hot methanol, followed with hot acetone, and then dried at 105°C for 2 hours. Batches of Celite were also treated in the same way. The adsorbent consisting of 3 g Silicic acid, 100 mesh, and 1 g of Celite was packed in a slurry of diethyl ether, and was allowed to settle in a 30 cm x 8 mm i.d. glass column with gentle

tapping. Successive portions of the suspension were added, and the column was vibrated with a hand vibrator until the slurry of adsorbent formed a bed about 10 to 15 times greater in height than in diameter. The Teflon stopcock was opened carefully, and the supernatant (diethyl ether) was drained into the bed of adsorbent. Aliquots containing 150 mg total lipids were dried under nitrogen and redissolved in about 1 ml of diethyl ether, and applied to the columns. Lipid samples were very carefully layered on the column with a Pasteur pipet without disturbing the adsorbent surface. The stopcock was opened until the sample solution had just drained into the surface of the adsorbent bed. The walls of the column above the adsorbent surface were carefully washed with a few drops of diethyl ether and again allowed to drain into the adsorbent by opening the stopcock. After the lipid samples were washed onto the bed of adsorbent and no lipid remained above the surface, the lipid components were removed from the column by eluting with solvents of gradually increasing polarity. Elution of neutral lipids was with 80 ml diethyl ether, glycolipids with 70 ml acetone, and finally, phospholipids with 50 ml of chloroform-methanol (1:1 v/v), followed by 40 ml methanol. The yield of each lipid fraction was determined gravimetrically using the electrobalance.

4. Lipid Separation by Thin-Layer Chromatography

a. Qualitative Identification of Potato Lipids

Aliquots containing 150 µg of total lipids were applied as double spots to 20 x 20 cm plates coated with 0.3 mm activated MN--Kieselgel N (no binder). For polar lipids, the plates were developed in

chloroform:methanol:acetic acid:water (170:30:20:5 v/v). The chromatograms were then vacuum-dried for 30 minutes. For non-specific identification of lipids, the dried chromatograms were routinely sprayed with 50% sulfuric acid, and charred at 170°C for 20 minutes. Lipids appeared as dark brown spots against a white background.

The identity of individual lipid spots was revealed by specific reagents. A chromogenic reagent prepared as described by Vaskovsky and Kostetsky (1968) was used for detection of phospholipids. Ammonium molybdate (16 g) was dissolved in 120 ml of water. A combination of 10 ml of mercury in 40 ml of concentrated hydrochloric acid was mixed with a portion of this solution (80 ml). This suspension was shaken for 30 minutes and filtered. The filtrate was mixed carefully with 200 ml of concentrated sulfuric acid and the remainder of the ammonium molybdate solution. After cooling, the above mixture was diluted with water to 1 liter. Phospholipids appeared as bluish spots on a white background immediately after being sprayed at room temperature with the above reagent. Glycolipids were identified by spraying the plate with a 0.5% solution of naphthol (freshly crystallized from hexane/chloroform) in methanol:water (1:1 v/v). The plates were air-dried and lightly sprayed with a fine mist of concentrated sulfuric acid. Glycolipids appeared as bluish purple spots upon heating the plates at 120°C. The steryl lipids were located by their purple color when the plates were sprayed with 50% sulfuric acid and warmed at 70°C for 10 minutes.

For neutral lipids, potato total lipid was spotted on thin-layer plates along with spots of authentic standards. The plates were developed in a mixed solvent system. The first development was in a

solvent mixture of diethyl ether:benzene:ethanol:acetic acid (40:50:2:0.2 v/v) and the second in diethyl ether:hexane (6:94 v/v). After drying, the chromatograms were sprayed with 50% sulfuric acid and charred at 170°C. The spots matching the standards were identified.

b. Quantitative Lipid Analysis

(i) Phospholipids

The fraction of phospholipids collected by silicic acid column chromatography was further separated into individual constituents. Aliquots corresponding to a range of 1-10 µg phosphorus were applied to plates and developed in tanks containing chloroform:methanol:acetic acid:water (170:30:20:5 v/v). Authentic standards of phospholipids were also plated in a marker lane. After the chromatogram was developed, the marker lane was sprayed with the reagent of Vaskovsky and Kostetsky (1968). The areas corresponding to PI, PE, and PC were matched with spots on the marker lane and were scraped from the plates. Silica gel blanks adjacent to and corresponding to the area of each phospholipid spot were also scraped. These samples were digested at 250°C for 3 hours with 0.3 ml concentrated sulfuric acid, with occasional swirling of the tubes to break clumps of silica gel. Then a few drops of 30% hydrogen peroxide were added, and heating was continued for an additional hour (Parker and Peterson, 1965). After cooling, the digests were diluted to 4 ml with double distilled and deionized water, using the water to wash down the walls of the tube. Sulfite solution (0.1 ml) was then added, and the tubes shaken to acidify the lower walls of the tubes. 1 ml of molybdate solution

(2% w/v) was added directly into the acid, taking care not to touch the walls of the tube, and 10 mg of ascorbic acid were added and dissolved in the solution. The tubes were heated at 100°C for 10 minutes and cooled. The final volume was made up to 5.0 ml. The tubes were stoppered and shaken to mix the contents thoroughly. Absorption readings were taken at 830 nm (Morrison, 1964). The concentration of phosphorus was calculated from a calibration curve (0-10 µg range) prepared from inorganic phosphorus that was digested as above (Figure 6).

(ii) Galactolipids

Aliquots of the glycolipid fraction (10-100 µg galactose) were applied on a preparative plate of 0.5 mm MN-Kieselgel N. The plate was then developed in tanks containing a chloroform:acetone:water (30:60:2 v/v) solvent system. Standard galactolipids were spotted in the marker lanes of the same plate. After the chromatogram was developed, the marker lanes were sprayed with a dilute solution of iodine in methanol to visualize the galactolipids. The areas matching the MGDG and DGDG spots of the marker lane were scraped from the corresponding sample lanes and eluted with chloroform:methanol:water (4:1:0.1 v/v). The eluted extract was evaporated to dryness under nitrogen and then treated at 100°C for 1 hour with 2 ml of 3 N sulfuric acid. Galactose from the hydrolyzed lipid was determined quantitatively by the phenol--sulfuric acid method of Dubois et al. (1956). To the cooled hydrolyzed lipid solution, 1 ml of 5% aqueous phenol and 4 ml of concentrated sulfuric acid were added, the stream of acid being directed against the liquid surface in order to obtain good mixing. The contents were

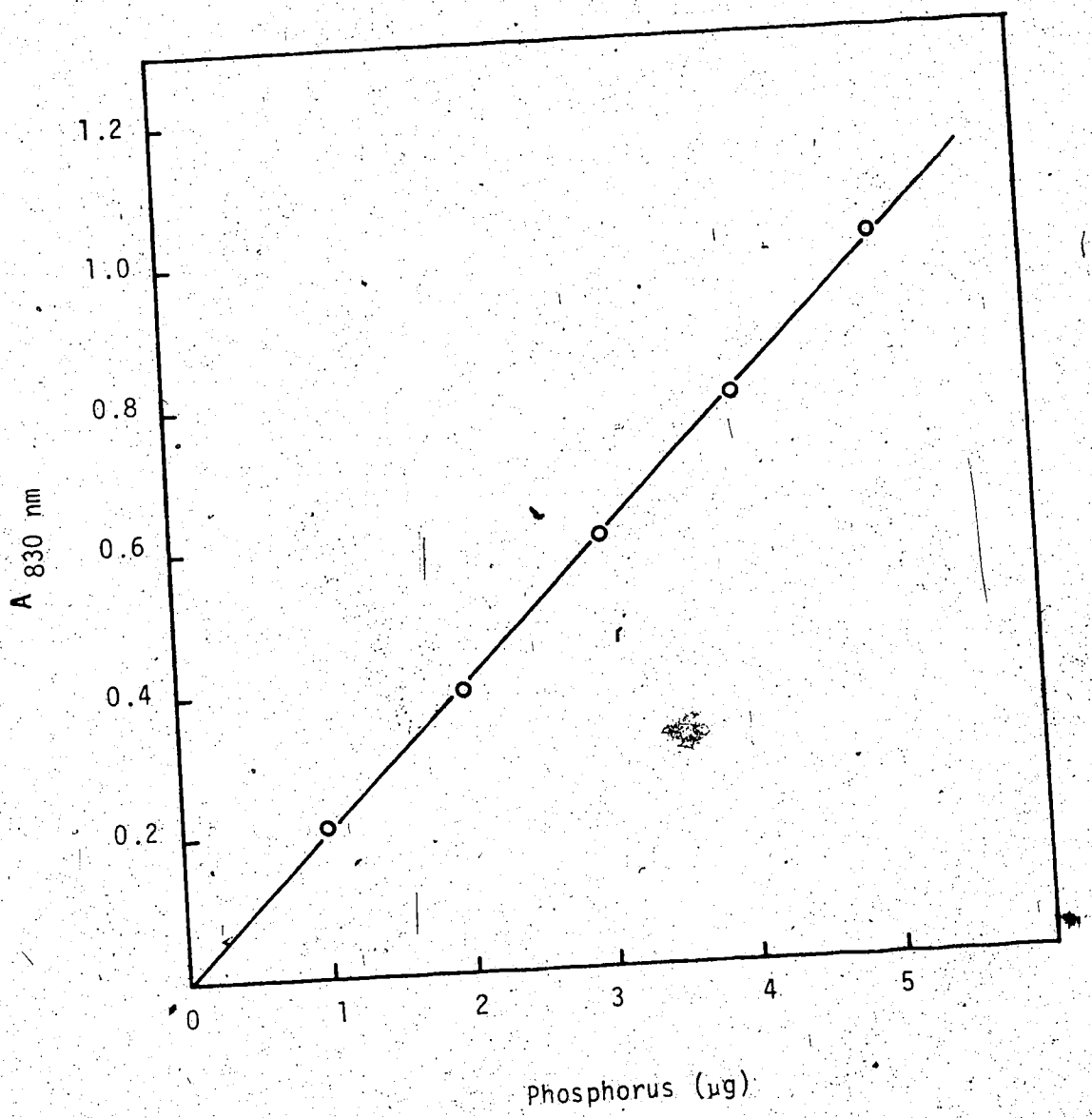


Figure 6. Calibration curve for phosphorus determination. The method was that of Morrison (1964). Readings were taken in 1 cm cell using a Bausch and Lomb Model 20 Spectrophotometer.

then mixed with a vortex vibrator, kept at room temperature for 15 minutes, and the absorbance read at 490 nm. Galactose concentrations were calculated from the standard curve shown in Figure 7. The contents of the other galactolipids were obtained by subtracting the values of MGDG plus DGDG from the total galactolipid fraction.

(iii) Neutral and Steryl Lipids

Bands (10 mg) of neutral lipids were developed on a preparative plate applying a double development method of Freeman and West (1966). The first development was in a solvent mixture of diethyl ether:benzene: ethanol:acetic acid (40:50:2:0.2 v/v) and the second in diethyl ether:hexane (6:94 v/v). Standard TG, FFA and steryl lipids were spotted in the marker lanes. These lipids were identified by spraying the marker lane with a solution of iodine in methanol, and corresponding areas were scraped from the unexposed sample lanes and eluted with diethyl ether. Each isolated fraction was quantitated by taking it to a constant weight after the ether was removed under nitrogen.

Sterols from various steryl lipids were isolated for further analysis. SE was refluxed for 30 minutes under nitrogen with 0.2 N sodium methoxide in methanol. The mixture was then cooled, water was added, and the free sterol present was extracted with diethyl ether. Sterols from ESG and SG were hydrolyzed for 4 hours in boiling anhydrous methanol containing 5% hydrochloric acid. The free sterol was separated from these reaction mixtures on preparative plates using a developing solvent of diethyl ether:hexane:acetic acid (10:90:1 v/v). The free sterol zone was then recovered in diethyl ether and further purified by a clean-up procedure using a glass column, 30 cm x 2.8 cm i.d., filled

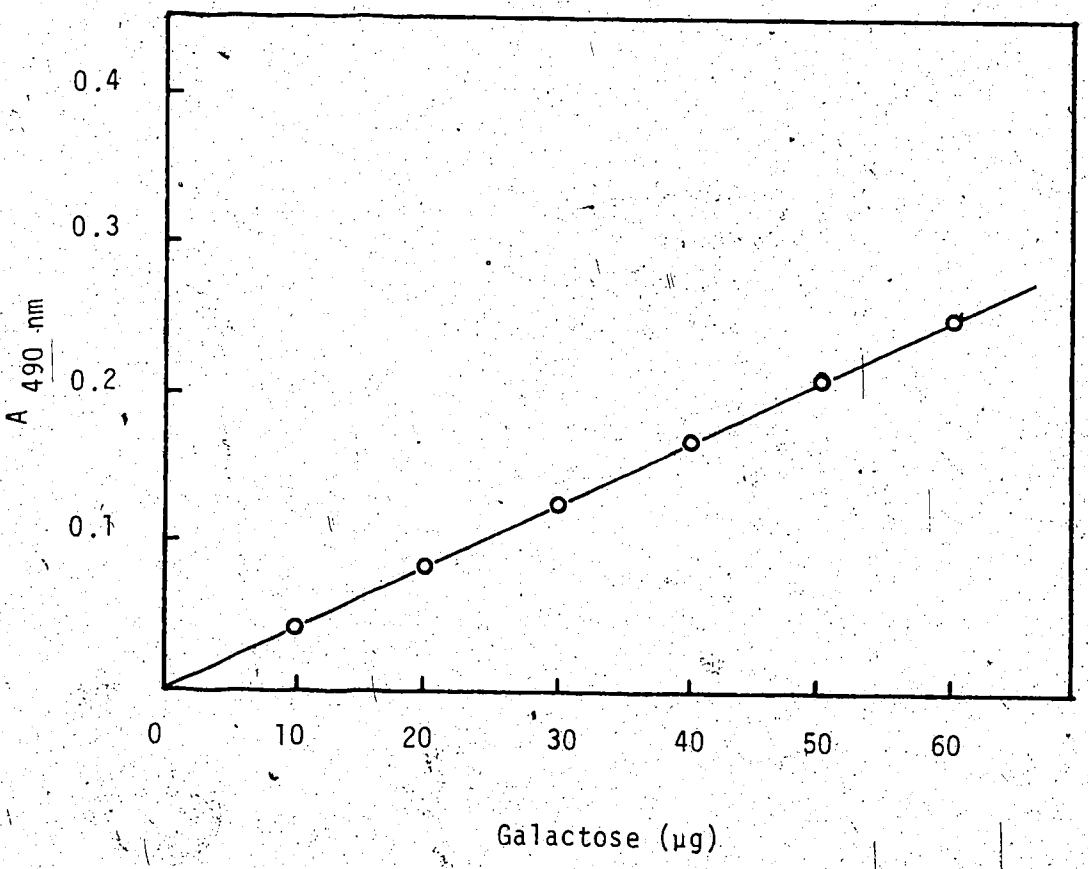


Figure 7. Calibration curve for galactose determination. The method was that of Dubois et al. (1956). Readings were taken in 1 cm cell using a Pye Unicam SP 1800 Spectrophotometer.

with 50 g of florisil (deactivated with 2.5 g water) and topped with a 2 cm layer of anhydrous sodium sulfate. The sterol was applied to the column in a petroleum ether solution. A "clean-up" elution followed with 300 ml of methylene chloride, the effluent being discarded. Sterols were then eluted with 300 ml ethyl acetate: petroleum ether (25:75 v/v). The effluent was evaporated to dryness under nitrogen, and the residue was dissolved in 0.5 ml ethyl acetate containing cholestane as a standard (0.04 µg/ml). This solution was then analyzed by gas-liquid chromatography.

