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.

SOLVENT FRACTIONATION OF NEW ZEALAND MUTTON TALLOW

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biotechnology at Massey University

GRANT ALEXANDER RAMSAY

1981

ABSTRACT

Samples of inedible bulk mutton tallow were collected monthly throughout two killing seasons from one meat killing plant. These samples plus one sample from another plant, were analysed for fatty acid and triglyceride composition. In these samples, four fatty acids (myristic (14:0), palmitic (16:0), stearic (18:0) and oleic (18:1)) comprised 88.6% of the total fatty acids, and there was an average of 16% trisaturated triglycerides, 38% disaturated triglycerides and 46% of triglycerides with a greater degree of unsaturation. Overall, there was a significant decrease in the proportion of 14:0, and a significant increase in the proportion of 18:0, from November to June; and there was a significant difference in the mean proportion of 16:0, and also 18:1, between the two seasons. There was a significant difference in the proportion of cis monounsaturated triglycerides, and the more highly unsaturated triglycerides, between some of the different tallows analysed. There was a significant decrease in the proportion of 2-oleo disaturated triglycerides from November to June, with a range from 10.0% (May, 1978) to 20.5% (November, 1976).

An acetone fractionation scheme was developed with the main aim of concentrating these 2-oleo disaturated triglycerides into one fraction (the intermediate fraction) which may be useful as a cocoa butter replacer. The first precipitate (the hard fraction) was separated by filtration, and the filtrate was adjusted for solvent : fat ratio and then further cooled to precipitate the intermediate fraction. After separation of this precipitate, acetone was distilled from the filtrate to produce a final fraction (the soft fraction).

A screening experiment showed that the solvent : fat ratio at each crystallisation, the temperature to which the fat solution was cooled at each crystallisation, the water content of the acetone and the degree of agitation during crystallisation all affected the fractionation. The effect of these variables upon one sample of mutton tallow was studied, and mathematical models were developed to predict the yields of the three fractions and the melting properties of the intermediate fraction. The model predicting the melting properties of the intermediate

fraction was used to estimate the fractionation conditions which would give an intermediate fraction with melting properties most similar to those of cocoa butter. From this, a fractionation was performed with first and second crystallisation temperatures of 9.2°C and 5.2°C respectively, solvent to fat ratios at the first and second crystallisations of 1.0:1 and 10.0:1 respectively, a water concentration in the acetone of 0.6% and a defined agitation condition. The yields of the hard, intermediate and soft fractions were 34.5 wt %, 2.5 wt % and 63.0 wt % of the tallow respectively. The intermediate fraction contained 51.0% of 2-oleo disaturated triglycerides (compared to 68.9% in cocoa butter) and had very similar melting properties to cocoa butter. Then the fractionation scheme was modified to give a greater yield of the intermediate fraction (8.3 wt %) but the melting properties of this intermediate fraction were less similar to those of cocoa butter. This latter fractionation scheme was scaled up (from 20 g tallow to 200 g and 1 kg). On each scale an intermediate fraction with consistent yield and melting properties was obtained. The yields of the other two fractions varied, however, and overall there was a considerable difference in the behaviour of the fractionations on each scale. Attempts on the 1 kg scale to produce an intermediate fraction with properties similar to those of the best 20 g intermediate fraction (i.e. similar to cocoa butter) were unsuccessful. The highest proportion of the important 2-oleo disaturated triglycerides attained in a 1 kg scale intermediate fraction was 36.8%, and this fraction melted over a wider temperature range than cocoa butter. This intermediate fraction may be useful as a cocoa butter substitute in a coating chocolate, but is unlikely to be able to replace cocoa butter in chocolate. The hard fraction produced from this 1 kg fractionation (23.0 wt % of the tallow) showed promise in a baking shortening blend with butter, but was too hard to be useful as a pastry shortening. The soft fraction performed well as a deep-frying medium and in mayonnaise.

ACKNOWLEDGEMENTS

I wish to express my appreciation of the assistance of the following:

- Professor R.L. Earle and Dr's R.P. Garland and J.C. Hawke for their expert supervision and guidance.

- Dr M.D. Earle for her continued interest throughout the project, and for her valuable guidance in the preparation of this thesis.

- The Department of Scientific and Industrial Research and New Zealand Pharmaceuticals Ltd. for funding this research.

- The Massey University Food Technology Research Centre and Cadbury Schweppes Hudson Ltd. for testing the fractionation products.

- Waitaki N.Z. Refrigerating Ltd. for the supply of mutton tallow from their Smithfield meat killing plant.

- The Ocean Beach Freezing Co. Ltd for the supply of mutton tallow.

- The staff, and especially Dr R. Norris, of the Milkfat and Butter Section of the New Zealand Dairy Research Institute for the loan of equipment and for general advice and encouragement throughout this work.

CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
CONTENTS	v
LIST OF FIGURES	xiii
LIST OF TABLES	xx
1. PRELUDE	1
1.1 Plastic Shortenings	1
1.2 Liquid Shortenings	2
1.3 Fluid Shortenings	2
1.4 Frying Shortenings	2
1.5 Margarine Shortenings	3
1.6 Salad Oils	3
1.7 Cocoa Butter Replacer Fats	3
1.8 Development of New Zealand Mutton Tallow as a Source of Edible Oils and Fats	6
2. LITERATURE REVEIW	8
2.1 Introduction	8
2.2 Melting and Solidification of Fats	8
2.2.1 The Effect of Chemical Structure Upon the Melting of Triglycerides	8
2.2.2 Solid/Liquid Phase Behaviour of Mixtures of Triglycerides	9
2.2.2.1 Solid Solutions	10
2.2.2.2 Crystalline Compound Formation	12
2.2.2.3 Layer Crystals	12
2.2.3 Phase Behaviour of Natural and Commercial Fats	13
2.2.3.1 Phase Behaviour and Composition of Cocoa Butter	13
2.2.4 Polymorphism	16
2.2.5 Crystal Habit	18
2.2.6 Conclusions	19

Page

2.3 Fractionation Methods 19

2.3.1 Fractionation Crystallisation 19

2.3.2 Fractional Extraction 20

2.3.3 Liquid/Liquid Extraction 20

2.3.4 Comparison of Fractionation Methods 21

2.3.5 Comparison of Fractional Crystallisation Methods 22

2.4 Solvent Fractionation

2.4.1 Formation of the Solid Phase 23

2.4.2 Separation of the Liquid and Solid Phases 24

2.4.3 Factors Influencing the Nature of the Precipitate 25

2.4.3.1 Solvent Type 25

2.4.3.2 Water Content of the Solvent 27

2.4.3.3 Solvent/Fat Ratio 28

2.4.3.4 Cooling Rate 29

2.4.3.5 Degree of Agitation During Crystallisation 30

2.4.4 Conclusions on Solvent Fractionation 31

2.5 Tallow Composition 32

2.5.1 Fatty Acid Composition of Mutton Tallows 32

2.5.1.1 Breed 33

2.5.1.2 Body Site 33

2.5.1.3 Diet 34

2.5.1.4 Age 34

2.5.1.5 Season 35

2.5.1.6 Sex 35

2.5.2 Triglyceride Composition of Mutton Tallows 35

2.5.3 Comparison of Beef and Mutton Tallows 38

2.5.4 Variation in Tallow Composition 38

Page

2.6 Solvent Fractionation Schemes for Tallow 40
2.6.1 Introduction 40
2.6.2 Analytical Fractionation Schemes 40
2.6.3 Schemes to Produce Fractions for Specific Uses 42

2.7 General Conclusions from Literature Survey 45

3. METHODS OF ANALYSIS 47
3.1 Chemical Analysis 47
3.1.1 Thin Layer Chromatography (TLC) 47
3.1.1.1 Preparation and Development of Plates 47
3.1.1.2 Preparative TLC 48
3.1.1.3 Argentation TLC 48
3.1.1.4 TLC of 2-Monoglycerides 49
3.1.2 Fatty Acid Analysis 49
3.1.2.1 Preparation of Fatty Acid Methyl Esters 50
3.1.2.2 Gas Liquid Chromatography (GLC) 50
3.1.3 Quantitative Measurement of Triglyceride Bands 50
3.1.4 Preparation of 2-Monoglycerides 51

3.2 Thermal Analysis-Differential Scanning Calorimetry (DSC) 51

4. TRIGLYCERIDE AND FATTY ACID ANALYSIS OF SELECTED NEW ZEALAND MUTTON TALLOWS 54
4.1 Introduction 54
4.2 Methods of Collection and Analysis of the Tallows 54
4.3 Fatty Acids in the Tallows Analysed 55
4.4 Identification of Triglycerides in Mutton Tallow 61
4.5 Proportion of the Different Triglyceride Types in the Tallows Analysed 63
4.6 Proportion of 2-oleo Disaturated Triglycerides 66
4.7 Conclusions 68

Page

5. DEVELOPMENT OF A SOLVENT FRACTIONATION SCHEME FOR NEW ZEALAND MUTTON TALLOW 70

5.1 Introduction 70
5.2 Outline of the Fractionation Method 71
5.3 The Independent Variables 71
5.3.1 First Crystallisation Temperature 72
5.3.2 Second Crystallisation Temperature 72
5.3.3 Solvent to Fat Ratio at Each Crystallisation 72
5.3.4 Water Content of the Acetone 72
5.3.5 Agitation Speed 73
5.3.6 Crystallisation Time 73
5.3.7 Tallow Source 73
5.4 Dependent Variables 74
5.5 Screening Experiment 75
5.5.1 Experiment Design 75
5.5.2 Results of the Screening Experiment 76
5.5.3 Empirical Equations Relating Dependent and independent Variables 76
5.5.3.1 Yield of the Hard Fraction 78
5.5.3.2 Yield of the Intermediate Fraction 79
5.5.3.3 Yield of the Soft Fraction 79
5.5.3.4 CBLF of the Intermediate Fraction 80
5.5.3.5 Residual Plots 81
5.5.4 Main Effects 81
5.5.5 Interactive and Quadratic Effects 82
5.5.6 Choosing Variables for Future Experimentation 83
5.5.7 Conclusions from the Screening Experiment 84

6. OPTIMISING THE MELTING PROPERTIES OF THE INTERMEDIATE FRACTION 85

6.1 Introduction 85
6.2 Fractionation Method 85

Page

6.3 Experimental Design 85
6.4 Levels of the Independent Variables 87
6.5 Results 91
6.6 Empirical Equations Relating Dependent and Independent Variables 91
6.7 Decoding the Models 93
6.8 Interpretation of the Models 94
6.8.1 Isolated Effect of the Variables 94
6.8.2 Consideration of the Combined Effect of the Six Independent Variables 96
6.9 Comparison of Results of the Screening Experiment and this Experiment 99
6.10 Extrapolation of Some of the Independent Variables 101
6.11 Further Study of the Effect of Altering S_1 and S_2 101
6.12 Stages of Fractionation 102
6.13 Consideration of Further Experiments 102
6.14 Conclusions 103

7. FURTHER STUDY OF THE INDEPENDENT VARIABLES AROUND THE PREVIOUS PREDICTED OPTIMUM 105
7.1 Introduction 105
7.2 Methods 105
7.3 Experimental Design 106
7.4 Levels of the Independent Variables 106
7.5 Results 107
7.6 Empirical Equations Relating Dependent to Independent Variables 107
7.6.1 Residuals from the Fitted Models 108
7.6.2 Isolated Effect of the Variables 112
7.7 Interpretation of the Results 113
7.8 Stages of the Fractionation 115
7.9 Temperature History of the Fat Solution During Crystallisation 116
7.10 Conclusions 117

Page

8. SCALE-UP OF THE FRACTIONATION 118

8.1 Introduction 118

8.2 Fractionation of Two Hundred Grams of Tallow 118

8.2.1 Methods 118

8.2.2 Results 120

8.2.3 Yields of the Three Fractions 121

8.3 Fractionation of One Kilogram of Tallow 121

8.3.1 Methods 121

8.3.2 Results 123

8.3.3 Comparison of the One Kilogram Fractionations with those of the Smaller Scale Fractionations 124

8.3.4 Further Attempts to Fractionate One Kilogram of Tallow 125

8.4 Comparison of all Fractionations 127

8.5 Conclusions 131

8.6 Discussion and Conclusions on the Fractionation Method 132

9. EVALUATION OF THE FRACTIONATION PRODUCTS 134

9.1 Introduction 134

9.2 Fatty Acid Composition of the Fractions 135

9.2.1 Hard Fractions 135

9.2.2 Intermediate Fractions 137

9.2.3 Soft Fractions 137

9.3 Proportion of Each of the Triglyceride Groups 137

9.4 Fatty Acid Composition of the Triglyceride Groups 140

9.5 Proportion of 2-oleo Disaturated Triglycerides in the Intermediate Fractions 141

9.6 Differential Scanning Calorimetry 142

9.6.1 Hard Fractions 142

9.6.2 Intermediate Fractions 142

9.6.3 Soft Fractions 143

9.7 Comparison with Other Fats 143

Page

9.7.1 Hard Fractions 143
9.7.2 Intermediate Fractions 144
9.7.3 Soft Fractions 148

9.8 Summary of Composition of the Fractions 151

9.9 Further Testing of the Fractions 152
9.9.1 Evaluation of the Soft Tallow Fraction as a Frying Medium 153
9.9.2 Evalution of the Soft Tallow Fraction for Mayonnaise Manufacture 153
9.9.3 Evaluation of Tallow Fractions for use in Baking 154
9.9.4 Evaluation of the Hard Tallow Fraction as a Pastry Fat 155
9.9.5 Nuclear Magnetic Resonance (NMR) of the Intermediate Fraction 155

9.10 Discussion and Conclusions on Uses for the Fractions 156

10. GENERAL CONCLUSIONS AND CONSIDERATIONS FOR FUTURE WORK 159

APPENDICES

1 Polymorphism of Triglycerides 162

2 Solvents which have been used for Solvent Fractionation of Fat Substances 165

3 Results of the Triglyceride and Fatty Acid Analyses of Selected New Zealand Mutton Tallows 167

4 Regression Equations and Regression Statistics from the Screening Experiment 181

5 Regression Equations and Regression Statistics from the Central Composite Design; and an Example of the Method used to compare Observed Responses with Responses Predicted from the Fitted Equations. 185

6 Regression Equations and Regression Statistics from the Box and Behnken Design (Chapter 7) 190

Page

7 Results of the Fatty Acid and Triglyceride Analyses of Some Selected Tallow Fractions 193

8 Further Aspects of Tallow Quality. 204

ABBREVIATIONS AND NOMENCLATURE 207

REFERENCES 209

LIST OF FIGURES

Figure	Title	Between pp.
2.1	Phase diagram of two triglyceride species which form ideal liquid and solid solutions	10-11
2.2	Binary phase diagram for a system exhibiting incomplete solid solubility	10-11
2.3	Binary eutectic phase diagram	11-12
2.4	Binary monotectic phase diagram	11-12
2.5	Differential scanning calorimeter (DSC) diagram for two samples of cocoa butter (a) pressed from shell-free West African cocoa (b) pressed from shell-free Brazilian cocoa	13-14
2.6	Fractionation scheme of Riemenschneider *et al*	40-41
2.7	Crystallisation sequence for separating sheep body triglycerides into six fractions of widely different iodine value	40-41
2.8	Unilever's fractionation scheme for mutton tallow	42-43
2.9	Fractionation scheme of Luddy *et al*	43-44
3.1	General scheme for analysis of the fat samples	47-48
3.2	Calculating the CBLF of a fat sample giving a DSC profile with only one peak	53-54
4.1	A plot of the proportion of 14:0 and 18:0 in the tallows analysed against the month the tallow was produced, and the least squares regression for the same	58-59
4.2	Argentation TLC of tallow triglycerides	61-62

LIST OF FIGURES (continued)

Figure	Title	Between pp.
4.3	Seasonal variation in the proportion of trisaturated, trans monounsaturated, cis monounsaturated and more unsaturated triglycerides in mutton tallows	63-64
4.4	Seasonal trend in the proportion of 2-oleo disaturated triglycerides in mutton tallows from the Smithfield works, and the least-squares linear regression line for this data	66-67
5.1	Outline of the two-stage solvent fractionation process	71-72
5.2	Half-normal plot for the yield of hard fraction	78-79
5.3	Half-normal plot for the yield of the intermediate fraction	78-79
5.4	Half-normal plot for the yield of the soft fraction	79-80
5.5	Half-normal plot for the CBLF of the intermediate fraction	79-80
5.6	Plot of residual v. Y for the yield of the hard fraction	81-82
5.7	Plot of residual v. Y for the yield of the intermediate fraction	81-82
5.8	Plot of residual v. Y for the yield of the soft fraction	81-82
5.9	Plot of residual v. Y for the CBLF of the intermediate fraction	81-82
5.10	Effect of fitting quadratic terms to a regression equation	83-84

LIST OF FIGURES (continued)

Figure	Title	Between pp.
6.1	Plot of residual v. Y for the yield of hard fraction model	92-93
6.2	Plot of residual v. Y for the yield of intermediate fraction model	92-93
6.3	Plot of residual v. Y for the yield of soft fraction model	92-93
6.4	Plot of residual v. Y for the CBLF of the intermediate fraction model	92-93
6.5	The isolated effect of T_1 on the dependent variables	
	(a) Yield of the hard fraction	94-95
	(b) Yield of the intermediate fraction	94-95
	(c) Yield of the soft fraction	94-95
	(d) CBLF of the intermediate fraction	94-95
6.6	The isolated effect of T_2 on the dependent variables	
	(a) Yield of the intermediate fraction	95-96
	(b) Yield of the soft fraction	95-96
	(c) CBLF of the intermediate fraction	95-96
6.7	The isolated effect of S_1 on the dependent variables	
	(a) Yield of the hard fraction	95-96
	(b) Yield of the intermediate fraction	95-96
	(c) Yield of the soft fraction	95-96
	(d) CBLF of the intermediate fraction	95-96

LIST OF FIGURES (continued)

Figure	Title	Between pp.
6.8	The isolated effect of S_2 on the dependent variables	
	(a) Yield of the intermediate fraction	95-96
	(b) Yield of the soft fraction	95-96
	(c) CBLF of the intermediate fraction	95-96
6.9	The isolated effect of W on the dependent variables	
	(a) Yield of the hard fraction	96-97
	(b) Yield of the intermediate fraction	96-97
	(c) Yield of the soft fraction	96-97
	(d) CBLF of the intermediate fraction	96-97
6.10	The isolated effect of A on the dependent variables	
	(a) Yield of the hard fraction	96-97
	(b) Yield of the intermediate fraction	96-97
	(c) Yield of the soft fraction	96-97
	(d) CBLF of the intermediate fraction	96-97
6.11	DSC profile of the intermediate fraction produced under the predicted optimum conditions and the DSC profile of a cocoa butter sample	97-98
6.12	Comparison of the DSC profiles of the intermediate fraction produced under the conditions of section 6.10 and cocoa butter	101-102
7.1	The isolated effect of T_1 on the dependent variables	
	(a) Yield of hard fraction	112-113
	(b) Yield of intermediate fraction	112-113
	(c) Yield of the soft fraction	112-113
	(d) CBLF of the intermediate fraction	112-113

LIST OF FIGURES (continued)

Figure	Title	Between pp.
7.2	The isolated effect of T_2 on the dependent variables	
	(a) Yield of intermediate fraction	112-113
	(b) Yield of soft fraction	112-113
	(c) CBLF of the intermediate fraction	112-113
7.3	The isolated effect of S_1 on the dependent variables	
	(a) Yield of the hard fraction	112-113
	(b) Yield of the intermediate fraction	112-113
	(c) Yield of the soft fraction	112-113
	(d) CBLF of the intermediate fraction	112-113
7.4	The isolated effect of S_2 on the dependent variables	
	(a) Yield of the intermediate fraction	112-113
	(b) Yield of the soft fraction	112-113
	(c) CBLF of the intermediate fraction	112-113
7.5	DSC profile of the intermediate tallow fraction produced with $T_1 = 9.2^{o}C$, $T_2 = 5.2^{o}C$, $S_1 = 4:1$, $S_2 = 12:1$; and the DSC profile of a cocoa butter sample	114-115
7.6	DSC profile of the intermediate tallow fraction produced with $T_1 = 9.2^{o}C$, $T_2 = 5.2^{o}C$, $S_1 = 4:1$, $S_2 = 6:1$; and the DSC profile of a cocoa butter sample	114-115
7.7	Temperature history of the fat solution during crystallisation	
	(a) First crystallisation	116-117
	(b) Second crystallisation	116-117

LIST OF FIGURES (continued)

Figure	Title	Between pp.
8.1	Cooling curves for the fractionation of 200 g of tallow	
	(a) First crystallisation	119-120
	(b) Second crystallisation	119-120
8.2	DSC profile of the intermediate tallow fraction produced by fractionating 200 g of tallow with air cooling; and the DSC profile of a cocoa butter sample	120-121
8.3	Apparatus used for the fractionation of 1 kg of tallow	122-123
8.4	Cooling curves for the fractionation of 1 kg of tallow	
	(a) First crystallisation	122-123
	(b) Second crystallisation	122-123
8.5	DSC profile of the intermediate fraction produced by fractionating 1 kg of tallow with stirrer speed of 25 rpm; and the DSC profile of a cocoa butter sample	123-124
8.6	DSC profiles of the intermediate fractions produced by fractionating 1 kg of tallow with T_1 = 11.5°C and slow and fast cooling rates; and the DSC profile of a cocoa butter sample	126-127
8.7	The effect of varying S_1 upon the yield of hard fraction	127-128

LIST OF FIGURES (continued)

Figure	Title	Between pp.
8.8	DSC profile of the intermediate fraction produced by fractionating 1 kg of tallow with S_1 = 1:1; and the DSC profile of a cocoa butter sample	127-128
8.9	DSC profile of the intermediate tallow fraction produced by fractionating 1 kg of tallow with S_1 = 1.5:1; and the DSC profile of a cocoa butter sample	127-128
9.1	DSC profiles of the hard fractions produced from 1 kg of tallow, and of a fraction of Luddy et al, 1977	143-144
9.2	DSC profile of the soft fraction produced by fractionating 1 kg of tallow with S_1 = 4:1, the two softest fractions produced by Luddy et al (1977) and a commercial salad oil (Luddy et al, 1977)	148-149

LIST OF TABLES

Table	Title	Page
2.1	Fatty acid composition of cocoa butter	15
2.2	Triglyceride composition of cocoa butter	15
2.3	Composition of the disaturated triglycerides in cocoa butter	16
2.4	Fatty acid composition of mutton tallows from various countries	33
2.5	Triglyceride composition of mutton tallows from various countries	36
2.6	Fatty acid composition of beef tallows from various countries	37
2.7	Triglyceride composition of beef tallows from various countries	37
2.8	Triglyceride composition of fractions by the scheme of Riemenschneider, Luddy, Swain and Ault (1946)	41
2.9	Triglyceride composition of fractions by the scheme of Hilditch and Shrivastava (1949)	41
2.10	Composition of products from solvent fractionation of Japanese mutton tallow	44
4.1	Total fatty acid composition of mutton tallows from the 1976/1977 season	56
4.2	Total fatty acid composition of mutton tallows from the 1977/1978 season	57
4.3	Comparison of the minimum, maximum and average proportion of selected fatty acids in the Smithfield tallows with published values	60

LIST OF TABLES (continued)

Table	Title	Page
4.4	Comparison of the fatty acid composition of mutton tallow (Smithfield November 1976 1½ R) determined by direct analysis and by calculation from the amount of each triglyceride group separated by argentation TLC and their respective fatty acid compositions	62
4.5	Comparison of the minimum, maximum and average proportion of the different triglyceride groups in the Smithfield tallows analysed with published values	65
4.6	Fatty acid composition at the 2-position of tallow triglycerides (Ocean Beach, May 1977) determined by direct analysis and by analysis of the triglyceride groups separated by argentation TLC	67
5.1	Definition of independent variables selected for the screening experiment	74
5.2	Effects determined using a 2^{8-4}_{IV} fold-over design	76
5.3	The experimental design used and the responses to the independent variables	77
6.1	Levels of the independent variables	87
6.2	The experimental design and the responses of the dependent variables	88
6.3	Fitted models for each of the response variables	92
6.4	Decoded models for each of the response variables	93
6.5	Predicted and measured values of the four response variables at the predicted optimum of the CBLF model	98

LIST OF TABLES (continued)

Table	Title	Page
6.6	Comparison of the significant terms in the fitted empirical models for the screening experiment and the central composite design	99
6.7	Levels of S_1 and S_2 and yields of fractions produced	102
7.1	Levels of the independent variables	107
7.2	The experimental design and the values of the response variables obtained	109
7.3	Empirical models for the response variables	110
7.4	Residuals from the fitted models	111
7.5	Summary of the effect of altering S_1 and S_2 around the optimum	115
8.1	Yields and characteristics of fractions obtained from 20 g and 200 g tallow using similar temperature and solvent : fat ratios	121
8.2	The effect of agitation speed and cooling rate upon the fractionation of 1 kg of tallow	124
8.3	A comparison of the yields of the three fractions produced from the fractionations of different amounts of tallow	125
8.4	The fractionation of 1 kg of tallow with different levels of T_1 or S_1	128
8.5	Comparison of the results of selected fractions from each of the three scales	130
9.1	Fatty acid composition of tallow and fractions obtained by solvent crystallisation	136
9.2	Proportions of the different triglyceride groups in each of the fractions and the original tallow	138

LIST OF TABLES (continued)

Table	Title	Page
9.3	Proportion of 2-oleo disaturated triglycerides in the intermediate fractions analysed, and the original tallow	141
9.4	Fatty acid composition of the 200 g hard fraction, the 1 kg hard fraction with S_1 = 4:1, and fraction 2 by Luddy et al (1977) from North American beef tallow (see figure 2.9)	143
9.5	Fatty acid compositions of each of the intermediate fractions analysed, cocoa butter and several fats which have been used to replace all or a portion of cocoa butter in chocolate or chocolate-type products.	145
9.6	Triglyceride composition of each of the intermediate fractions analysed, cocoa butter and some cocoa butter replacer fats	146
9.7	Fatty acid composition of the soft fraction produced by fractionating 1 kg of tallow (S_1 = 4:1), two North American beef tallow fractions (fractions 4 and 5 from figure 2.9), a commercial soyabean salad oil, zero-erucic acid rapeseed oil and a commercial shortening for heavy-duty frying (Sheppard, Iverson and Weihrauch, 1978)	150

CHAPTER 1
PRELUDE

In the year ending June 1979, New Zealand exported 90,000 tonnes of tallow. Ninety-seven per cent of this was inedible mixed beef and mutton tallow which was sold at an average price of only $473 per tonne.

In the same period, total imports of oils and fats were about 22,230 tonnes at an average price of approximately $760 per tonne (New Zealand Official Yearbook, 1980). Thus it would be advantageous if New Zealand could exploit this large domestic fat resource of tallow to a greater extent to reduce the need to import fats and oils.

Tallow has a great number of component triglycerides which exhibit a variety of properties. This makes tallow a product with very limited uses (Luddy, Hampson & Herb, 1977).

Fractionation of fats is a well-established industrial practice. Fractionation of a fat can give products with a triglyceride composition and physical properties which differ markedly from those of the starting material (Swern, 1964). Fractionation has been applied to many fats, including tallow, but there is little information on the nature of the products obtainable from fractionating New Zealand mutton tallow. The aim of this work was to study the nature of the fats which could be produced by fractionating New Zealand mutton tallow.

Tallow fractionation overseas, particularly beef tallow fractionation, has given products suitable for a wide variety of uses (Dobson, 1967; Weiss, 1970; Luddy, Hampson, Herb & Rothbart, 1973). Tallow or tallow fractions have been used in the typical edible fats discussed in the following sections.

1.1 PLASTIC SHORTENINGS

The original subject of the term shortening was any solid fatty material which exhibits a shortening effect in baked products. However, in the U.S.A. all fats and oils are now

called shortenings to distinguish them from margarines and other high fat content products which also contain non-fat materials, and this terminology will be used here. Shortenings may also contain emulsifying agents (Weiss, 1970).

Plastic shortenings are usually prepared by blending a relatively large oil fraction (the base stock) with a minor proportion of a hard fat (plasticiser). The softening action of the base stock is balanced with the hardening action of the plasticiser to obtain a shortening of the desired consistency and plastic range (Thompson, 1963).

The plasticiser is usually prepared by fractionation or hydrogenation. The latter process produces a high proportion of trans isomers, and because nutritive value of these has been questioned it may be prudent to minimise their levels in foods (Melnick and Gooding, 1960; Mishkel and Spritz, 1969; Luddy et al, 1973). For this reason, fractionation is often the preferred process.

1.2 LIQUID SHORTENINGS

Liquid shortenings are pourable and clear (Weiss, 1970). They are convenient to use and are generally suitable for all shortening requirements except where plasticity is required (Swern, 1964).

1.3 FLUID SHORTENINGS

An ideal fluid shortening is a stable suspension of solid discrete particles of fats in oil at a temperature of 15° to 40°C. The suspension should remain fluid throughout this temperature range without any significant change in viscosity and without setting of the solid fraction (Swern, 1964).

1.4 FRYING SHORTENINGS

Frying shortenings must have low free fat acidity and high smoke point, and should not produce objectionable odours during frying (Weiss, 1970). They are normally deodorised before use (Swern, 1964). Liquid frying shortenings have the

advantages that they can be poured and filtered when cold and can be heated without the danger of burning on hot coils. However they generally have lower resistance to oxidation than plastic shortenings.

1.5 MARGARINE SHORTENINGS

Margarines are shortenings in emulsion form. The purpose for which the margarine is used determines the properties required of the shortening. The shortening in a table margarine must be plastic and spread easily at room temperature, but should melt completely in the mouth.

Bakery margarines are used in baking in the same manner as shortenings, but usually impart more flavour and odour to the product. They are not required to melt at body temperature, and a shortening with a wide plastic range is generally used (Swern, 1964; Weiss, 1970).

1.6 SALAD OILS

Salad oils are liquid at room temperature and do not cloud or solidify at normal household refrigeration temperatures (4.5-10°C). An oil which becomes solid in the refrigerator is inconvenient to handle and less suitable for mayonnaise manufacture (Weiss, 1970).

The standard test for evaluating salad oils is the cold test of the American Oil Chemist's Society which requires the oil to remain clear after holding at 0°C for 5½ hours (Swern, 1964; American Oil Chemist's Society, 1973).

Salad oils are normally deodorised, and both dark and light coloured oils are produced (Swern, 1964).

1.7 COCOA BUTTER REPLACER FATS

Cocoa (or cacao) butter is a pale yellow solid fat obtained by hydraulic expression or solvent extraction of the whole beans, or the nib (cotyledon), of the tropical plant Theobroma cacao. Cocoa butter has very unique physical

properties for a fat. It melts very sharply at a temperature just below body temperature, but at lower temperatures is quite firm, does not feel greasy to the touch, and exhibits a distinctly brittle fracture below 20°C. The main use of cocoa butter is in the manufacture of solid chocolate confectionery and coatings for chocolates, candies and baked goods (Swern, 1964; Minifie, 1970; Weiss, 1970) where it binds the other ingredients and imparts desirable melting properties upon the product (Feuge, 1964; Soeters, 1970).

The high price of cocoa butter relevant to other fats, price fluctuations and unstable supply have resulted in many attempts to produce cocoa butter alternatives from cheaper fat sources, either for part or total replacement (Farr, 1954; Feuge and Lovegren, 1958; Crossley, Paul, Pardun and Soeters, 1960 A & B, 1961 A & B; Best, Soeters, Davies and Paul, 1961; Landmann, Lovegren and Feuge, 1961; Spadaro, Lovegren, Feuge and Patton, 1961; Sinnema, 1962, 1963, 1967; Decossas, Koltun, Spadaro, Feuge, Pollard and Patton, 1962; Arnold, 1963; Loders and Nucoline, 1964; Kawada and Matsui, 1968; Akiya, 1970; Babayan, 1970; Ryberg, 1970 & 1971; Errboe, Braemer-Madsen and Andersen, 1971; Kassabian, 1971; Bhambani, Shitole and Kane, 1972; Feuge, Gajee and Lovegren, 1973; Lovegren, Gajee, Gray and Feuge, 1973; Luddy, Hampson, Herb and Rothbart, 1973, 1976, 1978; Hasman and Zielinski, 1974; Wolf, 1975; Luddy, Rothbart, Hampson, Miller and Alt, 1976; Thomas and Paulicka, 1976; Anonymous, 1977; Minowa, Masyoshi, Toyoshima, Yasuo, Yasuda and Nozomi, 1977; Luddy, Hampson and Herb, 1977; Luddy, Hampson and Koos, 1979; Baliga and Shitole, 1981). A fermentation process has even been developed to produce a cocoa butter replacer (Fuji Oil Co., 1975, 1976). It has been predicted that the cost of cocoa butter will continue to increase dramatically until at least 1990 (Kooyker and Gustafson, 1977).

The principal ingredients of chocolate and chocolate coatings are chocolate liquor or cocoa powder, sugar and additional cocoa butter.

In many countries, including New Zealand, the use of cocoa butter alternative fats in chocolate is prohibited by law. However, they can be utilised to produce chocolate-like products, particularly for coatings (Feuge, 1964; Minifie, 1970; New Zealand Department of Health, 1973).

The extra added cocoa butter in chocolate is the fat for which an alternative is desired. The presence of some cocoa butter in chocolate or chocolate coatings is unavoidable since the desired flavour can only be achieved by the use of chocolate liquor or cocoa powder (Johnston, 1972). While cocoa powder from which the cocoa butter is removed by solvent extraction can be used with a cocoa butter alternative to produce a "chocolate" containing no cocoa butter, and this avoids the problem of fat compatability (Sheppard, Iverson and Weihrauch, 1978), the extraction process removes other compounds, especially anti-oxidants, from the cocoa powder. This leads to flavour deterioration, and such products are odourless because of the lack of cocoa butter (Minifie, 1970; Weiss, 1970).

Three different types of cocoa butter alternative fats may be distinguished:

(i) an equivalent - this can be mixed with cocoa butter in any proportion without altering the melting characteristics, and has itself similar melting properties;

(ii) an extender - this can be used as a part replacement for cocoa butter, but does not necessarily result in similar melting properties over the whole range of addition, and

(iii) a substitute - this alters the melting properties of cocoa butter and may itself have different melting characteristics, but can be used as a coating fat in certain circumstances (Paulicka, 1970, 1973; Chapman, Akehurst and Wright, 1971).

Coatings made with fats softer than cocoa butter are required for cakes, frozen desserts and ice cream where the cocoa butter ingredient of chocolate sets too hard and produces a brittle coating which flakes off.

Coatings made with fats harder than cocoa butter also have some uses. A coating fat with a large proportion of high-melting triglycerides extends the plastic range too far above body temperature and a waxy sensation on the palate results. However these may be used in candy products where the primary eating sensation is derived from the centres and the coatings are relatively thin, or for candy bar and biscuit enrobing where the melting point of chocolate is too low, especially in summer. Here it is not necessary for the coatings to melt completely in the mouth as they are masticated with solid components (Weiss, 1970; Dallow, 1974).

Hence, as well as demand for a cocoa butter equivalent, cocoa butter extenders and substitutes are useful in some circumstances.

1.8 DEVELOPMENT OF NEW ZEALAND MUTTON TALLOW AS A SOURCE OF EDIBLE OILS AND FATS

New Zealand tallows, especially beef tallow, are used directly in frying and are also used on a small scale in blends of fats as margarine and frying fats. They are not particularly suitable, however, because of their high melting points, and New Zealand imports large quantities of vegetable oils.

From overseas experience in fractionating tallows, it might be possible to separate New Zealand mutton into an oil fraction which could replace at least part of the imported vegetable oils; soft and hard fractions which could be blended to give margarines and shortenings for baking and table use; and a cocoa butter replacer which could replace some of the imported cocoa butter. As well as import substitution, export of these specialist fats, alone or blended with milkfat or other fats, could develop.

To gain insight into the composition of New Zealand mutton tallow, and hence the possible composition of products from it, mutton tallow samples were collected from throughout two seasons and analysed for fatty acid and triglyceride composition. From this study, a laboratory-scale fractionation scheme was developed to produce three fractions from New Zealand mutton tallow. The process was optimised for the yield and melting properties of the fraction which was likely to prove the most valuable - a fat with melting properties similar to those of cocoa butter.

To produce sufficient quantities of the three fractions for testing of their properties in different commercial applications, attempts were made to increase the scale of the process to fractionate 1 kg of tallow per run. Sufficient of each of the three products from this scale were produced for further testing as cooking and baking fats by the Food Technology Research Centre (Massey University) and as a cocoa butter substitue by Cadbury Schweppes Hudson Limited (Dunedin).

The triglyceride and fatty acid composition of these fractions was also determined and compared to that of the original tallow and certain commercial fats produced for specific uses.

CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

The aim of this literature review was to study the relationship between the chemical composition and the phase behaviour of fats in general as a background to understanding the behaviour of the triglycerides in tallow. The first section describes the melting and solidification properties of fats and how chemical composition and polymorphism affect these properties. The fatty acid and triglyceride compositions of mutton and beef tallows from around the world were studied to determine the factors that cause differences in their composition and properties, and to evaluate the feasibility of producing products with specified compositions and properties from tallow.

The methods by which fractionation can produce fats with specific melting properties, by concentrating triglycerides of suitable composition in the various fractions, were reviewed; and in particular fractionation schemes already used on tallows overseas were studied to see if they were applicable to New Zealand mutton tallow.

2.2 MELTING AND SOLIDIFICATION OF FATS

This section reviews the effects of chemical structure on the melting of pure triglycerides, the differences between the melting of triglycerides separately and in mixtures, polymorphism of triglycerides, triglyceride crystal habit and how these effects are transmitted to natural and commercial fats.

The unusual composition and corresponding unusual melting properties of cocoa butter are also reviewed.

2.2.1 The Effect of Chemical Structure Upon the Melting of Triglycerides

The melting point of a pure triglyceride depends upon:

(1) degree of saturation of the constituent fatty acids,

(2) length of the fatty acid chains,

(3) cis-trans isomerism of unsaturated fatty acids,

(4) positioning of the fatty acids within the triglyceride molecule,

(5) positioning of the double bonds within the fatty acid (Litchfield, 1972).

For simple triglycerides (those containing only one fatty acid species) there is a decrease in the melting point with increased unsaturation and decreased chain length. Trans isomers melt at higher temperatures than the corresponding cis isomers.

With mixed triglycerides, the pattern is very complex. In trisaturated mixed triglycerides, the melting point is generally lowered as either the difference between the length of the terminal chains or the maximum difference in chain length between the middle and terminal chains is increased. A similar pattern exists for the mixed unsaturated triglycerides, but the number, nature and positioning of the double bonds are also significant factors, with an unsaturated acid in a terminal position lowering the melting point more than a similar fatty acid in the 2-position.

This great number of interacting factors makes the prediction of melting point from triglyceride structure very difficult (Bailey, 1950).

