Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

SURVEY OF THE FATTY ACID CONTENT OF NATIVE NEW ZEALAND PLANTS

A thesis in partial fulfillment of the requirements for the degree of Master of Science in Nutritional Science at Massey University

ZIRSHA WHAREMATE 2003

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.

Acknowledgements

I would like to thank all my supervisors, but particularly Dr John MacIntosh and Dr Kay Rutherfurd-Markwick for their patient, on-going advice and support throughout this lengthy research period. I also acknowledge the assistance of other staff from the Institute of Food, Nutrition and Human Health, including the Nutrition lab staff for allowing me to invade their space and use their equipment, **M**r Shane Rutherfurd for his assistance with understanding the computer programs and Dr Kathy E. Kitson for her invaluable support and encouragement throughout the years it has taken to complete my degrees. Much aroha also to Mr Nick Roskruge who put up with the numerous reports and letters of support required as my Kaiāwhina. Kia ora e hoa.

The financial assistance and support from The Foundation of Research Science and Technology was instrumental in my endeavours into a Masters degree without which, I could not have even begun. Kia ora to all the staff involved, particularly Dr Jim MacMillan.

Lastly, I give many, many thanks to my families (-both in-laws and out-laws) for encouraging me to keep at it and providing me with opportunities to work at it. Much aroha to you all for your unwavering support, assistance, love, patience and faith in me. To my Tāne, Jasen Wharemate and my daughter Szharei, this work is dedicated to you. For without your support and sacrifice, it would have never been finished. Arohatinotinonui ki a kōrua.

Table of Contents

ABSTR	RACT			Page ii
ACKN	OWLEI	OGEMEN	TS	iv
TABLI	E OF CO	ONTENTS	5	v
LIST C	F ABB	REVIATI	ONS	xi
LIST C	F FIGU	IRES		xiii
LIST C	F TAB	LES		xiv
CHAP	FER 1	INTR	ODUCTION	1
1.1 AI	MS OF T	HIS PROJ	ЕСТ	1
1.2 Lľ	ГERATU	RE REVIE	W AND BACKGROUND	2
1.2.1	Classifie	cation of Li	pids	3
1.2.2	Biologic	al Function	s of Lipids	5
	1.2.2.1	Lipids as 1	Energy, Food and Carbon Source	5
	1.2.2.2	Structural	Role of Lipids in Membranes	6
	1.2.2.3	Communi	cation and Transport Roles of Lipids	7
		1.2.2.3.1	Nervous System	7
		1.2.2.3.2	Hormones, Pheromones and Allelochemicals	8
	1.2.2.4	Selected I	ipids Involved in Lipid Transport,	
		Digestion	and Absorption	8
	1.2.2.5	Protective	Role of Lipids	10
		1.2.2.5.1	Waterproofing	10
		1.2.2.5.2	Insulation and Heat Production	10
		1.2.2.5.3	Structural Support	11

vi

				Page
1.2.3	Structure of Triacylglycerols and Fatty Acids			
	1.2.3.1	Triacylgly	cerols	12
	1.2.3.2	Fatty Acid	s	13
1.2.4	Nomenc	lature, Clas	ssification and	
	Distribu	tion/Occur	rence of Fatty Acids	15
	1.2.4.1	Nomencla	ture	15
	1.2.4.2	Classificat	ion	15
		1.2.4.2.1	Degree of Unsaturation	15
		1.2.4.2.2	Chain Length	16
		1.2.4.2.3	Solubility and Melting Points	20
	1.2.4.3	Distributio	on and/or Occurrence of Fatty Acids	20
		1.2.4.3.1	Saturated Fatty Acids	21
		1.2.4.3.2	Mono- and Polyunsaturated Fatty Acids	21
		1.2.4.3.3	Dietary Sources of Essential and	
			Conditionally Essential Fatty Acids	22
1.2.5	Special	Groups of F	atty Acids in Health, Nutrition and Disease	24
	1.2.5.1	Essential a	and Conditionally Essential Dietary Fatty Acids	24
	1.2.5.2	Eicosanoid	is	27
	1.2.5.3	Dietary Fa	tty Acids and Disease Implications	29
		1.2.5.3.1	The Influence of Dietary Fatty Acids on	
			Blood Lipid, Lipoprotein Concentrations	30
		1.2.5.3.2	Influence of Dietary Fatty Acids on Eicosanoid	ł
			Production	31
		1.2.5.3.3	Dietary Polyunsaturated Fatty Acids	
			and Lipid Peroxidation	31
1.2.6	Comme	rcial Applic	ations of Fatty Acids	33
	1.2.6.1	Features o	f Plant Lipids	33
	1.2.6.2	Oilseeds		34
	1.2.6.3	Commerce	ally Important sources of Fats and Oils	35