5. Fatty Acid Analysis

Transesterification procedures were used to obtain FAME of the lipid fractions collected from column chromatography, or of individual lipid constituents recovered from thin-layer plates. Neutral lipid fractions of 5-20 mg (and much less for SE, TG, and ESG) were refluxed gently for 2 hours in capped tubes with 2 ml of a methanol:benzene:sulfuric acid mixture (20:10:1 v/v). PI, PE, PC, and MGDG samples were gently refluxed for 10 minutes with 1 ml of boron-trifluoride-methanol solution (14% w/v), as recommended by Morrison and Smith (1964). The DGDG sample was treated for 30 minutes to ensure complete esterification. The esters were isolated from the reaction mixture after it was diluted with water and extracted with hexane. The extract was washed with 0.2 N potassium carbonate and water. The residual water was removed with anhydrous sodium sulfate, and the esters (concentrated in a stream of nitrogen) were analyzed by gas-liquid chromatography on a Bendix Model 2500 gas chromatograph equipped with a flame ionization detector. Chromatography was performed

by injecting an aliquot of the sample into a U-shaped glass column (1.8 m x 3 mm i.d.) packed with 15% EGS polymer coated on acid washed 100/120 mesh Chromosorb P. The operating temperatures were: column 185°C, injector port 220°C, and detector 245°C. Nitrogen was used as a carrier gas, at a flow rate of 60 ml per minute. The fatty acids, as their methyl esters, were identified by means of a calibration curve of equivalent chain length of fatty acids versus their retention times (Figure 8), and by comparison with standard methyl esters. Quantitation was by peak area integration.

6. Sterol Analysis

This was done by gas chromatographic-mass spectral analysis. A Varian gas chromatograph Model 1200, fitted with an "electron impact" ionization detector was used with U-shaped glass columns, 82 cm x 3 mm i.d., packed with 10% methyl-silicone (OV-101) and trifluoropropyl-methyl-silicone (QF-1) in a ratio of 2:3 on an inert support of Gas Chrom Q 80/100 mesh. Isothermal separation at 260°C was done at a helium flow rate of 7 ml per minute. The individual eluted sterols were transferred to an AEI mass spectrometer 2, which had the following operating conditions: accelerating voltage, 80 V; scan rate, 50 a.m.u. per second; oscillographic recorder, 0-450 a.m.u.; sensitivity range, 0.1-1.0; and linear scan mode. The relative retention times were determined for each standard sterol.

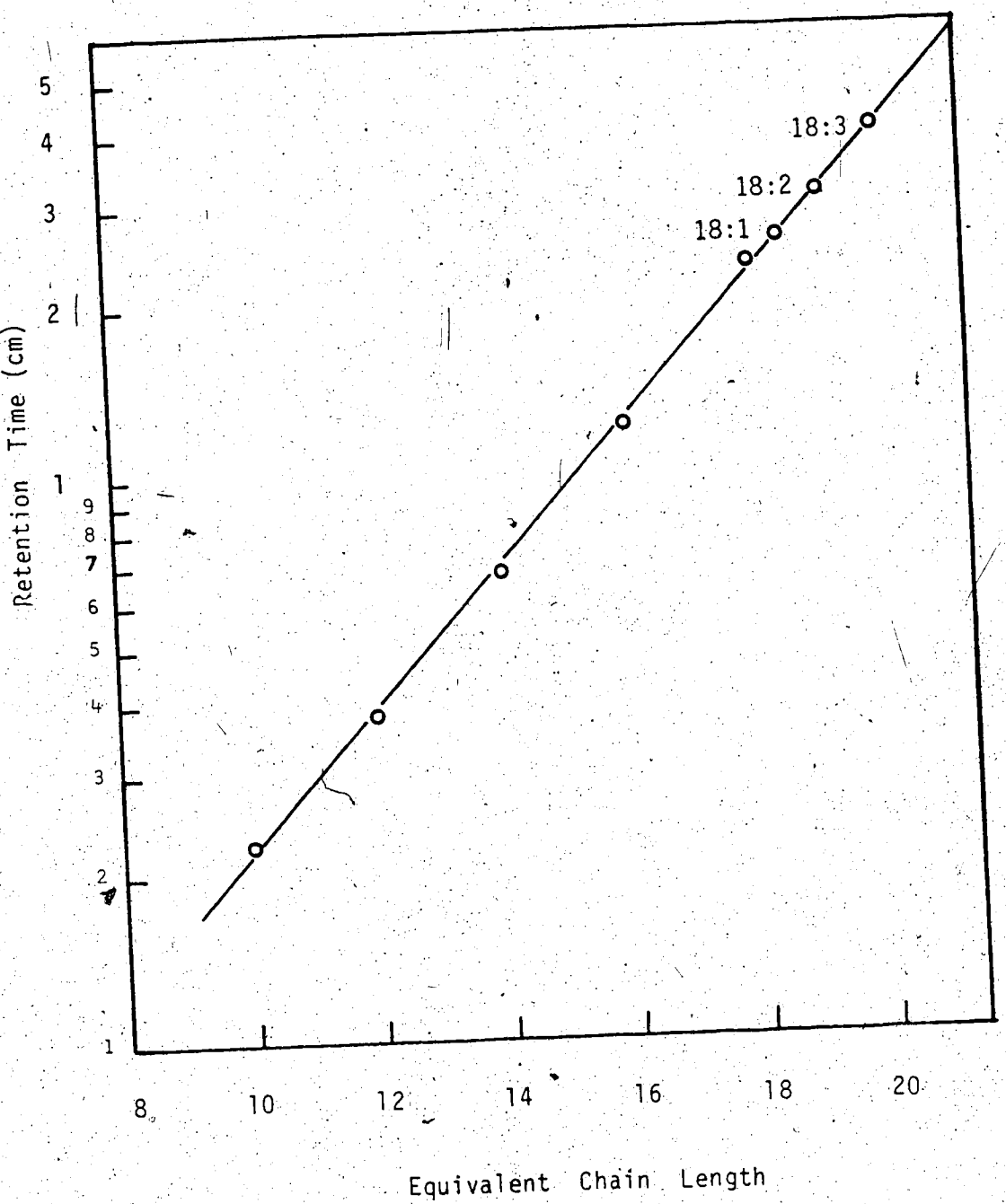


Figure 8. Retention time of standard FAME versus equivalent chain length using EGS as liquid phase. Gas chromatograph Bendix 2500; isothermal separation 185°C, N_2 flow rate 60 ml/min. Pure samples of FAME were obtained from Applied Science Laboratories, Inc., State College, Penn.

C. Isolation of Cell Membranes and Organelles, and Lipid Analysis

1. Isolation of Membranes and Organelles

a. Amyloplasts (Starch Grains)

Potato tubers (200 g) prechilled to 4°C were peeled, sliced (ca. 1 - 2 mm) and homogenized in 400 ml of grinding medium consisting of 0.4 M sucrose, 50 μM nupercaine hydrochloride, 0.5 mM sodium 2-mercaptobenzothiazole and 0.1 M Tris-chloride buffer, pH 7.8, at medium speed for 1 minute at 4°C using a type 45 Virtis homogenizer. The homogenate was filtered through 2 layers of Miracloth and centrifuged at 2,000 x g for 7 minutes to sediment the starch grains. The purity of the starch grains was checked under the light microscope.

b. Plasmalemma, Mitochondria, and Peroxisomes

The post-amyloplast supernatant was centrifuged at 21,000 x g for 15 minutes. The pellet obtained was carefully washed with buffer and fractionated into purified fractions by discontinuous sucrose density gradient centrifugation. Routinely, the crude mitochondrial suspension (approximately 2 ml per centrifuge tube) was layered on top of discontinuous sucrose gradients, and centrifuged in a swinging bucket rotor at 54,000 x g for 50 minutes. The gradients were prepared similar to Douce et al. (1972) by layering sucrose solutions containing 0.05 M Tris-chloride buffer, pH 7.8, into centrifuge tubes in this sequence of concentrations, starting from the bottom of the tubes: 1.8 M (8 ml), 1.45 M (8 ml), 1.2 M (8 ml), 0.9 M (4 ml), and 0.6 M (4 ml). Membranes and organelles separated as distinct bands. These bands were plasmalemma (in 1.0 M sucrose), pure mitochnodria (in 1.35 M sucrose), and peroxisomes (in 1.6 M sucrose). The

fractionated bands were collected with the help of a syringe and diluted with 0.05 M Tris-chloride buffer, pH 7.8, containing 50 μM nupercaine, to a final sucrose concentration of 0.3 M. Centrifugation at 30,000 x g for 30 minutes was done to collect the individual pellets, which were suspended in a known volume of 0.4 M sucrose containing 0.05 M Tris-chloride buffer, pH 7.8, with the help of a loosely fitted glass homogenizer.

c. Microsomes

The post crude mitochondrial (21,000 x g) supernatant was used to isolate microsomes. The supernatant was centrifuged at 105,000 x g for 90 minutes. After centrifugation, the microsomal pellet was washed by suspending it in a known volume of 0.4 M sucrose containing 50 μM nupercaine, 0.05 M Tris-chloride buffer, pH 7.8, and centrifuging it again at 105,000 x g for 90 minutes. The washed microsomal pellet was suspended in a small volume of the above buffer medium. A flow diagram for the isolation procedure is given in Figure 9.

d. Cell Wall

Potato tubers were peeled, washed, cut along the long axis and cut into 1 mm thick slices. Slices (20 g) were mechanically disintegrated with the Virtis homogenizer at full speed (20,000 rpm) for 10 minutes in 50 ml of ice-cold 0.5% sodium sulfite, 0.5 M Tris-chloride buffer, pH 7.8, containing 50 μM nupercaine and 0.5 mM sodium 2-mercaptobenzothiazole. The glass container was put in a trough of ice-cold water to maintain the temperature below 5°C.

The slurry was transferred quantitatively into a beaker.

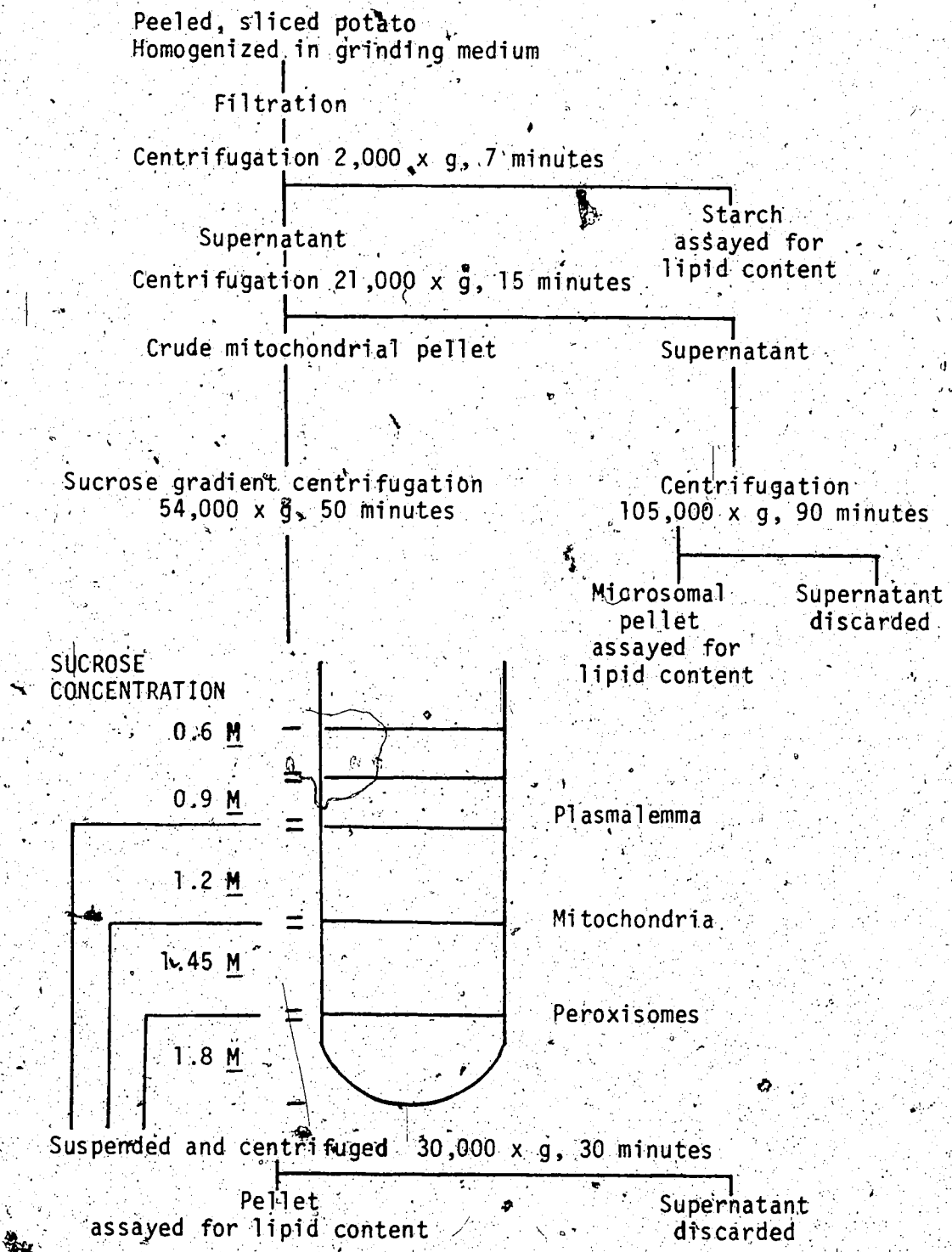


Figure 9. Flow diagram for isolation of starch, plasmalemma, mitochondria, peroxisomes and microsomes.

Combined slurries were transferred to 4 layers of a 200 mesh polyester sieve cloth, squeezed immediately, washed several times with buffer, and then thoroughly washed from entrapped starch grains with demineralized water. Washings followed by intermittent squeezing of the residue were continued until no free starch grains were evident under a polarized-light microscope. The cell wall material after final filtration was squeezed dry and weighed. The moisture content of this semiwet material was determined by drying it at 105°C for 2 hours, then at 95°C overnight, until a constant weight.

2. Lipid Analysis

For extraction of total lipids from organelles and membranes, the isolated cellular fractions (plasmalemma, mitochondria, peroxisomes, microsomes, starch grains and cell walls) were first treated with boiling isopropanol and then further extracted with chloroform-methanol (2:1 v/v). The basic procedure for lipid analysis was essentially the same as described in section B of Experimental.

However, for the separation of neutral lipids, the solvent system of hexane:diethyl ether:formic acid (40:20:1 v/v) (Khan and Kolattukudy, 1973) was used instead of the double development method of Freeman and West (1966).

Quantitative determination of phospholipids in these lipid samples was routinely performed by a convenient and rapid colorimetric method (Raheja et al., 1973). In this procedure, phospholipid phosphorus determination did not involve the acid digestion of the lipid. The phospholipids, after separation by thin-layer chromatography and elution from the silica gel, were heated with a slightly

modified chromogenic spray reagent of Vaskovsky and Kostetsky (1968). The modified chromogenic reagent was prepared by adding 45 ml of methanol, 5 ml of chloroform, and 20 ml of water to 25 ml of the original reagent solution of Vaskovsky and Kostetsky (1968). For colorimetric determination, the lipid sample (1-10 µg phospholipid phosphorus) in chloroform was added to a test tube and the solvent was evaporated; 0.4 ml of chloroform and 0.1 ml of chromogenic solution were added. The tubes were placed in a boiling water bath for 1-1.5 minutes. After cooling to room temperature, the tubes were allowed to stand for 5 minutes, then 5 ml of chloroform were added and the mixture was shaken gently. The contents of each tube were transferred to a 15 ml separatory funnel. After standing for 30 minutes, the lower, chloroform layer was removed and the absorbance at 710 nm was taken against a blank using a Bausch & Lomb Spectronic 20 spectrophotometer with a 1P40 tube and a red filter. The concentration of phosphorus was calculated from a calibration curve (0-10 µg range) prepared by using purified potato PC and PE as standard.

D. Assay for Mitochondrial Viability

The respiratory control ratio (r.c.r.) and ADP/O value were measured. The assay medium consisted of 0.3 M mannitol, 4 mM $MgCl_2$, 5 mM H_3PO_4, 0.075% BSA (w/v), 50 mM TES, pH 7.2, with 8 mM succinate as a substrate. Oxygen uptake was measured polarographically as outlined under enzyme assay in Section F. After the addition of 100 µl mitochondrial suspension to 2.9 ml of the assay medium, 0.3 mM of ADP was added at least twice to record more than one cycle for r.c.r. and ADP/O values (Haydar and Hadziyev, 1974).

E. Transmission Electron Microscopy (TEM)

The principle of transmission electron microscopy involves placing the prepared specimen between a source of electrons and an imaging system. In the following section, details of the specimen preparation for TEM analysis are described.