2.2.2 Solid/Liquid Phase Behaviour of Mixtures of Triglycerides

In a mixture of triglycerides, the melting point of any one triglyceride component is affected by the other components and may deviate considerably from that of the pure triglyceride (Bailey, 1950). If the solubilities of individual triglycerides in mixtures even approached those of the pure compounds, then excellent separation would be possible by either selective crystallisation or solubilisation (Brown, 1955; Brown and Kolb, 1955).

Crystallisation of complex triglyceride mixtures produces crystals containing more than one kind of molecule. There are three processes by which this may occur (Bailey, 1950; Sambuc, 1964; Rossell, 1973; Jacobsberg and Jacqmain, 1976): solid solutions, crystalline compounds and layer crystals.

2.2.2.1 Solid Solutions

Solid solutions, in which molecules of one constituent enter the lattice of the other and are uniformly distributed throughout it, occur when there is chemical similarity between the molecules involved and there is no great difference in their sizes (Maron and Prutton, 1969).

In a complex mixture of triglycerides, such as a natural or commercial fat, there are many triglycerides capable of forming solid solutions, and the formation of a pure triglyceride crystal is not possible. Similarly, the partial solubilisation of such a fat does not produce a liquid phase containing a pure triglyceride species, but produces a mixture of triglycerides.

The phase diagram for triglycerides which form ideal liquid and solid solutions is shown in Figure 2.1.

If a liquid mixture of composition x is cooled from a, solidification will begin, in the absence of supercooling, at b, with the first solid having the composition represented by c, and will end at d, with the last material solidifying having the composition d. If the system is cooled to point f, the separating solid and liquid will have the respective compositions h and g only if cooling is rapid enough to avoid crystallisation in the interval bf. If it is cooled slowly, only the last material solidifying will have the composition h - the rest will vary in composition from h to c. If such fractionation occurs, the system will not be in equilibrium at f. The two phases will eventually approach g and h in composition, but the attainment of this equilibrium may take a long time to occur - months or even years (Bailey, 1950; Sambuc, 1964).

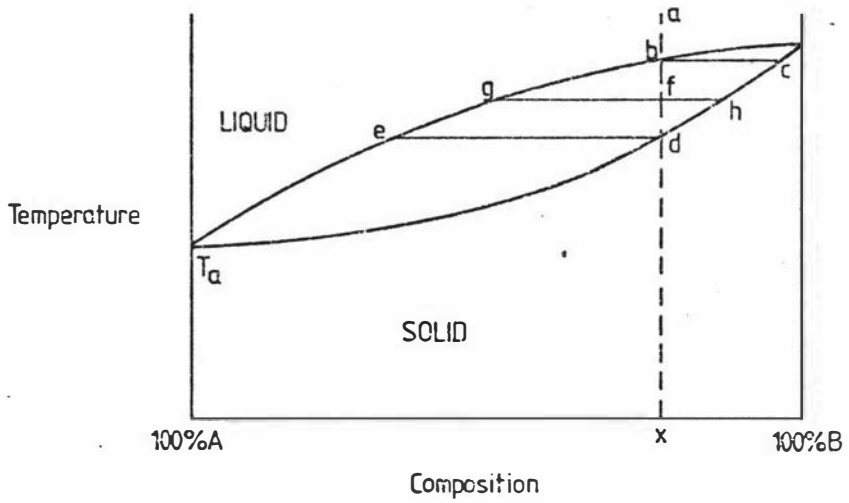


Figure 2·1 : Phase diagram of two triglyceride species which form ideal liquid and solid solutions.

(Sambuc, 1964)

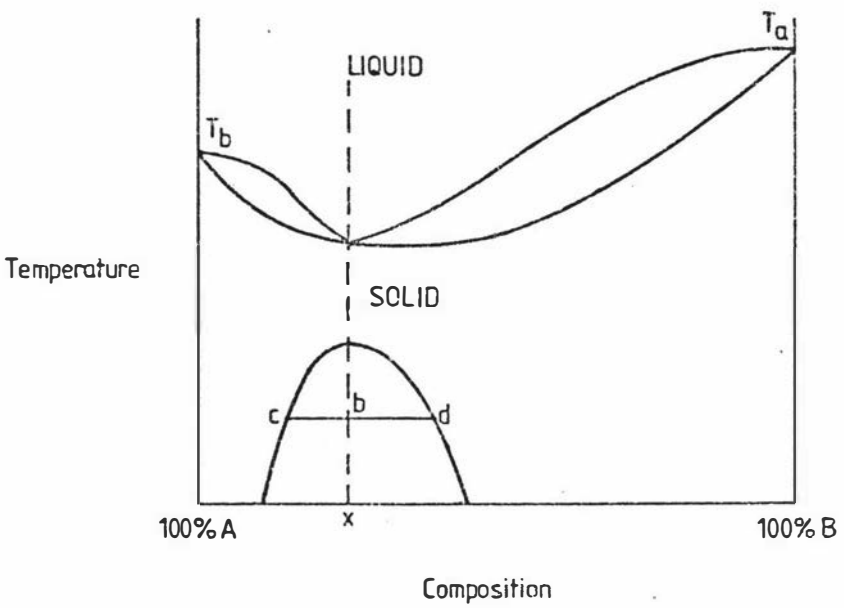


Figure 2·2 : Binary phase diagram for a system exhibiting incomplete solid solubility.

(Bailey, 1950)

A system where complete intersolubility occurs is rare for fatty substances (Bailey, 1950), though mixtures of ESS and SSS and POS and SOS (E = elaidic acid, S = stearic acid, P = palmitic acid, O = oleic acid) do show continuous solid solubility (Rossell, 1967; Jacobsberg & Jacqmain, 1976). The occurrence of limited solid solubility is more common and has two main effects:

(i) the formation of a solubility gap;

(ii) depression of the solidus and liquidus lines, causing a minimum for each.

A phase diagram for a simple 2-component system exhibiting incomplete solid solubility, and the occurrence of these characteristics, is shown in Figure 2.2.

A system of composition x brought to the temperature represented by b will separate into two solid phases of compositions c and d.

A more common system for triglyceride mixtures exhibiting limited solid solubility is where the solubility gap extends up into the liquid region - this is known as the eutectic system. The phase diagram for a binary eutectic system is shown in Figure 2.3.

The point E in this figure is called the eutectic point, and it is the lowest temperature for appearance of the liquid. A mixture of composition corresponding to the eutectic point melts as a pure compound. For all compositions between y and z, melting begins at this point. Outside this zone, heating or melting proceeds as for a system showing ideal solid solubility.

Binary triglyceride mixtures which exhibit eutectic behaviour include PPP and SSS, EEE and SOS, POS and PSO, PPP LLL and PPP and SOS (P = palmitic acid, S = stearic acid, E = elaidic acid, O = oleic acid, L = lauric acid), (Rossell, 1967 and 1973; Jacobsberg and Jacqmain, 1976).

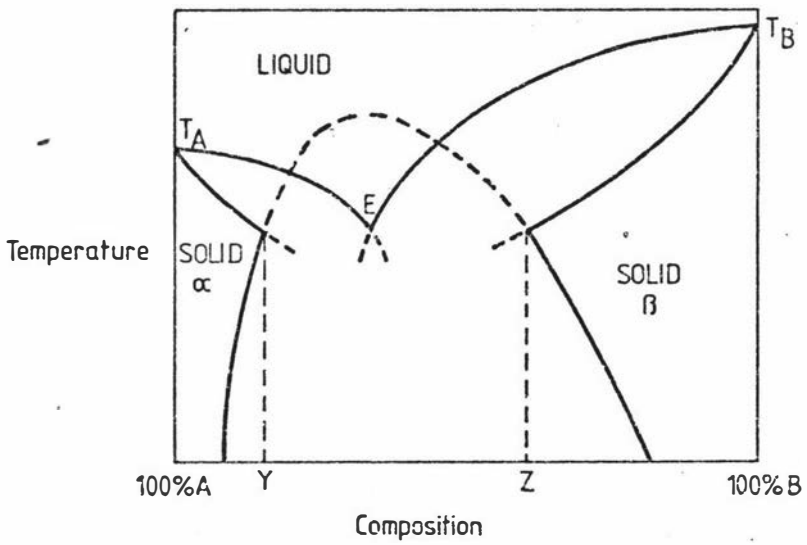


Figure 2·3 : Binary eutectic phase diagram

(Sambuc, 1964)

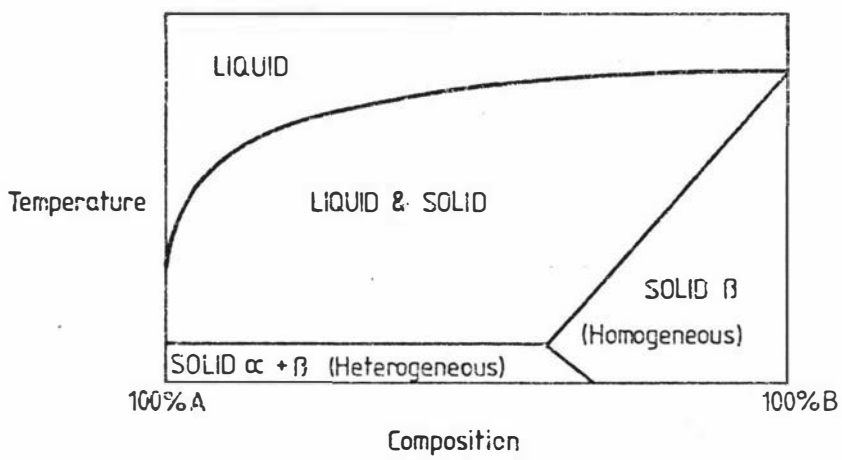


Figure 2·4 : Binary monotectic phase diagram

(Rossell, 1973; Jacobsberg and Jacqmain, 1976)

With triglyceride mixtures a special case of eutectic, the monotectic, in which the eutectic point coincides with one of the components, is often encountered. A monotectic phase diagram for a binary mixture is shown in Figure 2.4. Binary triglyceride mixtures which show monotectic behaviour include SSS and OOO, SSS and LLL, PPP and POP and SOS and SSS (Rossell, 1973; Jacobsberg and Jacqmain, 1976).

A further type of behaviour in triglyceride mixtures in which incomplete solid solubility occurs is peritectic behaviour, where a solid solution of one type is transformed to a solid solution of another type at a definite temperature (Bailey, 1950; Hannewijk, Haighton and Hendrikse, 1964). Binary mixtures of SOS and SOO exhibit peritectic behaviour (Rossell, 1967).

2.2.2.2 Crystalline Compound Formation

This unusual behaviour has been observed between some triglyceride pairs. Chemical compounds are not envisaged, but rather crystalline interactions and preferential crystal structures. In each case where this effect has been observed between triglyceride pairs, a fatty acid at the 2-position in one molecule is repeated at the 1- and 3-positions of the other molecule, and equal proportions of the two molecules have been required to obtain the effect. This suggests that there is some underlying cause, such as chain pairing, to explain the behaviour (Rossell, 1973). It can cause the inclusion of higher than expected proportions of relatively unsaturated triglycerides in stearine fractions produced by crystallisation e.g. in solvent fractionation of palm oil, compound formation between sn - OPO and sn - POP causes high proportions of sn-OPO to be crystallised with the stearine fraction (Berger, 1977). Compound behaviour has also been observed in a mixture of SSO and SOS (Rossell, 1973).

2.2.2.3 Layer Crystals

Because of the similarity in structure and properties of different triglycerides it is possible, when a fat is cooled slowly, for the layers of one to deposit readily on the crystal surfaces of another, thus producing crystals with a layered structure. The high melting point triglycerides will

tend to the inner layers, and the low melting to the outer layers, even if solid intersolubility prevents the separation of the different members in pure form.

2.2.3 Phase Behaviour of Natural and Commercial Fats

The types of phase behaviour observed between triglyceride pairs are transmitted to natural and commercial fats in which these triglycerides are major constituents (Berger, 1977). However the mixture of triglycerides present in any natural or commercial fat is too complex to allow accurate prediction of their phase behaviour from their triglyceride composition (Rossell, 1973; Berger, 1977). For instance tallow, with ten major fatty acids, can have a theoretical maximum of 550 triglyceride types (not including optical isomers). Assuming 65% of these occur, there will be about 350 different triglyceride species present (Litchfield, 1972). The attainment of physical data for such a system is obviously impractical. Even if phase data were available for triglycerides of a natural fat, its applicability to any real situation would be severely limited because of the difficulty of obtaining equilibrium in fatty substances. Phase diagrams are only applicable to systems in equilibrium, but this is very difficult to obtain with fats. Once mixed crystals are formed, equilibrium can only be established by molecular diffusion, but the diffusion of long chain molecules in a solid is very slow and complete homogenisation may take months or even years. The very poor thermal conductivity of fats and their tendency towards polymorphism add to the difficulty of obtaining equilibrium (Bailey, 1950).

2.2.3.1 Phase Behaviour and Composition of Cocoa Butter

Cocoa butter has very unique physical properties for a fat (see section 1.7). The differential scanning calorimeter (DSC) melting profiles of two samples of cocoa butter are presented in figure 2.5. The temperature melting range of cocoa butter is very small compared to other fats.

The distinctive physical properties of cocoa butter are due to its unique triglyceride composition (Hilditch, 1964; Soeters, 1970; Chapman, Akehurst and Wright, 1971; Huyghebaert and Hendrickx, 1971; Padley, Paulussen, Soeters and Tresser, 1972; Dugan , 1976; Gordon, Padley and Timms, 1979). While

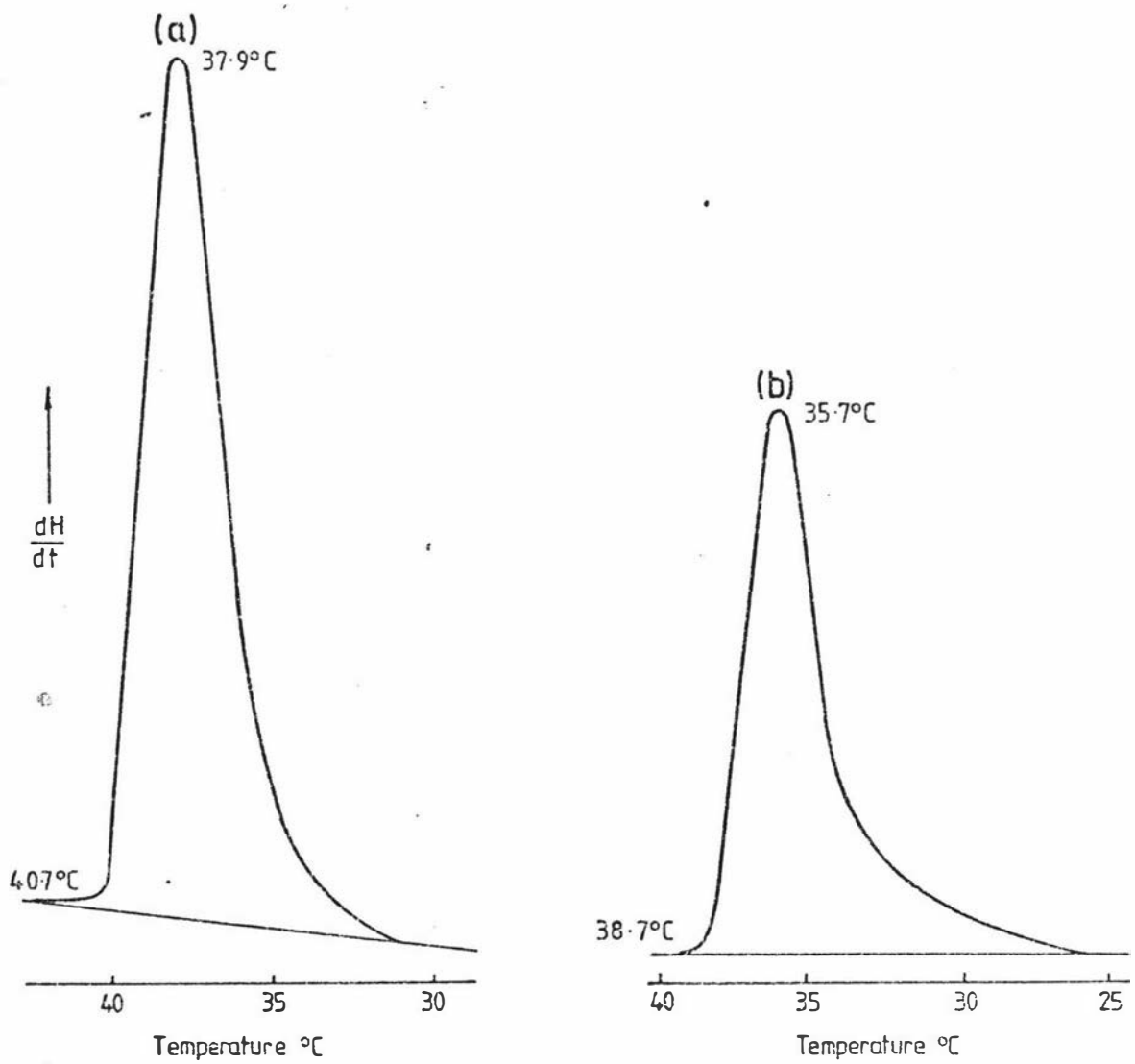


Figure 2·5: Differential scanning calorimeter (DSC) diagram for two samples of cocoa butter. (Johnston, 1972)

(a) pressed from shell-free West African cocoa

(b) pressed from shell-free Brazilian cocoa

the fatty acid composition (see Table 2.1) is similar to that of some other fats, including tallow, (Luddy et al, 1973) the triglyceride composition (see Table 2.2) is unusual. It has a high content of disaturated triglycerides, and in particular 2-oleo disaturated triglycerides. A cocoa butter sample analysed by Sampugna and Jensen (1969) contained 74.1% of disaturated triglycerides, and 93% of these had oleate at the 2-position (see Table 2.3).

There is almost identical thermal behaviour between a cocoa butter sample and a ternary mixture of the main 2-oleo disaturated triglycerides. (Chapman et al, 1971). The quality of inferior cocoa butter can be improved by the addition of 2-oleo disaturated triglycerides (Padley et al, 1972).

Table 2.1: Fatty acid composition of cocoa butter

SOURCE	FATTY ACID (MOLE % IN COCOA BUTTER)								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	OTHERS
Hilditch and Williams (1964)	-	26.2	-	34.4	37.3	2.1	-	-	-
Thomas and Paulicka (1976)	0.1	25.8	0.3	34.5	35.3	2.9	-	-	1.1
Chapman, Akehurst and Wright (1971)	-	24.4	-	35.0	36.3	2.8	-	1.0	0.5
Willie and Lutton (1966)	-	25.5	-	36.5	35.0	2.0	1.0	-	-
Lovegren, Gray and Feuge (1976)	tr	28.4	0.1	32.1	36.1	3.0	-	0.3	tr
Ryberg (1970)	tr	26.0	1.0	32.0	34.0	4.0	-	2.0	1.0
Gordon, Padley and Timms (1979)	tr	25.0	-	35.0	37.0	3.0	-	-	1.0
Hampson, Luddy and Rothbart (1975)	1.0	24.0	-	35.0	38.0	2.0	-	-	-
Sheppard, Iverson and Weihrauch (1978)*	0.1	25.4	0.4	33.2	32.6	2.8	0.2	0.9	-
Brockerhoff and Yurkowski (1966)	-	24.1	0.4	35.0	36.0	3.4	-	1.1	-
Average (calculated)	0.1	25.5	0.2	34.3	35.8	2.8	0.1	0.5	0.4

* Mean of 158 analyses.

Table 2.2: Triglyceride composition of cocoa butter

SOURCE	TG GROUP (MOLE % OF COCOA BUTTER)			
	TRISATURATED	DISATURATED	MONOSATURATED	TRIUNSATURATED
Kawada and Matsui (1968)	1.9	77.1	16.5	4.5
Hampson, Luddy and Rothbart (1975)	2.0	84.0	12.0	2.0
Jurriens and Kroesen (1965)	2.2	78.3	16.0	3.5
Swern (1964)	2.5	76.9	20.4	-
Hilditch and Williams (1964)	2.0	77.0	21.0	-
	2.5	83.0	13.5	1.0
Average (calculated)	2.2	79.4	16.6	1.8

Table 2.3: Composition of the disaturated triglycerides in cocoa butter (Sampugna and Jensen, 1969)

TG	(MOLE % of cocoa butter)
sn-SOP	16.3
sn-POS	16.5
POP	12.1
SOS	24.2
sn-OPP	0.0
sn-PPO	1.1
sn-PSO	0.3
sn-OSP	0.0
sn-OPS	0.0
sn-SPO	2.1
sn-SSO	0.8
sn-OSS	0.7

2.2.4 Polymorphism

Fatty acids and glycerides, in common with virtually all long chain compounds and many other substances, exhibit the phenomenon of polymorphism or existence in more than one crystal form (Bailey, 1950).

Polymorphism arises from different patterns of molecular packing in the crystal.

The packing of the hydrocarbon chains in a triglyceride dominates its solid state structure. The chains are arranged in the triglyceride molecule in an extended planar zig-zag conformation with their axes aligned parallel to one another (Norris, 1977). The relative orientation of these zig-zag planes between adjacent molecules is an important factor in differentiating polymorphic forms (Lutton, 1972). A more detailed account of triglyceride polymorphism is given in Appendix I.

Although fats are complicated mixtures of triglycerides they often display polymorphic behaviour similar to that of the component triglycerides (Bailey, 1950).

Polymorphism in natural fats is generally most pronounced when they are relatively simple in composition and consist predominantly of one triglyceride or one class of closely-related triglycerides. For instance cocoa butter, which consists predominantly of 2-oleo disaturated triglycerides with very similar structures, is a strongly polymorphic fat. Six polymorphic states, named I to VI in order of increasing melting point, have been observed for cocoa butter (Willie and Lutton, 1966; Witzel and Becker, 1969; Huyghebaert and Hendrickx, 1971). Polymorphic behaviour is observed to a lesser extent in beef fat, which has a more heterogeneous triglyceride structure, but is observed to a greater extent after the fat has been fractionally crystallised to yield an oleo oil of much more uniform composition. Similarly, whereas partially hydrogenated cottonseed oil shows little polymorphism, highly hydrogenated cottonseed oil is quite marked in polymorphic behaviour, and it is even more pronounced in hydrogenated oils consisting predominantly of 18 carbon acids which form mostly tristearin after complete hydrogenation (Bailey, 1950).

In fats, the end group may be distorted to allow accommodation of chains of different lengths. The longer chains may be bent near the ends of adjacent shorter chains or they may penetrate into other layers of molecules. In complex triglyceride mixtures, the β' form is more stable than in pure mono-acid triglycerides. Fats in which the chain lengths show little variation are β tending (Norris, 1977).

The polymorphic form in which a fat crystal is obtained from the melt depends upon the temperature at which crystallisation occurs. If cooled quickly, the melt can be super-cooled to a temperature just below the melting point of the lowest melting form, but only by very little. For practical purposes, the point at which solidification begins is the melting point of the lowest polymorphic form. If the melt is cooled slowly, crystallisation in other forms occurs. Crystals of a low melting form can be transformed by holding the material at a temperature above the melting point of that form and allowing melting and re-solidification to occur. Transformation will also occur without melting, the rate of transformation being greater the closer the temperature is to the melting point of

the polymorphic form, and increasing with decreasing chain length of the molecules. At temperatures well below the melting point of an unstable form, transition proceeds very slowly, and the unstable form may persist almost indefinitely. Because of the low rate of heat transfer in fats, it is usually impossible to get pure unstable forms except in thin layers. Crystallisation of fats from solvents almost always produces crystals in the most stable form (Bailey, 1950).

A polymorphic transition can be considered as a real phase change like melting or vaporisation as it involves reconstruction of the crystal lattice and is accompanied by discontinuities in the heat content, the specific volume and certain other properties of the material. Hence a measure of any of these properties over a range of temperatures can be used to study the phase behaviour (including polymorphic behaviour) of a substance (Bailey, 1950; Mullin, 1961; Chapman, 1962).

Since the composition of mixed crystals, as well as their polymorphic form, is dependent upon the rate of cooling, the thermal behaviour of fats is very complicated (Norris, 1977).

2.2.5 Crystal Habit

Although triglycerides normally only pack in the above forms, the relative sizes of the faces of a particular crystal can vary considerably, causing great variance in the external shape or habit of a crystal. The crystals may grow more rapidly, or be stunted, in one direction; thus an elongated growth of the prismatic habit gives a needle-shaped (acicular) habit while a stunted growth gives a flat plate-like crystal (tabular, platy or flaky habit). The crystals of fatty acids and glycerides usually appear as rather elongated needles, though this can vary considerably depending upon such factors as the nature of the solvent or the presence of impurities. Many fatty compounds grow as fans or spherulites consisting of needles radiating outwards from a common centre. Crystals usually assume their most highly developed and characteristic forms when they are grown slowly from a melt or solution only slightly supercooled, and in which there is free circulation of the liquid about the crystals. Rapidly formed crystals tend to grow without the

development of all faces, as there are usually mechanical restrictions upon growth in all directions (Bailey, 1950; Mullin, 1961).

2.2.6 Conclusions

The melting behaviour of natural and commercial fats is extremely complicated and it is not possible to accurately predict the behaviour of any such fat from the properties of the individual component triglycerides. However chemical composition of a fat does give a broad indication of the type of behaviour which can be expected. In order to get a cocoa butter substitute from tallow, the fraction must contain a high proportion of 2-oleo disaturated triglycerides; oils must contain a high proportion of unsaturated fatty acids; and very hard fats must contain a high proportion of saturated fatty acids.

2.3 FRACTIONATION METHODS

Any components of a fat that differ considerably in melting point can be preferentially separated by crystallising out the higher melting component or dissolving the lower melting component and separating the two phases.

Three methods by which this is achieved are:

(1) fractional crystallisation;

(2) fractional extraction;

(3) liquid/liquid extraction.

2.3.1 Fractional Crystallisation

Fractional crystallisation is a separation process where some component triglycerides are preferentially crystallised and then separated from the liquid phase.

Three fractional crystallisation processes are used:

(a) dry fractionation - this involves cooling the fat to a temperature which allows the desired fraction of higher melting triglycerides to crystallise and then separating it from the melt.

(b) solvent fractionation - the fat is dissolved in an organic solvent and cooled to precipitate the desired triglycerides. "There is no particular distinction between the processes of melting and solution. Both are processes of dissolution governed by the principles applying to all solid - liquid phase transformations. Hence a triglyceride fraction crystallised from solvent at a high temperature will have a higher melting point than a fraction crystallised at a lower temperature" (Bailey, 1950).

(c) detergent fractionation - the partially crystallised fat mass is mixed with an aqueous solution of a surface active agent which replaces the oil phase on the surface of the crystals. The crystals and the aqueous solution form a suspension which can be separated from the liquid oil phase by centrifugation (Seugé and Vinconneau, 1975; Braae, 1976). The water may be replaced by an ammonia solution adjusted to a density between the densities of the solid and liquid phases (Poot, Dijkshoorn, Haighton and Verburg, 1975).

2.3.2 Fractional Extraction

Instead of crystallising the higher melting fraction from a melt or organic solution of the fat, it is possible to preferentially dissolve the lower melting fraction of the solid fat and separate the two phases (Sinnema, 1963).

2.3.3 Liquid - Liquid Extraction

In the liquid - liquid extraction process a fat is brought to equilibrium in contact with a solvent with which it is incompletely miscible and the two phases are allowed to separate. The portion of fat dissolved in the solvent phase will be different in composition from that remaining in the fat phase.

(Hixson and Bockelmann, 1942; Gloyer, 1948; Pratt, 1953; Naudet, 1964; Unilever, 1974). Liquid - liquid extraction can be used to get good separation, but the process is very laborious. If it is performed in batch, then many steps are required - the degree of separation from a single stage is rarely significant in producing fractions of commercial importance (Gloyer and Stewart, 1950). A single-stage liquid—liquid extraction gives far poorer separation than a single-stage crystallisation (Goss, 1949).

In practice, liquid - liquid extraction is performed continuously in tall columns in which the solvent and fat flow countercurrently. The fat is fed in half-way up the column and the solvent from the top. A portion of the extract is recycled to the column to increase the efficiency of separation. Alternatively a second solvent, miscible with the oil but immiscible with the first solvent, may be fed into the column (Swern, 1964; Wisniak and Barrientos, 1967; Parsons, 1975; Contreras, Migliaro & Raffo, 1971).

2.3.4 Comparison of Fractionation Methods

The claimed advantages of fractional extraction over fractional crystallisation are that at a given temperature the lower-melting fraction dissolves much more quickly than the harder fraction crystallises in fractional crystallisation, occluded lower-melting components are extracted, unsaponifiable constituents are removed, it is easier to carry out continuously and the product is more constant than with fractional crystallisation (Arnold, 1963; Sinnema 1963 & 1967).

The main disadvantage is that the crystals entering the solid fraction are not normally as pure as crystals entering the solid fraction in a solvent fractionation process. In solvent fractionation, the crystals are grown from a solvent and this greatly reduces the occurrence of mixed crystals, enabling purer products to be obtained (Bailey, 1950; Kreulen, 1976). However, in a fractional extraction process the crystals entering the solid phase have not grown in this manner and hence mixed crystal formation is enhanced.

Liquid - liquid extraction might offer possibilities for future developments, but the only important method of fat fractionation on a commercial scale up to now is by fractional crystallisation (Kreulen, 1976). The application of liquid - liquid extraction with fatty products has been very limited (Naudet, 1964) and recent literature does not reveal any further substantial development of liquid - liquid or fractional extraction processes.

Hence fractional crystallisation appears to be the most promising process for fractionating tallow in this work where good separation of a specific group of triglycerides is required in order to produce a cocoa butter replacer.

2.3.5 Comparison of Fractional Crystallisation Methods

Solvent fractionation gives better separation than detergent fractionation, and detergent fractionation gives better separation than dry fractionation (Bernardini, 1968; Kassabian, 1974; Loncin, 1976; Berger, 1977). Crystallisation from solvents can produce a number of fat products which are impossible to obtain by dry fractionation (Bailey, 1950). Detergent fractionation gives poorer separation of palm oil than does solvent fractionation (Construzioni Meccaniche Bernardini; Kassabian, 1974).

With centrifugation an essential operation in detergent fractionation, both capital and running costs are high (Taylor, 1973). Other disadvantages of detergent fractionation over solvent fractionation are that the solid fraction in detergent fractionation is crystallised from the melt, which enhances the degree of mixed crystal formation (Bailey, 1950; Kreulen, 1976), and despite extensive washing it is impossible to completely eliminate detergent from the fractions (Koslowsky and Letan, 1975). Commercial problems have occurred in the disposal of fats containing even traces of detergent (Construzioni Meccaniche Bernardini). Detergent fractionation has been used in some other processes (Unilever, 1966; Taylor, 1973; Poot, Dijkshoorn, Haighton and Verburg, 1975; Stein and Hartmann, 1975; Rek, 1977; Bussey, Ryan, Gray and Zabik, 1981) to give

adequate separation, but it appears that none of these have led to commercial application.

Disadvantages of solvent fractionation are that complex and thus expensive equipment is required, comprising leakproof and flameproof installations, and safety equipment when the solvents are inflammable (Taylor, 1973; Societe Pour L'Equipment des Industries Chimiques Speichim, 1975). Organic solvents are also generally expensive, and if they have high vapour pressure (as does acetone) high losses occur (Aarhus Oliefabrik, 1964). Hence while solvent fractionation gives better resolution of fractions than detergent or dry fractionations, it is also probably the most expensive fractional crystallisation process.

However because it was desired in this work to concentrate a very specific group of triglycerides into the intermediate fraction, it was thought that it was probably necessary to use the most efficient fractionation process. Thus solvent fractionation was chosen as the method for further study in this work.

2.4 SOLVENT FRACTIONATION

Two successive stages can be distinguished in the solvent fractionation process:

(1) formation of the solid phase
  (a) cooling of the liquid to supersaturation
  (b) progressive growth of the crystals.

(2) separation of the liquid and solid phases.

2.4.1 Formation of the Solid Phase

The object of this step is to produce a crystalline phase of the desired triglyceride composition which can be handled easily and which separates clearly from the mother liquor containing the liquid triglycerides. The nature of the crystals from the first stage affects the degree of separation of the crystals possible in stage two. The triglyceride purity of the crystals formed in stage one is severely limited by the

phase characteristics of triglyceride mixtures which prevents the growth of pure crystals - see section 2.2.

2.4.2 Separation of the Liquid and Solid Phases

Because fat crystals are voluminous and entrap large quantities of the mother liquor, mechanical separation of the two phases in step two is difficult and this also restricts the degree of separation possible (Magnusson and Hammond, 1959; Coppa-Zucarri, 1971). The higher the solvent:fat ratio, the less mother liquor is entrapped because of dilution (Brown, 1955). Washing of the separated crystals with fresh solvent also helps to decrease the degree of mechanical entrainment (Stevens, 1952; Pratt, 1956).

Filtration, centrifugation and decantation are all used to separate the precipitated triglycerides from the liquid. The form which the crystals must be in to achieve the best separation depends upon the separation method used.

For filtration to be used successfully, it is essential to grow distinct large crystals through which the liquid will flow in the filter. This allows for fast filtration rates without blocking the filter (James, 1943; Viarengo & Pascilli, 1973) and also for adequate washing of the crystals (Muckerheide, 1950).

Centrifugation has been used with some solvent fractionation processes (James 1943; Rubin, Teasdale and Mertens, 1959; Kassabian, 1972; Cavanagh, 1976; Tatsumi, Hashimoto, Terashima and Matsuo, 1977) but filtration is the method usually used with solvent fraction (Kreulen, 1976). Koslowsky (1975) and Koslowsky and Letan (1975) used isopropanol with a "natural additive" (unspecified) to fractionate palm oil. The solvent was incorporated in the crystals (like water is in organic compounds as the water of crystallisation) and decreased the density of them to such an extent that they floated on the surface of the oil and were removable by decantation. The solvent:fat ration was 1:1 (v/w).

However Kassabian (1974) found that this was suited only to small scale operation. The resolution was not good enough for large-scale, continuous, commercial operation.

In some cottonseed oil winterisation processes, settling of the crystals was achieved by using high cooling rates to produce small crystals and by using high solvent to fat ratios to reduce the viscosity of the oil. The crystals were thus able to be separated by decantation (Cavanagh, 1959). However, centrifugation gave more efficient separation (Cavanagh, 1976). Farr's (1954) processes utilized both filtration and decantation with fractionations, using acetone, of winterised cottonseed oil bottoms, but filtration was the preferred method. McGuine and Moss (1960) used a combined filtration/ decantation process.

2.4.3 Factors Influencing the Nature of the Precipitate

There are several factors which can influence the nature of the precipitate:

(1) nature of the solvent - both solvent type and water content of the solvent.

(2) solvent to fat ratio.

(3) cooling rate.

(4) degree of agitation during crystallisation.

2.4.3.1 Solvent Type

Many solvents, both polar and non-polar, have been used for solvent fractionation - see Appendix 2.

The crystals obtained from polar solvents are generally granular, needle-like crystals with good filtering and solvent washing properties, while those from non-polar solvents are flat, pearly, plate-type crystals or amorphous slimy precipitates not suitable for separation by filtration (James, 1943; Demmerle, 1947; Muckerheide, 1950; Boucher and Skau, 1952; Subrahmanyam and Achaya, 1958; Aarhus Oliefabrik, 1964).

When Muckerheide (1950) fractionated lard from isopropyl acetate, filtration was achieved in 1 minute compared to 1½ hours when a non-polar petroleum solvent was used.

While a precipitate of distinct large crystals such as that obtained from polar solvents is suitable for normal filtration, it is not suitable for separation by continuous centrifugation as such crystals will pack or interlock and not flow through the centrifuge. For this purpose the solids are required in a flowable form analagous to a very viscous liquid so that they flow through the centrifuge. This can be achieved by using non-polar solvents (which are generally cheaper) or by adjusting other crystallising conditions (especially rate of chilling and degree of agitation) with polar solvents (James, 1943). However such crystals are not in the best form for subsequent washing (Muckerheide, 1950).

An advantage of polar solvents is that triglycerides are less soluble in them than in non-polar solvents and hence higher crystallisation temperatures can be used, thus reducing refrigeration costs. By adding isopropanol to acetone, the latter's solvent action is diluted and fractionation can occur at higher temperatures (Kassabian, 1971).

As well as producing crystals in a form suitable for the separation method to be used, a solvent must be capable of giving good separation of the desired triglycerides. Hilditch (1964) performed and reviewed a vast number of solvent fractionation processes upon a wide variety of fats mainly as an analytical technique. He concluded that acetone generally gave the best fractionating power. Acetone is the solvent most often used in practice (Cording, Willard, Edwards and Eskew, 1953; Kreulen, 1976). However petroleum ether is better than acetone for the separation of the saturated and unsaturated fractions of kusum oil (Kundu, 1970). Construzioni Meccaniche Bernardini obtained the highest yield and best quality of liquid oil from palm oil fractionation using hexane, but Kassabian (1974) found that this gave poor resolution. Boucher and Skau (1951) found that acetone was more suitable than hexane in every way for the fractionation of cottonseed oil.

Thus overall acetone is the most widely recommended solvent where filtration is the method of separating the phases. However, there are certain fats for which other solvents appear to be more suitable.

2.4.3.2 Water Content of the Solvent

The water content of a solvent is very important because even small amounts can dramatically change a solvent's properties. Those solvents which give fat crystals suitable for filtering are generally capable of dissolving water from 1 to 15% by weight at 20°C (Muckerheide, 1950). High water concentrations allow the crystallisation to be carried out at higher temperatures, thus reducing refrigeration costs, but this may be detrimental to the crystallisation. In the fractionation of white grease (a product similar to tallow but of lower melting point) from acetone, separation was seriously impaired with water concentrations in excess of 2%, though acetone with a water concentration less than this gave the best separation of a number of solvents (Cording, Willard, Edwards and Eskew, 1953). A decrease in resolution with increasing water concentration is general for most solvents (Cording et al, 1953). Cavanagh (1959) claimed that minute quantities of moisture may act as crystal nuclei for the hard fraction and hence be beneficial. However McGuine (1960) said that water in the solvent slows down crystal agglomeration and hinders the operation of a crystalliser. The presence of water does not affect the type of crystal formed (Muckerheide, 1950).

McGuine (1960) recommended that the water content of the solvent should not exceed 5%, while Rubin et al (1959) said that 4% was the upper limit. Demmerle (1947), Hilditch and Williams (1964) and Luddy et al (1973) all recommended the use of "anhydrous" acetone, with Demmerle defining "anhydrous" as being less than 2% water.

Acetone is capable of dissolving water at levels much higher than those recommended and hence the water content must be carefully controlled. It would be preferable from this point of view to use a solvent in which water is less soluble (e.g. methyl isobutyl ketone, isopropyl acetate, ether acetate). When these solvents become water saturated, further water

settles out in the oil-solvent solution, thus automatically controlling the water concentration once saturation is achieved (Cording et al, 1953; Rubin et al, 1959).