				Page
	1.2.6.4	Fatty Acid	Properties-their Commercial Potential	
		and Uses i	n Industry	35
		1.2.6.4.1	Edible Fats and Oils	36
		1.2.6.4.2	Non-edible Fats and Oils	37
	1.2.6.5	Determina	ation of Marketability	41
1.2.7	7 Discussi	on on New	Zealand Native Flora	43
	1.2.7.1	Uses of N	ative New Zealand Plants	44
	1.2.7.2	Edible Us	es of NZ Native Plants	45
		1.2.7.2.1	Food	45
		1.2.7.2.2	Beverages	46
		1.2.7.2.3	Flavourings and Others	46
	1.2.7.3	Non-edibl	e Uses of NZ Native Plants	47
		1.2.7.3.1	Wood, Timber and Other Agricultural Uses	47
		1.2.7.3.2	Medicinal/Pharmaceutical/Biological Activities	47
		1.2.7.3.3	Dyestuffs, Colouring Matters, Resins and	
			Tannins	49
		1.2.7.3.4	Fragrances, Cosmetics	50
	1.2.7.4	Work Dor	ne on the Fatty Acid Composition of	
		New Zeal	and's Native Seed Oils	50
1.3 S	SUMMARY	AND AIM	IS FOR RESEARCH	52

СНАР	TER 2	MATERIALS AND METHODS	54
2.1 M	ATERIAI	LS	54
2.1.1 Plant Mater		laterial	54
	2.1.1.1	Collection	54
	2.1.1.2	Preparation	57
2.1.2	Chemic	als and Equipment	58
	2.1.2.1	General	58

vii

				P	age
		2.1.2.2	Fatty Acid	Methyl Ester Preparation and Analysis	58
		2.1.2.3	Gas Chrom	atography	58
		2.1.2.4	Hydrogena	tion	58
		2.1.2.5	Thin-Layer	Chromatography	59
2.2	MH	ETHODS			60
2	.2.1	Oil Extra	iction Meth	ods	60
		2.2.1.1	Isopropano	l / Methanol / Chloroform Extraction	61
		2.2.1.2	Soxtec/Sox	chlet Lipid Extraction	61
			2.2.1.2.1	General procedure	61
		2.2.1.3	Fatty Acid	Methyl Esters Preparation and Extraction	62
			2.2.1.3.1	Introduction	62
			2.2.1.3.2	FAMES Preparation Method	63
2	.2.2	Methods	for Lipid S	eparation and Identification	64
		2.2.2.1	Hydrogena	tion of FAMES	64
		2.2.2.2	Thin-layer	Chromatography (TLC)	64
			2.2.2.2.1	Standard TLC	64
			2.2.2.2.2	Silver Nitrate TLC (Argentation)	66
		2.2.2.3	Analysis of	f Fatty Acid Composition by	
			Gas Liquid	l Chromatography	66
			2.2.2.3.1	Shimadzu GC-8A / 15% EGSS-X column	67
			2.2.2.3.2	Hewlett-Packard 5890 Series II / BPX70 column	67
			2.2.2.3.3	FAMES Standards	68
			2.2.2.3.4	Quantification of Fatty Acids	68
2	.2.3	Presenta	tion of Res	ults	69