1. Preparation of Samples and Their Treatment with Specific Solvents

The raw potato tuber was peeled, halved along the short axis, and the parallel cuts obtained were then sliced to remove the cortex zone, which was cut into cubes ca. 1 mm thick with stainless steel blades, and immersed in 0.1 M phosphate buffer, pH 7.8, containing 50 μM nupercaine hydrochloride. Approximately 0.5 g of the tissue was kept as a control, while approximately 3 g were placed into each of five 125-ml Erlenmeyer flasks containing 30 ml of the following solvents for lipid extraction: (a) petroleum ether, (b) acetone, (c) chloroform:methanol (2:1 v/v), (d) [redacted]thanol (1:1 v/v), and (e) butanol saturated with water. The flasks were then well-stoppered and agitated continuously for 1 hour at room temperature using a Burrell wrist-action shaker. The various solvents were then decanted and replaced by 30 ml of fresh solvents. The lipid extraction process was repeated for another hour, and the samples were kept overnight. The samples were again subjected to a final extraction with 30 ml of fresh solvents for an hour, and then left to air dry. For the cooked potato sample, the tuber was sliced longitudinally into two halves, and then precooked at 70°C for 20 minutes in water, cooled for 10 minutes in running tap water, then steam-cooked for 30 minutes, and finally cooled in tap water. The cortex zone of the

cooked tuber was also removed from these samples.

An appropriate amount of each sample was transferred into glass vials containing 3% glutaraldehyde in the same buffer, and then aspirated for 10-15 minutes, or until the tissue sank to the bottom of the vials. Glutaraldehyde was used because it penetrated rapidly into the tissue and stabilized any carbohydrate present inside the cell or cellular organelles. Phosphate buffer was used because it did not react chemically with the fixative solution, was not toxic to the tissue, and maintained the pH of 7.8 at which the activity of lipid--degrading enzymes was minimum. Fixation was carried out for 6 hours at 4 °C. The fixed material was washed thoroughly by gentle agitation in 3 changes of the same buffer, each lasting 30 minutes. Washing of the excess glutaraldehyde was necessary to prevent its reaction with osmium tetroxide. The washed samples were post-fixed with 2% osmium tetroxide in the same buffer, and fixation was continued for an additional 6 hours at 4°C. During this time, lipids and proteins were fixed and stabilized by reaction with the reagent. Osmium tetroxide preserved lipids, firstly, by forming addition compounds with unsaturated fatty acid chains, and, secondly, by its solubility in triglycerides. Thus, it not only preserved the fine structure of the tissues and their cellular components, but also provided electron contrast by its physical density. Though it was very poor at preserving carbohydrates, it was extremely good at preserving the phospholipoprotein membrane skeleton of the cell. In addition, it did not harden, embrittle, shrink, or swell the fixed tissue. After fixation, the samples were again rinsed three times with phosphate

buffer at 30 minutes intervals. The fixed tissue was dehydrated in graded concentrations of ethanol, (50%, 70%, 80%, and 95%) at 30 minute intervals. Ethanol was used because it did not harden the tissue, but did make it brittle for subsequent ultra-thin sectioning. All above treatments were carried out at 4°C. The samples were then treated overnight at 4°C with 98.6% ethanol, followed by another change of 98.6% ethanol, for 1 hour at room temperature to ensure that all moisture was removed from the tissue. This was essential because infiltration by propylene oxide would be incomplete and unsatisfactory if moisture was present. Henceforth, all subsequent treatments were carried out at room temperature. The 98.6% ethanol was then replaced with propylene oxide:98.6% ethanol (1:1 v/v), and the dehydrated samples were kept in this mixture for 45 minutes to permit further dehydration. They were then infiltrated with three changes of propylene oxide at 45 minutes intervals, with periodic stirring. The propylene oxide was then discarded and quickly replaced with a propylene oxide:araldite mixture (1:1 v/v), the latter consisted of dodecenyl succinic anhydride, araldite resin 502, and DMP-30 (49:49:2 v/v). Infiltration was continued for 3 days with occasional stirring to facilitate vaporization of propylene oxide. Propylene oxide was used because it was completely miscible with the epoxy resins used for later embedding purposes. Also, it was extremely volatile and had a low viscosity, so it penetrated the tissue very rapidly, carrying with it the resin monomer, and then evaporated, leaving the monomer behind. The samples were transferred from the vials to flat silicone rubber molds, properly aligned, and then embedded in araldite mixture, so that the

cells were sufficiently stiffened for thin sectioning. Polymerization was carried out at 65°C for 36 hours, during which the infiltrating fluid permeating the tissue was hardened so that it formed a solid matrix which supported the tissue rigidly without disturbing any of the spatial relationships. The processes of dehydration and infiltration were carried out as rapidly as possible since all the reagents used were powerful lipid solvents, and would remove a significant amount of lipid even after fixation with osmium tetroxide.

The solid matrix incorporating potato tissue was thinly cut into sections of approximately 600-700 Å thickness, using a Porter--Blum ultramicrotome equipped with a glass knife. This ultramicrotome can give sufficient precision and reliability under optimal operating conditions; sections in long unbroken ribbons can also be obtained easily. The glass knife was used, since glass is homogeneous and hard without being excessively brittle, and is therefore an ideal material for use as a cutting edge. The individual sections were then transferred and mounted on Formvar (0.2% in ethylene chloride) coated 200 mesh copper grids. Formvar is a plastic used to prepare support films, and the copper grids are used for accommodating the thin sections. The mounted samples were then stained with 2% aqueous uranyl acetate for 2 hours, and post-stained with 0.2% lead citrate (alkaline) for 4 minutes prior to examination under the electron microscope. Aqueous uranyl acetate contains uranyl ions of high atomic weight which are very effective in scattering electrons. Also, these ions combine in large amounts with nucleic acid and phosphate groups, thereby improving

the contrast of such organelles as the nucleus. Thus, the treatment with aqueous uranyl acetate, following double fixation with glutaraldehyde and osmium tetroxide, markedly improved the appearance of membraneous structures. Moreover, citrate did not interfere with staining, because anionic tissue binding sites apparently had a greater affinity for the lead cation than for the citrate. Most probably, citrate formed stable complexes with the cationic alkaline lead salts.

In the above section-staining, the electron scattering power (contrast) of the tissue constituents was increased by reaction of the mounted sections with solutions of heavy metals (section stains). Hence, staining not only increased the observed contrast and facilitated focussing, but also provided a possible means of obtaining cytochemical information about cell components.

The grids were then examined with a Phillips EM-200 transmission electron microscope, operated at an accelerating voltage of 60 kV, and images of the sections, known as "electron micrographs", were recorded using Kodak 35 mm black and white film.

Finally, the size of each of the cell organelles and membrane systems was determined from electron micrographs of known magnification with the aid of a measuring magnifier. A minimum of five random measurements were made. Statistical analyses were based on a confidence limit of 95%.

2. Precautions Pertinent to Potato Tissues for Transmission Electron Microscopy Studies

Several precautions are necessary during preparation of potato specimens for transmission electron microscopy. Since osmium

tetroxide solution penetrates slowly, potato tuber must be cut into very small cubes (less than 1 mm edge) so that the fixative solution can penetrate through the tissue and its membraneous structures. A buffer is needed to maintain the pH of the fixative solution at the physiological value. It also takes care of any dilution of the fixative caused by mixing with the tissue fluid, since the salts in the buffer can increase the tonicity of the fixative sufficiently to prevent osmotic shrinkage or swelling. After fixation, disruption of structure by autolysis, or attack by microorganisms must be prevented. In addition, various processes involved in dehydrating and embedding must not remove or add any components, or distort the relationships of the cell organelles and membrane systems. During sectioning, potato tissues must be cut very thin to eliminate structural overlap, and ensure that the electron beam can penetrate through the section and higher resolution can be obtained as a result of the reduction of scattering or electron loss. However, a decrease in section thickness is accompanied by a decrease in contrast. The cell must be stiffened sufficiently so that it is not damaged by the sharp cutting edge during sectioning.

F. Enzymic Breakdown of Lipid Substrates

1. Source of Enzymes

Peeled and diced whole tuber tissue (100 g) was homogenized at high speed at 4°C in a Virtis homogenizer with 200 ml of 0.1 M acetate buffer, pH 5.5, containing 2 mM sodium metabisulfite. The crude homogenate was filtered through two layers of Miracloth and centrifuged at 15,000 x g for 30 minutes. The supernatant was used as

the source of crude enzymes (Galliard, 1970). In assays in which the polyphenol oxidase darkening effect was inhibited and the change in LOX activity monitored, sodium metabisulfite was replaced by other inhibitors in the homogenization medium. Sodium mercaptobenzothiazole and p-chloromercuribenzoate were used at 0.1 mM, and sodium dithionite, cysteine hydrochloride, and sodium cyanide at the 2 mM level.

2. Substrates

The activity of LOX was followed by using 1 mM freshly distilled linoleic acid (b.p. 149-150°C at 0.4 mm Hg), in the form of its ammonium salt, in 0.1 M acetate buffer, pH 5.5. The effect of pH on activity was studied using 0.1 M buffers: sodium acetate (pH 3.0-5.5), potassium phosphate (pH 6.0-7.25), and Tris-chloride (pH 7.5-9.5).

The coupled LAH-LOX activity measurements were monitored in substrates consisting of purified lipids isolated from whole potato tuber. Fractions of NL, phospho- and glycolipids, as well as individual lipid compounds such as PC, PE, PI, MGDG, DGDG, SE, and ESG, were used as substrates; and were emulsified in phosphate buffer, pH 7.0, by Triton X-100 (0.2%), enhanced by ultrasonication for 30 seconds. The lipid weight used was adjusted so that the combined concentration of the linoleic and linolenic acid substrate was 1 mM.

Nupercaine, used as a LAH inhibitor, was prepared as a 15 mM aqueous stock solution, and was added in levels of 50-450 μM to 6 ml lipid substrate before incubation.

3. Enzyme Assay

LOX-catalyzed oxidation of the lipids was followed

polarographically in a Model 53 Biological Oxygen Monitor equipped with a Clark electrode. Air-saturated substrates (3 ml) were stirred continuously at 25°C and, after equilibrium was attained, the enzyme extracts were injected. Activities were calculated from initial rates of oxygen uptake, assuming an initial dissolved oxygen concentration of 240 μM/liter at 25°C. Preliminary assays with at least four concentrations of enzyme extracts were used to establish the linear relationship with oxygen uptake. One unit of LOX activity corresponded to an uptake of 1 μmol O_2/minute. Enzyme substrates or inbibitors were omitted in control assays. The effect of temperature on enzyme activity was recorded in the range of 15-35°C.

IV. RESULTS

A. Lipids in Raw Potato Tuber

Data presented in the following sections were obtained by extracting lipids from whole raw potato tubers.

1. Total Lipids.

It was found that extraction of potato lipids could be best achieved by using the method of Folch et al. (1957). Fractionation of lipid classes on silicic acid-Celite columns did not provide complete separation, since neutral lipids (NL) contained a portion of ESG, and glycolipids (GL) some SG. Therefore, further separation by thin-layer chromatography (TLC) was required. The results in Figure 10 show a clear-cut separation of polar lipids on a thin-layer chromatogram developed in a solvent mixture of chloroform:methanol:acetic acid:water (170:30:20:5 v/v). The R_f values of lipid classes in decreasing order were: NL 0.86; ESG 0.74; MGDG 0.69; SG 0.53; PE 0.36; DGDG 0.23; PC 0.14; and PI 0.10. In the above solvent system, the polar classes of potato lipids could be distinctly separated, while all the NL moved close to the solvent front as one spot. Quantitative separation of each type of lipid using selective development will be described in subsequent sections. It would be worthwhile to state here that, after application of lipids on thin-layer plates as a narrow band, they can be developed in any appropriate solvent mixture. A uniform spraying with sulfuric acid and subsequent charring would produce bands of individual lipid. These bands could be measured densitometrically. A densitometric estimation of a thin-layer chromatogram developed in the solvent system described above is given in Table 1. However, numerous separations showed that densitometric results were not sufficiently reliable or reproducible,

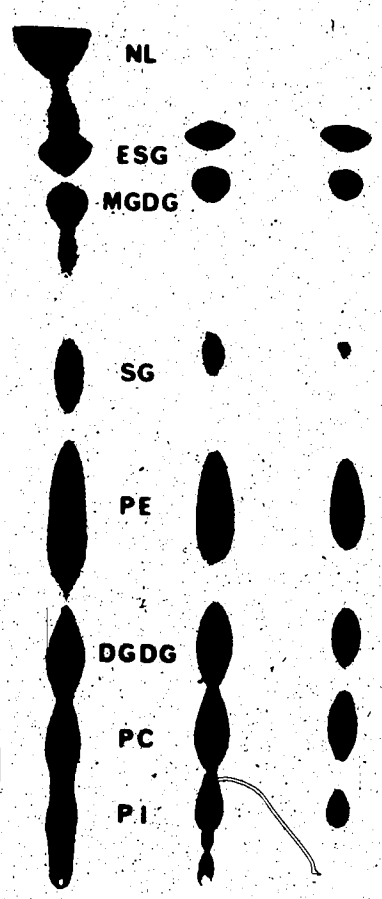


Figure 10. TLC separation of potato polar lipids. NL = neutral lipid; MGDG and DGDG = mono- and di-galactosyl diglycerides; SG = steryl glucoside; ESG = esterified steryl glucoside; PC, PE, and PI = phosphatidyl -choline, -ethanolamine and inositol, respectively.

Figure 11. TLC separation of potato neutral lipids with double development. FS, SE = free and esterified sterol; TG = triglyceride; FFA = free fatty acids; SG = steryl glucoside; ESG = esterified steryl glucoside; U = unknown.

Table 1. Polar lipid composition based on densitometric determination.

Lipid*	Raw potatoes	Steam cooked and mashed	Dehydrated granules
Esterified steryl glucoside	15.1	19.1	22.5
Monogalactosyl diglyceride	9.6	10.6	10.7
Steryl glucoside	8.2	9.1	6.2
Phosphatidyl ethanolamine	24.9	23.0	21.6
Digalactosyl diglyceride	15.8	14.4	13.2
Phosphatidyl choline	17.1	17.3	19.4
Phosphatidyl inositol	9.2	6.5	6.5

*Listed in order of their decreasing R_f values. The results represent the percent of individual major polar lipids with respect to the total amount of polar lipids recorded densitometrically.

so they were used only to gain preliminary semiquantitative data. An accurate analysis required lipid recovery from plates followed by gravimetric or colorimetric determinations. Thus, it was found that the total lipids (TL) in raw potato represented 0.16% on a fresh, or 0.65% on a dry weight basis. Their composition is given in Tables 2 and 7.

2. Phospholipids

A major portion (52%) of the TL was in the phospholipid fraction. The pure phospholipid fraction eluted from the silicic acid-Celite column was further separated into individual phospholipid components by TLC. Thin-layer chromatographic separation of potato phospholipids in a solvent mixture of chloroform:methanol:acetic acid: water (170:30:20:5 v/v) is shown in Figure 13. The R_f values of individual phospholipids, in decreasing order, were: PE 0.41; PC 0.18; and PI 0.08. Quantitative determination by phosphorus estimation showed that PC was the major phospholipid, followed by PE. PI was also present in substantial quantities. The relative amounts of individual phospholipids (PC, PE, and PI) present in the TL are presented in Table 2. Other phospholipids such as DPG, PS, etc. were present only in trace amounts. Therefore, their individual values are not incorporated in the table; instead their combined content has been included.

The relative compositions of fatty acid moieties present in these phospholipids are reported in Table 2. PC, the major phospholipid fraction, contained mostly linoleic (18:2), followed by palmitic (16:0), and linolenic (18:3) acids. Small amounts of stearic (18:0) and oleic (18:1) acids were also present. PE also contained linoleic acid as its major fatty acid; however, the relative amount of palmitic acid was

Table 2. Composition of lipids and fatty acids in raw potato tuber.