2.4.3.3 Solvent/Fat Ratio

Solvent to fat ratio also has an important effect upon the nature of the precipitate obtained. Crystal habit is altered under conditions of high fat concentration as there are usually mechanical restrictions upon growth in all directions (Bailey, 1950), and this may inhibit the efficiency of the separation process. The polarity of the solvent is also affected if too great a concentration of fat in solvent is used. The solid triglycerides are then precipitated from a solvent containing a large proportion of dissolved liquid triglycerides; such a mixture is different in polarity from the solvent itself (Muckerheide, 1950). Similarly, too great a concentration of fat increases the amount of liquid triglycerides entrapped within the solid precipitate. Low solvent to fat ratios may cause handling problems, if the volume of precipitate is sufficient to produce a thick pasty slurry which is difficult to pump. Hence the maximum fat concentration is dictated to a certain degree by the solid triglyceride content of the fat being processed. The lower the concentration of fat, the more selective is the fractionation, though this may only be because of dilution of the entrapped oil (James, 1943). High solvent: fat ratios decrease the viscosity and allow for faster filtration rates (Pratt, 1956; Swern, 1964).

The upper limit of solvent ratio is dictated by economics. Capacity of the equipment and refrigeration and solvent recovery costs all increase as the solvent to fat ratio increases.

Solvent to fat ratios which have been used range from 0.1:1 (v/w) (Rubin et al, 1959) to 20:1 (v/w) (Pramuk, Whiting and McLaren, 1953; Sinnema, 1962; Luddy et al, 1973) though most fractionations operate at ratios of 2:1 to 5:1. Rubin et al (1959) and Mertens, Rubin and Teasdale (1961) used a

0.1:1 ratio for the winterisation of cottonseed oil with acetone, and this resulted in very short crystallisation times. However Weiss (1967) found that this produced a viscous miscella which was not much better than a solvent-free system.

Kawada and Matsui (1968) found that the best beef tallow fractions were obtained with a solvent to fat ratio of 3:1. Muckerheide (1950) recommended a 3.3:1 ratio and Spadaro, Lovegren, Feuge and Patton (1961) who used acetone to produce a confectionery fat from hydrogenated cottonseed oil, found that a 4:1 ratio was optimum. Spadaro et al (1961) experimented with ratios as high as 30:1, but no appreciable difference in iodine values or yields of their fractions was obtained with ratios greater than 4:1. Hilditch and Williams (1964) advised 10:1, but this was mainly for small-scale analytical purposes where resolution was the main criterion.

2.4.3.4 Cooling Rate

In any crystallisation where filtration is the means of separation, the aim is to produce a small number of nuclei around which the crystal formation grows larger in size as cooling is continued. If a vast number of nuclei are formed then filtration will be difficult due to the mass of small crystals. On the other hand if the crystals group together in clumps, then the liquid phase will be occluded, the result being poor separation and yield. To enable proper growth to take place, the cooling rate must be commensurate with the growth rate and mobility of nuclei (Weiss, 1967; Taylor, 1973).

The readiness with which the different polymorphic forms crystallise is in reverse order of their stability, hence the least stable form appears in a strongly undercooled material as less entropy of activation is involved in the formation of the less stable, less highly ordered crystals (Bailey, 1950).

In the presence of a suitable solvent the tendency for a fat to supercool is very small, at least partly because of the reduction in viscosity, and crystals of the most stable polymorphic form, which are large and easily-separable, are usually produced even at relatively high cooling rates. This decreases

the retention time in both the crystalliser and the filter (Bailey, 1950; Kreulen, 1976).

Triglyceride solutions in polar solvents can be cooled very rapidly and still produce precipitates which can be easily filtered (Muckerheide, 1950). Supercooling is observed to a greater extent in cottonseed oil/hexane solutions than in cottonseed oil/acetone or cottonseed oil/acetone/hexane solutions (Boucher and Skau, 1951) and this may at least partly explain the generally poorer filtering properties of fatty materials crystallised from non-polar solvents. Gee (1948) claimed that chilling fats at 1.1 to 1.5°C min^{-1} in both polar and non-polar solvents gave precipitates suitable for filtering, and Lacey and Leaders (1951) found that cooling fats in propane at 0.9°C min^{-1} produced easily-filterable crystals.

Muckerheide (1950) recommended a cooling rate of greater than 0.75°C min^{-1} to minimise holding time and mechanical damage to the crystals. The cooling rate is also important economically as it determines the size of equipment (Demmerle, 1947).

Cooling rate also affects the composition of the crystals formed (Bailey, 1950; Hinnekens, 1976).

The surface area to volume ratio of a crystalliser may also influence the crystallisation not only because the increased surface area will allow the solution to be cooled more quickly, but because solid surfaces can present points favourable for the initiation of crystallisation. The material of which the crystalliser is made may also be important (Bailey, 1950; Hinnekens, 1976).

2.4.3.5 Degree of Agitation During Crystallisation

If during cooling a crystal is broken up, then each of the fragments may act as a nucleus for further crystal growth. Shattering of crystals into many pieces produces a large number of nuclei and a large number of small crystals which are difficult to filter are formed.

Agitation during crystallisation is important as it helps contact all of the oil with the crystal nuclei to produce uniform and fast crystallisation and aids heat transfer. However it is important to use low shear rates or crystal attrition and an unfilterable precipitate results (Demmerle, 1947; Muckerheide, 1950; Rubin et al, 1959; McGuine, 1960; Taylor, 1973; Viarengo and Pasculli, 1973). To maintain good heat transfer throughout the crystallisation, scraped-surface crystallisers are required (Demmerle, 1947; Muckerheide, 1950). Muckerheide (1950) recommended that the cooling surface be scraped no more than once every three to six seconds to minimise the amount of mechanical working.

Agitation also reduces the degree of supercooling required before nucleation occurs (Berger, 1977).

2.4.4 Conclusions on Solvent Fractionation

Solvent fractionation seems to be the most promising process to produce useful fractions from New Zealand mutton tallow.

The choice of crystallisation temperature(s), solvent and solvent to fat ratio(s), cooling rate and degree of agitation all affect the nature of the products from a solvent fractionation process - both the yields and properties of the products, and also the suitability of the crystals for the separation process to be used. These variables can all be altered to give products with the desired properties, but the ultimate limiting factor in achieving this is the phase behaviour of the system.

It is not possible from the literature to accurately predict the effect of the above variables upon any specific fractionation process, and hence it is necessary to examine their influence upon any process developed in this work.

2.5 TALLOW COMPOSITION

Tallow is a triglyceride mixture produced as a by-product of the meat industry by the rendering of meat tissue.

There are several main types of tallow produced in New Zealand - inedible beef, inedible mutton, inedible mixed, edible beef and edible mixed. As well, several minor tallows are produced, including margarine grade, neatsfoot oil, K grade and edible mutton. Each of the main inedible tallows is further graded according to colour.

The composition of tallow is influenced by both the composition of the depot fats on the animals entering the meat killing plant and the practices followed during processing of the animal.

The body fat of animals entering meat killing plants is influenced by season, the species of animal, the position of the fat on the animal, diet, age and sex of the animal (Shorland, 1953; Cramer and Marchello, 1964; Hilditch, 1964; Duncan and Garton, 1967; Garton and Duncan, 1965, 1969).

2.5.1 Fatty Acid Composition of Mutton Tallows

The fatty acid compositions of mutton tallows from various countries are presented in Table 2.4. It can be seen that there is wide variation in the proportion of some fatty acids between tallows of the different countries, but also between different tallow samples from Australia.

The effects of season, breed , position on the body, diet, age and sex upon the fatty acid composition of sheep depot fats are discussed below:

Table 2.4: Fatty acid composition of mutton tallows from various countries

COUNTRY OF ORIGIN	FATTY ACID (MOLE % IN TALLOW)										
	12:0	14:0	16:0	18:0	20:0	14:1	16:1	18:1	18:2	⩾ 20 unsatd	Others
India[a]	-	2.9	27.8	27.7	1.5	0.4	2.7	33.0	3.4	0.6	-
South America[a]	-	1.0	21.0	30.0	-	-	-	43.0	5.0	-	-
North America[c]	5.2	0.8	23.6	24.5	0.8	0.3	2.5	35.3	4.0	-	3.0
Australia[a]	-	2.0	25.0	23.0	-	-	-	47.0	3.0	-	1.0
Australia[d]	0.1	2.6	20.4	25.6	-	0.8	2.5	37.7	4.3	0.4	5.6
Australia[a]	-	4.0	25.0	31.0	-	-	-	36.0	4.0	-	-
Australia[e]	-	3.3	25.5	22.2	-	-	4.7	38.3	2.2	1.6	-
England[b]	-	4.6	24.6	28.1	2.4	-	-	36.0	4.3	-	-
Average	0.7	2.7	24.1	26.5	0.6	0.2	1.6	38.3	3.8	0.3	1.2

a- Hilditch & Williams (1964); b - Swern (1964); c - Thomas and Paulicka (1976); d - Craske and Edwards (1970); e - Herbert and Kearney (1975).

2.5.1.1 Breed

Fatty acid analysis of subcutaneous and perinephric fats from sheep grazed on New Zealand pastures shows small variation in the content of individual fatty acids from corresponding tissues between animals of the same and of different breed, suggesting that environmental factors are more important than breed in determining the composition of sheep depot fats (Hansen and Czochanska, 1976).

2.5.1.2 Body Site

Variation in fatty acid composition between different tissues within the same adult animal is well illustrated (Duncan and Garton, 1967; Christie and Moore, 1971; L'estrange and Mulvihill, 1975; Hansen and Czochanska, 1976) with a general

tendency towards unsaturation in the fats on the outside of the body. In the most exposed tissues (legs, ears) oleic acid can account for as much as 60 to 70% of the total fatty acids present. However regardless of their location in the body, all sheep triglycerides have saturated fatty acids preferentially distributed to the 1- and 3- positions, and unsaturated (mainly oleic) predominantly in the 2- position (Duncan and Garton, 1967).

Fatty acid analysis of triglycerides from thirteen different body sites of sheep showed the oleic acid content to range from 27.0% (adrenals) to 41.2% (liver), the elaidic acid content from 1.6% (plasma) to 4.6% (heart), the palmitic acid content from 29.6% (testes) to 21.4% (perineal fat) and stearic acid from 12.2% (liver) to 34.7% (perineal fat) (Christie and Moore, 1971).

2.5.1.3 Diet

New Zealand sheep fed on ryegrass have a significantly higher percentage of total unsaturated fatty acids in their subcutaneous fat and their longissimus dorsi muscle fat than sheep fed on white clover (Sherland, Barton, Cramer and Czochanska, 1967). Groups of sheep fed diets leading to high proportions of trans fatty acids or polyunsaturated fatty acids in the lipids of their rumen content have similarly high proportions of these fatty acids incorporated into the triglycerides of their depot fats (Garton and Duncan, 1969; Herbert and Kearney, 1975; Hawke, Morrison and Wood, 1977).

2.5.1.4 Age

Triglycerides from the perinephric and subcutaneous tissues of neonatal lambs are very similar in fatty acid composition to each other and also to the composition of the subcutaneous tissue of the grown animal. As an animal matures, there is an increasingly selective deposition of saturated and trans unsaturated fatty acids to the perirenal rather than the subcutaneous fat, and inside subcutaneous rather than outside subcutaneous. (Callow, 1958; Sink, Watkins, Ziegler and Miller, 1964; Garton and Duncan, 1969).

However the distribution of fatty acids within sheep triglycerides is similar irrespective of age, with saturated acids predominantly in the 1- and 3- positions, and unsaturated (almost entirely oleic) predominantly in the 2- position (Garton and Duncan, 1965, 1969).

Scott, Setchell and Bassett (1967) said there was a lower concentration of oleic acid in the heart, liver and kidneys of adult sheep tissue than fetal tissue, but the opposite with brain tissue.

2.5.1.5 Season

Season influences sheep fat composition, with maximum iodine number occurring in Summer and the minimum iodine number and maximum melting point occurring in Winter. The oleic acid content of lamb fat decreases by 14% from Spring to Winter for perinephric fat, and 10% for subcutaneous fat. The concentration of stearic acid increases correspondingly, and palmitic acid remains about constant. The melting point of both subcutaneous and perinephric fats increases progressively from Spring to Winter (L'estrange and Mulvihill, 1975).

2.5.1.6 Sex

Sex also influences fatty acid composition, with female sheep having greater amounts of all fatty acids containing sixteen acyl carbon atoms, regardless of degree of saturation, and lower percentages of all acids with less than sixteen carbon atoms (Cramer and Marchello, 1964).

2.5.2 Triglyceride Composition of Mutton Tallows

The properties of a fat depend not only upon total fatty acid content, but also upon the distribution of the fatty acids within the triglycerides.

Few mutton tallows have been analysed for triglyceride composition, and there is considerable disparity between the results published. The triglyceride compositions of a number

Table 2.5: Triglyceride composition of mutton tallows from various countries

COUNTRY OF ORIGIN	TRIGLYCERIDE GROUP (MOLE % OF TALLOW)			
	Tri-saturated	Di-saturated	Mono-saturated	Triunsaturated
Japan[c]	18.0	47.1	24.3	10.6
Indian[a]	28.0	29.0	40.0	3.0
English[b]	26.0	30-52	0-44	-
North American[a]	15.0	46.0	36.8	2.2
North American[a]	15.0	42.0	38.0	5.0
Average (excluding English tallow)	19.0	41.0	34.8	5.2

a - Hilditch and Williams (1964);
b - Swern (1964);
c - Kawada and Matsui (1968).

of tallows are presented in Table 2.5. These results show there to be about 40% of disaturated triglycerides in mutton tallow. It is a common feature of most animal fats that oleic acid is preferentially (but not exclusively) distributed to the 2- position of the triglycerides (Hilditch and Williams, 1964; Brockerhoff, 1966; Brockerhoff, Hoyle and Wolmark, 1966; Litchfield, 1972) and Garton and Duncan (1965, 1969) and Duncan and Garton (1967) have shown this to hold for sheep fats irrespective of their position in the body or age of the animal. Hence the disaturated triglyceride fraction of mutton tallow probably contains a significant quantity of 2-oleo disaturated triglycerides.

Table 2.6: Fatty acid composition of beef tallows from various countries

COUNTRY OF ORIGIN	FATTY ACID (MOLE % OF TALLOW)									
	12:0	14:0	16:0	18:0	20:0	14:1	16:1	18:1	18:2	20-22 unsatd.
North America[b]	-	6.3	27.4	14.1	-	-	-	49.6	2.5	-
England[b]	0.2	3.1	24.9	24.1	0.8	0.4	2.4	41.8	1.8	0.5
India[b]	0.2	3.7	37.1	29.4	1.2	0.4	1.0	25.9	0.9	0.2
India[a]	-	5.7	33.4	27.9	0.5	1.9		29.0	1.5	0.1
India[a]	-	5.9	40.8	25.5	0.7	2.8		22.9	1.1	0.3
North America[d]	2.0	4.0	27.0	14.0	-	2.0	5.0	42.0	2.0	-
North America[b]	-	5.0	30.0	25.0	1.0	-	-	37.0	2.0	-
Average	0.3	4.8	31.5	22.9	0.6	0.6	1.7	35.5	1.7	0.2

a - Hilditch and Williams, 1964; b - Swern, 1964; c - Kawada and Matsui, 1968; d - Luddy et al, 1973.

Table 2.7: Triglyceride composition of beef tallows from various countries

COUNTRY OF ORIGIN	TRIGLYCERIDE GROUP (MOLE % OF TALLOW)			
	Tri saturated	Di-saturated	Mono-saturated	Tri-unsaturated
Japan[c]	13.8	43.3	26.9	16.0
North America[d]	8.0	40.0	40.0	12.0
North America[b]	13.9	22-54	0-64	0-3
England[b]	15.5	31.0	53.0	-
India[a]	28.0	51.0	20.0	-
India[a]	36.0	52.0	12.0	-
India[a]	18.0	41.0	41.0	-
Average	19.0	42.3	32.1	4.2

2.5.3 Comparison of Beef and Mutton Tallows

The compositions of beef tallows from various countries are presented in Tables 2.6 and 2.7. The range of concentrations of fatty acids and triglyceride groups in mutton and beef tallows overlap each other, so there is no consistent difference in their composition. However, beef tallow appears to be generally slightly richer in palmitic acid, and generally mutton tallow has a slightly higher melting point than beef tallow (Swern, 1964; Pattinson, 1975; Patterson, 1976).

Beef tallow is expected to have a significant amount of 2-oleo disaturated triglycerides for the same reasons as mutton tallow, and significant amounts of these triglycerides have been determined in beef tallow (Luddy et al, 1973; Patterson, 1976).

2.5.4 Variation in Tallow Composition

The published analyses of tallows from various countries (see Sections 2.5.1 and 2.5.2) show that there is considerable variation in the composition of both mutton and beef tallows.

There is no published information on the composition of New Zealand tallows, though some analyses of individual body fats of New Zealand sheep have been published (Shorland, 1948; Hansen and Czochanska, 1976; Hawke, Morrison and Wood, 1977). However Swern (1964) found no consistent differences in either iodine number or firmness among edible tallows from the United States, South America, Australia or New Zealand. Thus it seems likely that New Zealand tallow has a similar composition to overseas tallows, and thus similar fractions would be able to be separated from it.

From the studies on sheep depot fats which showed that the composition of some depot fats varied with the age of the sheep, it would be expected that the composition of mutton tallows produced early in the killing season, when young (10 week or so) lambs are being killed, would be different from the mutton tallows produced later in the season when older lambs and sheep are being killed. Similarly, the trimming practises

followed in any particular establishment affect to a considerable extent the composition and consistency of commercial tallows (Swern, 1964).

Because of the large number of variables which affect both the composition and incidence of raw materials to rendering, it would be expected that variation in the composition of tallows even within one country would be quite large, and Ashton (1975) claims that there is little standardisation of tallows produced in New Zealand. This is confirmed by the variation in the fatty acid compositions of different Australian mutton tallow samples (see Table 2.4) and in the variation in fatty acid and triglyceride compositions of different Indian beef tallow samples (see Tables 2.6 and 2.7). Swern (1964) said there was considerable variation in the hardness of different lots of commercial North American edible tallow, and it is impossible to maintain the level of tallow constant in blended shortenings and still maintain a product of uniform consistency.

Because there are seasonal changes in the composition of some sheep depot fats (see Section 2.5.1.5), there is possibly a change in diet throughout a season as grass growing conditions change, and the lamb to sheep ratio alters throughout a killing season, it seems likely that the variation in tallow composition follows some seasonal pattern, but there is far too little information available to be able to predict the nature of any overall seasonal effect on tallow composition.

Luddy et al (1973), however, claimed that North American beef tallow was "a reasonably uniform product" with not much difference in the differential scanning calorimeter (DSC) profiles of various tallows. Solvent fractionation of different tallows produced similar proportions of the various fractions.

Normal variation in the composition of palm oil has a considerable effect on solvent fractionation of palm oil (Berger, 1977). Any variation in tallow composition may have similar results.

2.6 SOLVENT FRACTIONATION SCHEMES FOR TALLOW

2.6.1 Introduction

Tallow is composed of a great number of constituent triglycerides which exhibit a wide range of physical properties (Luddy et al, 1973). Solvent fractionation can partially separate these triglycerides, and the literature cites several fractionation schemes for tallow.

These fractionation schemes have been developed for one of two purposes:

(1) To concentrate groups of similar triglycerides as an aid to analysis;

(2) To produce fractions with properties suited to specific uses;

2.6.2 Analytical Fractionation Schemes

Riemenschneider, Luddy, Swain and Ault's (1946) scheme used successive solvent fractionations with acetone to produce seven fractions from edible mutton tallow as a means of analysis (see Figure 2.6). The triglyceride composition of the fractions is given in Table 2.8. It was assumed that each of the fractions contained only two of each of the triglyceride classes of trisaturated, disaturated, monosaturated or triunsaturated triglycerides.

Fraction P_4 had a yield of 38% of the original tallow (w/w) and contained 92% disaturated triglycerides and 8% monosaturated triglycerides. This is a composition very similar to that of cocoa butter, but no analysis of the fatty acids at the 2-position of these triglycerides was given.

Hiditch and Shrivastava's (1949) crystallisation scheme used acetone and diethyl ether at different stages to produce six fractions of widely different iodine values from sheep body triglycerides. The solvent: fat ratio was 10:1 (v/w) at each stage. See Figure 2.7 for the crystallisation sequence, and Table 2.9 for the triglyceride composition of the products.

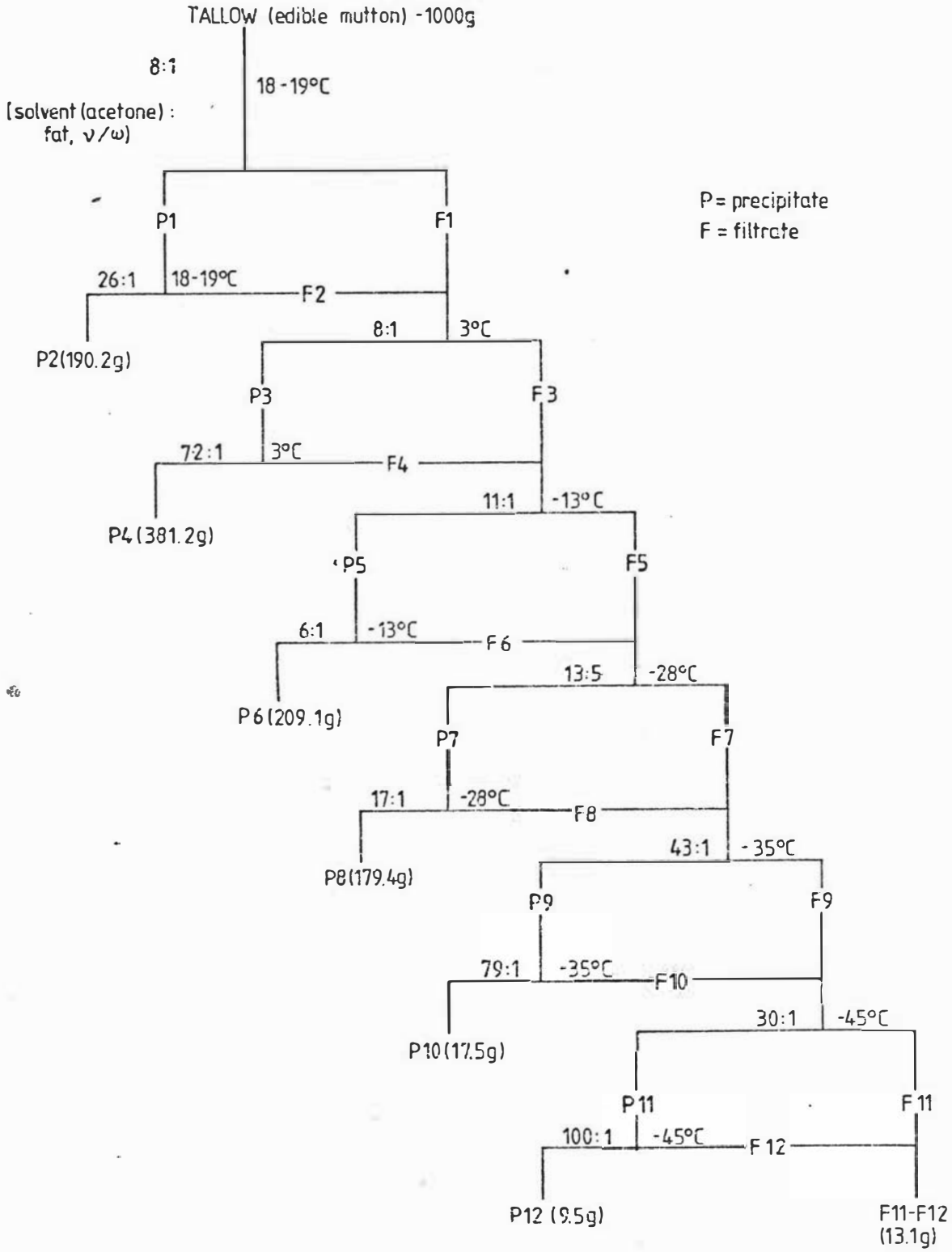


Figure 2·6 : Fractionation scheme of Riemenschneider et al (1946)

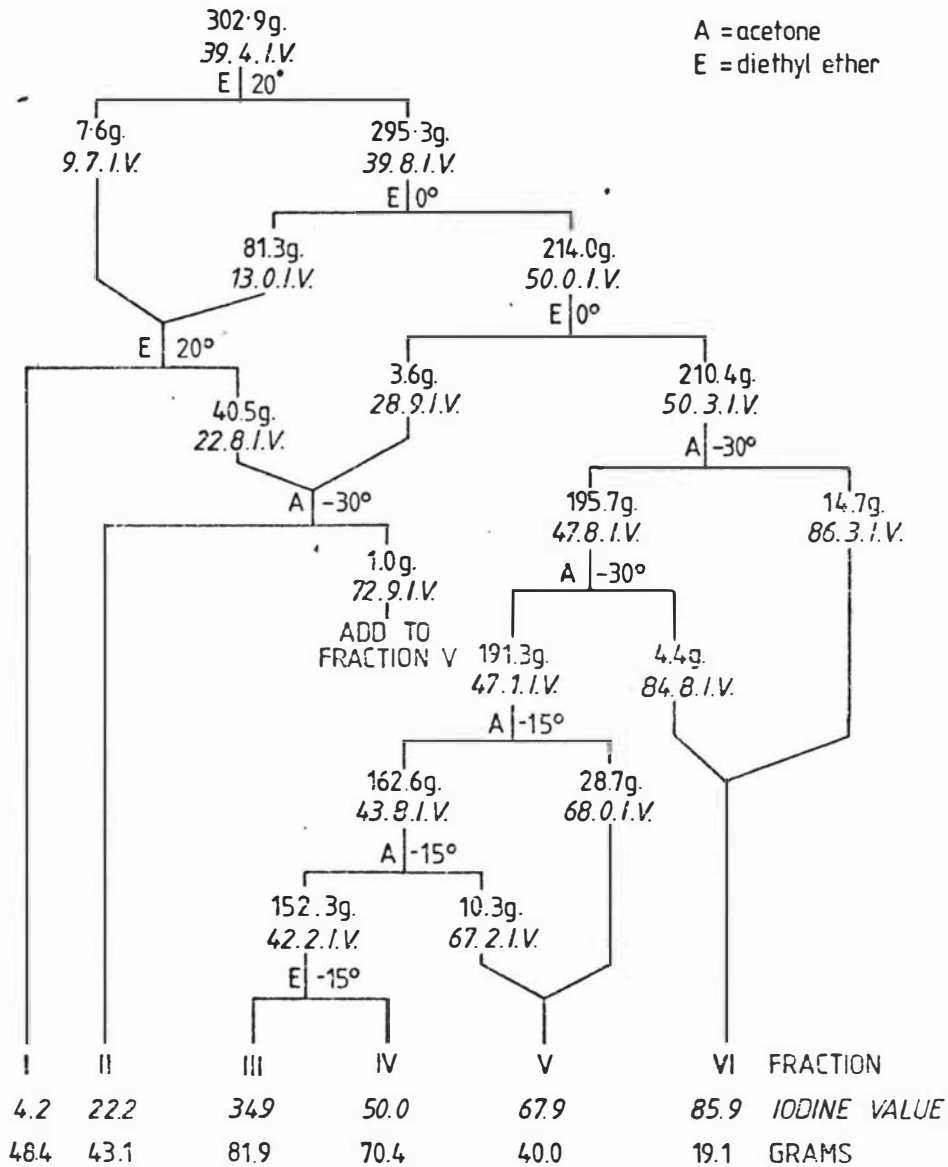


Figure 2·7 : Crystallisation sequence for separating sheep body Triglycerides into six fractions of widely different iodine value.

(Hilditch and Shrivastava, 1949)

Table 2.8: Triglyceride composition of fractions by the scheme of Riemenschneider et al (1946)

Fraction No.	Mole % of tallow	Triglyceride group (mole % of fraction)			
		Tri-saturated	Di-saturated	Mono-saturated	Triunsaturated
P_2	19.0	77.5	22.5	-	-
P_4	38.1	-	92.3	7.7	-
P_6	20.9	-	28.6	71.4	-
P_8	17.9	-	24.8	75.2	-
P_{10}	1.8	-	-	63.9	36.1
P_{12}	1.0	-	-	45.5	54.5
F_{11}-F_{12}	1.3	-	-	14.0	86.0
Total tallow (mole %)		14.9	46.0	36.9	2.2

Table 2.9: Triglyceride composition of fractions by the scheme of Hilditch and Shrivastava (1949)

Fraction No.	Mole % of tallow	Triglyceride group (mole % of fraction)			
		Tri-saturated	Di-saturated	Mono-saturated	Triunsaturated
I	16.0	84.4	15.6	-	-
II	14.5	35.2	56.4	8.4	-
III	27.1	23.2	42.3	34.5	-
IV	23.0	13.0	23.4	63.9	-
V	13.2	-	8.1	91.9	-
VI	6.2	-	-	54.6	45.4
Total tallow (mole %)		28.0	28.5	40.7	2.8

2.6.3 Schemes to Produce Fractions for Specific Uses

The scheme of Farr (1954) entailed crystallising tallow in acetone (3:1, acetone: tallow (v/w)) at room temperature then filtering and washing the precipitate. The filtrate was then held at -1°C for four hours and filtered to give a crystalline fraction suitable as a coating fat.

Unilever's process (Crossley, Paul, Pardun and Soeters, 1960) fractionated mutton or beef tallows by the scheme shown in Figure 2.8. With mutton tallow, the precipitate P_3 comprised 17.3% (w/w) of the initial tallow and it was claimed that it could be used in chocolate to replace 41% of the added cocoa butter (15% total fat) without adversely affecting appearance, taste or snap. A similar fractionation of beef tallow produced a fraction with a yield of 26% of the tallow by weight and which was claimed to be able to satisfactorily replace 68% of the added cocoa butter (25% total fat) in chocolate.

Kawada and Matsui's (1968) fractionation method to produce a fraction with a high concentration of 2-oleo disaturated triglycerides from Japanese mutton tallow involved crystallising the fat from acetone, 1- or 2- nitropropane or hexane and filtering off the crystals as a first fraction, then further cooling the filtrate to form more crystals and removing these as the second fraction. This second fraction had a high concentration of 2-oleo disaturated triglycerides. The best results were obtained using 2- nitropropane and a solvent: fat ratio of 3:1 (v/w) and crystallisation temperatures of 10 to 11°C and 3 to 4°C for the first and second crystallisations respectively. This produced a second fraction with a yield of 33.5% of the original tallow (w/w) and containing 77.7% of disaturated triglycerides. Of these disaturated triglycerides, 64.1% had oleic acid in the 2-position and 3.0% had palmitoleic acid in the 2-position. With acetone under the same conditions the second fraction had a 30.2% yield and contained 69.3% of disaturated triglycerides, 56.1% of which had oleic acid in the 2-poisition, and 3.0% had palmitoleic acid in the 2-position. With n-hexane, the yield of the second fraction was 28.5% and this contained 75.8% disaturated triglycerides, 52.5% of which had oleic acid in the 2-position, and 2.4% had palmitoleic acid

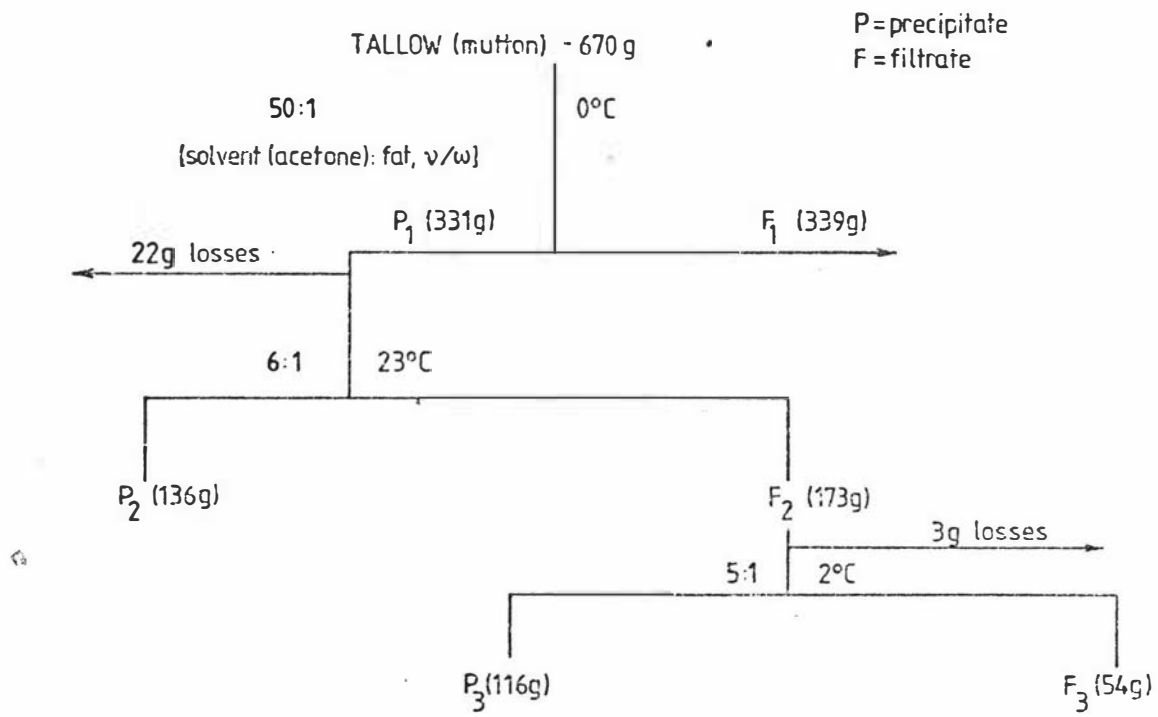


Figure 2·8 : Unilever's fractionation scheme for mutton tallow.
(1960)

in the 2-position.

Changing the crystallisation temperatures to 18°C and 10°C and using 1- nitropropane and acetone produced the following results (see Table 2.10). The fat solution was held for 30 minutes at the first crystallisation temperature and 60 minutes at the second. It was proposed that fraction 2 could be used as a cocoa butter replacer in chocolate, but there was no mention of tests to determine its effectiveness as such.

In the process of Luddy et al (1973, 1976, 1978) North America beef tallow was separated into five fractions by solvent fractionation using acetone. The fractionation scheme is shown in Figure 2.9.

Fraction 3 had a triglyceride composition and thermal properties very similar to cocoa butter. It contained 90% disaturated triglycerides, 8% monosaturated and 2% trisaturated, and showed excellent compatability with cocoa butter over all ranges of addition. A test coating where the tallow fraction comprised 93% of the total fat, with the remaining 7% coming from the cocoa butter in the cocoa powder, had excellent flavour, gloss, snap, mould release and bloom resistance. Blending of fraction 3 with other fats produced a coating fat with softer or harder properties for specialty uses.

Fractions 1 and 2 were hard fats which could be used, without hydrogenation, for hardening shortenings and margarine fats, and fraction 5, which accounted for 60% of the tallow, was a liquid fat which could be used in formulations of salad oils, margarines, liquid and plastic shortenings as well as for non-food uses such as the manufacture of synthetic sperm oil, foam plastics, lotions, creams and ointments in cosmetics and pharmaceuticals. It remained liquid at 7°C and at 4.5°C after the addition of a crystal inhibitor. It could be further improved as a salad oil by the removal of 2 to 3% of the higher melting triglycerides or by trans-esterification.

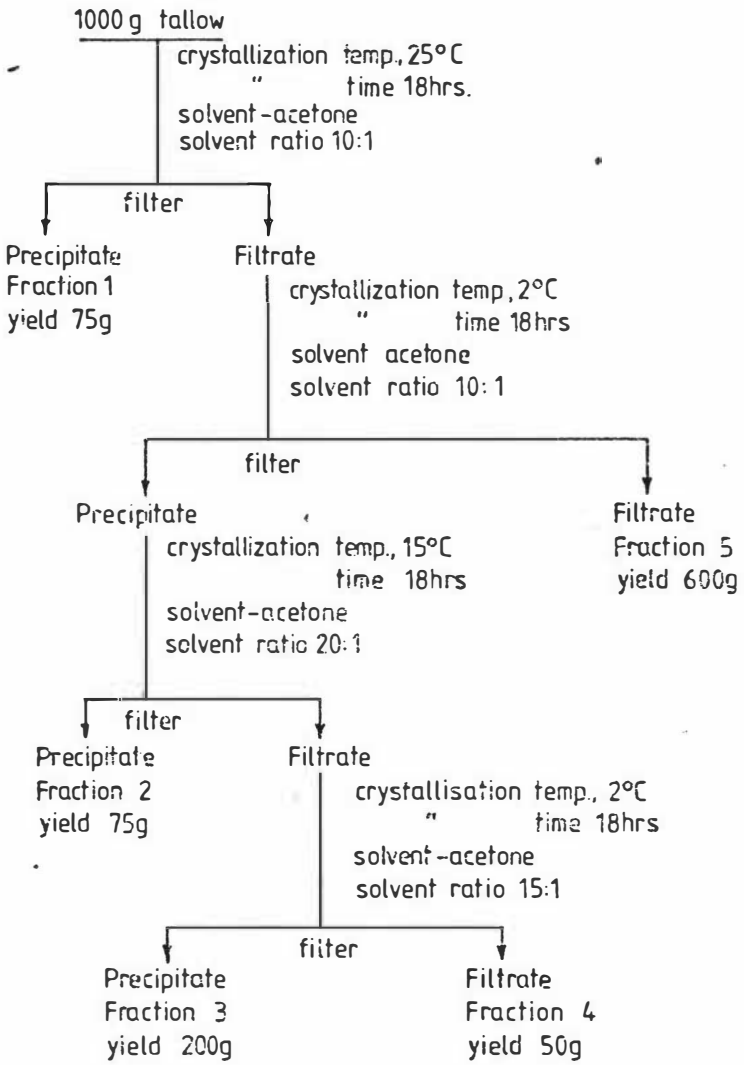


Figure 2·9 : Fractionation scheme of Luddy et al

(1976, 1977, 1978)

Table 2.10: Composition of products from solvent fractionation of Japanese mutton tallow (Kawada and Matsui, 1968)

Experiment No.	Solvent	Solvent:fat (v/w)	Fraction No.	Yield (% tallow, w/w)	Iodine value	Triglyceride Composition (mole % of fraction)			
						Trisatd.	Disatd.	Monosatd.	More Unsatd.
1	1-nitro-propane	10.0	1	28.7	23.7				
			2	29.9	37.9	2.1	79.1 (0.44*)	11.0	7.8
			3	40.4	60.9				
2	1-nitro-propane	7.5	1	28.9	23.7				
			2	27.9	36.2	0.8	76.5 (0.56*)	15.2	7.5
			3	43.7	58.2				
3	1-nitro-propane	3.0	1	35.0	24.2				
			2	26.7	38.6	0.8	75.6 (0.69*)	16.3	7.3
			3	39.3	58.6				
4	acetone	10.0	1	24.6	15.5				
			2	35.1	36.8	5.9	75.7 (0.48*)	12.3	6.1
			3	39.7	59.2				
5	acetone	7.5	1	24.6	15.5				
			2	31.1	36.6	5.7	77.8 (0.52*)	12.3	4.2
			3	41.7	58.2				
6	acetone	3.0	1	35.7	22.4				
			2	32.0	40.4	5.7	72.2 (0.51*)	13.8	8.3
			3	32.9	61.4				

* Proportion of disaturated triglycerides which have oleic acid in the 2-position.

