SURVEY OF THE FATTY ACID CONTENT OF NATIVE NEW ZEALAND PLANTS

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Abstract

Fatty acids are monocarboxylic acids, which primarily exist in the form of mixed triacylglycerides and are widely distributed as esters in natural fats, oils and waxes throughout the animal and plant kingdoms. As well as providing daily requirements for essential fatty acids and energy in animals and a source of carbon in plant seeds, fatty acids are important components of a number of macromolecules, structures or organs such as phospholipids, hormones, cellular membranes or adipose tissue. The New Zealand flora includes a wide range of unique species only found in New Zealand, which have the potential to be the source of rare or novel compounds that may be used in the food, chemical and pharmaceutical industries. Native New Zealand plants have been surveyed for a number of plant constituents including alkaloids, essential oils, tannins, steroids, toxic compound and dye or colouring materials. However relatively little work has been carried out to survey the non-volatile oils and fats, such as fatty acids, contained within the native species (Cambie, 1986).

In this study, a preliminary investigation of the fatty acid content of seeds or fruits from 46 native New Zealand plants has been carried out, with an emphasis on finding and reporting unusual, or commercially viable proportions of fatty acids or seed lipid compositions. 26 out of the initial 46 species were found to have a potentially useful proportion of a particular fatty acid or group of fatty acids. Based on the historical or other potential uses of the plants, ease of growth, NZ-wide distribution and total fatty acid content per gram of dried seed weight, it was concluded that of the 46 species analysed those with the greatest commercial viability overall included the NZ flax, cabbage tree, five finger, patē/seven finger, wineberry, kōhia, tītoki, kahikatea and tōtara.

A further eight species would warrant further investigation if improvements in ease of growth, cultivation and nation-wide distribution were increased (snowberry, karo, miro, horoeka, Chatham Island forget-me-not, and the Marlborough rock daisy) or if useful properties other than as an ornamental plant were found (native rock lily, māhoe) since they all contained a potentially useful proportion of a particular fatty acid or group of fatty acids and >10% total fatty acid per gram of dried seed weight.

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List of Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
DGLA	Dihomo gamma-linolenic acid
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EGGS-X	Ethylene glycol succinate
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FAMES	Fatty acid methyl ester(s)
FFA	Free fatty acid(s)
GLA	Gamma linolenic acid
GLC	Gas liquid chromatography
H/C	Hydrocarbon
HDL	High-density lipoprotein(s)
IUPAC	International Union of Pure and Applied Chemistry
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acid(s)
LDL	Low-density lipoprotein(s)
mg/g	milligrams per gram
MUFA	Monoenoic/monounsaturated fatty acid(s)
MW	Molecular weight

-OH	Hydroxyl side chain
O	Oxygen
PUFA	Polyenoic/polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
TAG	Triacylglycerol(s)
TFA	Total fatty acid
TLC	Thin-layer chromatography
UFA	Unsaturated fatty acid(s)
UV	Ultra violet
VLDL	Very low density lipoprotein(s)

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1 Introduction

1.1 AIMS OF THIS PROJECT

The New Zealand flora includes a wide range of unique species, which have the potential to provide a source of compounds that may be useful in the food, chemical and pharmaceutical industries. About 80% of New Zealand's species of higher plants and 85% of seed plants are endemic (Godley, 1976; Cambie, 1986). This high proportion of endemism provides opportunities for unique research as useful or novel plant constituents may be found which are unique to species of New Zealand. Studies to date have surveyed New Zealand plants for constituents such as alkaloids, essential oils, tannins, steroids, toxic compounds and dye or colouring materials. However, relatively little work has been carried out on the non-volatile oils and fats from native species (Cambie, 1986).

The goal of this research project was to study the fatty acid composition of seed lipids found in indigenous New Zealand plants, with an emphasis on finding and characterising unusual or commercially viable fatty acids or seed lipid compositions. The number of plants indigenous to NZ, combined with their relative fatty acid composition, requires time and scope too great to cover comprehensively in a single Master of Science research project, thus this study provides a basis for future detailed work.

Vegetable fats or oils generally occur in greatest quantity either in the fleshy part of the fruit, such as in avocado, and/or in the seed such as in sunflower, safflower or rapeseed oils (Eckey, 1954). The lipid fraction of plant seeds is a concentrated source of fatty acids, as they are primarily made up of triacylglycerols. Seed lipids may also contain minor contributions from free fatty acids, mono- and diacylglycerols, glycolipids (i.e. galactolipids), phospholipids (i.e. phosphatidyl choline), sterols (i.e. cholesterol), vitamins, antioxidants, pigments and hydrocarbons. (Eckey, 1954). This study has concentrated primarily on examining the fatty acid composition of seeds and/or fruit from native New Zealand plants that were readily available or suggested as potentially worthwhile by one of Massey University's resident botanists, Professor David Fountain.

1.2 LITERATURE REVIEW AND BACKGROUND

Lipids are dynamic, complex metabolic biochemicals that are linked directly or indirectly with virtually every structure and metabolic function in the body. They are an important source of energy as well as being a major component of the structure of cell and organelle membranes. As membrane constituents, they help control the integrity of tissues as well as the flux of chemicals into and out of the cell. They are involved in a wide range of physiological pathways including glandular secretions and muscle recovery. They are necessary for growth, tissue repair and reproduction. They help provide culinary interest, satiety, carry fat-soluble vitamins and are precursors of various hormones as well as highly potent eicosanoids. Lipids are also implicated in the susceptibility to, and recovery from, disease (Wysong, 1990).

In Sections 1.2.1 and 1.2.2, a general overview of the classification and biological function of lipids will be discussed. This will be followed by a more detailed overview on the structure, nomenclature, classification, distribution, occurrence, nutritional relevance and commercial applications of triacylglycerols (TAG) and fatty acids (FA) in Sections 1.2.3 to 1.2.6, with particular emphasis being placed on the fatty acids of seed lipids. Finally, a brief summary of the description, history and uses of New Zealand's native flora will be discussed along with research that has already been done on the non-volatile (seed) oils and fats of New Zealand's native plants (Section 1.2.7). These sections also explain the background and relevance of this research project.

1.2.1 Classification of Lipids.

The term "lipids" encompasses an enormous range of organic compounds with diverse structures and biological functions, which are found in plants, animals and micro-organisms. Lipids are greasy to the touch and are distinguished from other biologically produced organic materials by their solubility. The structures of lipids are primarily hydrocarbon-like and thus they are insoluble in water but are soluble in ether, and similar non-polar solvents (Hadley, 1985). There are many ways to classify lipids but the system presented by White *et al.*, (1973) is the basis of the classification system followed throughout this thesis:

CLASSIFICATION OF LIPIDS

I. *Fatty acids (Hydrocarbon, (H/C) chain with terminal carboxylic acid)*

II. *Lipids containing glycerol (glycerolipids): have glycerol as a backbone.*

- a. Neutral fats
  1. Mono-, di-, and triacylglycerols
  2. Glycerol esters (batyl and selachyl alcohol)
  3. Glycosylglycerols/Glycolipids (galactosyl diacylglycerol)
- b. Phosphoglycerides (Contain glycerol, FA, inorganic phosphate & an organic base or polyhydroxyl compound)
  1. Phosphatidates (phosphatidylcholine, -serine, -ethanolamine)
  2. Diphosphatidylglycerols, phosphoinositides

III. *Lipids not containing glycerol*

- a. Sphingolipids (Contain sphingosine or a closely related compound) Ceramides, Sphingomyelins & Glycosphingolipids (i.e. cerebrosides)
- b. Aliphatic alcohols (H/C chain with -OH gps., i.e. stearyl alcohol)
- c. Wax esters (FA esterified to aliphatic alcohols, i.e. beeswax)
- d. Hydrocarbons (long, un/branched chains of H/C, i.e. paraffins)
- e. Terpenes (consist of isoprene units, and often O, i.e. citronellal)
- f. Steroids (derivatives of a sterane ring, i.e. cholesterol, bile acids)
- g. Prostaglandins

IV. *Lipids combined with other classes of compounds*: (proteolipids, phosphatidopeptides, lipoproteins, -amino acids & -polysaccharides).

Lipids are also sometimes referred to in general terms as "fats" or "oils". This distinction is based on the physical state of the lipid at room temperature. Fats (like tallow or lard) are usually distinguished from oils (such as olive or canola) based on their melting point. Fats are solid at room temperature (20°C) while oils are liquids. Often however, fats and oils are general terms used when referring to esters of fatty acids with glycerol. These lipids are the most important from the point of view of quantity, wideness of distribution, food value and commercial interest (Bloor, 1943). Some lipids may also be classified according to their overall charge. For example neutral fats are those with an overall neutral charge, such as TAG, as opposed to phospholipids, which carry the negatively charged phosphate group.

1.2.2 Biological Functions of Lipids

Apart from providing an important energy, food and carbon source for living organisms, key biological functions of lipids include their structural roles, (the organisation and function of all cellular membranes), their communication roles (such as signalling, hormonal and defense-related activities) and their protective roles (such as in waterproofing, protection against mechanical injury, padding of important internal organs and providing thermal insulation). Lipids also have many other biological roles such as in the maintenance of good health and nutrition. Raised or lowered levels of certain lipids can have profound implications in diseases such as high blood cholesterol in heart disease. However due to the broad areas that these subjects cover, only specific lipid groups involved in lipid transport, absorption and digestion will be briefly covered in this section. Health, nutrition and disease implications with specific reference to selected fatty acid groups will be discussed in Section 1.2.5.

1.2.2.1 Lipids as an energy, food and carbon source

Daily requirements for energy are variable according to factors such as the age, size, health, activity level and metabolic needs of individual organisms. In animals, lipids, particularly TAG, provide an important, space-saving, concentrated source of potential fuel that can be harvested through oxidation of the FA. About 9 kcal of energy per gram of fat can be produced through FA oxidation as compared with 4 kcal/g of carbohydrate or protein (Eckey, 1954; Linder, 1991).

Although carbohydrates are generally the preferred energy source, energy derived from FA oxidation is important when carbohydrate levels are low such as in conditions of fasting or starvation. Fatty acids can also be metabolised into ketone bodies, which are a more water-soluble form of fuel, produced particularly under such conditions. During these times, ketone bodies are the preferred energy source for the central nervous system and the brain (Stryer, 1988; Linder, 1991; Gurr, 1999). Lipids comprise one of the three major classes of food components and, with proteins and carbohydrates, are the major natural components of all living organisms (Eckey, 1954; Hilditch & Williams, 1964).

Plant seeds such as sunflower, safflower or rapeseed and some fruits such as avocado or olive, are an abundant source of lipid, and in particular, TAG. Some plants may accumulate as much as 75% of their seed weight as lipids. In contrast to animals, seed TAG do not serve as an energy store but mainly as a carbon store to supply the carbon required for biosynthetic processes during seed germination. The purpose of TAG in fruit is to attract animals to consume these fruits in order to distribute their seeds. TAG have an advantage over carbohydrates as storage compounds because their weight/carbon ratio is much lower. Carbon stored in seeds as fat requires less than half the weight as when stored as starch and having a low weight is advantageous for seed dispersal (Heldt, 1999).

1.2.2.2 Structural role of lipids in membranes.

The most important structural role of lipids is in their contribution to the fluidity, stability, structure and function of all membranes. Phospholipids are major components of cell membranes as well as the membranes of cell components such as mitochondria. Major lipid species that contribute to the composition of cell membranes, (other than glycerophospholipids such as lecithin), include galactolipids and glycosphingolipids (plants); and cholesterol and sphingomyelins (animals).

Lecithin and other similiar amphiphillic lipids consist of a hydrophillic head and a hydrophobic tail. Hence they are soluble in both water and lipid and are thus able to maintain the continuity between the water and lipid phases both inside and outside the cell. This is achieved by forming lipid bilayers in which the hydrocarbon tails are held together by hydrophobic interactions and the hydrophillic heads protrude into the aqueous phase and form electrostatic and hydrogen-bonding attractions with water molecules (Heldt, 1999). Many of the special properties and functions of biological membranes, including lipid-protein interactions, are due to this amphipathic nature of lipids. This amphiphillic property of lipids is also important in the digestion and absorption of dietary lipids and transport of lipids throughout an organism.

Membrane lipids containing mainly saturated fatty acids (SFA) form a very regular lipid bilayer as the hydrocarbon chains of SFA pack well together. The kinks caused in the hydrocarbon chain by *cis*-carbon-carbon double bonds in unsaturated fatty acids result in disturbances in the lipid bilayer and lead to an increase in the fluidity of the membrane (Heldt, 1999). Steroids, (i.e. cholesterol in eukaryote membranes) are also key regulators of membrane fluidity. Cholesterol prevents the crystallization of fatty acids by fitting in between them (increasing fluidity) but it also sterically blocks large motions of fatty acid chains (decreasing fluidity).

Other factors such as the length of the fatty acid chains, the nature of the polar head group and the homogeneity of the lipid constituents also affect the fluidity of the membrane (Stryer, 1988). Variation in membrane fluidity is important in order to control the entry and exit of various substances, adjust to temperature fluctuations and provide a means of communication between areas that the membranes are separating (Cullis & Hope, 1985).

There are great differences in the lipid composition of the various membranes between organisms, within an organism or cell, between different organelle membranes and even within the same membrane. Much of this has to do with the availability of membrane components, lipid constituents in the membrane and the function of the cell or organelle (Heldt, 1999).

1.2.2.3 Communication and transport roles of lipids.

1.2.2.3.1 Nervous system

Important lipids of the nervous system include various phosphatidates, sphingomyelin, cholesterol and cerebrosides. Lipids are involved directly or indirectly in the conduction of nerve impulses (synaptic membrane lipids such as gangliosides), in the insulation of nerve cells (i.e. lipid-rich myelin sheath), in the functioning of receptors and binding of neurotransmitters (Hadley, 1985).

1.2.2.3.2 Hormones, Pheromones and Allelochemicals

Chemical communication is the most widespread mode of information transfer among organisms. Lipids are well suited for communicative roles as semiochemicals such as pheromones and allelochemicals. These semiochemicals may function in a number of ways including acting as deterrents, attractants or stimulants. Lipids are particularly useful as airborne chemical messengers due to the large molecular diversity of volatile lipid molecules that can fall within the predicted optimum size range of 80 to 300MW. This diversity is increased by the ability of lipids to form relatively stable structural and geometric isomers (Hadley, 1985). Lipids are also involved in cell-to-cell chemical communication (i.e. lipid hormones). One important group of lipids that function in this respect are the prostaglandins, a family of 20 carbon fatty acid derived compounds that contain a cyclopentane ring in the middle of the fatty acid chain.

Prostaglandins are involved in a wide range of biological activities such as those described in Figure 1.4 (See Section 1.2.5). Steroid hormones synthesized from cholesterol such as androgens and estrogens are another group of lipid messengers. These steroid hormones are involved in roles including the regulation and development of sexual behaviour and secondary sex characteristics. Due to their relative insolubility in water, small size and ability to pass through lipid bilayers of target cells, steroid hormones persist in the blood for longer periods of time than hydrophilic hormones, thus enabling them to mediate longer lasting responses (Hadley, 1985; Stryer, 1988).

1.2.2.4 Selected Lipids Involved in Lipid Transport, Digestion and Absorption

As lipids do not dissolve in water, yet many biochemical reactions normally occur in an aqueous medium, special strategies are needed for processes involving the metabolism, digestion/absorption and transport of lipids. In higher animals, phospholipids, bile salts, specific proteins, cholesterol-carrying lipoproteins and other lipid containing structures such as chylomicrons and micelles are employed to assist in lipid metabolism and transport. The amphiphilic property of these various lipids or lipid-containing molecules allows dietary fat emulsion, solubilisation, absorption and transport.

Phospholipids assist in the stabilization of the oil-in-water emulsion in the stomach and are a major component of the structures involved in lipid digestion, transport and assimilation. Stabilizing lipid particles with a coat of amphiphilic compounds, to form lipoproteins, has solved the problem of lipid transport via the blood to their various destinations. The lipoprotein particles that are assembled in the enterocyte following the intake of dietary lipids are called chylomicrons. In well-nourished humans, the chylomicrons containing these exogenous lipids are generally taken to the adipose tissue where some of the TAG contained within, are unloaded. The apoprotein (protein moieties of the lipoprotein), named apo-B48, is unique to the enterocyte and identifies particles carrying lipids of exogenous (dietary) origin (Stryer, 1988; Linder, 1991; Gurr, 1999).

Another type of lipoprotein, (very low density lipoprotein or VLDL) carries mainly TAG synthesized in the liver as opposed to those absorbed from the diet. In man, cholesterol is carried primarily in smaller particles called low-density lipoproteins (LDL), so-called because the higher ratio of lipid to protein gives them a low density. High-density lipoprotein (HDL), has more protein than lipid (hence its name). All lipoproteins contain combinations of cholesterol, cholesterol esters, TAG, phospholipids and proteins but the proportions differ. These various proportions confer different densities, (and hence their names), that allow separations to be made by centrifugation (Gurr, 1999).

Despite its bad publicity, the lipid cholesterol is an essential nutrient. Current recommendations for cholesterol intake suggest less than 300mg daily (Bremer, 1994) and a plasma cholesterol level of less than 5.2mmol/L (National Heart Foundation, 1988). It has a principal function in the formation of all interior and exterior cell membranes, is involved in lipid transport and among other roles, is a substrate for the formation of bile salts, steroid hormones and Vitamin D3 (Stryer, 1988; Linder, 1991; Gurr, 1999). Concentrations of some of the lipids and structures involved in lipid transport, (particularly blood cholesterol and lipoprotein structures), are measured in the blood to study the influence of certain dietary lipids with relevance to their health or disease implications.

Using these measures, recommendations for improvement of health or disease prevention are often made based on the results found, although there has been a great deal of debate surrounding their validity (Gurr, 1999). This is discussed further in Section 1.2.5.3.

1.2.2.5 Protective Role of Lipids.

1.2.2.5.1 Waterproofing

Maintaining water balance is a critical problem for all organisms regardless of their habitat. Formation of a tough external layer or barrier is vital in terrestrial plants and animals where there is a dessication potential and access to free water is limited. Waxes and other highly non-polar hydrocarbons provide vital waterproofing on outer surfaces due to their ability to repel water and form an outer protective coating on some parts of plants and for some animals, particularly arthropods (cuticulin layers).

These lipids are usually highly saturated with long carbon chains and thus are not easily lost through volatilisation; they provide thermal stability and are protected to some extent against degradation by oxygen and micro-organisms. They can also provide an effective deterrent against predation due to the tough exterior which they can form (Hadley, 1985).

1.2.2.5.2 Insulation and Heat Production

Lipids help maintain body temperature through both their insulating effects and the heat generated from their oxidation. A large percentage of energy is lost as heat in the complete oxidation of fatty acids to carbon dioxide and water. This heat production is important in most birds and mammals. "Brown fat" in particular has unique heat-producing qualities that are especially prominent in species that hibernate. Subcutaneous deposits of lipids insulate animals against the cold because of the low rate of heat transfer in fats, a property especially important to animals commonly found in cold climates. Seals and whales, for example, accumulate a large amount of subcutaneous fat (blubber) to protect them against heat loss in their cold, watery environment (Hadley, 1985).

1.2.2.5.3 Structural support

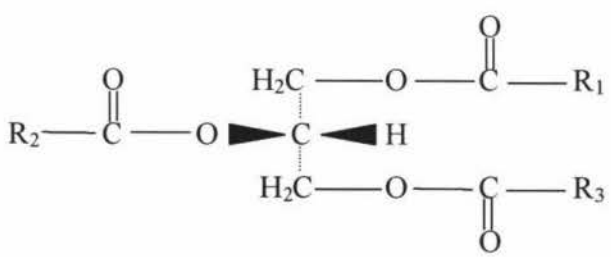
Adipose tissue is the storage form of fats in animals. It is a specialised form of loose connective tissue that is widely distributed throughout the body. Apart from being a potential energy source it also provides structural support or padding for the protection of vital organs such as the heart, kidneys and eyeballs.

1.2.3 Structure of Triacylglycerols and Fatty Acids

It is the fatty acids from the acylglyceride group of lipids, especially plant seed lipids, that are of particular interest in this research and hence these are discussed further in Sections 1.2.3 to 1.2.7. Glycerolipids are a broad group of lipids that include any esters of the trihydric alcohol, glycerol, and between one and three fatty acids. Collectively, these glycerolipids are the most important constituents of plant membranes and oils. Three major categories of glycerolipids are found in plants, i.e. simple acylglycerols, phospholipids and glycolipids. The simple acylglycerols are the major components of seed storage oils, the most common of these in seed oils being the triacylglycerols (Murphy, 1999).

1.2.3.1 Triacylglycerols

A triacylglycerol (TAG) consists of three fatty acids esterified to a glycerol backbone, whereas a diacylglycerol and a monoacylglycerol consist of two and one fatty acid(s) respectively esterified to a glycerol backbone. Triacylglycerols are the chief constituents of natural fats and oils and are the major storage lipids in animals and plants. Collectively, they constitute the most important and abundant source of fatty acids for humans as TAG account for 95-98% of the fat ingested in all forms of food (Linder, 1991). Glycerol-containing lipids such as TAG are numbered according to the IUPAC convention (IUPAC, 1960), and drawn as a Fischer projection as in Fig 1.1 below.



1,2,3-triacyl-*sn*-glycerol

FIGURE 1.1 Structure of a Triacylglyceride

(Where the groups R_1, R_2, and R_3 represent the hydrocarbon chains of the fatty acids and the prefix *sn*- describes the use of the IUPAC/Fischer projection convention.)

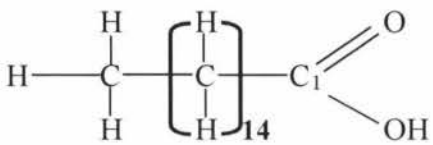
Simple acylglycerides contain only one type of acyl group, mixed acylglycerides contain two or more kinds of acyl groups. Mixed acylglycerides are the most common type, as a different FA is usually involved in each of the three TAG ester functions. An unsaturated acid is often present in the middle ester of the TAG. For a more detailed characterisation of TAG composition in fats and oils, one can determine the amount of individual fatty acids and their positions on the glycerol backbone. This can be accomplished by using an enzymatic 2-position fatty acid assay followed by gas liquid chromatography (GLC) analysis.

1.2.3.2 Fatty acids

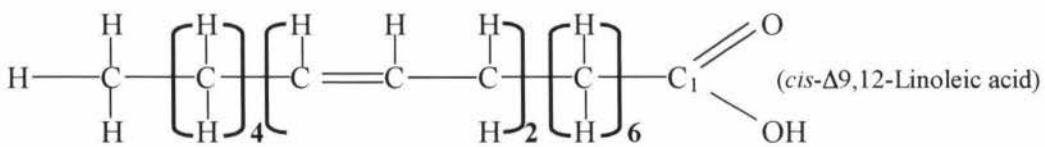
Fatty acids are monocarboxylic acids linked to a hydrocarbon chain and are represented by the formula RCOOH, in which R represents the hydrocarbon chain. The chain is usually an unbranched straight-chain or normal hydrocarbon group containing an even number of carbons ranging from twelve to twenty carbons (predominantly 16 or 18 chain lengths) and frequently containing one or more *cis*-olefinic linkages. Structural features such as cycloalkyl, conjugated polyene, methoxy, epoxyl, keto, fluoro and hydroxyl substitutes are more rarely encountered, almost always in plant rather than animal tissues. Apart from ricinoleic acid, which contains a hydroxyl group, fatty acids with these groups will not be discussed to any great extent in this thesis.

Fatty acids occur almost exclusively in nature in the form of esters and are seldom found in the free state. The hydrocarbon chains of the FA may be completely saturated (thus termed saturated fatty acids or SFA), or may contain one or more ethylenic/double bonds (termed unsaturated fatty acids or UFA). Discussion here will generally concentrate on the SFA and UFA with carbon chain lengths ranging from twelve to twenty four carbons as these are the straight-chain acids most frequently found in seed fats and therefore of particular interest in this study.

If a double bond is present in the hydrocarbon chain, the configuration is almost always *cis*. If one double bond is present in the chain, it is termed a monoenoic/monounsaturated fatty acid (MUFA), if containing more than one double bond, it may be termed a dienoic, trienoic or a polyenoic/polyunsaturated fatty acid (PUFA) depending on the number of double bonds. Though the term PUFA may be used for fatty acids with four or more double bonds, in the nutritional arena, PUFA generally refers to fatty acids with two or more double bonds. Figure 1.2 shows palmitic and linoleic acids, two of the more common fatty acids existing in nature and examples of a saturated and unsaturated fatty acid respectively.



(Palmitic acid)



(*cis*-Δ9,12-Linoleic acid)

FIGURE 1.2 Structures of Palmitic and Linoleic Fatty Acids

(Where the first carbon atom (C_1) is numbered beginning from the carboxyl terminal).

1.2.4 Nomenclature, Classification and Distribution/Occurrence of Fatty Acids

1.2.4.1 Nomenclature

Fatty acids are named in a number of ways. Three common methods, which will be used in this thesis, include the systematic, common and shorthand designation of fatty acids. Tables 1.1 and 1.2 describe the major ways by which the fatty acids of interest in this research study are named. For convenience in this thesis, the common name and/or shorthand designations described in those tables will generally be the fatty acid nomenclature used. Note the terms or symbols (***delta-***, or Δ) or (***omega-*** , ω-, or ***n-***) denote the positions of the ethylenic bonds numbered from the terminal carboxyl or methyl end of the carbon chain respectively.

1.2.4.2 Classification

Lipids in general, or groups of lipids such as fatty acids, can be classified according to their chemical structure, solubilities, and rate of turnover. The fatty acids are a large group of aliphatic carboxylic acids, so termed because of their widespread occurrence in natural fats, oils and allied substances. Various series of fatty acids have many properties in common, thus groupings such as the saturated fatty acids, the various unsaturated fatty acids, the hydroxy or conjugated acids, represent a classification based on certain differences in chemical structure (Ralston, 1948). Fatty acids may also be grouped according to their degree of unsaturation, importance in the diet, chain lengths, solubility and melting points. These groupings also define characteristics or properties that are important in their roles as lipids (Section 1.2.3) or reasons for use commercially or otherwise (Sections 1.2.5 & 1.2.6). Some of these groupings will be discussed below.

1.2.4.2.1 Degree of Unsaturation

The presence of one or more double bonds in the hydrocarbon chains of FA distinguishes unsaturated fatty acids from those of the saturated series. The properties of unsaturated fatty acids are more complex as they may exhibit those of their saturated counterparts as well as those typical of unsaturated carbon-to-carbon linkages. In this series the phenomena of both positional and geometric isomerism are also encountered.

In this thesis, discussion will mainly concentrate on the saturated and unsaturated fatty acids of particular interest to this research work, as described in Sections 1.2.4 to 1.2.7 and Tables 1.1 & 1.2.

1.2.4.2.2 Chain Length

Fatty acids may also be grouped according to their hydrocarbon chain length. However the groupings are somewhat variable between authoritative sources and hence are defined throughout this study. In general however they fall into the following groups:

- Very-Short-chain fatty acids: Fatty acids with 2-6 carbon chain lengths
- Short-chain fatty acids: Fatty acids with 8-10 carbon chain lengths
- Medium-chain fatty acids: Fatty acids with 12-14 carbon chain lengths
- Long-chain fatty acids: Fatty acids with 16-20 carbon chain lengths
- Very long-chain fatty acids: Fatty acids with 22^{+}carbon chain length

TABLE 1.1 Description and Sources of Important Saturated Fatty Acids

Systematic Name	Formula	Common Name	Shorthand Notation	Description, Sources
Butyric	$CH_3(CH_2)_2CO_2H$		4:0	Volatile? Energy source in ruminants? Found in appreciable quantities in cow's milk.
Hexanoic	$CH_3(CH_2)_4CO_2H$		6:0	Volatile? Energy source in ruminants? Found in appreciable quantities in cow's milk.
Octanoic	$CH_3(CH_2)_6CO_2H$	Caprylic	8:0	Colourless liquid, rancid odour, slightly sol. in water. Sol. in organic solvents. Wide distrib. in animal and vegetable fats. Best-known sources; coconut, palm kernel oils.
Decanoic	$CH_3(CH_2)_8CO_2H$	Capric	10:0	Low-melting solid, v.slightly sol. in water. Sol. in organic solvents. Wide distrib. in animal and vegetable fats. Best-known sources; coconut, palm kernel oils. High % of fatty acids in elm-seed oil.
Dodecanoic	$CH_3(CH_2)_{10}CO_2H$	Lauric	12:0	Crystalline solid, faint, fatty odour, sol. in acetone, ethanol, ether. Abundant in nature, major constituent of oils from palm family, coconut oil. Major industry uses in cosmetics, detergents.
Tetradecanoic	$CH_3(CH_2)_{12}CO_2H$	Myristic	14:0	Odourless, crystalline solid, insol. in water, sol. in acetone, ether, chloroform, glacial acetic acid. Wide distrib. in animal, marine and vegetable fats. Major fatty acid component of Myristicaceae and seed fats of the palm family. Minor comp. of vege oils i.e. cottonseed, walnut, olive oils. Major industry use in detergents.
Hexadecanoic	$CH_3(CH_2)_{14}CO_2H$	Palmitic	16:0	Odourless, waxy, crystalline solid, insol. in water, sol in hot ethanol and ether. Present and often substantial component in essentially all the oils and fats of animal, vegetable and marine origin. Most abundant saturated fatty acid. Major industry use as a component of edible oil.
Heptadecanoic	$CH_3(CH_2)_{15}CO_2H$	Margaric/Daturic	17:0	Odourless, waxy, crystalline solid. Insol. in water, sol. in ethanol, ether and glacial acetic acid. Wide controversy as to its existence in natural products.
Octadecanoic	$CH_3(CH_2)_{16}CO_2H$	Stearic	18:0	Waxy solid, insol. in water, sol. in ether, benzene, carbon disulfide. Wide distrib. in animal and vegetable fats, though less abundant than palmitic. Large component of hydrogenated animal and vegetable fats. Major industry uses in edible oils and also in soap production.
Eicosanoic	$CH_3(CH_2)_{18}CO_2H$	Arachidic	20:0	Waxy solid. Soluble in chloroform, ether and benzene. Wide distrib. in nature though generally minor constituent. Appreciable component of certain hydrog. fish oils.
Docosanoic	$CH_3(CH_2)_{20}CO_2H$	Behenic	22:0	Waxy solid. Reasonably sol. in ethanol and ether. Not abundant in nature, minor component of many seed fats i.e. rape, peanut, mustard seed, trace amounts in several animal milk fats and marine oils.
Tetracosanoic	$CH_3(CH_2)_{22}CO_2H$	Lignoceric	24:0	Slightly sol. in ethanol. Found in small amounts in many vegetable oils, particularly seed fats i.e., coffee bean, sunflower, rape seed.

TABLE 1.2 Description and Sources of Important Unsaturated Fatty Acids

Systematic Name	Formula	Common Name	Shorthand Notation	Description, Sources
Cis-Δ9-hexadecenoic	$C_{16}H_{30}O_2$	Palmitoleic	16:1 (n-7)	Found in appreciable amounts in animal, vegetable and marine oils. Major component of marine oils, also of olive, tea seed, peanut and palm oils.
Cis-Δ9-octadecenoic	$C_{18}H_{34}O_2$	Oleic	18:1 (n-9)	Most important monounsat. FA. Has almost universal occurrence, more abundant than any other FA. Large component of olive and almond oils. Major uses in edible oils and as a lubricant.
12-Hydroxy-Cis-Δ9-octadecenoic	$C_{18}H_{34}O_3$	Ricinoleic	18:1,12 OH (n-9)	Major use in Pharmaceuticals and in plastics.
Cis-Δ11-eicosenoic	$C_{20}H_{38}O_2$	Gondoic	20:1 (n-9)	Found in jojoba oil.
Cis-Δ9-eicosenoic	$C_{20}H_{38}O_2$	Gadoleic	20:1 (n-11)	Common component of marine and fish oils. Major use in lubricating oils.
Cis-Δ13-docosenoic	$C_{22}H_{42}O_2$	Erucic	22:1 (n-9)	Common component of seed fats, especially of cruciferae family such as mustard and rape seed oils. Possible toxicity in mammals. Major uses in plastics and lubricating oils.
Cis-Δ9,12-octadecadienoic	$C_{18}H_{32}O_2$	Linoleic	18:2 (n-6)	Essential FA (EFA) for most animal diets (including humans). Has wide distribution in vegetable kingdom, especially seed oils. Major constituent of seed oils. Characteristic of the drying and semi-drying oils.
Cis-Δ11,14-eicosadienoic	$C_{20}H_{36}O_2$		20:2 (n-6)	
Cis-Δ8,11-eicosadienoic	$C_{20}H_{36}O_2$		20:2 (n-9)	
Cis-Δ9,12,15-octadecatrienoic	$C_{18}H_{30}O_2$	Alpha-linolenic	18:3 (n-3)	EFA for humans. Major component in linseed, walnut, cedar nut, fig seed and pine seed oils. Is a colourless liquid, soluble in pet. ether, acetone, ethanol and ether. Major uses in paints and drying agents.
Cis-Δ6,9,12-octadecatrienoic	$C_{18}H_{30}O_2$	Gamma-linolenic	18:3 (n-6)	May be considered a "conditional EFA" at times. Found in appreciable amounts in evening primrose oil. Is a bright-yellow, pleasant-smelling liquid. Major use is in health products.
Cis-Δ8,11,14-eicosatrienoic	$C_{20}H_{34}O_2$	Dihomo-delta linolenic	20:3 (n-6)	Important intermediate in arachidonic acid production.
Cis-Δ5,8,11-eicosatrienoic	$C_{20}H_{34}O_2$		20:3 (n-9)	

TABLE 1.2 (cont.) Description and Sources of Important Unsaturated Fatty Acids

Systematic Name	Formula	Common Name	Shorthand Notation	Description, Sources
Cis-Δ6,9,12,15-octadecatetraenoic	$C_{18}H_{28}O_2$	Stearidonic	18:4 (n-3)	
Cis-Δ8,11,14,17-eicosatetraenoic	$C_{20}H_{32}O_2$		20:4 (n-3)	
Cis-Δ5,8,11,14-eicosatetraenoic	$C_{20}H_{32}O_2$	Arachidonic	20:4 (n-6)	May be considered a "conditional EFA".
Cis-Δ5,8,11,14,17-eicosapentaenoic	$C_{20}H_{30}O_2$	Eicosapentaenoic	20:5 (n-3)	May be considered a "conditional EFA".
Cis-Δ4,7,10,13,16,19-docosahexaenoic	$C_{22}H_{32}O_2$	Docosahexaenoic	22:6 (n-3)	May be considered a "conditional EFA".

Sources of information were: Gurr (1999), Ralston (1948), Wysong (1990) and Walton (1993).

1.2.4.2.3 Solubility and Melting Points

In general, water solubility of the fatty acids decreases with an increase in the number of carbon atoms. The boiling and melting points increase with each successive addition of a CH_2 group (as the packing becomes tighter), whereas in general, with an increasing number of *cis*-double bonds or kinks in the chain preventing tight packing, the melting point decreases (See Table1.3). The saturated fatty acids range from volatile liquids to waxy solids as the series is ascended and the intermediate members show all graduations between these two extremes (Ralston, 1948). Unsaturated fatty acids also show these graduations depending on their degree of unsaturation and chain length.

TABLE 1.3 Influence of Chain Length and Double Bonds on the Melting Points of Fatty Acids

Fatty acid	Chain length : double bonds			Melting point
Lauric acid	12	:	0	44°C
Myristic acid	14	:	0	54°C
Palmitic acid	16	:	0	63°C
Stearic acid	18	:	0	70°C
Oleic acid	18	:	1	13°-16 °C
Linoleic acid	18	:	2	-5°C
α-Linolenic acid	18	:	3	-16°C

(Table adapted from Gurr & Harwood, 1991).

1.2.4.3 Distribution and/or Occurrence of Fatty Acids

As previously mentioned, saturated and unsaturated fatty acids are widely distributed as esters in natural fats, oils, and waxes throughout the animal and plant kingdoms and primarily exist in the form of mixed triacylglycerides. The bulk of FA of TAG from seed oils and animal fats comprise C16 and C18 acids with a smaller proportion of fatty acids of shorter or longer chain lengths (Bhati, 1987).

Tables 1.1 and 1.2 and the discussion following describe some of the more interesting and important points of the distribution, occurrence and sources of fatty acids of interest to this research.

1.2.4.3.1 Saturated Fatty Acids

Palmitic acid is the most abundant and widely distributed of the saturated fatty acids (SFA). This is probably due to the fact that it is the primary product of fatty acid synthesis. Palmitic acid is a major component of many fats and waxes and found in practically every fatty substance investigated. Stearic acid, though less abundant in nature than palmitic, is also an important constituent of all animal fats, as well as many vegetable fats and waxes.