Lipid	Wt % of total lipids*	Fatty acid composition (% of total fatty acids)**				
		16:0	18:0	18:1	18:2	18:3
Total phospholipid	51.9					
PC	25.0	18.2	2.2	1.3	62.3	16.0
PE	16.8	24.2	2.7	1.2	62.8	9.0
PI	9.1	36.4	4.0	1.0	46.3	12.3
Total galactolipid	20.0					
MGDG	5.8	4.2	2.3	0.5	54.6	38.3
DGDG	12.4	11.5	8.9	2.0	54.6	23.1
Other galactolipids	1.8					
Total steryl lipid	15.7					
ESG	4.3	59.1	9.1	1.6	25.9	4.2
SG	4.4					
FS	2.3					
SE	4.7	60.3	3.8	tr	25.9	9.9
Neutral lipids***		21.6	6.4	2.0	48.7	21.4
FFA	2.3					
TG	5.1	17.9	3.9	3.4	47.1	27.6
Other neutral lipids	5.2					
Total lipids	100.0	20.8	2.7	0.9	57.7	17.9

*Based on gravimetric results.

**Standard deviations: $C_{16:0}$, 0.2 to 2.5; $C_{18:0}$, 0.1 to 0.6; $C_{18:1}$, 0.1 to 0.3; $C_{18:2}$, 0.6 to 4.2; $C_{18:3}$, 0.5 to 1.5.

***Lipid fraction eluted with diethyl ether from silicic acid-Celite column.

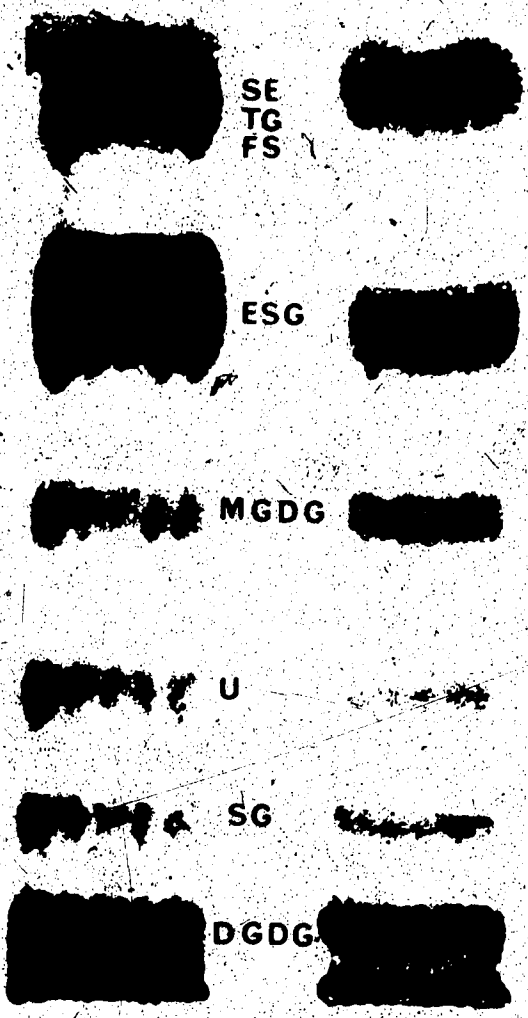


Figure 12. TLC separation of MGDG and DGDG from potato glycolipids. MGDG and DGDG = mono- and digalactosyl diglycerides; TG = triglyceride; FS, SE = free and esterified sterol; SG = steryl glucoside; ESG = esterified steryl glucoside; U = unknown.

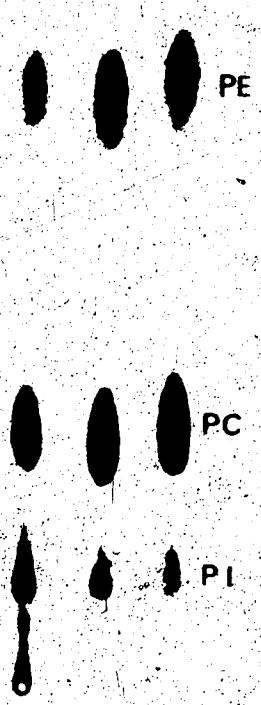


Figure 13. TLC separation of potato phospholipids. PC, PE, and PI = phosphatidyl -choline, -ethanolamine and inositol, respectively.

greater than in PC, while the amount of linolenic acid was less. In the PI fraction, linoleic acid was again the major acyl moiety, but this fraction characteristically had more palmitic and stearic acids than the other two phospholipids. Basically the same complement of fatty acids was present in all the phospholipid classes, but each had a characteristic make-up of these acids.

3. Galactolipids

The result of thin-layer separation of the glycolipid fraction using the solvent system of chloroform:acetone:water (30:60:2 v/v) is presented in Figure 12. In this solvent system, DGDG remained close to the start line, while MGDG moved away and acquired an R_f value of 0.45. Other constituents of the glycolipid fraction had the following R_f values: SG 0.18; unknown 0.30; and ESG 0.68. When the TL was developed in this solvent system, the R_f values were unchanged. In this system, the phospholipid constituents of the TL remained at the start line, while SE, TG, and FS moved close to the solvent front (R_f value of 0.90).

Quantitative results for the galactolipids present in the TL are reported in Table 2. These lipids were 20% of the TL by weight. Their contents were arrived at by estimating galactose contents of these lipids. The amount of each galactolipid was determined by calculation, making use of known molecular weights of these galactolipids. The galactolipid fraction contained mostly digalactosyl and monogalactosyl diglycerides. More than half was present as DGDG while only one-third was MGDG. Measurable quantities of polygalactolipids were also revealed by semi-quantitative TLC and, also, by quantitative estimation of lipid sugar after acid hydrolysis. Since these polygalactolipids were not characterized any further, their value in Table 2 is provided under

"other galactolipids".

The relative composition of fatty acid moieties present in each galactolipid is also reported in Table 2. The major galactolipid fraction, DGDG, contained predominantly linoleic and linolenic acids, followed by palmitic and stearic acids; a small amount of oleic acid was also present. In the MGDG fraction, the content of linoleic acid was similar to that of DGDG, but this fraction contained characteristically higher amounts of linolenic acid than DGDG. The contents of the saturated fatty acids, on the other hand, were considerably less in the MGDG fraction as compared to the DGDG fraction. Hence, the MGDG fraction was relatively more unsaturated than the DGDG fraction. In fact, MGDG was the most unsaturated of all the lipid fractions.

4. Steryl Lipids

TLC of the TL and fractions isolated by silicic acid chromatography in various solvent systems revealed the presence of steryl lipids in these fractions. Identification of steryl lipids on thin-layer chromatograms was done by uniformly spraying them with sterol-specific spray reagent (a mixture of concentrated sulfuric acid and acetic acid, 1:1 v/v). Heating the plate at 90°C for 15 minutes produced characteristic red spots on a white background for steryl lipids. The steryl lipids identified were FS, SE, SG, and ESG. The spots of these lipids were confirmed by plating authentic standards on the side of the plate. Further characterization of each steryl lipid was done by isolating the individual lipids and chemically analysing them for various moieties. The FS fraction gave a positive Liberman-Burchard reaction (Galliard et al., 1975). The isolated lipid of the SE fraction was subjected to alkaline hydrolysis (by refluxing for 30

minutes under nitrogen with 0.2 N sodium methoxide in methanol). After the mixture was cooled, water was added. The fatty acid and sterol moieties were selectively extracted with diethyl ether and identified by TLC in hexane:diethyl ether:formic acid (40:10:1-v/v). The SG fraction on acid hydrolysis (for 4 hours in boiling anhydrous methanol containing 5% hydrochloric acid) gave sterol and sugar moieties. In the case of ESG, the components were identified by double hydrolysis. The first, alkaline hydrolysis, yielded fatty acids and SG. In the second, acid hydrolysis, the isolated SG gave FS and sugar moieties.

Quantitative results for the steryl lipids present in the TL are reported in Table 2. These lipids constituted 16% by weight of the TL. They were present almost in equal amounts (about 4% each) as SE, SG, and ESG; FS was also present, but amounted to only half as much as the other steryl lipids.

Among these steryl lipids, only ESG and SE contained fatty acid moieties. The relative composition of their fatty acids is given in Table 2. Both steryl lipids contained predominantly palmitic acid (about 60%), followed by linoleic acid; linolenic and stearic acids were present as minor components. The acyl moieties of these steryl lipids varied only in their minor fatty acid components, e.g., ESG contained relatively more stearic and less linolenic acid than SE. Due to the presence of higher amounts of palmitic acid in these two steryl lipid fractions than any other lipids, they appeared to be the most saturated potato lipids.

The nature of the sterols present in steryl lipids was established by gas-liquid chromatography coupled with mass spectro-

metry. Mass spectral peak analysis of standard sterols was simultaneously conducted and compared with potato sterols. The base peak of cholesterol coincided with that of β-sitosterol. In spite of this, the fragmentation pattern was typical for each sterol. These techniques provided identification of cholesterol, stigmasterol and β-sitosterol (Table 3). The quantitative composition of sterols in various steryl lipids is given in Table 4. The predominant sterol in all the steryl lipids was β-sitosterol; cholesterol was present mostly in SE and SG fractions.

5. Other Neutral Lipids

The NL could also be separated into distinct bands on thin-layer plates (Figure 11) by using the double development method of Freeman and West (1966). In this technique the purpose of the second development was to separate the TG and the SE fractions of the TL. Development as described above gave the following R_f values in decreasing order: SE 0.89; TG 0.77; FFA 0.51; unknown 0.32; ESG 0.12; and SG 0.02.

Quantitative analysis of the NL contents of the TL is shown in Table 2. NL were only about 12-13% by weight of TL. Among them TG was about 5% and FFA about 2%. Unidentified NL made up the remaining 5%. No mono- or diglycerides were detected.

The relative composition of fatty acid moieties present in TG is also reported in Table 2. This NL fraction contained mostly linoleic and linolenic acids, followed by palmitic acid; small amounts of stearic and oleic acids were also present. The TG had 74.4% of polyunsaturated and 21.8% saturated acids. Essentially the

Table 3. Gas-liquid chromatography - mass spectral data of potato sterols.

Peak No.	Retention time (min)		Relative retention time		Mass spectral data								Identity
	I*	II**	I	II	Parent peak (M^+)	Base peak	m/e***						
1	1.40	4.40	1.00	1.00									Cholestane (internal standard)
2	3.70	7.46	2.64	1.69	386	81	107	95	69	105	145	93	Cholesterol
3	5.30	10.13	3.79	2.30	412	55	81	69	97	67	255	95 145	Stigmasterol
4	6.90	11.50	4.93	2.61	414	81	95	107	105	67	145	93	β-sitosterol

*Determined on Varian Gas Chromatograph M-1200.

**Determined on a Hewlett Packard Gas Chromatograph Model 7620, equipped with FID. Columns used: 96 cm x 6 mm pyrex glass, packed with 10% GE SF 96 on Gas Chrom Q 80/100 mesh: column temperature 260°C; injection port and FID detector at 300°C; Carrier gas Nitrogen 90 ml/min.

***In order of decreasing relative intensities.

Table 4. Sterol composition of steryl lipids.

Sterol	Nonpolar lipids		Polar lipids	
	FS	SE	ESG	SG
Cholesterol	11.4*	24.0	3.3	16.7
Stigmasterol	7.9	n/d**	14.6	16.7
β-Sitosterol	80.7	76.0	82.1	66.7

*As % of the total peak areas from gas-liquid chromatography.

**Below the detection limit.

same complement of fatty acids was present as in other lipid fractions, however, TG had its own characteristic make-up of these acids.

B. Lipid Composition of Processed Potatoes

Processing of raw potatoes into dehydrated granules by the freeze-thaw method primarily involves: (1) steam-cooking/hot mashing, (2) dehydration. The results for the above two processing steps will be presented in the following section.

1. Steam-Cooking/Hot Mashing Steps

The lipid composition of potatoes obtained after steam-cooking/hot mashing appears to [illegible]ite similar to that of raw potatoes (Table 5 versus Table 2). T[illegible] individual fractions showed that phospholipids were still the major lipids, followed by galactolipids, steryl lipids and neutral lipids. The fatty acid composition of each isolated lipid fraction are also reported in Table 5. The data in fatty acid composition columns also appear to be very similar to those reported for raw potatoes (Table 2). In most lipid fractions, the major fatty acids were still linoleic, palmitic, and linolenic acids.

2. Dehydration and Granulation Step

The composition of lipids isolated after dehydration and granulation is reported in Table 6. Analysis of the TL in individual lipid fractions by TLC (Table 6) showed that phospholipids remained the major potato lipids, followed by galacto-, steryl and neutral lipids. The relative composition of individual lipid classes in these lipid fractions appeared to be the same as reported for raw potatoes or after steam-cooking/hot mashing steps. Fatty acid analysis of the acyl lipids isolated by TLC (Table 6) showed that dehydration and granulation steps of processing did not change the fatty acid

Table 5. Composition of lipids and fatty acids in steam-cooked and mashed potatoes.

Lipid	Wt % of total lipids	Fatty acid composition (% of total fatty acids)				
		16:0	18:0	18:1	18:2	18:3
Total phospholipid	53.2					
PC	27.0	18.6	2.9	1.7	61.2	15.6
PE	15.8	25.1	3.4	1.6	60.8	9.1
PI	9.0	40.5	5.3	2.6	40.6	11.0
Total galactolipid	22.6					
MGDG	7.6	3.6	2.8	0.9	54.8	37.9
DGDG	13.0	11.4	10.0	1.8	54.7	22.1
Other galactolipids	2.0					
Total steryl lipid	14.3					
ESG	4.5	58.1	12.2	2.3	23.9	3.5
SG	4.3					
FS	1.3					
SE	4.2	61.8	4.9	tr	24.9	8.5
Neutral lipids		22.7	6.7	2.8	44.3	23.5
FFA	2.0					
TG	4.3	17.8	5.2	4.5	46.2	26.3
Other neutral lipids	3.5					
Total lipids	100.0	20.1	4.8	0.9	59.0	15.2

Table 6. Composition of lipids and fatty acids in dehydrated granules.

Lipid	Wt % of total lipids	Fatty acid composition (% of total fatty acids)				
		16:0	18:0	18:1	18:2	18:3
Total phospholipid	51.1					
PC	26.3	19.0	4.8	1.4	60.2	14.5
PE	14.9	26.1	4.1	1.8	58.2	9.8
PI	8.4	44.2	5.3	1.2	39.0	10.3
Total galactolipid	22.7					
MGDG	8.9	4.6	2.4	1.0	53.1	38.9
DGDG	11.9	11.6	10.1	3.1	54.0	21.2
Other galactolipids	1.9					
Total steryl lipid	14.8					
ESG	4.5	58.5	16.4	2.7	19.1	3.2
SG	4.4					
FS	1.8	62.3	4.6	tr	24.3	8.8
SE	4.1	28.0	12.1	1.5	41.4	17.1
Neutral lipids						
FFA	2.1	17.7	5.5	4.3	46.2	26.5
TG	4.4					
Other neutral lipid	4.8					
Total lipids	100.0	17.7	8.7	1.0	59.1	13.6

composition to any significant extent from that of raw or steam-cooked potatoes. The major fatty acids in most lipid fractions of the dehydrated granules were still linoleic, palmitic, and linolenic acids.

From these results, it is evident that the relative composition of potato lipids did not change by any remarkable extent during processing.

C. Effect of Processing on the Lipid Content of Potatoes

The analysis of the relative composition of processed potatoes described in the previous section showed that the steps involved in processing did not have detrimental effects on lipids. However, relative composition values could be misleading if they are not analyzed in terms of absolute amounts of lipids isolated. For this purpose, the data for the lipid contents of raw and processed potatoes (at both steps of processing) were obtained and expressed on a dry weight basis (Table 7). Analysis of data in Table 7 showed that steam-cooking/hot mashing steps brought about a 12.9% loss of the TL. PE and PI were the most affected phospholipids during the cooking-mashing steps. They decreased by 17.8% and 14.6%, respectively, while PC decreased by only 5.8%. The MGDG fraction of the galactolipids increased quite significantly (15%) during these processing steps, whereas the DGDG fraction decreased by about 8%. The steryl lipids were also reduced significantly (20%) during these processing steps. Among these lipids, the loss was most prominent in FS (51%), followed by SE (22%), and SG (14%), while ESG was the least affected (8%). Among the NL, the loss of TG was about 25%. This loss, together with those found for other lipids, was not accompanied by any increase in the content of tuber FFA. On the

Table 7. Lipids in raw and processed potatoes.