More recently, this four-step process has been reduced to two steps with a simultaneous reduction in process time and solvent: fat ratio (Elias, 1979; Luddy, Hampson and Koos, 1979). Three products were obtained: a solid fraction, a confectionery fat and an oil. The yield of the confectionery fat was 40% higher than by the four-step process, but it had a wider melting range. A satisfactory chocolate coating has been made using the fraction. Details of the process have not been published.

2.7 GENERAL CONCLUSIONS FROM LITERATURE SURVEY

The literature survey has shown that there are many factors which influence the composition of tallow. Tallows from different countries had a wide variation in their compositions, and there was a similar variation in the compositions of different tallow samples from within the same country. There were no reported analyses of New Zealand mutton tallow, so it was not possible to ascertain definitely if it is similar in composition to tallows from other countries. However the iodine number and firmness of New Zealand tallows have been found to be similar to those of overseas tallows. All of the tallows with analyses reported in the literature contained a significant quantity of disaturated triglycerides. A high proportion of the disaturated triglycerides found in tallows are likely to have oleic acid at the 2-position.

Seasonal trends in the compositions of certain individual sheep body fats have been discovered, but there was no similar information on seasonal patterns in tallow composition.

The composition of New Zealand mutton tallow must be determined in this work to confirm the occurrence of certain desirable triglyceride types, especially the 2-oleo disaturated triglycerides, and hence determine the feasibility of producing fractions with specific compositions. It is also important to determine if there is a seasonal trend in the proportion of any triglyceride type.

However the degree to which any desired triglyceride type can be concentrated into a specific fraction is limited by the complicated phase characteristics of triglyceride mixtures and the efficiency of separation of any fractionation method. Solvent fractionation is the method most likely to give the best separation of any specific triglyceride type, and acetone is the most widely used and recommended solvent.

Four reported solvent fractionation schemes have been applied to tallows overseas to produce fractions with specific properties. All were designed to produce a cocoa butter alternative fat as a priority because of the high demand (and hence price) for a cocoa butter alternative fat and because of the concentration of 2-oleo disaturated triglycerides in tallow. Harder and softer fractions were also produced. Two of these schemes involved 2-stage fractionation procedures (i.e. two crystallisations), one a 3-stage and one a 4-stage, though the 4-stage process was subsequently modified to a 2-stage process. It was claimed that each of these processes could produce a fraction capable of replacing at least part of the added cocoa butter in chocolate-like products. Other uses were found for some of the other fractions.

CHAPTER 3
METHODS OF ANALYSIS

The methods described in this chapter were used to determine the fatty acid and triglyceride structures of tallows (see Chapter 4) and their fractionation products (see Chapter 9).

The method of Differential Scanning Calorimetry (DSC), used to examine the thermal properties of selected fractions, is also presented here.

3.1 CHEMICAL ANALYSIS

A summary of the scheme used to analyse whole tallow or fractionation products is given in figure 3.1.

3.1.1 Thin Layer Chromatography (TLC)

Preparative TLC was used to isolate triglycerides from tallow samples.

Argentation TLC was used to resolve triglycerides on the basis of unsaturation and cis-trans isomerism.

3.1.1.1 Preparation and Development of Plates

Silica gel G suspended in either distilled water or 10% $AgNO_3$ solution in the ratio of 1:2 (w/v) was spread on to the plates (20 x 20 cm) to a thickness of 0.5 mm (for analytical plates) or 0.7 mm (for preparative separations).

The layers were left to dry ($AgNO_3$/silica gel in the dark) for 15 minutes then activated for two hours at 120°C.

The appropriate solvent was added to a depth of 0.5 cm to chromatographic tanks, lined with filter paper, 30 minutes before chromatography to saturate the tank space with solvent vapour.

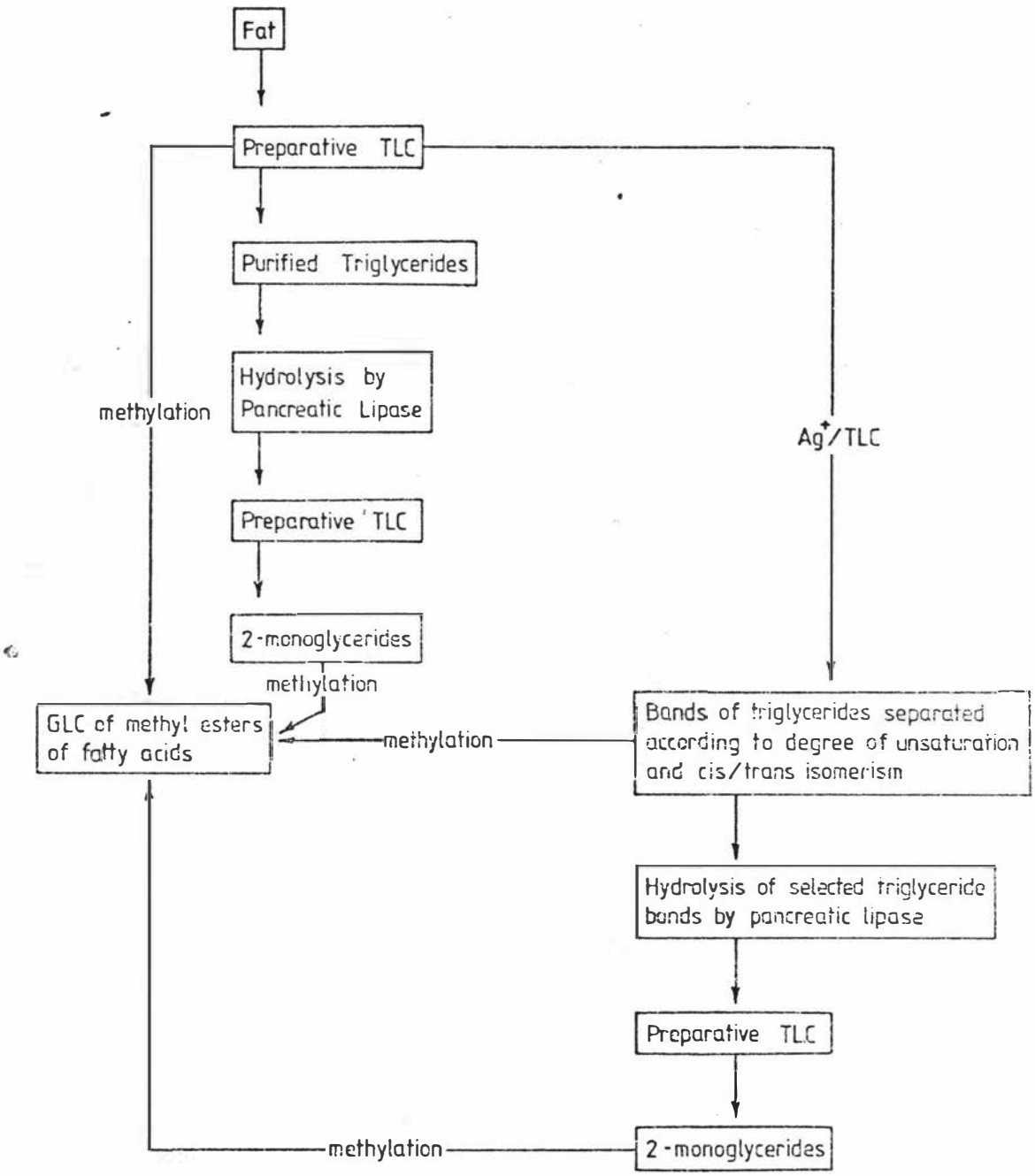


Figure 3·1 : General scheme for analysis of the fat samples

3.1.1.2 Preparative TLC

The tallow sample was dissolved in hexane (1:3 w/v) and applied with a 100 µl syringe as a series of over-lapping spots about 2 cm from the edge of the adsorbent layer. A standard triglyceride was run to help identify the triglyceride band.

The layer was developed by allowing the hexane : diethyl ether (4:1, v/v) solvent front to rise to 2 cm from the top of the adsorbent layer. After drying at room temperature, the edges of the plate containing the standard were sprayed with a 0.05% (w/v) 2'7' dichlorofluorescein/methanol solution and viewed under U.V. light to locate the position of the triglycerides.

The triglyceride band was scraped into a centrifuge tube and extracted from the silica gel with 10 ml diethyl ether by slurrying on a Vortex mixer. After brief centrifugation, the solvent layer was drawn off with a Pasteur pipette. The residue was extracted twice more with 5 ml diethyl ether.

3.1.1.3 Argentation TLC

Triglycerides were resolved on the basis of unsaturation and cis-trans isomerism on layers of silica gel impregnated with 20% (w/w) $AgNO_3$.

The basis of separation was the weak interaction between Ag^+ and the π-electrons of double and triple bonds. The Ag^+/olefin complex is of sufficiently low energy that it can be made and broken during standard lipid chromatographic procedures. Exposure to $AgNO_3$ does not produce any chemical alteration of normal triglycerides; hence the fractions separated can be recovered unaltered from the impregnated adsorbent for further use. Trans double bonds form weaker π-complexes than cis double bonds, hence cis-trans isomers can be separated by silver ion adsorption chromatography (Litchfield, 1972).

About 10 mg of triglycerides, dissolved in $CHCl_3$, were applied as a series of over-lapping spots to the plate. The developing solvent was chloroform/methanol (99.5:0.5, v/v).

Triglyceride bands were revealed as for preparative TLC, and were identified by reference to standard triglycerides (glycerol tripalmitate, sn-glycerol-1-palmitate-2-oleate-3-stearate, sn-glycerol-3-stearate-1,2-oleate and glycerol trioleate) used as markers.

After removing the fluorescent bands from the plate into centrifuge tubes, 1% NaCl in methanol/water (9:1, v/v) was added until the red colour of the silver-dichlorofluoroscein complex disappeared (about 1.5 ml). Ten ml diethyl ether/methanol (9:1, v/v) was added, followed by slurrying on a Vortex mixer. After brief centrifugation and removal of the solvent layer with a Pasteur pipette, the residue was extracted twice more with 5 ml portions of diethyl ether/methanol (9:1, v/v) (Hill, Husbands and Lands, 1968; Morrison, 1976).

Solvent was removed under a stream of N_2 and the residue redissolved in hexane and washed several times with small volumes of water to remove traces of dichlorofluoroscein and $AgNO_3$ prior to gas liquid chromatography (GLC) analysis.

3.1.1.4 TLC of 2-Monoglycerides

2-monoglycerides were separated from fatty acids and glycerides by dissolving in $CHCl_3$, applying to a thin layer of silica gel G, and developing in hexane: diethyl ether (1:1, v/v).

The monoglyceride band was identified by comparison with a standard monoglyceride after spraying with aqueous Rhodamine 6G (0.01% w/v). The monoglycerides were recovered from the TLC plates as described above for triglycerides.

3.1.2 Fatty Acid Analysis

Fatty acids were converted to fatty acid methyl esters before being analysed by Gas Liquid Chromatography (GLC).

3.1.2.1 Preparation of Fatty Acid Methyl Esters

One ml of 0.5M methanolic NaOH was added to 2-5 mg of triglyceride (or monoglyceride) in a 25 ml roundbottom flask. The flask was attached to a water condenser and heated on a sand bath, to gently reflux the contents, for two minutes. One ml 14% (w/v) boron trifluoride in methanol was added through the condenser and the reflux continued for a further two minutes, after which 2 ml hexane was added followed by refluxing for a further 30 seconds. After cooling the flask in iced water, sufficient water was added to bring the hexane layer into the neck of the flask. The hexane layer was transferred to a glass-stoppered centrifuge tube using a Pasteur pipette, and concentrated by evaporation under N_2.2 to 5 µl of the sample were used for GLC (Van Wijngaarden, 1967; Morrison, 1976).

3.1.2.2 Gas Liquid Chromatography (GLC)

Fatty acids were analysed as their methyl esters on a Packard gas chromatograph fitted with a hydrogen flame ionisation detector (Jamieson, 1970). The glass column (180 cm x 0.25 cm i.d.) was packed with 12% diethylene glycol succinate polyester (DEGS) (Hi - Eff 1B 530-G) or 10% EGSS-X on Chromosorb Q. The "funnel coating method" (McNair and Bonelli, 1968) was used for the preparation of the column packing. Column conditioning was carried out at 200°C for 12 hours with a N_2 flow of 10 ml/min.

During normal operation the chromatograph was run with column and inlet temperatures of 160 and 205°C respectively, and a nitrogen flow of 25 ml/min.

3.1.3 Quantitative Measurement of Triglyceride Bands

The relative quantities of each of the triglyceride bands separated by $AgNO_3$/silica gel TLC were determined through the use of an internal standard. A known quantity of heptadecanoic acid was added to each of the triglyceride samples before methylation, and the sum of the areas of each of the fatty acid peaks from GLC was compared with the peak area for the heptadecanoic acid to give an estimate of the relative proportion of each triglyceride band.

3.1.4 Preparation of 2-Monoglycerides

The fatty acid composition at the 2-position of some triglycerides was determined by selective enzymatic hydrolysis of the fatty acids at the 1- and 3-positions to produce the representative 2-monoglycerides. The specific action of pancreatic lipase for the ester groups at the 1- and 3-positions of triglycerides is nearly absolute (Riemenschneider, Luddy, Barford, Herb and Magidman, 1964).

About 40 mg of triglycerides were dissolved in 0.1 ml hexane and incubated for 70 seconds at 37°C, with vigorous shaking, with 15 mg pancreatic lipase (previously extracted with diethyl ether - Sampugna, Jensen, Parry and Krewson, 1964) dispersed in 1.0 ml 0.05M-tris buffer (pH 8.0), 0.2 ml 0.2% (w/v) sodium cholate and 0.1 ml 22% (w/v) $CaCl_2$. The reaction was stopped by the addition of 1.5 ml ethanol. The pH of the solution was adjusted to 4.0 with 1M-HCl and the mixture extracted with three 20 ml portions of diethyl ether. The total ether extract was washed with 2 ml portions of water until the washings were neutral. The ether was evaporated at room temperature under N_2 and the lipid residue re-dissolved in chloroform (Taylor and Hawke, 1975; Morrison, 1976).

3.2 THERMAL ANALYSIS - DIFFERENTIAL SCANNING CALORIMETRY (DSC)

A differential scanning calorimeter (Perkin Elmer DSC Model 1B) was used to carry out the thermal analysis of selected fractionation products.

Differential scanning calorimetry is a technique whereby the sample and an inert reference material are subjected to a controlled heating programme (linear with time), and the differential energy required to keep the two samples at the same temperature is measured. This is achieved through the use, for sample and reference, of nearly identical copper holder cups containing a platinum resistance thermometer, a temperature programmed heater and an auxiliary heater. The temperature programmer heats the holders at a constant, pre-selected, rate. A temperature averaging network detects any differences in temperature beween the holders and supplies current to the

auxiliary heaters to maintain a zero differential. When an endothermic transition occurs in the sample the energy absorbed is replenished by this network. This energy input is a direct measure of the transition energy of the sample as a function of time. The net enthalpy flow rate and the average temperature of the two cups are plotted on a recorder as the ordinate and abscissa respectively. When only sensible heat changes occur in the sample as it is heated, the DSC plot appears as a relatively straight, horizontal, line. Any exothermic or endothermic change in the sample causes deviation from this base-line, and peaks are obtained on the plot. The area of the peak is proportional to the enthalpy change of the sample (O'Neill, 1964; Driscoll, Duling and Magnotta, 1968; Perkin-Elmer, 1969; Rek, 1972; Daniels, 1973; Wendlandt, 1974).

The temperature axis of the instrument was calibrated using diphenyl, diphenylamine, indium, n-octane and tin, and the differential temperature network was calibrated using twin samples of indium and then diphenylamine.

A fat sample of about 9 mg (Luddy et al, 1973; Deroanne, 1977; Kawamura, 1979) was sealed in an aluminium sample pan and loaded into the instrument. An empty aluminium pan served as the reference (Daniels, 1973; Gray, Lovegren and Feuge, 1973; Luddy et al, 1973). The low temperature Dewar flask sample cover was put in place and the sample area flushed with N_2 gas. The N_2 flow-rate throughout was 15 ml.min^{-1} (Luddy et al, 1973). The sample cover was filled with liquid N_2 and the sample and reference were cooled to 240K. After equilibriation they were heated to 325K at a rate of 8 K .min^{-1} (Lovegren, Gray and Feuge, 1971; Luddy et al, 1973).

It was particularly desired in this work to produce a fraction with melting properties, and hence a DSC profile, similar to that of cocoa butter (see section 2.2.3.1). There are two distinguishing features of the cocoa butter DSC profile - its narrow melting range and its "melting point" of 36°C (see Figure 2.5).

Thus a peak parameter, the "cocoa butter likeness factor" (C.B.L.F.), was developed to compare the DSC profiles of the tallow fractions to that of cocoa butter.

$$\text{CBLF } (^{\circ}C) = \sum_{i=1}^{n} \left\{ \left(|36 - T_{im}| \right) + \left(T_{i1} - T_{i2} \right) \right\} \frac{A_i}{A_T}$$

where,

n = number of separate peaks on the DSC curve of the sample

T_{i1} = temperature of final deviation of peak i from the base-line.

T_{i2} = temperature of initial deviation of peak i from the base-line.

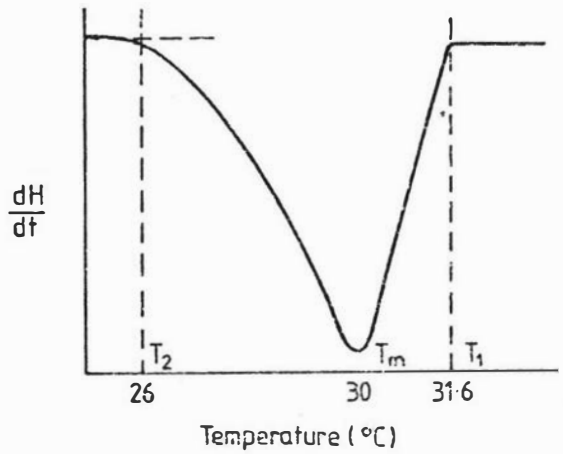
T_{im} = "melting point" of peak i - taken as the temperature at which peak maximum occurs.

A_i = area of peak i.

A_T = sum of areas of all peaks for the sample.

See Figure 3.2 for an example of determining the CBLF of a sample giving a DSC profile with only one peak.

The CBLF is the sum of the temperature range (in $^{\circ}C$) over which the sample melts and the absolute value of the difference in temperature (in $^{\circ}C$) between the temperature of DSC peak maximum and $36^{\circ}C$, the temperature of DSC peak maximum for a sample of cocoa butter obtained from a N.Z. chocolate manufacturer. A portion of this cocoa butter sample was analysed on the DSC immediately after each tallow sample so that a comparison of their melting properties could be made under near-identical conditions. The CBLF of this cocoa butter sample was 6.5, but there is variation in the properties of different batches of cocoa butter (Johnston, 1972). When a tallow sample gave a DSC profile with more than one peak, the CBLF of each peak was calculated, and the overall CBLF for the sample was determined by summing the product of the CBLF for each peak and the area of that peak expressed as a fraction of the sum of peak areas for the sample.



$$C.B.L.F. = \sum_{i=1}^{n} [(|36 - T_{im}|) + (T_{i1} - T_{i2})] \frac{A_i}{A_T}$$

$$C.B.L.F. = |36 - T_m| + (T_1 - T_2) \qquad \left[\text{as } \frac{A_i}{A_T} = 1\right]$$

$$= |36 - 30| + (31{\cdot}6 - 26)$$

$$= 6{\cdot}0 + 5{\cdot}6$$

$$= 11{\cdot}6$$

Figure 3·2 : Calculating the C.B.L.F. of a fat sample giving a D.S.C. profile with only one peak.

CHAPTER 4

TRIGLYCERIDE AND FATTY ACID ANALYSIS OF SELECTED NEW ZEALAND MUTTON TALLOWS

4.1 INTRODUCTION

This chapter studies the overall seasonal changes in mutton tallow from one meat killing plant, but makes no attempt to elucidate the effects of the individual variables (see Section 2.5), influencing tallow composition.

A knowledge of the fatty acid and triglyceride composition and how it varies throughout a season was needed to determine the feasibility of producing fractions with a specified composition from New Zealand mutton tallow, and if there is a time of year when tallow with a maximum concentration of any particular triglyceride type or fatty acid is produced.

4.2 METHODS OF COLLECTION AND ANALYSIS OF THE TALLOWS

The samples for analysis were inedible mutton tallows from two meat killing plants -- one in Canterbury and the other in Southland - which were only killing sheep. Therefore, mutton tallow was collected free from any beef tallow. Samples from the Smithfield meat killing plant in Canterbury were collected throughout the 1976/1977 and 1977/1978 seasons. The sample from the Ocean Beach meat killing plant in Southland was collected in May, 1977. A wet rendering process was used at the Canterbury plant and a dry rendering process at the Southland plant. All of the tallow samples were collected from the molten bulk tallow immediately prior to its being transferred to railway tankers.

Tallows are graded according to colour by matching with colour standards. Two grades of tallow are produced at the Smithfield meat killing plant - ½ R and 1½ R. The fatty acid analysis of the November, January and March 1977 Smithfield tallows was performed upon both 1½ R and ½ R graded samples to determine if there was any difference in the composition of the different grades.

The tallow samples were purified by TLC (see Section 3.1.1.2) and then separated into triglyceride groups, according to degree of unsaturation and cis-trans isomerism, by argentation TLC (see Section 3.1.1.3). The relative proportion of each triglyceride group and their overall fatty acid compositions, as well as the fatty acid composition of the whole tallows, were determined by the methods outlined in sections 3.1.3 and 3.1.2 respectively. Some selected triglyceride groups were analysed for fatty acid composition at the 2-position using pancreatic lipase hydrolysis (see Section 3.1.4). The overall scheme for the tallow analysis is shown in Figure 3.1.

4.3 FATTY ACIDS IN THE TALLOWS ANALYSED

The total fatty acid composition of the tallow samples is shown in Tables 4.1 and 4.2. The fatty acids were calculated as mole percentages of the total tallow. Four analyses were done on the November 1977 1½ R tallow and these were used to determine the experimental variance in the fatty acid analyses. This experimental variance was used to test the significance of the difference in proportions of the four main fatty acids (14:0, 16:0, 18:0 and 18:1) in different tallows from throughout the same season and between seasons. Because only one sample of each tallow was taken, it was not possible to determine the variation due to sampling.

It was found in both seasons that the concentration of each of the four main fatty acids (14:0, 16:0, 18:0 and 18:1) in different tallows from the same season varied significantly at the 5 per cent level. The concentration of 14:0 appeared to decrease as the season progressed, and the concentration of 18:0 to increase. The concentration of each of these fatty acids was linearly regressed, by the least squares method, against the month in which the tallows were produced. The months were numbered from 1 (November) to 8 (June). All of the analyses from both of the seasons were included in the regressions. The regression equation for the proportion of 14:0 (mole %) in the tallows was:

proportion of 14:0 in the tallow = (7.4 - 0.7 (month)) (mole %).

Table 4.1: Total fatty acid composition of mutton tallows from the 1976/1977 season

Tallow	Fatty Acid (mole % in tallow)							
	Smithfield 1½R, November 1976	Smithfield ½R, November 1976	Smithfield 1½R, December 1976	Smithfield 1½R, January 1977	Smithfield ½R, January 1977	Smithfield 1½R, March 1977	Smithfield ½R, March 1977	Ocean Beach, May 1977
Fatty Acid								
14:0	5.9	4.8	7.6	3.4	4.5	2.8	2.7	3.0
iso 15:0+14:1	0.7	0.7	0.8	0.5	0.8	0.5	0.7	0.5
15:0	0.9	0.9	1.2	0.8	0.7	0.4	1.2	0.8
iso 16:0	0.2	0.2	0.2	0.1	0.1	0.6	0.6	0.1
16:0	22.2	20.8	22.8	20.5	19.8	20.8	20.6	20.4
16:1	4.9	6.1	5.1	2.5	4.2	4.5	4.5	4.1
17:0	2.5	3.4	1.6	2.7	1.9	2.2	2.1	2.1
17:1	1.0	1.4	0.9	0.9	0.4	0.7	0.7	0.4
18:0	20.2	21.0	18.5	24.0	24.2	26.4	26.1	26.3
18:1	40.6	38.8	39.0	40.6	40.2	37.6	37.3	39.1
18:2	0.9	1.9	2.3	4.0	3.2	3.5	3.5	3.2

Table 4.2: Total fatty acid composition of mutton tallows from the 1977/1978 season

Tallow	Smithfield 1½R, November 1977 *	Smithfield 1½R, January 1978	Smithfield 1½R February 1978	Smithfield 1½R, March 1978	Smithfield 1½R, April 1978	Smithfield 1½R, May 1978	Smithfield 1½R, June 1978
Fatty Acid							
14:0	7.7	5.3	5.6	3.3	4.5	3.1	2.9
iso 15:0 + 14:1	0.8	0.5	0.8	0.5	0.8	0.4	0.7
15:0	0.9	1.2	1.2	0.7	0.8	0.8	0.8
iso 16:0	0.3	0.3	0.5	0.4	0.5	0.5	0.4
16:0	24.2	26.3	26.0	23.8	23.4	22.6	25.5
16:1	3.4	3.4	2.6	3.5	3.2	3.5	4.0
17:0	2.3	2.2	0.2	2.0	1.7	1.8	2.4
17:1	0.8	1.7	0.7	1.0	0.8	1.0	0.8
18:0	22.2	23.1	22.5	28.8	24.9	26.7	26.9
18:1	34.4	34.4	37.8	33.1	36.6	36.3	33.7
18:2	3.0	1.6	2.1	2.9	2.8	3.3	1.9

* Average of four analyses.

The slope of this line was found to be significantly different from zero at the 0.2% level, so there was a significant overall decrease in the proportion of 14:0 as the seasons progressed.

The regression equation for the proportion of 18:0 (mole %) in the tallows was:
proportion of 18:0 = (20.5 + 0.9 (month))
in the tallow (mole %).

The slope of this line was found to be significantly different from zero at the 0.2% level, so there was a significant overall increase in the proportion of 18:0 as the seasons progressed.

A plot of the proportion of each of these fatty acids against the month of production of the tallow, and the regression lines for each fatty acid, are shown in Figure 4.1. The regression lines appeared to fit the observed data well. The linear correlation coefficient between the proportions of 14:0 and 18:0 was -0.77. This was significant at the 0.2% level.

There did not seem to be any overall seasonal pattern in the variation of 16:0 or 18:1. The slope of the regression lines for the proportion of each of these fatty acids in the tallows against the month of production of the tallows were not significantly different from zero at the 5 per cent level.

There was no significant linear correlation found at the 5% level between the proportion of 16:0 with 18:1, 14:0 or 18:0; nor between 18:1 with 14:0 or 18:0 - i.e. the changes in proportions of these fatty acids throughout the seasons were unrelated.

The mean proportion of each of the four main fatty acids in the 1976/1977 tallows was tested against the mean proportion of the same fatty acids in the 1977/1978 tallows. There was not found to be a significant overall difference in the mean proportion of 14:0 between tallows of the different seasons at the 5% level (4.4% for 1976/1977, 5.6% for 1977/1978); nor for 18:0 (23.3% for 1976/1977, 24.2% for 1977/1978).

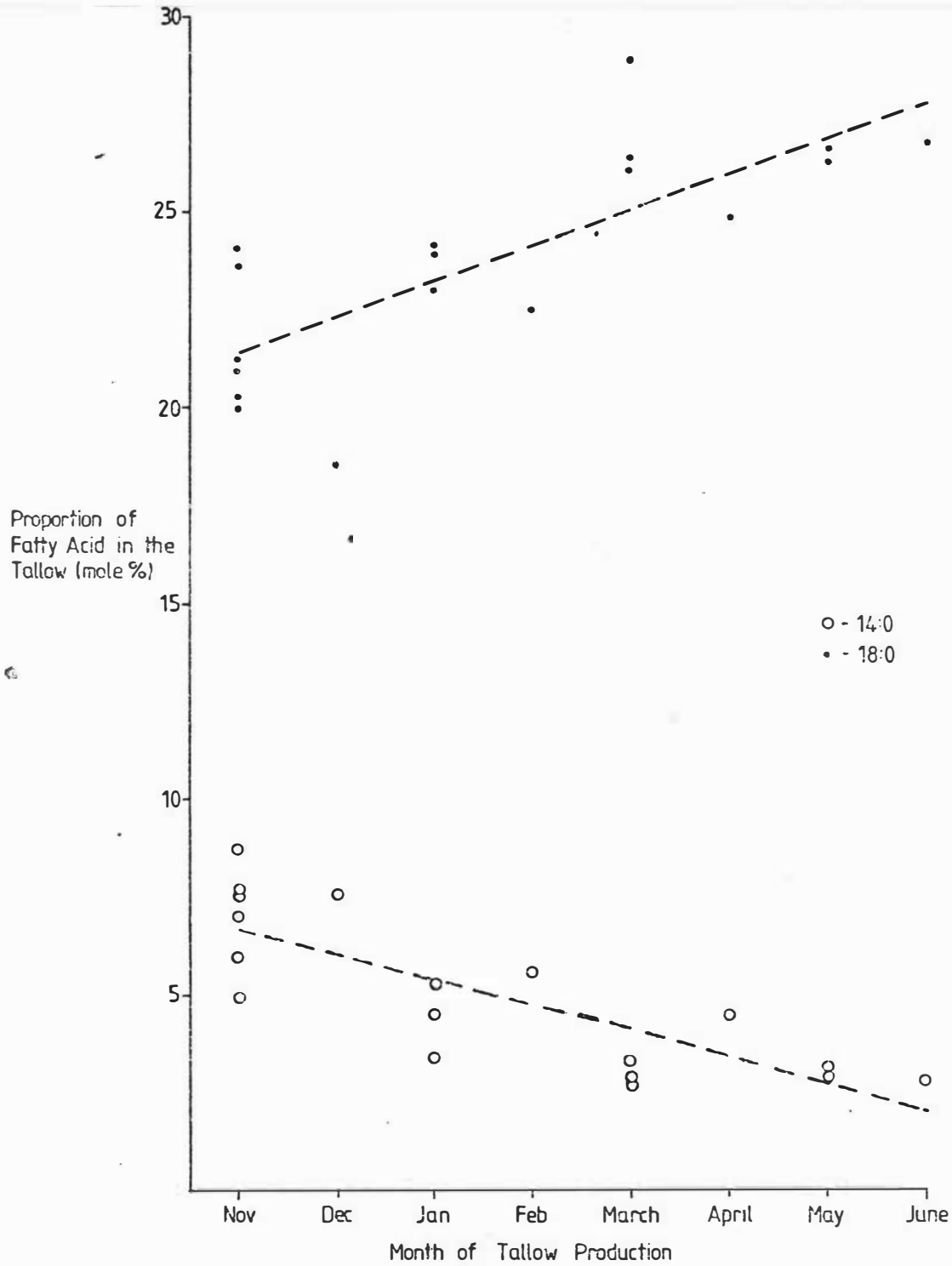


Figure 4·1 : A plot of the proportion of 14:0 and 18:0 in the tallows analysed against the month the tallow was produced, and the least squares regression for the same.

However there was a significant difference (at the 5% level) in the mean proportion of 16:0 in the tallows from the two seasons (21.0% for 1976/1977, 24.4% for 1977/1978); and also for 18:1 (39.2% for 1976/1977; 34.9% for 1977/1978).

The four fatty acids considered above comprise, on average, 88.6% of the total fatty acids found in the tallows. Of the other seven fatty acids quantified in the tallows, 16:1 was the most abundant with an average proportion of 4.0% and then 18:2 with an average proportion of 2.7%. As well as the eleven fatty acids tabulated, traces of two fatty acids which were eluted from the GLC column before 14:0 were present. These were tentatively identified as 12:0 and iso - 14:0. Trace amounts of two fatty acids which were eluted from the GLC column after 18:2 were also present. These were tentatively identified as 18:3 and 20:0, or an unsaturated 20 carbon chain length fatty acid.

There was a significant difference in the proportion of 14:0 between the different colour grades of Canterbury November 1976 and January 1977 tallows - higher in November 1976 1½ R than November 1976 ½ R, and lower in January 1977 1½ R, than January 1977 ½ R. There was also a significant difference in the proportion of 16:0 and 18:1 between the two grades of November 1976 tallow. There were no other significant differences in the proportions of the main fatty acids between any of the comparable ½ R and 1½ R grade tallows. There is no deliberate selection of raw materials entering the two grades of tallow at the Smithfield meat killing plant (Stedman, 1981), so these differences would be as a result of random variation in the incidence of raw materials.

The minimum, maximum and average proportions of the six main fatty acids in the tallows analysed are given in Table 4.3 with similar data from published fatty acid analyses of mutton tallows (see Section 2.5).

Overall, there is close agreement in fatty acid composition between the Smithfield tallows analysed and the published analyses, with the average proportion of each of the six fatty acids considered in the Smithfield tallows being within the range of proportions reported in the literature.

Table 4.3: Comparison of the minimum, maximum and average proportion of selected fatty acids in the Smithfield tallows with published values

Fatty Acid	Fatty Acid (mole % in tallow)					
	Smithfield Tallows (from Tables 4.1 and 4.2)			Published Values (from Table 2.4)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
14:0	2.7	7.6	4.5	0.8	4.6	2.7
16:0	19.8	26.3	22.6	20.4	27.8	24.1
16:1	2.5	6.1	4.0	0.0	4.7	3.1
18:0	18.5	28.8	24.1	22.2	31.0	26.5
18:1	33.1	40.6	37.3	33.0	47.0	38.3
18:2	0.9	4.0	2.7	2.2	5.0	3.8

4.4 IDENTIFICATION OF TRIGLYCERIDES IN MUTTON TALLOW

Argentation TLC resolved the tallow triglycerides into five groups (see Figure 4.2).

Fatty acid analysis showed that group one triglycerides contained only saturated fatty acids - i.e. they were tri-saturated triglycerides.

Fatty acid analysis of group 2 and 3 triglycerides revealed a composition of approximately one-third monounsaturated fatty acids and two-thirds saturated fatty acids, consistent with the triglycerides in these groups containing one monounsaturated fatty acid and two saturated fatty acids. The monounsaturated fatty acid in each of the group 2 triglycerides was probably a trans isomer, and that of the group 3 triglycerides a cis isomer.

Fatty acid analysis of groups 4 and 5 triglycerides showed that they contained approximately one-third saturated fatty acids and approximately two-thirds monounsaturated fatty acids. Thus most of the triglycerides in these groups would have been diunsaturated - i.e. they would have contained one saturated fatty acid and two monounsaturated fatty acids. One or both of the unsaturated fatty acids in the group 4 triglycerides was probably trans unsaturated to separate them from cis unsaturated fatty acids in the group 5 triglycerides on the silver-impregnated plates.

Groups 4 and 5 also contained a small proportion of triglycerides containg polyunsaturated fatty acids. The polyunsaturated fatty acids from group 4 probably contained one or two trans double bonds, as normally the affinity of Ag^+ for triglycerides with more than one double bond is greater when the double bonds are concentrated in one fatty acid (Litchfield, 1972), and thus without considering the effect of cis/trans isomers, the polyunsaturated fatty acids would be expected to be in triglyceride group 5.

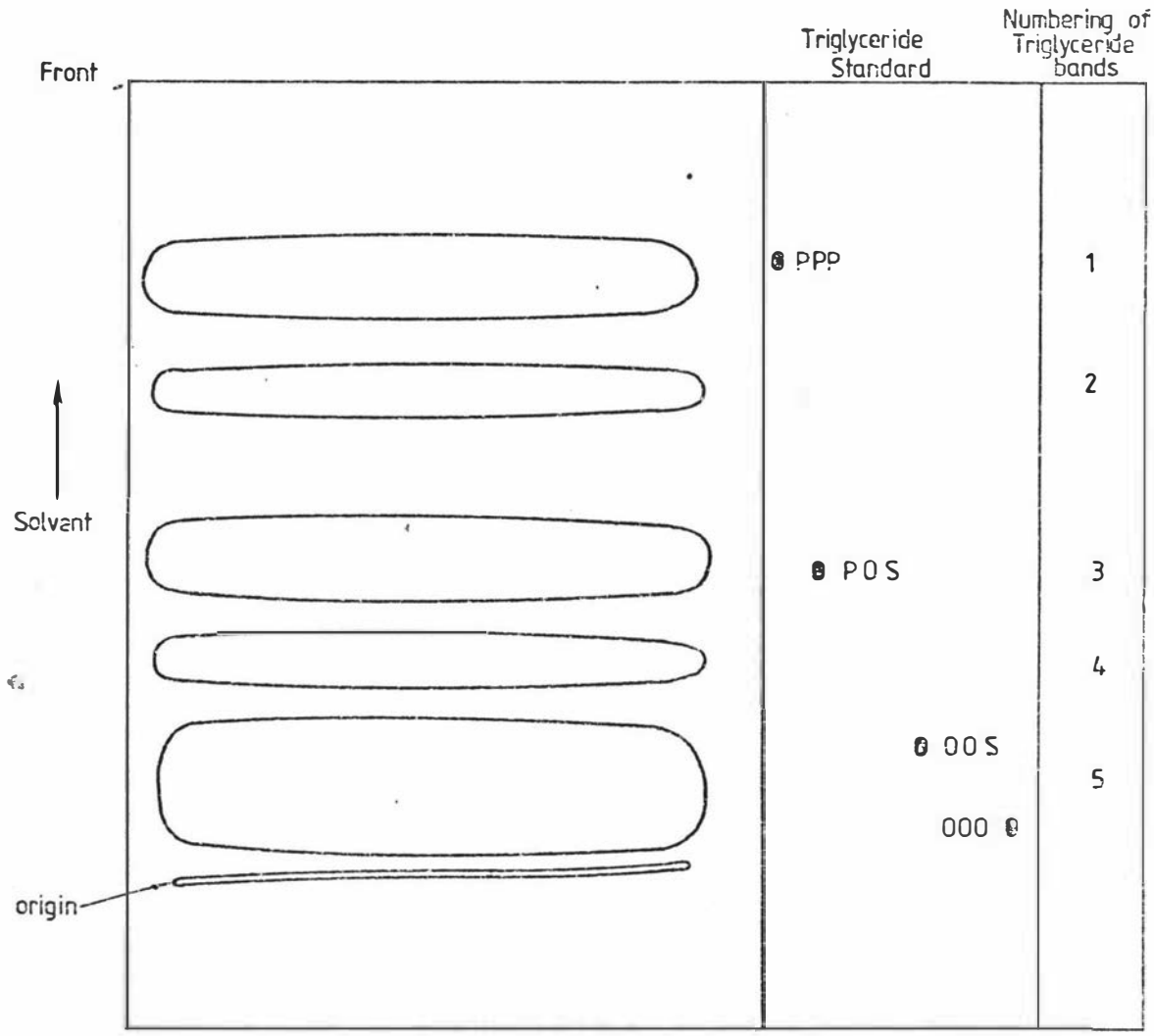


Figure 4·2 : Argentation TLC of tallow triglycerides.