CHAPTER 3 RESULTS	AND	DISCUSSION	70
-------------------	-----	------------	----

3.1	INTRODUCTION	70
0.1	In TRODUCTION	10

viii

ix

					Page
3.2	LI	PID QUA	NTIFICAT	TON AND COMPONENTS	71
3	3.2.1	Isoprop	anol / Meth	anol / Chloroform Lipid Extraction	71
		Followe	d by TLC A	Analysis	
3	3.2.2	Soxhlet/	Soxtec Lipi	d Extraction Followed by TLC Analysis	72
3	3.2.3	Summa	ry		73
3.3	FA	TTY AC	ID ANALY	SIS	74
3	3.3.1	Introdu	ction		74
3	3.3.2	Determi	nation of th	e Parent Fatty Acid Carbon by	
		Hydroge	enation		75
		3.3.1.1	Proportion	ns of C20:0 and C18:3(n-3)	75
		3.3.1.2	Presence of	of C15, C17 and very-long chain	
			Polyunsati	urated Fatty Acids	77
		3.3.1.3	Summary		77
3	3.3.3	Analysis	of Kahika	tea FAMES by TLC	79
		3.3.2.1	Initial Pur	ification	79
		3.3.2.2	Separation	of Kahikatea FAMES by Silver Nitrate	
			TLC Anal	ysis	80
		3.3.2.3	Summary		81
3	3.3.4	Fatty Ac	cid Profile o	of the 46 Native Species	82
		3.3.4.1	Groupings	of Plant Species	89
			3.3.4.1.1	Group 1. Results of species with high levels	
				of polyunsaturated fatty acids	90
			3.3.4.1.2	Group 2. Results of species with high levels	
				of monounsaturated fatty acids	92
			3.3.4.1.3	Group 3. Results of species with high levels	
				of saturated fatty acids	94
			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 group	ed 95

				Page
3.4	CH	LANGES	IN FATTY ACID COMPOSITION DURING	
	DE	VELOP	MENTAL PERIODS	97
3.5	DI	SCUSSIC	ON OF THE COMMERCIAL POTENTIAL OF	
	TH	IE 46 NA	TIVE SPECIES	103
3	.5.1	Introdu	ction	103
3.5.2 Comparison of Valuable Fatty Acid Composition in			rison of Valuable Fatty Acid Composition in	
		Comme	rcially Used Vegetable Oils with Native Equivalents	103
		3.5.2.1	Species with high contributions from saturated fatty acids	104
		3.5.2.2	Species with valuable contributions from	
			monounsaturated fatty acids	108
		3.5.2.3	Species with valuable contributions from	
			polyunsaturated fatty acids	111
		3.5.2.4	Species with high contributions from other fatty acids	
			or groups	115
		3.5.2.5	Summary	117
3	.5.3	Compa	rison of the Total Oil % in Commercially Used	
		Vegetat	ole Oils with Native Equivalents	118
		3.5.3.1	Summary	118
3	.5.4	A Sumr	nary of the Historical and Current Economic Uses	
		in 26 Na	ative Species of Interest	119
3	.5.5	A Sumr	nary of the Cultivation Requirements and	
		Distribu	tion in 26 Native species of Interest	127
3.6	CC	ONCLUS	IONS AND SUMMARY OF RESULTS	131

CHAPTER 4	CONCLUSIONS AND FUTURE WORK	134
APPENDIX		137
Appendix I		138
Appendix II		141
REFERENCES		142

x

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
0	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)