Lipid	Weight in mg per 100 g dry weight*				
	Raw potatoes	Steam-cooked and mashed potatoes		Dehydrated potato granules	
Total phospholipid	335.2	299.9	(10.5)**	282.3	(15.8)
PC	161.3	151.9	(5.8)	145.1	(10.0)
PE	108.6	89.3	(17.8)	82.4	(24.1)
PI	59.1	50.5	(14.6)	46.7	(21.0)
Total galactolipid	128.6	127.1	(1.2)	125.2	(2.6)
MGDG	37.3	42.8	(+14.7)	49.0	(+31.4)
DGDG	79.9	73.3	(8.3)	65.8	(17.6)
Other galactolipids	11.4	11.0	(3.5)	10.4	(8.8)
Total steryl lipid	101.3	80.8	(20.2)	81.9	(19.2)
ESG	27.7	25.5	(7.9)	24.9	(10.1)
SG	28.1	24.1	(14.2)	24.1	(14.2)
FS	14.8	7.2	(51.3)	9.7	(34.5)
SE	30.6	24.0	(21.6)	23.2	(24.2)
FFA	15.0	11.3	(24.7)	11.5	(23.3)
TG	32.8	24.4	(25.6)	24.4	(25.6)
Other neutral lipids	33.4	19.7	(41.0)	26.7	(20.1)
Total lipids	646.5	563.2	(12.9)	551.7	(14.7)

*An average of three determinations, which did not differ more than ± 2.5%.

**Values in parentheses are percentage losses of lipids as compared to raw potatoes. For MGDG a plus sign indicates an increase.

contrary, the FFA decreased by 25% from their initial amount in raw tuber.

The TL content of the freshly produced granules compared to the TL content in raw tuber showed a 14.7% lipid loss throughout the process (Table 7). This would mean an additional loss of only 1.8% commencing with the freeze-thaw step up to the end of granule processing. However, a comparison of lipid contents with the previous steps of processing showed that, during dehydration-granulation steps, the losses were mainly in the phospholipid fractions. The only lipid fraction which showed an increase in its content was, again, the MGDG fraction. The losses in the amounts of most lipids during these dehydration-granulation steps appeared to be low in spite of the fact that the dehydration step provided a moisture vaporization of 73% of the moist mash load.

Fatty acid analysis showed that the cooking and mashing steps caused only a slight decrease in the sum of polyunsaturated acids. This resulted in a small decrease (0.24 unit) in the unsaturation ratio of TL (Table 8). This decrease could not be ascribed to any particular lipid since there was a non-preferential reduction of lipids. Of particular interest was the finding that MGDG in the cooked and mashed tuber retained its original fatty acid composition and, thus, its high unsaturation ratio.

The fatty acid analysis of lipids in dehydrated potato granules showed little change in the unsaturation ratio from that of steam-cooked mash. Only minor losses in most of the lipids were observed. It would appear that during the entire process, the unsaturation ratios would undergo very little change. However, the

Table 8. Changes in the degree of unsaturation of fatty acids in lipids during processing into dehydrated granules.

Samples	Unsaturation ratio*									
	Total lipids	Nonpolar lipids			Phospholipids			Glycolipids		
		NL**	TG	SE	PC	PE	PI	MGDG	DGDG	ESG
Raw potato	3.22	2.50	3.43	0.56	3.84	2.65	1.45	14.29	3.81	0.44
Steam-cooked and mashed potato	2.98	2.31	3.15	0.50	3.57	2.45	1.13	14.48	3.59	0.39
Dehydrated potato granules	2.75	1.46	3.14	0.49	3.14	2.25	0.99	13.14	3.47	0.30

*(Linoleic + linolenic acid)/(palmitic + stearic acid).

**Lipid fraction eluted with diethyl ether from silicic acid-Celite column.

NL fraction showed a substantial decrease in its unsatuartion ratio during dehydration-granulation steps, probably because NL in the cells were not bound and could have been oxidized during drying.

Since the unsaturation ratios of lipids in freshly processed granules remained very high, off-flavor development in dehydrated granules during any prolonged storage or shipping could be a potential problem. In light of the results given, any of these lipids containing unsaturated fatty acids could produce rancid off-flavor; however, those containing the most unsaturated fatty acids, e.g., galactolipids and phosphqlipids, would be the likeliest source of the problem. Off--flavor development as a result of oxidation of unsaturated fatty acids would also be greatly dependent on the location of lipids in the processed products. It would be equally important to know whether processing had brought about any spatial rearrangement in the location of lipids or lipid-containing systems from those present in raw tuber.

D. Enzymic Breakdown of Tuber Lipids

Homogenization of potato tuber at room temperature or even 4°C brings about a rapid enzymic breakdown of phospho- and galactolipids. Tuber LOX activity in some commercially grown Alberta potato cultivars is presented in Table 9. The crude enzyme activity did not correlate with tuber specific gravity. High enzyme activity was retained at 0°C even after 1 week, while a small decrease in activity was obtained with room temperature storage of the extract. The LOX pH response curve revealed an optimum activity at pH 5.7 and no activity at pH 8.0 (Figure 14). A decrease from pH 7.6 to pH 7.4 brought about a 5-fold increase in enzyme activity, while decreases to pH 7.2 and 7.0 gave 15-fold and 20-fold rises, respectively. The remarkable enzyme activity

Table 9. LOX activity in extracts of some Alberta grown potato cultivars

Cultivar*	% of total potato acreage	Specific gravity	Enzyme units (O_2-uptake, µmol/min) per g fresh weight**			
			Fresh extract	After 24 hr at 0°C	After 24 hr at 22°C	After 1 week at 0°C
Irish Cobbler	1.0	1.089 ± .005	0.60	0.60	0.55	0.51
Netted Gem	73.2	1.096 ± .002	0.87	0.87	0.71	0.71
Norgold Russet	1.5	1.088 ± .004	3.28	3.17	2.89	3.09
Norland	8.5	1.077 ± .003	1.19	1.19	1.19	1.19
Warba (white)	1.2	1.086 ± .001	1.00	1.00	0.83	0.87

*All potatoes were freshly harvested tubers of an average size of 200 g; the acreage is for 1977 harvest, while the specific gravity data are averages for the past 5 years.

**Values reported in this and following Tables are averages of at least two analyses with a relative deviation of less than 3.0%.

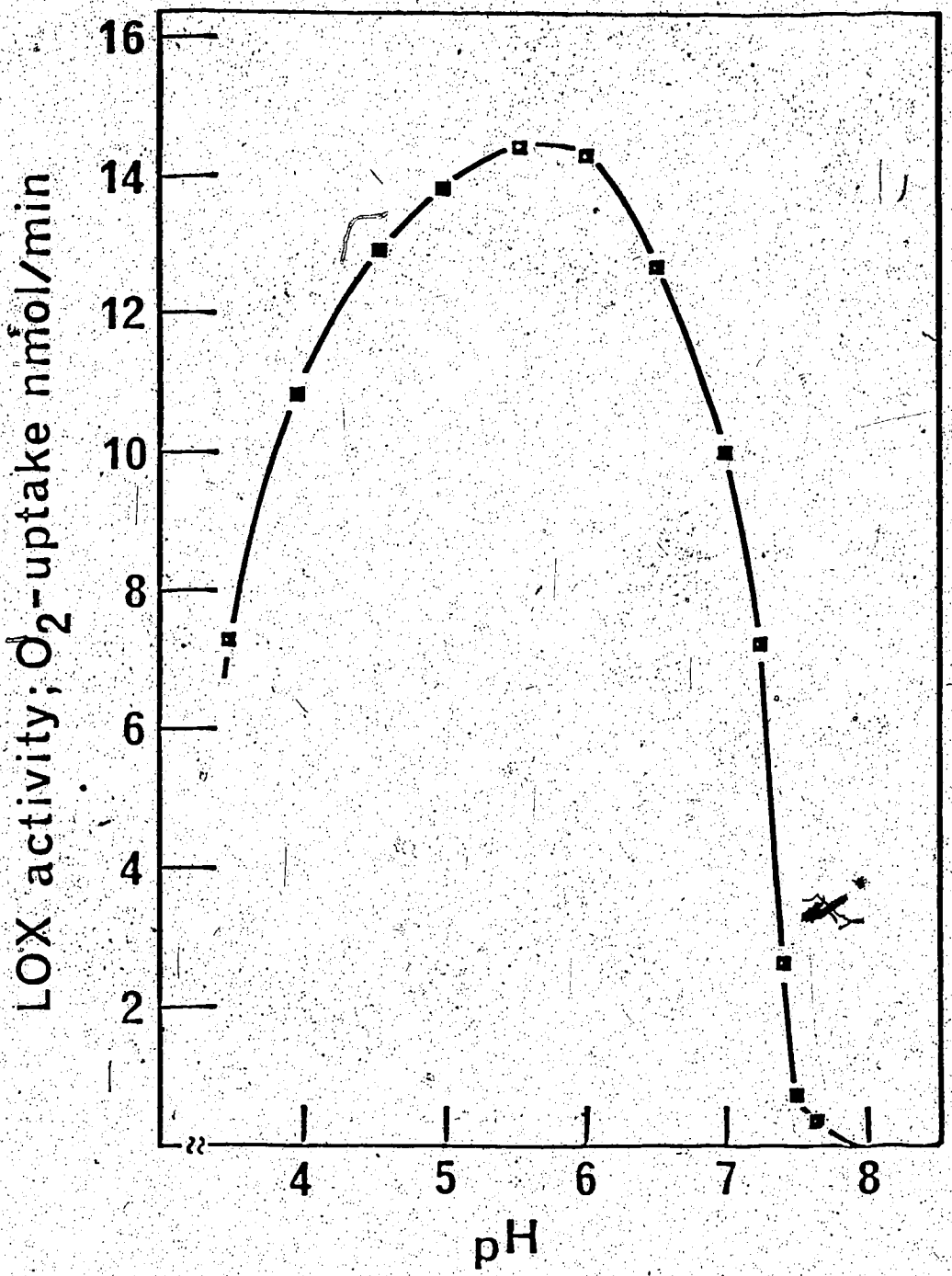


Figure 14. The effect of pH on potato lipoxygenase (LOX) activity at 25° C.
0.1 M buffers used: Na-acetate (pH 3.0-5.5), K-phosphate (pH 6.0-7.25), and Tris-chloride (pH 7.5-9.5).

at low temperatures was enhanced at higher temperatures. A Q_{10} value of 1.8-2.3 was found in the range of 15-35°C. These results suggested that buffers of pH 6.7-7.4 would not be suitable for avoiding high LOX activity. The buffer of pH 7.8 gave an optimum suppression of LOX activity without affecting membrane integrity, as monitored by mitochondrial viability assay and electron microscopy.

Crude LOX activity was unaffected by any of the sulfite or thiol inhibitors. 2 mM dithionite brought about enzyme activation instead of suppression. The enzyme was unaffected by cyanide. However, since inhibition was consistently obtained in the presence of mercaptobenzothiazole (Table 10), it was used throughout the study.

TLC of lipids extracted from tuber homogenate immediately, or 5 and 10 minutes following tissue homogenization at 0°C showed a gradual disappearance of phospho- and galactolipids. After 10 minutes, the homogenate was practically depleted of MGDG and, to a lesser extent, of phospholipid constituents. The most susceptible to hydrolysis was MGDG, much more so than DGDG and most of the phospholipids. Negligible changes were observed among steryl lipids, and only a small decrease in PI and TG. A sharp decrease of the last two was visible only with homogenates kept at 20°C. All decreases were associated with an increase in the content of FFA when homogenates were prepared in buffer of pH 7.8, or in content of hydroperoxides (conjugated cis-trans; A_{max} =235) when prepared in buffer of pH 7.2. No monoacyl-galactolipid, lysophospholipids, phosphatidic acid, or mono- or diglycerides could be detected.

Potato tuber phospholipase activity was suppressed by 60% when pure phospholipid-fraction was used, while its galactolipase

Table 10. LOX activity as affected by some common inhibitors added to enzyme extraction medium*

Inhibitor	Enzyme activity (O_2-uptake, nmol/min)
Na-sulfite	11.0
Na-metabisulfite	11.0
Na-dithionite	19.1
Na-cyanide	11.3
Cysteine hydrochloride	10.8
Na 2-mercaptobenzothiazole	7.6
p-chloromercuribenzoate (PCMB)	16.2

*The enzyme extract from potato tubers, *cv.* Netted Gem. For extract preparation and inhibitor levels see: Experimental. Control value (no inhibitor) 11.0.

activity was suppressed by 36% when the glycolipid fraction was used as a substrate (Table 11). The highest inhibition, 60-70%, of activity was observed with pure PC and PE, a moderate suppression with pure DGDG (47%), and slightly less with MGDG (36%). Negligible enzyme activity was found with pure steryl lipids and TG as substrates, and this activity was retained in the presence of nupercaine.

Effective suppression of LAH activity was achieved at 50 μM levels of nupercaine. Increasing the level up to 450 μM did not result in additional suppression.

The effect of temperature on LAH activity was recorded with PC as a substrate. The activity responded to increasing temperature, with a Q_{10} close to 1.8 found between 15-35°C. Complete inactivation was obtained at 70°C after 25 minutes.

As seen from Table 12, an isolation procedure applied with no precautions against lipid breakdown, and completed within 3 hours, brought about average losses of 18.9% of phospholipids, 30.2% of glycolipids, and only 2.5% of TG selected as a marker for the NL fraction. When the procedure was repeated in the presence of nupercaine, the losses were less by an average 60% for phospholipids and 35% for glycolipids, while TG was unchanged. These results proved that a significant difference exists in rates of breakdown between free and membrane-bound lipids in tuber homogenates, and that only a portion of the lipids is readily accessible to enzyme attack, which, as reflected by the extent of suppression by nupercaine, simulates that on the free lipids.

Table 11. The effect of nupercaine on coupled LAH-LOX activity

Lipid substrate*	Enzyme activity (O_2-uptake, nmol/min)**		
	Nupercaine		Suppression
	Omitted	Added	%
PL	11.9	4.8	60
PC	9.6	2.9	70
PE	4.8	1.8	62
PI	5.8	4.0	31
GL	5.3	3.4	36
MGDG	8.1	4.3	47
DGDG	10.1	6.5	36
ESG	1.4	1.4	0
NL	1.4	1.4	0
TG	1.4	1.4	0
SE	1.4	1.4	0

*Enzyme substrates used were pure lipids isolated from potato tuber, *cv.* Netted Gem.

**The enzyme activity was followed over a 15 min period. Incubation of enzyme extract for up to 2 hr with nupercaine did not result in further suppression.

Table 12. Lipid losses during isolation of membrane and subcellular particles from tuber *cv.* Netted Gem*

Lipid source	PL	GL	TG	FFA
	mg/100 g fresh tuber			
Whole peeled tuber	78.2	30.5	8.0	3.6
Cell wall	0.9	0.6	0.8	0.8
Microsomes	29.2	14.3	2.2	2.5
Mitochondria	27.7	3.1	1.3	2.3
Peroxisomes	1.4	0.4	0.8	0.8
Plasmalemma	4.1	2.9	2.6	1.6
Starch	0.1	0	0.1	1.1
Lipids total	63.4	21.3	7.8	9.1
Loss %	18.9	30.2	2.5	253(gain)

*No precautions were taken against enzymic lipid breakdown. Isolation was completed within 3 hr in a medium of TRIS-Cl buffer pH 7.2, containing 0.4 M sucrose and 4 mM Na-sulfite.

E. Lipids and Their Association with Cellular Components of the Potato

The results in the preceding sections showed that the potato tuber contained predominantly polar lipids (phospho- and glycolipids). These kinds of lipids in many biological systems exist mainly as constituents of bio-membranes. In order to characterize the nature and location of these lipids in raw and processed potatoes, experiments were conducted to determine: (i) the in situ morphology of cellular components; (ii) the proximate nature of lipids associated with them; (iii) a complete analysis of lipids present within each cellular component. The in situ morphology and proximate lipid analysis of cellular components was best studied with the help of transmission electron microscopy. For a complete characterization of their lipids, each cellular fraction was isolated and purified by techniques of differential and density gradient centrifugations. In the following sections, these results are described in detail.

1. Transmission Electron Microscopy of Cellular Components

Morphological observation of cellular components was made by examining properly processed sections through a cell of potato tuber under a transmission electron microscope. A photoelectron micrograph (Figure 15) shows a thick region of cell wall (CW), lined with plasmalemma membrane (PM). Rows of parallel fibers were discernible in the cell wall region. The plasmalemma membrane, however, appeared as a single membrane, and was slightly drawn towards the cell wall. A thin layer of cytoplasm (C) underlayed the cell wall. The cytoplasmic region was separated from the vacuolar region by a membrane layer (vacuolar

Figure 15. Transmission electron micrograph of a section of potato cells, showing a mitochondrion (M), cytoplasm (C), cell wall (CW), plasmalemma (PM), vacuole (V) and its membrane (VM).