Saturated acids containing more than eighteen carbon atoms are usually present only as minor components in animal and vegetable fats; however, such fatty acids frequently constitute major components of plant and insect waxes. SFA containing less than sixteen carbon atoms are frequently found in vegetable oils but with the exception of milk fats, do not form major components of animal fats.

An interesting observation, which specifically applies to SFA, is that when one member of the series is present in large amounts in a fat, the next lower and the next higher homolog are also likely to be found. It is also generally accepted that most of the naturally occurring SFA contain a normal, unbranched chain of carbon atoms with an even number of carbon atoms (Ralston, 1948; Wan, 1991).

1.2.4.3.2 Mono- and Polyunsaturated Fatty Acids

Fatty acids with one or more double bonds within their hydrocarbon chain are known as ethylenic-, unsaturated-, polyenoic- or polyunsaturated fatty acids (PUFA). Collectively, they constitute a major percentage of the FA of vegetable oils and also occur in large amounts in animal fats and marine oils. Animal fats however, do not generally contain large amounts of fatty acids with more than one double bond. Large sources of PUFA with two or more double bonds are essentially confined to the vegetable and marine oils.

Marine and fish oils are characterized by large percentages of unsaturated fatty acids, substantial quantities of which contain twenty or more carbon atoms, frequently containing three or more ethylenic bonds. Oleic acid (a monoenoic fatty acid) is the most widespread and abundant unsaturated fatty acid of animals and plants. Dienoic fatty acids characterize the semi-drying and drying oils. Although they are present in marine and animal oils, they appear to be distinctively associated with oils of vegetable origin. Trienoic fatty acids (such as α-linolenic acid in linseed oil) are characteristic of the vegetable drying oils to which they impart the property of forming glossy, tough films upon exposure to the atmosphere which are useful in paint, varnishing and metal-protective industries. Oils with four or more double bonds occur to a limited extent in certain animal lipids (liver & brain), and are found in variable quantities in vegetable oils but are more likely found in aquatic sources.

1.2.4.3.3 Dietary sources of Essential and Conditionally Essential Fatty Acids

Edible sources of nutritionally important fatty acids such as those mentioned below are desirable and constitute an important reason for the search for potential new sources. If any of these FA are found in the seed oils being analysed, the potential then exists for further investigation and possible nutritional or commercial use. For example the oils may prove particularly worthwhile as a nutritional supplement of essential fatty acids, particularly for those with low enzyme production of, or little or no 6-desaturase activity (see Section 1.2.5).

Major dietary sources of linoleic acid (LA), a primary essential fatty acid (EFA), are seed oils such as sunflower, corn and soybean. These oils have an important role in the food industry as they are incorporated into margarine and cooking oils and therefore into a huge range of food products. The other major essential fatty acid, α-linolenic acid (ALA), is synthesized only in higher plants, algae and phytoplankton (particularly in chloroplasts). However the main dietary sources are certain seed oils (e.g rapeseed and soybean oil).

Deficiency of ALA and particularly LA is very uncommon nowadays due to their widespread occurrence in nature and incorporation into many food products. Only about 1% of the total dietary energy needs to be provided by LA and 0.2% by ALA to avoid deficiency (Gurr, 1991). Because of the enormous mass of green plants both on land and in the oceans, and since ALA contributes to over half the FA of the lipids of chloroplast membranes, it is probably the predominant fatty acid on the planet (Gurr, 1991).

In certain animals such as cats and on specific occasions such as when individuals lack sufficient levels of key enzymes involved in polyunsaturated fatty acid (PUFA) production or metabolism, certain PUFA may also be regarded as essential fatty acids or "conditionally essential fatty acids" (See Section 1.2.5). These include γ-linolenic acid (GLA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dietary sources of GLA are mainly from seed oils such as evening primrose, blackcurrant or borage oils. Significant quantities are also found in human milk.

The major dietary source of AA is from the membranous tissues of animal products, such as eggs, meats or fish oils for those diets high in fish. In the human diet, the major sources of EPA and DHA are through the oils of fatty fish, which contain much greater quantities of the long chain n-3 fatty acids (Gurr, 1991). Optimum intakes of total PUFA, in order to maintain appropriate blood lipid levels are deemed to be around 6-6.5% of the total dietary energy, with an individual maximal intake of 10% (Gurr, 1991).

1.2.5 Special Groups of Fatty Acids in Health, Nutrition and Disease

1.2.5.1 Essential, and Conditionally Essential Dietary Fatty Acids.

As well as the daily requirements for energy, many animals including man, require certain lipids and specific fatty acids to provide optimal health and nutrition and prevent deficiencies such as the development of skin lesions, scaliness, poor hair growth, and low growth rates. Lipids, FA or other nutrients that cannot be synthesized from other compounds in an organism, but are absolutely needed for life, are termed essential nutrients and must be supplied in the diet or via another organism that can supply it. Most FA with varying degrees of unsaturation and chain lengths are able to be synthesized in animal tissues by various elongation and desaturation reactions. For example mammalian tissues are able to convert saturated fatty acids into monounsaturated fatty acids through the action of a desaturase that produces a *cis*-Δ9-monoene -such as in the conversion of stearic acid, 18:0 into oleic acid, 18:1, *cis*-Δ9 (Stryer, 1988; Linder, 1991; Gurr, 1999).

Further desaturations are possible on the carboxyl side of the 9-double bond, (i.e. on carbons 4-8) but the ability to desaturate on the methyl side of the 9-double bond has been "lost" by animals including man. Polyunsaturated fatty acids with double bonds at positions 12 and 15 perform many vital functions in biological membranes such as contributing to 'membrane fluidity' and acting as precursors of long-chain polyunsaturated fatty acids (LCPUFA) such as arachidonic acid. Generally, only plants are able to insert double bonds at these positions. Thus for animals, FA containing double bonds at positions 12 & 15 must be supplied in the diet (Stryer, 1988; Linder, 1991; Gurr, 1999).

Two important fatty acids containing double bonds at positions 12 and/or 15 are provided in the form of linoleic acid (LA), (n-6)18:2, Δ9,12 and α-linolenic acid (ALA), (n-3)18:3, Δ9,12,15. These are the primary dietary essential fatty acids. They have a wide distribution throughout the vegetable kingdom, especially in seed oils and are the parent acids of the n-6 and n-3 families of important LCPUFA (See Figure 1.3 for the major n-3 and n-6 FA biosynthetic pathways).

Some polyunsaturated fatty acids or PUFA, although not dietary "essential fatty acids" in the sense that they cannot be synthesized by the organism, are however essential nutrients to normal life and are sometimes referred to as "conditionally essential fatty acids". Apart from roles in membrane fluidity, PUFA are important precursors for further elongation and desaturation products. PUFA also influence the metabolism of lipoproteins that carry lipids in the blood, thereby regulating the levels and types of blood lipoproteins. Recently roles for PUFA in the regulation of gene transcription have also been recognized (Gurr, 1999).

Inclusion of these conditionally essential fatty acids in the diet is particularly relevant in those who lack or have very low rates of activity of key enzymes involved in PUFA production or metabolism. For this reason, γ-linolenic acid and arachidonic acid may at times be termed conditionally essential fatty acids. Differences in levels and activity of a particular 6-desaturase enzyme occur between animal species. This enzyme converts linoleic acid to γ-linolenic acid (n-6)-18:3, **all** *cis*-Δ6,9,12 in the n-6 pathway. Cats for example lack the 6-desaturase and cannot synthesize γ-linolenic acid or arachidonic acid, (n-6)-20:4, **all** *cis*-Δ5,8,11,14. This 6-desaturase often has low activity and possibly low levels in human tissues and is probably the rate-limiting step in the whole sequence from linoleic to arachidonic acid. Arachidonic acid (AA) is the main product of the n-6 pathway, is a precursor for the n-6 eicosanoids and is the principal n-6 PUFA in most mammalian cell membranes. The importance of AA can be deduced from the fact that membrane concentrations of AA are maintained in the face of relatively severe dietary linoleic restriction and are depleted only after a long period of linoleic deficiency (Gurr, 1999).

Long-chain PUFA products of α-linolenic acid, (the 'omega-3', ω-3 or n-3 FA) may also be referred to as conditionally essential fatty acids. Of particular importance are eicosapentaenoic acid, (n-3)-20:5, **all** *cis*-Δ5,8,11,14,15,17 and docosahexaenoic acid, (n-3)-22:6, **all** *cis*-Δ4,7,10,13,16,19. These n-3 FA are especially abundant in brain phospholipids and are essential for the development and function of the brain and retina, as well as sperm. They also provide the precursors for the n-3 family of eicosanoids (Gurr, 1999).

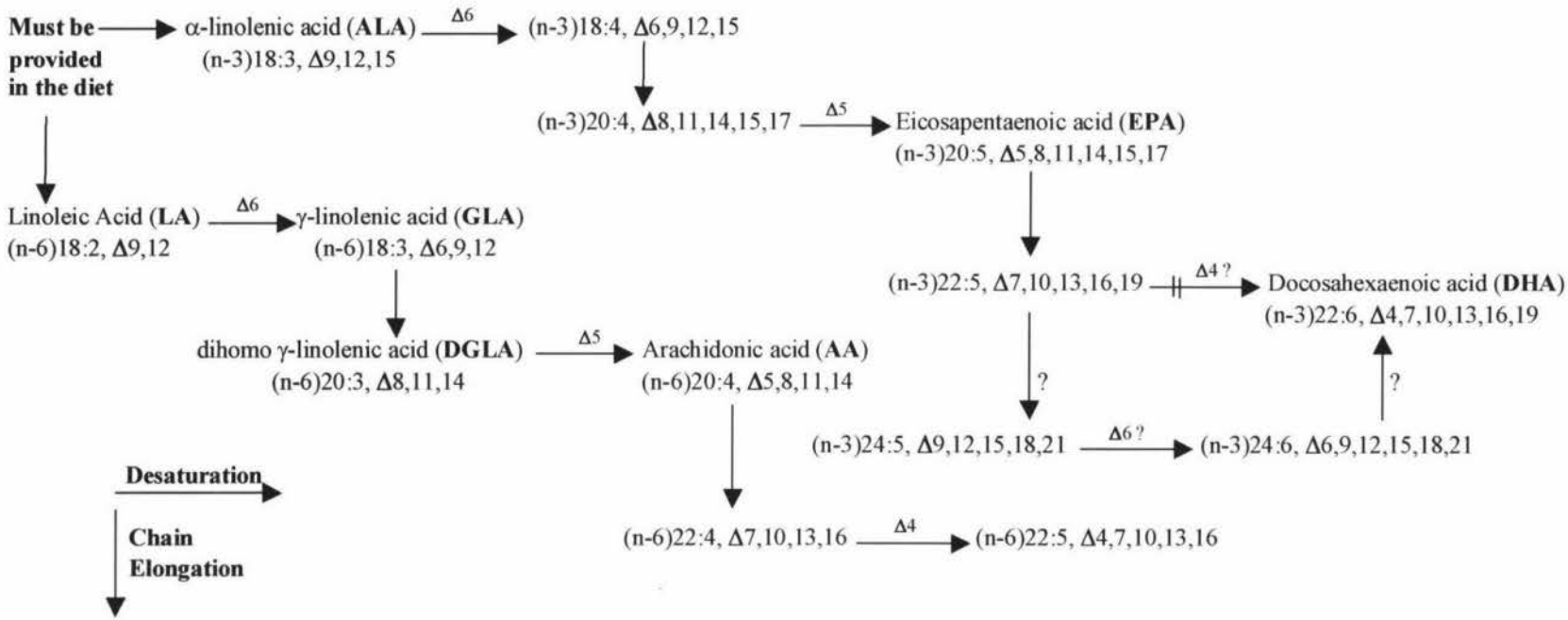


FIGURE 1.3 Major Biosynthetic Pathways of the (n-6) and (n-3) Essential Fatty Acids in Animal Tissues.

Note: **?** signifies hypothesized pathways of conversion from EPA to DHA (Adapted from Cook, 1985).

Linoleic acid is the parent of the n-6 family and must be provided in the diet of animals. The main importance of the n-6 pathway is to provide a pool of membrane DGLA and AA and sufficient precursors to continually maintain a supply of long-chain polyunsaturated fatty acids (LCPUFA) such as the n-6 family of eicosanoids, required in metabolic regulation. α-Linolenic acid (ALA) must also be provided in the diet and is the parent of the n-3 family of LCPUFA, which includes EPA, DHA and the n-3 family of eicosanoids.

1.2.5.2 Eicosanoids

Eicosanoids are so-named because they are derived from C_{20} polyunsaturated fatty acids such as arachidonic acid (AA) and eicosapentaenoic acid (EPA), which represent the main precursors of the n-6 and n-3 family of eicosanoids respectively. Specific prostaglandins, leukotrienes, and thromboxanes are produced from these two families. They are hormone-like substances secreted for short-range action upon neighbouring tissues (Linder, 1991; Gurr, 1999).

The specific prostaglandins, thromboxanes and leukotrienes produced depends on the starting material which is mostly AA, but can also be EPA or (indirectly) docosahexaenoic acid (DHA). The prostanoid products of AA and EPA/DHA have different and sometimes antagonistic effects. Some functions are unique to either the n-3 or n-6 eicosanoids so that one family cannot always substitute for the other and the members of the n-3 and n-6 families cannot be interconverted in the human body (Linder, 1991; Gurr, 1999). These two families of polyunsaturates compete for common enzymes involved in their metabolism and thus the n-3/n-6 ratio in the diet is crucial to their relative composition in tissues and ability to perform their allotted functions. This ratio has also been linked to the pathophysiology of some diseases (See Section 1.2.5.3).

Prostaglandins are produced in all parts of the body and have been implicated in activities from the induction of labour, to inflammation, blood pressure maintenance, and headaches. Leukotrienes and thromboxanes are alternative products involved in platelet aggregation and the inflammatory response. Leukotrienes, especially those derived from AA, are chemoattractants for phagocytes and polymorphonuclear leukocytes in inflammation and during plaque formation in arteries (Linder, 1991; Gurr, 1999). Figure 1.4 shows some of the metabolic effects of these compounds.

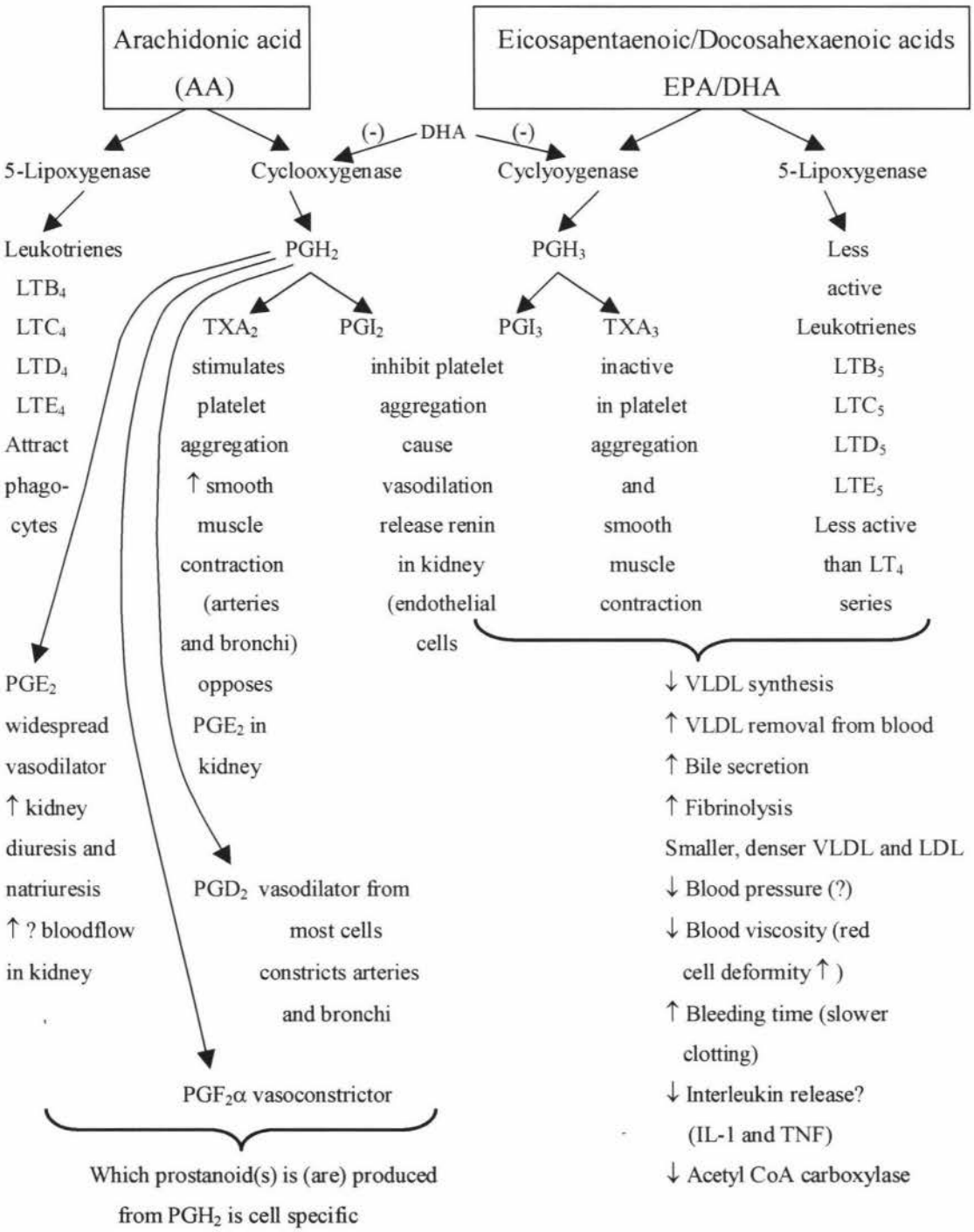


FIGURE 1.4 Metabolic Effects of Eicosanoids

PG(x) = groups of prostaglandins, LT(x)=groups of leukotrienes, TXA= Thromboxanes, IL-1 = Interleukin 1, TNF = Tumour Necrosis Factor, VLDL = very low density lipoproteins, LDL = low density lipoproteins, ↑ = increases/stimulates, ↓ = decreases, (-) = inhibits reaction (Adapted from Linder, 1991).

1.2.5.3 Dietary Fatty Acids and Disease Implications

Much research and emphasis has gone into defining the relative importance of lipids as a whole and lipids as individual compounds with respect to health and disease. Indeed, some scientific advice regarding certain facets of lipid nutrition are over-looked, whereas other advice is over-emphasized, such as in commercial interests in order to market food products as providing health benefits. There is currently much emphasis in public health policy on health promotion and prevention of illness. Prevention of chronic diseases such as diseases of the heart and vascular systems, cancer, diabetes and obesity is of primary concern. Smoking, drinking, physical activity and nutritional factors are among those considered of major importance in terms of modifiable behaviours for disease prevention. Within nutritional aspects, much focus is placed on the modification of dietary fat intake.

Most of what we term 'dietary fat' consists of triacylglycerols, and therefore esterified fatty acids. Triacylglycerols (TAG) account for 95-98% of the fat ingested in all forms of food. Other dietary lipids include esters such as phosphoglycerides, galactosyldiacylglycerols, cholesteryl and retinyl esters and other non-saponifiable compounds such as cholesterol, plant sterols, cholecalciferol, carotenoids, tocols and many other minor lipid soluble compounds (Gurr, 1999).

The types of lipids found in an individual animal vary according to the animal species and the composition of fats in the food it consumes. Fats used by or stored in animal tissues come from two sources – diet and enzymatic synthesis. The lipids synthesized from carbohydrates or proteins are characteristic of the animal species, whereas those synthesized from dietary fats are characteristic of the food ingested (Wysong, 1990). Therefore, the composition and level of dietary fats can contribute to various health and disease states, by determining the composition of the fat that accumulates in the organs and tissues of the body. A diet high in unsaturated fats will tend to result in the accumulation and deposition of more unsaturated TAG and vice versa.

1.2.5.3.1 The Influence of Dietary Fatty Acids on Blood Lipid, Lipoprotein Concentrations

In healthy animals, levels of lipids in the blood such as cholesterol are controlled within a range and are generally synthesized only as required by the animal's needs and availability from the diet. Problems occur due to the inability of the animal to keep within these levels. This may be due to many things including ineffective control or receptor systems, overloading of cholesterol levels from overproduction endogenously, ineffective removal and/or high dietary cholesterol intakes. Sustained high blood lipid levels such as in hypercholesterolemia are a "risk factor" linked to atherosclerosis (Watkins et al., 1996). A raised LDL-cholesterol (high cholesterol & lipid/protein ratio) is regarded as a major risk factor for coronary heart diease, whereas a raised HDL-cholesterol (low lipid/protein ratio) is thought to protect against the disease (Watkins *et al.*, 1996). Sustained high blood lipid levels leads to the accumulation of fatty deposits around the heart and within blood vessels. This leads to the narrowing of blood vessels and decreased efficiency of blood flow and oxygen throughout the body.

The nature of the dietary fat (i.e. distribution of FA on the three positions of the glycerol backbone of TAG or degree of unsaturation and chain length of the FA) and dietary cholesterol itself, influences the concentration of lipids in the blood like cholesterol and cholesterol-carrying lipoproteins. In general, saturated fatty acids (SFA) tend to raise, and polyunsaturated fatty acids tend to lower the concentration of blood cholesterol. Variations even occur within fatty acid groups. SFA with chain lengths C12, C14 and C16 seem to be the more effective in raising plasma total cholesterol and LDL-cholesterol compared to SFA with other lengths (Watkins *et al.*, 1996). Lower-chain length SFA (2-10 carbon atoms) do not have a great effect on plasma cholesterol as they are absorbed directly and quickly metabolised unlike the longer-chain acids which are absorbed as 'chylomicrons'.

As with SFA, PUFA also have variable effects. Intake of linoleic acid (LA) in the dietary mix seems to reduce the extent to which SFA raise cholesterol and is proposed to maintain a low concentration of cholesterol in the blood, mainly in the LDL fraction.

As a result, increased LA dietary intakes have been recommended for lowering blood cholesterol as a preventative measure against coronary heart disease. PUFA of the n-6 family lowers LDL-cholesterol, while the n-3 family tend to lower TAG-rich lipoproteins (VLDL) levels (Watkins *et al.*, 1996).

1.2.5.3.2 Influence of Dietary Fatty Acids on Eicosanoid Production.

Some of the differing effects of the n-6 and n-3 long-chain polyunsaturates used in certain disease treatments have much to do with their relative eicosanoid productions. Observation of the Inuit (Eskimo) diet which is high in fish oils rich in EPA and DHA, found that while on their traditional high fat and cholesterol diet, they did not generally suffer from atherosclerosis (Bang & Dyerberg, 1973, 1980). The hypothesis explaining this observation is based on the preferential formation of prostaglandins, thromboxanes and leukotrienes from the n-3 rather than the n-6 family due to the substrate competition of the cyclooxygenase and lipoxygenase enzymes involved in the metabolism of these families. In general, n-3 eicosanoids have weaker activities in such processes as platelet aggregation, blood clotting and blood vessel constriction, thus reducing the risk of thrombosis. In an increased n-3/n-6 PUFA ratio, the strongly pro-inflammatory eicosanoids from the n-6 family would be reduced and replaced by the weakly inflammatory n-3 eicosanoids, which provides potential value in the treatment of rheumatoid arthritis (Kremer *et al.*, 1985).

1.2.5.3.3 Dietary Polyunsaturated Fatty Acids and Lipid Peroxidation.

Lipid peroxidation is initiated when a hydrogen atom is removed from a lipid molecule to generate a lipid radical. Further interaction with oxygen generates peroxy and hydroperoxy radicals that are capable of further radical formation and propagation until termination occurs for example, by interaction with an antioxidant. When lipid peroxidation occurs in food lipids, the results are rancidity, deterioration in product quality, a shorter shelf life and lower nutritional benefits. When it occurs in an uncontrolled manner amongst cell membrane constituents, or in the conversion of long-chain polyunsaturates into eicosanoid products, much damage can occur.

Many chronic diseases including athersclerosis, thrombosis, cancer and respiratory diseases have been linked with such damage (Stryer, 1988; Linder, 1991; Gurr, 1999).

As mentioned earlier, certain unsaturated fatty acids are essential nutrients. Dietary guidelines recommend a reduced intake of saturated fatty acids with an increased intake of unsaturated fatty acids. However, owing to the ease with which poly- and unsaturated fatty acids undergo free-radical formation, much debate continues regarding appropriate dietary guidelines and recommendations for safe levels in the diet (Stryer, 1988; Linder, 1991; Gurr, 1999).

1.2.6 Commercial Applications of Fatty Acids

Vegetable and animal oils or fats have a vast array of commercial applications in industries supplying edible and non-edible products. Some of these include: being components of edible foods (such as in spreads and cooking oils or in the feed stock industry); providing drying and semi-drying agents in paints and protective coatings; and being components of detergents, soaps, sunscreen semulants, lotions and lubricants (Luhs & Friedt, 1994a). Others have potential uses in pharmacological and cosmetic industries (Luhs & Freidt, 1994a). Some of these applications will be discussed in this Section. As this research is based on the FA found in plant lipids (particularly seed lipids), discussion will mainly concentrate on features of vegetable and seed oils, as a whole and those FA, fats and oils important in industry.

1.2.6.1 Features of Plant Lipids

The bulk of fatty acids in plant oils, particularly seed oils comprise of fatty acids with carbon chain lengths of C16 and C18. The principal unsaturated acids of vegetable origin contain eighteen carbons and a structural similarity to oleic acid (double bond between carbon 9 and 10). However in the polyenoic group, C20 and C22 acids appear to predominate (Luhs & Freidt, 1994a). Another interesting fact is the tendency of certain plant species to synthesize specific unsaturated fatty acids to the extent that the presence of these acids characterises oils from these sources. For example, linseed oil contains a large percentage of linolenic and linoleic; erucic acid characterises rapeseed oil and eleostearic acid distinguishes tung oil.

While the relative proportions of the various acids in the oils obtained from certain plants may differ according to the conditions of growth, climate or other factors (Chu & Sheldon, 1979), the ability to synthesize these characteristic acids is apparently an inherent property of the plant itself. This property has commercial potential, particularly if these fatty acids are commercially important or novel.

Seeds from botanically related species often produce similar oils (Gunstone, 1967), thus a related plant species growing in another country may provide a source of important fatty acids for that country. The major oilcrops of world importance include the *Brassicaceae, Compositae, Malvaceae, Palmae* and *Papilionaceae* families. Thus native New Zealand species belonging to these families may be of particular worth for investigation.

1.2.6.2 Oilseeds

Oilseeds are a class of plant in which relatively large amounts of lipid are stored in the seed tissues. They are generally the most commercially important oil plants and include species such as soybean, sunflower, safflower, rapeseed, canola, palm, sesame, linseed and cottonseed. The amount of lipid stored can range from 10 to 20% in species such as maize and soybean to 50% in sunflower and rapeseed. Oilseed products are utilised for a variety of edible and non-edible applications such as those discussed later in this section. For example, the ancient Persians used sesame oil in cooking, as body massage oil, for illumination, in cosmetics and as a lubricant in simple machines (Murphy, 1999). Oils from safflower varieties may be used as a heat-transfer medium in cooking oils, in production of semi-solid margarines, in a variety of processed foods or as a drying oil in paints. At present, most oilseed crops are utilized for edible purposes. Their seed storage lipids, which are normally made up of TAG, are the basis of most cooking oils and margarines and are components of about half of all processed food products sold in supermarkets.

Unlike membrane lipids, storage lipids such as those found in oilseeds, often have huge diversity in their fatty acid compositions –a property valuable for a wide range of possible commercial applications. Oilseed storage lipid compositions are also able to be manipulated using techniques such as genetic engineering, without obvious detriment to the plant or oil yield –providing the opportunity for a specific end-use or niche in the market (Hammond, 1991).

1.2.6.3 Commercially Important Sources of Fats and Oils

Edible vegetable fats and oils important in world commerce include soybean, sunflower, safflower, rapeseed, canola, palm, palm kernel, olive, sesame, peanut, cottonseed, corn, rice bran, carob, coconut and cocoa butter. Important edible animal fats and oils include fish oils such as herring, sardine and meneheden, whale oil and animal fats such as lard, tallow and milkfat.

Important fats and oils used for non-edible industrial purposes include castor bean, crambe, flax, linseed, rapeseed, jojoba seed, tall and tung nut (Hammond, 1991; Chen, 1991). Many of the fats and oils from the sources listed above are used in both edible and non-edible industries and are in production for a variety of reasons such as those discussed below and listed in Table 1.4.

1.2.6.4 Fatty Acid Properties-their Commercial Potential and Uses in Industry

Natural fats and oils have a wide range of properties that are useful in industry. Some are valued for their properties in edible products such as melting behaviour, flavour, flavour stability and nutritional advantage. Some serve conveniently as a heating medium and are frequently used to cook and preserve food. Their reversible solid-to-liquid phase transition properties allow them to function as pastry fat, frying shortenings and in confectionery applications. Their ability to dissolve colour and flavour or lubrication plays an important role in making food palatable and desirable (Wan, 1991b).

Properties valuable in non-edible uses include the ability of some lipids to dry quickly while forming a tough flexible film (paint and protective coatings). Some are important functional components of lubricating greases in metal works. Their foaming properties are useful in detergent and surfactant fields. Others function as softeners, plasticizers or emulsifiers (soap, grease and rubber manufacture) (Zilch, 1991).

The desirable properties of lipids in industry stem from the composition of FA and TAG contained in the fat or oil and their physical or chemical properties (Browse *et al.*, 1998). For example, olive oil is valued for its flavour stability (due to its relatively small amount of PUFA prone to oxidation), its fluidity (due to its high percentage of oleic acid), and flavour stability, as it is not subject to off-flavours produced on hydrolysis of short- and medium-chain (6-14 carbons) fatty acids (Hammond, 1991).

Structural diversity of triacylglyceride molecules due to variable positions of fatty acids on the triacylglyceride also contribute important properties such as melting points of the fat or oil. Cocoa butter for example is valued for its sharp melting point near human body temperature. This melting behaviour is due to its fatty composition as well as its triacylglyceride structures (Hammond, 1991).

1.2.6.4.1 Edible Fats and Oils.

The most important edible fats and oils are those containing palmitic, stearic, oleic, linoleic acid and to a smaller extent, α-linolenic acid (Harwood, 1998b; Murphy, 1999). Of these five FA, oleic and linoleic are the most valuable. Palmitic and stearic acids are saturated lipids and are important components in margarines and chocolate formation. However, publicity concerning possible adverse nutritional consequences of diets high in saturated fatty acids mentioned in Section 1.2.5 has reduced the appeal of oils high in such FA. Oleic acid is relatively stable at high temperatures, and thus oils high in oleic acid are suitable for cooking and other processes where heating is required. Linoleic acid (LA) is the major "polyunsaturated" fatty acid in foods and since LA is an essential nutrient for humans, it is a desirable component for many food applications. However, LA has limited stability when heated (Harwood, 1998b; Murphy, 1999).

Oils high in polyunsaturated fatty acids also have limited shelf life and are prone to undesirable odours and flavours and a decrease in nutritional value when subjected to oxidation. Oil-plants containing high levels of oleic and linoleic acids include rapeseed, sunflower, soybean and olive oils.

1.2.6.4.2 Non-Edible Fats and Oils

Almost any type of FA is of potential use for non-edible oil products. Very-short chain fatty acids can be used as fuels, or as detergents. Highly PUFA's are used as drying agents such as in paints and varnishes. Hydroxylated FA can be used for cosmetics and plastics manufacture. Very long chain FA are often used as lubricating oils. High-erucic rapeseed oil, although not valuable as an edible oil (due to its proposed toxicity), has special uses in lubricants.

High-erucic oils such as this may also be useful in other ways as erucic acid can be oxidized at its double bond to produce the dicarboxylic brassylic acid, which can be made into a type of nylon that is particularly tough and suitable for production of mechanical gears (Sonntag, 1995). Plant oils, a renewable resource, may eventually come to replace many of the mineral oils, which are presently extracted from non-renewable fossil reserves. These fossil reserves will eventually be depleted, perhaps even during this century (Murphy, 1999).

The fatty acids of vegetable and seed oils vary greatly in number and structure but those of quantitative importance generally have an even number of carbon atoms and are between 12 to 24 carbon atoms in length. Table 1.4 describes the major reasons for and against the use of major fatty acids in industry, and their major commercial animal or vegetable sources.

TABLE 1.4 Major Fatty Acids and Their Industrial Uses.

Fatty Acid	Major Commercial Sources	Reasons for and Major Uses in Industry		Reasons Against Use in Industry
		Edible	Non-Edible	
4:0-6:0	Milk fat, coconut oil	Easily metabolized, edible.	Greater volatility-fuels?	
8:0-10:0	Coconut, palm kernel oils, milk fats	Easily metabolized, edible. ↑Fluidity, oxidative and flavour stability in free form.	Fuels. Foaming, surfactant, detergent properties	Form off-flavours on hydrolysis
Lauric (12:0)	Coconut, palm kernel oils	↑Fluidity, oxidative and flavour stability.	Surfactant, detergent properties. Soaps, Cosmetics.	Form off-flavours on hydrolysis, may raise blood lipids.
Myristic (14:0)	Coconut, palm kernel oils, butter	↑Oxidative and flavour stability.	Detergents	May raise blood lipids.
Palmitic (16:0)	Animal fats, cocoa butter, palm, cottonseed oils	Relatively stable at high temps. Included in a large range of produced foods i.e. Cooking fats, spreads, chocolate, confectionery		May raise blood lipids. Nutritional advice to decrease SFA consumption
Palmitoleic (16:1)	Cotton seed, tall oils, milkfats, beef tallow	↑Fluidity? Reasonable oxidative and flavour stability. Nutritive-health value		
Stearic (18:0)	Animal fats, cocoa butter, high-18:0 soybean, oilseed oils	High temp. stability, heat-transfer medium. (Deep frying, high temp. food process.) Included in a large range of produced foods i.e. cooking fats, spreads, chocolate, confectionery production	Soap production	May raise blood lipids. Nutritional advice to decrease SFA consumption

TABLE 1.4cont. Major Fatty Acids and Their Industrial Uses.

Fatty Acid	Major Commercial Sources	Reasons for and Major Uses in Industry		Reasons Against Use in Industry
		Edible	Non-Edible	
Oleic (18:1n-9)	Animal fats, high-18:1 safflower/sunflower, low-erucic rapeseed, low-18:3 soybean, olive, palm, peanut, sesame, tall oils, cocoa butter	High temp. stability, heat-transfer medium. (Deep frying, high temp. food process.) ↑Fluidity. Reasonable oxidative and flavour stability? Included in a large range of produced foods i.e. cooking oils, spreads, chocolate, confect. Nutritive-health value.		
Ricinoleic (12-hydroxy, 18:1n-9)	Castor bean oil	Laxative	Drying oil, lubricant, surface protection, plastics, cosmetics, pharmaceuticals	
Linoleic (18:2n-6)	Soybean, linseed, tall, safflower, sunflower, cottonseed, peanut, corn, sesame	Essential Fatty Acid for humans, animal diets	Semi/drying agent in paints, varnishes and other protective surfaces and coatings.	Relatively high oxidative instability. Production of off-flavours and low shelf-life in foods.
α-Linolenic (18:3n3)	Linseed oil, borage, blackcurrant seed oil.	Essential Fatty Acid for humans, animal diets	Drying agent in paints, varnishes and other protective surfaces and coatings.	High oxidative instability. Production of off-flavours and low shelf-life in foods.

TABLE 1.4 cont. Major Fatty Acids and Their Industrial Uses.

Fatty Acid	Major Commercial Sources	Reasons for and Major Uses in Industry		Reasons Against Use in Industry
		Edible	Non-Edible	
Eleostearic (18:3n-5)	Tung oil.		Drying agent in paints, varnishes, lacquers and other protective surfaces and coatings.	
Arachidic (20:0)	Peanut, herring oil.	Nutritive-health value. Important precursor of eicosanoid products.		
Gadoleic (20:1n-11)	Rapeseed, crambe, meneheden fish oil.		Lubricating oils, greases.	
Arachidonic (20:4n-6)	Oils of "fatty" fish i.e. herring, meneheden.	Nutritive-health value. Eicosanoid, membrane lipid precursor.		High oxidative instability.
Eicosapentaenoic (EPA, 20:5n-3)	Oils of "fatty" fish i.e. herring, meneheden.	Nutritive-health value. Eicosanoid precursor.		High oxidative instability.
Erucic (22:1n-9)	High-erucic rapeseed, crambe, fish oils.		Lubricants, nylon, plastic production. Surface protection, solvent softeners for textiles.	Possible toxic effects in high amounts.
Docosahexaenoic (DHA, 22:6n-3)	Oils of "fatty" fish i.e. herring, meneheden.	Nutritive-health value. Eicosanoid precursor.		High oxidative instability.