Group 5 triglycerides from all the tallows contained, on average, 0.74 double bonds per fatty acid. The group 5 triglycerides of each tallow analysed had greater than 0.66 double bonds per fatty acid, the value for triglycerides containing one saturated fatty acid and two monounsaturated fatty acids. Thus group 5 includes some triglycerides with greater than two double bonds per molecule.

Details of the fatty acid analysis of each of the triglyceride groups from each of the tallows are given in Appendix 3.

As a check on the analytical and separation procedures, the fatty acid composition of the tallow was calculated from the fatty acid analysis and proportion of each triglyceride group and compared with the fatty acid composition obtained by direct analysis of the tallow. As an example, the fatty acid composition of the Smithfield November 1976 1½ R mutton tallow as determined by each of these methods is presented in Table 4.4.

Table 4.4: Comparison of the fatty acid composition of mutton tallow (Smithfield November 1976 1½ R) determined by direct analysis and by calculation from the amount of each triglyceride group separated by argentation TLC and their respective fatty acid compositions

	Fatty Acid (mole % in tallow)	
	Direct analysis	Calculated from analysis of fractions
Fatty Acid		
14:0	5.9	6.3
iso - 15:0 + 14:1	0.7	1.0
15:0	0.9	1.2
iso - 16:0	0.2	0.2
16:0	22.2	23.9
16:1	4.9	3.8
17:0	2.5	-
17:1	1.0	1.2
18:0	20.2	21.8
18:1	40.6	39.1
18:2	0.9	1.5

Each of the other tallows analysed showed similarly good agreement between the fatty acid compositions determined directly and indirectly (see Appendix 3).

As 17:0 was the internal standard used to determine the proportion of each of the triglyceride components this minor component has been omitted.

4.5 PROPORTION OF THE DIFFERENT TRIGLYCERIDE TYPES IN THE TALLOWS ANALYSED

The proportions of trisaturated, cis monounsaturated, trans monounsaturated and the more unsaturated triglycerides in Smithfield tallows from throughout the two seasons are plotted in Figure 4.3. The proportion of trisaturated triglycerides ranged from 13.3% (January 1977) to 19.7% (June 1978), trans monounsaturated triglycerides from 6.9% (November 1976) to 12.1% (December 1976), cis monounsaturated triglycerides from 22.8% (March 1977) to 33.0% (November 1976) and more unsaturated triglycerides from 39.6% (March 1978) to 53.2% (March 1977).

The triglyceride analysis of the Smithfield November 1976 tallow was duplicated. This was used to estimate the analytical variance in the triglyceride analyses, and a t-test was used to determine if there was a significant difference between the extreme concentrations of the different triglyceride groups. However as the analysis of the November 1976 tallow was the only triglyceride analysis repeated, there was only one degree of freedom available for the t-test.

The differences between the measured proportions of trisaturated triglycerides in different tallows were not significant at the 5% level. Similarly, the differences between the measured proportions of trans monounsaturated triglycerides in different tallows were not significant at the 5% level.

However, there was a significant difference between the extreme measured levels of cis monounsaturated triglycerides (22.8% for March 1977 tallow, and 33.0% for November 1976 tallow) and the extreme measured levels of the more unsaturated

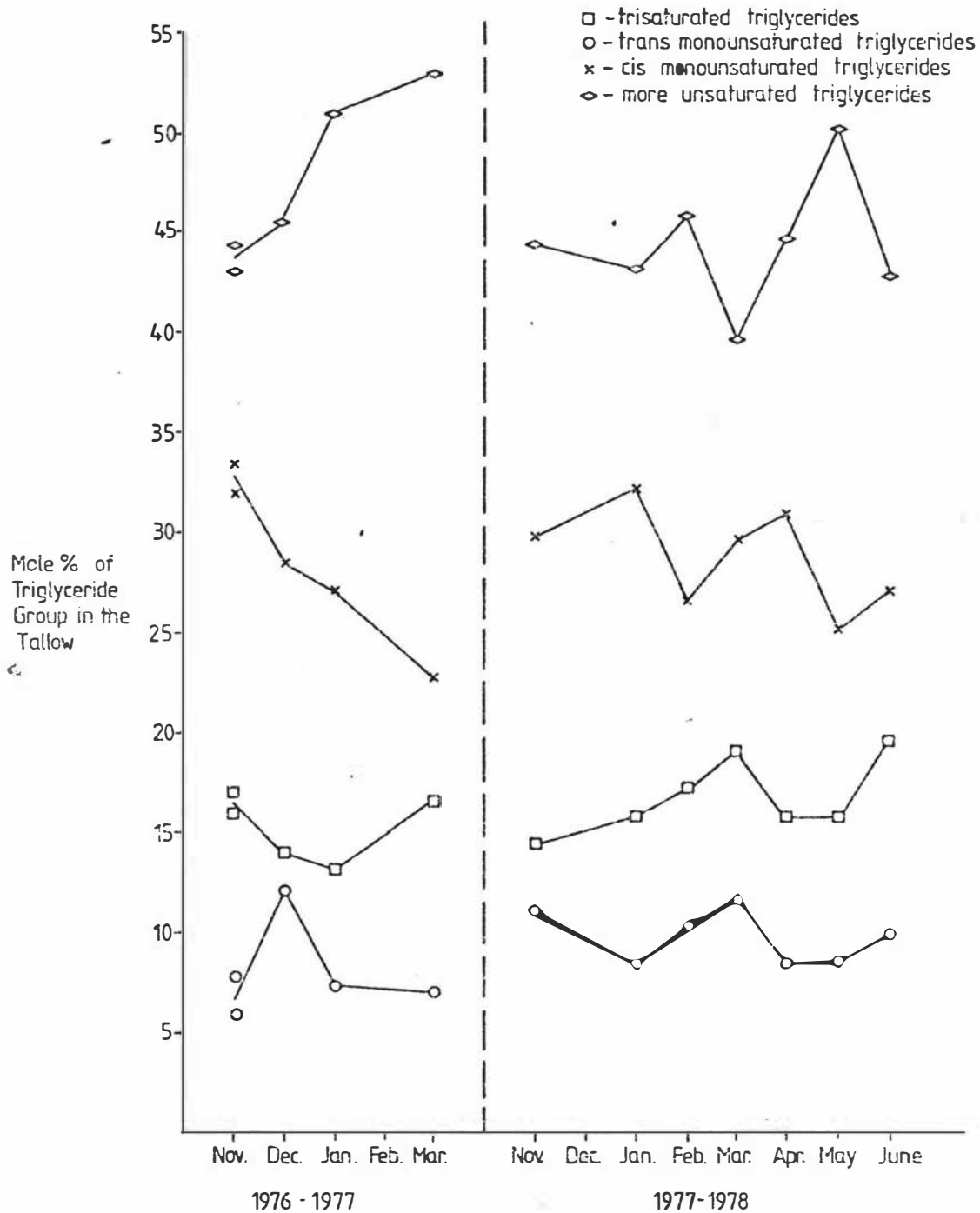


Figure 4·3 : Seasonal variation in the proportion of trisaturated, trans monounsaturated, cis monounsaturated and more unsaturated Triglycerides in mutton tallows.

triglycerides (39.6% for March 1978, and 53.2% for March 1977).

Although there was a month to month increase in the proportion of the most unsaturated triglycerides as the 1976/1977 killing season progressed, and a similar but less distinct trend in 1977/1978, the slope of the regression line for the combined data from both seasons was not significantly different from zero at the 5% level.

Similarly there appeared to be a month to month decrease in the proportion of cis monounsaturated triglycerides for both seasons as the seasons progressed, with this trend being most apparent in the 1976/1977 tallows, but again the slope of the regression line for the combined data was not significantly different from zero at the 5% level. However, there was a significant inverse correlation between the proportions of these two triglyceride groups at the 1% level (correlation coefficient = -0.74).

There was not a significant overall difference in the mean concentration of any of the four triglyceride groups between tallows of the different seasons at the 5% level.

The minimum, maximum and average values for the proportion of trisaturated, disaturated and unsaturated triglycerides from the Smithfield tallows analysed are given in Table 4.5 along with similar data from published triglyceride analyses of mutton tallows (see Section 2.5).

The Smithfield tallows analysed had a similar overall composition to that already published, but had a slightly lower than average concentration of trisaturated and disaturated triglycerides and a higher concentration of triglycerides with more than one double bond. However, the number of samples analysed (both in this work and in the literature) is too small to allow any firm conclusions to be drawn.

Assuming that the unsaturated fatty acids in the group 2 triglycerides were all trans unsaturated, and that each of the triglycerides in group 4 contained one trans double bond, this gave an average proportion of trans fatty acids in the tallows

Table 4.5: Comparison of the minimum, maximum and average proportion of the different triglyceride groups in the Smithfield tallows analysed with published values

Triglyceride Group	Proportion of Triglyceride Group (mole % of tallow)					
	Smithfield Tallows			Published Values (from Table 2.5)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Trisaturated	14.0 (Jan. 1977)	19.7 (June 1978)	16.2	15.0 (Nth American tallow)	28.0 (Indian tallow)	19.0
Disaturated	29.9 (March 1977)	41.4 (March 1978)	37.5	29.0 (Indian tallow)	47.1 (Japanese tallow)	41.0
More unsaturated	39.6 (March 1978)	53.2 (March 1977)	46.3	34.9 (Japanese tallow)	43.0 (Indian and Nth American tallows)	40.0

of 6.8%, with a range of 5.3% to 8.0%. This agrees with the results of van Beers (1961) who found that the content of trans fatty acids in tallows ranged from 5 to 10%.

4.6 PROPORTION OF 2-OLEO DISATURATED TRIGLYCERIDES

The proportion of 2-oleo-disaturated triglycerides (and in particular sn - SOS, sn - POS and sn - POP as discussed in the literature review) in the tallow samples is important because of the interest in producing a cocoa butter replacer fat. The total proportion of 2-oleo disaturated triglycerides was estimated from the product of the percentage of cis monounsaturated triglycerides (group 3 from argentation TLC) in the tallow and the percentage of oleic acid at the 2-position of these triglycerides. The concentration of 2-oleo disaturated triglycerides in each of the Smithfield tallows from throughout the seasons are shown in Figure 4.4. As palmitic and stearic fatty acids were the two main saturated fatty acids, it seems reasonable to assume that the relative proportions of 2-oleo disaturated triglycerides between different tallows was a good indication of the relative proportions of the total amount of sn-SOS, sn-POS, and sn-POP in the same tallows. Accordingly, the level of 2-oleo disaturated triglycerides was taken as an indication of the relative value of each tallow as a raw material for cocoa butter replacer production.

The overall range of 2-oleo disaturated triglycerides in the Smithfield tallows analysed varied from a minumum of 10.0% for May 1973 tallow to a maximum of 20.5% for November 1976 tallow. As a check on the analyses, each of the five triglyceride groups separated by argentation TLC from the Ocean Beach May 1977 tallow, as well as the whole tallow, were analysed for fatty acid composition at the 2-position. The fatty acid composition at the 2-position of the whole tallow was also calculated from the proportion of each triglyceride group and the fatty acid composition at the 2-position of each triglyceride group. These results were then compared with the proportion of fatty acid at the 2-position of the whole tallow as determined by direct measurement. The two sets of results, which are presented for comparison in Table 4.6, were in good agreement.

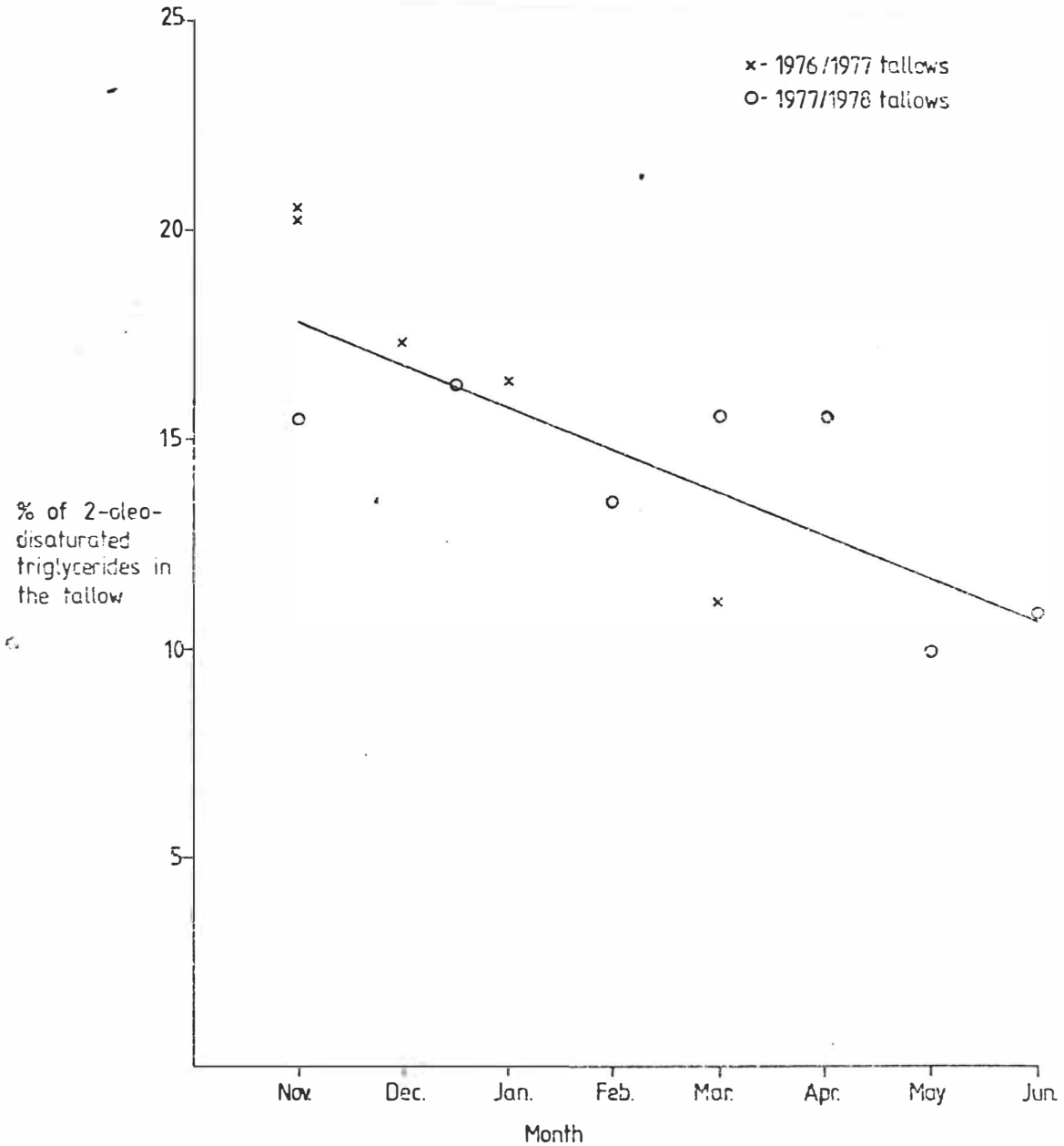


Figure 4·4 : Seasonal trend in the proportion of 2-oleo-disaturated triglycerides in mutton tallows from the Smithfield works, and the least-squares linear regression line for this data.

Table 4.6: Fatty acid composition at the 2-position of tallow triglycerides (Ocean Beach, May 1977) determined by direct analysis and by analysis of the triglyceride groups separated by argentation TLC

Fatty Acid	Direct Analysis	Calculated Composition
14:0	5.0	5.2
iso - 15:0 + 14:1	2.1	1.6
15:0	1.3	0.8
iso - 16:0	0.3	0.2
16:0	12.0	12.9
16:1	4.4	4.1
17:0	2.2	1.6
17:1	0.8	1.3
18:0	14.6	13.7
18:1	52.0	52.1
18:2	5.3	6.4

There was a marked decrease (from 20.5% to 11.2%) in the proportion of 2-oleo disaturated triglycerides in the Canterbury tallows as the 1976/1977 season progressed.

However an isolated May tallow from Southland contained 15.7% of 2-oleo disaturated triglycerides. This increase may have been due to a number of factors as the tallow was from a different meat killing plant in a different area and a different rendering process was used.

Such a marked overall trend in the proportion of 2-oleo disaturated triglycerides was not apparent in the 1977/1978 tallows although there was a decrease in the proportion of these triglycerides in the late-season samples. The overall range (10.0% to 16.3%) was less than for the 1976/1977 tallows.

The proportion of 2-oleo disaturated triglycerides in the Smithfield tallows for the two seasons was regressed linearly by the least squares method against the month of production

of the tallow. The months were numbered from 1 (November) to 8 (June).

The regression equation was:

proportion of 2-oleo disaturated triglycerides in the tallow (mole %) = (18.8 - 1.0 (Month))

This regression line is plotted in Figure 4.4 with the measured results. It appears to fit the observed data well.

The slope coefficient of the regression equation (-1.0) was found to be significantly different from zero at the 0.2% level. Thus overall for the two seasons, there was a significant decrease in the proportion of 2-oleo-disaturated triglycerides in the tallows as the seasons progressed.

4.7 CONCLUSIONS

(1) Four fatty acids (14:0, 16:0, 18:0 and 18:1) comprised, on average, 88.6% of the total fatty acids in the tallows. There were significant differences in the proportions of these fatty acids in different tallows from the same season.

(2) The combined data for analyses upon tallows from both seasons showed:

(a) There was a significant decrease in the proportion of 14:0 and a significant increase in the concentration of 18:0 from November to June.

(b) There was a significant difference in the mean proportion of 16:0, and also for 18:1, between the two seasons.

(c) There was a significant difference in the proportion of cis-monounsaturated triglycerides and the more highly unsaturated triglycerides between some of the different tallows analysed.

(3) The average proportion of each of the six most abundant fatty acids in the Smithfield tallows was within the range of proportions reported in the literature. Also, the

average proportion of the trisaturated, disaturated and more unsaturated triglycerides in the Smithfield tallows was within the range of proportions reported in the literature.

(4) Each of the tallows contained a significant proportion of 2-oleo disaturated triglycerides, and thus New Zealand mutton tallow has potential as a cocoa butter replacer fat if these triglycerides can be separated.

(5) There was a significant decrease in the proportion of 2-oleo disaturated triglycerides in the Smithfield tallows from November to June, with a range from 10.0% (May 1978) to 20.5% (November 1976).

(6) Each of the tallows also contained more highly saturated and more highly unsaturated triglycerides. Separation of these would produce fractions with properties very different from those of the original tallow, and these may be useful for a variety of purposes.

CHAPTER 5

DEVELOPMENT OF A SOLVENT FRACTIONATION SCHEME FOR NEW ZEALAND MUTTON TALLOW

5.1 INTRODUCTION

Several methods for the solvent fractionation of tallow to produce fractions with desirable melting properties have been reported, (see 2.6.3) but none specifically for New Zealand mutton tallow. These methods all produced a fraction intended for use as a "cocoa butter replacer" fat, plus other higher and lower melting fractions.

When the four-stage solvent fractionation method of Luddy et al (1973 , 1976, 1978), developed for North American beef tallow, was applied to New Zealand mutton tallow the yields and melting properties of each of the fractions obtained were widely different from those reported by Luddy et al. The scheme was considered not to be suitable for New Zealand mutton tallow.

Hence, it was decided to develop a new solvent fractionation process for New Zealand mutton tallow. Kawada and Matsui (1968) successfully used a two-stage solvent fractionation scheme to produce a fraction from Japanese mutton tallow which contained a high proportion of 2-oleo disaturated triglycerides and it was suggested that it could be used to replace part of the cocoa butter in chocolate; and Farr (1954) also used a two-stage solvent fractionation scheme with tallow to produce a coating fat (see Section 2.6.3). Because of the simplicity and thus reduced costs of a two-stage solvent fractionation process, and because of the success of the above two-stage solvent fractionation methods, it was decided to develop a two-stage solvent fractionation process for New Zealand mutton tallow. A study was made of the different variables - crystallisation temperatures, solvent to fat ratios, tallow source, agitation, water content of the acetone and crystallisation time - to find the optimum conditions for a two-stage fractionation of New Zealand mutton tallow. Acetone was the only solvent used, and filtration was used to recover the crystals.

5.2 OUTLINE OF THE FRACTIONATION METHOD

An outline of the fractionation process is shown in Figure 5.1.

Fifty g of tallow were dissolved in the appropriate amount of acetone in a stoppered 250 ml conical flask, heated to 40°C and then cooled by holding for the desired period in a constant temperature room.

The crystals were filtered under vacuum on a Buchner funnel at the temperature of crystallisation, and were then washed on the filter with 100 ml of acetone at this temperature and with the same water content as the acetone used for the crystallisation.

5.3 THE INDEPENDENT VARIABLES

Variables which are likely to affect the nature and proportion of each of the fractions produced by a two-stage solvent fractionation process include:

- temperature for first crystallisation
- temperature for second crystallisation
- solvent to fat ratio at first crystallisation
- solvent to fat ratio at second crystallisation
- proportion of water in the solvent
- degree of agitation during crystallisation
- time of holding before filtering
- tallow composition
- rate of cooling the fat solution.

The rate of cooling the fat solution was difficult to control as an independent variable because it was affected by the degree of agitation and the crystallisation temperature. Hence its effect was not considered at this stage.

A full investigation of each of the remaining eight process variables and their interactive effects would still have required considerable experimental work. Therefore a screening experiment in which the variables were studied at two levels was conducted to determine which of these were important for further study.

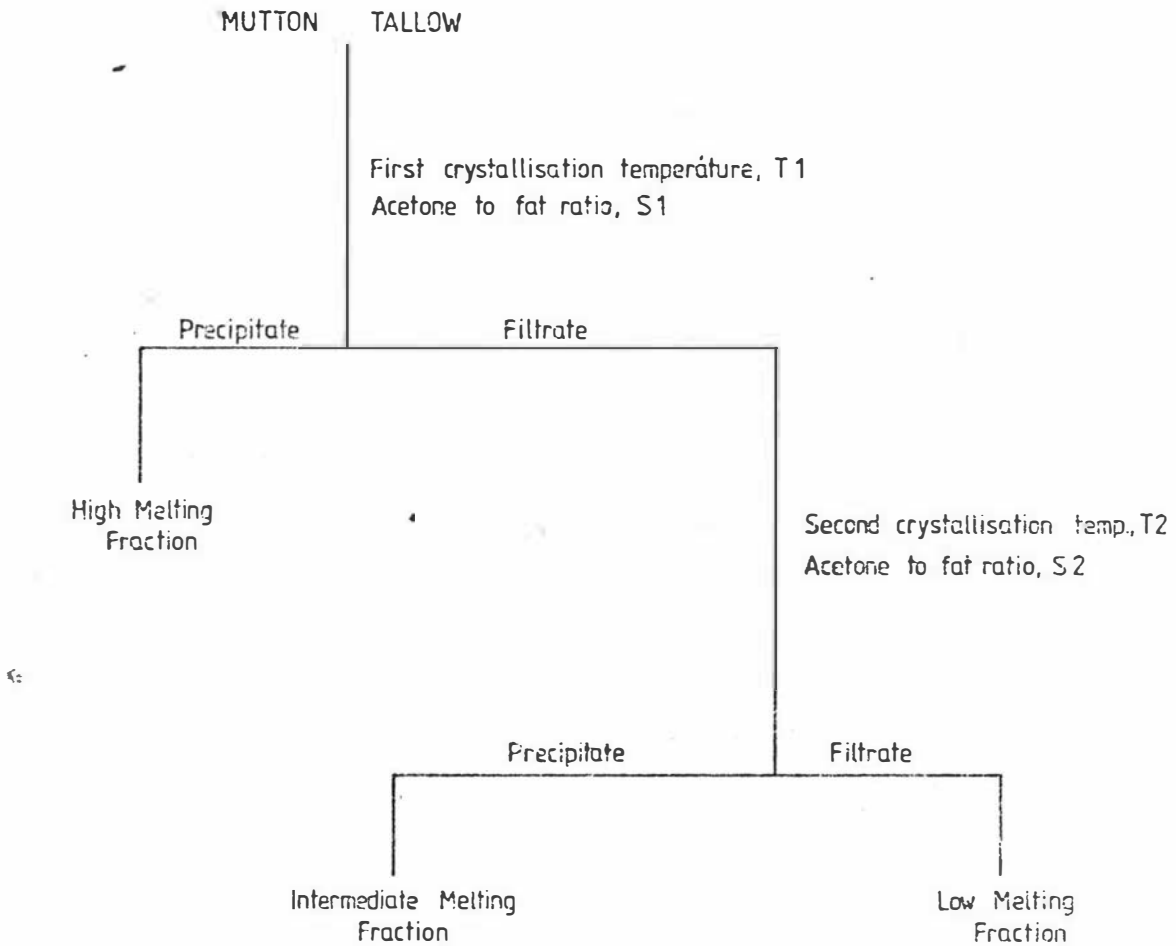


Figure 5·1 : Outline of the two-stage solvent fractionation process.

5.3.1 First Crystallisation Temperature (T_1)

Kawada and Matsui (1968) produced a cocoa butter replacer fat from Japanese mutton tallow using a two-stage solvent fractionation process with acetone and a first crystallisation temperature of 10-11°C. Their alternative scheme used a first crystallisation temperature of 18°C, and by adjusting other variables the intermediate fraction was still suitable as a cocoa butter replacer fat.

Farr (1954) performed the first crystallisation at room temperature in a similar process to produce a coating fat (see Section 2.6.3).

The analytical solvent fractionation scheme of Riemenschneider et al (1946) used two initial crystallisations at 18-19°C to remove all of the trisaturated triglycerides and a portion of the disaturated triglycerides from mutton tallow (see Section 2.6.2).

Therefore for this screening experiment 11°C and 20°C were chosen as the first crystallisation temperatures.

5.3.2 Second Crystallisation Temperature (T_2)

Second crystallisation temperatures of 0°C and 8°C were chosen (cf. with 3 to 4°C and 0°C used by Kawada and Matsui (1968) and Farr (1954) respectively).

5.3.3 Solvent to Fat Ratio at Each Crystallisation (S_1 and S_2)

Although solvent to fat ratios from 0.1:1 (v/w) to 20:1 (v/w) have been used, most fat fractionations work at ratios of 2:1 to 5:1 (see Section 2.4.3.3). These were the levels set for the screening experiment.

5.3.4 Water Content of the Acetone (W)

Several workers have recommended the use of acetone with a water content of less than 2% (Section 2.4.3.2). This was used as the high level for the screening experiment, and the low level was set at 0.5%.

5.3.5 Agitation Speed (A)

There is little quantitative data as to the optimum agitation speed for solvent fractionation and it was not possible to select a constant agitation speed as this decreased as the deposition of fat crystals increased the viscosity of the solution. The applied voltages of 180 and 160 volts to the stirrer motor were used as the independent variables.

5.3.6 Crystallisation Time (t)

Preliminary experiments showed that there was no further crystallisation of the fat solution after 60 minutes in the constant temperature room.

The holding times for this experiment were set at 30 minutes and 60 minutes.

5.3.7 Tallow Source (M)

A Smithfield tallow (November 1976 1½ R) containing 20.5% 2-oleo disaturated triglycerides and an Ocean Beach tallow (May 1977) containing 15.5% 2-oleo disaturated triglycerides were chosen for this experiment.

The eight independent variables selected for the screening experiment, and their levels, are summarised in Table 5.1.

Table 5.1: Definition of independent variables selected for the screening experiment

VARIABLE	SYMBOL	CODED LEVEL	
	-	-1	+1
First Crystallisation Temperature (^{o}C)	T_1	11^{o}C	20^{o}C
Second Crystallisation Temperature (^{o}C)	T_2	0^{o}C	8^{o}C
Solvent to Fat Ratio (v/w) - First Crystallisation	S_1	2:1	5:1
Solvent to Fat Ratio (v/w) - Second Crystallisation	S_2	2:1	5:1
Water Content of Acetone (% v/v)	W	0.5	2.0
Agitation Speed (volts)	A	160	180
Crystallisation time (minutes)	t	30	60
Tallow Source	M	Smithfield, Nov. 1976.	Ocean Beach, May 1977

5.4 DEPENDENT VARIABLES

The major aim of the fractionation was to produce a satisfactory cocoa butter replacer fat - i.e. a fraction with a triglyceride composition similar to cocoa butter which would melt like cocoa butter and be compatible with cocoa butter. Ideally, the triglyceride composition of each of the fractions should have been determined to fully understand the effect of the variables upon the fractions produced.

Such analyses were prohibitively time-consuming, and consequently the dependent variables chosen were the yield of the three fractions and the similarity between the DSC profile of the intermediate fraction and the DSC profile of cocoa butter. This similarity was quantified using the cocoa butter likeness factor (CBLF) - see Section 3.2.

The yield of each fraction was measured by weighing the recovered crystals after drying in a vacuum dessicator.

5.5 SCREENING EXPERIMENT

The aim of this experiment was to determine which of the preceding eight independent variables were important for further study.

5.5.1 Experiment Design

A fractional design of resolution IV was chosen to screen the eight variables. A resolution IV design has the properties that no main effect is confounded with any other main effect or two - factor interaction, but two - factor interactions are confounded with one another. Thus a resolution IV design could determine which of the eight main effects were significant as well as determine the significant groups of two - factor interactions.

It is a general principle of fractional factorial designs that if any fractional is replicated with reversed signs, then all alias links between main effects and two - factor interactions are broken (Box and Hunter, 1961). Hence by switching the signs of all the variables in a 2^{7-4}_{III} design (a seven - factor, 1/16th replicated design of resolution III with each of the variables tested at two levels) and adding the resultant design to the original fraction an aggregate design is produced which uses sixteen runs and makes it possible to estimate all main effects clear of the two - factor interactions. This is a 2^{7-3}_{IV} design. (A resolution III design has the properties that no main effects are confounded with any other main effect, but main effects are confounded with two - factor interactions and two - factor interactions with one another). The above 2^{7-3}_{IV} design can be further improved to a 2^{8-4}_{IV} design by switching the signs of the elements corresponding to the identity column and associating the resulting set of eight positive and eight negative signs with an eigth factor.

Such a design is called a "fold over" design (Box and Hunter, 1961). See Table 5.3 for the experimental design.

Ignoring interactions between three or more factors, which are confounded with the main effects, the sixteen quantities which can be estimated from the experimental design are shown

in Table 5.2:

Table 5.2: Effects determined using a 2^{8-4}_{IV} fold-over design

Average							
T_1							
T_2							
S_1							
S_2							
W							
A							
t							
M							
T_1T_2	+	S_1t	+	S_2M	+	WA	
T_1S_1	+	T_2t	+	WM	+	S_2A	
T_1S_2	+	T_2M	+	S_1A	+	Wt	
T_1W	+	S_1M	+	T_2A	+	S_2t	
T_1A	+	tM	+	S_1S_2	+	T_2W	
T_1t	+	T_2S_1	+	AM	+	S_2W	
T_1M	+	T_2S_2	+	S_1W	+	At	

5.5.2 Results of the Screening Experiment

The yields of each of the three fractions, adjusted to 100% total yield, are given in Table 5.3. The CBLF of the DSC profiles of each of the intermediate fractions are also presented in Table 5.3.

5.5.3 Empirical Equations Relating Dependent and Independent Variables

The data for the yields of the three fractions and the CBLF of the intermediate fractions were read into the Massey University Burrough's B6700 computer and analysed using the Minitab statistical package. This was done by multiple regression of the coded linear terms and chains of two - factor interactions of the independent variables to the dependent variables.

Table 5.3: The experimental design used and the responses to the independent variables

								DEPENDENT VARIABLES			
T_1	T_2	S_1	S_2	W	A	t	M	Yield of hard fraction (wt % of tallow)	Yield of intermediate fraction (wt % of tallow)	Yield of soft fraction (wt % of tallow)	CBLF of intermediate fraction
-1	-1	-1	-1	-1	-1	-1	-1	27.1	18.6	54.3	11.0
+1	-1	-1	+1	+1	-1	+1	-1	13.6	59.8	26.6	18.5
-1	+1	-1	+1	-1	+1	+1	-1	27.8	3.8	68.4	14.0
+1	+1	-1	-1	+1	+1	-1	-1	13.0	40.0	47.0	24.3
-1	-1	+1	-1	+1	+1	+1	-1	21.8	30.2	48.0	24.8
+1	-1	+1	+1	-1	+1	-1	-1	0.4	22.6	77.0	26.5
-1	+1	+1	+1	+1	-1	-1	-1	25.4	7.6	67.0	13.7
+1	+1	+1	-1	-1	-1	+1	-1	11.1	18.2	70.7	20.5
-1	-1	-1	+1	+1	+1	-1	+1	53.8	11.6	34.6	13.3
+1	-1	-1	-1	-1	+1	+1	+1	5.4	44.4	50.2	21.3
-1	+1	-1	-1	+1	-1	+1	+1	48.6	22.0	29.4	11.7
+1	+1	-1	+1	-1	-1	-1	+1	4.8	18.0	77.2	18.5
-1	-1	+1	+1	-1	-1	+1	+1	18.2	9.6	72.2	11.9
+1	-1	+1	-1	+1	-1	-1	+1	11.2	52.0	36.8	29.2
-1	+1	+1	-1	-1	+1	-1	+1	20.0	11.4	68.6	14.5
+1	+1	+1	+1	+1	+1	+1	+1	7.6	47.6	44.8	25.0

Because fifteen effects (eight main effects and seven chains of two-factor interactions) plus the constant were estimated from the sixteen runs it was not possible to apply a statistical test to the coefficients of each effect to determine their significance (no degrees of freedom to estimate error). Hence a half-normal probability plot was used to estimate the significance of each of the terms.

A half-normal plot is a plot of the magnitude of each effect against its order number on half-normal probability paper. The null hypothesis is that each of the fifteen treatment effects is a normal variate with zero mean and constant variance. If this was true, all of the points would lie on a straight line. Any effects which are significant would violate the null hypothesis and would not be on this straight line through the other points (Daniel, 1959; Belz, 1973).

Each of the four dependent variables were then regressed against those terms which appeared, from the half-normal plots, to be significant. By relegating all other terms to the residual, there were then sufficient degrees of freedom available to test significance of the remaining terms by the t-test.

5.5.3.1 Yield of the Hard Fraction

The regression equation for the yield of hard fraction is shown in Table A4.1, Appendix 4. The half-normal plot of the regression coefficients is shown in Figure 5.2. From this, T_1, W, S_1 and the $(T_1S_1 + T_2t + WM + S_2A)$, $(T_1M + T_2S_2 + S_1W + At)$ and $(T_1W + S_1M + T_2A + S_2t)$ chains of two-factor interactions appear to lie off the straight line through the other points. The yield data were regressed against these six terms. The resulting equation was:

$$\text{Yield of hard fraction (\%)} = 19.4 - 11.0\,(T_1) - 4.9\,(S_1) + 5.0\,(W) + 4.1(T_1S_1 + T_2t + WM + S_2A) - 2.1\,(T_1W + S_1M + T_2A + S_2t) + 3.6\,(T_1M + T_2S_2 + S_1W + At)$$

Each of these six terms were significant, by the t-test, at the 5% level.

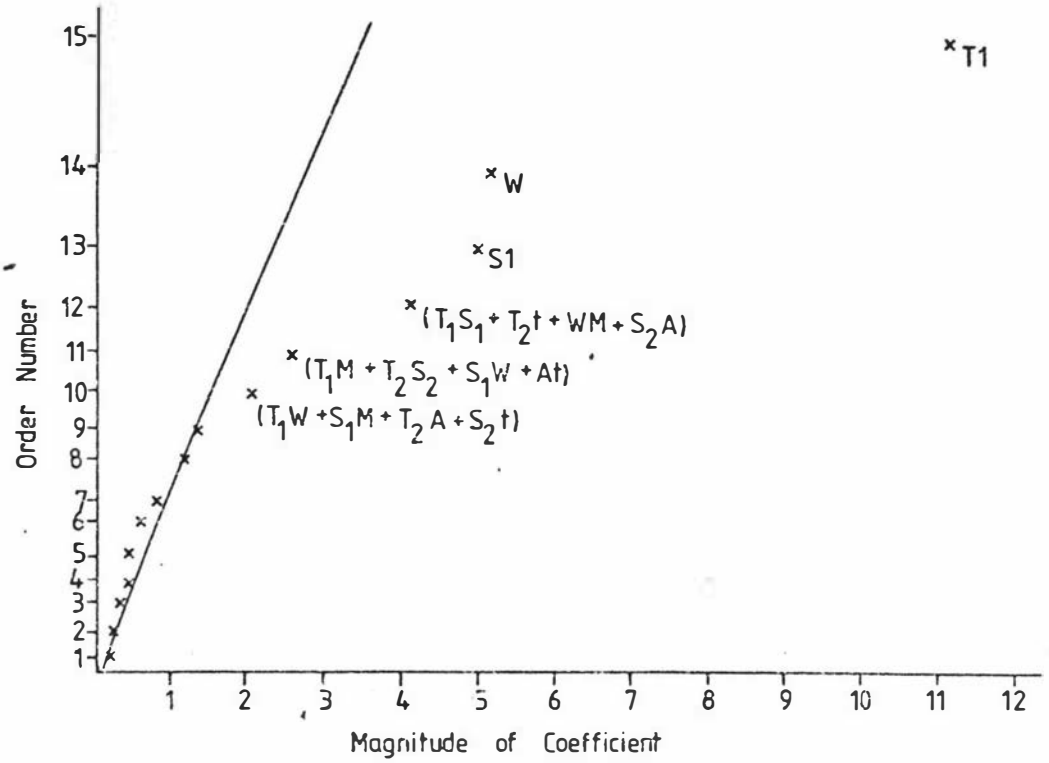


Figure 5·2 : Half-normal plot for the yield of hard fraction

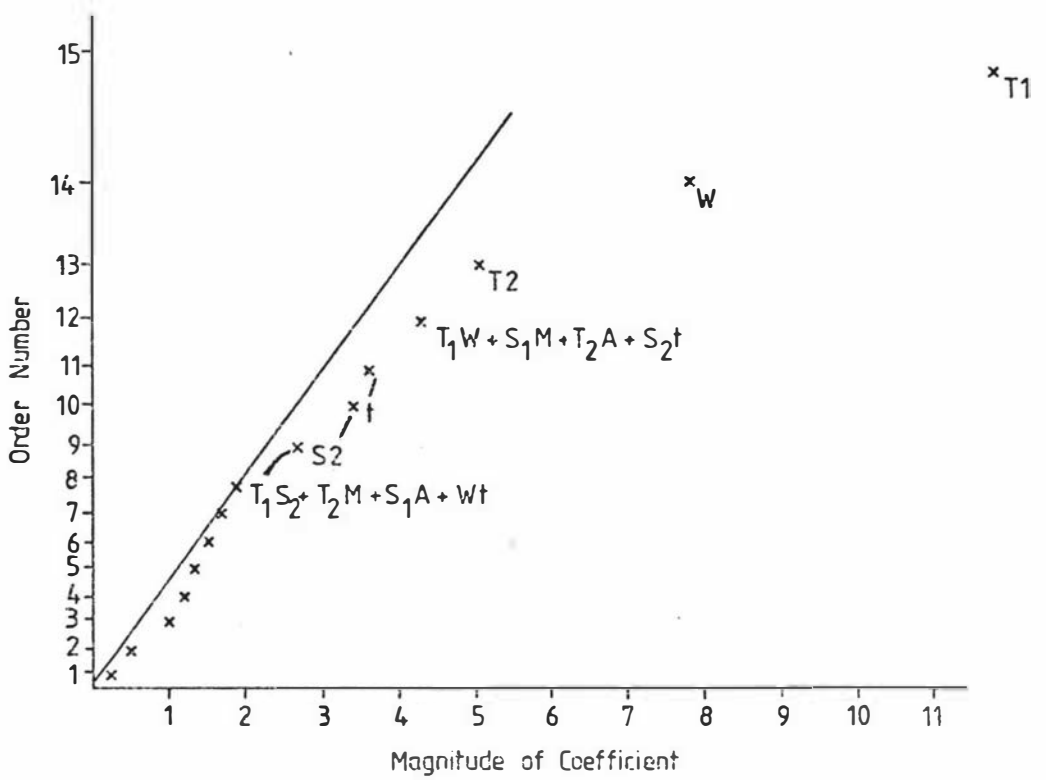


Figure 5·3 : Half-normal plot for the yield of the intermediate fraction

The regression statistics for this equation are presented in Table A4.1, Appendix 4.