Figure 16. Transmission electron micrograph showing higher magnification of a section of tuber cells. Endoplasmic reticulum (ER), a mitochondrion (M), cell wall (CW), cytoplasm (C), plasmalemma (PM), and ribosomes (r) are shown.

membrane, VM). The vacuole occupied a large volume of the cell. The cytoplasmic region contained mitochondria (M) which were surrounded by double membranes. These double membranes of mitochondria are more easily seen in Figure 16. This electron micrograph also reveals endoplasmic reticulum (ER) as a component of the cytoplasmic region. Usually, they are attached numerous ribosomes (r), which appear as black dots on micrographs. Electron micrographs of amyloplasts *in situ* showed the usual image of patches of a dense material within the grain, and a discrete membrane around the grain (Figure 17). The matrix of the starch grain (SG) did not appear as uniform as the cytoplasm, and at places showed dense foldings (Figure 17). Occasionally a large starch grain within the cell protruded into the vacuolar space (V) (Figure 18). Fragmentation of the amyloplast membrane was observed with the majority of the amyloplasts, and it was found to be more pronounced in tuber samples stored at 4°C. Highly magnified electron micrographs of the cytoplasmic region (Figures 19 a & b, 20) also revealed the presence of small spherical and oval-shaped units of peroxisomes (Px), containing some cuboidal inclusions (protein crystals), and lipid bodies (LB). Peroxisomes were only found in large numbers in peripheral cortex cells of the dormant tuber. Intercellular space (IS) and middle lamella (ML), imbedded between the cell wall, were also discernible (Figure 21).

Morphological changes during processing were only studied with steam-cooked samples. An electron micrograph of steam-cooked potato tuber cells showed dramatic structural changes (Figure 22). In cooked tissue, the major area of the cell was occupied by gelled starch.

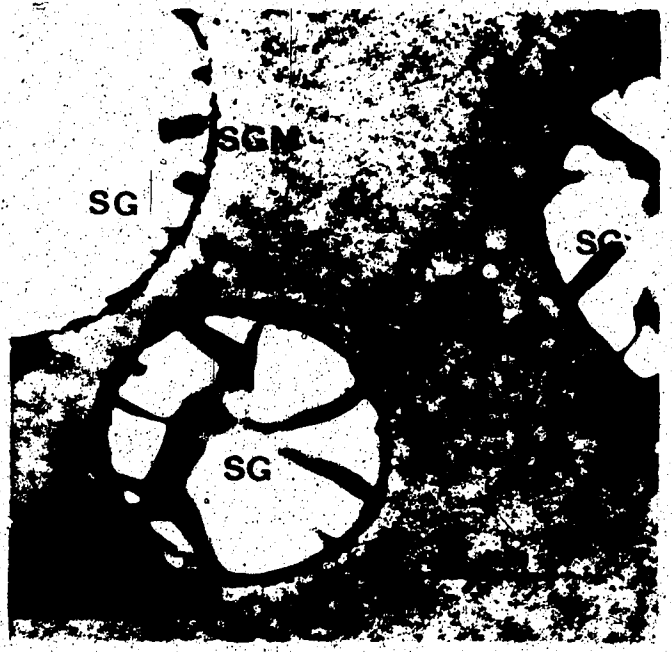


Figure 17. Transmission electron micrograph of amyloplast (starch grain) membranes, showing starch grains (SG) and their membranes (SGM). Cytoplasm (C) and a mitochondrion (M) are shown.

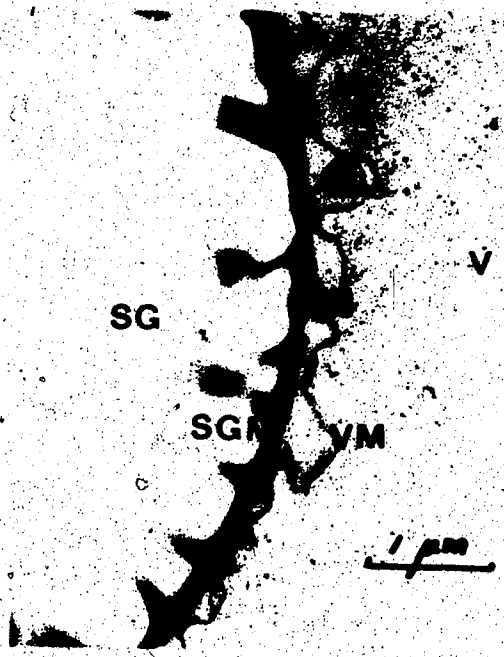


Figure 18. Transmission electron micrograph showing higher magnification of amyloplast. A starch grain (SG), its membrane (SGM), vacuole (V) and its membrane (VM) are shown.

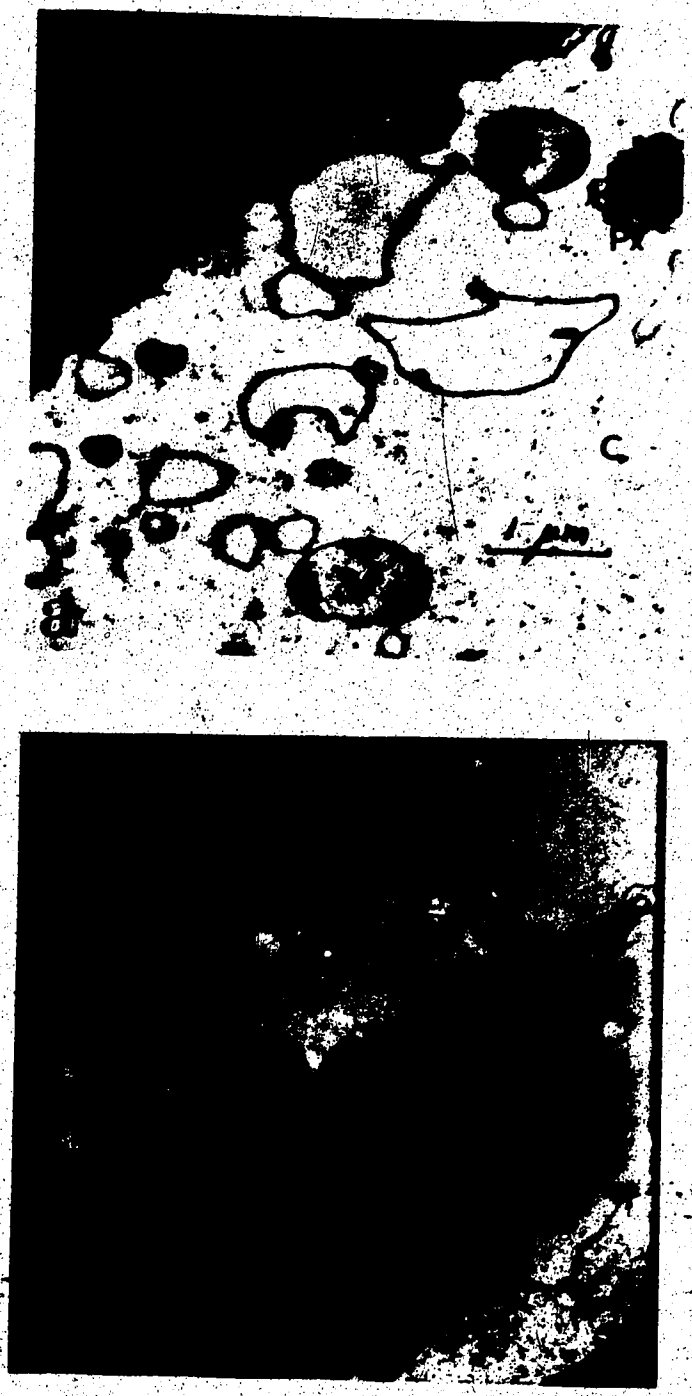


Figure 19 a & b. Transmission electron micrographs of peroxisomes. Peroxisomes (Px), cell wall (CW), cytoplasm (C), a lipid body (LB), mitochondria (M), plasmalemma (PM), vacuole (V) and its membrane (VM) are shown.

Figure 20. Transmission electron micrograph of a lipid body. A lipid body (LB), cell wall (CW) and plasmalemma (PM) are shown.

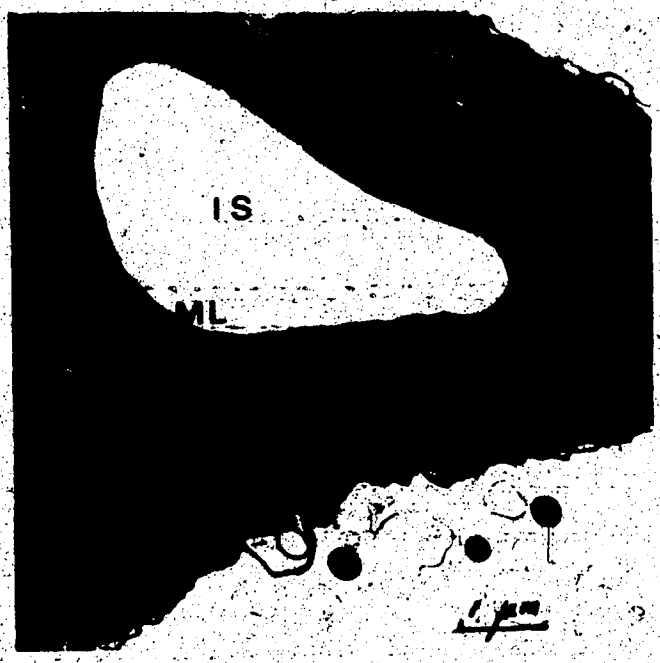


Figure 21. Transmission electron micrograph of intercellular space. Intercellular space (IS), middle lamella (ML) and cell wall (CW) are shown.

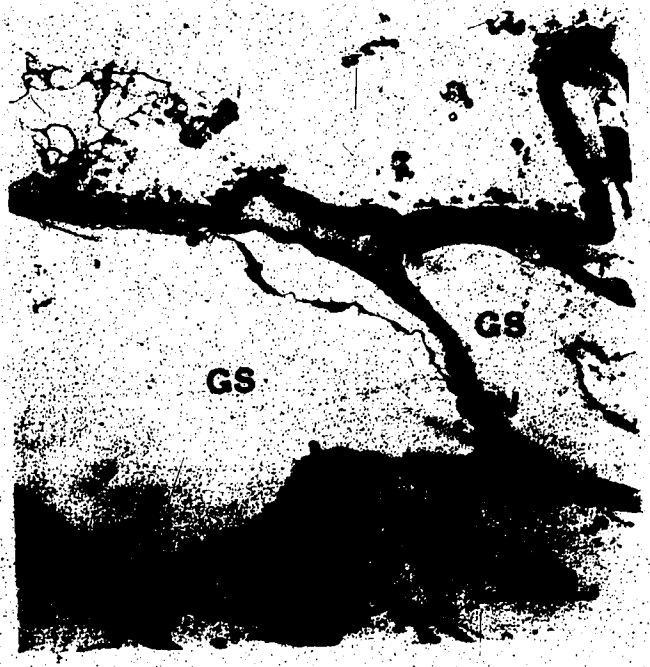


Figure 22. Transmission electron micrograph of a section of cooked potato cells. Gelatinized starch (GS) and cell wall (CW) are shown.

The cell wall also appeared to be swollen or gelled in places, while all the cytoplasmic structures were left as denatured masses of indefinite shape lying between the gelled starch and the cell wall. The gelling of starch and cell wall structures appeared to have sandwiched the denatured cytoplasmic material as a membrane-like boundary around the gelled starch. It is evident from the above electron micrograph that, although steam-cooking had not resulted in cell rupture, it had substantially altered the spatial organization of cellular structures and, probably, their constituents. The swollen, gelled starch did not appear to contain any imbedded cellular constituents. These physico-chemical changes in cellular structures by steam-cooking rendered any differentiation of cellular membranes very difficult, and, thus, limited the usefulness of transmission electron microscopy for studies of subsequent steps of processing.

The above results provided a general picture of various morphological structures in potato tuber cells. At this point, it was felt that it would be useful to determine the size dimensions of some of these cellular membranes and organelles. This information could have its bearing on the nature of membrane constituents or lipid species. Generally, all the lipid-containing membranes would consist of two dark lines separated by a light line, thus possessing a so-called unit membrane structure in which the two dense lines would represent osmium--fixed proteins, while the intervening space would be occupied with the long chain fatty acids of the lipids. The single unit membrane occurring around vacuoles (VM) was determined to have a thickness of 82±15 Å, while that of plasmalemma was close to 80 Å, with the innermost line facing the cytoplasmic matrix being 28±7 Å, the intervening space

26±3 Å, and the membrane facing the cell wall 26±4 Å. Double unit membranes found in the endoplasmic reticulum were each 24±2 Å thick, while the electron transparent space between each pair of the membranes was 23±7 Å. Double unit membranes around spherical or ellipsoidal mitochondria had a thick outer membrane of 45±10 Å, and a thin inner membrane of 30±9 Å. The average size of mitochondria was 0.90±0.24 μm in length, and 0.67±0.18 μm in width. A double unit membrane structure around the starch grains of matured tuber could not be easily resolved. The membrane structure showed evidence of disintegration, with some thin layers apparently peeling away from each other. Such a membrane had a thickness of 199±59 Å. A population of peroxisomes, small spherical units, with an average length of 0.78±0.23 μm and width of 0.64±0.09 μm (a size close to that of mitochondria), had only a single unit membrane structure which was 67±12 Å thick.

2. Proximate Nature of Lipids Associated with Cellular Components

From the results described, it is evident that a potato tuber cell contains membranes and organelles which contain lipid constituents. A novel experimental approach was used to recognize the *in situ* nature of the lipids. For this purpose, transmission electron microscopy was done after a series of selective extractions of cellular lipids with solvents of known extraction characteristics. Extraction of a particular type of lipid with a specific solvent would render characteristic changes in that membrane or organelle where it was primarily present. The values of dielectric constant, ε, of the solvent system used are provided in Table 13 to show their elutropic nature. Electron micrographs (Figures 23 a & b) prepared after treatment of tissue with petroleum ether, a nonpolar solvent with a low dielectric constant

Table 13. Dielectric constants for lipid solvents used in the cell membrane electron microscopy study.

Lipid solvents	ε	°C	$\alpha \times 10$ *
Petroleum ether	1.89	25	
Diethyl ether	4.34	20	0.217
Chloroform:methanol (2:1 v/v)	16.15	25	
Chloroform	4.81	20	0.160
1-Butanol	17.80	20	0.300
	17.10	25	0.335
Acetone	20.70	25	0.205
Ethanol	24.30	25	0.270
Methanol	32.63	25	0.264

*α, temperature coefficient (of the dielectric constant)
$= d \log_{10} \varepsilon/dt$

From: Handbook of Chem. & Phys., 55th Ed., 1974-75.

Figure 23a. Transmission electron micrograph of potato tissue extracted with petroleum ether. Endoplasmic reticulum (ER), a mitochondrion (M) and cell wall (CW) are shown.

Figure 23b. Transmission electron micrograph of peroxisomes treated with petroleum ether. Peroxisomes (Px) and a mitochondrion (M) are shown.

of 1.89, showed removal mostly of neutral lipid-containing membrane systems, such as those of peroxisomes, plasmalemma, cell walls and lipid bodies; while other membrane systems, such as mitochondria and endoplasmic reticulum, were not affected as much, suggesting the polar nature of their lipids. Extraction of tissue with acetone, a poor phospholipid solvent, resulted in electron micrographs (Figures 24 a & b) showing a complete wash out of lipids around amyloplast membranes, and those of peroxisomes, cell walls and lipid bodies. In this treatment, fragments of vacuolar membranes (VM) were still seen, while plasmalemma was mostly deformed, and the mitochondrial membranes were also not distinguishable. Since acetone is known to be a good solvent for many lipids, especially the glycolipids, disintegration of many cellular structures by its treatment could mean that most of these membranes contained glycolipids as their most important lipid constitutent. Acetone treatment would probably disintegrate cellular structures containing other lipids, such as phospholipids, if those structures require a complement of glyco- and neutral lipids for normal membrane morphology. This would explain why many of these membrane structures contain varying complements of phospho-, glyco- and neutral lipids. Treatment of tissue with polar solvents such as n-butanol saturated with water, produced electron micrographs (Figures 25 a & b) in which all the cellular structures were completely disorganized. Solvents such as water-saturated butanol, chloroform-methanol (2:1 v/v), methanol-ethanol (1:1 v/v) produced similar kinds of cellular disintegration. A complete disintegration by these solvents of structures containing polar lipids could be mainly due to the ability of these

Figure 24 a & b. Transmission electron micrographs of potato tissue extracted with acetone. Starch grain (SG), vacuolar membrane (VM), cytoplasm (C), cell wall (CW) and middle lamella (ML) are shown.