Note: ? = Suggested use or potential property

Information obtained from Browse *et al.*, 1998; Chen, 1991; Hammond, 1991; Harwood, 1998b; Murphy, 1999; Sonntag, 1995; Wan, 1991b; Zilch, 1991.

1.2.6.5 Determination of Marketability

Many factors contribute to whether a potential new species of an oil-producing plant will be commercially viable. Some of these, such as the properties of the fatty acids the oils contain, have already been discussed. Apart from the potential uses of the oil itself, a new oil-producing crop must have enough value to bear the costs of encouraging its production and be able to hold its place on the market and in trade channels. Unless destined for a smaller speciality market, the introduction of a new species can be quite difficult because oil production requires considerable scale to be efficient. Large areas of land would need to be devoted to crop growth, markets would need to be developed, machinery may need to be adapted for harvesting and years of development may be required for it to have a hope of competing with the already established crops. However, manipulation of the fatty acid composition of vegetable oils has in recent years provided the means for the introduction of new or improved species.

A homogenous or specific FA composition in oils is desirable in industrial processes. Techniques such as plant breeding, induced mutation and genetic engineering are being used to manipulate the seed oil quality and composition. The objective has been to produce a whole new series of new seed varieties, each of which will contain a different but relatively homogenous oil composition targeted towards a specific end use (Murphy, 1999). Soybean, oil palm, canola (low-erucic rapeseed), sunflower and safflower are examples of vegetable oils in which the fatty acid composition has been modified for a specific end use.

Efficiency, yield of the oil production and ease in responding to changing market trends are important factors when considering commercial viability. For example palm oil is produced with great efficiency in yield per acre compared with other oilseed crops. However palm oil is a product of a tree which take several years to grow and will keep producing for a number of years regardless of the change in market for the oil. Growers of soybeans and rape can respond more readily to short-term trends and market predictions, as they are annual plants.

The possibility of commercial viability would also increase if the plant source had other potential uses apart from in the oil-trade. Several fats and oils are by-products of industries other than the oil-trade, for example tall oil is a by-product of paper manufacture. Soybeans, besides oil, produce a valuable by-product in the form of a high-protein meal, important for the animal feed industry. However, the amount of oil produced as a by-product of another process may be restricted by the demand or availability for the main product. For example, fish oil production depends on the size of the fish catch and the resources devoted to fishing, while the market for cotton limits cottonseed oil production.

1.2.7 Discussion on New Zealand Native Flora

From the similarity and identity of some plants from the flora of New Zealand with those of the southern part of Chile, together with paleobotanical evidence from Antartica, it would appear that in past geological times New Zealand was connected through Antartica to South America and possibly Australia. However, due to the long period of separation from other land masses, evolutionary processes and the variety of geographical features of the New Zealand landscape, a unique flora and fauna has been produced (Fleming, 1978; Cambie 1986).

In 1961, a reasonable estimate for the number of indigenous New Zealand vascular flora was close to 2000 species (Alan, 1961). Thirty to forty years later, this estimate is still likely to be about right (Cambie, 1986). It consists of approximately 166 ferns and fern allies, 20 gymnosperms, 1304 dicotyledons and 500 monocotyledons -including 171 grasses. As well as vascular plants, the indigenous flora contains approximately 800 marine macroalgae, 750 marine microalgae, 15 freshwater *Characeae*, 2076 freshwater microalgae, 2500 fungi, 500 liverworts, 15 hornworts, 525 mosses, and 1500 species of lichens (Alan, 1961; Cambie, 1986; Moore & Edgar, 1970).

Although the number of native plants in New Zealand is small compared with that of our neighbouring Pacific countries such as China and Indonesia, our plant life has unusual features, which are significant in assessing any potential utilisation or economic value. (Cambie,1986). According to geological evidence, the land mass of New Zealand has existed for more than 100 million years, and compared to the forests on other land masses of the world, the New Zealand forests are most like the Mesozoic forests of Gondwanaland (Fleming, 1978). Early ancestors of our living kauri, podocarp and beech forest plants found in fossil remains, possibly date back to ancient forests some 100-250 million years ago (Fleming, 1978).

Before the arrival of the European settlers approximately 80% of this land was covered with virgin bush. Today, most of this has been destroyed and replaced by farmland (Cambie, 1986). A high degree of endemism still exists however. Among the remaining native flora from algae to flowering plants, at least 2500 are not to be found elsewhere, (Martin, 1961), about 80% of New Zealand's species of higher plants and 85% of seed plants are endemic (Cambie, 1986; Godley 1976).

1.2.7.1 Uses of Native New Zealand Plants

This high degree of endemism not only gives reason to treasure and conserve our native flora but it provides many opportunities for unique research. Despite the distinctive nature of our native flora, or because of it, very few native plants have been exploited commercially (Haase, 1990). In many cases, quicker growing, more economic introduced plants have been substituted for their native equivalent. For example, potato has substituted for the native fern root (arūhe) and the kumara brought to NZ by the early Maori. The radiata pine has substituted native conifers for timber requirements (Brooker *et al.*, 1998).

Although the uses of NZ vascular plants have been investigated more extensively than the lower plants, a diverse range of constituents has been revealed. These include (among others), alkaloids, toxic principles, essential oils, colouring matters, dyestuffs, tannins and steroids. More detailed descriptions of constituents isolated from the New Zealand native flora can be found in the New Zealand Phytochemical Registers listed by Brooker *et al.*, 1963, 1966 and Cambie 1976, 1988 and 1996.

Other recent works that have studied and reviewed the constituents and/or uses of a large number of native plant species include Cooper & Cambie 1991; Buisson 1979; Brooker *et al.*, 1998; Brooker, 1986 and Crowe, 1997a. Various researchers have provided information on specific groups of constituents in native plants and include studies such as Cain *et al.*, 1961, 1962; Cambie *et al.*, 1961 (alkaloids, saponins, leukoanthocyanidins); Bailey & Pain, 1971 (monosaccharides); Russell & Fenemore 1970 (ecdysones); Calder *et al.*, 1986; Bloor 1993, 1995; Kellam *et al.*, 1992; Singh *et al.*, 1978 (anti -bacterial, -fungal, -viral and other biological activities); Breitweiser & Ward, 1993 (flavonoids).

The phytochemical registers and works mentioned above provide a more detailed list of other current research works on native plant constituents and their researchers. The following is a brief summary of some of the more economic uses and potential uses (past and present) of New Zealand's native plants. Emphasis is placed on those species analysed in this research work.

In most cases, it was the wood, bark, gum or leaves of the plant, rather than the fruit or seed that were used for healing, medicinal, art, craftwork, building or ceremonial purposes by the Māori or early European. However, as discussed in Section 1.2.7.2, the fruit from a number of species were a valuable addition to the available food supply. The native plant-derived oils most commonly used by Māori or early European came from the seed oils of tītoki, kōhia and miro hence these species will be worth investigating for lipid and fatty acid composition. These seed oils were used for a variety of purposes from ceremonial, healing and cosmetic use, to being a fuel source for lamps. Essential oil extracts from native species with scented flowers, leaves or gum (such as mairehau and tarata) were also extracted in order to add fragrance to the above seed oils (Brooker, 1986; Cooper and Cambie, 1991; Crowe, 1992, 1994, 1997a; Riley, 1997).

1.2.7.2 Edible Uses of NZ Native Plants

1.2.7.2.1 Food

Some of the most important native edible plants in the past have included aruhe (bracken fern root), varieties of the *Cordyline* species (i.e. *C. australis*, tī kauka, or cabbage tree), nīkau palm, kiokio (fern fronds), NZ "cress", "spinach" & "celery" and various fruits and berries (Brooker, 1986). Introduced plants have since replaced these as a major source of food, however in bush survival, many of these have proven valuable (Crowe, 1997). Some of these, including the *Cordyline* species, are still being investigated for potential food uses. The roots of the *Cordyline* species, particularly the *C. australis* have a high content of fructose in their roots. Fankhauser & Brasch, 1985 and more recently Harris & Mann, 1994 have studied this species for their potential as a fructose-producing crop.

The fruits or berries of many native species including the wineberry, tātāramoa, mingimingi, snowberry, konini, hīnau, kahikatea, and kiekie have distinctive but acceptable flavours. However, many of the native fruits or berries are small, have too many seeds to make pleasant eating or have large kernels in relation to the amount of flesh. A number also have an undesirable turpentine flavour (miro, tawa, taraire, tītoki) or require careful preparation to remove harmful constituents before they are eaten (karaka, tutu, poroporo) (Brooker *et al*, 1998; Crowe, 1997; Cambie, 1986, Brooker, 1986). Nevertheless, as indicated by Laing and Blackwell (1964), there is probably more scope for development of these fruits and berries as many of the cultivated fruits of today have been developed from equally dubious progenitors.

1.2.7.2.2 Beverages

Plants used for beverages have included the juice from wineberry, karaeo, tutu and kiekie fruit or berries. Sap extracts from mataī, rimu and supplejack vines have been used to produce beers. Coffee and herbal teas have also been made from certain plants. These have included mānuka, kānuka, kawakawa, bidibidi, different coprosma species, native dandelion and NZ flax seeds (Brooker *et al.*, 1998). By far the most important economic beverage has been produced from the tītoki berries. Tītoki-flavoured liqueur is still being used commercially in the beverage line and is called Ti.Toki liquer (Ti-Toki Ltd, 2003).

1.2.7.2.3 Flavourings and Others

Plants such as hangehange, tutu berries and honeys from various plants have been used for flavourings. In fact honeys such as those from rewarewa, pohutukawa, tāwari, kāmahi, NZ flax and mānuka are among those currently being produced today. Native seaweed is a good source of minerals, particularly potassium and iodine, but species such as the NZ native red seaweed are more likely to be used to produce agar (Brooker, 1986). Special interest lately has also centred on the n-3 polyunsaturated fatty acid levels in common New Zealand finfish and shellfish such as the green-lipped mussel (Vlieg & Body, 1988).

1.2.7.3 Non-edible Uses of NZ Native Plants

1.2.7.3.1 Wood, timber and other agricultural uses

Kauri, rimu, tōtara, miro, kahikatea and other native conifers were milled, and still are though less extensively, for construction purposes. A small industry also exists in producing carved or turned souvenirs or furniture from native woods for the tourist trade (Brooker *et al.*, 1998). Many native NZ plants are popular garden plants in this country and overseas. Research has gone into searching for species more suitable for conditions in various countries and for the mini pot-plant trade (Hobbs 1994, 1995). Flax fibre is used in craft industries and its essential oils in some herbal remedies. Alkaloids, toxic principles and other constituents from native plants have shown various potential uses in pest control. These include constituents from poroporo, karaka, immature kōhia and tutu. Of particular interest is the conversion of *Solanum* alkaloids into ecdysones as these have potential uses as insecticides.

1.2.7.3.2 Medicinal/Pharmaceutical/Biological activities

It has been said that 25% of today's pharmaceuticals are derived from plant sources, which suggests that more could be found by investigating unusual or uncommon species such as those endemic to NZ (Brooker *et al.*, 1998). A vast number of native plant species were valued and used by early Māori for their healing and herbal properties (Crowe 1992, 1994; Williams, 1996; Riley, 1997). Evidence of their validity and practice could be shown by the fact that the pre-European Māori were a fit and healthy race at contact time, with but few diseases (Riley, 1997).

Historically, practical use and information on the correct preparation or application of these plants were restricted to the tōhunga of the tribe. Information was carefully passed on to a select few deemed worthy of the knowledge. Unfortunately, certain aspects of Rongoā Māori or the knowledge and application of various native species in healing and medicine has been lost to the majority of the current Māori population. Thankfully though, known aspects of Rongoā Māori are still currently practised, taught and recognised as a valuable source of knowledge. Standards for practise have been developed with support from Ngā Ringa Whakahaere o Te Iwi Māori (the National Body of Traditional Māori Healers) and the Health Funding Authority (Ministry of Health, 1999).

Investigation of the chemical constituents potentially responsible for those healing qualities in native New Zealand plants have been carried out by a number of researchers. Brooker, Cambie and Cooper have provided a valuable compilation of some of the uses, chemical constituents and possible related pharmacology of native species (Brooker *et al.*, 1998). Work by researchers such as Dr Meto Leach at the University of Waikato, NZ, is being carried out to link the chemical constituents and biological activity of traditional medicines obtained from native plants of a certain area. Species such as kawakawa, karemu, miro, NZ flax, kareao and kohekohe are among a few of those used in healing salves and hand balm being marketed currently (http://www.oceanorganics.co.nz/Herbal_Healing.htm, retrieved 01/02/03).

Some of the most economically useful constituents found in NZ plants include certain alkaloids for their medicinal purposes (Brooker *et al.*, 1998), and other compounds such as isoprenoids, which display various biological activities (Calder *et al.*, 1986; Kellam *et al.*, 1992; Allen *et al.*, 1999) or aromatic properties. Alkaloids of particular importance include *Solanum*-derived constituents. Solasodine, a steroidal alkaloid, is used as a source for the production of physiologically active steroids, while other *Solanum* alkaloids have valuable antibiotic, antifungal and insect-control activities (Cambie, 1986). Alkaloids from the NZ *Sophora* (kōwhai) species are of interest due to their antifungal activity against brown rot in stonefruit. Falcarindiol, an alkaloid produced from the patē or seven-finger plant (*Schefflera digitata*), is being utilised as an antifungal agent (Muir *et al.*, 1982; http://www.irl.cri.nz/get/services/nat_prod.htm, retrieved 20/01/2003).

Essential oils from native species have also been investigated and include kauri (*Araucariaceae*); tōtara, rimu, miro (*Podocarpaceae*); tarata, kōhūhū, black māpou (*Pittosporaceae*); beech, mānuka, rata, horopito, kawakawa, ngaio, ramarama and ferns. Those of the native gymnosperm species such as the *Araucariaceae* and *Podocarpaceae* are chief among those studied. For example, **Totarol**^{TM} is a natural aromatic diterpenoid, which has antibiotic and antioxidant properties. It is found in many *Podocarp* species (cypress, juniper, rosemary, rimu) but the most abundant source is the heartwood of tōtara. (Bendall 1995; http://www.essentiallynz.com/totarol.cfm, retrieved 20/01/2003).

New Zealand "tea-tree", or mānuka, is plentiful and fast growing. Although its main use is as firewood, the leaves of the mānuka and kānuka have been used for brewing teas. More recently, essential oils from the leaves, and honey from the flowers are being harvested for their anti-fungal and anti-bacterial elements (Brooker *et al.*, 1998). Leptosperm, an antibiotic agent is one of the active factors in the oil and is also found in some of the honeys from these plants. Research led by Professor Dick Merz in conjunction with Waikato University, found antiseptic, antibiotic, antifungal, anaesthetic and other properties in tea-tree oil (correspondence received by B. R. Jordan from Professor D.F. Merz, 3/12/1994).

1.2.7.3.3 Dyestuffs, colouring matters, resins and tannins

Many native species were used historically by pre-European Maori and early settlers to produce dyestuffs and pigments for clothing manufacture and dyeing of fibres for such things as tukutuku or kōwhaiwhai designs. The bark of the hīnau plant was used to produce a black dye; blue-black colours were obtained from the tutu and makomako; red colours were obtained from the tānekaha and toatoa; while the yellow colours came from the pūriri or *Coprosma* species. The mud surrounding the mataī and kahikatea trees was also used as a mordant (Crowe, 1992; 1994; Cooper & Cambie, 1991).

Resins and gums from kauri (agathic acid) and *Podocarpaceae* have been used in varnishes, linoleum production, rubber compounding, adhesives, paper coatings, ink and even chewing gum (Cooper & Cambie, 1991). However the gum from kauri cannot be taken from live trees without killing them. Currently, Manool, a resin from the NZ pink pine species, is being produced for use as an odorant in the fragrance industry (Douglas *et al.*, 1994). Certain phenolics and flavonoids are being produced from NZ bryophytes, ferns, conifers and some *Hebe* species for use in insecticides (Douglas *et al.*, 1994). Native species most important in tannin production included tānekaha, tōwai and tāwhero.

1.2.7.3.4 Fragrances, cosmetics

Common species used for the production of fragrances include those from the *Rutaceae* family. Only a few NZ native species belong to this family and they include mairehau and whārangi. Others used for fragrance production include mānuka, kōhūhū, tarata, taramea, aromatic ferns, raukawa, karetu and koromiko (Cambie, 1986; Cooper & Cambie, 1991).

1.2.7.4 Work Done on the Fatty Acid Composition of New Zealand's Native Seed Oils

Except for Isabel Morice's extensive examination of the fatty acid composition of seed oils from various native species, as listed below, relatively little work has been carried out on the non-volatile oils and fats from native New Zealand seeding plants (Cambie, 1986). The fruit or seed oils examined by Morice included certain members from the following families: *Agavaceae* (1962, 1965); *Juncaceae* (1967a); *Liliaceae* (1967b, 1969a, 1975b); *Iridaceae* (1969b); *Hypoxidaceae*, *Smilaceae* and *Philesiaceae* (1970); *Palmae*, *Elaeocarpaceae*, *Oleaceae* (1975a) and *Cyperaceae* (1977). Most of these species were monocotyledons. Further detail of the lipid and fatty acid composition in these species can be found in Appendix One.

Native fruit or seed oils examined by other researchers include members from the following families: *Sapindaceae*- oil of the scarlet aril and seed oil of tītoki- *Alectryon excelsus* (Brooker, 1957; Brooker & Eyres, 1981); seed oil of akeake- *Dodonea viscosa* (Brooker & Eyres, 1981); *Meliaceae*- seed oil of kohekohe- *Dysoxylum spectabile* (Brooker, 1960; Kleiman *et al.*, 1984); *Passifloraceae*- seed oil of the kohia, the New Zealand Passionfruit, *Passiflora tetrandra* (Brooker, 1960); *Corynocarpaceae*- the kernel and kernel husks of the karaka berry- *Corynocarpus laevigatus* (Body, 1983). (See also Appendix One for further detail of these works).

Upon survey of the results obtained from the above species it can be assumed that a variety of fatty acids are likely to exist within the fruit or seed oils of native New Zealand plants. Certain members of the *Agavaceae*, *Liliaceae*, *Sapindaceae* and *Passifloraceae* families contained 10% or greater percentages of oil.

As expected, linoleic, oleic and palmitic acids were the major components of the total fatty acid content. Saturated fatty acids ranged from C10 to C28 in the even carbon chain series and C15 to C27 in the uneven carbon chain series. Monoenoic fatty acids included all even and uneven carbon chain series from C15:1 to C24:1 except for those from C19 and C23 parent acids. Polyenoic fatty acids were almost solely from the C18 parent acid. Previous studies also show that potentially valuable sources of linoleic acid are likely to be found within the *Agavaceae* (*Cordyline* and *Phormium* species), *Liliaceae* and *Cyperaceae* families, while α- and γ-linolenic fatty acids are likely to be found in the *Aizoceae* (Aoki, 1982) and in *Desmoneuron* & *Isoneuron* sections of the *Liliaceae* genera of *Collospermum* and *Astelia* (Morice, 1967b; 1975b).

Research on extracts from leaves, stems, heart & sapwood, surface waxes and other native plant components have at times listed fatty acid and triacylglyceride compositions but as this is outside the scope of this research, these will not be discussed. Instead, one would be directed to the aforementioned New Zealand Phytochemical Registers prepared by Brooker, Cambie and other researchers for further information.

Other native species that may be potentially worthwhile lipid or fatty acid sources would be those belonging to important commercial vegetable oil-producing families. No native species of note belong to the *Brassicaceae* (rapeseed) family but 29 genera such as *Celmisia* or *Olearia* (58 and 32 of which are endemic) belong to the *Compositae* (i.e. sunflower) family. Common *Olearia* species include akeake and rangiora. Three genera *Hibiscus, Hoheria* and *Plagianthus* of the *Malvaceae* family (i.e. cotton) occur in New Zealand. In the *Hoheria* genus, five are endemic to New Zealand and include the lacebark species. Ribbonwood (mānatu), is one of the two endemic species belonging to the *Plagianthus* genus, while mikoikoi is one of endemic species belonging to the *Hibiscus* genus. The only native species belonging to the *Palmae* (i.e coconut or palm/kernel) family of note, is the nīkau palm of the *Rhopalostylis* genus. Eight genera and 49 species of the *Papilionaceae* family (i.e peanut and soybean) occur in New Zealand. Some of these include *Sophora* (kōwhai); *Chordospartium, Notospartium* and *Carmichaelia* (native brooms).

1.3 SUMMARY AND AIMS FOR RESEARCH

Lipids are dynamic, complex metabolic biochemicals that are linked directly or indirectly with virtually every structure and metabolic function in the body. Apart from providing an important energy, food and carbon source for living organisms, key biological functions of lipids include the organisation and function of all cellular membranes, their communication roles (such as signalling, hormonal and defense-related activities) and their protective roles (such as in waterproofing, protection against mechanical injury, padding of important internal organs and providing thermal insulation).

Lipids also have many other biological roles such as in the maintenance of good health and nutrition. Triacylglycerols are the chief constituents of natural fats and oils and so account for 95-98% of the dietary fat ingested in the human diet. These dietary fats help provide essential fatty acids, culinary interest, satiety and carry fat-soluble vitamins. Triacylglycerols consist of three fatty acids esterified to glycerol. Fatty acids are an important source of energy or carbon as well as being a major component of the structure of cell and organelle membranes. Fatty acids are the building blocks of larger lipid compounds such as hormones and eicosanoids, which are involved in key physiological functions. However, particular lipids or fatty acids are also implicated in the susceptibility to, and recovery from disease.

Vegetable fats or oils generally occur in greatest quantity either in the fleshy part of the fruit, and/or in the seed. The lipid fraction of plant seeds is a concentrated source of fatty acids as they are primarily made up of triacylglycerols. Natural fats and oils have a wide range of properties that are useful in industry. Some are valued for their properties in edible products such as melting behaviour, flavour, flavour stability and nutritional advantage. Some serve conveniently as a heating medium and are frequently used to cook and preserve food. Their reversible solid-to-liquid phase transition properties allow them to function as pastry fat, frying shortenings and in confectionery applications. Their ability to dissolve colour and flavour or lubrication plays an important role in making food palatable and desirable.

Properties valuable in non-edible uses include the ability of some lipids to dry quickly while forming a tough flexible film (paint and protective coatings). Some are important functional components of lubricating greases in metal works. Their foaming properties are useful in detergent and surfactant fields. Others function as softeners, plasticizers or emulsifiers (soap, grease and rubber manufacture). Many of these desirable properties stem from the physical or chemical properties of the fatty acid and triacylglyceride mix contained in the oil.

In summary, lipids such as triacylglycerides and fatty acids, are beneficial from a physiological, biological, nutritional and industrial perspective and can be naturally found in large quantities in the fruit flesh or seed tissues of plants. A large majority of the native New Zealand vascular flora are endemic to New Zealand. This high proportion of endemism provides opportunities for unique research as useful or novel plant constituents may be found which are unique to species of New Zealand and can be commercially exploited for the benefit of the New Zealand economy.

To date, relatively little work has been carried out to survey the non-volatile oil and fat content of native species. From those investigations that have been carried out, results have shown that a wide variety of fatty acids are likely to be present. Certain members of the *Agavaceae*, *Liliaceae*, *Sapindaceae* and *Passifloraceae* families contained potentially valuable percentages of oil. These studies also showed that good sources of essential fatty acids such as linoleic and α-linolenic fatty acids are likely to be found.

Thus the aim of this project was to collect seed and/or fruit from New Zealand native species and analyse the oil content in order to determine whether any of the native species had a fatty acid or lipid composition that would warrant further investigation for potential nutritional, health or industrial applications. Collection of material would be from as many taxonomical families as possible but would focus on native species that were readily available, belonging to the same taxonomical families as those from commercial vegetable oils or suggested as being potentially worthwhile by a botanical expert.

2 Materials and Methods

2.1 MATERIALS

2.1.1 Plant material

A total of 46 native New Zealand plant species from 31 botanical families were surveyed for their seed or fruit lipid content and composition. The majority (40) of the species were flowering plants (*Angiospermae*) of which 33 were dicotyledon and 7 were monocotyledon. Of the remaining 6, 4 were gymnosperm and 2 were of marine or aquatic origin. A larger number of dicotyledon species were analysed due to the fact that these plants were easily accessible and much less was known about this group of plants in comparison to monocotyledons. A detailed list of the various names of these species can be found in Table 2.1.

Plants of marine or aquatic origin are more likely than higher, land-based species to contain greater levels of longer chain, polyunsaturated fatty acids. Biological products of longer-chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), 20:5(n3) and docosahexaenoic acid (DHA), 22:6(n3) are reported to have health benefits in the treatment of high blood lipid or cholesterol as discussed in section 1.2.5. Hence, it was deemed worthwhile to also investigate the fatty acid composition of native plant species of aquatic or marine origin. Unfortunately, due to time restrictions, only 2 plants of marine or aquatic origin were analysed.

2.1.1.1 Collection

Fruits and seeds of native New Zealand higher plants were collected on the basis of convenience in locality, family variance of plant species, and suspected potential oil source according to family group of commercial vegetable oils and suggestions made by Professor David Fountain, a botanical expert in native plants at Massey University, Palmerston North. However, when interesting results were obtained from a particular plant species, other members within the same botanical family were also investigated wherever possible. This was the case with the *Araliaceae, Podocarpaceae* and *Pittosporaceae* families.

Samples were obtained directly from the living flora and analysed as soon as possible after collection. However, fruiting and thus collection times were variable and occurred over a two to three year period. At times this required initial samples to be kept frozen or under cool storage until enough material was available to be analysed.

The land-based native species were collected mainly from within the wider Manawatu area of New Zealand. This included the Massey University grounds, the Victoria Esplanade and local gardens -all within Palmerston North, and forest areas within the Pohangina Valley, Ashhurst. The aquatic fern was collected from local Palmerston North garden ponds while samples of the neptune's necklace was collected from the upper Wellington westcoast beaches. Excellent illustrated field-guide texts by Crowe (1992, 1994) and Salmon (1996) provided the main basis for initial identification of native plants, however, Professor David Fountain and Dr John McIntosh from Massey University also assisted with the identification of the species once collected.

Fruit or seed samples unable to be collected in sufficient quantities in the above areas were purchased from New Zealand Tree Seeds, (Rangiora, NZ) and Proseed New Zealand, (RD1, Amberley, NZ). These included the following species: *Prumnopitys ferruginea* (miro/brown pine); *Gaultheria antipoda* (tāwiniwini/snowberry); *Myoporum laetum* (ngaio); *Pittosporum tenuifolium* (kōhūhū) and *Pittosporum tenuifolium colens* (black māpau/māpou).

Table 2.1 below lists the scientific, common and Māori names of those species that were collected and analysed in this research. For the sake of simplicity in the thesis, the plant species will generally be referred to by their common names. Note that the Māori names used vary between different tribal areas of New Zealand and in some cases, the same Māori name is used for two different species of plant. In such cases, I have listed those Māori names most commonly used.

TABLE 2.1 Names of Native Plants Collected and Analysed

SCIENTIFIC NAME		COMMON NAME	MĀORI NAME
Family	**Species**		
Agavaceae	*Cordyline australis/banskii*	Cabbage Tree	Tī Kōuka / Tī Ngahere
Agavaceae	*Phormium tenax*	NZ Flax	Harakeke / Whatariki
Araliaceae	*Pseudopanax arboreus/laetus*	Five Finger	Whauwhaupaku / Puahou
Araliaceae	*Pseudopanax crassifolium*	Lancewood	Horoeka
Araliaceae	*Schefflera digitata*	Patē / Seven Finger	Patē
Asteraceae	*Pachystegia insignis*	Marlborough Rock Daisy	
Azollaceae	*Azolla rubra/filiculoides*	NZ aquatic fern	Karerarera / Retoreto
Boraginaceae	*Myosotidium hortensia*	Chatham Is. Forget-Me-Not	Kopakopa
Cornaceae	*Griselinia lucida*	Puka / Shining Broadleaf	Puka / Kāpuka / Pāpāuma
Corynocarpaceae	*Corynocarpus laevigatus*	Karaka	Karaka
Elaeocarpaceae	*Aristotelia serrata*	Wineberry	Makomako
Elaeocarpaceae	*Elaeocarpus dentatus*	Hīnau	Hīnau
Ericaceae (Heath)	*Gaultheria antipoda*	Snowberry	Tāwiniwini
Hormosiraceae	*Hormosira banskii*	Neptune's Necklace	
Irideceae	*Libertia ixioides/grandiflora*	Native NZ Iris	Mīkoikoi
Lauraceae	*Beilschmiedia tawa*	Tawa	Tawa
Liliaceae	*Arthropodium cirratum/paniculatum*	Native Rock Lily	Rengarenga
Liliaceae	*Dianella intermedia*	NZ Blueberry	Tūrutu
Malvaceae	*Hibiscus trionum*	Perenial Hibiscus	
Malvaceae	*Hoheria augustifolia*	Narrow-Leaved Lacebark	Houhere
Malvaceae	*Plagianathus regius*	Ribbonwood	Manatu
Monimiaceae	*Hedycarya arborea*	Pigeonwood	Porokaiwhiri
Myoporaceae	*Myoporum laetum*	Ngaio	Ngaio
Myrsinaceae	*Myrsine australis*	Red Matipo / Māpau / Māpou	Matipo / Māpau / Māpou
Palmaceae	*Rhopalostylis sapida*	Nīkau Palm	Nīkau
Papilionaceae	*Carmichaelia aligera?*	Native Broom	Maukoro
Papilionaceae	*Clianthus puniceus rosea*	Red Kākā Beak	Kowhai-Ngutu-Kākā
Papilionaceae	*Sophora microphylla*	Kōwhai	Kōwhai
Passifloraceae	*Passiflora tetrandra*	Kōhia / Native Passionfruit	Kōhia
Piperaceae	*Macropiper excelsum*	Kawakawa / Pepperwood	Kawakawa
Pittosporaceae	*Pittosporum eugenioides*	Lemonwood	Tarata
Pittosporaceae	*Pittosporum tenu. ssp colensoi*	Black Māpau	Māpau/Māpou
Pittosporaceae	*Pittosporum tenuifolium*	Kōhūhū	Kōhūhū
Pittosporaceae	*Pittosprorum crassifolium*	Turpentine tree	Karo
Podocarpaceae	*Dacrycarpus dacrydiodes*	Kahikatea / White Pine	Kahikatea
Podocarpaceae	*Dacrydium cupressium*	Rimu / Red pine	Rimu
Podocarpaceae	*Podocarpus tōtara*	Tōtara	Tōtara
Podocarpaceae	*Prumnopitys ferruginea*	Miro / Brown Pine	Miro
Proteaceae	*Knightia excelsa*	Rewarewa / NZ Honeysuckle	Rewarewa
Rosaceae	*Acaena novae zealandiae*	Bidibid	Piripiri
Rubiaceae	*Coprosma lucida/robusta*	Karamū / red-fruited coprosma	Karamū/Kākāramu
Rutaceae	*Melicope ternata*	Wharangi/Three finger	Wharangi/Koheriki
Sapindaeceae	*Alectryon excelsus*	Tītoki	Tītoki
Smilaceae	*Ripogonum scandens*	Supplejack	Kareao
Verbenaceae	*Vitex lucens*	Pūriri	Pūriri
Violaceae	*Melicytus ramiflorus*	Māhoe / Whiteywood	Māhoe

2.1.1.2 Preparation

Once collected, the larger fruits were pulped and washed in water to extract the seeds. Some species such as the karaka, pūriri, kōhia/NZ passionfruit, miro/brown pine and hīnau, required removal of tough outer husks or seed coatings. In such cases, the inner kernels were analysed for lipid content. Other species such as bidibid, māhoe/whiteywood, five finger, patē/seven finger, wineberry and ribbonwood had small fruits and tiny seeds. In these species, the whole fruit was dried, ground and analysed. Fruits, seeds or kernels were then air-dried or oven-dried at 55°C, cooled and stored in a dessicator over silica gel until required. Depending on the size and density of the fruits or seeds collected, the dried plant material was sliced, mulched or reduced but ultimately ground to approximately a 1-2mm granular size through a 1-2mm metal screen/sieve. Marine or aquatic species were washed in distilled water, air or freeze dried and then ground to a 1-2mm granular size as above. Care was taken to provide thorough mixing to ensure uniformity of the final sample. Ground material was kept in closed vials and stored in a cool, dark place prior to analysis.

2.1.2 Chemicals and Equipment

2.1.2.1 General

Unless otherwise specified, chemicals used in the experiments were of laboratory or analytical reagent grade and purchased through New Zealand distributors of BDH Laboratory Supplies, England; AJAX Chemicals, Australia; May and Baker Ltd., England; Aldrich Chemical Company Inc., USA; or Sigma Chemical Company, USA. Organic solvents were either redistilled at Massey University or were of analytical grade and purchased through the distributors mentioned above.

2.1.2.2 Fatty Acid Methyl Ester (FAMES) Preparation and Analysis

All except three fatty acid standards and fatty acid methyl ester chemicals were obtained from Sigma chemicals. Exceptions included behenic (docosanoic) and myristic (tetradecanoic) acids (which were obtained from BDH Laboratory Supplies, England) and lignoceric (tetracosanoic) acid (which was obtained from Fluka AG, Chemische Fabrik, Buchs SG, Germany). 10mg/ml of pentadecanoic acid, (approx 99%, Sigma) in dry toluene was used as an internal standard. Activated 4Å molecular sieves (sodium alumino-silicate, 8-12 mesh beads, Sigma) were added to the distilled toluene and AR grade methanol solvents used in the preparation and storage of FAMES to remove water. The methanolic hydrogen chloride was prepared fresh in a stoppered glass conical flask by adding 10% v/v acetyl chloride (98%, Aldrich Chemical Company Inc., USA) dropwise, with shaking, into dry methanol, cooling in an icebath. Florisil (synthetic magnesia-silica gel adsorbent, 60-100mesh, Hopkin & Williams Ltd, England) was used to remove pigments.

2.1.2.3 Gas Chromatography

For gas liquid chromatography analysis, a 2.6m packed column (15% ethylene glycol succinate -EGSS-X) on chromosorb W a/w, 80-120 mesh (Waters Associates Inc., USA) and a 30m, 70% Bicyanopropyl polysiloxane – BPX70 capilliary column (SGE, USA) was used.

2.1.2.4 Hydrogenation

The Palladium / 10% Carbon catalyst used in the hydrogenation procedure was of lab grade and purchased from the KOCH-LIGHT chemical company, England.

2.1.2.5 Thin-Layer Chromatography

Standard silica thin-layer chromatography plates were made with Silica Gel G (Kieselgel $60HF_{254}$, AR grade), purchased from MERCK, (Germany). The silver nitrate used in the preparation of silver nitrate-TLC plates was of AR grade and purchased from May & Baker, (Australia), while the silver nitrate TLC adsorbent used was a 13% calcium sulphate/0.5hydrate, silica chromolay, which was also purchased from May and Baker, Australia.

2.2 METHODS

2.2.1 Oil Extraction Methods

Before analysis of lipids can take place, it is first necessary to extract them from their tissue matrices in a relatively pure state. Lipids extracted from seed oils are likely to contain predominantly simple lipids such as fatty acids and acylglycerides, while lipids extracted from other plant tissues are likely to include more complex lipids such as those associated with membranous and exterior tissues.

During oil extraction, lipid-soluble compounds such as pigments and compounds of polyisoprenoid origin such as sterols, tocopherols, carotenoids and chlorophyll will extract into the oil. Non-lipid contaminants such as sugars, amino acids, urea and salts are also likely to be extracted. A wide variety of techniques such as the "Folch wash" described in Section 2.2.1.1 and the use of silica adsorbents such as florisil (Section 2.2.1.3) are employed for the purpose of eliminating unwanted contaminants.

Various solvents or solvent combinations have been suggested as extractants for lipids. However, the techniques employed have much to do with the desired end result or product. For example, the aim of the isopropanol/chloroform extraction method described in Section 2.2.1.1 was to first extract and then examine the *main lipid classes* on thin layer-chromatography. This was only done in a few species and the results are discussed in Section 3.2. The aim of the oil extraction using the soxtec/soxhlet systems (Section 2.2.1.2) was to estimate the total oil/fat content. Only a few species were tested in this way and the results are also discussed in Section 3.2.