While it is impossible to say which of the individual two-factor interactions were responsible for the significance of the chains of two-factor interactions, each of the three significant chains contained an interaction of two of the three significant main effects (T_1S_1, T_1W and S_1W respectively). It seems probable that these three interactions were responsible for the significance of the chains.

5.5.3.2 Yield of the Intermediate Fraction

The regression equation for the yield of the intermediate fraction is shown in Table A4.2, Appendix 4. The half-normal plot of the regression coefficients is shown in Figure 5.3. From this, T_1, T_2, S_2, W, t and the ($T_1W + S_1M + T_2A + S_2t$) and ($T_1S_2 + T_2M + S_1A + Wt$) chains of two-factor interactions appear to lie off the straight line through the other points. The intermediate fraction yield data were regressed against these seven terms. The resulting equation was:

Yield of Intermediate Fraction (%) = 26.1 + 11.7 (T_1) - 5.0 (T_2) - 3.5 (S_2) + 7.8 (W) + 3.4 (t) + 2.7 ($T_1S_2 + T_2M + S_1A + Wt$) + 4.3 ($T_1W + S_1M + T_2A + S_2t$)

Each of these seven terms was significant, by the t-test, at the 5% level.

The regression statistics for this equation are presented in Table A4.2, Appendix 4.

Each of the significant chains of two-factor interactions contained two interactions of the significant main effects (T_1S_2 and Wt in one chain, and T_1W and S_2t in the other chain). It is probable that one or both of these interactions in each chain were responsible for the significance of the chains.

5.5.3.3 Yield of the Soft Fraction

The regression equation for the yield of the soft fraction is shown in Table A4.3, Appendix 4. The half-normal plot for the regression coefficients is shown in Figure 5.4.

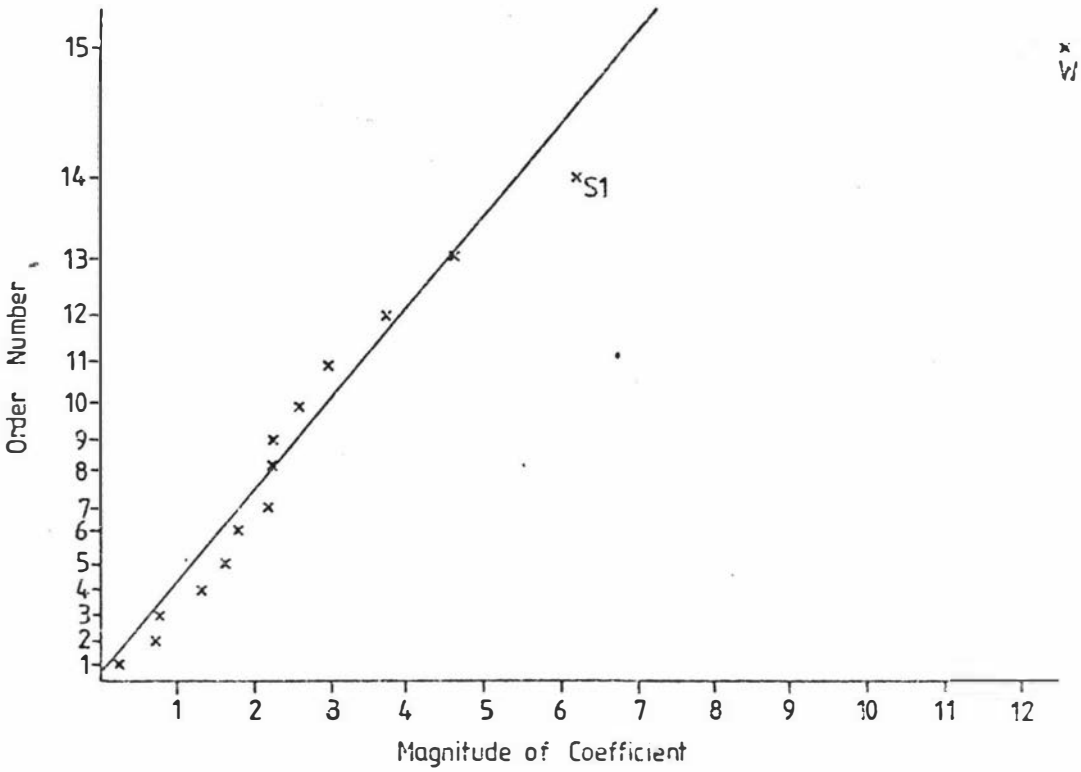


Figure 5·4: Half-normal plot for the yield of the soft fraction.

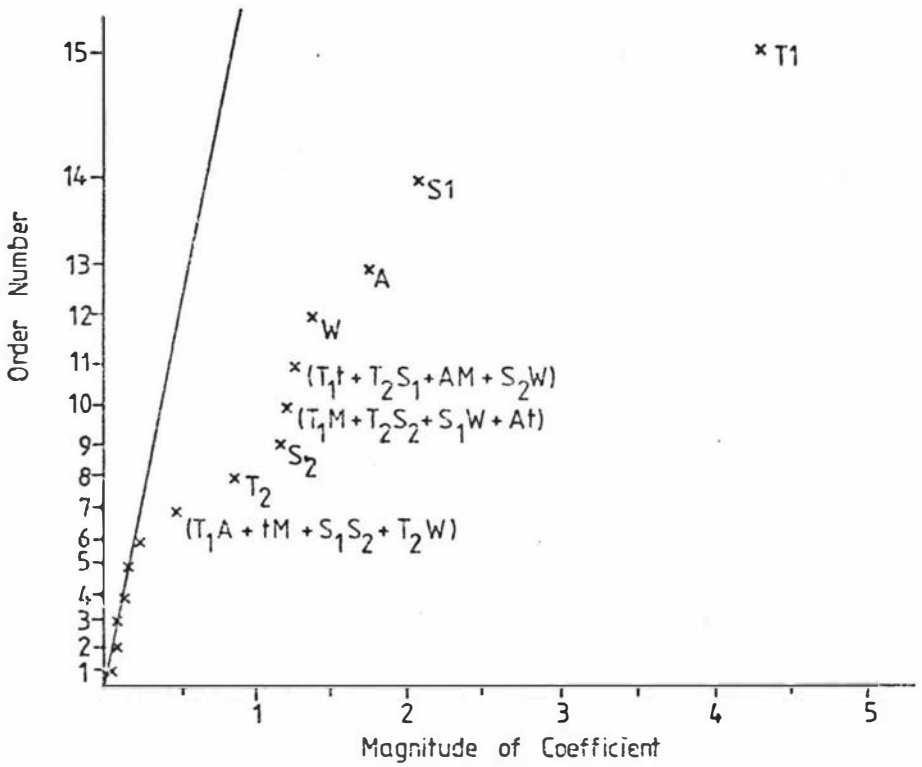


Figure 5·5: Half-normal plot for the CBLF of the intermediate fraction.

From this, W and S_1 were the only terms which appeared to lie off the line through the other points. The soft fraction yield data were regressed against these terms. The resulting equation was:

Yield of soft fraction (%) = 54.6 + 6.1 (S_1) - 12.8 (W)

Both of these terms were significant, by the t-test, at the 5% level.

The regression statistics for this equation are presented in Table A4.3, Appendix 4.

Though only two terms were shown to be significant by this method, it seems likely that all of the terms affecting the yield of the first two fractions would influence the yield of the soft fraction, as it is the final filtrate from the fractionation. If this was so, then a great number of the terms in the soft fraction equation should be significant. Unfortunately, a half-normal plot is not suitable for determining the significant terms of an equation where most of the terms are significant as there are not enough terms left to give an accurate linear graph for the smaller constraints. Hence, it is possible that a much greater number of these fifteen terms were significant than are apparent from the half-normal plot.

It is also possible, though, that the terms affecting the yield of the two precipitated fractions, but not of the soft fraction, only influenced the relative proportions of the two precipitated fractions and did not affect the quantity of the soft fraction.

5.5.3.4 CBLF of the Intermediate Fraction

The regression equation for the CBLF of the intermediate fraction is shown in Table A4.4, Appendix 4. The half-normal plot for the regression coefficients is shown in Figure 5.5. From this, T_1, T_2, S_1, S_2, W, A and the ($T_1t + T_2S_1 + AM + S_2W$), ($T_1M + T_2S_2 + S_1W + At$) and ($T_1A + tM + S_1S_2 + T_2W$) chains of two-factor interactions appeared to lie off the straight line through the other points. The CBLF data were regressed against these terms. The resulting equation was:

$$CBLF = 18.7 + 4.3\,(T_1) - 0.9(T_2) + 2.1\,(S_1) - 1.2\,(S_2) + 1.4\,(W) + 1.7(A) - 0.5\,(T_1A + tM + S_1S_2 + T_2W) - 1.3\,(T_1t + T_2S_1 + AM + S_2W) - 1.2\,(T_1M + T_2S_2 + S_1W + At)$$

Each of these terms was significant, by the t-test, at the 5% level.

The regression statistics for this equation are presented in Table A4.4, Appendix 4.

5.5.3.5 Residual Plots

Plots of the residual versus Y for the simplified regression equations for each of the four response variables are given in Figures 5.6 to 5.9.

The apparent random distribution of the points in each of the graphs indicates that there were no gross errors in the regression equations fitted to the data (Chatfield, 1970).

5.5.4 Main Effects

It was possible to study the effect of each independent variable alone and therefore obtain some indication of the region where the optimum level of each independent variable might be. However this does not account for interactive effects between the variables, and each independent variable was only studied at two levels.

The highest yield of the hard fraction was obtained with T_1 and S_1 set at their low levels, and with W at its high level. The highest yield of the intermediate fraction was obtained with T_1, W and t at their high levels, and with T_2 and S_2 at their low levels. S_1 at its high level and W at its low level gave the best yield of the soft fraction. The best CBLF values were obtained with T_1, S_1, W and A at their low levels, and with T_2 and S_2 at their high levels.

The monthly source of the tallow (M) was the only main effect which was not shown to significantly affect either the yield of the fractions or melting properties of the intermediate

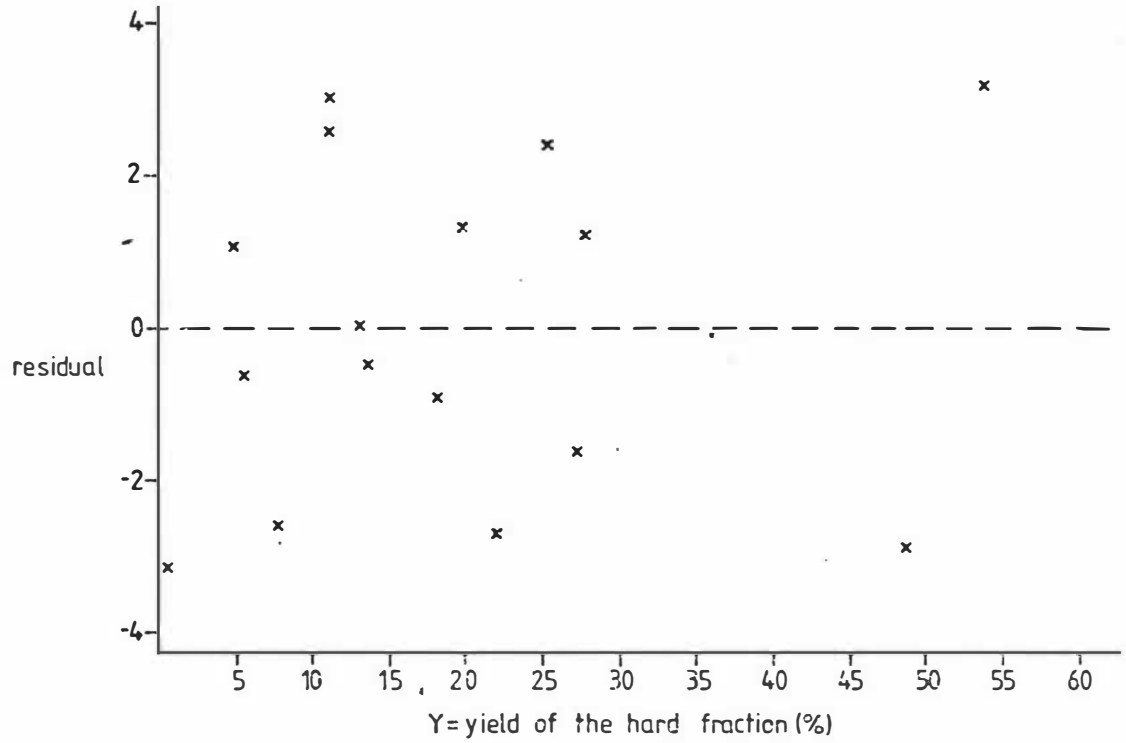


Figure 5·6: Plot of residual v. Y for the yield of the hard fraction.

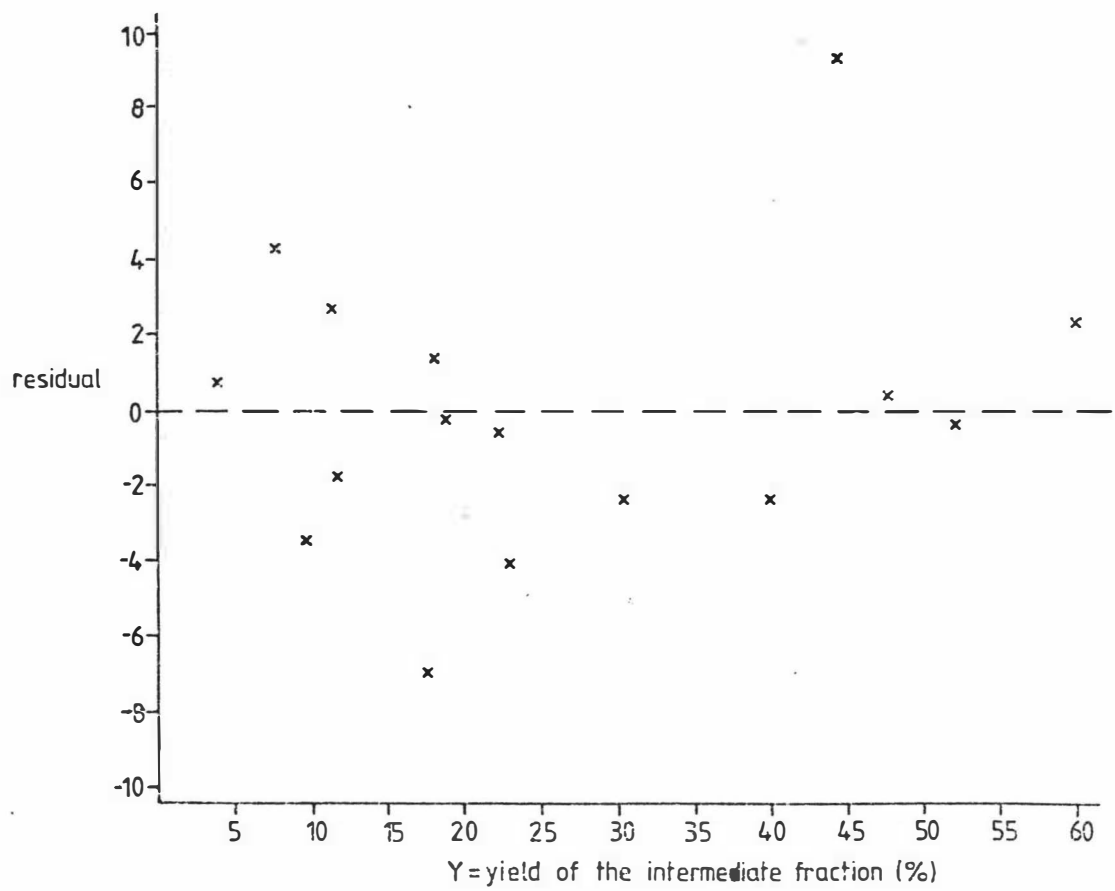


Figure 5·7: Plot of residual v. Y for the yield of the intermediate fraction.

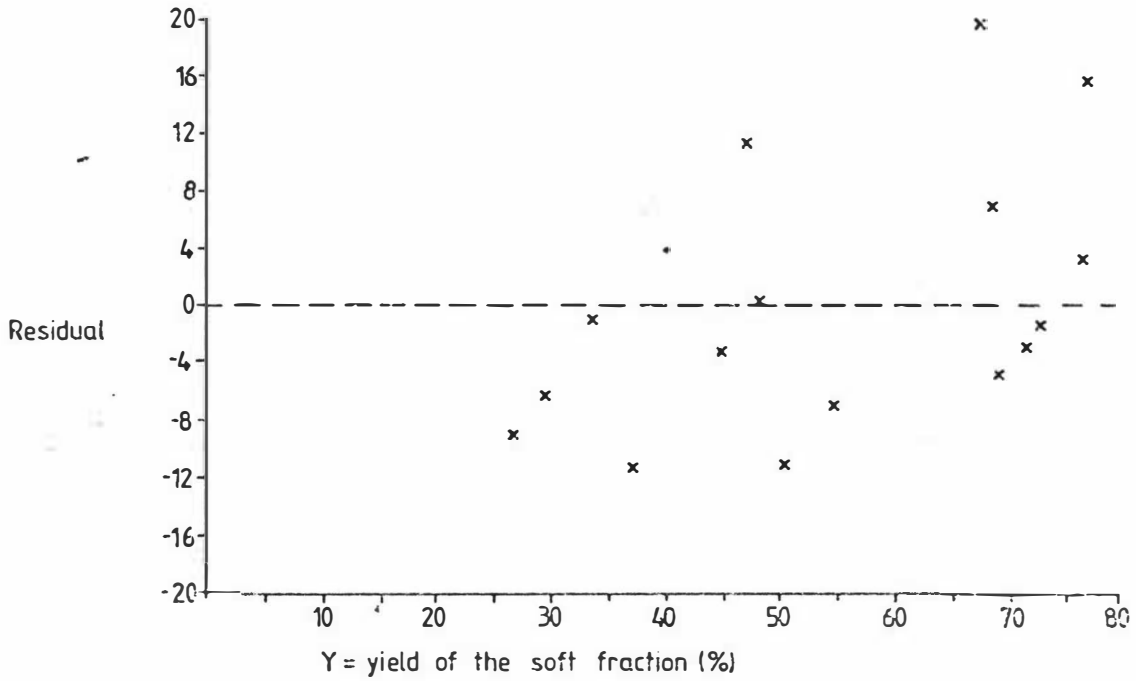


Figure 5·8 : Plot of residual vs Y for yield of the soft fraction.

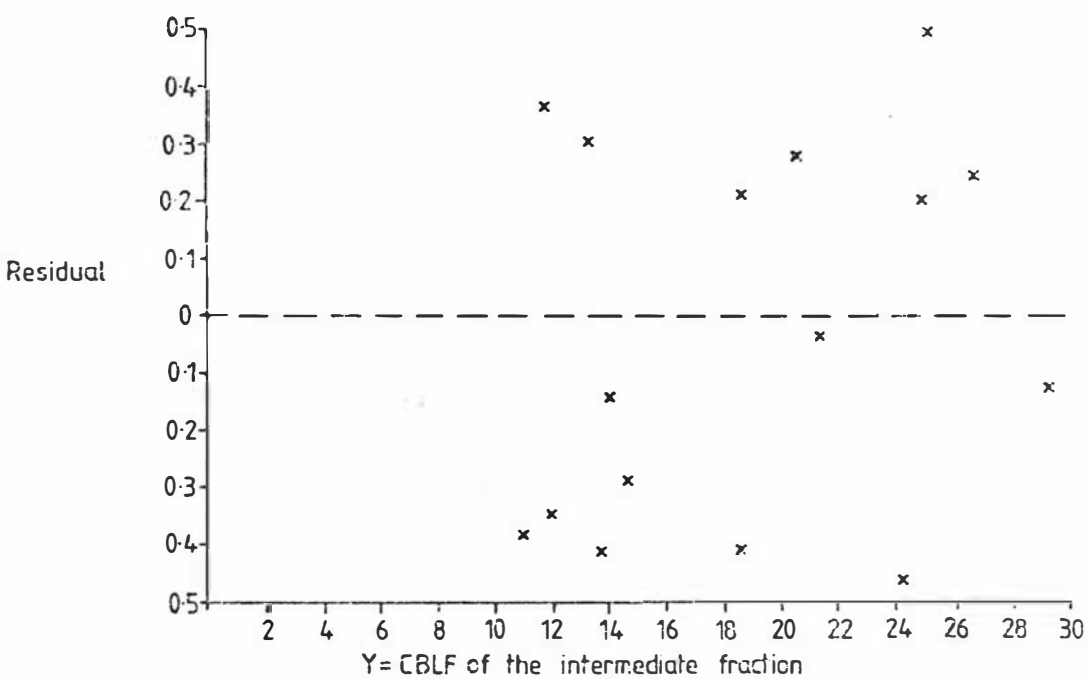


Figure 5·9 : Plot of residual vs Y for the CBLF of the intermediate fraction.

fraction. This was a surprising result, as it was suspected that the composition of the starting material would affect the products to some extent. However the two tallows chosen, despite being from the beginning and end of the season, did not differ in composition as much as some of the mid-season tallows differed in composition from the November tallow (see Chapter 4). It may have been better to use one of these mid-season tallows and the November tallow to study the effect of tallow composition as the difference in composition would have been greater. However at the time that these experiments were commenced, the November 1976 and May 1977 mutton tallows were the only ones available in sufficient quantity.

In a fold-over design, the main effects are confounded with three-factor and higher-order interactions. It is possible that the apparent significance of a main effect was actually due to one of these higher-order interactions.

5.5.5 Interative and Quadratic Effects

While significant chains of two-factor interactions were found for three of the four response variables, there is a possibility that some of the two-factor interactions in other chains may have been significant. It is conceivable that a significant two-factor interaction could have been cancelled out by another significant two-factor interaction, of similar magnitude but opposite sign, in the same chain.

In each of the regression equations where significant chains of two-factor interactions were discovered, there was at least one interaction in each of the significant chains between two main effects which were also shown to be significant in that equation. This does not necessarily imply that these were the terms causing the interactive chain to appear significant, however. Some of the other independent variables in the chain which did not have a significant main effect may have had a significant interactive effect.

The design used cannot predict quadratic effects as each of the variables are examined at only two levels. It is possible that some of the variables which were not shown to significantly affect one or more of the response variables may

have had quadratic terms which would have appeared significant if the independent variables were examined at more levels. In the example in Figure 5.10, examination of the response variable from a two-level design with the independent variable set at the -1 and 1 levels only would have predicted that the independent variable did not influence the response, however there is a latent quadratic effect.

Thus it is possible that some of the variables which have not appeared significant in these experiments may have significant quadratic effects which would influence the response if the independent variables were set at different levels in later experiments.

5.5.6 Choosing Variables for Future Experimentation

The aim of the next stage of experimentation was to examine in more detail the effect of those variables shown from the screening experiment to significantly affect the yields of the fractions produced and the melting properties of the intermediate fraction.

T_1, T_2, S_1, S_2, W and A were all shown to significantly affect the melting properties of the intermediate fraction, and all except A have been shown to also affect the yield of one or more of the fractions. Hence all of these variables were considered worthy of further study.

The variable t was shown to affect only the yield of the intermediate fraction, and was kept constant for the next stage of experimentation. For further experiments it was set at the upper level used in the screening experiment (60 minutes) as this produced the highest yield of the intermediate fraction.

While the main effect of M was not shown by this experiment to affect any of the response variables, the possibility of it having an effect in future experiments could not be ignored - this may occur either through interactive effects not shown to be significant in the screening experiment, through quadratic effects or when set at levels different from those chosen for the screening experiment.

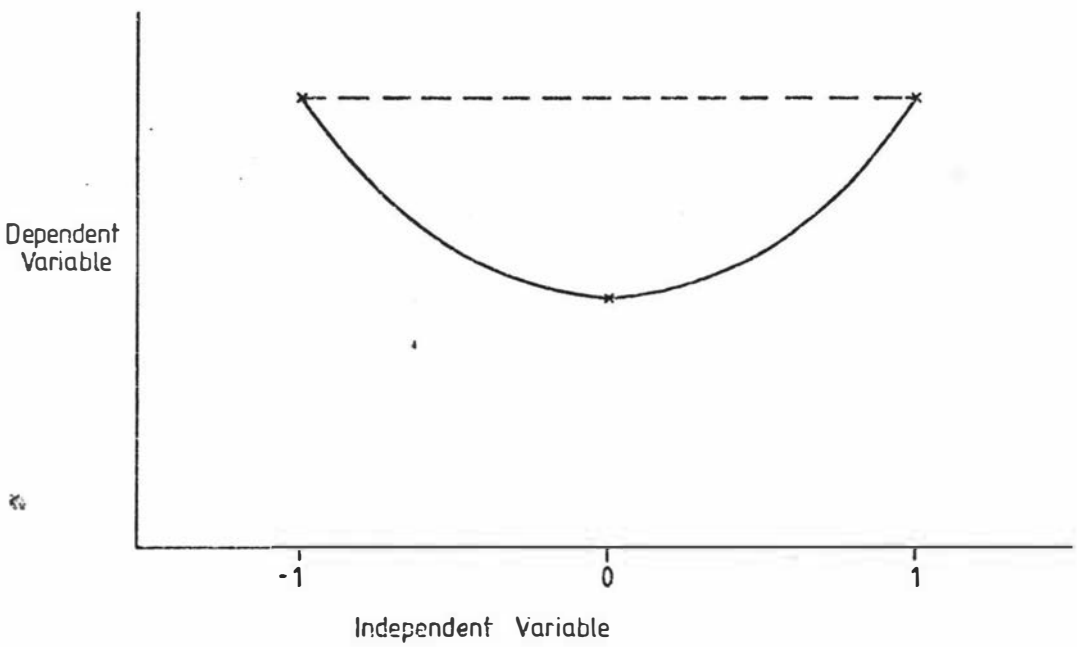


Figure 5·10 : Effect of fitting quadratic terms to a regression equation.

Thus in future experiments the tallow source was identical for all runs.

5.5.7 Conclusions from the Screening Experiment

(1) The first crystallisation temperature (T_1), the second crystallisation temperature (T_2), the solvent to fat ratio at the first crystallisation (S_1), the solvent to fat ratio at the second crystallisation (S_2), the water content of the acetone (W), the degree of agitation during crystallisation (A) and the ($T_1t + T_2S_1 + AM + S_2W$), ($T_1M + T_2S_2 + S_1W + At$) and ($T_1A + tM + S_1S_2 + T_2W$) chains of two-factor interactions were shown to significantly affect the melting properties of the intermediate fraction.

(2) T_1, S_1, W and the ($T_1S_1 + T_2t + WM + S_2A$), ($T_1M + T_2S_2 + S_1W + At$) and ($T_1W + S_1M + T_2A + S_2t$) chains of two-factor interactions were shown to significantly affect the yield of the hard fraction.

(3) T_1, T_2, S_2, W and t, and the ($T_1W + S_1M + T_2A + S_2t$) and ($T_1S_2 + T_2M + S_1A + Wt$) chains of two-factor interactions were shown to significantly affect the yield of the intermediate fraction.

(4) S_1 and W were found to significantly influence the yield of the soft fraction.

(5) The variables T_1, T_2, S_1, S_2, W and A were chosen for more detailed study of their influence upon the response variables.

CHAPTER 6

OPTIMISING THE MELTING PROPERTIES OF THE INTERMEDIATE FRACTION

6.1 INTRODUCTION

This experiment was designed to further investigate the effect of the six variables which the screening experiment showed to affect the yield of the three fractions and the CBLF value of the intermediate fraction, and hence to produce an intermediate fraction with a very low CBLF value (i.e. with melting properties similar to cocoa butter).

A mathematical model was fitted for each of the four response variables to predict the crystallisation scheme's response to changing levels of the six independent variables. The CBLF model was optimised to produce a fraction with a low CBLF value.

6.2 FRACTIONATION METHOD

The fractionations were carried out as for the screening experiment except that 20 g of tallow were used, and the fat solutions were held in the constant temperature room for 60 minutes before filtering.

The November 1976 Smithfield mutton tallow was found to have a high level of 2-oleo disaturated triglyceries (20.5%) so a large quantity of November 1977 Smithfield mutton tallow was obtained for these experiments. The bulk tallow was melted before sampling.

6.3 EXPERIMENTAL DESIGN

It was felt that a model of at least second order would be required to adequately represent the crystallisation scheme. To fit a second order response surface, each variable must be examined at three levels at least. A full factorial 3^k design would have required, in this case, $3^6 (= 729)$ experimental runs (Myers, 1971). Box and Wilson (1951) have devised a workable alternative to the 3^k factorial system with the development of composite designs, a special case of which are the central composite designs. These are first order factorial designs

augmented by additional "star" or "axial" points which allow estimation of the coefficients of a second order surface. The choice of value of the "star" points determines the properties of the experimental design.

Central composite designs are composed of three sets of points (Myers, 1971):

(i) the cube points (n_c) at $x_i = \pm 1$. Six independent variables were considered and a design consisting of a half-replicate 2^6 was chosen, giving thirty-two cube points ($n_c = 32$).

(ii) the star or axial points (n_a) are located two per axis at a distance $\pm \alpha$ from the origin. With six dependent variables, six axes are required and therefore $n_a = 12$. To achieve rotatability, $\alpha^4 = n_c$, so a rotatable half-replicate 2^6 central composite design has $\alpha = \sqrt[4]{32} = 2.378$;

(iii) the centre points (n_o) located at the origin. To achieve orthogonality,

$$n_o + n_a = \frac{4\alpha^2(n_c + \alpha^2)}{n_c}$$

$$n_o = 14.62 \simeq 15$$

Hence to achieve the properties of rotatability and near-orthogonality the design required N_T total runs, where

$$N_T = n_c + n_o + n_a = 59$$

See Table 6.2 for the design layout. Such a design has the benefits of economy, orthogonality and rotatability while estimating the second order effects (Davies, 1978). No main effect or two-factor interaction is confounded with any other main effect or two-factor interaction. Effects higher than second order cannot be estimated due to confounding.

Orthogonality means that the design has an X'X matrix where all terms off the leading diagonal are equal to zero, providing an ease of computation and uncorrelated estimates of the response model coefficients.

Rotatability means that the variance of the estimated response is a function only of the distance from the centre of

the design and not of the direction.

6.4 LEVELS OF THE INDEPENDENT VARIABLES

The central composite design required each variable to be set at five levels; -2.378, -1, 0, 1 and 2.378 in coded terms.

From consideration of the levels of the independent variables used in the screening experiment and their effect upon the dependent variables, and from the literature survey, the following levels of the independent variables were chosen (see Table 6.1).

Table 6.1: Levels of the independent variables

	CODED LEVEL				
SYMBOL	-2.378	-1	0	+1	+2.378
T_1 (^oC)	9.2	12	14	16	18.8
T_2 (^oC)	1.2	4	6	8	10.8
S_1	1.6	3	4	5	6.4
S_2	1.6	3	4	5	6.4
W (%)	0.15	0.7	1.1	1.5	2.05
A (volts)	146.22	160	170	180	193.78

Table 6.2: The experimental design and the responses of the dependent variables

RUN	T_1	T_2	S_1	S_2	W	A	YIELD OF HARD FRACTION (WT % OF TALLOW)	YIELD OF INTERMEDIATE FRACTION (WT % OF TALLOW)	YIELD OF SOFT FRACTION (WT % OF TALLOW)	CBLF OF INTERMEDIATE FRACTION
1	+1	+1	+1	+1	+1	+1	12.5	7.0	80.5	15.5
2	-1	+1	+1	+1	+1	-1	23.0	3.0	74.0	14.0
3	+1	-1	+1	+1	+1	-1	21.0	18.5	60.5	18.0
4	-1	-1	+1	+1	+1	+1	26.5	15.5	58.0	17.0
5	+1	+1	-1	+1	+1	-1	17.0	16.0	67.0	17.0
6	-1	+1	-1	+1	+1	+1	41.0	2.5	56.5	18.0
7	+1	-1	-1	+1	+1	+1	14.0	18.0	68.0	14.0
8	-1	-1	-1	+1	+1	-1	41.5	15.5	43.0	14.0
9	+1	+1	+1	-1	+1	-1	13.0	12.5	74.5	21.0
10	-1	+1	+1	-1	+1	+1	31.0	0.1	68.9	19.0
11	+1	-1	+1	-1	+1	+1	13.5	27.5	59.0	18.0
12	-1	-1	+1	-1	+1	-1	32.0	13.5	54.5	20.0
13	+1	+1	-1	-1	+1	+1	27.0	5.0	68.0	16.5
14	-1	+1	-1	-1	+1	-1	39.5	1.0	59.5	12.5
15	+1	-1	-1	-1	+1	-1	15.0	11.5	73.5	14.0
16	-1	-1	-1	-1	+1	+1	27.5	11.0	61.5	20.5
17	+1	+1	+1	+1	-1	-1	24.5	6.0	69.5	23.5
18	-1	+1	+1	+1	-1	+1	21.0	2.0	77.0	19.0
19	+1	-1	+1	+1	-1	+1	4.5	6.5	89.0	21.5
20	-1	-1	+1	+1	-1	-1	20.0	15.0	65.0	14.5
21	+1	+1	-1	+1	-1	+1	10.5	6.5	83.0	22.5
22	-1	+1	-1	+1	-1	-1	20.5	1.0	78.5	13.0

TABLE 6.2 (continued)

RUN	T_1	T_2	S_1	S_2	W	A	YIELD OF HARD FRACTION (WT % OF TALLOW)	YIELD OF INTERMEDIATE (WT % OF TALLOW)	YIELD OF SOFT FRACTION (WT % OF TALLOW)	CBLF OF INTER-MEDIATE FRACTION
23	+1	-1	-1	+1	-1	-1	24.0	10.5	65.5	15.0
24	-1	-1	-1	+1	-1	+1	26.5	2.0	71.5	14.0
25	+1	+1	+1	-1	-1	+1	18.5	12.5	69.0	24.0
26	-1	+1	+1	-1	-1	-1	11.0	2.0	87.0	15.0
27	+1	-1	+1	-1	-1	-1	9.5	31.0	59.5	20.0
28	-1	-1	+1	-1	-1	+1	17.5	24.5	58.0	18.0
29	+1	+1	-1	-1	-1	-1	16.0	20.5	63.5	22.5
30	-1	+1	-1	-1	-1	+1	24.0	0.1	75.9	21.0
31	+1	-1	-1	-1	-1	+1	22.5	20.5	57.0	17.0
32	-1	-1	-1	-1	-1	-1	25.5	9.0	65.5	13.0
33	-2.378	0	0	0	0	0	38.5	0.3	61.2	11.0
34	2.378	0	0	0	0	0	7.5	16.0	76.5	19.0
35	0	-2.378	0	0	0	0	19.0	19.0	62.0	26.5
36	0	2.378	0	0	0	0	20.0	1.5	78.5	25.0
37	0	0	-2.378	0	0	0	25.0	2.0	73.0	16.5
38	0	0	2.378	0	0	0	27.5	6.5	66.0	19.5
39	0	0	0	-2.378	0	0	19.5	7.5	73.0	18.0
40	0	0	0	2.378	0	0	17.5	8.5	74.0	16.5
41	0	0	0	0	-2.378	0	15.0	11.0	74.0	24.5
42	0	0	0	0	2.378	0	39.5	20.0	40.5	22.0
43	0	0	0	0	0	-2.378	27.0	7.3	65.7	13.0
44	0	0	0	0	0	2.378	9.5	13.5	77.0	20.5

CONTINUED

Table 6.2: (continued)

RUN	T_1	T_2	S_1	S_2	W	A	YIELD OF HARD FRACTION (WT % OF TALLOW)	YIELD OF INTERMEDIATE FRACTION (WT % OF TALLOW)	YIELD OF SOFT FRACTION (WT % OF TALLOW	CBLF OF INTERMEDIATE FRACTION
45	0	0	0	0	0	0	14.0	15.5	70.5	17.0
46	0	0	0	0	0	0	11.5	13.5	75.0	21.0
47	0	0	0	0	0	0	21.5	11.0	67.5	16.5
48	0	0	0	0	0	0	25.0	14.5	60.5	13.5
49	0	0	0	0	0	0	20.5	5.5	74.0	17.0
50	0	0	0	0	0	0	25.0	7.0	68.0	18.0
51	0	0	0	0	0	0	22.5	15.5	62.0	17.0
52	0	0	0	0	0	0	19.5	12.5	68.0	17.5
53	0	0	0	0	0	0	14.0	7.5	78.5	18.5
54	0	0	0	0	0	0	21.5	4.5	74.0	16.5
55	0	0	0	0	0	0	20.5	5.0	74.5	16.5
56	0	0	0	0	0	0	18.5	4.0	77.5	15.5
57	0	0	0	0	0	0	23.0	10.0	67.0	14.0
58	0	0	0	0	0	0	19.0	7.5	73.5	17.0
59	0	0	0	0	0	0	28.5	7.5	64.0	13.0

6.5 RESULTS

The measured yields of each of the three fractions and the CBLF of the intermediate fractions are presented in Table 6.2.

6.6 EMPIRICAL EQUATIONS RELATING DEPENDENT AND INDEPENDENT VARIABLES

An empirical mathematical model was fitted to each of the dependent variables by multiple regression, using Minitab, of each of the coded linear, quadratic and first order interaction terms.

The computer print-out for each regression contained a regression coefficient for each variable, the standard deviation of each coefficient and the Student's t-statistic of each coefficient. Coefficients with t-statistics not significant at the 5% level were discarded and only those with significant t-statistics contributed to the final model.

The models developed for each of the response variables are presented in Table 6.3.

The adequacy of fit of each of the models to the observed data was assessed using the F-ratio between the lack-of-fit mean square and the experimental error mean square.