List of Figures

Figure		Page
1.1	Structure of a Triacylglyceride	12
1.2	Structures of Palmitic and Linoleic Fatty Acids	14
1.3	Major Biosynthetic Pathways of the (n-6) and (n-3)	
	Essential Fatty Acids in Animal Tissues	26
1.4	Metabolic Effects of Eicosanoids	28
2.1	Acid-Catalysed Esterification of Fatty Acids and O-acyl Lipids	63
2.2	Schematic TLC Separation of Simple Lipids on Silica gel G.	65
3.1	Schematic TLC Separation of Seed Lipids using the Isopropanol /	
	Methanol / Chloroform Extraction Method	71
3.2	Analysis of Kahikatea FAMES by TLC	79
3.3	Separation of Kahikatea FAMES into Fatty Acid Groups	80
3.4	Identification of Enoic Fatty Acids in Kahikatea FAMES	81
3.6	Changes in Fatty Acid Distribution in Totara During Development	
	and Storage (mg/g dry weight)	101
3.7	Changes in Fatty Acid Distribution in Totara During Development	
	and Storage (% weight of Total Fatty Acid)	101
3.8	Changes in Fatty Acid Distribution in Kahikatea During Developme	ent
	and Storage (mg/g dry weight)	102
3.9	Changes in Fatty Acid Distribution in Kahikatea During Developme	ent
	and Storage (% weight of Total Fatty Acid)	102

List of Tables

Table		Page
1.1	Description and Sources of Important Saturated Fatty Acids	17
1.2	Description and Sources of Important Unsaturated Fatty Acids	18
1.3	Influence of Chain Length and Double Bonds on the Melting	
	Points of Fatty Acids	20
1.4	Major Fatty Acids and Their Industrial Uses	38
2.1	Names of Native Plants Collected and Analysed	56
3.1	Results of Ether Lipid Extraction and Standard TLC Analysis	72
3.2	Determination of the Parent Fatty Acid Carbon by Hydrogenation	76
3.3	Fatty Acid Profile of 46 NZ Native Species	84
3.4	Group 1a (20:2-20:4 Eicosenoic Acids)	90
3.5	Group 1b (n-6 and n-3 C18:3, Linolenic Acids)	91
3.6	Group 1c (18:2n-6, Linoleic Acid)	91
3.7	Group 2a (C22:1n-9, Erucic Acid)	92
3.8	Group 2b (C20:1n-11, Gadoleic Acid)	92
3.9	Group 2c (C18:1n-9, Oleic Acid)	93
3.10	Group 2d (C16:1n-7, Palmitoleic Acid)	93
3.11	Group 3b (C18:0, Stearic and C16:0, Palmitic Acids)	94
3.12	Group 3a (C20:0, Arachidic Acid)	94
3.13	Group 3c (C12:0, Lauric and C14:0, Myristic Acids)	95
3.14	Group 4a and 4b (C17, or C15 Acids)	96
3.15	Group 5 Other Species (not significant compared to other species)	96
3.16	Changes in Fatty Acid Distribution in Totara and Kahikatea	
	During Growth and Storage Periods	100
3.17	Comparison of Commercial Vegetable Oils High in Palmitic,	
	Myristic and Lauric Fatty Acids with Potential Native NZ Equivalent	nts 107

Table		Page
3.18	Comparison of Commercial Vegetable Oils High in Erucic	
	and Oleic Fatty Acids with Potential Native NZ Equivalents	110
3.19	Comparison of Commercial Vegetable Oils High in Alpha	
	and Gamma-Linolenic Fatty Acids with Potential Native	
	NZ Equivalents	112
3.20	Comparison of Commercial Vegetable Oils with High and	
	Medium Levels of Linoleic Acid with Potential Native	
	NZ Equivalents	114
3.21	Comparison of Premium Commercial Vegetable Oils with	
	Potential Native NZ Equivalents	116
3.22	Historical and Current Uses of 26 Native Species with Fatty Acids	
	or Fatty Acid Groups of Interest	121
3.23	Cultivation of 26 Native Species with Fatty Acids or Fatty Acid	
	Groups of Interest	129