The major aim of this research was to survey the composition of the seed oils. Since seed oil is composed predominantly of fatty acid-containing acylglycerols, phospholipids, gycolipids or free fatty acids, the major method employed to analyse the seed oil content was through the formation and extraction of methylated fatty acid derivatives, collectively named fatty acid methyl esters (FAMES). The method for this procedure is described in Section 2.2.1.3. Forty-six native species were analysed in this way and the results are presented in Section 3.3.

2.2.1.1 Isopropanol / Methanol / Chloroform Extraction

The following procedure was adapted from the methods described by Nichols (1963). Plant tissues were macerated with 50 parts (w/w) isopropanol so as to inhibit lipase activity and remove the more polar fats. The mixture was shaken and allowed to stand for 30 minutes prior to being filtered through Whatman No.1 or No.541 filter paper. 40ml of chloroform-isopropanol (1:1, v/v) was washed over the solid, and the solid was taken up in 45ml of chloroform-methanol (2:1, v/v), allowed to stand overnight in a fumehood and then given a "Folch" wash as described below.

Folch wash

The solid prepared as described in Section 2.2.1.1, was filtered and sequentially washed with 20ml chloroform and 10ml methanol. The combined filtrates were retained and transferred to a measuring cylinder, and then a 0.25 volume of 2M potassium chloride was added. This mixture was shaken well, allowed to settle and then the top layer was aspirated and discarded. A 0.25 volume of methanol-water (1:1, v/v) was added to the remaining layer, then the mixture was again shaken well and the top layer aspirated and discarded (Folch *et al.*, 1957).

Following the Folch wash, the anhydrous sodium sulphate was added to the resulting chloroform/methanol sample to remove excess water then the sample was filtered through Whatman filter paper containing a small amount of sodium sulphate into 500ml round-bottom glass flasks. Chloroform was evaporated off at 47°C using a rotavapour condensor system, (Buchi laboratoriums, Sweden). The fat residue remaining in the flask was dissolved in a minimal volume of dichloromethane and stored in stoppered, glass vials until analysis by thin-layer chromatography.

2.2.1.2 Soxtec/Soxhlet Lipid Extraction

2.2.1.2.1 General procedure

2-3g duplicates of previously dried, ground (1-2mm) seed material were weighed (W1b) into extraction thimbles and plugged with cotton wool to prevent loss of sample during solvent extraction. Extraction vessels (metal extraction cups or 250ml round bottom flasks) with 2-3 glass boiling beads were heated in an oven at ~55°C for 30mins, cooled in a dessicator over silica gel and then weighed (W2). 50mls of petroleum ether (bp60°C-80°C) or diethyl ether was poured into the extraction vessels and the thimbles containing the samples were refluxed for 60 minutes in a Tecator HT6 soxtec system with a Tecator HT 1043 extraction unit; or a soxhlet fat extraction unit.

(The soxhlet system was composed of glass condensers, 250ml round bottom flasks and quick-fit adapters; rubber tubing; and a graduated heating element). After removal of the solvent by evaporation, the extracted lipid remaining in the extraction vessels was cooled in a dessicator over silica gel and then weighed (W3).

Only where results were to be expressed as % fat *on a fresh weight basis* was an initial fresh weight (W1a) taken into account before the sample was dried overnight in an oven at ~55°C. In most cases, results of fat were expressed per gram of dried weight of the seed material and so the initial weight (W1a), was not recorded. The fat percentage per gram of dried plant material was calculated by subtracting the cup weight before extraction (W2), from the cup weight after extraction (W3), then dividing by the dried sample weight (W1b) and finally, multiplying that result by 100.

$$\% \text{ Fat} = \left(\frac{(\text{W3}) - (\text{W2})}{\text{Sample weight (W1b)}} \right) \text{ x } 100$$

NB. If the fat percentage per gram of fresh weight was required, weight (W1a) would replace (W1b) in the above equation.

2.2.1.3 Fatty Acid Methyl Esters (FAMES) Preparation and Extraction

2.2.1.3.1 Introduction

As discussed in Section 1.2.4, the longer the carbon chain length and greater the degree of unsaturation within the fatty acid carbon chain, the higher the subsequent boiling point. The formation of methylated lipid derivatives provides a relatively cheap but convenient means for decreasing the molecular weight of large lipid compounds and lowering the melting and boiling point of those non-volatile lipids such that they can be measured by gas liquid chromatography (GLC).

Acid-catalysed methyl esterification of the free fatty acids or transesterification of O-acyl lipids such as triglycerides is achieved by heating them with a large excess of anhydrous methanol and an acidic reagent as catalyst. Adding excess methanol pushes the equilibrium in favour of FAMES formation (Christie, 1990).

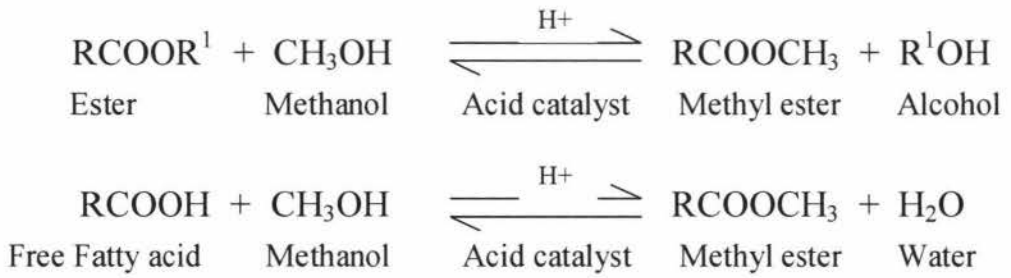


Figure 2.1 Acid-Catalysed Esterification of Fatty Acids and O-acyl Lipids

Many methods are available to produce FAMES derivatives, but an adaption of the 2 hour, acid-catalysed, one-step extraction - transesterification procedures, developed by Sukhija and Palmquist (1988), was used in this research and is described below.

2.2.1.3.2 FAMES Preparation Method

100 – 800mg of dried plant sample was accurately weighed into glass culture tubes (10cm x1cm) with screw-top, rubber-lined lids. (Where plant material was of sufficient quantity, plant samples were weighed in duplicate). To this was added 2ml of dry toluene, 0.5ml of the internal standard and 3ml of freshly prepared methanolic hydrogen chloride. The tubes were tightly stoppered and heated at 70°C in a waterbath for 2 hours. Each tube was gently mixed 3-4 times during this time and if solvent had escaped, 2ml of dry toluene was added to help ensure complete methylation. The tubes containing the derivatised samples were then cooled in iced water and 5ml of a 6.0% potassium carbonate solution, followed by 2 ml of dry toluene was added. The tubes were inverted several times, then centrifuged in an IEC centra GP8R centrifuge, with a swinging bucket rotor at 2000rpm (~850g max) for 5 – 7 minutes.

The top fatty acid-containing layer was transferred into clean, dry culture tubes and the water and pigments were removed in sequence by adding small amounts (~0.2-0.5g) of anhydrous sodium sulphate followed by florisil. For further purification in order to limit degradation during storage, the extracted FAMES now in anhydrous toluene, were filtered through Whatman No. 1 filter paper and glass wool over florisil and anhydrous calcium chloride. The samples were then stored at 4°C in dark-pigmented glass vials (4.5cm x 0.8cm) with screw-top, rubber-lined lids until required.

2.2.2 Methods for Lipid Separation and Identification

A number of methods were employed to estimate and identify the lipid compositions obtained using the extraction methods described in the previous section. Hydrogenation, thin-layer chromatography (TLC) and argentation were some techniques used to assist in the separation and identification of lipid groups in a few species, however, the separation, identification and quantification of seed lipids was primarily achieved through applying the technique of gas liquid chromatography (GLC).

2.2.2.1 Hydrogenation of FAMES

FAMES samples which had unidentified peaks or produced traces in which fatty acids were likely to overlap within the same peak (for example 18:3(n-3) and 20:0 on the EGGS-X column), were reduced to the parent, saturated fatty acids, by shaking under pressure, in an atmosphere of Hydrogen for 15 hours in the presence of a Palladium/Carbon catalyst.

The reduced sample was then centrifuged (MSE minor centrifuge, England) at low speed (~50gmax) for 15 minutes then separated from the charcoal by addition of 2mls of diethylether to the charcoal pellet. After mixing then re-centrifuging as above, the diethylether/FAMES mixture was then bubbled under oxygen-free nitrogen gas to remove excess diethylether solvent. The reduced FAMES were then re-examined by GLC, thus enabling one to distinguish to some extent between overlapping fatty acids of different chain lengths and the relative proportions of the saturated parent fatty acid.

2.2.2.2 Thin-Layer Chromatography (TLC)

2.2.2.2.1 Standard TLC

The lipids obtained from solvent extraction were separated on 0.25mm Silica Gel G plates and identified under UV light using a dichloroflourescein indicator. 30g of silica powder in 90mls of water was shaken in a screw top bottle, and spread over 20cm square glass plates to a depth of 0.25mm using a TLC plate spreader. The plates were left to set and dry at room temperature then stored until needed. TLC samples and standards were dissolved in minimal volumes of dichloromethane. About 2-5 µl volumes of each standard or sample were taken up in thin glass capilliaries and 5-6mm diameter spots, approximately 1.5-2cm apart and 3cm from the bottom edge, were applied on to TLC plates that had been previously activated at ~55°C, overnight.

In general, three solvent components were used in an 80:20:1 ratio; with heptane, toluene or hexane as the non-polar component; diethylether as the polar component; and formic or acetic acid to improve resolution. Approximately 100mls of the combined solvent mixture was poured into a chromatography tank, and the plate was allowed to develop for 1-2 hours or until the solvent front had reached 2cm from the top of the silica edge. The plate was carefully removed from the tank and allowed to air dry in a fumehood until all the solvent had evaporated.

The lipids were then located with a spray of 0.1% (w/v) solution of 2,7 dichlorofluorescein in 95% ethanol and examined under ultraviolet light. General lipid groups were separated out, with cholesterol esters migrating to the solvent front, followed by triacylglycerols, free fatty acids, cholesterol, diacylglycerols, monoacylglycerols and phospholipids with other polar lipids as shown below in Fig 2.2.

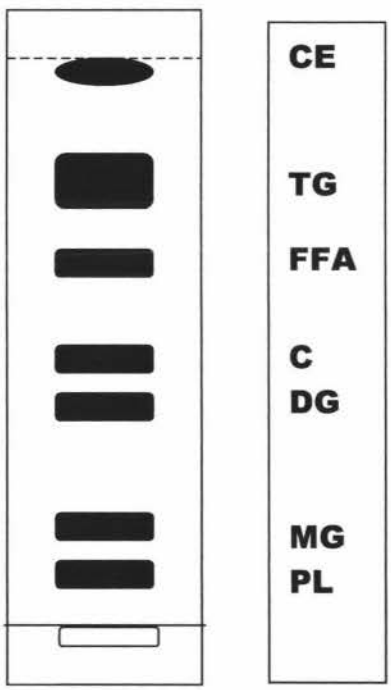


FIGURE 2.2 Schematic TLC Separation of Simple Lipids on Silica gel G

Key: CE = cholesterol esters, TG = triacylglycerols, FFA = free fatty acids, C = cholesterol, DG = diacylglycerols, MG = monoacylglycerols, PL, phospho- or polar lipids. —— represents the origin and ------ the solvent front on Figure 2.2.

2.2.2.2.2 Silver Nitrate TLC (Argentation)

TLC on silica gel impregnated with silver nitrate is of value due to the facility with which the double bonds in the alkyl chains of fatty acids form polar complexes reversibly with silver compounds. Thus fatty acids can be separated according to both the number and configuration of their double bonds and sometimes, with care, according to the position of the double bonds in the alkyl chain (Christie, 1994).

100mls of a 5% silver nitrate solution was prepared in a glass, foil-covered bottle. Then 50g of a silica gel / 13% calcium sulphate chromolay was added to the silver nitrate solution. 0.25mm TLC plates were spread and air-dried in a darkened room. The plates were then covered with foil until they were ready to be used. Prior to use, the foil covered plates were activated in an 105°C oven for 30-60minutes. Plates were used as soon as possible after preparation due to darkening of the adsorbent layer upon exposure to light.

Fatty acid methyl ester samples and standards were then applied to the plates and developed using a heptane: ethylacetate: acetic acid (90: 4: 3) solvent system in a dark room. The lipids were located with a spray of 0.1% 2,7 dichlorofluorescein in 95% ethanol and examined under UV light. The lipids were separated into various degrees of unsaturation and bands obtained should obtain the following classes of esters (in order of decreasing mobilities): saturated, monoenoic, dienoic, trienoic etc. Bands can then be scraped off the plate and filtered through Whatman No. 1 filter paper with diethylether into glass vials. Diethylether is then evaporated off and the lipid redissolved into capped vials with a minimal amount of dichloromethane. Lipids extracted from the different bands can then be re-examined by gas liquid chromatography.

2.2.2.3 Analysis of Fatty Acid Composition by Gas Liquid Chromatography

Separation, identification and quantification of FAMES was predominantly estimated with the Shimadzu GC-8A gas chromatograph and a 2.6m, 15% ethylene glycol succinate –(EGSS-X) column on chromosorb W. For initial fatty acid identification, 15% EGSS-X packed columns give good separation of fatty acid esters of a given chain-length that differ in degree of unsaturation and in most cases, those that differ only in positions or configurations of the double bonds. The principle disadvantage of this column is that there is co-elution of certain fatty acids.

With the EGSS-X /chromosorb W column used in this research, co-elution of C18:3(n-3) with C20:0 and possibly C20:4(n-6) with C22:1(n-9) seemed to occur. Thus further investigation on a Hewlett-Packard Series II gas chromatograph with a 30m, 70% Biscyanopropyl polysiloxane -(BPX70) capilliary column was carried out in potentially useful native species that produced a large C18:3(n-3)/C20:0 peak or had other suspected overlaps with the EGSS-X /chromosorb W column.

2.2.2.3.1 Shimadzu GC-8A / 15% EGSS-X column

The unknown fatty acid compositions from each plant species were analysed by injecting 4 to 5µl (depending on probable lipid concentration) of the FAMES samples prepared as described in Section 2.2.1.3, into the gas chromatograph. Nitrogen acted as the carrier gas with the pressure rate maintained between 80-180 kPa and 170°C – 200°C isothermal conditions. Higher temperatures were used to speed up the retention times for long-chain, and a higher degree of unsaturated FAMES. The temperatures of the injection and detector ports were maintained at 200°C. Peaks were detected using a flame ionisation detector and expressed by chart recorder tracings and integration into the Waters, MAXIMA computer program. (The Shimadzu GC-8A gas chromatograph was simultaneously attached to a Sekonic SS-250F chart recorder, and a Shimadzu GC-8A integrator interface which was linked to Maxima, Waters data display computer software).

2.2.2.3.2 Hewlett-Packard 5890 Series II / BPX70 column

0.1-0.2µl samples were injected using a Hewlett-Packard 7673 GC/SFC injector. Hydrogen acted as the carrier gas with a flow rate of 6.92ml/minute. Oven temperature was initially set to 120°C and was raised in 5°C/min increments to 175°C where it remained for the rest of the run. Injector and detector temperatures were both maintained at 190°C. A flame ionisation detector was used to detect fatty acids in the carrier gas. The Hewlett-Packard 5890 Series II Plus gas chromatograph settings, run sequence and data display was automatically integrated and controlled using Hewlett-Packard Series II ChemStation software.

2.2.2.3.3 FAMES Standards

The internal standard, C15:0, and other fatty acid methyl esters or standards either prepared using the method described in Section 2.2.1.3, or purchased from Sigma as FAMES standard combinations, were used to assist in the identification of unknown peaks. This was achieved by running unknown plant and then standard samples subsequently and under the same conditions, then comparing the retention times of the standard against those of the plant samples.

Allocation of unknown peaks appearing between those of known fatty acid standards were estimated relative to the expected retention time of fatty acids in that range due to chain length, position of enoic bonds and number of enoic bonds. For example, appropriate standards between the range of C20:1(n-11) to C20:4(n-6) were unavailable and thus allocation of peaks appearing between the standard retention times of gadoleic (C20:1n-11) and arachidonic (C20:4n-6) fatty acids were estimated.

2.2.2.3.4 Quantification of Fatty Acids

The quantity of individual fatty acids present (mg/g of dried seed material), were determined manually, or through automatic integration software available through the Waters, Maxima or Hewlett-Packard Series II ChemStation Software. By using the following formula, the peak area (peak height x peak width at half height) of each fatty acid could be converted to mg of fatty acid per gram of dried material.

$$\frac{\text{fatty acid weight (mg)}}{\text{seed weight (g)}} = \frac{\text{fatty acid peak area}}{\text{area of } C_{15} \text{ peak}} \div \text{Seed dry weight} \times \frac{15}{\text{fatty acid carbon no.}} \times 20.6 \times \frac{\text{molecular weight}}{1000}$$

The Total Fatty Acid content per gram of dried material could then be found by calculating the sum of all the individual fatty acids.

2.2.3 Presentation of Results

Note that the aim of this research was to provide a *preliminary survey* of the fatty acid composition in as wide a range of native seeding plants as possible, rather than to provide a definite fatty acid composition of each species by analysing multiple samples of the same species in different locations, stages of development or growth environment etc. Duplicate samples were unable to be carried out on some species due to the small amount of material able to be collected (snowberry, Marlborough rock daisy). In addition, a second initial gas chromatography analysis was not carried out on species where the first results from the gas chromatography analysis suggested that the fatty acid composition was deemed to be of low commercial potential (low total fatty acid content or lower proportion of a commercially valuable fatty acid compared to other native species analysed). This was the case for kareao, kōwhai, ngaio, Neptune's necklace, whārangi, karamū, houhere, porokaiwhiri, hinau, and ribbonwood.

For the remaining species, the results of the fatty acid proportions from duplicates or a greater of samples from each species were within 10% of the average result (Table 3.3). A greater number of samples from a wider range of plants from each species is recommended before fatty acid proportions in these native species can be definitively stated.

3 Results and Discussion

3.1 INTRODUCTION

As samples from each plant species were collected, preliminary gas liquid chromatography (GLC) fatty acid analysis was carried out on the relevant fatty acid methyl derivatives (FAMES), prepared as described in Section 2.2.1.3. Particular notice was taken of those species needing further verification of unknown, unusual or co-eluting fatty acid peaks, and those containing fatty acids of potential health or commercial benefit, such as linoleic (C18:2n-6), alpha-linolenic (C18:3n-3), gamma-linolenic (C18:3n-6), oleic (C18:1n-9), palmitic (C16:0) or erucic acids (C22:1n-9). An analysis of lipid components and estimation of total lipid content was also carried out on a number of species. Results of these findings are discussed in Section 3.2.

Species noted as requiring further investigation due to unidentified peaks were analysed using other gas liquid chromatography systems, by hydrogenation techniques and by thin-layer chromatography techniques such as argentation. The results of the latter two techniques are discussed in Sections 3.3.2 and 3.3.3 respectively. The combined data from the gas liquid chromatography, hydrogenation and argentation techniques is presented in Table 3.1 where the fatty acid profiles of the 46 native species is presented. These results are discussed in Section 3.3.4.

The results from a small survey analysing the changes in fatty acid composition over developmental or storage periods in tōtara and kahikatea are presented and discussed in Section 3.4. The commercial or otherwise potential of the 46 native seed or fruit lipids is assessed in Section 3.5 by matching the native species against commercial vegetable oils known for containing valuable levels of particular fatty acids, or with comparable fatty acid composition, and by discussing any of the 46 native species of particular interest relative to other past, present or potential uses of the plant and then discussing their distribution and cultivation needs. In the conclusion, (Section 3.6), a summary of the results and discussions is presented.

3.2 LIPID QUANTIFICATION AND COMPONENTS

3.2.1 Isopropanol / Methanol /Chloroform Lipid Extraction followed by TLC Analysis

Only the turpentine, lemonwood, tītoki and NZ flax samples were analysed using the Isopropanol/Methanol/Chloroform extraction method described in Section 2.2.1.1. The results of this method of extraction showed that the majority of the seed lipids contained in these species were acylglycerols, free fatty acids or cholesterol esters. A schematic diagram of the results of the standard silica gel G -TLC plates using a hexane : diethyl ether : acetic acid solvent system in an 80 : 20 : 1 ratio (as described in 2.2.2.2), is shown below.

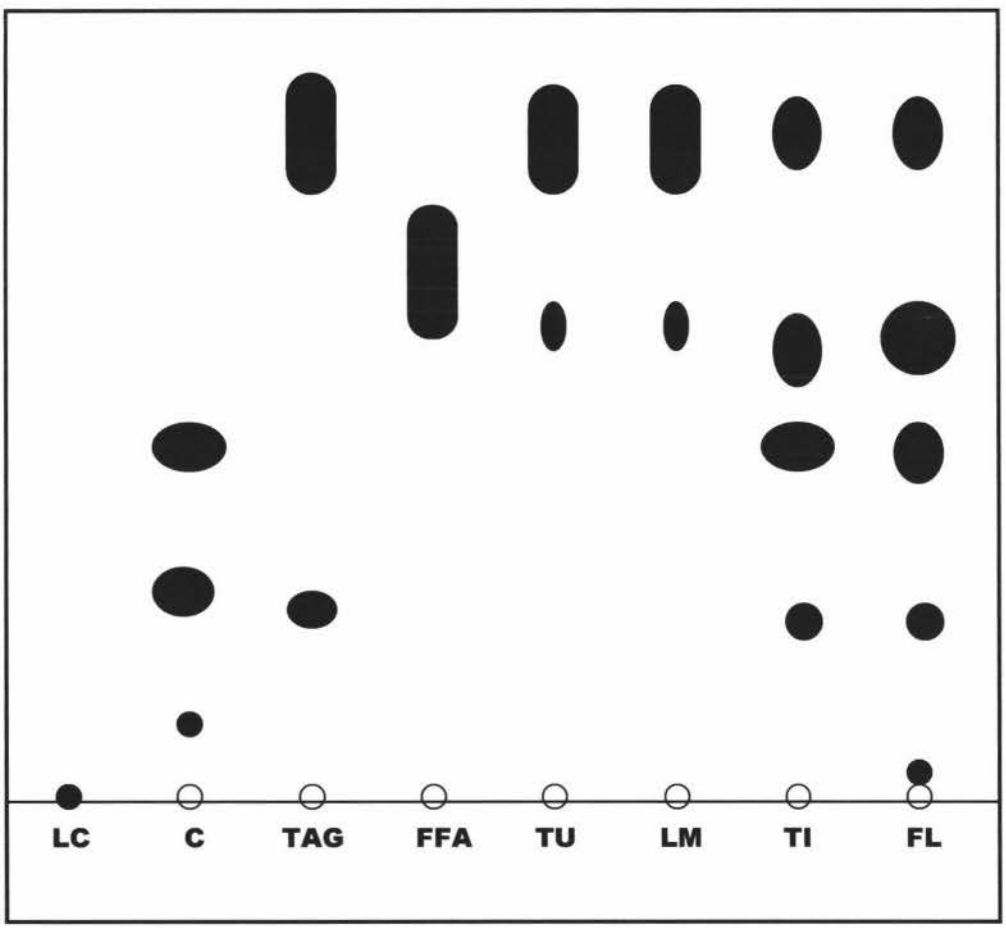


FIGURE 3.1 Schematic TLC Separation of Seed Lipids using the Isopropanol / Methanol /Chloroform Extraction Method.

Key: LC = Lecithin std; C = Cholesterol std; TAG = Triacylglycerol (tripalmitin) std; FFA = Free fatty acid (palmitic acid) std; TU = Turpentine; LM = Lemonwood; TI = Tītoki; FL = Flax.

3.2.2 Soxhlet/Soxtec Lipid Extraction followed by TLC Analysis

Seed or fruit lipid extraction by diethyl ether and/or petroleum ether using a soxhlet/soxtec analytical system was carried out on fourteen native species. These were the aquatic fern, cabbage tree, Chatham Island forget-me-not, five-finger, flax, kahikatea, kōhia/NZ passionfruit, kōwhai, lacebark, lemonwood, native broom, tawa (fruit flesh), tītoki and turpentine species. Initial extractions involved petroleum ether extraction in a soxhlet or soxtec apparatus but were later substituted for the diethyl ether extraction in a soxtec apparatus due to the better overall extraction and reproducibility. In most of these 14 species, TLC analysis (as described in Section 2.2.2.2) was carried out in order to determine which of the lipid components might be present in the seed oils. The initial aim was to perform these extractions and separations on all 46 species, but due to time constraints or lack of seed material, this was only done on the above listed species. Results from all lipid extractions or TLC analysis as described, have been included where appropriate in Table 3.1 and are shown below.

TABLE 3.1 Results of Ether Lipid Extraction and Standard TLC Analysis

Native Species	% Lipid/dry wt. using pet. ether	% Lipid/dry wt. using diethyl ether	Lipid components by TLC
Aquatic fern	ND	4.8	TAG (tr)
Cabbage Tree	ND	ND	TAG (maj), FFA, sterols (tr)
Forget-me-not	21.6	ND	TAG(maj), FFA, sterols, PL (tr)
Five finger	ND	17.7	TAG(maj), FFA, sterols, DAG?
Flax	ND	12	FFA, TAG, sterols, DAG, PL (tr)
Kahikatea	27.1	35.4	
Kōhia	ND	ND	TAG(maj), orange pigment
Kōwhai	2.9	3.9	TAG(maj)
Lacebark	ND	0.7	TAG(maj)
Lemonwood	5.9	7.2	TAG(maj), FFA, sterols (tr)
Native Broom	2.1	1.6	TAG(maj), FFA
Tawa (flesh)	0.4	ND	
Tītoki	34.8	38.3	
Turpentine	12.3	ND	

KEY: wt = weight; pet = petroleum; TLC = thin layer chromatography; ND = not determined; TAG = triacylglycerols; tr = trace; maj = major group; FFA = free fatty acid; DAG = diacylglycerol; PL = phospholipid or other highly polar lipid.

3.2.3 Summary

Results from the TLC analysis of the fourteen species extracted using isopropanol/methanol/chloroform, petroleum ether or diethyl ether, confirm that triacylglycerols make up a large proportion of their seed lipids. In most cases, triacylglycerols were the major component, followed by free fatty acids, sterols di- or mono-acylglycerols and lastly phospholipids or other lipids or lipid-soluble compounds of similar polarity. In tītoki and flax seed oils however, a greater contribution of the seed oil seems to be in the form of sterols or free fatty acids, with smaller contributions from triacyl- and di-acylglycerols. An even smaller proportion of the seed oil from flax may come from monoacylglycerols or polar components such as a seed pigment.

Excluding the aquatic fern and tawa, the seed lipid content recorded from the twelve remaining species showed significant variation between species (0.6-0.7% in native broom or lacebark and 35-38% in kahikatea or tītoki). The solvent used in the extraction also produced variation. For example, in the lemonwood seed oil, 5.9% fat was attained from the petroleum ether extraction whereas 7.2% fat was attained from the diethyl ether fat extraction. In most cases, the lipid percentages obtained from diethylether extraction were greater. It is unclear as to why this did not occur in the native broom extractions but this may have been due to a loss of lipid during the diethylether extraction. These differences are likely attributable to the affinity of various lipid groups for the relative solvent used in the extraction. Diethyl ether extracts a larger range of lipids from non-polar lipid groups such as TAG to more polar lipids or compounds such as phospholipids, whereas petroleum ether will extract the more non-polar range of lipids such as TAG and longer chain hydrocarbon compounds.

In conclusion, a wide range in total seed lipid content and lipid composition from the rest of the 46 native species would be expected, although triacylglycerols are likely to be the major seed lipid component. Lipid analysis using different techniques or solvent extraction methods is useful in gaining an initial estimation of the major lipid groups and seed lipid content. However, the type and amount of lipid extracted will depend on the affinity of the various lipid groups for the solvent used in the extraction process. All this being said, care in ensuring uniformity of the initial sample and minimal loss of lipid during extraction will also help reproducibility.

3.3 FATTY ACID ANALYSIS

3.3.1 Introduction

The first step in the fatty acid analysis was to extract the fatty acids from the seed, kernel or fruit tissues. The method of extraction is described in Section 2.2.1.3. Using this procedure, acid-catalysed methyl esterification of free fatty acids or transesterification of O-acyl lipids such as triaylglycerides is achieved. The products, called fatty acid methyl esters (FAMES), have lower relative melting and boiling points that enables the fatty acids to be separated and measured by gas liquid column chromatography (GLC).

Once the FAMES from a native species had been extracted, preliminary analysis of the sample was carried out on a Shimadzu GC-8A gas chromatograph with a 2.6m, 15% ethylene glycol succinate (EGSS-X), on chromosorb W a/w column. Peaks were quantified and identified as explained in Section 2.2.2.3. FAMES from native species of potential interest, but with a chromatogram containing unidentified or suspected overlapping peaks, were then analysed by hydrogenation (Section 3.3.2), measured by silver nitrate thin-layer chromatography (Section 3.3.3), and/or run on the Hewlett-Packard 5890 Series II gas chromatograph using a 30m, 70% bicyanopropyl polysiloxane (BPX70) capillary column.

The results obtained from all these methods were combined to provide the most likely proportion of fatty acids for each of the 46 native species. The final results are presented later in Section 3.3.4. The native species were then allocated into groupings according to similar levels or unusual contributions from a common fatty acid (Section 3.3.4.1).

3.3.2 Determination of the Parent Fatty Acid Carbon by Hydrogenation

Using variable mobile or stationary phase column materials, gas liquid chromatography has enabled the separation of the native seed lipid FAMES sufficiently to identify most straight-chain fatty acids contained within the samples. Hydrogenation of previously prepared FAMES provided a quick and convenient method for further verification or identification of unknown peaks. In the process of hydrogenation, unsaturated straight-chain FAMES are reduced to their parent, saturated FAMES. They can then be reanalysed on the GLC to determine the likely proportion of fatty acids belonging to each series and will provide a separation between straight and branched-chain fatty acids.

Hydrogenation was utilised in this research for two reasons: Firstly, to separate and identify relative proportions of fatty acids with overlapping peaks (such as in the case of the C20:0 and C18:3 peaks produced from the EGGS-X column used in this research); and secondly, to aid in the identification of unknown peaks such as confirming the presence of C15 and C17 fatty acids and also confirming that some of the long-chain fatty acid peaks obtained from *Podocarpaceae* species, were in fact, unsaturated C20 fatty acids. The fifteen native species analysed in this way were patē/seven finger, puka / shining broadleaf, karaka, red matipo, native broom, kōhia/NZ passionfruit, kawakawa, turpentine tree, tītoki, native flax, NZ blueberry, kahikatea and tōtara. Table 3.2 lists the results of the fifteen species analysed by hydrogenation.

3.3.1.1 Proportions of C20:0 and C18:3(n-3)

In most of the 15 species, it was difficult to determine the correct proportions of the C18:3(n-3) and C20:0 acids due to the small total amount attributable to the original overlapping peaks. It is likely that small amounts of each acid are present in each of these species. According to the combined weight of the C18 or C20 acids after hydrogenation, the initial overlapping peaks in patē, puka, kōhia, māhoe, native flax, kahikatea and tōtara were most likely attributable to only C18:3(n-3) (due to higher combined wt % of C18 following hydrogenation); while in karaka, matipo, native broom, tītoki and NZ blueberry, it was most likely C20:0. The remaining species kawakawa, lemonwood and turpentine had approximately equal proportions.

TABLE 3.2 Determination of the Parent Fatty Acid Carbon by Hydrogenation

Plant Name	10:0 % TFA	10:x % TFA	12:0 % TFA	12:x % TFA	14:0 % TFA	15:0 or 15:x % TFA	16:0 % TFA	16:1 % TFA	17:0 % TFA	18:0 % TFA	18:1 % TFA	18:2 % TFA	18:3a or 20:0 % TFA	20:0 % TFA	20:1 % TFA	20:2 % TFA	20:3 % TFA	20:4 % TFA	22:0 % TFA	22:1 % TFA	Ax % TFA	TFA mg/g
Patē			0.2		0.1		5.4	0.7	0.1	0.9	83.8	7.6	0.5	0.1	0.5							**205**
Patē (hyd)			0.3		0.1		6.5		0.2	91.2	0.7	0.4		0.6								**210**
Puka			0.8		0.6		19.6	0.8	2.2	4.4	58.3	11.3	1.4		0.6							**72**
Puka (hyd)			0.8		0.5		19.9		2.3	74.1	0.3	1.0		1.0								**77**
Karaka			0.5		0.2	0.1	16.0	0.5	0.2	5.2	28.8	44.5	3.2		0.7							**81**
Karaka (hyd)			0.5		0.1		19.7		0.1	75.2		0.4		3.9								**94**
Matipo			2.8		0.5		11.6	0.9	0.2	3.3	40.8	17.5	8.0		14.4							**42**
Matipo (hyd)			3.4		0.4		13.0		0.0	56.3	7.8			19.0								**50**
Native Broom			0.4		0.4	0.4	23.0	9.7	0.8	5.1	17.1	32.7	10.1		0.4							**26**
Native Broom (hyd)			0.4		2.2		32.8		1.1	52.2		0.4		10.9								**27**
Kōhia			0.2		0.3		9.8	0.4	0.5	4.8	19.5	63.3	1.1									**271**
Kōhia (hyd)			0.3		0.3	0.4	10.3		0.7	83.8	2.7	1.0		0.4								**273**
Kawakawa	1.0		3.1		28.7		6.1	0.3	0.3	7.2	21.5	9.2	6.1		12.3					4.1		**29**
Kawakawa (hyd)	1.0		3.0		29.1		7.0		0.3	37.5		3.0		15.1					4.0			**30**
Lemonwood			0.4		0.8		7.4	2.1	0.1	1.9	31.9	13.1	3.9		17.4		0.5		0.9	18.3	1.4	**77**
Lemonwood (hyd)	0.3		0.6		1.0	0.3	9.6		0.3	46.7	1.3	0.5		20.0					19.5			**77**
Turpentine			0.7				5.7		0.7	2.0	29.9	13.9	1.8		23.7		0.6			19.1	1.8	**109**
Turpentine (hyd)			0.7				6.4		0.8	45.0				25.5					21.5			**107**
Tītoki	0.03		0.1				4.2	0.9	0.1	0.9	46.6	4.0	11.8		31.5							**292**
Tītoki (hyd)	0.03		0.1				5.5		0.1	52.7		0.1		41.5								**294**
Māhoe			0.8		0.3		15.4	0.5	0.4	2.4	20.3	58.8	1.0		0.2							**119**
Māhoe (hyd)			0.9		0.3	2.4	16.7		0.4	77.5	1.1	0.2		0.5								**128**
Native Flax			0.1		0.04		8.9	0.2	0.1	3.5	15.2	71.6	0.4									**284**
Native Flax (hyd)			0.2		0.03		9.0		0.1	90.6				0.1								**289**
NZ Blueberry			1.1		9.2		18.9			2.2	19.3	48.0	1.4									**74**
NZ Blueberry (hyd)			1.3	1.3	8.7	0.5	18.5	1.4	0.5	59.7	6.5	0.5		1.0								**77**
Kahikatea		0.1	1.9	0.1	0.4	0.3	8.1	0.4	0.4	2.6	27.3	28.2	3.4		1.0	0.9	19.0	2.1	1.4		2.4	**140**
Kahikatea (hyd)	0.1		2.2		0.3	0.4	8.4		0.3	59.2				24.1			1.8		1.6		1.8	**141**
Totara	1.8	0.5	1.0	0.7	0.3	0.5	4.8	0.1	0.1	4.4	29.8	23.8	5.9		1.1	0.7	15.2	8.4	0.5		0.3	**155**
Tōtara(hyd)	1.7	0.4	1.7		0.6	0.3	5.0		0.3	62.5		0.3		25.2			1.1		0.9			**157**

Key: TFA = Total Fatty Acid, x = unknown number of enoic bonds, 18:3a = alpha linolenic acid, Ax = unknown very-long chain fatty acid, hyd = hydrogenated

3.3.1.2 Presence of C15, C17 and very-long chain polyunsaturated fatty acids

Uneven straight chain fatty acids are generally an unusual occurrence in seed oils, but if present, are usually only found in trace amounts (<0.1% wt). Initial GLC tracings from the separation of the patē, native broom, kowhai and māhoe, supplejack and ngaio FAMES showed an additional hump attached to the standard C15:0 peak, or a peak most likely to be C15:1. Hydrogenations confirmed a number of the native species had trace amounts of C15 acids (Table 3.2). In māhoe, 2.4% of the total fatty acids were C15 acids, however, initial identification of C15 acids was not confirmed in patē and native broom. Kōwhai, supplejack and ngaio FAMES were not hydrogenated as they had no other fatty acids of interest. Initial GLC tracings also showed an unknown peak eluting between the C16:1 and C18:0 standard peaks in a number of native species, but were of particular note in patē, puka, native broom, kōhia, turpentine and māhoe. Hydrogenations of those six species confirmed the presence of C17 acids with 2.3% and 1.1% of the total fatty acids present in the puka and native broom respectively, but 0.2-0.8% in the other remaining four species (Table 3.2).