Thus,

$$F_{n_1,n_2} = \frac{\text{mean sum of squares due to lack of fit}}{\text{mean sum of squares due to pure error}}$$

$$= \frac{MS_{lof}}{MS_{pe}}$$

Table 6.3: Fitted models for each of the response variables

Yield of the hard fraction:

$$Y_H \text{ (\% by wt of tallow)} = 21.37 - 5.51(T_1) - 2.01(S_1) + 3.63(W) - 2.91(T_1W)$$

Yield of the intermediate fraction:

$$Y_I \text{ (\% by wt of tallow)} = 10.20 + 3.46(T_1) - 4.48(T_2) + 1.32(S_1) - 1.25(S_2) + 1.27(W^2) - 1.48(T_1S_2) - 1.92(T_2S_1) - 1.36(S_1S_2) + 2.64(S_2W)$$

Yield of the soft fraction:

$$Y_S \text{ (\% by wt of tallow)} = 68.43 + 2.06(T_1) + 4.22(T_2) - 4.32(W) - 2.41(W^2) + 2.07(T_1S_2) + 3.05(S_1W) - 2.36(S_2W) + 2.52(S_2A)$$

CBLF of the intermediate fraction:

$$CBLF = 17.69 + 1.31(T_1) + 0.51(T_2) + 0.94(S_1) - 0.58(S_2) - 0.76(W) + 1.07(A) - 0.54(T_1^2) + 1.36(T_2^2) + 1.01(W^2) + 0.77(T_1T_2) - 1.23(T_1W) - 1.02(T_1A) - 0.55(T_2S_1) - 0.92(T_2W)$$

If this ratio was not significant, the conclusion was that the errors about the fitted model (lack-of-fit) were of the same order of magnitude as those accounted for by error of observation (experimental error) and the model was considered an adequate representation of the data (Myers, 1971).

The MS_{pe} for each of the models was calculated by considering the variation between observations at the replicated centre-points. This was achieved by performing a one-way analysis of variance upon the data for each of the four response variables.

Tests for goodness-of-fit of each of the models are presented in Tables A5.1 to A5.4, Appendix 5, along with the regression statistics for each model.

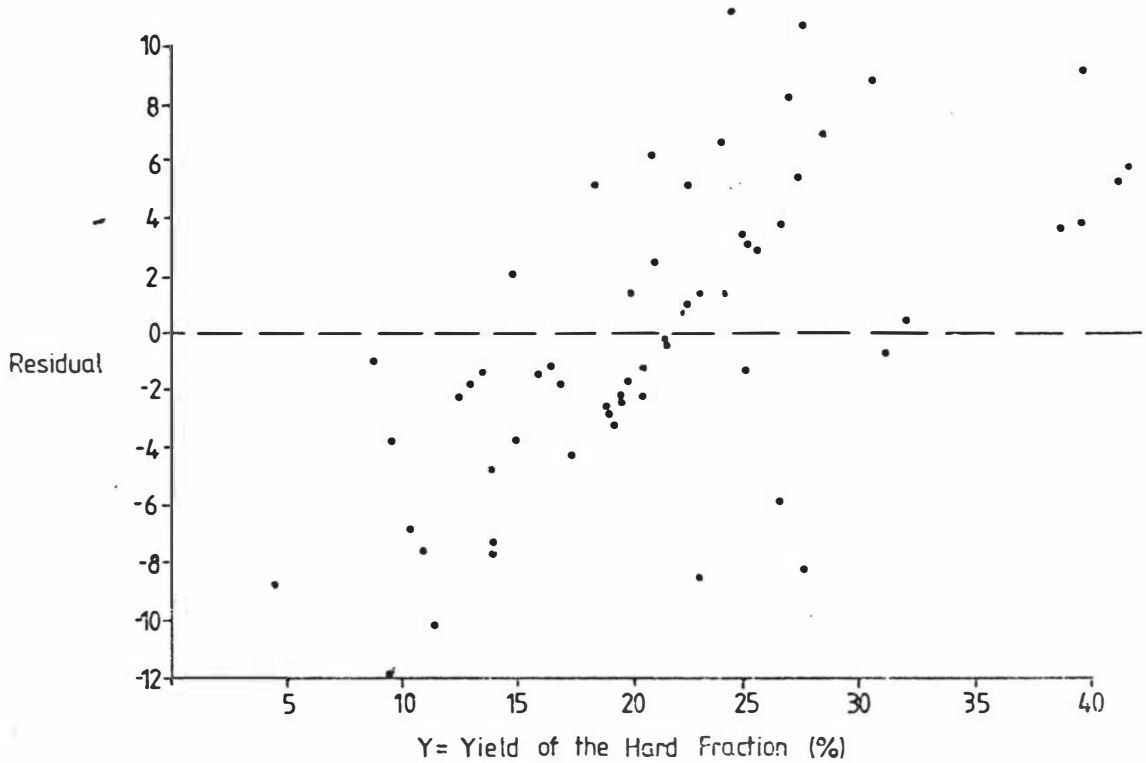


Figure 6·1 : Plot of residual vs Y for the yield of hard fraction model.

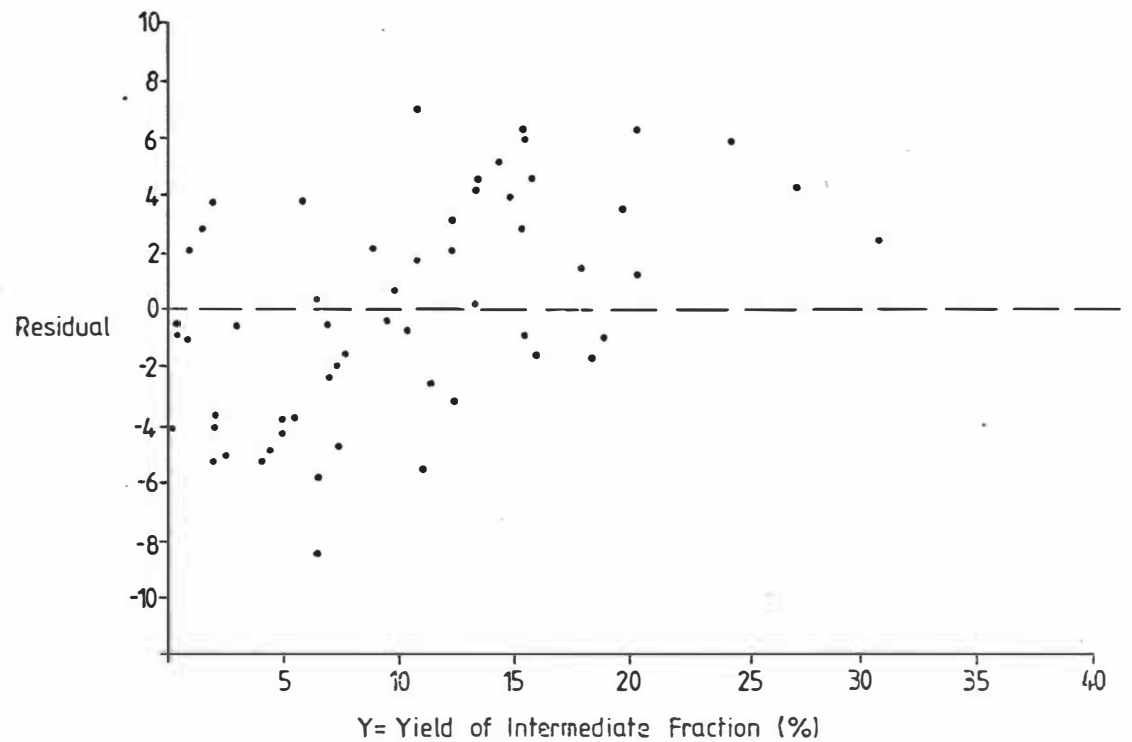


Figure 6·2 : Plot of residual vs. Y for the yield of intermediate fraction model.

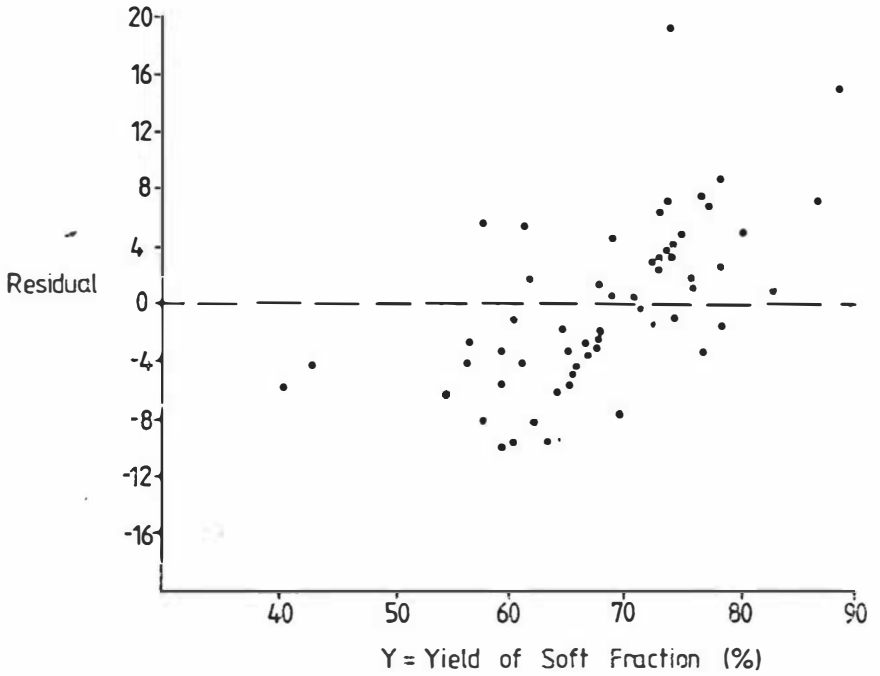


Figure 6·3 : Plot of residual vs. Y for the yield of soft fraction model.

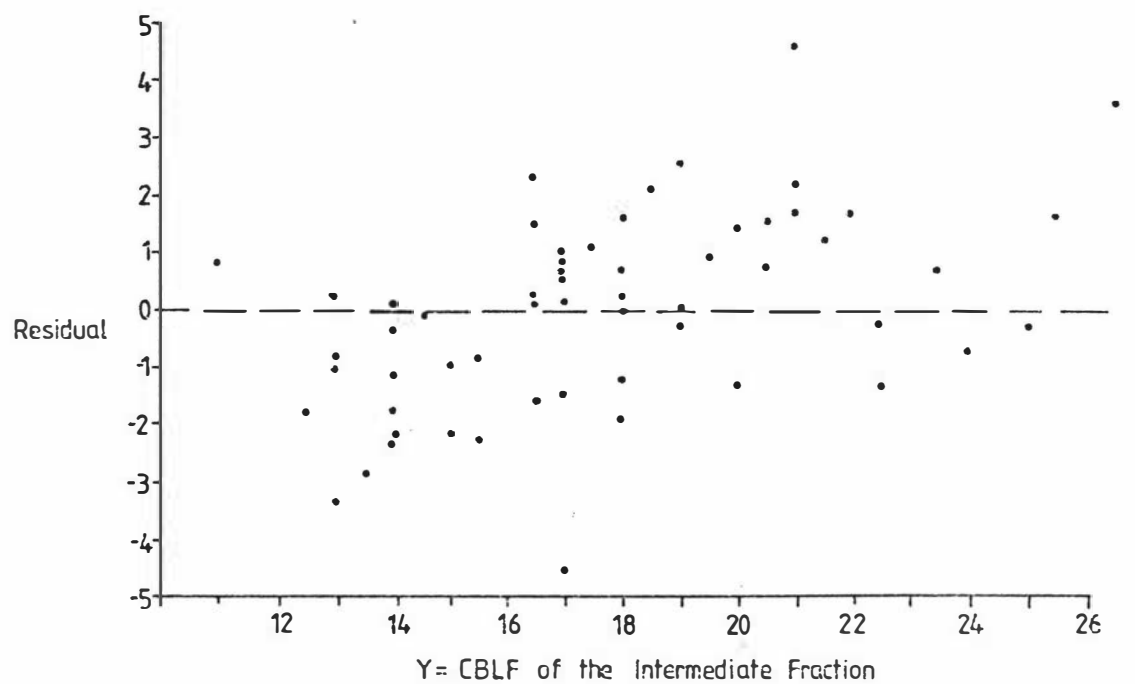


Figure 6·4 : Plot of residual vs. Y for the CBLF of the intermediate fraction model.

For each model, the F-ratio of MS_{lof} to MS_{pe} was not significant at the 5% level, so it was concluded that the models all adequately represented the observed data.

Residual plots for each of the four models are presented in Figures 6.1 to 6.4. In each case, the points appear to be randomly distributed.

6.7 DECODING THE MODELS

The above equations all predict the response values using the coded values of the independent variables. These equations can be manipulated to produce models which give the predicted response using uncoded independent variables (e.g. temperature in ^{o}C). Decoded models are generally easier to apply in practice.

The decoding was performed as outlined by Belz (1973) using Table XXIII of Fisher and Yates (1963).

The decoded models are given in Table 6.4.

Table 6.4: Decoded models for each of the response variables

Yield of the Hard Fraction:

$$Y_H = 2.07 + 1.24(T_1) - 2.01(S_1) + 59.93(W) - 3.63(T_1W)$$

Yield of the Intermediate Fraction:

$$Y_I = -49.39 + 4.69(T_1) + 1.60(T_2) + 12.52(S_1) + 7.29(S_2) - 43.86(W) + 7.94(W^2) - 0.74(T_1S_2) - 0.96(T_2S_1) - 1.36(S_1S_2) + 6.60(S_2W)$$

Yield of the Soft Fraction:

$$Y_S = 276.40 - 3.11(T_1) + 2.11(T_2) - 8.39(S_1) - 51.52(S_2) + 15.44(W) - 1.02(A) - 15.06(W^2) + 1.04(T_1S_2) + 7.63(S_1W) - 5.90(S_2W) + 0.26(S_2A)$$

CBLF of the Intermediate Fraction:

$$CBLF = -161.25 + 13.62(T_1) - 4.15(T_2) + 2.58(S_1) - 0.58(S_2) + 12.78(W) + 0.82(A) - 0.14(T_1^2) + 0.34(T_2^2) + 6.29(W^2) + 0.19(T_1T_2) - 1.54(T_1W) - 0.05(T_1A) - 0.27(T_2S_1) - 1.15(T_2W)$$

6.8 INTERPRETATION OF THE MODELS

6.8.1 Isolated Effect of the Variables

The simplest manner of interpreting the results was to examine the effect of one variable at a time. This was achieved by plotting the isolated effect of the independent variables on the dependent variables (see Figures 6.5 to 6.10). Such plots are also useful in visually assessing the adequacy of fit of the model to the data (McFarlane, 1979).

This presentation represents only a small proportion of the total information available from the model - it does not take into account any interactive effects in the models though some of these have been shown to be significant. Changes in the levels of the other independent variables occurring at the same time as the change in the isolated variable will affect the observed response and therefore it may differ from the predicted response.

By calculating the predicted response from the models for the variables at their various levels considered one at a time, and plotting this in figures 6.5 to 6.10, a visualisation of the degree of fit of the models was obtained.

An example calculation for the derivation of these effects is shown in Table A5.5, Appendix 5.

Figure 6.5 presents the isolated effect of T_1 on the yields of the three fractions and the CBLF of the intermediate fraction. In each case, good agreement was shown between the observed and the predicted data. The intermediate fraction with the best melting characteristics (CBLF = 11.0) was produced with T_1 set at its lowest level (9.2°C). This gave a yield of 39% hard fraction, 60% soft fraction and a very low yield of intermediate fraction. (see experiment 33).

The highest yield of intermediate fraction (16%) was obtained with T_1 set at its highest level.

The isolated effect of T_2 upon three response variables, as T_2 obviously cannot affect the yield of hard fraction, is

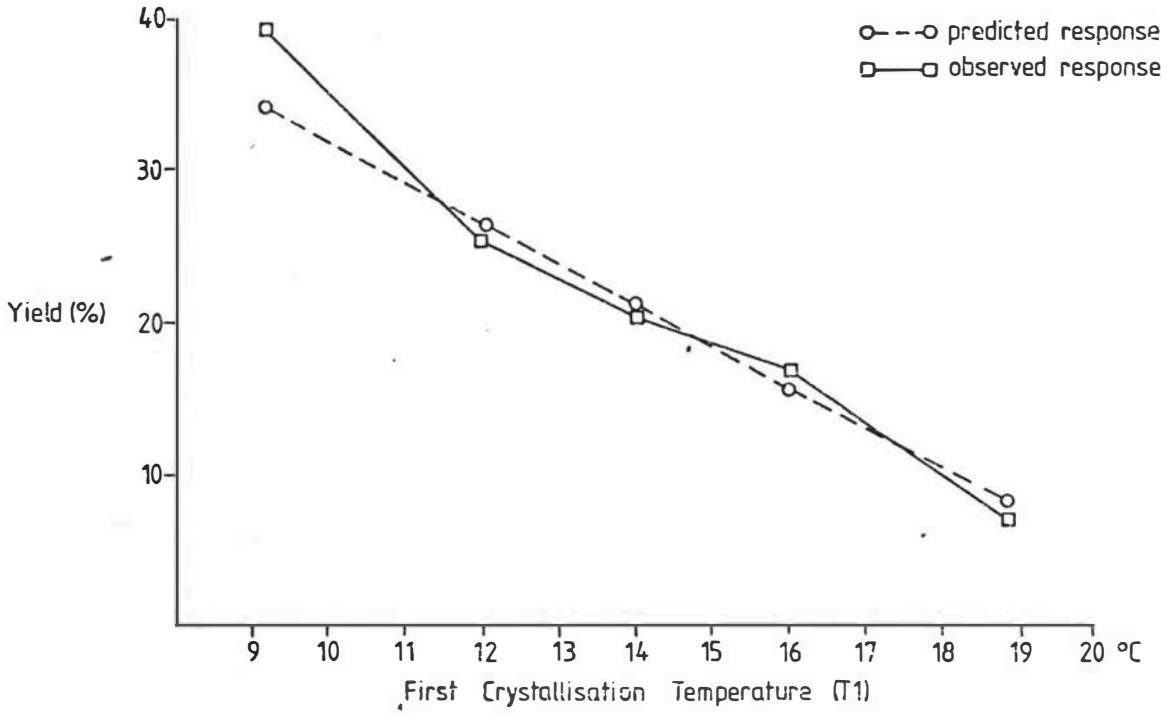
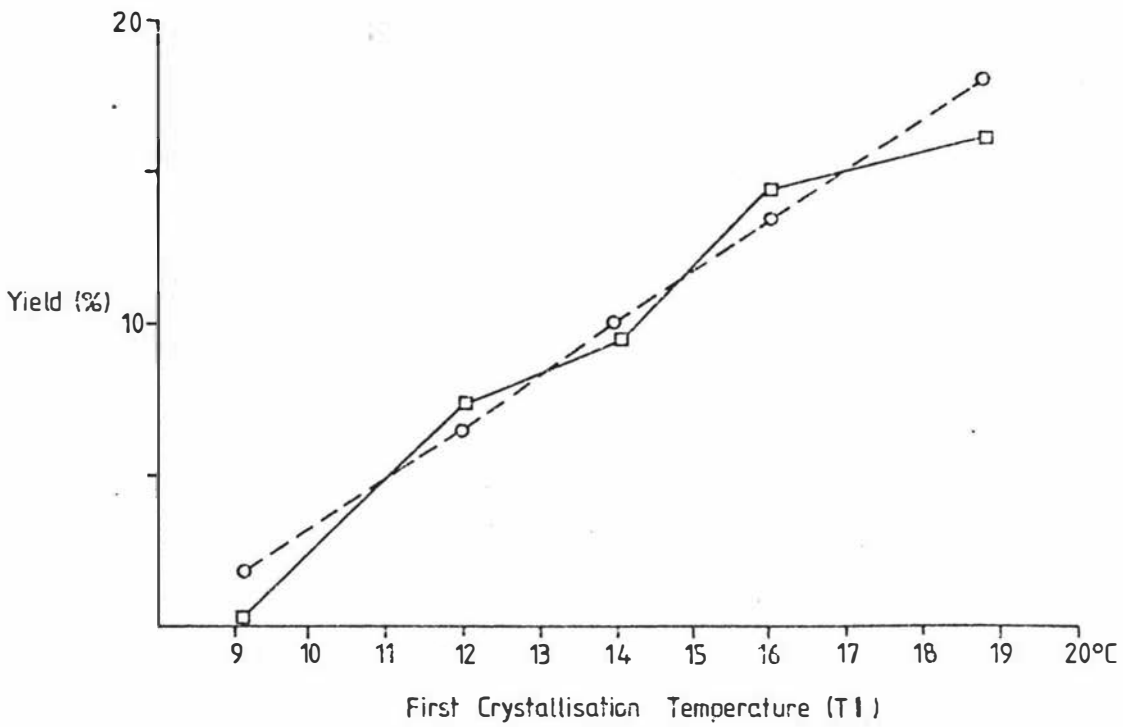
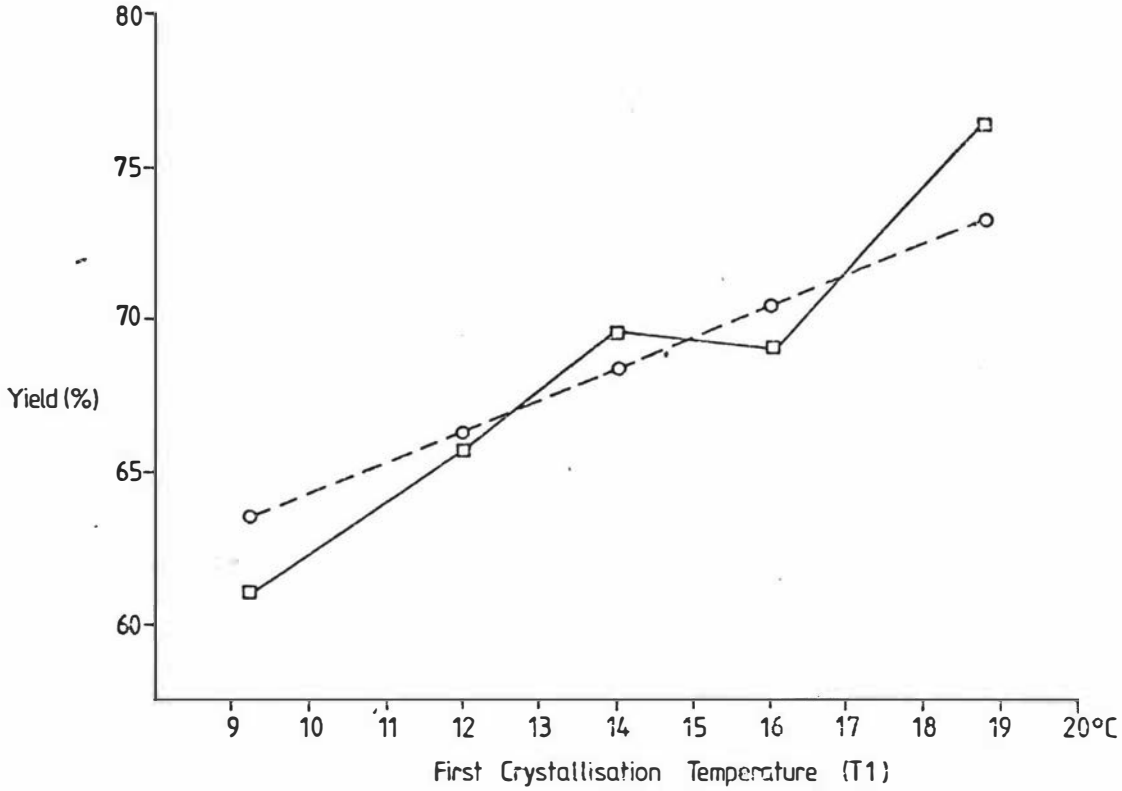


Figure 6·5 : The isolated effect of T1 on the dependent variables.

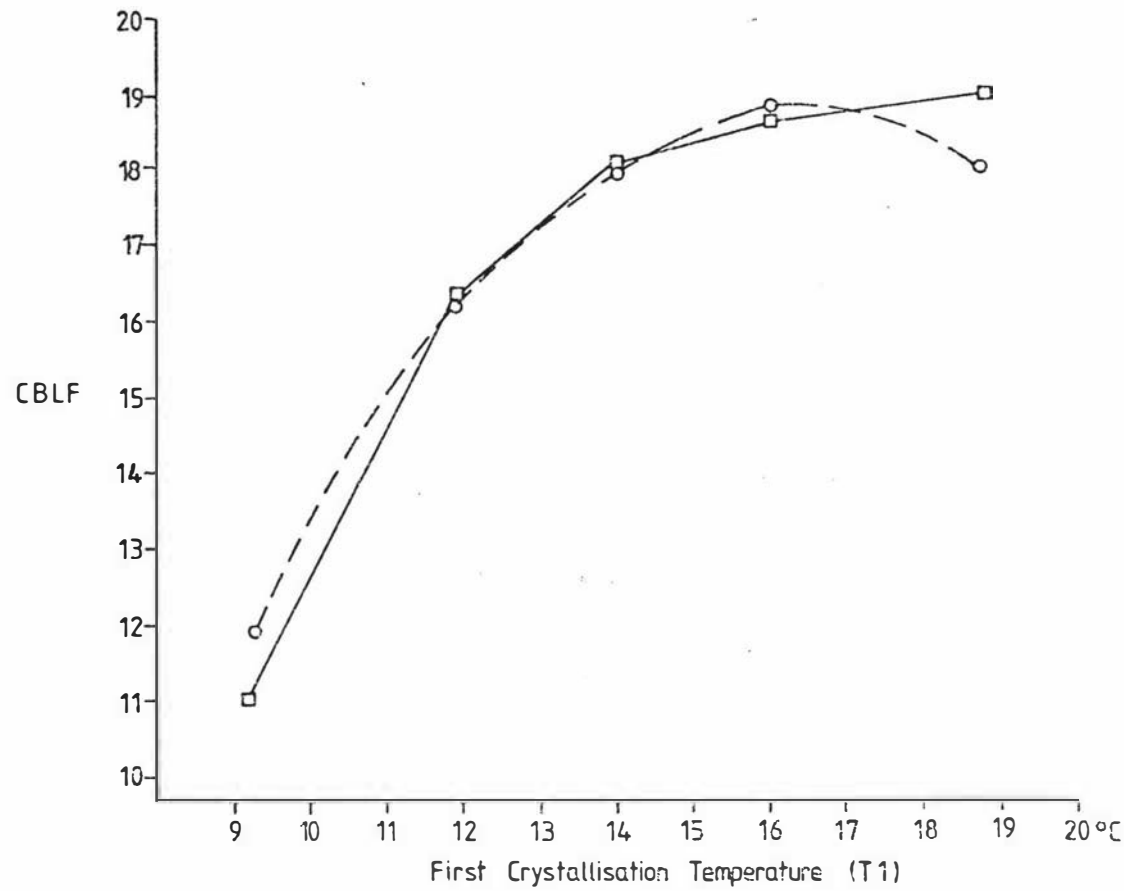
(a) Yield of the hard fraction.



(b) Yield of the intermediate fraction.



(c) Yield of the soft fraction.



(d) CBLF of the intermediate fraction.

shown in Figure 6.6. Again, good agreement between the observed and predicted responses was obtained. The best CBLF value of the intermediate fraction (CBLF = 16.5) was obtained with T_2 set at about 4 to 6°C. T_2 at this level gave average yields of about 11% and 68% of the intermediate and soft fractions respectively. The highest yield of intermediate fraction (19%) was obtained with T_2 set at its lowest level (1.6°C).

The isolated effect of S_1 upon the dependent variables is shown in Figure 6.7. The predicted response curve for the yield of the hard fraction fails to follow the observed increase in the yield of this fraction at the highest S_1 value. Similarly, the predicted curve for the yield of the intermediate fraction fails to follow the observed decrease in the yield of the intermediate fraction at the extreme S_1 levels. The fitted model for the yield of the soft fraction contains no main or quadratic effects for S_1, and hence the predicted response curve for the isolated effect of S_1 upon the yield of the soft fraction is an invariate line through the average yield. There appears to be poor agreement with the observed data at some levels (see Figure 6.7 c). However, the expanded ordinate scale in this plot exaggerates these discrepancies. The best CBLF value (16.5) was obtained with S_1 set at its lowest level (1.6:1). The hard, intermediate and soft fractions produced with S_1 = 1.6:1 had yields of 25%, 2% and 73% respectively. The best yield of intermediate fraction (13%) was obtained with S_1 set at 5:1.

The observed and predicted response curves for the isolated effect of S_2 on each of the three response variables are shown in Figure 6.8. The coded model for the yield of the soft fraction shows no main or quadratic effects for S_2, and hence the predicted curve for the isolated effect of S_2 upon the yield of the soft fraction is an invariate line through the average values. This fails to account for the high observed yield of soft fraction at the extreme S_2 levels, but the broad ordinate scale exaggerates the difference. There is good agreement between the observed and predicted curves for the

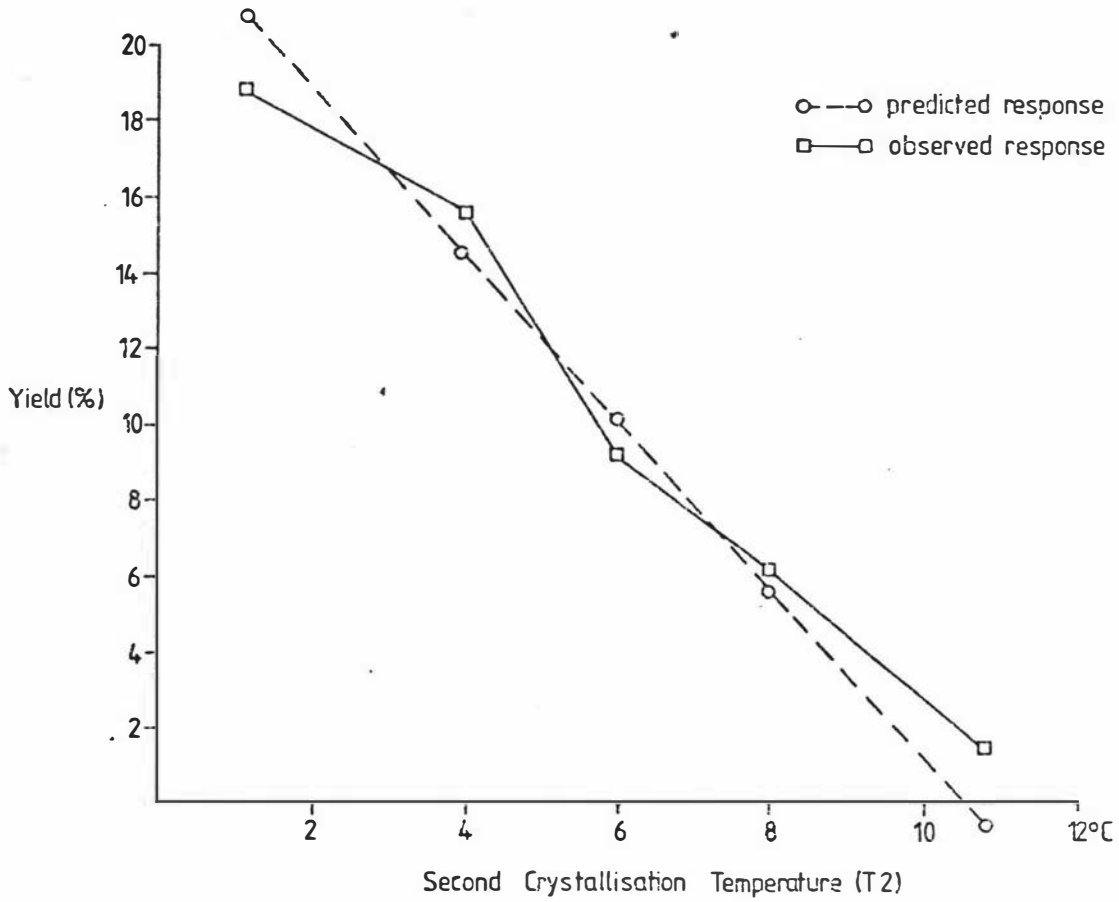
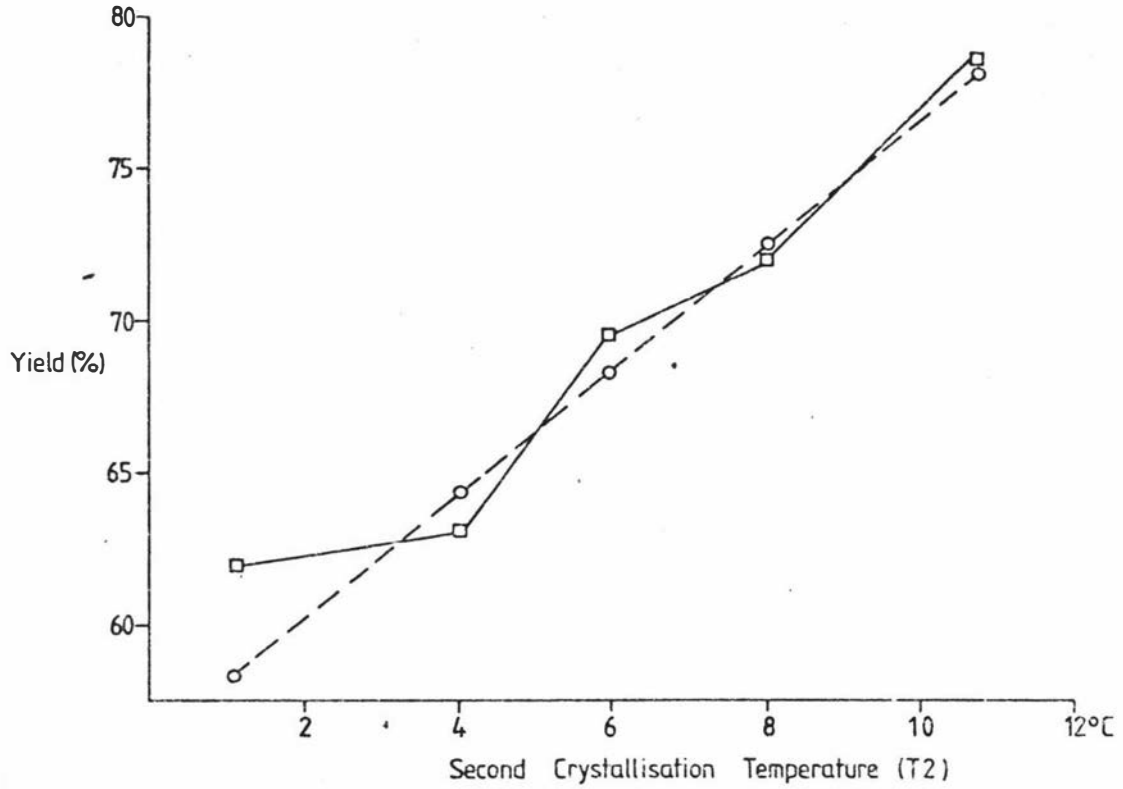
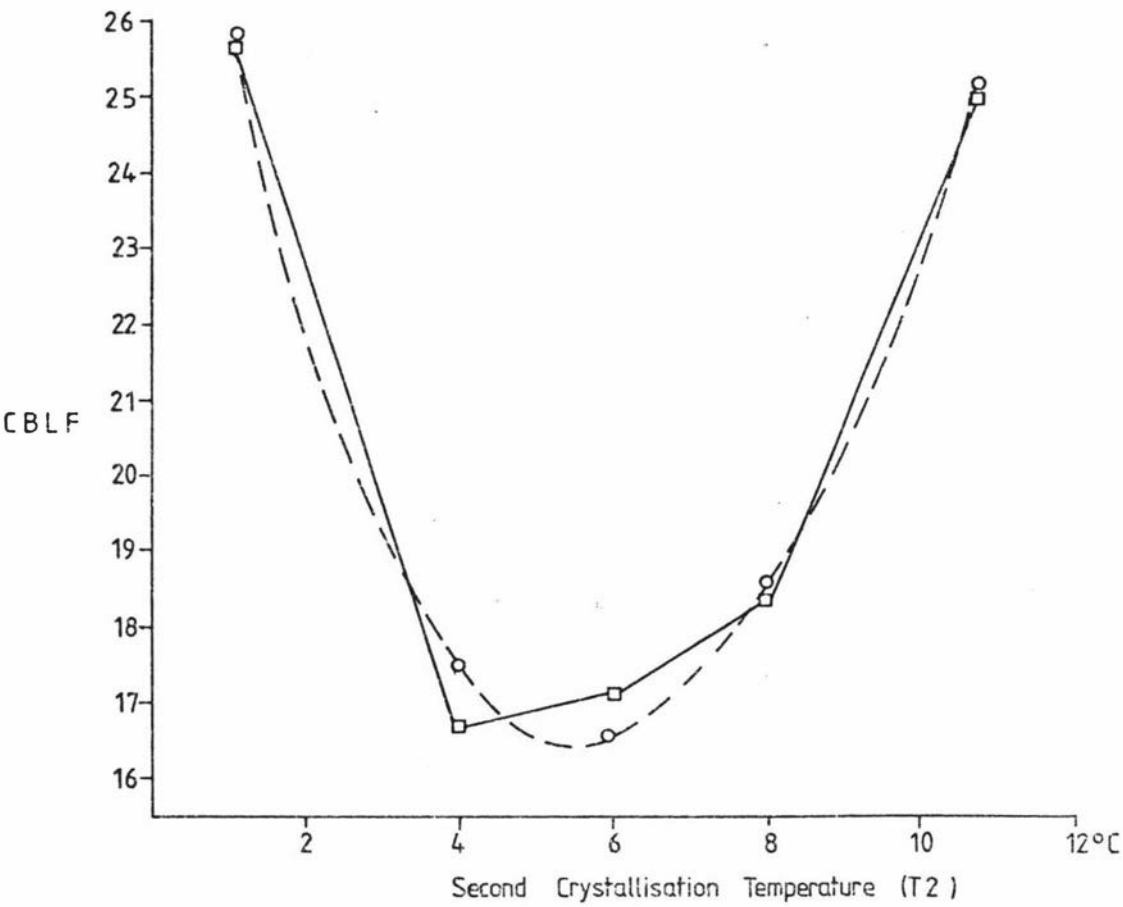


Figure 6·6 : The isolated effect of T2 on the dependent variables.
(a) Yield of the intermediate fraction



(b) Yield of the soft fraction.



(c) CBLF of the intermediate fraction.