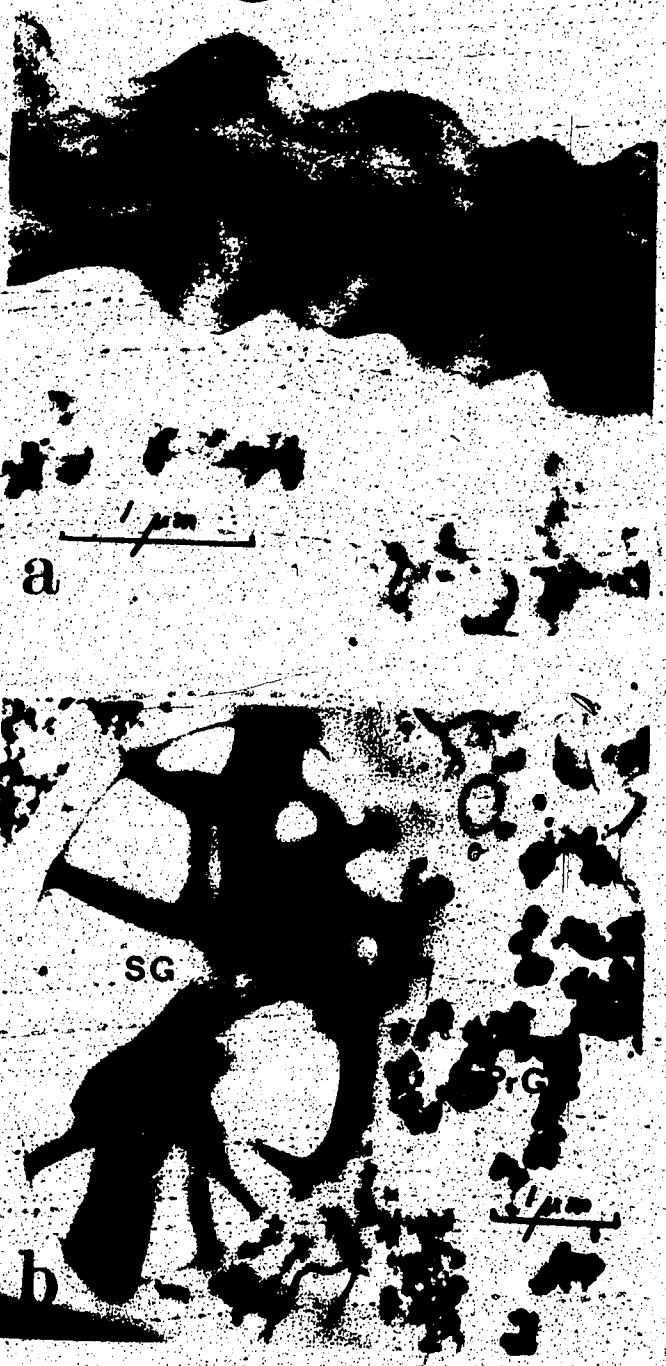


Figure 25 a & b. Transmission electron micrographs of potato tissue extracted with n-butanol saturated with water. Cell wall (CW), middle lamella (ML), starch grain (SG) and globular proteins (PrG) are shown.

solvents to solubilize the lipoprotein components of the membranes, and also to denaturing of the hydrophilic proteins of the cell. Such denaturation of hydrophilic globular proteins (PrG) is evident in Figure 25 b.

3. Lipids of Potato Cell Membranes and Organelles

The *in situ* analysis of the lipids in potato cellular components by transmission electron microscopy provided only limited information about the kind of polar and nonpolar components of the lipids. However, isolation of each cellular component, analysis of its lipids and fatty acids, and correlation of findings with those obtained with transmission electron microscopy permit a meaningful characterization of each lipid-containing cellular component. The results reported in the following sections specifically achieve the above purpose.

a. Composition of Lipids in Cellular Components of Potato Tuber

The composition of lipids isolated from lipid-containing cellular components is given in Tables 14-19. Lipomembranes present in mitochondrial, microsomal and plasmalemma fractions were rich in phospholipids. The relative composition of phospholipids in mitochondria was much higher than any other cellular organelle or membrane. Another characteristic feature of the mitochondrial fraction was its low content of glycolipids. On the other hand, the plasmalemma and the microsomal membranes were very rich in glycolipids. The lipids extracted from the peroxisomal and starch grain fractions, however, were mostly neutral in nature. These results clearly establish that the lipid composition of cellular organelles differed from one another. These observations necessitated a careful fractionation of individual

lipid components of each isolated cellular fraction. The results of such fractionations are described below.

(i) Microsomal Lipids

The lipid composition of microsomes is reported in Table 14. Preparations obtained in the presence of nupercaine had a slight excess of glycolipids over phospholipids. Phospholipids were the major lipid components of these lipomembranes. The major phospholipids present in microsomes were PC and PE, while PI and DPG were minor components. The glycolipid fraction was characterized by high contents of galactolipids, and it also contained significant proportions of SG and ESG. The NL in microsomes was identified in nearly equal proportions of TG, FFA, FS and SE. Preparations obtained in the absence of nupercaine had consistently less galactolipids, more steryl lipids, and a high phospholipid content.

Contents of fatty acid moieties present in total and isolated lipid fractions of microsomal membranes are also reported in Table 14. The major fatty acid in the TL was linoleic acid, followed by palmitic and linolenic acids. The composition of fatty acids in the major phospholipids, PC and PE, appeared quite similar to the TL, however PI contained significantly more palmitic acid. Among the glycolipids, the ESG fraction was also rich in palmitic acid while in the MGDG fraction linoleic and linolenic acids were the predominant fatty acids. MGDG differed from the other galactolipid, DGDG, in having significantly more linolenic but less palmitic acid. The MGDG fraction thus appeared to contain the highest amount of linolenic acid among all

Table 14. Lipid composition of potato tuber microsomal preparation

Lipid	Isolation procedure*			Fatty acid as % of total acids					UR***
	A	B	C	16:0	18:0	18:1	18:2	18:3	
TL (mg/g microsomes dry matter)	-	268.3	350.4						
	Weight % of membrane total lipid								
TL	100	100	100	24.7	6.8	2.0	49.6	15.4**	2.1
PL	45.9	43.9	41.7						
PC	20.1	20.2	19.5	25.4	3.8	1.4	59.0	10.4	2.4
PE	14.3	13.6	13.0	27.3	5.2	1.1	58.4	8.0	2.0
PI	6.8	6.5	5.9	40.2	4.7	1.9	46.0	7.2	1.2
DPG	0.9	1.1	1.0	30.0	8.9	0.8	45.3	15.0	1.6
Others	3.8	2.5	2.3						
GL	35.1	37.1	42.7						
MGDG	4.8	6.3	10.9	7.3	1.8	1.1	62.0	27.8	9.9
DGDG	10.3	10.3	15.3	20.6	4.5	2.3	59.8	12.8	2.9
SG	12.0	12.0	10.0						
ESG	8.0	8.5	6.5	59.3	7.6	4.3	21.6	7.2	0.4
NL	19.0	19.0	15.6						
FFA	3.6	4.4	4.7	45.4	20.7	7.0	21.4	5.6	0.4
TG	3.1	3.1	3.2	22.0	4.0	3.2	45.8	25.0	2.7
FS	3.0	3.0	2.0						
SE	4.0	4.0	3.0	60	4.1	tr	26.0	9.9	0.6
U	5.3	4.5	2.7						

*In this and following Tables membranes and subcellular particles were isolated from potato homogenate in (A) 0.5 M Tris-Cl buffer pH 7.2; (B) 0.1 M Tris-Cl buffer pH 7.8 without and (C) with 50 μM nupercaine as an enzyme inhibitor.

**TL also included 1.4% 20:1.

***Unsaturation ratio: (18:2 + 18:3)/(16:0 + 18:0).

the acyl lipids of this or any other membrane lipid. Thus almost 90% of the acyl moieties of this lipid fraction were unsaturated fatty acids. The compositions of fatty acids in the TG and FFA fractions of the NL also showed differences from the other lipids in the relative abundance of each chain length.

(ii) Mitochondrial Lipids

Mitochondria isolated by differential centrifugation, though tightly coupled (respiration control ratio, r.c.r., in the presence of succinate was 3.2-3.4, and ADP/O ratio was 1.3), appeared under the microscope to be contaminated to a great extent with other membranes, cell wall fragments, and plastids containing tiny starch grains. Extensive sucrose gradient purification increased the r.c.r. to near 5, and the ADP/O ratio to 1.7. Such a preparation, as shown by electron micrographs, had a homogeneous population of intact organelles, which were identical to those on micrographs of Douce et al. (1972), and McCarty et al. (1973), and appeared devoid of contaminants. The lipid composition of the preparation is given in Table 15. Lipids isolated from mitochondria showed a very high proportion of phospholipids. Mitochondrial phospholipids were mainly in the form of PC and PE. Mitochondria also contained a significant proportion of DPG, as well as some PI but relatively small amount of glycolipids. The NL of mitochondria contained mostly SE, while FFA and TG were present as minor components.

Analysis of the fatty acids in the mitochondrial TL showed linoleic acid as the most abundant fatty acid, followed by palmitic and linolenic acids. The major phospholipids, PC, PE and DPG, contained linoleic acid as their major fatty acid component. They, however,

Table 15. Lipid composition of potato tuber mitochondria

Lipid	Isolation procedure			Fatty acid as % of total acids					
	A	B	C	16:0	18:0	18:1	18:2	18:3	UR
TL (mg/g mitochondria dry matter)		205.3	215.2						
	Weight % of mitochondrial total lipid								
TL	100	100	100	19.4	4.7	2.4	59.6	12.1*	2.9
PL	85.5	83.2	79.2						
PC	38.3	37.8	36.0	17.5	4.0	4.8	60.3	13.3	3.4
PE	28.5	26.9	25.6	14.8	2.9	5.1	66.3	10.9	4.4
PI	7.0	6.7	6.4	37.2	10.1	3.4	37.3	12.0	1.0
DPG	11.7	11.8	11.2	5.1	3.0	6.2	66.4	18.7	10.5
GL	6.4	8.7	11.8						
MGDG	0.7	1.5	1.9						
DGDG	1.8	3.5	4.3						
SG	1.0	1.1	1.9						
ESG	2.9	2.6	3.7	46.7	32.6	4.6	13.2	2.9	0.2
NL	8.1	8.1	9.0						
FFA	2.4	1.7	2.1	35.6	10.2	8.7	41.3	4.2	1.0
TG	0.1	1.0	1.2	34.2	17.9	20.8	24.4	2.7	0.5
FS	0.8	0.6	0.7						
SE	2.8	3.2	4.0						
U	2.0	2.0	1.0						

*Also present were 12:0 (0.6%); 14:0 (0.4%); and 20:1 (0.8%).

differed considerably in the relative composition of their minor fatty acids (palmitic and linolenic). Fatty acid moieties in the TG and FFA fractions in mitochondria showed significantly high proportions of stearic (18:0) and oleic (18:1) acids.

(iii) Peroxisomal Lipids

The composition of lipids present in peroxisomes is reported in Table 16. These membranes contained mainly NL. The polar lipids (phospho- and glycolipids) were present in smaller quantities than in the other membranes. The predominant component of the NL fraction was SE. Other NL identified as minor components were FFA, TG, and FS. These results clearly show that the lipomembranes of peroxisomes would be mainly neutral in nature.

Fatty acid analysis of the TL of these membranes (Table 16) showed the predominance of linoleic acid, followed by palmitic, oleic, linolenic, and stearic acids.

(iv) Plasmalemmal Lipids

The composition of lipids isolated from plasmalemma membranes is reported in Table 17. These membranes were rich in polar lipids, mainly in the form of phospho- and glycolipids. The major phospholipids present were PC and PE. Like mitochondrial membranes, plasmalemma membranes contained a significant proportion of DPG but differed considerably in the relative proportions of other phospholipids. PI was found to be a minor component of plasmalemmal phospholipids. The glycolipid make-up of these membranes appeared quite distinct from the other membranes. ESG was the most abundant glycolipid. Another distinct feature of the glycolipids of these membranes was that they

Table 16. Lipid composition of potato tuber peroxisomes

Lipid	Isolation Procedure		
	A	B	C
TL (mg/g peroxisomes dry matter)	-	230.6	242.3
	Weight % of peroxisomes total lipids		
TL*	100	100	100
PL	15.6	14.5	13.9
PC	7.6	7.5	7.3
PE	3.5	3.0	2.9
PI	1.9	1.2	1.0
DPG	1.6	0.4	0.4
PG	1.0	2.4	2.3
GL	12.6	21.4	31.7
MGDG	1.7	5.8	7.5
DGDG	3.0	5.4	8.1
SG	2.5	4.4	6.6
ESG	5.4	5.8	9.5
NL	71.8	64.0	54.4
FFA	9.3	10.8	8.3
TG	9.1	8.2	6.4
FS	3.5	2.1	2.7
SE	45.3	37.5	32.4
U	4.6	5.4	4.6

*TL fatty acid composition as % of total acids: 2.5(12:0); 1.3 (14:0); 22.2(16:0); 5.0(16:1); 4.0(18:0); 11.0(18:1); 42.5(18:2); 10.9(18:3) and trace of 20:1. UR = 2.0.

Table 17. Lipid composition of potato tuber plasmalemma

Lipid	Isolation Procedure		
	A	B	C
TL (mg/g plasmalemma dry matter)	-	197.5	239.4
	Weight % of plasmalemma total lipid		
TL*	100	100	100
PL	40.4	28.8	34.9
PC	17.9	17.1	21.1
PE	13.4	6.3	8.0
PI	7.1	3.5	3.6
DPG	2.0	1.9	2.2
GL	31.5	51.6	49.6
MGDG	4.2	8.7	9.2
DGDG	5.3	11.0	11.6
SG	4.1	4.0	4.7
ESG	17.9	27.9	24.1
NL	28.1	19.6	15.5
FFA	3.1	2.5	3.2
TG	2.9	1.6	1.4
FS	2.7	1.5	0.8
SE	15.2	10.8	8.3
U	4.2	3.2	1.8

*TL fatty acid composition in % of total acids: traces of 12:0 and 14:0; 27.3(16:0); 7.7(18:0); 3.9(18:1); 47.9(18:2); 11.1(18:3); 2(20:1). UR = 1.7.

contained the two galactolipids, MGDG and DGDG, in nearly equal amounts. Among the NL, plasmalemma membranes contained SE as a major component. The other NL were present in smaller amounts.

Analysis of the fatty acid moieties present in plasmalemma membranes was done only with TL. The lipids of plasmalemma membranes contained linoleic and palmitic acids as their main fatty acids, but substantial amounts of minor components, such as linolenic and stearic acids, were also present. These results showed that the relative composition of the fatty acid present in plasmalemma membranes was unique and distinctly different from other membranes.

(v) Amyloplast (Starch Grain) Lipids

The lipid composition of amyloplasts is reported in Table 18. The content of phospholipids, with PC being predominant, was 16.3% of the membrane TL. Lipids of the glycolipid fraction were 26.2% and were enriched in galactolipids. NL were the main constituents of this fraction, which had a high content of sterols, the free non-esterified form being predominant. However, the high content of FFA and their oxidized forms suggested that all the precautions in amyloplast preparation still had not prevented enzymic lipid breakdown. Also, during the isolation of the amyloplasts, the starch grain membranes could have been exposed to air and oxidized.