GLC analysis of the FAMES produced from members of the *Pittosporaceae* and *Podocarpaceae* families produced unknown peaks between the C20:1(n-11), C20:4(n-6), C22:0 and C20:5(n-3) standard peaks. Results of the hydrogenation of the lemonwood and turpentine species (*Pittosporaceae* family), showed that these peaks were likely dienoic or higher, C20 acids (totalling 2-2.5% of the TFA) and dienoic or higher, C22 acids (totalling 2-2.2% of the TFA). In the kahikatea and tōtara species (*Podocarpaceae* family), the majority of the unknown long chain fatty acid peaks appeared to be C20 acids, which totalled around a quarter of the total fatty acid content in each species (Table 3.2).

3.3.1.3 Summary

Hydrogenation of the fatty acids from patē, puka, karaka, matipo, mative broom, kōhia, kawakawa, lemonwood, turpentine, tītoki, māhoe, native flax, NZ blueberry, kahikatea and tōtara has assisted in the verification that C15 and C17 uneven chain fatty acids are present in some of New Zealand's native seed oils. Of the 15 species analysed, C15 acids were present in 6, and C17 acids in 14 of the native species but the greatest amounts occurred in the māhoe (2.4%) and puka (2.3%) respectively.

Polyunsaturated C20 acids are likely present in members of the *Pittosporaceae* and *Podocarpaceae* families but are found in greater quantity in the latter. Analysis of the unknown peaks relative to those of the fatty acid standards, suggests that n-6 and/or n-3, polyenoic C20 fatty acids are also present in *Podocarpaceae* species.

3.3.3 Analysis of Kahikatea FAMES by TLC

3.3.2.1 Initial Purification

Once the FAMES from the dried, ground kahikatea seed material had been extracted, (see Section 2.2.1.3), a preliminary analysis of the kahikatea FAMES was carried out by TLC, to determine whether all the fatty acid esters previously attached to triacylglycerides had indeed been converted to free fatty acid methyl esters. The kahikatea FAMES had indeed been fully converted to free fatty acid methyl esters as shown in Figure 3.2, by the large, singular spot in line with a similar Rf to the palmitic acid standard spot.

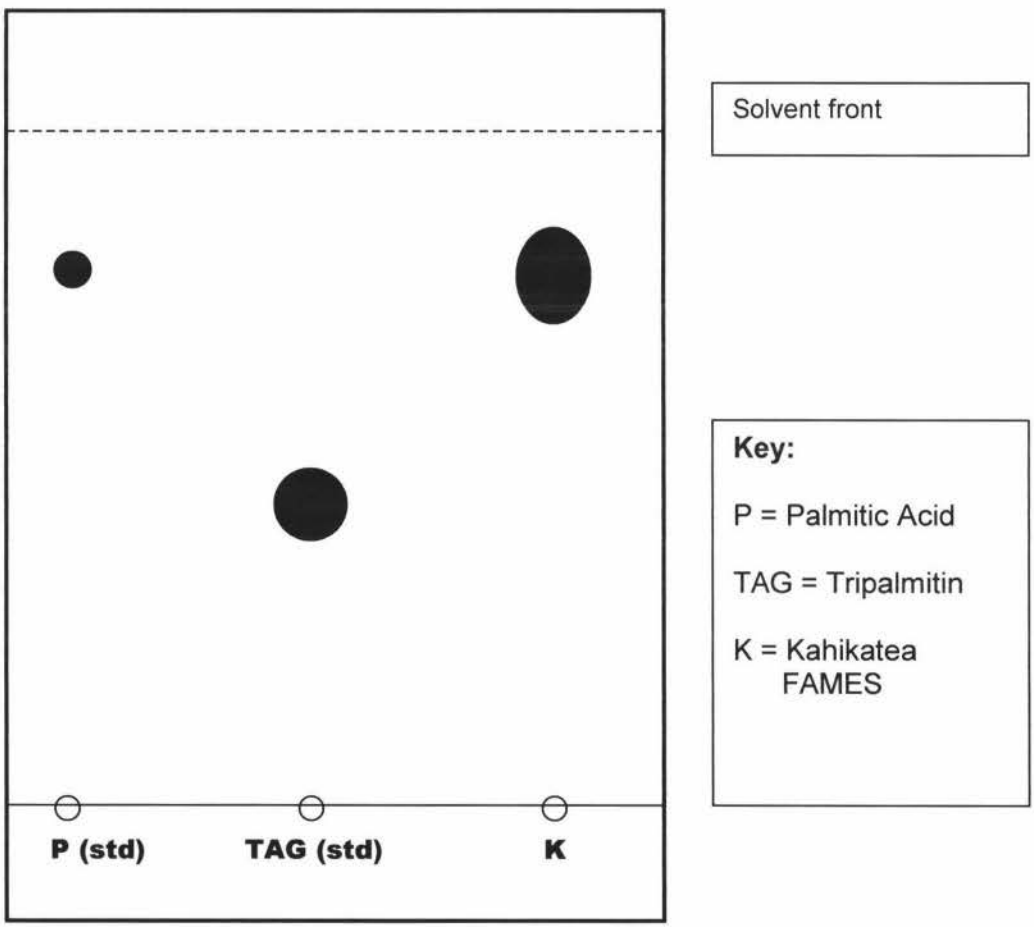


FIGURE 3.2 Analysis of Kahikatea FAMES by TLC

A small sample of the kahikatea FAMES was spotted on to a standard silica Gel G plate (see Section 2.2.2.2) with heptane : diethyl ether (90 : 6) as the developing solvent system.

3.3.2.2 Separation of Kahikatea FAMES by Silver Nitrate TLC Analysis

The GLC analysis of the original kahikatea FAMES showed that a number of peaks eluted between those corresponding to the C20:1(n-11), C20:4(n-6) and C22:0 and after the C22:1(n-9) fatty acid standard peaks. Hydrogenation results of the kahikatea FAMES showed that a significant amount (24 % wt) of C20 acids were present, while only a small percentage, (~1.6% wt) of C22 acids were present. Silver nitrate TLC analysis was used to separate the kahikatea FAMES according to the number of enoic bonds contained in each fatty acid chain. Once separated, each band containing FAMES with a similar number of enoic bonds could be carefully scraped off the TLC plate, extracted from the silica with diethyl ether, dissolved in dichloromethane and then rerun on GLC to achieve separation of the FAMES by chain length. Results of the silver nitrate analysis confirmed there were indeed saturated, monoenoic, dienoic, trienoic and likely tetraenoic fatty acids present within the kahikatea FAMES. Schematic representations of the TLC plates are shown in Figures 3.3 & 3.4.

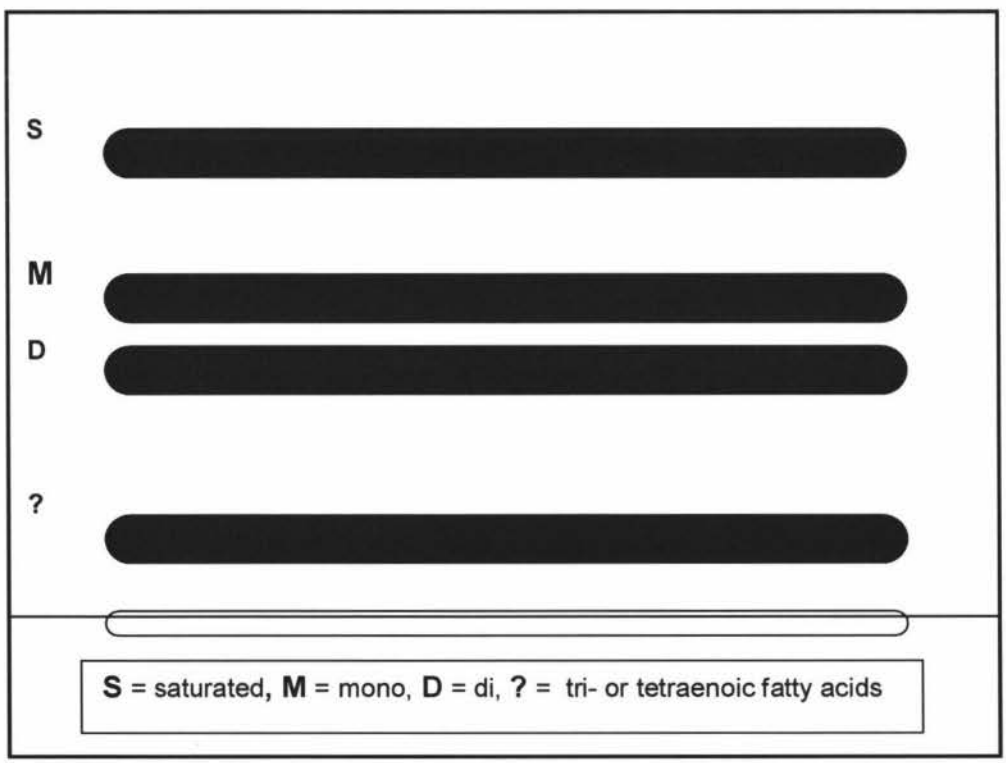


FIGURE 3.3 Separation of Kahikatea FAMES into Fatty Acid Groups

Kahikatea fames were gently spotted in a single streak across the $AgNO_3$ TLC plate and developed using a heptane : ethylacetate : acetic acid (90 : 4 : 3) solvent system. Bands were separated and extracted into different vials using dichloromethane then labelled K_S, K_M, K_{Di} and K_{Tr} respectively for further analysis (see Figure 3.3).

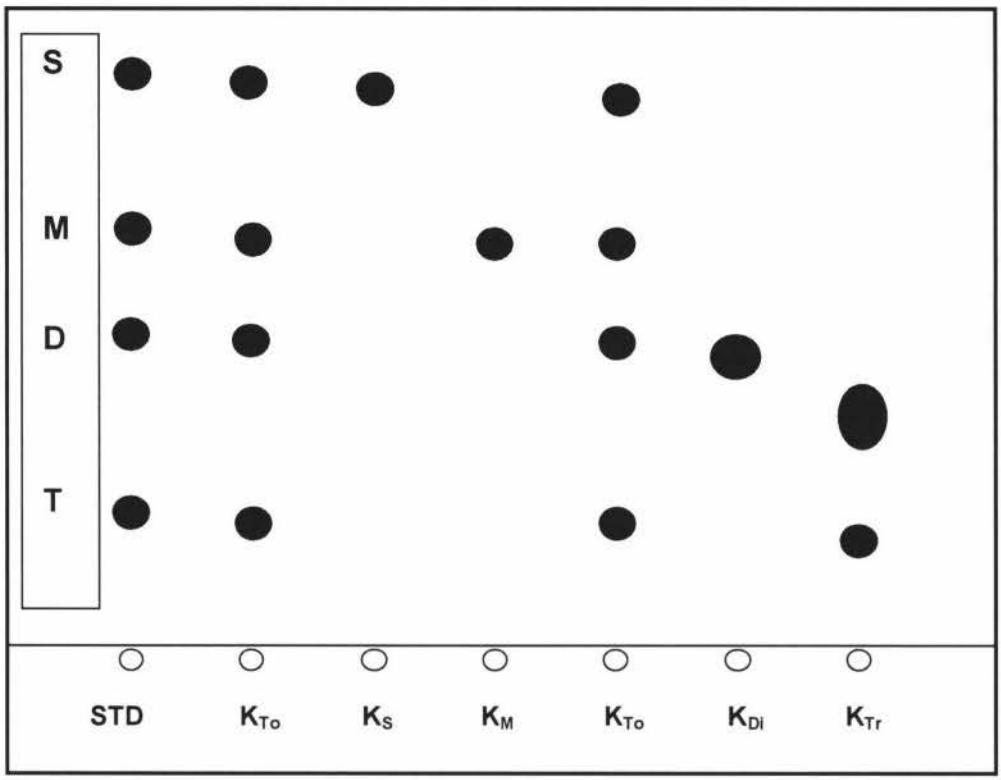


FIGURE 3.4 Identification of Enoic Fatty Acids in Kahikatea FAMES

A sample from each of the kahikatea FAMES labelled K_S, K_M, K_{Di} and K_{Tr} (separated previously) were spotted on to a $AgNO_3$ TLC plate along with the original Kahikatea FAMES (K_{To}) and a FAMES standard (STD), containing saturated (S), mono- (M), di- (D), and tri-enoic (T) fatty acids. The plate was again developed using a heptane : ethylacetate : acetic acid (90 : 4 : 3) solvent system.

3.3.2.3 Summary

Results of the silver nitrate analysis suggest there were indeed saturated, monoenoic, dienoic, trienoic and likely tetraenoic fatty acids present within the kahikatea FAMES. Since there are relatively little C18:3(n-3) or C22 acids present in kahikatea, any tri or tetraenoic fatty acids are most likely to be C20 acids.

3.3.4 Fatty Acid Profiles of the 46 Native Species

FAMES standards and the expected emersion sequence of the FAMES from the gas chromatograph were used to identify the majority of the fatty acid peaks. However, there were a number of unidentified peaks that were estimated based on the expected emergence of fatty acids with a retention time similar to those of nearby standards. These included peaks occurring between standards C20:1(n-11), C20:4(n-6), C22:0 and C22:1(n-9). Others were labelled as C:x (where C = the number of carbons in the chain and x = an unknown unsaturated or branched-chain isomer/derivative). Apart from C20:5n-3 and C22:6n-3, very long chain polyunsaturated fatty acids occurring after C22:1(n-9) were difficult to identify with certainty and were collectively labelled as **Ax** (where **A** and **x** represent an unknown carbon number and number of enoic bonds respectively).

Rewarewa, native blueberry and Neptune's necklace contained a small fatty acid peak that occurred between those of the oleic (18:1n-9) and linoleic (18:2n-6) acid standard peaks. This may be a branch-chain C18 acid but is likely not of commercial importance as other C18 fatty acids of importance in a nearby range include ricinoleic (12-hydroxy octadeca –9-enoic) and 9, 10 dihydroxy stearic acids, which are found in significant quantities (up to 80^{+}%) in castor oil. As the investigation of fatty acids in the native species did not extend to conjugated or hydroxy fatty acid groups, these groups will not be discussed further.

As expected, wide ranges of fatty acids were represented within the cohort of 46 native species tested. These included C8 to C24 acids in the even-carbon number saturated range, C15-C19 in the uneven chain acids, and also included unsaturated fatty acids with one to possibly four or five enoic bonds. In general however, the acids identified were C18 acids, and contained 0 - 3 enoic bonds. Common commercial vegetable oils and fats predominantly consist of saturated or unsaturated fatty acids with a carbon number ranging from 12 to 22 (Luhs & Friedt, 1994a). Once the component and proportions of fatty acids within each species was determined, the species were grouped according to similar concentrations of specific fatty acids. Groupings of the species are explained in Section 3.3.4.1.

The Total Fatty Acid (TFA) content of the 46 species varied from 4 – 357mg per gram (0.4 – 35.7% fatty acid) of dried plant material. A number of species contained a significant proportion (>20%) of fatty acid lipid per gram of dried weight, with the top eight species containing between 230 to 360 mg per gram. The top six quantitative fatty acid contributions, in order of highest frequency came from C18:2 (n-6), C18:1 (n-9), C16:0, C18:0, C18:3 (n-3) and C20:1(n-11) fatty acids respectively.

The fatty acid composition of seed or fruit oils from a number of these 46 species have already been analysed by other researchers. These include the seed oil from the Chatham Island forget-me-not, (McGill *et al.*, 2002); husk and kernel of the karaka, (Body, 1983); fruit coat and seed oil of the hīnau, (Morice, 1975a); seed oil of the kōhia/NZ passionfruit, (Brooker, 1960); seed oil of the tītoki, (Brooker 1957; Brooker & Eyres, 1981); fruit coat and seed oil of nīkau, (Morice, 1970 & 1975a); seed oils of NZ flax & cabbage tree, (Morice, 1962 & 1965); NZ blueberry & rock lily, (Morice 1969a); NZ iris (Morice 1969b); and supplejack, (Morice, 1970). Appendix I lists in detail, the fatty acid composition of these and other fruit or seed oils from native species previously studied by other researchers.

In general, the fatty acid % weights obtained for each of the above native species were similar to those obtained by other researchers. A difference in fatty acid percentage weights or total lipid percent is expected as the oil content within the same species will vary due to a number of reasons, including stage of seed development, ecological habitat of the plant used, soil conditions, experimental procedures and length of seed storage (Chu & Sheldon, 1979). Although seed oils are predominantly composed of fatty acid-containing compounds, other lipid or lipid-soluble compounds may be present. Hence, a measure of total seed lipid content is likely to be different to the total fatty acid content measured in the same species. Table 3.3 lists the fatty acid profiles of the 46 native species analysed. The contributions from each of the listed fatty acids are expressed as mg per gram of dried material and also as percentage weight of the total fatty acid content. Fatty acid values that are <0.1% are not included. Those significant in terms of being unusual, or comprising a high proportion of the TFA content are highlighted in blue.

TABLE 3.3 Fatty Acid Profile of 46 NZ Native Species

DICOTYLEDON																									
BOTANICAL FAMILY	COMMON NAME	Fatty acids*	10:0	10:x	12:0	12:x	14:0	15:x	16:0	16:1	17:0	18:0	18:1	18:1x	18:2	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:2	20:3	20:4	Ax	22:0	22:1	TFA
Araliaceae	Five Finger	mg/g			0.4		0.2		7.4	0.8	0.2	1.8	95		16		0.1									122
		%TFA			0.3		0.2		6	0.7	0.2	1.5	78		13		0.1									
	Lancewood	mg/g			0.5		1		6.1	1.1	0.1	1.4	163		12		1.2									186
		%TFA			0.2		0.5		3.3	0.6	0.1	0.8	87.5		6.5		0.6									
	Patē / Seven or Five Finger	mg/g	0.2		1.1		0.2		14	1.4	0.4	2	193		19		1.3	0.4	1.0							234
		%TFA	0.1		0.5		0.1		6	0.6	0.2	1	82		8		0.7	0.2	0.4							
Asteraceae	Marlborough Rock Daisy	mg/g	1.2		1.3		14		16			7.7	27		73	2					6.3					149
		%TFA	0.8		0.9		9.4		11			5.2	18		49	1.4					4.3					
Boraginaceae	Chatham Is. Forget-Me-Not[a]	mg/g							28.1	8.2		10.8	100		49.4	28.1	3.5		6.3		3.0		4.3		4.3	246
		%TFA							11.4	3.3		4.4	40.7		20.1	11.4	1.4		2.6		1.2		1.7		1.7	
Cornaceae	Puka / Shining Broadleaf	mg/g			0.6		0.4		15	1.0	1.8	4.9	42		9.6			1.0	0.5							77
		%TFA			0.8		0.5		19.5	1.3	2.3	6.4	54.5		12.5			1.3	0.6							
Corynocarpaceae	Karaka[a]	mg/g			0.4		0.1	0.1	14	0.4	0.2	4.3	24		38			2.6	0.6							85
		%TFA			0.5		0.1	0.1	17	0.5	0.2	5.0	28		45			3	0.7							
Elaeocarpaceae	Wineberry	mg/g			0.7		0.8		9.7	0.1		2.1	44		60		1.3	1		0.1	1.7					122
		%TFA			0.6		0.7		8	0.1		1.7	36.1		49.2		1.1	0.8		0.1	1.4					
	Hīnau[a]	mg/g			0.1		0.1		3	1.5		0.6	3.6		4.6		0.6									14
		%TFA			0.7		0.7		21.4	10.7		4.3	25.7		32.9		4.3									
Ericaceae (Heath)	Snowberry	mg/g			0.6		0.5		17		0.6	9.2	85		43		119									275
		%TFA			0.2		0.2		6.3		0.2	3.3	31		16		43.3									
Lauraceae	Tawa (flesh)	mg/g	0.2		0.5		0.6		0.8			0.3	0.7		0.8		0.1									4
		%TFA	5		12		15		20			7.5	18		20		2.5									
Malvaceae	Perennial Hibiscus	mg/g					44		16	5.7		8.9	27		41		1.3	2.6								147
		%TFA					30		11	3.9		6.1	19		28		0.9	1.8								
	Narrow-leaved Lacebark	mg/g			0.8				1.2						2.2											4
		%TFA			19				29						52											

TABLE 3.3 cont. Fatty Acid Profile of 46 NZ Native Species

DICOTYLEDON CONTINUED																										
BOTANICAL FAMILY	COMMON NAME	Fatty acids*	10:0	10:x	12:0	12:x	14:0	15:x	16:0	16:1	17:0	18:0	18:1	18:1x	18:2	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:2	20:3	20:4	Ax	22:0	22:1	TFA
Malvaceae	Ribbonwood	mg/g	0.1		0.4	0.1	0.3		7.5		0.2	1.6	4.8		24		3.2	2	0.7	2.6	0.2	1.4		1		50
		%TFA	0.1		0.8	0.2	0.6		15		0.4	3.2	9.5		48		6.4	4	1.4	5.2	0.4	2.8		2		
Monimiaceae	Pigeonwood	mg/g			0.4		0.1		2.3	0.1		0.5	2.5		5.7		0.9									13
		%TFA			3.2		0.9		18	0.9		3.9	20		46		7.3									
Myoporaceae	Ngaio	mg/g	0.1		0.5		0.1	0.6	2.3	0.1		0.7	4.7		15		1.6									26
		%TFA	0.3		1.9		0.4	2.3	8.9	0.4		2.7	18		59		6.2									
Myrsinaceae	Red Matipo / Māpau / Māpou	mg/g			2.1		0.2		5.3	0.4	0.1	1.6	21.7		8.7		2.4	2.9	7.7							53
		%TFA			4		0.4		10	0.8	0.2	3	41		16.5		4.5	5.4	14.5							
Papilionaceae	Native Broom	mg/g			0.3		0.1		6.9	4.2	0.5	1.7	5.6		13			3.2	0.2							36
		%TFA			0.8		0.3		19	12	1.3	4.7	16		36			9	0.6							
	Red Kaka Beak	mg/g			0.4		0.1		8.7		0.1	1.7	6.2		26		12.8		0.4	0.1	0.1					57
		%TFA			0.7		0.2		16		0.1	3	11		46		22.8		0.7	0.1	0.1					
	Kōwhai	mg/g			1.4			0.4	9.8			3.7	22		33											70
		%TFA			2.1			0.6	14			5.4	31		47											
Passifloraceae	Kōhia / Native Passionfruit[a]	mg/g			0.7		1.9	1.1	26	1.2	1.7	13	52		171		1.9	1.1								272
		%TFA			0.3		0.7	0.4	9.6	0.4	0.6	4.8	19		63		0.7	0.4								
Piperaceae	Pepperwood/Kawakawa	mg/g	0.3		0.7	0.1	14		2.4	0.1	0.1	0.6	8.6		4.9		0.6	1.0	3.5							37
		%TFA	0.8		1.9	0.3	38		6.5	0.3	0.3	1.6	23		13		1.6	2.7	9.5							
Pittosporaceae	Lemonwood	mg/g			0.3	0.1	0.5	0.2	5.6	1.6	0.1	1.3	25		10.5		1.0	1.6	15		0.7		1.0	0.5	13	78
		%TFA			0.4	0.1	0.6	0.3	7	2	0.1	1.7	32		14		1.3	2	19		0.9		1.3	0.6	17	
	Kōhūhū	mg/g			0.6		0.3	0.6	3.7	0.1		0.8	14		17		0.4		23		0.9		0.3		25	87
		%TFA			0.7		0.3	0.7	4.3	0.1		0.9	16.1		19.5		0.5		26.4		1		0.3		29	
	Black Māpau	mg/g			0.4		0.1		3.3			0.3	13		14		0.2		16		1.0		0.5		18	67
		%TFA			0.6		0.1		5.0			0.4	19.4		21		0.3		24		1.5		0.7		27	
	Turpentine tree	mg/g			0.8				8.1		0.9	2.2	32		17			2	25		0.6		2.0		23	114
		%TFA			0.6				7.1		0.8	1.9	28.1		15			1.8	22		0.5		1.8		20.2	

TABLE 3.3 cont. Fatty Acid Profile of 46 NZ Native Species

DICOTYLEDON CONTINUED																										
BOTANICAL FAMILY	COMMON NAME	Fatty acids*	10:0	10:x	12:0	12:x	14:0	15:x	16:0	16:1	17:0	18:0	18:1	18:1x	18:2	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:2	20:3	20:4	Ax	22:0	22:1	TFA
Proteaceae	Rewarewa / NZ Honeysuckle	mg/g	1.8		1.4		0.2		9	12		0.7	9.7	6.1	19.3		1.9				0.2			1.1		64
		%TFA	2.8		2.2		0.3		14.4	19		1.1	15.2	9.5	30.2		3				0.3			1.7		
Rosaceae	Bidibid	mg/g	0.8		0.1		0.1		4.3			2.4	9.5		22.2		26.9			0.1	0.7					67
		%TFA	1.2		0.1		0.1		6.4			3.6	14.2		33		40.1			0.1	1					
Rubiaceae	Karamū / red-fruited coprosma	mg/g			0.5				7.6				12		50											70
		%TFA			0.7				11				17		72											
Rutaceae	Wharangi / Three finger	mg/g							7.1				18		57											82
		%TFA							8.7				22		70											
Sapindaeceae	Tītoki[a]	mg/g	0.2		0.2				11	3.3	0.2	2.9	139		12		3.8	33	92							298
		%TFA	0.1		0.1				3.7	1.1	0.1	1	46.6		4.0		1.3	11	30.9							
Verbenaceae	Pūriri	mg/g			0.1		0.1		3.3	0.2	0.2	2.1	8.3		15		0.3	0.1								30
		%TFA			0.3		0.3		11	0.7	0.7	7.0	28		50		1	0.3								
Violaceae	Māhoe / Whiteywood	mg/g			4.6		1.2	3.2	16.9	0.6	0.1	5	26.7		70.7		1.7	0.6	0.4							132
		%TFA			3.5		0.9	2.4	12.8	0.4	0.1	3.8	20.2		53.5		1.3	0.4	0.3							

TABLE 3.3 cont. Fatty Acid Profile of 46 NZ Native Species

MONOCOTYLEDON																									
BOTANICAL FAMILY	COMMON NAME	Fatty acids*	10:0	10:x	12:0	12:x	14:0	15:x	16:0	16:1	17:0	18:0	18:1	18:1x	18:2	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:2	20:3	20:4	Ax	22:0	22:1	TFA
Agavaceae	Cabbage Tree[a]	mg/g			0.5				13	0.5	0.4	4.7	30		205		0.3									254
		%TFA			0.2				5	0.2	0.2	1.9	12		81		0.1									
	NZ Flax[a]	mg/g			0.5		0.2		36	1.9	0.2	14	61		242		0.8	0.5								357
		%TFA			0.1		0.1		10	0.6	0.1	3.9	17		68		0.2	0.1								
Iridaceae	NZ Iris[a]	mg/g	0.1		0.5		0.8		5.2	0.4		1	14		9.4		0.6									32
		%TFA	0.3		1.3		2.3		16	1		2.7	45		30		1.5									
Liliaceae	Native Ground Lily[a]	mg/g			1.1	0.2	0.2		15.2	0.5	0.1	1.6	18		129		0.2									166
		%TFA			0.7	0.1	0.1		9.2	0.3	0.1	1	10.8		77.7		0.1									
	NZ Blueberry[a]	mg/g			0.8		6.8	0.4	14			1.6	8.7	5.6	35.5		0.1	1.0								75
		%TFA			1.0		9.1	0.5	19			2.1	11.6	7.5	47.3		0.1	1.3								
Palmaceae	Nīkau Palm[a]	mg/g			1.5		2.1		7.4	0.4	0.5	1.2	9.7		5.9											29
		%TFA			5.2		7.2		25.5	1.4	1.7	4.1	33.4		21											
Smilaceae	Supplejack[a]	mg/g			0.7			2	1	0.1		3.2	13		25		2.5	3.1								51
		%TFA			1.4			4	2	0.2		6.3	26		49		4.9	6.1								

TABLE 3.3 cont. Fatty Acid Profile of 46 NZ Native Species

GYMNOSPERMAE																									
BOTANICAL FAMILY	COMMON NAME	Fatty acids*	10:0	10:x	12:0	12:x	14:0	15:x	16:0	16:1	17:0	18:0	18:1	18:1x	18:2	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:2	20:3	20:4	Ax	22:0	22:1	TFA
Podocarpaceae	Kahikatea / White Pine	mg/g	0.1		2.7	0.2	0.6	0.4	11	0.5	0.6	3.7	38		39		3.1	1.6	1.4	1.2	26	3	3.4	2		139
		%TFA	0.1		1.9	0.1	0.4	0.3	8	0.3	0.4	2.7	27.3		28.1		2.2	1.2	1	0.9	19	2.2	2.4	1.4		
	Rimu / Red pine	mg/g	0.6		0.1		0.2		1.7	0.1		0.5	1.5		0.9		0.4									6
		%TFA	10		1.7		3.3		28	1.7		8.3	25		15		6.7									
	Totara	mg/g	2.8	0.8	1.6	1.1	0.9	0.5	7.4	0.2	0.2	6.9	46		37		6.2	3	1.7	1.2	21.7	13	0.4	0.7		153
		%TFA	1.8	0.5	1.0	0.7	0.6	0.3	4.8	0.1	0.1	4.5	30		24		4.0	2	1.1	0.8	14	8.5	0.3	0.5		
	Miro / Brown Pine	mg/g			0.2				23		0.3	16	106		174		1.3	0.4		9	13					344
		%TFA			0.1				6.8		0.1	4.6	31		51		0.4	0.1		2.6	3.8					
MARINE or AQUATIC																									
Azollaceae	NZ aquatic fern	mg/g							6.7				2.1		1.0		3									13
		%TFA							52				16.2		8		23.1									
Hormosiraceae	Neptune's Necklace	mg/g			0.7		0.4		4.1	0.1		0.8	4.8	2.6	1.8		1.3							2.2	0.9	20
		%TFA			3.5		2.0		21	0.5		4.0	24	13	9		7							11	5	

KEY: * Designated by the number of carbon atoms followed by the number of double bonds. Only those fatty acids with >0.1% are listed in this table.

[a] = Seed oils studied by other researchers. (See Appendix I)

n = Number of carbon atoms from the methyl terminal end of the fatty acid hydrocarbon chain to the closest double bond

x = Unknown branched or enoic fatty acid

Ax = Unknown long chain fatty acid

TFA = Total Fatty Acid

3.3.4.1 Groupings of plant species

Each species was allocated a grouping according to similar higher levels or unusual contributions from a common fatty acid. Groups 1, 2, 3 and 4 included those species with higher levels or unusual contributions from polyunsaturated, monounsaturated, saturated and uneven-chain fatty acids respectively. Group 5 included those species that are unlikely to be of value as a potential fatty acid or lipid source compared to others that were analysed. Note these groupings were not exclusive, therefore a number of species such as the Chatham Island forget-me-not appear in more than one group. Tables 3.4 to 3.15 list the species grouped as explained below. The values are expressed as fatty acid weight (mg/g dry seed weight) and fatty acid percentage (weight % of the total fatty acid content). Comparison of all these native species with other valuable commercial oil species will be discussed in section 3.5.

Group 1, (significant in terms of relatively high levels of specified polyunsaturated fatty acids), was further divided into three subgroups: those with polyunsaturated eicosenoic (20:2-20:4) acids (Group 1a); those with alpha- (n-3), or gamma- (n-6), linolenic (18:3) acids, (Group 1b); and those with large quantities of linoleic (18:2n-6) acid, Group 1c (See Tables 3.4 to 3.6).

Group 2 (significant in terms of relatively high levels of specified monounsaturated fatty acids) was further divided into four subgroups: those with the greatest levels of erucic (22:1n-9) acid, (Group 2a); those with gadoleic (20:1n-11) acid, (Group 2b); those with oleic (18:1n-9) acid, (Group 2c); and those with the highest levels of palmitoleic (16:1n-7) acid, (Group 2d) (See Tables 3.7 to 3.10).

Group 3 (significant in terms of highest levels of specified saturated fatty acids) was divided into three subgroups: those with significant eicosanoic (20:0) acid, (Group 3a); those with highest levels of stearic (18:0) or palmitic (16:0) acids, (Group 3b); while Group 3c included those with greatest myristic (14:0) or lauric (12:0) acids (See Tables 3.11 to 3.13).

Group 4 was divided into two subgroups 4a and 4b, which included those with the highest levels of c17 or c15 acids respectively (Table 3.14).

3.3.4.1.1 Group 1. Results of species with high levels of polyunsaturated fatty acids

All of the 46 native species contained variable amounts of polyunsaturated fatty acids. Although vegetable oils containing high percentages of polyunsaturated fatty acids other than linoleic acid are generally unsuitable as an edible oil, benefits of such oils may be found in semi-drying or drying oil-based products such as paints, coatings, varnishes, linoleum, oilcloth, and printing inks.

The cosmetic, alternative health products or pharmaceutical industries may also find particular use for those high in n-3 or n-6 fatty acids. For example, oils containing precursor fatty acids to the n-3 or n-6 eicosanoid products (discussed in Section 1.2.5), may have relevance for humans, or animal species with an inability to produce sufficient quantities of these precursors. Such oils may also have relevance for the treatment of ischemic heart disease and thrombotic events in humans as supplementation of fish oils high in eicosapentaenoic acid (EPA or C20:5n-3) or docosahexaenoic acid (DHA or C22:6n-3) has been suggested by some researchers as a preventative measure for patients with a high risk of such occurrences (Bang & Dyerberg, 1973, 1980; Kremer *et al.*, 1985).

Six native species (Marlborough rock daisy, ribbonwood, kahikatea, tōtara and miro) had significant levels, (>5mg/g dry seed weight) of polyunsaturated eicosenoic acids. The latter three (all from the *Podocarpaceae* family) had the highest levels with greater than 20% of the total fatty acid weight percent in kahikatea and tōtara coming from these eicosenoic acids (See Table 3.4). This is unusual, as polyunsaturated eicosenoic acids are not usually found in these quantities in plant oils but are more common in fish oils.

TABLE 3.4 Group 1a (C20:2 – C20:4 Eicosenoic Acids)

		Kahikatea / White Pine	Marlborough Rock Daisy	Miro / Brown Pine	Ribbonwood	Totara
C20:2	*mg/g*	1.2		9.0	2.6	1.2
	%TFA	0.9		2.6	5.2	0.8
C20:3	*mg/g*	26	6.3	13	0.2	21.7
	%TFA	19	4.3	3.8	0.4	14
C20:4	*mg/g*	3.0			1.4	13
	%TFA	2.2			2.8	8.5
Total PUFA C20 acids	*%TFA*	*22.1*	*4.3*	*6.4*	*8.4*	*23.3*
TFA	***mg/g***	***139***	***149***	***344***	***50***	***153***

The most commonly found polyunsaturated fatty acid (PUFA), in the 46 native species tested, were linoleic and linolenic acids, as would be expected as these are the most common polyunsaturated fatty acids in seed oils. Of note were, wineberry, miro/brown pine, kōhia/NZ passionfruit, native ground lily and cabbage tree which had 50-80% weights of the total fatty acid (TFA) content as linoleic acid; red kaka beak, bidibid and snowberry which had 23, 40 and 43% TFA weights of α-linolenic acid respectively, and Chatham Island forget me-not which had an 11% TFA weight of γ-linolenic acid (See Table 3.5 and Table 3.6).

These levels of linoleic and linolenic acids in the native species are of interest as they compare favourably with commercial oils such as sunflower (60-80%), linseed (50%) and evening primrose oil (12%) known for their high seed oil proportions of linoleic, α-linolenic and γ-linolenic respectively.

TABLE 3.5 Group 1b (n-6 and n-3 C18:3, Linolenic Acids)

		Chatham Is. Forget-Me-Not	NZ Aquatic fern	Red Kaka Beak	Bidibid	Snowberry
C18:3 (n-6)	*mg/g*	28.1				
	%TFA	11.4				
C18:3 (n-3)	*mg/g*		3	12.8	26.9	119.0
	%TFA		23.1	22.8	40.1	43.3
TFA	***mg/g***	***246***	***13***	***57***	***67***	***275***

TABLE 3.6 Group 1c (C18:2n-6, Linoleic Acid)

		Cabbage Tree	Kōhia / Native Passionfruit	Miro / Brown Pine	Native Ground Lily	NZ Flax	Māhoe / Whiteywood
C18:2 (n-6)	*mg/g*	205	171	174.2	129	242	70.7
	%TFA	81	63	51	77.7	68	53.5
TFA	***mg/g***	***254***	***272***	***344***	***164***	***357***	***132***

3.3.4.1.2 Group 2. Results of species with high levels of monounsaturated fatty acids

The most common monounsaturated fatty acids (MFA) occurring in the 46 native species were C16:1n-7, C18:1n-9, C20:1n-11 and C22:1n-9. Of these four, the highest proportion from any one MFA usually came from C18:1(n-9), as would be expected, as it is the most common MFA in plant seed oils. The only exceptions were in the kōhūhū and black māpau; members of the *Pittosporaceae* family which both had higher proportions from the MFA erucic (C22:1n-9) and gadoleic (C20:1n-11) acids.

Oils with a high percentage of erucic acid are not as suitable as edible oils due to concerns about the cardiopathic potential of the fatty acid (Kramer *et al.*, 1983). However, these oils may have uses in industrial or non-food applications, such as in the manufacture of lubricants and fatty acid derivatives such as the erucamides. The highest TFA weight proportions (17 to 30% weight) of erucic acid were all found in the *Pittosporaceae* species (see Table 3.7).

TABLE 3.7 Group 2a (C22:1n-9, Erucic Acid)

	Black Māpau	Kōhūhū	Lemonwood	Turpentine Tree
mg/g	18	25	13	23
%TFA	27	29	17	20.2
TFA	***67***	***87***	***78***	***114***

All four *Pittosporaceae* species tested were also listed in those species with significant levels (19-31% weight) of gadoleic acid (see Table 3.8). Tītoki in particular contained 30.9% TFA of gadoleic acid. Although gadoleic acid is not one of the most important commercial fatty acids, it is commonly found in seed oils and has been used as a component of lubricating oils.

TABLE 3.8 Group 2b (C20:1n-11, Gadoleic Acid)

	Black Māpau	Kōhūhū	Lemonwood	Turpentine Tree	Tītoki	Red Matipo / Māpau / Māpou
mg/g	16	23	15	25	92	10
%TFA	24	26.4	19	22	30.9	18.5
TFA	***67***	***87***	***78***	***114***	***298***	***53***

Vegetable oils containing large quantities of oleic acid, (18:1n-9) are likely to have relevance in food industry applications as they have desirable antioxidant properties, do not undergo flavour reversion and are more heat stable than lower MW monounsaturates at higher temperatures, (thus making them more suitable as cooking oils). The native species with significant levels of oleic acid (30 to 88% wt of TFA) included miro / brown pine, Chatham Island forget-me-not, NZ iris, tītoki, and the three members of the *Araliaceae* family analysed: five-finger, patē/seven finger and lancewood. These latter three species also contained the highest contribution from oleic acid of all 46 species analysed, containing 78, 82 and 88% TFA weights of oleic acid respectively (see Table 3.9) and compare favourably with other commercial high-oleic oils such as olive oil (71%), high oleic safflower (80%) and canola (64%).

TABLE 3.9 Group 2c (C18:1n-9, Oleic Acid)

	Chatham Is. Forget-Me-Not	Five Finger	NZ Iris	Lancewood	Miro / Brown Pine	Patē / Seven Finger	Tītoki
mg/g	100	95	14	163	106	193	139.0
%TFA	40.7	78	45	87.5	31	82	46.6
TFA	***246***	***122***	***32***	***186***	***344***	***234***	***298***

The four species containing the highest contributions (mg/g dry wt) of palmitoleic acid, (C16:1 n-7) were Chatham Island forget-me-not, native broom, perennial hibiscus, and rewarewa/NZ honeysuckle, the results of which are listed in Table 3.10. Palmitoleic acid is also commonly distributed among vegetable oils and suitable for use in either food or non-food industries.

Table 3.10 Group 2d (C16:1n-7, Palmitoleic Acid)

	Chatham Is. Forget-Me-Not	Native Broom	Perennial Hibiscus	Rewarewa / NZ Honeysuckle
mg/g	8.2	4.2	5.7	12
%TFA	3.3	12	3.9	19
TFA	***246***	***36***	***147***	***64***

3.3.4.1.3 Group 3. Results of species with high levels of saturated fatty acids

Palmitic acid (C16:0), and stearic acid (C18:0), number among the most commonly distributed fatty acids in natural fat and oil sources. They have a great range of industrial applications in both food and non-food applications such as in the formation of edible fats and oils for biscuits, confectionery and other processed foods to the production of soaps. These two fatty acids also featured regularly among the top six quantitative fatty acids in the 46 species.

The highest amounts (mg/g dried seed material) of both palmitic and stearic acids respectively, were found in miro/brown pine (23 mg/g, 16 mg/g); kōhia/NZ passionfruit (26 mg/g, 13 mg/g); NZ flax (36 mg/g, 14 mg/g); and Chatham Island forget-me-not (28.1 mg/g, 10.8 mg/g) (See Table 3.11). The highest percentage weight of palmitic acid was found in the nīkau palm (25.5% TFA), which was to be expected, since members of the *Palmaceae* family are known for their significant proportion (often >40%) of palmitic acid (Orthoefer, 1996).

TABLE 3. 11 Group 3b (C18:0, Stearic and C16:0, Palmitic Acids)

		Nikau Palm	Chatham Is. Forget-Me-Not	Kōhia / Native Passionfruit	Miro / Brown Pine	NZ Flax
C18:0	mg/g	1.2	10.8	13	16	14
	%TFA	4.1	4.4	4.8	4.6	3.9
C16:0	mg/g	7.4	28.1	26	23	36
	%TFA	25.5	11.4	9.6	6.8	10
TFA	***mg/g***	***29***	***246***	***272***	***344***	***357***

Other saturated fatty acids such as arachidic (C20:0), myristic (C14:0), and lauric (C12:0) usually made up a minor proportion of the total fatty acid content in the native species, although 11% weight of arachidic acid was found in tītoki (see Table 3.12); and 30 and 38% weights of myristic acid were found in perennial hibiscus and kawakawa/pepperwood respectively (see Table 3.13).

TABLE 3. 12 Group 3a (C20:0, Arachidic Acid)

	Red Matipo / Māpau / Māpou	Supplejack	Native Broom	Tītoki
mg/g	2.9	3.1	3.2	33
%TFA	5.4	6.1	9	11
TFA	***53***	***51***	***36***	***298***

TABLE 3.13 Group 3c (C12:0, Lauric and C14:0, Myristic Acids)						
		Māhoe / Whiteywood	Marlborough Rock Daisy	NZ Blueberry	Kawakawa/ Pepperwood	Perennial Hibiscus
C14:0	mg/g		14	6.8	14	44
	%TFA		9.4	9.1	38	30
C12:0	mg/g	4.6				
	%TFA	3.5				
TFA	mg/g	132	149	75	37	147

Arachidic acid has a wide distribution in natural fats and oils, however it is usually a minor constituent and not one of the most important commercial fatty acids. Due to the high arachidic (11%) and gadoleic (31%) acid in tītoki, its seed oil may have more potential use as a lubricating oil rather than an edible oil.

Myristic and lauric acids also have a wide distribution in nature. As the names suggest, myristic acid is common in the *Myristicaceae* family, and lauric acid in the *Lauraceae* family, however the most valuable commercial sources of these fatty acids include those of the *Palmae* family and in particular coconut, babassu and palm kernel oils which contain 40-50% of lauric acid and 16-18% of myristic acid. The main industrial uses of these fatty acids is in non-food industries such as the cosmetic, soap or detergent industry (since they have detergent properties), although the palm oils also have applications in the food industry. None of the 46 species tested contained a comparable proportion of both of these fatty acids.

3.3.4.1.4 Group 4. Results of species with high levels of uneven-chain fatty acids and Group 5. Remaining native species not previously grouped

Uneven-chain fatty acids are generally uncommon in the seed oils of plants, are usually only found in trace amounts (< 0.1mg/g dried wt), and tend not to be of commercial significance. However, it was interesting to see that a number of native species were found to contain C17 acids such as margaric, C17:0 and suspected C15 acids. C17 acids were most pronounced in puka/shining broadleaf, (2.13% wt TFA); kōhia/NZ passionfruit (1.7mg/g dried wt); and native broom (1.3% wt TFA), while C15 acids were most pronounced in supplejack/kareao (4% wt TFA), māhoe/whiteywood (2.4%wt TFA) and ngaio (2.3%wt TFA) (see Table 3.14).

The remaining eleven native species not listed in any of the four previous groups did not contain large quantities of any particular fatty acids compared to other native species analysed. They were the hīnau, karaka, karamū, kōwhai, lacebark, pigeonwood, pūriri, rimu/red pine, tawa (fruit coat), whārangi/three finger and Neptune's necklace (see Table 3.15).

TABLE 3.14 Group 4a & 4b (C17, or C15 Acids)

		Kōhia / Native Passionfruit	Nīkau palm	Puka / Shining Broadleaf	Supplejack / Kareao	Māhoe / Whiteywood	Ngaio
C17	mg/g	1.7	0.5	1.8			
	%TFA	0.6	1.7	2.3			
C15	mg/g				2	3.2	0.6
	%TFA				4	2.4	2.3
TFA	mg/g	272	29	77	51	132	26

TABLE 3.15 Group 5: Other Species (Not significant compared with other species)

		Hīnau	Karaka	Karamū / red-fruited coprosma	Kōwhai	Narrow-leaved Lacebark	Pigeonwood	Pūriri	Rimu / Red pine	Tawa (fruit coat)	Neptune's necklace	Wharangi / Three finger
C10:0	mg/g								0.6	0.2		
	%TF								10	5		
C12:0	mg/g	0.1	0.4	0.5	1.4	0.8	0.4	0.1	0.1	0.5	0.7	
	%TF	0.7	0.5	0.7	2.1	19	3.2	0.3	1.7	12	3.5	
C14:0	mg/g	0.1	0.1				0.1	0.1	0.2	0.6	0.4	
	%TF	0.7	0.1				0.9	0.3	3.3	15	2.0	
C15:x	mg/g		0.1		0.4							
	%TF		0.1		0.6							
C16:0	mg/g	3	14	7.6	9.8	1.2	2.3	3.3	1.7	0.8	4.1	7.1
	%TF	21.4	17	11	14	29	18	11	28	20	21	8.7
C16:1	mg/g	1.5	0.4				0.1	0.2	0.1		0.1	
	%TF	10.7	0.5				0.9	0.7	1.7		0.5	
C17:0	mg/g		0.2					0.2				
	%TF		0.2					0.7				
C18:0	mg/g	0.6	4.3		3.7		0.5	2.1	0.5	0.3	0.8	
	%TF	4.3	5		5.4		3.9	7	8.3	7.5	4.0	
C18:1	mg/g	3.6	24	12	22		2.5	8.3	1.5	0.7	4.8	18
	%TF	25.6	28	17	31		20	28	25	18	24	22
C18:2	mg/g	4.6	38	50	33	2.2	5.7	15	0.9	0.8	1.8	57
	%TF	32.9	45	72	47	52	46	50	15	20	139	70
C18:3 (n-3)	mg/g	0.6					0.9	0.3	0.4	0.1	1.3	
	%TF	4.3					7.3	1	6.7	2.5	7	
C20:0	mg/g		2.6					0.1				
	%TF		3					0.3				
C20:1	mg/g		0.6									
	%TF		0.7									
TFA	mg/g	14	85	70	70	4	13	30	6	4	20	82

3.4 CHANGES IN FATTY ACID COMPOSITION DURING DEVELOPMENTAL PERIODS.

The fatty acids produced in any one type of oilseed may vary with geographic location, soil type, climate, moisture, temperature, maturity of the seed, and agricultural practice (Chu & Sheldon, 1979). A high level of polyunsaturated eicosenoic acids (potential health value), were found in members of the *Podocarpaceae*. As fatty acid seed composition is likely to change due to different stages of seed maturity and fruiting kahikatea and tōtara trees were conveniently located, it was deemed worthwhile to undertake a small-scale survey of changes in fatty acid composition in these two native species to see what, if any, changes might occur.

The tōtara samples were collected from a close group of trees and the kahikatea samples were collected from two trees located in the Esplanade gardens of Palmerston North. Determination of the different developmental periods was determined by the state of development of the fruit. In both of these species, the seed (usually a singular seed), sits atop a small fruit receptacle. Hence, if the sample analysed was labelled as being "green", this meant that the undeveloped fruit receptacle and the seed were both a green colour. "Ripe" samples of the seed were collected when the fruit receptacle was ripe (orange to red colour), and in the kahikatea, the seed had turned from green to black/dark purple. "Old" samples were collected when the fruit had fallen off the tree and had disintegrated, and the seed had started to shrivel.

In the tōtara, two sets of "ripe" data are presented. The "ripe (stored)" values of tōtara come from the tōtara samples used in all the initial analyses. These consisted of ripe seeds that had been freshly removed from ripe fruit still attached to the tree, then dried in a 60°C oven overnight, cooled in a dessicator over silica, then ground to 1mm and stored in a dessicator over a period of weeks while different analyses were taking place. The one designated in both species as "ripe (fresh)" included seeds collected, dried in an oven and cooled as above, but then 0.77-0.83g duplicates of the whole seed were weighed out. The seeds were not ground but crushed directly in a culture tube with a glass rod while soaking in 2mls of toluene -

ready for the FAMES preparation as described in Section 2.2.1.3. These samples were analysed within days of collection.

Since the total fatty acid (TFA) average obtained for the "ripe (fresh)" tōtara samples (253.2mg/g dried weight), was significantly different to the initial values obtained for tōtara FAMES samples (151.2, 152.5, 154.7 and 154.8 mg/g dried weight), these higher values were not included in the averaged value (153.3mg/g dried weight) recorded in Table 3.3. A difference in the TFA is assumed to be due to degradation or loss of fatty acids due to storage or grinding. Based on the results obtained here, other native samples that were oily, ground and stored over a long period until sufficient quantity of plant material was available, are also likely to have suffered from loss or degradation of lipids. By comparing the lower % TFA values attained here with the higher values recorded from studies of native seed oils by other researchers, this may have occurred with kōhia (27.2 % *cf* 39.8%); NZ Iris (3.2% *cf* 6.8-20.4%); native rock lily (16.6% *cf* 20-26%) and the nīkau palm (2.9% *cf* 3.7-5.5%).

From analysis of the results presented in Table 3.16 and Figures 3.1 to 3.4, it can be seen that seed fatty acid composition did change over these developmental or storage periods tested in both the tōtara and kahikatea. In the tōtara, fatty acid quantities (mg/g dried material) obtained for the "green" and "old" samples were generally less than 10mg/g in quantity and were generally less than those from the two "ripe" samples. All fatty acid values for the "green" samples were generally the least, with those from the "ripe (fresh)" samples, being the greatest.

When comparing the "ripe (fresh)" and "ripe (stored)" samples of tōtara, the quantity of each of the fatty acids were similar between the two, except for C18:1, C18:2, C20:3, C20:4 and Ax where the quantities of these acids in the "ripe (fresh)" sample was greater. It was interesting to see that the majority of the degradation or losses in fatty acid content between the two ripe tōtara samples occurred in mono- or polyunsaturated fatty acids. This may be due to the fact that fatty acids containing double bonds are less stable than their saturated counterparts over longer storage periods due to their greater light and oxidative sensitivity.

In the tōtara, it was also interesting to see that levels of short chain fatty acids such as C12 and C14 acids were higher in the "green" and "old" samples than in either of the "ripe" samples (See Figure 3.6). When comparing the proportion of the fatty acids (% weight of TFA) between the four samples, the "green" and "old" samples of tōtara had much higher proportions of short-medium chain acids but lower proportions of C18:1, C18:2, C20:3 and unknown long chain fatty acids (Ax) than those in the two "ripe" samples (See Figure 3.7).

In kahikatea, similar patterns to those in tōtara occurred in both quantity and proportion of fatty acids (Figures 3.8 and 3.9). However, a major difference in quantity and proportion of C20:4 was noticed between the two species, with ripe (fresh) tōtara containing far greater amounts (26.9mg/g, 11.4% wt) than the ripe (fresh) kahikatea (3mg/g, 2.2% wt). As all the kahikatea samples were collected after the initial analysis of the tōtara samples, none of the kahikatea samples were stored for very long prior to analysis and the oil was extracted by crushing the seeds directly into the culture tubes. Loss of oil or fatty acids due to storage or processing should have been minimal.

In summary, decreases in short chain fatty acids and increases in C18:1, C18:2 and polyunsaturated long-chain fatty acids (quantity and proportion) occurs in tōtara and kahikatea as the immature seed and fruit develops to maturity (becoming ripe). As the fruit falls from the tree, degrades and the seed is left, the level of all the fatty acids fall. It is assumed that the loss in fatty acids between the "ripe (fresh)" and "ripe (stored)" samples of tōtara was due to the difference in maturity of the seeds at collection; or as a result of fatty acid degradation or loss. This degradation or loss may have occurred due to the longer storage period, or due to differences in the grinding / crushing procedure.

Tōtara seeds contain a significant proportion (>20%) of oil, and upon grinding, the seed material became quite sticky. It is possible that some of the seed fat may have stuck to the inner compartments of the grinder where the seed was being processed. By crushing the seeds directly into the tubes, ready for FAMES analysis, the loss of total seed oil would have been minimised, thus increasing the overall content.

TABLE 3.16 Changes in Fatty Acid Distribution in Tōtara and Kahikatea During Growth and Storage Periods

Fatty Acid		Totara				Kahikatea	
		Green	*Ripe (Fresh)*	*Ripe (Stored)*	*Old*	*Green*	*Ripe (Fresh)*
C8:0	*mg/g*		0.2				
	%TFA		0.1				
C10:0	*mg/g*	1.5	3.4	2.8	2.3	0.5	0.1
	%TFA	5.0	1.4	1.8	3.6	0.6	0.1
C10:x	*mg/g*		1.0	0.8	0.2		
	%TFA		0.4	0.5	0.3		
C12:0	*mg/g*	3.0	2.5	1.6	8.2	8.9	2.7
	%TFA	9.9	1.1	1.0	13.0	10.5	1.9
C12:x	*mg/g*	1.3	0.8	1.1	3.8	5.4	0.2
	%TFA	4.3	0.3	0.7	6.0	6.4	0.1
C14:0	*mg/g*	2.1	1.6	0.9	3.1	4.4	0.6
	%TFA	7.0	0.7	0.6	4.9	5.2	0.4
C15:x	*mg/g*	1.0	1.6	0.5	2.5		0.4
	%TFA	3.3	0.7	0.3	4.0		0.3
C16:0	*mg/g*	2.3	8.5	7.4	2.0	5.1	11.0
	%TFA	7.6	3.6	4.8	3.2	6.0	7.9
C16:1	*mg/g*	0.7	1.7	0.2	0.8	0.9	0.5
	%TFA	2.3	0.7	0.1	1.3	1.1	0.4
C17:0	*mg/g*	0.8		0.2		0.3	0.6
	%TFA	2.6		0.1		0.4	0.4
C18:0	*mg/g*	0.5	3.9	6.9	1.0	1.5	3.7
	%TFA	1.7	1.6	4.5	1.6	1.8	2.7
C18:1	*mg/g*	3.4	57.9	46.0	10.8	14.6	38.0
	%TFA	11.3	24.6	30.0	17.1	17.3	27.4
C18:2	*mg/g*	4.6	66.8	37.0	10.4	14.7	39.0
	%TFA	15.2	28.4	24.1	16.5	17.4	28.2
C18:3a	*mg/g*	2.4	9.3	6.2	0.2	3.3	3.1
	%TFA	7.9	4.0	4.0	0.3	3.9	2.2
C20:0	*mg/g*	0.1	3.0	3.0	0.7	0.8	1.6
	%TFA	0.3	1.3	2.0	1.1	0.9	1.2
C20:1	*mg/g*	0.2	1.4	1.7	0.3	0.5	1.4
	%TFA	0.7	0.6	1.1	0.5	0.6	1.0
C20:2	*mg/g*		0.7	1.2		0.5	1.2
	%TFA		0.3	0.8		0.6	0.9
C20:3	*mg/g*	0.7	36.1	21.7	5.6	8.4	26.0
	%TFA	2.3	15.3	14.2	8.9	10.0	18.8
C20:4	*mg/g*	3.1	26.9	13.0	3.9	4.5	3.0
	%TFA	10.3	11.4	8.5	6.2	5.3	2.2
C22	*mg/g*	0.3	0.1	0.7	0.7	2.5	2.0
	%TFA	1.0	0.0	0.5	1.1	3.0	1.4
Ax	*mg/g*	2.2	8.1	0.4	6.6	7.6	3.4
	%TFA	7.3	3.4	0.3	10.5	9.0	2.5
TFA	**(mg/g)**	**30.2**	**235.2**	**153.3**	**63.1**	**84.4**	**138.5**

Key: C = Carbon; **TFA** = Total Fatty Acid; **x** = unknown unsaturated fatty acid; **Ax** = unknown very long chain fatty acid

FIGURE 3.6 Changes in Fatty Acid Distribution inTotara During Development & Storage

(mg /g dry wt)

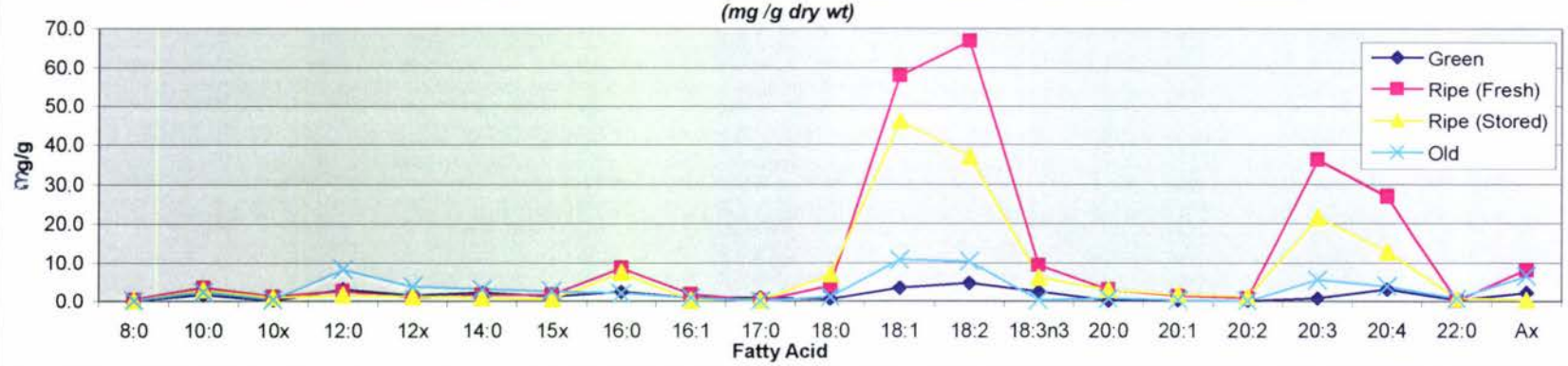


FIGURE 3.7 Changes in Fatty Acid Percentage in Totara During Development and Storage

(% weight of Total Fatty Acid)

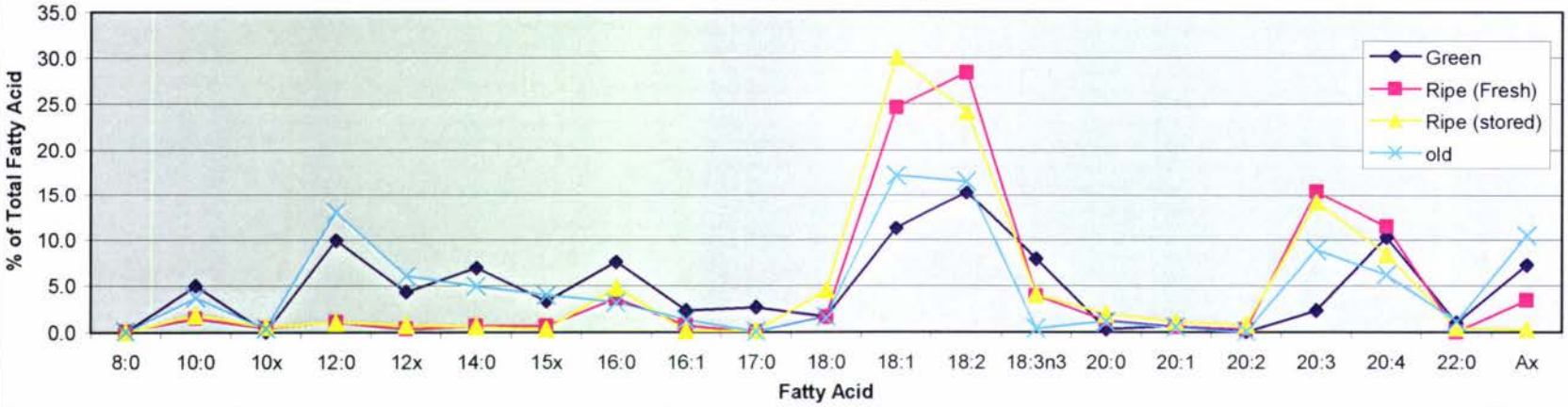


FIGURE 3.8 Changes in Fatty Acid Distribution in Kahikatea During Development
(mg / g dry wt)

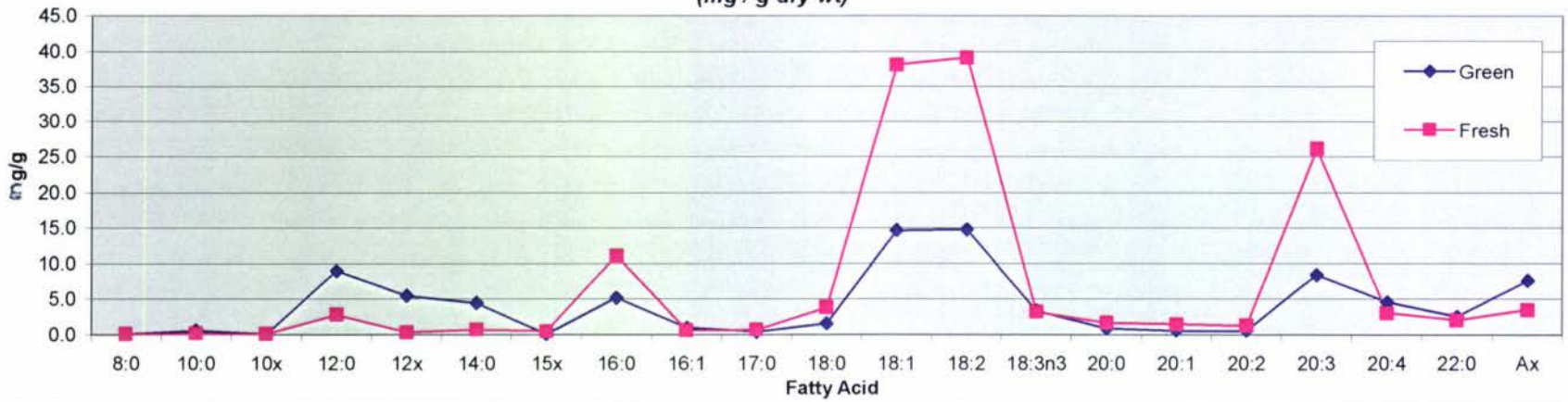
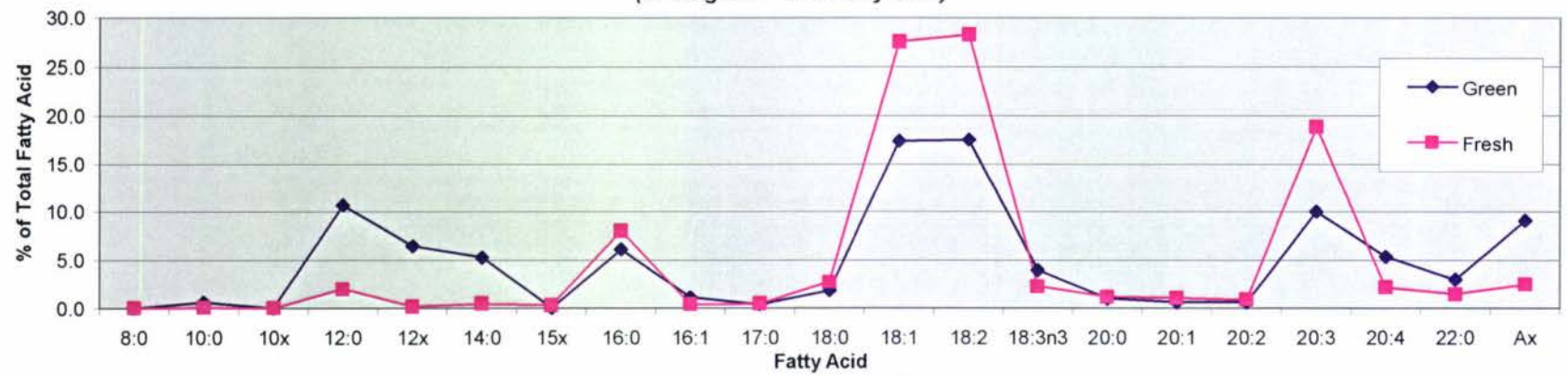


FIGURE 3.9 Changes in Fatty Acid Percentage in Kahikatea During Development
(% weight of Total Fatty Acid)



3.5 DISCUSSION OF THE COMMERCIAL POTENTIAL OF THE 46 NATIVE SPECIES

3.5.1 Introduction

Once the data had been collected and collated, a preliminary evaluation of any potential industrial or commercial uses of the 46 native seed oils studied was made. Note that an in-depth study of the commercial potential of these 46 native species is beyond the scope of this thesis. All 46 native species were first evaluated in terms of potentially valuable quantities of certain known valuable fatty acids or total oil percent compared to those in both edible and non-edible commercial vegetable oils (Sections 3.5.2 and 3.5.3). The native plants with the most potential as a fatty acid or oil source (26 species), were then evaluated for their historical and current uses, and cultivation requirements in order to determine any potential commercial value. Summaries of these findings are discussed in Sections 3.5.4 and 3.5.5 respectively.

3.5.2 Comparison of Valuable Fatty Acid Composition in Commercially Used Vegetable Oils with Native Equivalents

In the edible foods industry, vegetable oils may be classified according to a principal fatty acid or a characteristic fatty acid composition (Orthoefer, 1996). In the non-edible industry, the classification of oils is often based on the type of non-edible application they would be suited for, such as the drying, detergency, or saponification properties of the oil. These classifications also have much to do with the fatty acid or lipid composition of the oil. In the following discussion, common commercial vegetable oil sources with a high proportion (% weight) of a particular fatty acid or group of acids will be compared to native species of potential equivalence.

The main categories are those with potentially valuable contributions from:

1. Saturated fatty acids including lauric (C12:0), myristic (C14:0) or palmitic acid (C16:0)
2. Monounsaturated fatty acids including oleic acid (C18:1n-9 -high or medium levels) or erucic acid (C22:1)
3. Polyunsaturated fatty acids including medium and high levels of linoleic acid (C18:2n-6), and useful proportions from C18:3n-3 and/or C18:3n-6 linolenic acid
4. Other valuable fatty acids or fatty acid groups

3.5.2.1 Species with high contributions from saturated fatty acids

Although palmitic and lauric acids might be considered the most important commercial saturated fatty acids of all, the important saturated fatty acids in the edible or non-edible food industries can be divided into two groups according to their chain length. Palmitic acid (16:0) and stearic acids (18:0) can be included in the long-chain fatty acid range and lauric (C12:0) and myristic (C14:0) acids in the medium-chain range.

Oils with a high proportion of palmitic and stearic acid are more likely to be directed towards use in the edible foods industry and are included extensively in a range of processed foods such as cooking fats, spreads, chocolate, confectionery and packaged foods. Both fatty acids are relatively stable at high temperatures, (stearic being the more heat stable of the two) and are less likely to form off flavours or odours due to oxidative degradation. The main industrial uses of high palmitic or stearic acid oils or fats such as palm oil and animal fats include soap, candle or crayon manufacturing and in the oleochemical industry (Luhs & Friedt, 1994b).

The downside of these saturated fatty acids in the edible foods industry, is due to the fact that many nutritional advisory committees worldwide such as the NZ Dietetic Association (Bremer, 1994) and the Department of Health (1991a, 1991b), recommend that total fat intake should be reduced to less than 33-30% of the total energy intake and saturated fatty acids to less than 12% of the total energy intake. Consequently, the consumer demand for such oils and their sources has fallen (Wood, 1992).

Both stearic and palmitic acid are found in a variety of animal and plant sources in nature but of the two, palmitic acid is generally found in greater quantities in vegetable oils, while sources of stearic acid are more likely to be found in animal fats. Palm oil, (different from palm kernel oil), is the principal commercial palmitic acid oil and is obtained from the palm; *Elaesis guineensis* which contains 32 to 47% palmitic acid (Orthoefer, 1996). Other vegetable sources high in palmitic acid include cocoa butter (~26%) and cottonseed oil (~25%).

The only native sources of a comparable percentage weight of palmitic acid are the nīkau palm (~26%) and puka (~20%), (see Table 3.17). These are not likely to be of any commercial potential as an oil source of palmitic acid, as the TFA content in nīkau and puka are much lower (2.9% and 7.7% weights) than the commercial palms (>20%). However, a number of native species such as flax, kōhia and miro are a good oil source (27-36% wt TFA) and contain a large quantity of palmitic acid per gram of dried seed weight (23-36mg/g), (See Table 3.12). These latter species are more likely to be of commercial value. None of the 46 native species tested are likely to be potential sources of stearic acid compared to those levels able to be obtained from either cocoa butter (34.5%) or 12.3 – 24.5% in animal fats (White, 1992).

Oils with high proportions of medium (C12-C14) or short-chain fatty acids (C6-C10) have potential applications in both edible and non-edible applications, though are more likely to be used in the latter due to their greater susceptibility to undesirable flavour reversion in edible applications. Commercial vegetable oils with high proportions of these acids mainly include oils from the palms specifically coconut, babassu, and palm kernel oils. In these, the lauric acid content ranges from about 40 to 50%.

Edible food applications of oils such as coconut include use in confectionery, biscuits and hard butters. However, unpleasant off-flavours may be produced from oxidation and hydrolysation of these acids (Luhs & Friedt, 1994b).

Industrial uses of high lauric oils like coconut, include soap, shampoo, detergent and cleaning agent production, (helps maintain the balance of water solubility versus detergency, lathering characteristics); cosmetics (formulations are more easily absorbed due to low melting point, act as emollients in skin lotions); and in pharmaceutical industries (able to carry fat-soluble vitamins and other medicinal substances) (Luhs & Friedt, 1994a; Orthoefer, 1996).

None of the 46 native species studied could match the high lauric percentage of these palm oils but those with the highest lauric (māhoe /whiteywood, 3.5%) and myristic percentage weights: (Marlborough rock daisy, 9.4%; NZ blueberry, 9.1%; kawakawa/pepperwood, 38%; perennial hibiscus, 30%) are listed in Table 3.17. Since the percentage weights of the native species do not compare favourably with those of the commercial oils, which are high in lauric or myristic acids, no further discussion regarding the potential use of these native species as a source of these two fatty acids will be made.

TABLE 3.17 Comparison of Commercial Vegetable Oils High in Palmitic, Myristic and Lauric Fatty Acids with Potential Native NZ Equivalents.

	12:0	14:0	16:0	18:0	20:0	18:1	18:2 (n-6)	18:3 (n-3)	Others	TFA (mg/g)
High palmitic, (16:0) plant oils										
Palm	0.3	1.1	**45.1**	4.7	0.2	38.8	9.4	0.3	16:1	
Cocoa butter		0.1	**25.8**	**34.5**	1.1	35.3	2.9		16:1	
Cottonseed		0.9	**24.7**	2.3	0.1	17.6	53.3	0.3	16:1, 17	
Nīkau Palm	*5.2*	*7.2*	***25.5***	*4.1*		*33.4*	*21*		*16:1, 17*	*29*
Puka	*0.8*	*0.5*	***19.5***	*6.4*	*1.3*	*54.5*	*12.5*		*16:1, 17, 20:1*	*77*
High medium-chain saturated fatty acid (12:0, 14:0) plant oils										
Coconut	**48.5**	**17.6**	8.4	2.5	0.1	6.5	1.5		6, 8, 10	
Palm kernel	**49.6**	**16**	8	2.4	0.1	13.7	2.0		6, 8, 10	
Babassu	**44.2**	**15.8**	8.6	2.9	0.1	15.1	1.7		6, 8, 10	
Māhoe / Whiteywood	***3.5***	***0.9***	*12.8*	*3.8*	*0.4*	*20.2*	*53.5*	*1.3*	*15x, 16:1, 17, 20:1*	*132*
Marlborough Rock Daisy	***0.9***	***9.4***	*11*	*5.2*		*18*	*49*		*10, 18:3(n-6) 20x*	*149*
Perennial Hibiscus		***30***	*11*	*6.1*	*1.8*	*19*	*28*	*0.9*	*16:1*	*147*
Kawakawa	***1.9***	***38***	*6.5*	*1.6*	*2.7*	*23*	*13*	*1.6*	*10, 12x, 16:1, 17, 20:1*	*37*
NZ Blueberry	***1***	***9.1***	*19*	*2.1*	*1.3*	*11.6*	*47.3*	*0.1*	*15x, 18:1x*	*75*

Key : TFA = Total Fatty Acids, x = unknown fatty acid. Source of data for commercial oils was White, 1992 (p. 238). See also Appendix II.