The fatty acid composition of amyloplast membrane revealed an unsaturation ratio (UR) of 1.4 for the large-size grains, the predominant acid being linoleic, followed by palmitic, linolenic, stearic, and oleic acids. However, the fatty acid composition of extensively degraded membranes, obtained by grain gravity sedimentation

Table 18. Lipid composition of potato starch (amyloplast) membranes

Lipid	Isolation Procedure		
	A	B	C
TL (mg/g starch dry matter)	-	1.09	1.60
	Weight % of membrane total lipid		
TL*	100	100	100
PL	0	9.1	16.3
PC		6.4	11.9
PE		1.8	2.5
PI		0.9	1.9
GL	0	24.8	26.2
MGDG		7.3	7.5
DGDG		3.7	5.6
SG	tr	2.8	3.7
ESG	tr	11.0	9.4
NL	100	66.1	57.5
FFA	17.1	10.1	12.5
TG	13.1	12.8	11.9
FS	10.5	20.3	18.0
SE	13.0	11.5	8.8
$U_1+U_2+U_3$	45.7	11.4	6.2

*TL fatty acid composition in % of total acids: 0.7(12:0); 0.8 (14:0); 22.4(16:0); 4.3(16:1); 14.1(18:0); 4.0(18:1); 37.8(18:2); 14.3(18:3); 1.6(20:1). UR = 1.4.

fasting one hour, was in percent of total acids: 80(16:0), 2.7(18:0), 3.3(18:1), 10.2(18:2), and 4.2(18:3).

(vi) Cell Wall Lipids

The lipid composition of cell wall is reported in Table 19. NL were the major constituents of cell wall. Among them, SE was the predominant component at 40% of the TL. TG and FFA were other prominent components of the NL. Besides these NL, diglyceride was also identified in measurable quantities in the lipids of the cell wall. Polar lipids (phospholipids and glycolipids) were also detected in cell wall lipids, but in greatly reduced amounts relative to other cellular membranes. The major polar lipid of the cell wall was ESG, while PI, PC, and PE were present in almost equal but smaller proportions. Another distinct feature of the cell wall lipids was higher levels of MGDG as compared to DGDG.

Analysis of the fatty acid moieties present in the cell wall acyl lipids also showed a completely different make-up than any other cellular structures. The major acyl moiety in the cell wall lipids was palmitic acid (60%); however, there were substantial quantities of linoleic, linolenic, and stearic acids. Oleic acid was a minor component.

Table 19. Lipid composition of potato tuber cell wall preparations

Total lipid (mg/g cell wall dry matter) 3.08*					
PL		GL		NL	
As weight % of total lipid					
Total PL	9.4	Total GL	19.4	Total NL	71.2
PC	3.3	MGDG	4.6	FFA	9.1
PE	3.1	DGDG	1.9	TG	9.4
PI	3.0	SG	3.1	DG**	3.8
		ESG	9.8	SE	40.2
				U	8.7

*TL fatty acid composition in % of total acids: 59.3 (16:0); 7.9 (18:0); 3.4 (18:1); 19.9 (18:2); 9.5 (18:3). UR = 0.4.

**Diglyceride

V. DISCUSSION AND CONCLUSION

In this study, it was found that lipid was only a trace component of potato tubers. The cultivar 'Netted Gem' contained 0.65% lipids on a dry weight basis.

The major lipids found were phospholipids, followed by galactolipids, steryl lipids and NL. The major phospholipid was PC, followed by PE and PI. Galactolipids were found to be predominantly DGDG and MGDG. The steryl lipids identified were FS, SE, SG and ESG. NL were only minor constituents of potato lipids, while TG was the major component of this fraction. The predominance of polar lipids (phospho- and glycolipids) in the tuber is an indication that tuber lipids are primarily structural elements of cellular organelles and lipomembranes. They were rich in polyunsaturated fatty acids.

Potato lipids contained mostly linoleic, linolenic, and palmitic acids. Stearic and oleic acids were also present to a lesser extent. Basically the same complement of fatty acids was present in all the lipids classes, but each had its characteristic make-up of these acids. The galactolipids, MGDG and DGDG, containing predominantly linoleic and linolenic acids, were the most unsaturated potato lipids. On the other hand, the steryl lipids, ESG and SE, which were composed mainly of palmitic acid, were the most saturated potato lipids.

The sterol composition of steryl lipids was established by gas-liquid chromatography coupled with mass spectrometry. The presence of cholesterol, stigmasterol and β-sitosterol was confirmed

by their characteristic gas-liquid chromatography retention times and also by their characteristic mass spectrometry fragmentation patterns. β-sitosterol was found to be the predominant sterol in all the steryl lipids, whereas cholesterol was mainly present in SE and SG fractions.

The relative amounts of individual lipids did not change significantly during processing. However, their absolute amounts were decreased substantially during processing; the major loss occurred during the steam-cooking/hot mashing step. This loss was mainly in the phospholipid fractions; PE and PI were the most affected lipids. However, the loss of various lipids was not accompanied by an increase in the content of FFA. On the contrary, the FFA decreased substantially. This finding would suggest that the lipids were lost in cooking as a result of enzymic hydrolysis and degradation, and steam-distillation of lipid-breakdown products, since pure TG and other lipids were not distillable under simulated conditions of cooking and/or mashing.

The relative fatty acid composition of TL only changed slightly during processing: there was a slight decrease in linoleic acid accompanied by a slight increase in stearic and palmitic acids. Thus, there was only a slight decrease in the sum of the polyunsaturated fatty acids. Also, the fatty acid composition of the acyl lipids isolated by TLC did not change to any significant extent during processing into dehydrated granules. Hence, the unsaturation ratio of lipids in freshly processed granules remained very high, so off-flavor development in dehydrated granules would not be surprising. In light of the results, galactolipids and phospholipids, which

contained the most unsaturated fatty acids, would be the major source of the problem.

A comprehensive study of potato lipids and changes in their constituents and fatty acid composition during granule processing by a freeze-thaw process was presented in this study. The findings could be very useful to potato processors, since lipids are known to be precursors of rancidity in potato products.

It was suggested that environment was often more important for autoxidation than lipid composition (Walter et al., 1972). Hence, the location of phospholipids and galactolipids would be an important factor in lipid oxidation and degradation.

It was observed from preliminary thin-layer chromatographic studies of lipids extracted from tuber homogenate that MGDG was most susceptible to enzymic hydrolysis, much more so than DGDG and most of the phospholipids. Negligible changes were observed among steryl lipids, and only small decreases in PI and TG. Since monoacyl galactolipid, lysophospholipids, PA, or mono- or diglycerides could not be detected, it was concluded that phospholipase D activity in tuber homogenate was low or negligible, and that LAH's (comprising phospholipases A_1 and A_2 and galactolipase) and LOX were jointly involved in lipid destruction. While LOX was suppressed at pH 7.8 and in the presence of mercaptobenzothiazole, LAH's were unaffected. Hence, compositional data on lipids of tuber membranes and subcellular particles are highly unreliable unless lipid analysis was performed by controlling the activity of LAH's. It was found in the enzyme studies that nupercaine not only suppressed phospholipase, but also inhibited galactolipase activity as well.

The proximate nature of lipids associated with cellular components was determined by transmission electron microscopic examination after a series of selective extractions of cellular lipids with solvents of known extraction characteristics. Also, in situ morphology of cellular components and their relative spatial orientation were determined. Results showed that the lipoprotein membranes of the cell and cellular organelles contained varying complements of phospho-, glyco- and neutral lipids, with the exception of the tonoplast, which probably has a unique lipid composition or contains certain unidentified lipids which are not affected by most of the solvent systems used in this study. In addition, morphological studies of steam-cooked tuber cells showed dramatic structural changes. However, steam-cooking did not result in cell distention or cell rupture; instead, substantial changes in the spatial organization of cellular structures and their constituents were evident.

In the isolation of cell membranes and organelles, precautions were taken to prevent lipid changes as affected by enzymic degradation. Homogenization of potato tissues at room temperature or even 4°C brings about a rapid enzymic breakdown of phospho- and galactolipids, and the released FFA are subjected to a high rate of peroxidation by tuber LOX; the oxidized products ranging from hydroperoxides of linoleic and linolenic acids to low molecular weight breakdown products (Galliard, 1970, Galliard and Phillips, 1971). Hence, in the isolation of cell membranes and organelles, a buffer of pH 7.8, containing mercaptobenzothiazole to suppress LOX activity, was used. Nupercaine was also added to suppress LAH activity. This seemed to be quite successful as

there was no substantial increase in the FFA content, nor were oxidation products of lipids detected. However, in the lengthy isolation of starch grains, lipid oxidation and degradation was inevitable.

The subcellular fraction, plasmalemma, was difficult to prepare. During homogenization of potato tissues, the plasmalemma was ruptured either into large fragments that co-sedimented in the gradients with nuclei and mitochondria, or into small vesicles that co-sedimented with microsomes. Plasmalemma contained high amounts of steryl lipids compared to the other subcellular fractions. It is known that sterol molecules can complex phospholipids in monolayers and thus decrease the fluidity and the permeability of the lipid phase. It has been suggested that high concentration of sterols in the plasmalemma helps restrict the permeability of this membrane, which is directly exposed to the external medium (Mazliak, 1977). Plasmalemma membranes, prepared from an initial 2,000 x g pellet from tuber homogenates, contained the usual large amount of PC, PE, and polyunsaturated fatty acids. This preparation reduced the degradation of lipids by phospholipase, which could easily have occurred during long centrifugation times in the preparation method starting from the microsomal pellet. The galactolipid content was greatly influenced by LAH of the tuber enzymes, and its highest content (20.8%) was found in membranes prepared in the presence of nupercaine. Also, if nupercaine was not used during the preparation of plasmalemma membranes, there was an increase in palmitic acid by up to 35%, accompanied by a decrease of linoleic and linolenic acids.

The microsomal preparations in the presence of nupercaine had higher contents of galactolipids than in its absence. Of interest are the results for recovered FFA. An accumulation of palmitic and stearic acids and a decrease in polyunsaturated acids suggested that lipid breakdown occurred despite precautions taken against lipid-degrading enzymes.

A simple purification on a sucrose gradient did not yield pure peroxisomes. However, additional centrifugation on a shallow sucrose gradient of 1.16-1.29 g/cm^3, with the band at 1.24-1.25 (Tchang et al., 1978), gave satisfactory results.

The presence of MGDG and DGDG in mitochondria is greatly disputed. Schwertner and Biale (1975) claimed that potato tuber mitochondrial lipids differed from other plant mitochondria by their high content of galactolipids (22.8% of TL), and also by their free and esterified sterols, the predominant being NL. Their phopholipid fraction was only 56% of mitochondrial TL. Contrary to these results, Mannella and Bonner (1975) found, by a heptadecanoic acid recovery test, that phospholipids were the only significant acyl lipid moiety within potato mitochondria, but confirmed the presence of sterols in outer mitochondrial membranes. McCarty et al. (1973) could not detect galactolipids in lightly purified potato mitochondria, and their possible presence was ascribed to plastid contamination. My experience showed that the crude mitochondrial pellet collected by centrifugation of the tuber homogenate obtained after filtration through Miracloth could be purified by a discontinuous sucrose gradient centrifugation. Again, in the absence of nupercaine, an increase in phospholipids and

steryl lipids occurred at the expense of enzymic breakdown of galactolipids. This implies that mitochondrial lipid results devoid of MGDG constituents might be questionable.

Isolation of amyloplasts from tuber homogenate by squeezing through Miracloth yielded grains enriched in size below 20 µm, while using a 200 mesh polyester sieve cloth resulted in amyloplasts enriched in large oval-shaped grains (20-38 µm in short axis, and 40-90 µm in long axis). The lipid yields differed between these two preparations, and were consistently higher for small grains. Isolation in the presence of nupercaine increased the yield by 32%. Amyloplast isolation in the absence of nupercaine, by free gravity sedimentation lasting from 30 minutes to 2 hours, brought about a complete loss of membrane phospho- and glycolipids. In such starch preparations, only the NL fraction was present, half of it already being in the form of FFA oxidized to various degrees. No lyso derivatives were found among the degradation products. Thus, the only fairly reliable starch preparation was that obtained by low-speed centrifugation within 5-7 minutes in a buffer of pH 7.8 containing nupercaine.

Potato cell wall preparations also contained lipids. When thorough washing with intermittent sonication was repeated many times, the lipid content did not change. Microscopic examination showed the absence of starch and plasmalemma contaminants. In addition, such preparations had the expected polygalacturonide content, degree of esterification, pectin methylesterase activity, and ability to release protein after polygalacturonase treatment -- all properties of a sound

preparation. Therefore, it appeared that the extracted lipids might be an integral part of the wall structure. This lipid content was 0.31% of the cell wall dry matter, or 2.3% of tuber TL.

Analysis of isolated cellular components confirmed that lipids in potato exist as structural components of cellular membranes and organelles. Also, each cellular fraction has its characteristic lipid composition. Most of the TL were present in the microsomal membranes, followed by mitochondria and plasmalemma. Most polar lipids (phospho- and glycolipids) were associated with membranes (mitochondria and endoplasmic reticulum). Other cellular organelles, starch grains, and cell walls consisted mainly of saturated NL.

Distribution of the lipid classes showed that cell wall and adjacent plasmalemma were highest in steryl lipids, while phospholipid was predominant in cytoplasm, and glyco- and neutral lipids in starch grains. Again, each cellular structure contained a distinctly different complement of fatty acids. Moreover, cellular membranes contained significant amounts of unsaturated lipids and, so, would be potential sites of oxidative degradation.

The unsaturation degrees associated with membranes and/or organelles are of great importance from the processing point of view, since they can contribute to a rapid oxidation of lipids in processed granules. As shown in Table 20, such a contribution would be least expected from cell walls with an unsaturation ratio (UR) of 0.4, in which palmitic acid was predominant (Table 19). Again, a great contribution was not expected from starch grains, since their membranes are highly saturated. Plasmalemma (UR = 1.7), peroxisomes (UR = 2.0)

Table 20. The unsaturation degree of lipids associated with tuber cell membranes and organelles.

Membrane system	Unsaturation ratio*
Mitochondria	2.9
Endoplasmic reticulum (microsomes)	2.1
Peroxisomes	2.0
Cell walls	0.4
Plasmalemma	1.7
Starch grains (amyloplasts)	1.4

*Unsaturation ratio: (linoleic + linolenic acids)/(palmitic + stearic acids).

and microsomes (endoplasmic reticulum, UR = 2.1) would be rated as moderate contributors to lipid oxidation. The real contribution to rapid oxidation might be expected from mitochondria, with an unsaturation ratio of 2.9.

These findings and those for lipid-degrading enzymes can be used to clarify the fate of lipids in commercial potato granule processing. Lipid degradation and peroxidation start instantly after slicing, and continue during the rising temperature period of the precooking step performed at 70°C. By the end of precooking, the enzymes are inactivated, and enzymic lipid breakdown is stopped.

Since processing did not appreciably change the lipid composition of potato tuber tissues (Tables 2, 5 and 6), it is probable that the nature of lipids, the type of association with cellular components, and the morphological changes in the spatial organization of these cellular constituents would be important determinants in controlling the quality of processed potato products.

During cooking and subsequent steps, the lipids are embedded and/or spread over gelled starch, denatured protein, and cellulose--pectin matrices. Lipids of membranes in cooked potato cells exist as a free component sandwiched between gelled starch and cell wall, as observed by transmission electron microscopy (Figure 22). Their oxidation would be partially prevented during processing since they are not exposed directly to the surrounding air. Upon dehydration, however, this spatial arrangement of lipid constituents would not be effectively protected because both starch and cell wall in dehydrated state would act only as spreading matrices for the lipid, thus leaving lipids exposed to oxidative attack. Such a possibility would be

greatly minimized if lipids in the processed product existed in combination with proteins (Khan and Hadziyev, 1978).

In dehydrated granules the oxidation rate of phospholipids would be affected by the nature of the protein, and that of the bulk of glycolipids by the starch matrix. This implies that the extent of off-flavor rise in granules would be a resultant not only of lipid structure and unsaturation, but also of the extent of enzymic breakdown, and of the matrix to which the lipid is attached.

Further investigation of autoxidation of phospho-, glyco- and neutral lipids on different matrices, such as dehydrated cell walls, proteins, starch and pectic substances, could provide information on the lipid oxidation rate and the effect of various matrices on lipid oxidation in dehydrated potato systems. In additon, studies of the modes of application of phenolic antioxidants and their effects on lipid oxidation in these dehydrated systems could provide important insights into the prevention of rancidity in dehydrated potato products.

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