3.5.2.2 Species with valuable contributions from monounsaturated fatty acids

Vegetable oils with monounsaturated fatty acids of particular commercial interest would be those with large quantities of either erucic acid (22:1n-9) or oleic acid (18:1n-9). Generally speaking, seed oils with high proportions of erucic acid (40% or greater) are used in non-edible applications, as high-erucic acid oils have been suspected to be nutritionally undesirable due to concerns about the cardiopathic potential of this acid (Kramer *et al.*, 1983). High erucic acid varieties are mainly produced for the manufacture of fatty acid derivatives or oleochemicals such as erucamide, which is used in the production of plastics (Sonntag, 1995).

Commercial vegetable oil sources of erucic acid are mainly found among the *Brassica* oilseeds, such as rapeseed. These generally contain about 38-54% of their total fatty acids as erucic, but cultivars with >50% erucic acid, are also being developed (Uppström, 1995). All members of the *Pittosporaceae* family analysed (kōhūhū, black māpau, karo/turpentine and tarata/lemonwood), contained significant quantities of erucic acid (17-29% wt TFA) as shown in Table 3.18. This family may have potential commercial use, as they are quick growing and plentiful in New Zealand (see Section 3.5.5), and may be able to be modified through plant breeding techniques to produce higher quantities of erucic acid.

Commercially important vegetable oils with high proportions of oleic acid (60-80^{+}%) include olive oil and high oleic safflower, sunflower and canola varieties. Peanut (groundnut), rice bran and sesame oils are examples of important vegetable oils with medium (40-60%) proportions of oleic acid.

Native species of comparable proportions of oleic acid included five finger, lancewood and patē/seven finger in the higher range and tītoki, Chatham Island forget-me-not, red matipo and NZ iris in the medium-oleic range (see Table 3.18). As discussed later in Section 3.5.5, all except the red matipo and the NZ iris are good oil sources (>12% TFA content) and all except the forget-me-not have a wide distribution in New Zealand and are relatively easy to grow or propagate and thus may have potential commercially.

The main uses of oils high in oleic acid are in the edible food industry as they confer desirable high temperature and heat stability qualities, have reasonable oxidative and flavour stability as well as nutritive and health value. Dietary monounsaturated fatty acids have reported health and nutritional benefits such as in lowering plasma total and low-density lipoprotein (LDL) cholesterol (Mattson & Grundy, 1985) and may have a protective effect against the oxidation of LDL as some evidence has shown that the uptake of LDL and the formation of fatty streaks in the intima wall of large blood vessels, which is considered an early lesion of atherosclerosis, is enhanced by the oxidation of LDL (Steinberg *et al.*, 1989; Reaven *et al.*, 1991).

TABLE 3.18 Comparison of Commercial Vegetable Oils High in Erucic and Oleic Fatty Acids with Potential Native NZ Equivalents.

	14:0	16:0	18:0	18:1	20:1	22:1	18:2(n-6)	18:3(n-3)	Others	TFA (mg/g)
High erucic acid (22:1) plant oils										
¤Rapeseed (high erucic)	0.1	3.0	0.8-1.5	10-21	6.3-12.2	**38.6-52.3**	13.5-13.9	9.1-9.8s	16:1, 20, 20:2, 22, 22:2, 24	
*Crambe		2-2.8	.0.5-0.8	20.1-20.9	3.5-3.8	**57.9-60.7**	6.2-9.8	2.6-5.5	16:1, 22, 22:2	
Kōhūhū	*0.3*	*4.3*	*0.9*	*16.1*	*26.4*	***29***	*19.5*	*0.5*	*12, 15x, 16:1, 20x*	*87*
Black Māpau	*0.1*	*5.0*	*0.4*	*19.4*	*24*	***27***	*21*	*0.3*	*12, 20x*	*67*
Karo/Turpentine		*7.1*	*1.9*	*28.1*	*22*	***20.2***	*15*		*12, 17, 20x*	*114*
Tarata/Lemonwood	*0.6*	*7*	*1.7*	*32*	*19*	***17***	*14*	*1.3*	*12,12x, 16:1, 17, 20, 20x, 22*	*78*
High and medium oleic acid (18:1) plant oils										
Olive		13.7	2.5	**71.1**			10	0.6	16:1, 20	
Safflower (high oleic)	0.1	5.5	2.2	**79.7**			12	0.2	16:1, 20	
Canola (low erucic rapeseed)		3.9	1.9	**64.1**	1.0		18.7	9.2	16:1, 20, 22, 24	
Five finger	*0.2*	*6*	*1.5*	***78***			*13*	*0.1*	*12, 16:1, 17*	*122*
Lancewood	*0.5*	*3.3*	*0.8*	***87.5***			*6.5*	*0.6*	*12, 16:1, 17*	*186*
Patē/Seven finger	*0.1*	*6*	*1*	***82***	*0.4*		*8*	*0.7*	*10, 12, 16:1, 17, 20*	*234*
Groundnut (peanut)	-	6-8	4-6	**52-60**	3.3		21-25	0	20, 24	
Rice bran oil	0.5	16.4	2.1	**43.8**	0.4		34	1.1	12, 16:1, 20 22, 24	
Sesame oil		9.9	5.2	**41.2**			43.3	0.2	16:1	
Tītoki		*3.7*	*1*	***46.6***	*30.9*		*4*	*1.3*	*10, 12, 16:1, 17, 20*	*298*
Chatham Is Forget me not		*11.4*	*4.4*	***40.7***	*2.6*	*1.7*	*20.1*	*1.4*	*16:1, 20x 18:3(n-6),*	*246*
Red Matipo	*0.4*	*10*	*3*	***41***	*14.5*		*16.5*	*4.5*	*12, 16:1, 17, 20*	*53*
NZ Iris	*2.3*	*16*	*2.7*	***45***			*30*	*1.5*	*10, 12, 16:1*	*32*

Key : TFA = Total Fatty Acids, x = unknown fatty acid. Sources of data for commercial oils: ¤ = Uppström, 1995, (pg 220); * = Weiss, 2000, (p306); and White, 1992, (p. 238). for the rest. See also Appendix II.

3.5.2.3 Species with valuable contributions from polyunsaturated fatty acids

In general, vegetable oils with a high degree of polyunsaturated fatty acids (PUFA) are more suitable for industrial, non-edible consumer applications such as in paint, varnishing or other types of coatings or cosmetic industries. The use of highly polyunsaturated fatty acids in the edible industry is limited as oils high in such fatty acids lead to problems with oxidative rancidity, reduction in shelf life of the oil or product, and development of off flavours and odours after prolonged storage or repeated frying use.

Linseed oils are known for their high percentage (~52.7% wt) of n-3 or α-linolenic acid (ALA). Soybean, hempseed, wheat germ and canola oils are also listed among those with high levels of ALA. The application of linseed oil and other high proportion PUFA oils in the paint and coating industries is due to the ability of the unsaturated fatty acids to polymerise, cross-link, and form quick-drying, glossy films after they have been applied. Thus sometimes they are referred to as drying or semi-drying oils (Orthoefer, 1996). Native species with significant (>20% TFA) % weights of ALA include snowberry (43%), bidibid (40%) and red kākā (23%). As discussed in Section 3.5.5, all three native species may have commercial potential: snowberry has a high TFA content (27.5%) and a wide NZ distribution; bidibid is fast-growing, has a wide distribution, and other members of the endemic *Acanae* genus are popular house plants; and although red kākā has a low % weight TFA in it's seed oil (5.7%), and is rare in naturally-grown areas, it is extremely popular as a garden plant and cultivation efforts are improving it's distribution.

Blackcurrant seed, borage seed and evening primrose oils are among those valued for their high content (12-30%) of n-6 or γ-linolenic acid (GLA). Human milk also contains GLA (Scott, 1989). Oils high in GLA have potential uses in cosmetic, herbal and skin-care treatments including atopic eczema, rheumatoid arthritis, dry, scaling skin and premenstrual syndrome (Horrobin, D, 1989; Kast RE, 2001; Rieger M, 1996; Zurier *et al.*, 2001). The only native species tested that might be of particular interest as a source of GLA is the Chatham Island forget-me-not seed oil which has ~11% wt of GLA and a significant (24% wt TFA) total oil content. Examples of commercial vegetable oils high in either ALA or GLA are shown in Table 3.19 along with potentially equivalent native species.

TABLE 3.19 Comparison of Commercial Vegetable Oils High in Alpha and Gamma-Linolenic Fatty Acids with Potential Native NZ Equivalents

	14:0	16:0	18:0	20:0	18:1	18:2 (n-6)	18:3 (n-6)	18:3 (n-3)	Others	TFA mg/g
High Gamma- Linolenic Oils (18:3n-6)										
*Blackcurrant seed oil		7	2		10	49	**30 (mostly n-6)**			
*Borage seed oil		9	3		14	38	**25 (mostly n-6)**			
*Evening Primrose oil		6	2		8	71	**12 (mostly n-6)**			
Chatham Is Forget me not		*11.4*	*4.4*		*40.7*	*20.1*	***11.4***		*16:1, 20x, 22:1*	*246*
High Alpha- Linolenic Oils (18:3n-3)										
Linseed		4.8	4.7		19.9	15.9		**52.7**		
Soybean	0.1	11	4	0.3	23.4	53.2		**7.8**	16:1, 22	
Canola		3.9	1.9	0.6	64	19		**9.2**	16:1, 20:1, 22, 24	
Snowberry	*0.2*	*6.3*	*3.3*		*31*	*16*		***43.3***	*12, 17*	*275*
Bidibid	*0.1*	*6.4*	*3.6*		*14.2*	*33*		***40.1***	*10, 12, 20x*	*67*
Red Kaka	*0.2*	*16*	*3*		*11*	*46*		***22.8***	*12, 17, 20:1, 20x*	*57*

Key : TFA = Total Fatty Acids, x = unknown fatty acid. Sources of data for commercial oils: * = Weiss, 2000, (p306), and White, 1992, (p. 238) for the rest. See also Appendix II.

Commercial vegetable oils with significant proportions (>60%) of linoleic acid include sunflower (68%) and high linoleic safflower (78%) oils. Native equivalents might include the NZ cabbage tree (81%), the native ground lily (78%) the NZ flax varieties (~68%) and the kōhia/NZ passionfruit (~63%). Valuable commercial medium-level (40-60%) linoleic acid oils include soybean, cottonseed, sesame and maize oils. Equivalent native species might include māhoe (54%), miro (51%), wineberry (49%) and the Marlborough rock daisy (49%) (see Table 3.20).

All of these native species may be of commercial potential as they are all good oil sources (>12% TFA content), all except the Marlborough rock daisy have a wide distribution and all except the daisy and miro are relatively easy to grow (see Section 3.5.5). As discussed in Section 3.5.5, a study by I.M.Morice in 1965 revealed the yield of oil and linoleic acid in NZ cabbage tree and NZ flax varieties were comparable to that attained by soybean and maize. Thus these two species in particular should be further investigated for potential as an oil and linoleic acid crop.

TABLE 3.20 Comparison of Commercial Vegetable Oils With High and Medium Levels of Linoleic Acid with Potential Native NZ Equivalents

	14:0	16:0	16:1	18:0	20:0	18:1	18:2 (n-6)	18:3 (n-3)	Others	TFA mg/g
High (>60%) linoleic acid (18:2n-6) oils										
Safflower (high linoleic)	0.1	6.5		2.4	0.2	13.1	**77.7**			
Sunflower	0.2	6.8	0.1	4.7	0.4	18.6	**68.2**	0.5	12	
NZ Cabbage Tree		*5*	*0.2*	*1.9*		*12*	***81***	*0.1*	*12, 17*	*254*
Native Ground Lily	*0.1*	*9.2*	*0.3*	*1*		*10.8*	***78***	*0.1*	*12, 12x, 17*	*166*
NZ Flax	*0.1*	*10*	*0.6*	*3.9*	*0.1*	*17*	***68***	*0.2*	*12, 17*	*357*
Kōhia / NZ passionfruit	*0.7*	*9.6*	*0.4*	*4.8*	*0.4*	*19*	***63***	*0.7*	*12,15x, 17*	*272*
Medium (>40%, <60%) linoleic acid (18:2n-6) oils										
Corn		12	0.1	2.2	0.1	28	**57**	0.9		
Soybean	0.1	11	0.1	4	0.3	23	**53**	7.8	22	
Cottonseed	0.9	25	0.7	2.3	0.1	18	**53**	0.3	17	
Sesame		9.9	0.3	5.2		41	**43**	0.2		
Māhoe	*0.9*	*12.8*	*0.4*	*3.8*	*0.4*	*20.2*	***53.5***	*1.3*	*12,15x,17,20:1*	*132*
Miro		*6.8*		*4.6*	*0.1*	*31*	***51***	*0.4*	*12,17,20:2,20:3*	*344*
Wineberry	*0.7*	*8*	*0.1*	*1.7*	*0.8*	*36.1*	***49.2***	*1.1*	*12,20:2,20:3*	*122*
Marlborough Rock Daisy	*9.4*	*11*		*5.2*		*18*	***49***	*1.4*	*10,12,20:3*	*149*

Key : TFA = Total Fatty Acids, x = unknown fatty acid. Source of data for commercial oils was White, 1992, (p. 238). See also Appendix II.

3.5.2.4 Species with high contributions from other fatty acids or groups

Sources of oils with significant oleic acid (20-80^{+}%) are even more valuable if they also contain significant levels of linoleic acid (~10-60%), low levels of saturated acids (<20%) and minimum levels of highly unsaturated acids such as linolenic acids (<2%), or other fats (<5%). Examples of commercial oils classed within this group include corn, cottonseed, peanut, olive, sunflower (high oleic), sesame, safflower (high oleic), and rice bran oil.

This oleic-linoleic group is the most widely used and adaptable of all the fats and oils. They are considered the premium oils since in the edible foods industry, they have desirable antioxidant properties and do not undergo flavour reversion. They may also be hydrogenated to plastic fats with varying degrees of hardness. Non-edible applications of these premium oils are in the manufacture of soaps, cosmetics and lotions (Orthoefer, 1996).

Native species that might be classed in this group, and hence have potential commercial significance, include wineberry, NZ flax and kōhia/NZ passionfruit (similar composition to corn and cottonseed oil); NZ iris (similar composition to peanut oil); and five finger, patē/seven finger and lancewood (similar composition to olive oil and high oleic varieties of sunflower or safflower oils) (see Table 3.21).

Oils containing high proportions of n-3 or n-6 polyunsaturated fatty acids such as linoleic or linolenic acids, or their further elongation and desaturation products such as arachidonic acid (AA or 20:4n-6), eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:5n-3), may be desirable from a nutritional or health-promoting standpoint due to their dietary essentiality, importance in cell membranes and other organs, or in the production of eicosanoid products (see Section 1.2.5).

TABLE 3.21 Comparison of Premium Commercial Vegetable Oils with Potential Native NZ Equivalents

	% SFA	% MFA	% PUFA	% 18:3n-3	% Other fats	% Fat
Corn	13	25	62	<1		
Cottonseed	27	19	54	0.3		~20
Wineberry	*11.8*	*36.2*	*50.7*	*1.1*	*?*	*~12*
NZ Flax	*15.2*	*17.6*	*68.2*	*0.2*	*?*	*~36*
Kōhia / NZ passionfruit	*16.4*	*19.4*	*63.7*	*0.7*	*?*	*~27*
Peanut	13	49	33		5	48
NZ Iris	*22.6*	*46*	*31.5*	*1.5*	*?*	*~3*
Olive	14	77	9	0.6		
High oleic sunflower	10	80	10	0.2		45
High oleic safflower	9	62-80	10-28	0.2	1	
Five finger	*8.2*	*78.7*	*13.1*	*0.1*	*?*	*~12*
Lancewood	*4.9*	*88.1*	*7.1*	*0.6*	*?*	*~19*
Patē	*9.9*	*83*	*8.7*	*0.7*	*?*	*~23*

Key : SFA = Saturated Fatty Acids, MFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acid, 18:3 n-3 = α-linolenic acid. Source of data for commercial oils was of Jandacek, 1992, (pg 402).

Common edible sources of linoleic and linolenic acids are found in a wide range of natural sources in the animal and vegetable kingdoms. Arachidonic acid can be obtained from meat or other membranous tissues, but good sources of EPA and DHA are generally limited to fish oils. The use of fish oils in edible products is limited due to the generally undesirable taste and odour imparted from the use of such oils. Therefore, vegetable sources of precursors of these long-chain PUFA may have potential use in human or feline dietary supplementation, pharmaceutical applications or medical purposes as discussed previously in Section 1.2.4.3.3 and in Section 1.2.5.

Seed oils from tōtara and kahikatea and other members of the *Podocarpaceae* family may contain n-3 or n-6 precursors as shown by their high level of eicosenoic polyunsaturated fatty acids which account for ~20 weight % of their total fatty acid (see Table 3.4, p90 shown previously). If so, members of this family may have potential applications in the herbal, pharmaceutical or medical companies. However, a vigorous analysis of the suitability of these oils as edible oil would need to be carried out before they could be considered for animal or human consumption.

3.5.2.5 Summary

Native species of particular interest with regard to having a high proportion of one, or a group of fatty acids include: nīkau palm and puka (high palmitic); kōhūhū, black māpau, turpentine, lemonwood (high erucic); five finger, lancewood, patē (high oleic & potential premium oil); tītoki, red matipo (medium oleic); Chatham Is. forget-me-not (medium oleic & high γ-linolenic); NZ Iris (medium oleic & potential premium oil); snowberry, bidibid, red kaka (high α-linolenic); cabbage tree, native rock lily, (high linoleic); NZ flax, kōhia (high linoleic & potential premium oil); māhoe, miro, Marlborough rock daisy (medium linoleic); tōtara, kahikatea (potential high n-3 or n-6 PUFA); and wineberry (medium linoleic & potential premium oil). These 26 species are likely to have the most commercial potential and were further evaluated for their potential by evaluating their seed lipid content (Section 3.5.3), previous and current uses of the plants (Section 3.5.4), as well as their New Zealand distribution and cultivation requirements (Section 3.5.5).

3.5.3 Comparison of the Total Oil % in Commercially Used Vegetable Oils with Native Equivalents

According to reports by the United States Division of Agriculture (USDA) in November 2002, the 7 major oilseeds of importance in the world supply and distribution include soybean (*Papilionaceae or Papilionoidae*), palm kernel (*Palmae*), rapeseed (*Brassicaceae*), sunflowerseed (*Compositae*), cottonseed (*Malvaceae*), groundnut or peanut (*Papilionaceae*) and copra (*Palmae*) which is the oil extracted from the dried coconut meat (USDA: FAS, 2002). These major oilseeds generally contain 20 to 64^{+} % weights of oil. The bulk of these vegetable oils, (more than 90%), is directed to food uses, predominantly in the form of margarines, shortenings, salad oils and for frying purposes (Luhs & Friedt, 1994a).

Of the 46 native species analysed, none belonged to the *Brassicaceae* or *Compositae* families, but 3 species (native broom, red kaka beak, kōwhai) belonged to the *Papilionaceae* family; 1 (nīkau palm) belonged to the *Palmae* family; and 3 (perennial hibiscus, narrow-leaved lacebark and ribbonwood) belonged to the *Malvaceae* family. None of these 7 native species contained greater than a 20% weight of oil in the samples analysed. However, eight other native species did contain more than 20 percent of their dried weight as fatty acid lipids (10 if including the kahikatea petroleum ether (27%), or diethyl ether (35%) total fat extractions discussed in Section 3.2.2 or the "ripe–fresh" weight of tōtara (24%), discussed in Section 3.4 previously). The initial eight species in order of increasing % fat per dry weight were; patē/seven finger (23%), Chatham Island forget-me-not (25%), NZ cabbage tree (25%), kōhia/NZ passionfruit (27%), snowberry (28%), tītoki (30%), miro (34%), and the NZ flax (36%).

3.5.3.1 Summary

All ten of these species have a potentially high proportion (~20% wt TFA or greater) of lipid and have already been mentioned in Section 3.5.2 previously for also containing a high proportion of a particular fatty acid or group of acids. Thus, these species are of particular interest for further commercial evaluation (see Section 3.5.4 and Section 3.5.5 following).

3.5.4 A Summary of the Historical and Current Economic Uses in 26 Native Species of Interest

Of the 46 native species analysed, 26 species have been chosen for their potentially useful proportion of a particular fatty acid or groups of acids (Section 3.5.2), or for their potentially significant (>20%) total fat percentage weight (Section 3.5.3). These are the NZ cabbage tree and NZ flax (*Agavaceae*); five finger, lancewood and patē/seven finger (*Araliaceae*); Marlborough rock daisy (*Asteraceae*); Chatham Island forget-me-not (*Boraginaceae*); puka (*Cornaceae*); wineberry (*Elaeocarpaceae*); snowberry (*Ericaceae*); NZ iris (*Iridaceae*); native rock lily (*Liliaceae*); red matipo (*Myrsinaceae*); nīkau palm (*Palmaceae*); red kākā (*Papilionaceae*); kōhia/NZ passionfriut (*Passifloraceae*); kōhūhū, black māpau, karo/turpentine and tarata/lemonwood (*Pittosporaceae*); miro, tōtara and kahikatea (*Podocarpaceae*); bidibid (*Rosaceae*); tītoki (*Sapindaceae*); and māhoe/whiteywood (*Violaceae*).

Historically, all 26 of these species were likely to be used by Māori and pre-European for a variety of purposes, but little information was available for some of these species such as the Chatham Island forget-me-not, Marlborough rock daisy, red kākā beak and black māpau. The first two are only naturally found in the areas they are named after, red kākā (endangered) has been quite rare until recent cultivation efforts and black māpau is likely to be a recent hybrid of kōhūhū.

In most cases, it was the wood, bark, gum or leaves of the plants that were used for healing, medicinal, art, craftwork, building or ceremonial purposes by the Māori or early European. However, the fruit of a number of these 26 species (tōtara, kahikatea, miro, tītoki, wineberry, snowberry and kōhia) were also eaten where possible. The most commonly used seed oils by Māori or early European came from the tītoki, kōhia and miro. These seed oils were used for a variety of purposes from ceremonial, healing and cosmetic use, to being a fuel source for lamps. Essential oil extracts from native species with scented flowers, leaves or gum (such as kōhūhū and tarata) were used to perfume the above seed oils (Brooker, 1986; Cooper & Cambie, 1991; Crowe, 1992, 1994, 1997a; Riley, 1997).

Nowadays, the most common uses of these 26 native plants are as ornamental house and garden plants for national and international markets (Harris & Heenan, 1992). The popularity of growing native plant species in home and city gardens has increased in the last decade such that a greater variety of native species are being cultivated and are available for purchase from market garden suppliers (Hobbs, 1994). One nursery, "Naturally Native" specialises in providing and producing New Zealand native plants and products (http://www.naturallynative.co.nz/index.html, retrieved 28/01/2003). With the public interest in "GE-free" and "natural products" increasing, small niche markets for herbal remedies made from chemical-free, home-grown natural products are proving more lucrative in New Zealand and overseas markets, particularly Asia (Chadwick, 2001; Scoop, 2002; Boase, 2002).

Biological properties of the extracts from various parts of these 26 plants have and are still being investigated for potential use. Some such as the flax, cabbage tree, five finger, seven finger, wineberry, snowberry, red matipo, kōhia, tītoki, *Pittosporaceae* and *Podocarpaceae* species have shown potential for antibacterial, antibiotic, enzyme inhibitory and other biological activities (Calder *et al.*,1986; Bloor, 1995; Kellam *et al.*, 1992; Lis-Balchin *et al.*, 1996; Lorimer *et al.*, 1995). Kōhūhū is listed as having potential lipoxygenase activity (Allen *et al.*, 1999) and the cabbage tree has been surveyed for potential commercial value as a crop for fructose (Harris & Mann, 1994).

Those species with the most commercial potential are those which have ornamental or garden value as well as potential for biological activity, health or nutritive value. All 26 species have ornamental or garden value (Cave & Paddison, 1999; Fisher & Forde, 1994; Matthews, 1993), hence flax, cabbage tree, five finger, seven finger, wineberry, snowberry, red matipo, kōhia, tītoki, tōtara, kahikatea and all four *Pittosporaceae* have more commercial potential due to their biological activities (Calder *et al.*,1986; Bloor, 1995; Kellam *et al.*, 1992; Lis-Balchin *et al.*, 1996; Lorimer *et al.*, 1995), fructose content (Harris & Mann, 1994) or lipid content. Table 3.22 lists the historical and current uses of these 26 species. Note that this is not a comprehensive list of all uses, but of those most commonly known.

TABLE 3. 22 Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest						
Native Plant		**Historical Uses**		**Current Uses**		**Sources**
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Agavaceae	**NZ Cabbage Tree**	Leaves brewed as tea for dysentery. Impt food source for early Māori (young shoots, tap roots, core of trunk).	Weaving, crafts, paper.	High level of fructose in roots.	Ornamental plant, leaves for crafts and weaving, antibacterial properties.	Crowe, 1992, p. 6; Harris & Mann, 1994
				Potential as a crop for linoleic oil		Morice, 1965
				Potential for antibacterial properties		Calder *et al.*, 1986
Agavaceae	**NZ Flax**	Used by Māori as a laxative, for menstrual complaints, bring on abortion, alleviate poisoning, purgative, relieve stomach and toothache. Nectar used as sweetening, drink.	Used for binding up wounds, splints, fibre used for weaving, clothing, rope. Applied to boils, tumours, abscesses, wounds, bruises, swelling, ringworm, and rheumatism. Gum used for sticking plaster, letters.	Seed oil is a source of essential fatty acid for herbal remedies.	Ornamental plant, weaving, fibre.	Crowe, 1990, p. 97; Riley, 1997, pp. 126-136
		All parts of the flax plant were used by early Māori.				
				Potential for antibacterial properties.		Calder *et al.*, 1986
				Potential for biological activity.		Bloor, 1995
				Potential as a crop for linoleic acid.		Morice, 1965
Araliaceae	**Puahou, Whauwhaupaku, Five finger**		Wool dyes made from berries, water-carrying containers made from bark.		Ornamental plant.	Crowe, 1992, p. 11
		Gum chewed to alleviate bad breath.	Flowers had aromatic uses.			Riley, 1997, pp. 501-2
				Potential for enzyme inhibitory properties.		Kellam *et al.*, 1992
Araliaceae	**Horoeka, Lancewood**		As a source of timber, though not as durable as other woods. Mid ribs of juvenile leaves used as a needle for mending, flexible trunks used as stock/horse whips		Ornamental plant.	Crowe, 1992, p. 24
		Gum-like fluid under bark used to relieve after-effects of diarrhoea.	Wood and leaves used as bird spears.			Riley, 1997, p. 144

TABLE 3. 22 continued. Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest

Native Plant		Historical Uses		Current Uses		Sources
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Araliaceae	**Patē/ Seven Finger**		Māori used the sap to heal ringworm and sores caused by tuberculosis of the lymph glands. Newborn babies were wrapped in these leaves, kindling used to make fire by friction, dye and writing ink produced from berries.		Ornamental plant, which attracts birds. Leaves contain falcarindiol, which is an anti-fungal agent.	Crowe, 1992, p. 10 Muir *et al.*, 1992, Riley, 1997, p. 330
				Boiled leaves are used as a slimming aid.		Riley, 1997, p. 330
				Potential for antibacterial properties.		Calder *et al.*, 1986
Asteraceae	**Marlborough rock daisy**		Ornamental plant.		Ornamental plant.	Cooper & Cambie, 1991, pp. 15, 32; Matthews, 1993, p. 46
Boraginaceae	**Chatham Is. Forget-me-not**	Note: This plant was historically endemic to the Chatham Islands. Hence a lack of information exists on its use.			Ornamental plant.	Matthews, 1993, p. 43
Cornaceae	**Puka / Shining broadleaf**	Māori ate the seeds of the related *Griselinia littoralis* but they are bitter.	Inner bark of *G. littoralis* was used for Tuberculosis. Wood was used for timber.		Ornamental plant, which grows easily and is tolerant to winds and salt-spray.	Crowe, 1994, p. 58, Crowe, 1992, p. 50
			Stem used for sore stomach, some venereal diseases.			Riley, 1997, p. 176
Elaeocarpaceae	**Wineberry**	Wine, jams and jellies were produced from the berries. The Māori ate the berries.	Source of blue/black dye, aristoteline and other alkaloids.			Cooper & Cambie, 1991
		Liquid from boiled leaves was drunken to relieve painful joints.	Leaves or bark used by Māori for sore eyes, treating burns and relief from arthritis.			Riley, 1997, pp. 266-7; Macdonald, 1973, p. 50;
				Potential for enzyme inhibitory activities.		Kellam *et al.*, 1992

TABLE 3. 22 continued. Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest						
Native Plant		**Historical Uses**		**Current Uses**		**Sources**
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Ericaceae	**Snowberry**	Infusion of leaves used to treat asthma; leaf oil used for rheumatism; was an aid for nursing mothers; berries eaten as food.	Leaves used as a poultice on wounds, cuts.		Ornamental plant.	Crowe, 1994, p. 40
				Potential for antibacterial properties.		Calder et.al., 1986
Iridaceae	**Native Iris**		Was recommended for papermaking.		Ornamental plant.	Crowe, 1994, p. 14
Liliaceae	**Native Rock/Ground Lily**	Rhizomes cooked as food by Māori.			Ornamental plant.	Crowe, 1990, p. 110
Myrsinaceae	**Red Matipo**	Leaves used by the Māori for a relief of toothache.	Branches used in Māori ceremonial use. Leaves contain rutin, which is used in relief of arthritis. Plant also contains embelin used elsewhere as a remedy for skin disease, intestinal worms and a general tonic. Timber used in furniture.		An ornamental tree, which is quick growing, hardy and grows easily from seed or cuttings.	Crowe, 1992, p. 48
		Used to treat toothache.	Considered as a sacred tree and parts of the plant used as a dye.		Ornamental tree.	Riley, 1997, pp. 284-5
				Potential for biological activities		Bloor, 1995
Palmaceae	**Nīkau Palm**	Flowers and heart of nīkau eaten by the Māori. Bulbous above the trunk was pierced to extract fluid.	Extracts from leaves were used to ease childbirth. Leaves were also used in crafts, weaving and as rooftops by Māori.		Ornamental tree, though it is slow growing.	Crowe, 1992, p. 7
			Hard seeds used to make jewellery, or as ammunition.			Riley, 1997, p. 311
Papilionaceae	**Red Kākā**		Ornamental plant.		Ornamental plant, generally easy to grow from cuttings or seed.	Cooper & Cambie, 1991, pp. 19-40; Matthews, 1993, p. 16

TABLE 3. 22 continued. Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest

Native Plant		Historical Uses		Current Uses		Sources
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Passifloraceae	**Kōhia / NZ Passionfruit**	Fruit eaten by Māori.				Cooper & Cambie, 1991, p. 103
		Gum from stem for chewing, berry juice mixed with flax for flatulence.	Vines for binding, slow-burning wood useful. Oil extracted from seeds for application to sores, wounds, and chapped nipples, anointing body, base for perfumed oil.			Crowe, 1994, p. 34; Brooker *et al.*,1998, pp. 89, 183; Riley, 1991, p. 213-6
				Potential for antibiotic properties.		Calder *et al.*, 1986
Pittosporaceae	**Lemonwood / Tarata**	Gum chewed to combat bad breath and other mouth complaints, good honey produced from flowers.	Oil from leaves and flowers, and gum used in perfumes and scenting oils. Was used for Māori ceremonial purposes, as an aphrodisiac, insect repellent, or to treat rheumatism. Gum used as a glue and ingredient in paint.			Riley, 1997, p. 216-7; p. 441-3
			Ornamental tree.		Potential use of essential oil in perfumes and fragrances.	Cooper and Cambie, 1991, pp. 19-40, 152, 154
				Potential for enzyme inhibitory activities.		Kellam *et al.*, 1992
				Potential for antibiotic properties.		Calder *et al.*, 1986
				Potential for biological activity.		Bloor, 1995
Pittosporaceae	**Black Māpau**	Note: No information was found specifically for this species but closely related to kōhūhū, see uses of kōhūhū below.			Ornamental tree.	See below for kōhūhū
Pittosporaceae	**Kōhūhū**	Tree gum used as chewing gum and other mouth complaints.	Used by Māori in perfumes, scenting oils, treating scabies, eczema, and other skin complaints and to make dyes. Strong timber but lacks durability.		Ornamental, hedge tree, which is hardy and grows easily.	Crowe, 1992, p. 49; Riley, 1997, pp. 216-7
			Ornamental tree, use in dye production, and tanneries.		Potential use of essential oil in perfumes and fragrances .	Cooper and Cambie, 1991, pp. 19-40, 135, 152, 154
				Lypoxygenase activity.		Allen *et al.*, 1999
				Potential for biological activity.		Bloor, 1995

TABLE 3. 22 continued. Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest						
Native Plant		**Historical Uses**		**Current Uses**		**Sources**
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Pittosporaceae	**Turpentine / Karo**	Often hybridises with kōhūhū, thus uses of karo by Māori may be similar to those of the kōhūhū, see above. Seed oil was used to rub on the head to encourage hair growth. Gum from fruit, seeds and tree used as glue.				Riley, 1997, pp. 216-7
			Ornamental, shelter tree, stabilises inland sand dunes.		Ornamental, shelter and stabilising tree, easy to grow and resistant to winds and salt spray. Bird attractant.	Crowe, 1992, p. 56
					Potential for saponins, polyeyne ketone and polyeyne alcohol.	Cambie, 1986
				Potential for enzyme inhibitory activities.		Kellam *et al.*, 1992
Podocarpaceae	**Kahikatea**	Fruits eaten by Māori, beer made from sap by early settlers.	Wood used for timber, bowls, canoe, tools, peat, weapons, and papermaking. Mud surrounding tree used as a dye mordant.		Resin acids, podocarpic acid (promotes bile flow) for use in fragrance or pharmaceutical use.	Cooper & Cambie, 1991, pp. 8, 11, 61, 69, 85, 103, 107, 122, 134, 144, 203
		Berry as diuretic; gum for chewing; beer from sap; leaves for urinary complaints.	Leaves and wood used for application to bruises; burnt wood used in tattooing, ceremonial uses. Butter boxes & hair combs made from wood.			Riley, 1991, pp. 159-160; Williams, 1996, p. 18; Crowe, 1992, p. 40
				Potential for enzyme inhibitory activity.		Kellam *et al.*, 1992
				Potential for biological activity.		Bloor, 1995
				Potential for antibiotic activity.		Calder *et al.*, 1986
Podocarpaceae	**Tōtara**	Fruit eaten by Māori.	Timber used for canoe, carvings, bowls, tools, and weapons. Ornamental tree.		Podocarpic acid and resin acids as kahikatea above. Ornamental tree.	Cooper & Cambie, 1991, pp. 10, 26, 61, 69, 103, 122; Crowe, 1992, p. 46
		Berry eaten as laxative, infusion prepared from inner bark or leaves used for lowering fever.	Wood used as splint, produce fire; smoke from wood used to clear venereal disease & skin complaints.		Wood used in carving, furniture making. Dye is obtained from bark.	Riley, 1991, pp. 474-6; Williams, 1996, p. 74; Crowe, 1992, p. 46
				Potential for enzyme inhibitory activity.		Kellam *et al.*, 1992
				Potential for biological activity.		Bloor, 1995
				Potential for antibiotic activity.		Calder *et al.*, 1986

TABLE 3. 22 continued. Historical and Current Uses of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest						
Native Plant		**Historical Uses**		**Current Uses**		**Sources**
Family	**Species**	**Edible**	**Non-edible**	**Edible**	**Non-edible**	
Podocarpaceae	**Miro / Brown Pine**	Fruit eaten by Māori; infusion from inner bark or leaves used as an antiseptic, to treat gonorrhoea, stomachache and fever. Sore throat was relieved by inhaling the aromatic gum.	Seed oil or leaves used as insecticide, to stop bleeding, for skin complaints; oil for anointing or base for perfumed oil; gum applied to wounds (gum seals wounds and stops bleeding), ulcers.		Furniture making, carving.	Cooper & Cambie, 1991, pp. 10, 103; Williams, 1996, p. 4; Riley, 1991, pp. 298-300; Crowe, 1992, p. 39
Rosaceae	**Bidibid**	Tea made from infusion of dried leaves. Infusion used as a tonic to cure headache, nausea, kidney complaints, constipation or bladder complaints, rheumatism, dysentery, and gallstones and as a blood purifier.	Ornamental indoor and outdoor groundcover plant. Used for dyeing wool.		Certain *Acaena* species are used as mat-forming plants in rock gardens.	Riley, 1997, pp. 339-342; Crow, 1994, p. 22; Matthews, 1993, p. 7
Sapindaceae	**Tītoki**	Māori ate fruit.	Seed oil had ceremonial use, was used as oil for hair, lamps, and lubricants, soothing and healing purposes. Timber used for strength and elastic properties (axe handles).	Liqueur produced from fruit.	Ornamental tree, oil contains cyanogenic glycosides, which may have potential pharmaceutical use.	Crowe, 1992, p. 26; Cooper & Cambie, 1991, pp. 16, 105, 108, 127, 142-3
		Berries sometimes eaten to aid blood spitting in tuberculosis, seed oil used as a laxative.	Leaves and seed oil used as insecticide, oil for ears, eyes, body, sore breasts, hair, sores, bruises, rheumatism, soothe skin complaints, insect bites.			Williams, 1996, p. 71; Riley, 1991, pp. 461-3; Brooker *et al.*, 1998, p. 218;
				Potential biological activity.		Bloor, 1995
				Potential for antibiotic activity.		Calder *et al.*, 1986
Violaceae	**Māhoe / Whiteywood**		Soft wood aided in fire making, charcoal from wood used as gunpowder, liquid from boiled leaves applied to relieve scabies rheumatism or burns. Leaves produced green dye.		Ornamental tree, has perfumed flowers.	Crowe, 1992, p. 26; Cooper & Cambie, 1991, p. 155; Williams, 1996, p. 33; Brooker *et al.*, 1998, p. 238
		Leaves helped promote flow of saliva and were used in the treatment of diarrhoea.	Soot from wood or berry juice used in tattooing; leaves applied to heal wounds, relieve pain, rheumatism, or skin disease. Inner bark frayed then applied to burns.			Riley, 1991, pp. 259-60

3.5.5 A Summary of the Cultivation Requirements and New Zealand Distribution of 26 Native Species of Interest

The seven major oil crops in the world's vegetable oil supply and distribution are soybean, rapeseed, cottonseed, peanut, sunflower seed, palm kernel and copra -dried coconut meat (FAS: COTS, November 2002). These species not only produce a high percentage of seed oil per weight (20-60+ %), but also can produce a high oil yield per hectare if grown in the optimum conditions (Luhs & Friedt, 1994). Therefore, in order to further evaluate the potential viability of extracting the seed or fruit oils from a potentially useful species, information on the required optimum growth conditions, cultivation and expected oil yield per plant or crop area would be important. In this section, the 26 species chosen for their potentially useful proportion of a particular fatty acid or group of acids will be briefly reviewed for their cultivation needs and common ecological distribution in New Zealand (see Table 3.23). Available data on the estimated oil yield per plant or crop area is unavailable for the majority of these species other than NZ flax and cabbage tree, hence this will not be discussed in this Section.

In 1965, Isobel Morice surveyed the yield of linoleic acid from the seed oils of NZ flax (*Phormium tenax*), and the NZ cabbage tree (*Cordyline australis*). It was estimated that 62-66kg and 26-28kg respectively of linoleic acid and 87kg and 33kg of oil, could be produced per acre. These yields of linoleic acid are comparable with those of soybean and maize, as is the oil yield from flax (Morice, 1965).

Apart from cultivation efforts throughout New Zealand, the Marlborough rock daisy and Chatham Island forget-me-not can only be found naturally in the South Island, red kākā only in the eastern areas of the North Island and karo only in the North Island. Native rock lily, nīkau palm, kōhia, kōhūhū, five finger and tītoki can be found naturally in the North Island and certain parts of the South Island. The rest of the 26 species can be found New Zealand wide. Most of the 26 species grow reasonably well from seed or cuttings, but some are short lived (wineberry, kākā), some are slow to germinate, mature or fruit (horoeka, snowberry, nīkau, miro) and the fruit of the nīkau, miro and kahikatea, take a year to mature.

Nīkau in particular would be unprofitable as a crop as the seeds are slow to germinate, it takes 20 years to grow a trunk, 30 years to flower, and the fruit take a year to ripen.

Nine of the 26 native species (five finger, horoeka, patē, wineberry, kōhia, kahikatea, tōtara, miro, māhoe) are dioecious (flowers are produced on the male and female plants), and so both male and female plants would need to be planted together to produce fruit in these species.

Based on the distribution in New Zealand, ease of cultivation or fruit production and percentage of oil (total fatty acid content per gram of dry seed weight), the species with the most potential to be grown as an oilseed or a particular fatty acid crop would be NZ cabbage tree, NZ flax, five finger, patē, wineberry, native rock lily, kōhia, kahikatea, tōtara, tītoki and māhoe. Once matured (snowberry, miro, horoeka) or more widely cultivated (karo, Chatham Island forget-me-not, Marlborough rock daisy), these six species may also provide good oil or fatty acid yields.

TABLE 3. 23 Cultivation of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest				
Family	**Species**	**TFA (% Dry wt)**	**Distribution / Ecological habitat**	**Cultivation**
Agavaceae	**NZ Cabbage Tree**	25.4	NZ-wide, below 600m. Common in swamps & flood plains.	Versatile and easy to grow in various conditions and soil - even in sand. Seeds germinate readily and can be grown from root cuttings. Fruits in late summer.
Agavaceae	**NZ Flax**	35.7	NZ wide distribution, mountain flax found in more alpine areas.	Is a vigorous, adaptable, highly versatile perennial. Will tolerate poor, dry-moist soil, coastal wind, full sun and is ok in sand. Fruits in summer.
Araliaceae	**Five finger**	12.2	NZ-wide except for Chat Is. and lowest part of Sth. Is. Grows below 760m.	Can grow in a wide range of soils and situations. Propagation easy by seed or cuttings. Rapid growth, useful for revegetation. Fruits on female in spring.
Araliaceae	**Horoeka / Lancewood**	18.6	NZ-wide. Below 760m.	Grows easily from seeds or young plants but is slow growing and matures after 15-20 years. Fruits on female in autumn/winter.
Araliaceae	**Patē / Seven Finger**	23.4	NZ-wide, below 1200m. Often found along edges of forest.	Easily grown from seed or cuttings. Best in good soil and some shade. Dioecious (needs both male and female trees to produce fruit). Fruits on female in autumn.
Asteraceae	**Marlborough rock daisy**	14.9	Found naturally only in steep rocky slopes of Marlborough.	Grown from seed or cuttings. Needs well-drained soil with open sun. Frost hardy, tolerates coastal wind. Fruits in late summer-autumn.
Boraginaceae	**Chatham Is. Forget-me-not**	24.6	Naturally found in Chat Is. On rocks or tussock grassland.	Easily grown from fresh seed but hard to maintain. Vulnerable to wind, needs ample moisture and should avoid hot sun. Fruits in summer-autumn.
Cornaceae	**Puka / Shining broadleaf**	7.7	NZ-wide, below 1000m. Often growing up in trees.	Easily grown from seed or semi-hardwood cuttings. Tolerates cold conditions, salt winds, most soil types but prefers full sunlight. Fruits summer-winter.
Elaeocarpaceae	**Wineberry**	12.2	NZ-wide, below 1050m. Common in regrowth forest.	Grown from seed or cuttings. Fast grower but short lived. Dioecious. Grows to approx. 9m in 20yrs. Fruits on female in late summer.
Ericaceae	**Snowberry**	27.5	NZ-wide, below 1500m.	Propagate from seed, rooted pieces and young plants but slow growing. Prefers a rich, moist soil. Fruits in summer and early autumn.
Irideceae	**Native Iris**	3.2	NZ-wide. Found naturally along tracksides and streams.	Propagate from seed or division. Capsules release seed quickly. Prefers well-drained soil, will tolerate open sun or light shade and rock gardens. Fruits in autumn-winter.
Liliaceae	**Native Rock Lily**	16.6	Occurs naturally near sea, mostly on cliffs and as far south as Kaikoura.	Grows easily in most soils and sites, adaptable perennial though frost tender. Fruits late summer.
Myrsinaceae	**Red Matipo**	5.3	NZ-wide, below 900m.	Fairly easily grown from seed or cuttings. Tolerates a wide range of conditions. Fruits in summer.
Palmaceae	**Nīkau Palm**	2.9	Upper Sth Is. And North Is, below 500m.	Seeds slow to germinate. Best planted in deep rich soil with shade and shelter. Doesn't flower until 30yrs old and fruits take a year to ripen. Takes 20 years to produce trunk. Fruits late summer-autumn.

TABLE 3. 23 cont. Cultivation of 26 Native Species with Fatty Acids or Fatty Acid Groups of Interest				
Family	**Species**	**TFA (% Dry wt)**	**Distribution / Ecological habitat**	**Cultivation**
Papilionaceae	**Red Kākā**	5.7	Naturally found from east cape to Lake Waikaremoana. Endangered species.	Easily propagated by seed or cuttings but relatively short-lived. Very rare plant though easy to grow in most soils. Fruits in spring-summer.
Passifloraceae	**Kōhia / NZ Passionfruit**	27.2	Upper half on Sth Is. And Nth Is., below 100m.	Propagated by seed or cuttings. Dioecious. Fruit on female vines only. Quick-growing in good soil, flowers best in full sun. Fruits in autumn.
Pittosporaceae	**Lemonwood/Tarata**	7.8	NZ-wide, below 600m. Grows naturally along streams and open forest.	Grows easily and quickly from ripe seed though not easily grown from cuttings. Have sweet-smelling flowers, fruits in late summer-autumn.
Pittosporaceae	**Black Māpau**	6.7	Likely similar to kōhūhū.	Grows easily from seed and can be raised from semi-hardwood cuttings. Fast growing in variable soil conditions. Fruits in late summer-autumn.
Pittosporaceae	**Kōhūhū**	8.7	All North Is and eastern half of Sth. Is., below 920m.	Best grown from seed. Won't tolerate water logging or extreme drought. Good as shrub, hedge or small tree. Fruits ripen in late autumn.
Pittosporaceae	**Turpentine/Karo**	11.4	North Is. only, mostly coastal.	Very easy to grow and tolerant of most conditions. Doesn't root easily from cuttings but transplants well. Seeds ripe on tree for up to 6 months. Fruits in autumn.
Podocarpaceae	**Kahikatea**	13.9	NZ-wide, below 600m. Common in swampy forest.	Propagation by seed or cuttings. Dioecious and fruit takes a year to mature. Fruit on female trees only in late summer-autumn.
Podocarpaceae	**Tōtara**	15.3	NZ-wide, below 600m.	Grows easily from fresh seed or tip cuttings. Very hardy, tolerant of both wet and dry conditions, though grows faster in good soil. Dioecious with fruit on female trees only in late summer-autumn.
Podocarpaceae	**Miro / Brown Pine**	34.4	NZ-wide, below 1000m.	Slow germination taking 3-5 years. Slow growing tree. Dioecious with fruit on female trees that take up to a year to ripen. Fruits throughout the year.
Rosaceae	**Bidibid**	6.7	NZ-wide, below 1000m.	Perennial, grows from seeds, cuttings or division. Tolerates a wide variety of soil types but susceptible to extremes. Fruits in summer.
Sapindaceae	**Tītoki**	29.8	Entire North Island and down to Christchurch, below 600m. Common in river flats.	Easiest to grow from seed taken from mature capsules, though these take a year to ripen. Sensitive to frosts. Fruits in early summer.
Violaceae	**Māhoe / Whiteywood**	13.2	NZ-wide, below 100m. Common in regrowth and coastal bush.	Best grown in sheltered area but will tolerate wind. Grows quickly to 5m or more. Dioecious. Fruits on female in late summer.

Key: TFA = Total Fatty Acid. Cultivation information for this table was obtained from Cave & Paddison, 1999; Crowe, 1992, 1994, 1997b; Fisher & Forde, 1994; Matthews, 1993; and Metcalf 1995.

3.6 CONCLUSIONS AND SUMMARY OF RESULTS

Forty-six native New Zealand plant species were surveyed to determine the fatty acid composition of the fruit, and/or seed oils. A variety of techniques including solvent extraction, thin-layer chromatography (TLC), gas-liquid chromatography (GLC), hydrogenation, argentation and acid-catalysed fatty acid methyl derivatization were employed to estimate the quantity and proportions of lipid and fatty acid content.

Initial lipid analysis by various solvent extractions and TLC separation in 14/46 species revealed that a wide range of total seed lipid content (~0.6 - 0.7% in native broom or lacebark; 35-38% in kahikatea or tītoki) could be expected; however, triacylglycerols were likely to be the major component in these oils. This was confirmed later by total fatty acid (TFA) analysis, which showed that the TFA content of the 46 species varied from 0.4 – 35.7% per gram of dried plant material. The lipid percent in the 7 major oil crops ranges from 20 – 60+ percent. In this analysis, 10/46 native species contained ~20% or greater lipid / dry weight. These were the patē/seven finger (23%), tōtara (24%), Chatham Island forget-me-not (25%), NZ cabbage tree (25%), kōhia/NZ passionfruit (27%), kahikatea (27-35%), snowberry (28%), tītoki (30%), miro (34%), and the NZ flax (36%).

Fatty acid analyses of the fruit or seed oils from these native species revealed a wide range of fatty acids. These included C8 to C24 in the saturated, straight chain range; C15-C19 in the uneven chain acids; and also included unsaturated fatty acids with one, to possibly four or five enoic bonds. In general however, the majority of the fatty acids present were C18 acids, and/or contained 0 – 3 enoic bonds. The top six quantitative fatty acid contributions, in order of highest frequency came from C18:2 (n-6), C18:1 (n-9), C16:0, C18:0, C18:3 (n-3) and C20:1(n-11) fatty acids respectively.

A number of native species showed comparable quantities of a particular fatty acid or group of acids when compared to common commercial seed oils. These included the nīkau palm and puka (high palmitic); kōhūhū, black māpau, turpentine, lemonwood (high erucic); five finger, lancewood, patē (high oleic & potential premium oil); tītoki, red matipo (medium oleic); Chatham Is. forget-me-not (medium oleic & high γ-linolenic); NZ Iris (medium oleic & potential premium oil); snowberry, bidibid, red kaka (high α-linolenic); cabbage tree, native rock lily, (high linoleic); NZ flax, kōhia (high linoleic & potential premium oil); māhoe, miro, Marlborough rock daisy (medium linoleic); tōtara, kahikatea (potential high n-3 or n-6 PUFA); and wineberry (medium linoleic & potential premium oil).

26 out of the 46 species, (chosen for their comparable or potentially useful proportion of total lipid, a particular fatty acid or groups of fatty acids), were then analysed for added commercial potential by looking at the past and present uses of the plant, their distribution throughout New Zealand and cultivation requirements. These were the NZ cabbage tree, NZ flax, (*Agavaceae*); five finger, lancewood, patē/seven finger, (*Araliaceae*); Marlborough rock daisy (*Asteraceae*); Chatham Island forget-me-not, (*Boraginaceae*); puka, (*Cornaceae*); wineberry, (*Elaeocarpaceae*); snowberry, (*Ericaceae*); NZ iris (*Iridaceae*); native rock lily (*Liliaceae*); red matipo (*Myrsinaceae*); nīkau palm (*Palmaceae*); red kākā (*Papilionaceae*); kōhia/NZ passionfriut (*Passifloraceae*); kōhūhū, black māpau, karo/turpentine, tarata/lemonwood (*Pittosporaceae*); miro, tōtara, kahikatea (*Podocarpaceae*); bidibid (*Rosaceae*); tītoki (*Sapindaceae*); and māhoe/whiteywood (*Violaceae*).

In the analysis of historical, current or potential uses of the above 26 species, (Section 3.5.4), all had ornamental or garden value, but 16 species: NZ flax, cabbage tree, five finger, patē/seven finger, wineberry, snowberry, red matipo, kōhia, tītoki, kōhūhū, karo, black māpou, lemonwood, tōtara, miro, and kahikatea had added value due to biological activity found in various parts of the plant (and hence pharmaceutical, herbal or oleo chemical potential), high fructose content in the roots (cabbage tree), honey production (NZ flax), value for timber (tōtara) or fibre (NZ flax), and high essential or conditionally essential fatty acid content (NZ flax, cabbage tree, wineberry, snowberry, kōhia, tōtara, miro and kahikatea).

Based on the distribution within New Zealand, ease of cultivation or fruit production and percentage of oil (total fatty acid content/g dry seed weight), (Section 3.5.4), those of the 26 native species tested with the most potential to be grown as an oilseed or a particular fatty acid crop would be the following 11 species: NZ flax, cabbage tree, five finger, patē/seven finger, wineberry, native rock lily, kōhia, kahikatea, tōtara, tītoki and māhoe. However, once matured or more widely cultivated, 6 further species: snowberry, miro, horoeka, karo, Chatham Island forget-me-not, and the Marlborough rock daisy may also provide good oil or fatty acid yields.

In conclusion, of the above 26 native New Zealand species they are more likely to have commercial potential, if apart from their valuable fatty acid or oil proportion, they are also popular as ornamental plants; have a use in an established market; have potential biological activities; have potential nutritive or health value; and are easy to propagate, grow and produce fruit. The nine fitting these criteria would include NZ flax, cabbage tree, five finger, patē/seven finger, wineberry, kōhia, tītoki, kahikatea and tōtara. The snowberry, karo, miro, horoeka, Chatham Island forget-me-not, and the Marlborough rock daisy species do not score as highly as the previous nine, but if improvements were made in ease of growth, cultivation and nation-wide distribution, these would also warrant further investigation. Native rock lily and māhoe score well as a potential fatty acid or oil source, NZ distribution and ease of growth, but do not score highly for other additional uses. Red matipo, kōhūhū, black māpou and tarata/lemonwood have a NZ- wide distribution, are easy to grow and may have biological activities but have less than 10% total fatty acid/g of dried seed weight.

4 Conclusions and Future Work

46 native New Zealand plant species were tested in this research study. From an analysis of the results from a survey of the seed fatty acid composition, 26 out of the 46 species were chosen for their potentially useful proportion of total fatty acid, or levels of a particular fatty acid or groups of fatty acids (See Sections 3.5.2 and 3.5.3). These 26 species were evaluated further by surveying their historical and current uses, and cultivation requirements in order to determine any potential commercial value.

Based on the ease of growth, NZ-wide distribution and total fatty acid content per gram of dried seed weight, the 11 with the most potential to be grown as an oilseed or a particular fatty acid crop were NZ flax, cabbage tree, five finger, patē, wineberry, native rock lily, kōhia, kahikatea, tōtara, tītoki and māhoe. However, once matured or more widely cultivated, snowberry, karo, miro, horoeka, Chatham Island forget-me-not, and the Marlborough rock daisy may also provide good oil or fatty acid yields.

New plant sources of oil are more likely to have the greatest commercial potential or viability if they also have other useful commercial properties such as discussed previously in the summary to Section 3. It was concluded that those with the most potential included the following nine species; NZ flax, cabbage tree, five finger, patē/seven finger, wineberry, kōhia, tītoki, kahikatea and tōtara. The remaining eight species would also warrant further investigation if improvements in ease of growth, cultivation and nation-wide distribution were increased (snowberry, karo, miro, horoeka, Chatham Island forget-me-not, and the Marlborough rock daisy) or if useful properties other than as an ornamental plant were found (native rock lily, māhoe), since they all have a >10% total fatty acid content/g dried seed weight.

With the recent advances in seed oil modification now available, there is potential in high-oil producing species, such as the 17 above-listed native species, for the total oil content to be increased, or the proportion of a fatty acid or group of fatty acids to be modified. Such modifications can be manipulated towards a specified end use. As an example, erucic acid was eliminated from the seed oil of different cruciferous crops such as rapeseed to produce prime edible oil known as canola oil (Friedt, 1994).

Much greater investigation into determining viability of these species as a source of oil or a fatty acid would need to be carried out. Feasibility studies such as determining the amount of seed per acre and thus oil yield per acre, land availability, machine requirements, ease in seed collection, best methods for oil extraction, and expected economic return would need to be carried out. Even more stringent tests would need to be carried out to determine whether a new oil source could be considered as a potential edible oil. This is outside the scope of this Masters research project, but as very little research has been carried out in these areas on any of these New Zealand native species except to a small extent on the NZ flax and cabbage tree, it may be an area for future research.

Further examination of other lipid classes or characteristics of the triacylglycerols such as determining the positions of the fatty acids on the glycerol backbone is another potential area of study. This can be accomplished by using an enzymatic 2-position fatty acid assay followed by gas liquid chromatography (GLC) analysis. This method would have been useful for further analysis of the seed oils obtained in this research but due to time constraints and the number of species analysed, it was not employed in this research study.

New Zealand may have difficulty breaking into the world oilseed production due to competition with the well-established overseas oilseed markets. However, there may be potential for these species to fit into local, small-business or niche market applications such as in herbal or natural product initiatives.

Based on the results of the small survey of oil yields from kahikatea and tōtara seeds over various developmental stages, it would also be worthwhile in native species of potential worth, to determine the best time for extraction of the oil in order to obtain the optimum oil or fatty acid yield. Such a survey would require more frequent analysis of oil content during seed development, a viable quantity of seed and close monitoring of environmental factors that could also affect the yield. It would also be worthwhile to experiment to find the best methods of oil extraction and seed storage to be able to obtain the greatest quantity of oil and minimise losses.

Another investigation that would be interesting is to determine whether the lipid content in native seeds or fruits, which are part of the diet of many of New Zealand's native bird population, have any bearing on their successful breeding. Of the four *Podocarpaceae* species studied, tōtara and kahikatea in particular have unusually high quantities of polyunsaturated eicosenoic fatty acids, some of which may be precursors to important prostaglandins. Native bird species such as the kea, kākā or kākāpō may be more likely to eat seeds from the native flora, though a number of bird species eat the fruit from native flora.

Taking into consideration the fact that 17 out of 46 native species tested may have promise as a fatty acid or oil source, and 15 out of 17 had other useful qualities, combined with the fact that 85% of native seeding plants are endemic to New Zealand, it can be considered worthwhile for further extensive examination of these and other non-volatile lipid compounds to be carried out on New Zealand native species. The potential to find unusual or novel compounds, only available in New Zealand plant species, is high. This not only improves the economic and commercial potential of these species, but also improves the likelihood of success for those willing to investigate them further.

Appendix

APPENDIX I Fatty Acid Composition of Previously Studied NZ Species

BOTANICAL FAMILY	GENUS / SPECIES	10:0	12:0	14:0	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3 (n6)	18:3 (n3)	19:0	20:0	20:1	20:2	21:0	21:1	22:0	22:1	23:0	24:0	24:1	Fat % Oil	REFERENCES
Agavaceae	Cordyline						3-8.0				0.5-1.5	5.3-14.6	79.5-89.1														22-37	Morice 1962, 1965.
	Phormium						6-11.0				1.3-2.5	10.5-15.5	75-81.3														27-31	Morice 1962, 1965.
Aizoaceae	Tetragonia (leaves)			0.4-1.7			14-17.6				0.4-0.9	9.7-11	11-13.1		56-58													Aoki *et al.*, 1982.
	Tetragonia (stems)			0.9-1.3			27-41				1-1.9	15.6-15.7	30.8-36.6		6-21													Aoki *et al.*, 1982.
Boraginaceae	Myosotidium																											McGill *et al.*, 2002.
Corynocarpaceae	Karaka (kernals-TAG fraction)			0.1	tr		13.1	tr	tr		7.2	27.2	45.3		1.1		4.2					1.4			0.4			Body, 1983.
	Karaka (husks-TAG fraction)			0.4	0.2	0.1	18.6	0.7	0.2	tr	4.4	20.7	41.8		9.7		1.6					0.9			0.7			Body, 1983.
	Karaka			0.7	0.7	0.2	35.7	0.8	1.0	0.1	5.1	14.1	15.9		3.9	0.2	1.8			0.6		2.6			3	5.2		Body, 1983.
Cyperaceae *(tribe Cariceae)*	Carex			0.2-0.4			7-12.7	0.2-0.4			1.8-4.0	18.3-32.1	46.8-68.4		1.6-2.5	0.1-0.3	0.5-0.7	0.3-0.4				0.4-0.9					2.4-8.5	Morice, 1977.
	Uncinia			tr-0.2			4.5-9.5	tr-0.3			1.2-4.5	12.9-24.2	62-78.1		0.1-1.1	tr-0.2	tr-0.7	0.1-0.6				0.1-0.9					5.9-19.5	Morice, 1977.
Cyperaceae *(tribe Cypereae)*	Cyperus			0.1			7.6	0.2			2	30.2	55.7		0.5	tr	0.6	0.7				2.4					18.4	Morice, 1977.
Cyperaceae *(tribe Rhyncosporaceae)*	Morelotia						6.2	0.1			1.8	41.4	49.8		0.3	0.1	0.1	0.1				tr					16.8	Morice, 1977.
	Gahnia		0.2-0.7	tr-0.4			6-32.4	tr-0.6			1.3-4.6	21.8-48.3	34.1-66.1		tr-0.1	0.1-2.3	tr-0.7	tr-1.0		tr-0.9	tr-1.5	0.3-0.8					2.3-17.3	Morice, 1977.
Cyperaceae *(tribe Scirpeae)*	Desmoschoenus	1.1	0.2-0.7	2.2			6.1	0.2			2.6	26.5	58.2		0.8	0.1	0.5	0.2				0.8			0.5		1.8	Morice, 1977.
Elaeocarpaceae	Elaeocarpus dentatus (coat)		0.2	1.4			23.8	0.8	tr	tr	3.7	36.8	26.1	1.1	4.5		0.2	0.3				0.2			0.9		0.8	Morice, 1975a.
	Elaeocarpus dentatus (seed)	0.1	0.1				23.3	10			3.4	31.5	31.2		0.3												5.2	Morice, 1975a.
Fagaceae (Nothofagus)	Red beech (leaf & sap)		1-2	2-4			22-34				6-10	45-50	4		1		1					2			1			Lloyd, 1975.
	Silver beech		1-2	4-8			22-47				11-12	14-45	2-10		2		1-5					1			1			Lloyd, 1975.
	Hard beech		1-2	4			31-38				3-9	30-42	4-5		1-2		4					1						Lloyd, 1975.
	Mountain beech		1-2	3-5			27-38				4-12	37-38	9-16		1		1					2						Lloyd, 1975.

APPENDIX 1 cont. Fatty Acid Composition of Previously Studied NZ Species

BOTANICAL FAMILY	GENUS / SPECIES	10:0	12:0	14:0	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3 (n6)	18:3 (n3)	19:0	20:0	20:1	20:2	21:0	21:1	22:0	22:1	23:0	24:0	24:1	Fat % Oil	REFERENCES
Gramineae	Cortaderia (surface wax)		22-28	19-30			32-39				8-15	6-8																Connor & Purdie, 1976.
Hypoxidaceae	Hypoxis						4.4				1.1	9.4	84		0.5		0.1	0.1				0.4					32.8	Morice, 1970.
Iridaceae	Libertia	tr-0.3	0.1-3.9	0.3-2.2	0-0.2	0-0.2	13.6-24.5	0-0.5	0-0.1		0.9-3.8	9-24.5	36.7-61.1		0.2-1.7												6.8-20.4	Morice 1969b.
Juncaceae	Juncus		tr-0.2	0.1-1.0	tr-0.4		6.3-18.7	0.2-2.3	tr-1.2	tr-1.6	1.2-4.2	21-50	34-57		0.5-4.6	tr-1.3	0.2-2.0	0.1-1.0	0-0.2			0.1-0.9	tr-0.5		tr-0.5		7.9-22.1	Morice, 1967a.
	Luzula		tr-0.2	0.3-0.7	tr-0.2		10.1-16.4	tr-0.8	tr-0.2	tr-0.2	1.4-1.9	33.6-54.8	28.3-45.9		0.3-0.6	0.1-0.5	0.1-0.4	1.5-3.5				0.3-0.7	0.5-1.3		0.2-0.5	0.7-2.1	2.8-5.0	Morice, 1967a.
Liliaceae *(tribe Asphodeleae)*	Asphodelus			0.5			5.8				3.7	33.8	56.2														21	Morice, 1969a.
	Arthropodium			tr			7.2-15.5	0.1-0.3	tr		0.7-1.4	10.1-18.3	69.2-80.4		0.2-0.5		tr-0.2	0.2-0.5				0-0.1					20-26	Morice, 1969a.
	Bulbinella		0-0.3	0-0.2	0-tr		5.5-10.4	tr-0.3	tr		0.7-2.9	6.6-21.7	65-87		tr-0.4		tr-0.3	tr-0.7				0-0.1	0-0.2				14-39	Morice, 1969a.
	Herpolirion			tr			5.8		tr		1.8	23.5	67.6		0.2		0.2	0.4				0.5					29.5	Morice, 1969a.
Liliaceae *(tribe Asphodeleae)*	Xeronema			tr			10.8		tr		1.8	12.6	73.5		1.0		0.1	0.2									35.3-36	Morice, 1969a.
Liliaceae *(tribe Dianelleae)*	Dianella Intermedia			0-0.1			4.6-5.3				2-3.3	13.8-24.0	68.8-78.8				tr-0.1	tr-0.4				0-0.2	0-0.2				34-36.0	Morice, 1969a.
Liliaceae *(tribe Iphigenieae)*	Iphigenia Novae Zealandiae		0.1	0.4.	0.2	0.1	19.9	17	0.4	0.1	2.0	33.5	25.8		0.5												19.1	Morice, 1969a.
Liliaceae *(tribe Milliganieae)*	Astelia						3.5-12.2	tr-0.7			0.7-3.4	4-30	54-82	0-29.5	0-0.8		tr-0.9	tr-1.1	tr-0.6			tr-0.3	tr-0.4				8.2-48.4	Morice, 1967b, 1975b
	Collospermum							3.6-4.0			1.1-1.5	8-11	70-71	12.5-14.4	0.1		0.1-0.2	0.1-0.3	0.1-0.2			0.1-0.2	0.1-0.8				43-49	Morice, 1967b, 1975b.
Meliaceae	Dysoxylum spectabile (moles%)		0.05	0.37	0.05		53.8	0.2	0.05	0.07	3.0	1.31	38.47		2.63												29.2	Brooker, 1960.
				0.4			51.3	0.4			4.8	3.3	35.6		3.1		0.2										34.1	Kleiman *et al.*, 1984.
Oleaceae	Nestegis (coat)		0.3	0.6			35.1	1.8	tr	tr	3.6	12.7	30.2		13.3		1.2										2.4	Morice, 1975a.
	Nestegis (seed)			0.6			11.1	0.6			1.8	67.9	16.2				1.2	0.6									5.0	Morice, 1975a.
Palmae	Rhopalostylis sapida (seed)	tr-0.1	3.3-9.4	8-17	tr-0.1	tr	23-26	tr-0.1	0-0.1	0-tr	1-5	24-33	17.6-39.5		0.2-0.5	tr-0.2	tr-0.2	0.1-0.3		0-0.3		0-0.1		0-0.3	0-0.1		3.7-5.5	Morice, 1970, 1975a.
	Rhopalostylis sapida (fruit coat)		0.1	0.2			24.9	1.4	0.1	tr	1.1	49.1	21.3		1.5		0.1	0.1				0.1					8.2	Morice, 1975a

APPENDIX 1 cont. Fatty Acid Composition of Previously Studied NZ Species

BOTANICAL FAMILY	GENUS / SPECIES	10:0	12:0	14:0	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3 (n6)	18:3 (n3)	19:0	20:0	20:1	20:2	21:0	21:1	22:0	22:1	23:0	24:0	24:1	Fat % Oil	REFERENCES
Palmae	Rhopalostylis baueri		0.1	0-0.1			25.9	1.6	0.1	0.1	1.2	52.4	15.6		1.2		tr	0.1				0.2			0.6		10.9	Morice, 1975a
Passiffloraceae	Passiflora tetrandra		tr	2.52			13.29	1.58	2.17		1.75	22.6	54.1		2.23												39.8	Brooker, 1960
Philesiaceae	Luzuriaga						12.2	0.4			1.2	14.2	36.6		0.8		0.2	15		0.1		0.2	12.2		tr	6.9	9.6	Morice, 1970
Sapindaceae	Alectyron excelsum		0.5-2	0.3-1.8	1.8		1.8-4.2	0.2-0.6			0.5-1.3	18.1-48.4	1.8-5.0		0.2-0.6		8.8-16.5	28.5-55				0.9-2.0	0.7-1.3				30-32	Brooker & Eyres, 1957, 1981
	Dodonea viscosa												57														17	Brooker & Eyres, 1981
Smilacaceae	Ripogonum	tr	tr-0.3	0.3-0.5	tr-0.2	0.1	25-31	1-1.2	tr-0.3	0.1	3-4	17-24	39-40		1.1-2.9	0.5-2.1	0.2-0.3	1.5-1.6		0.1		0.4-0.8	0.1	0.1-0.3	0.4-0.8		0.7-0.8	Morice 1970

Key: Tr = trace

Appendix II Typical Compositions and Chemical Constants of Common Edible Oils

	Butyric	Caproic	Caprylic	Capric	Undecanoic	Lauric	Tridecanoic	Myristic	Myristoleic	Pentadecanoic	Pentadecanoic	Palmitic	Palmitoleic	Margaric	Margaroleic	Stearic	Oleic	Linoleic	Linolenic	Nonadecanoic	Arachidic	Gadoleic	Eicosadienoic	Arachidonic	Behenic	Erucic	Docosadiennoic	Lignoceric	Iodine Value	Saponification Value
Carbon atom: double bonds	**4:0**	**6:0**	**8:0**	**10:0**	**11:0**	**12:0**	**13:0**	**14:0**	**14:1**	**15:0**	**15:1**	**16:0**	**16:1**	**17:0**	**17:1**	**18:0**	**18:1**	**18:2**	**18:3**	**19:0**	**20:0**	**20:1**	**20:2**	**20:4**	**22:0**	**22:1**	**22:2**	**24:0**		
Babassu		0	5	5.9		44		16				8.6				2.9	15	1.7			0.1								13-18	247-254
Canola oil												3.9	0.2			1.9	64	19	9.2		0.6	1			0.2			0.2	110-115	
Citrus seed oil						0.1		0.5				28	0.2			3.5	23	38	5.7		0.8								99-106	192-197
Cocoa butter								0.1				26	0.3			35	35	2.9			1.1								32-40	190-200
Coconut oil		1	8	6.4		49		18				8.4				2.5	6.5	1.5			0.1								7-13	248-264
Corn oil												12	0.1			2.2	28	57	0.9		0.1								110-128	186-196
Cottonseed oil								0.9				25	0.7	0.1		2.3	18	53	0.3		0.1								99-121	189-199
Linseed oil												4.8				4.7	20	16	53											
Oat oil								0.2				17	0.5			1.4	33	45			0.2	2.4							105-110	180-198
Olive oil												14	1.2			2.5	71	10	0.6		0.9								76-90	188-196
Palm oil						0.3		1.1				45	0.1			4.7	39	9.4	0.3		0.2								45-56	195-205
Palm kernel oil		0	4	4		50		16				8				2.4	14	2			0.1								14-24	243-255
Peanut oil								0.1				12	0.2	0.1		3.1	47	31			1.5	1.4	0.1		3			1	84-102	188-196
Rapeseed oil								0.1				2.8	0.2			1.3	24	15	7.3		0.7	12	0.6		0.4	35	0.3	1	97-110	168-183
Rice bran oil			0	0.1		0.4		0.5				16	0.3			2.1	44	34	1.1		0.5	0.4			0.2			0.1	92-109	181-195
Safflower oil								0.1				6.5				2.4	13	78			0.2								138-151	186-198
Safflower oil (high oleic)								0.1				5.5	0.1			2.2	80	12	0.2		0.2								85-93	185-195
Sesame oil												9.9	0.3			5.2	41	43	0.2										104-118	187-196
Soybean oil								0.1				11	0.1			4	23	53	7.8		0.3				0.1				125-138	188-195
Sunflower oil						0.5		0.2				6.8	0.1			4.7	19	68	0.5		0.4								122-139	186-196

Adapted from White, 1992, (p 238).

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