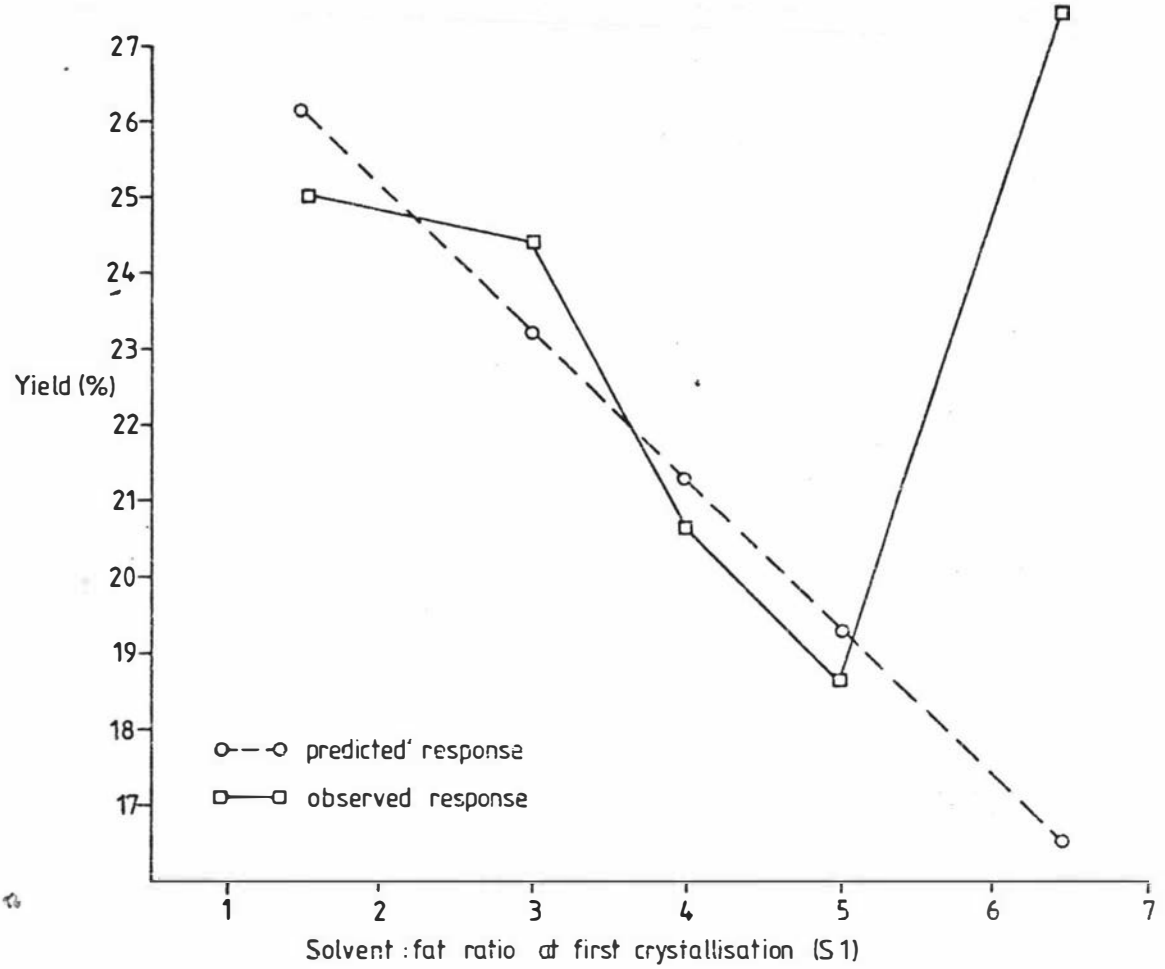
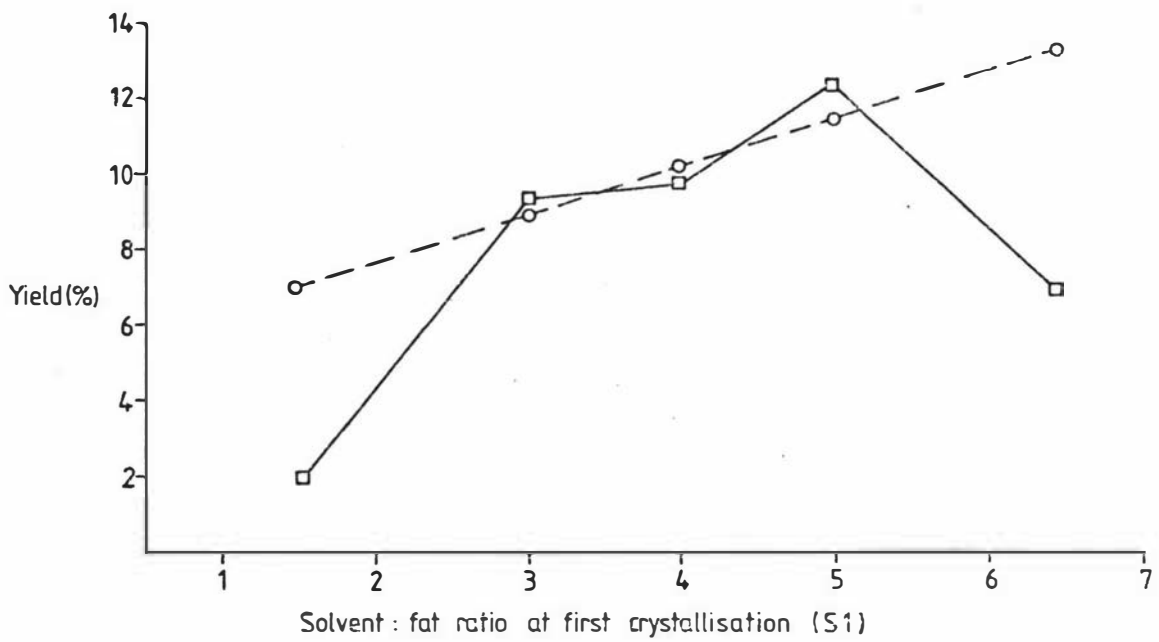
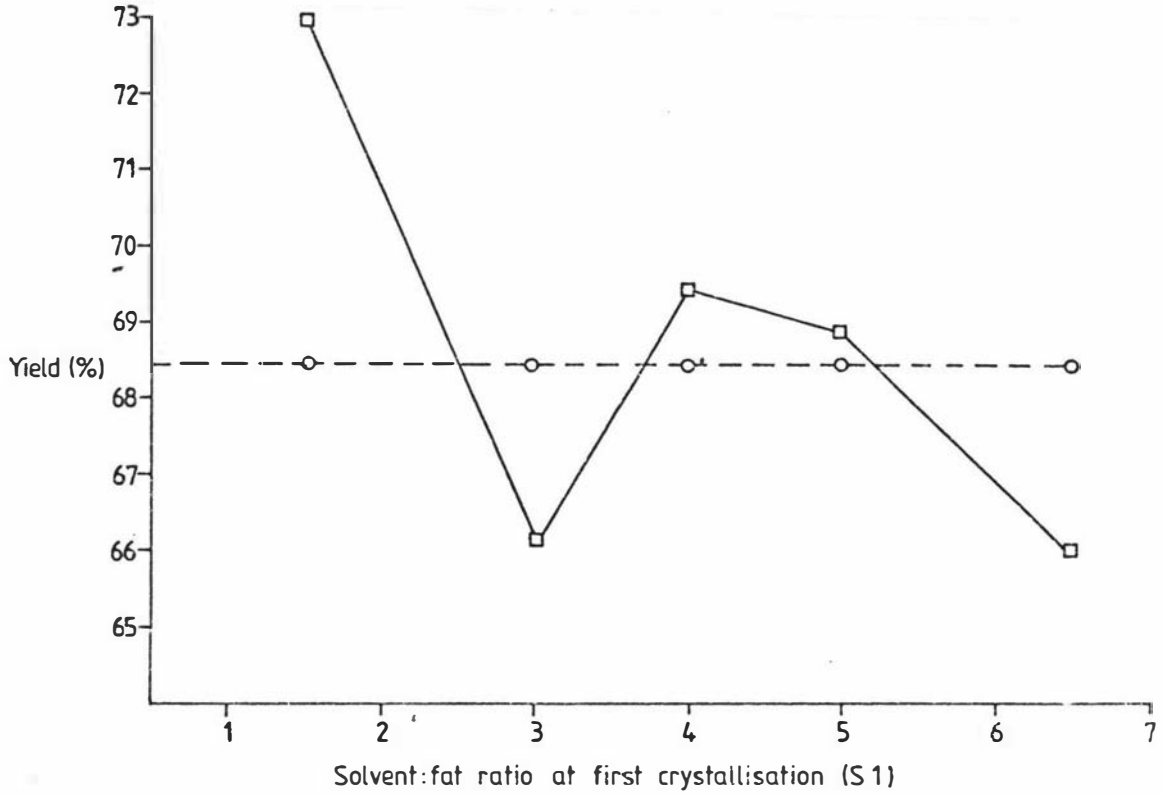


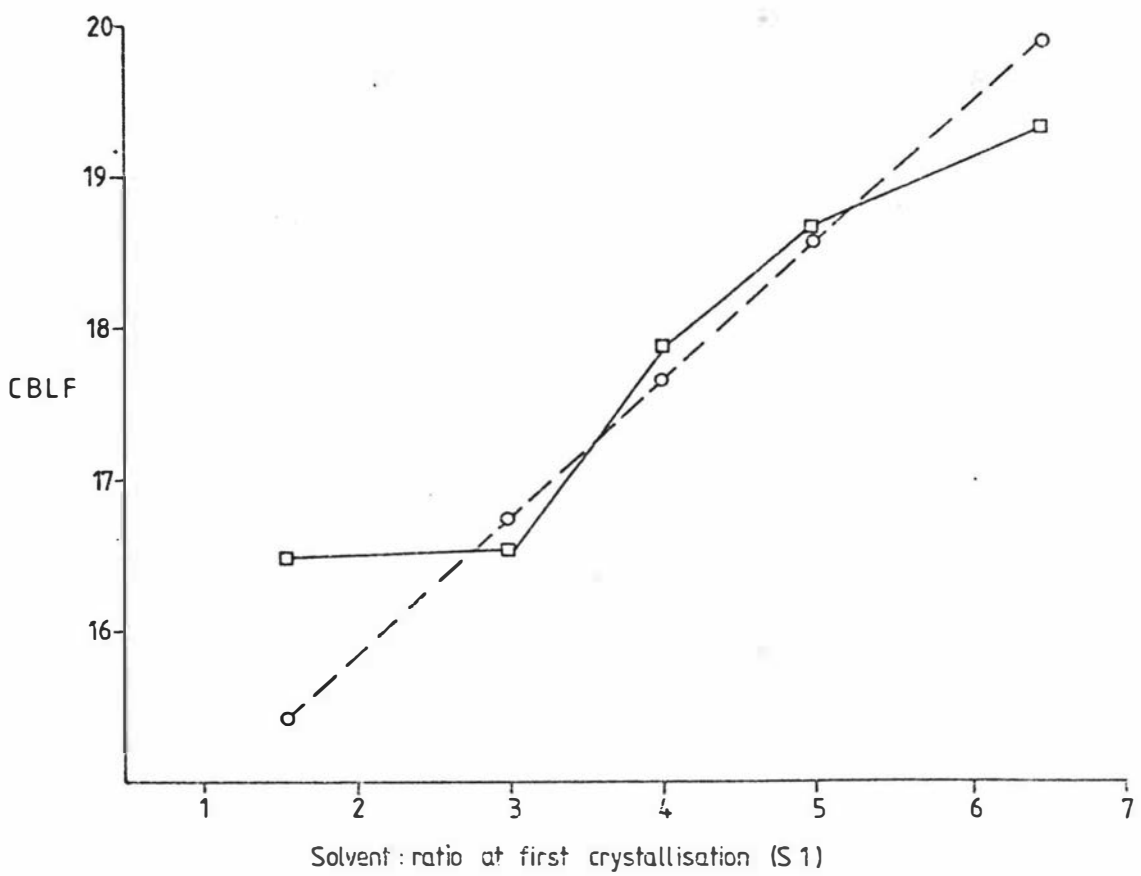
Figure 6·7 : The isolated effect of S1 on the dependent variables.
(a) Yield of the hard fraction.



(b) Yield of the intermediate fraction.



(c) Yield of the soft fraction.



(d) CBLF of the intermediate fraction.

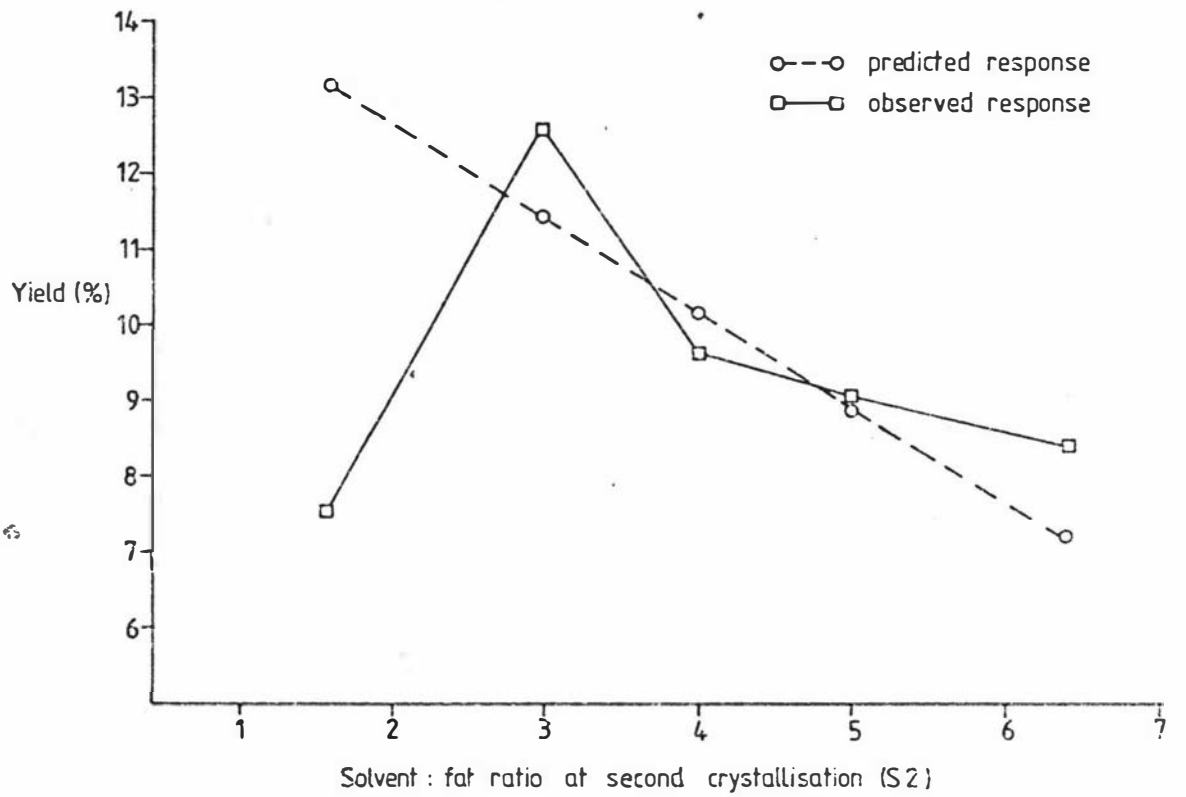
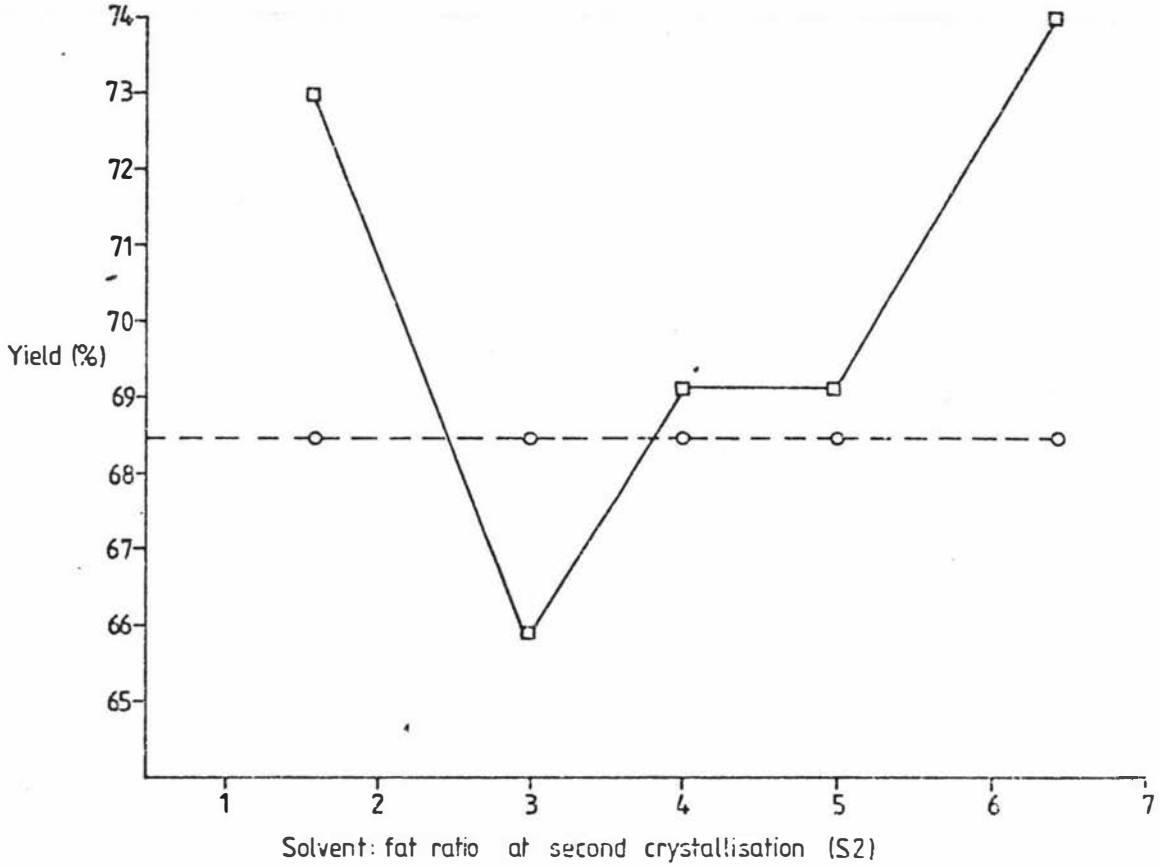
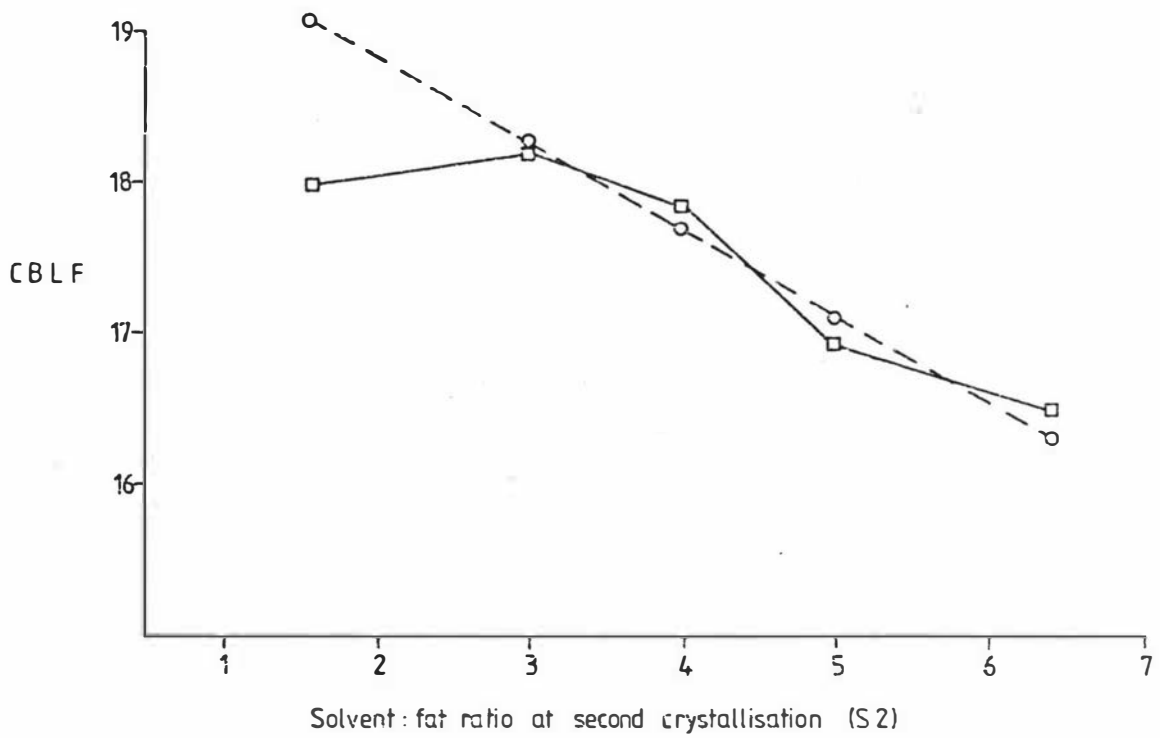


Figure 6·8: The isolated effect of S2 on the dependent variables.

(a) Yield of the intermediate fraction.



(b) Yield of the soft fraction.



(c) CBLF of the intermediate fraction.

isolated effect of S_2 upon the yield and CBLF of the intermediate fraction with the exception that the predicted curve for the yield of the intermediate fraction fails to follow the observed low value at the low S_2 value. The best-melting intermediate fraction (CBLF = 16.5) was obtained with S_2 at its highest level (6.4:1). S_2 at this level gave yields of the intermediate and soft fractions of 8.5% and 74% respectively. The highest yield of intermediate fraction (12.5%) was obtained when S_2 was set at 3:1.

The isolated effect of W is shown in Figure 6.9. There is good agreement between the observed and predicted curves for each of the response variables with the exception that the predicted curve for the yield of the intermediate fraction fails to allow for the low observed yield of the intermediate fraction at the low level of W. The best CBLF value (17.0) was obtained with W set at about 1.1%. W at this level gave average overall yields of the hard, intermediate and soft fractions of 20%, 9% and 71% respectively. The best yield of the intermediate fraction (20%) was obtained with a W value of 2.0%.

The fitted models for the yields of the three fractions do not show any significant main or quadratic effects for A. Figure 6.10 compares the observed and predicted curves for the isolated effect of A upon each of the response variables. There is poor agreement between the observed and predicted values of the yields of the hard and intermediate fractions at the extreme values of A. There is close similarity between the observed and predicted curves for the CBLF of the intermediate fraction, and also for the yield of the soft fraction, though the predicted curve for the yield of the soft fraction fails to account for the high observed yield at high agitation speed. The best CBLF value (13.0) was obtained at the lowest agitation speed. The yields of the three fractions at this agitation speed were 27%, 7% and 66% for the hard, intermediate and soft fractions respectively.

6.8.2 Consideration of the Combined Effect of the Six Independent Variables

In Section 6.8.1, the isolated effect of each of the independent variables upon each of the dependent variables was considered. This failed to account for the effects of interactions between the variables, even though the fitted

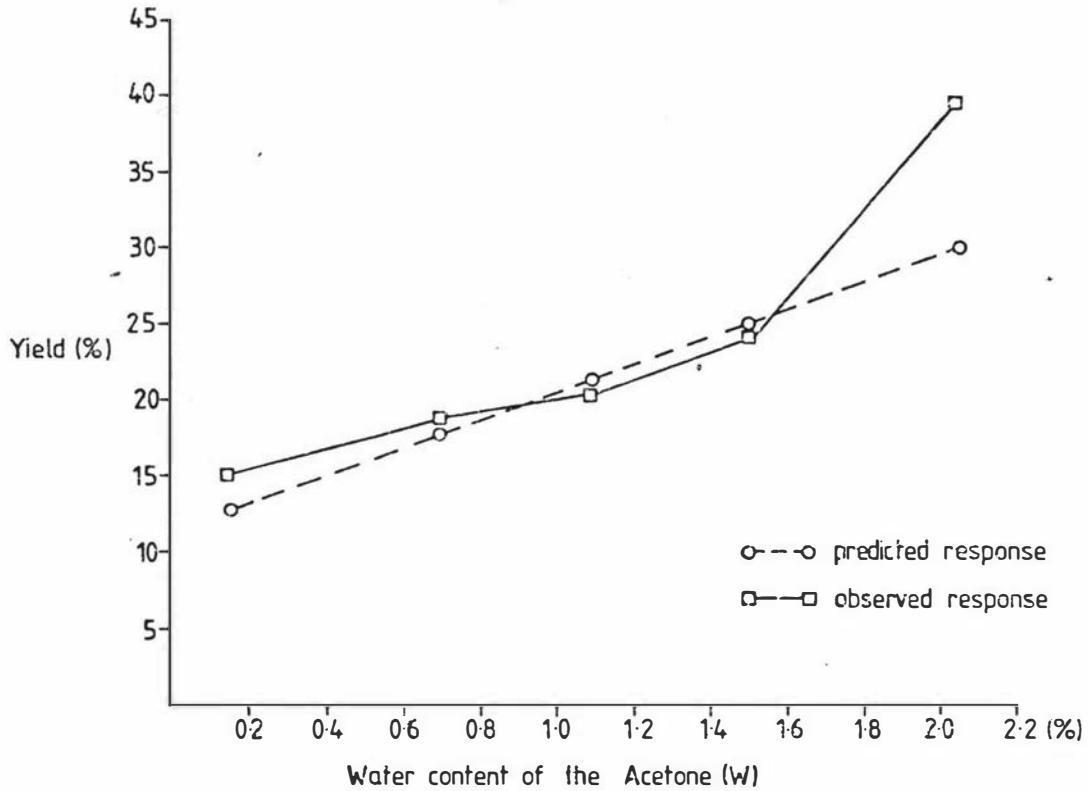
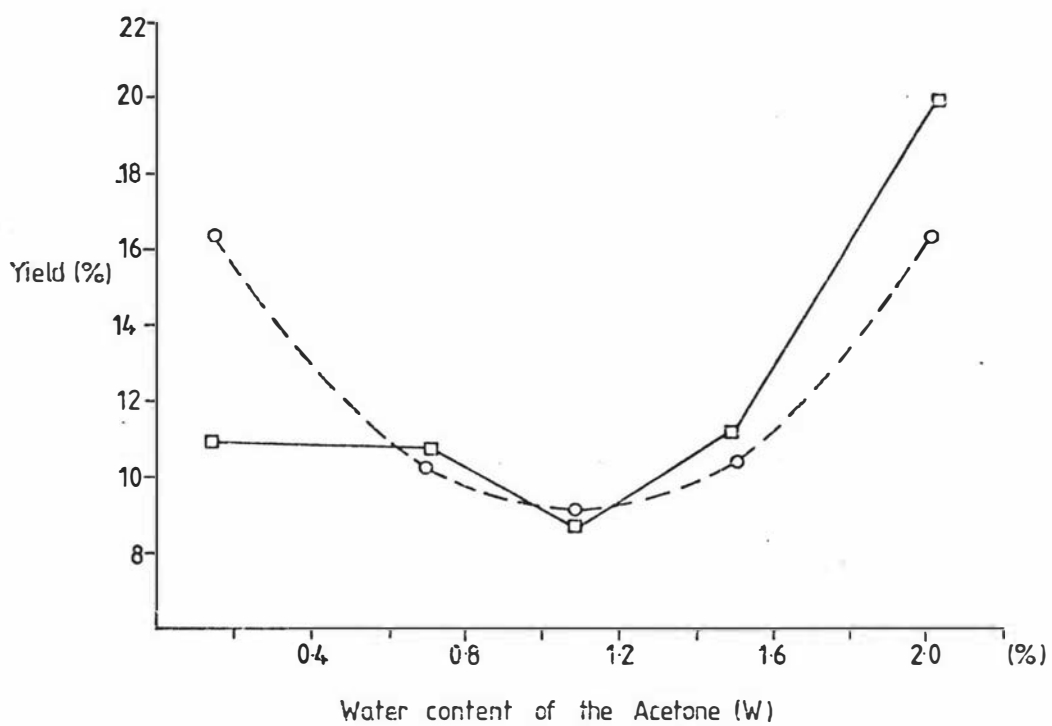
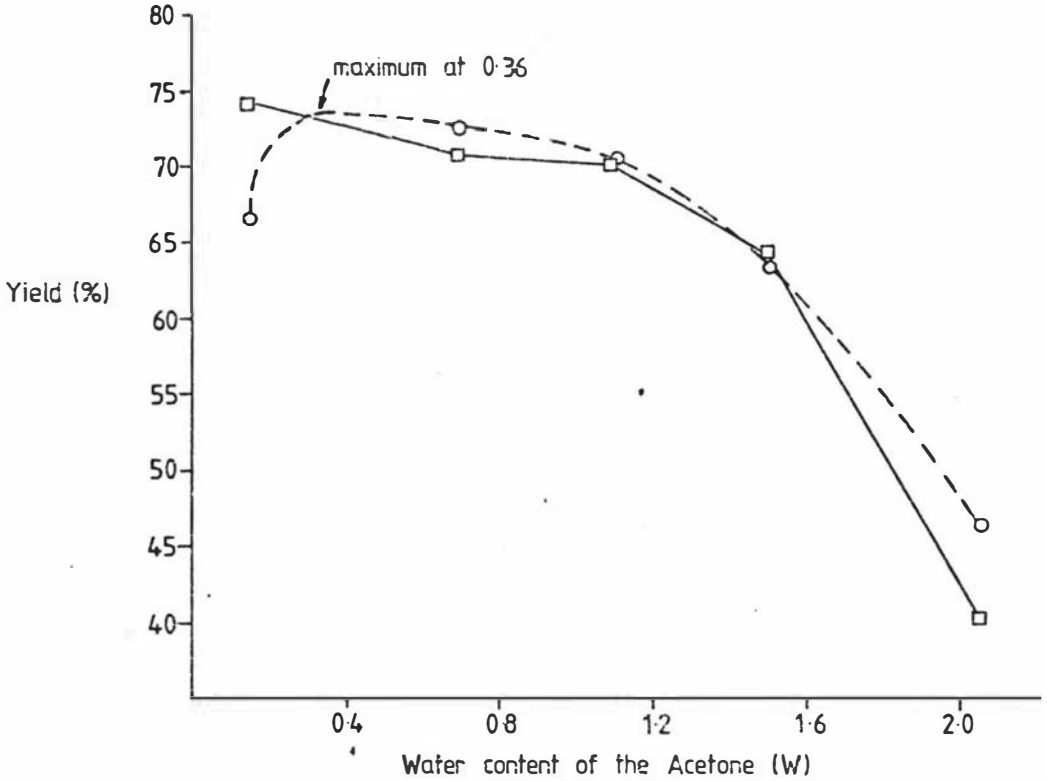


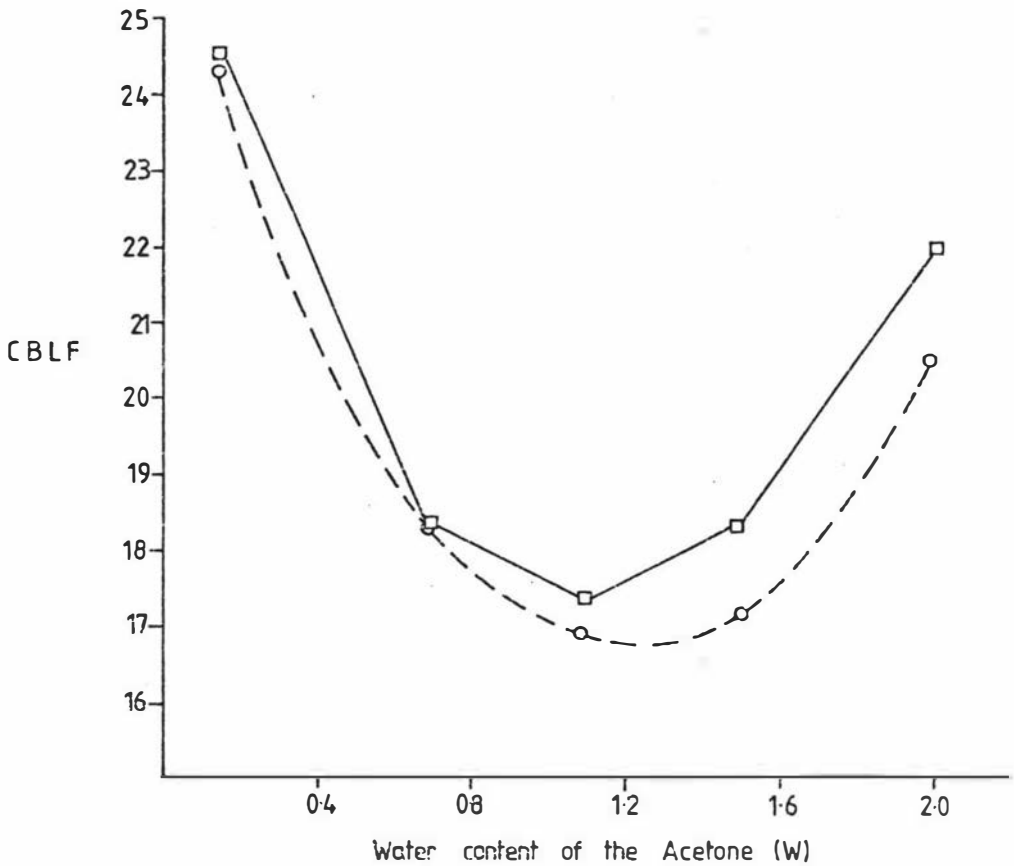
Figure 6·9 : The isolated effect of W on the dependent variables.
(a) Yield of the hard fraction.



(b) Yield of the intermediate fraction.



(c) Yield of the soft fraction.



(d) CBLF of the intermediate fraction.

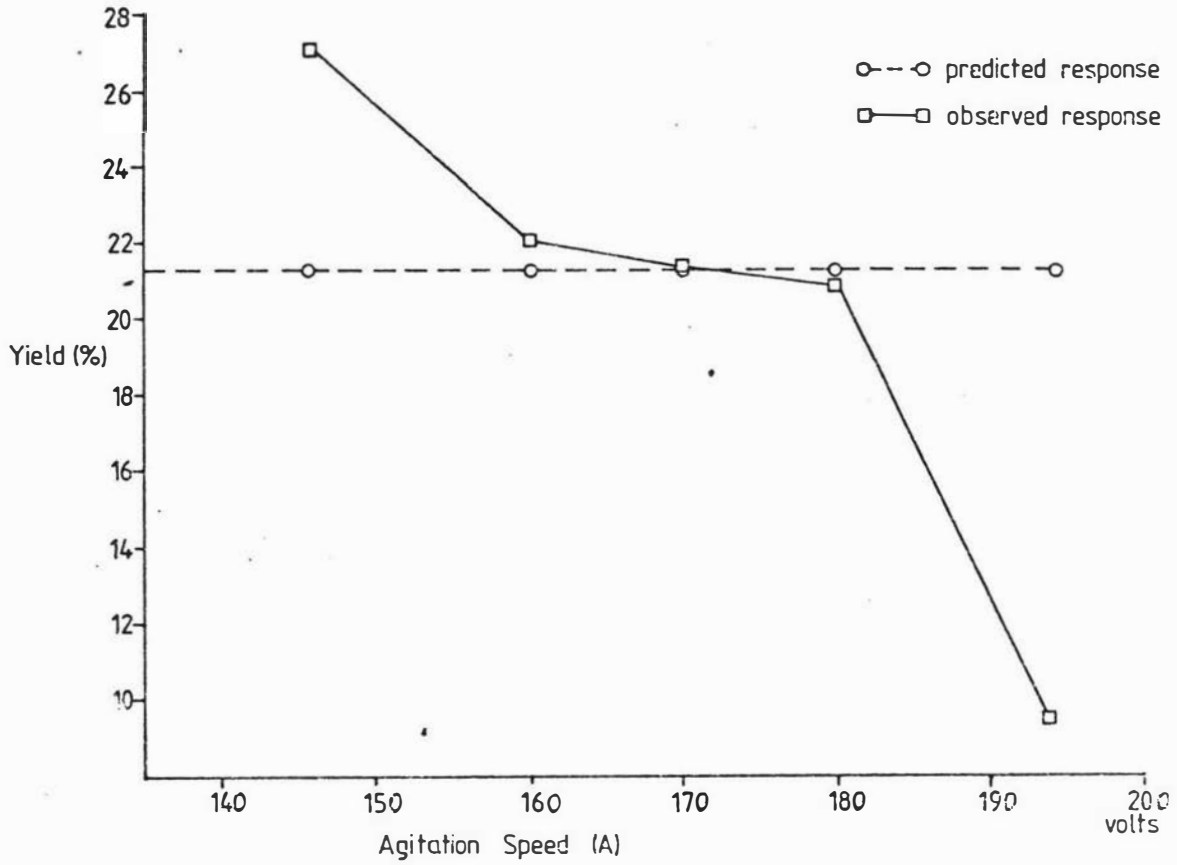
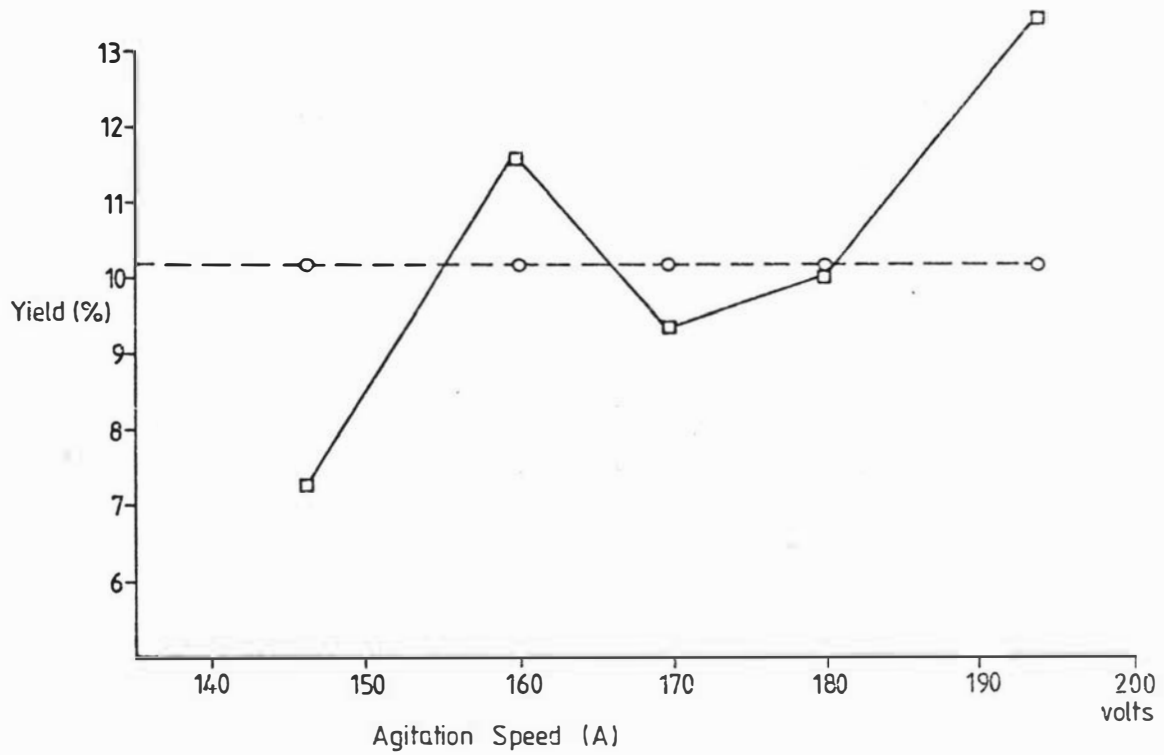
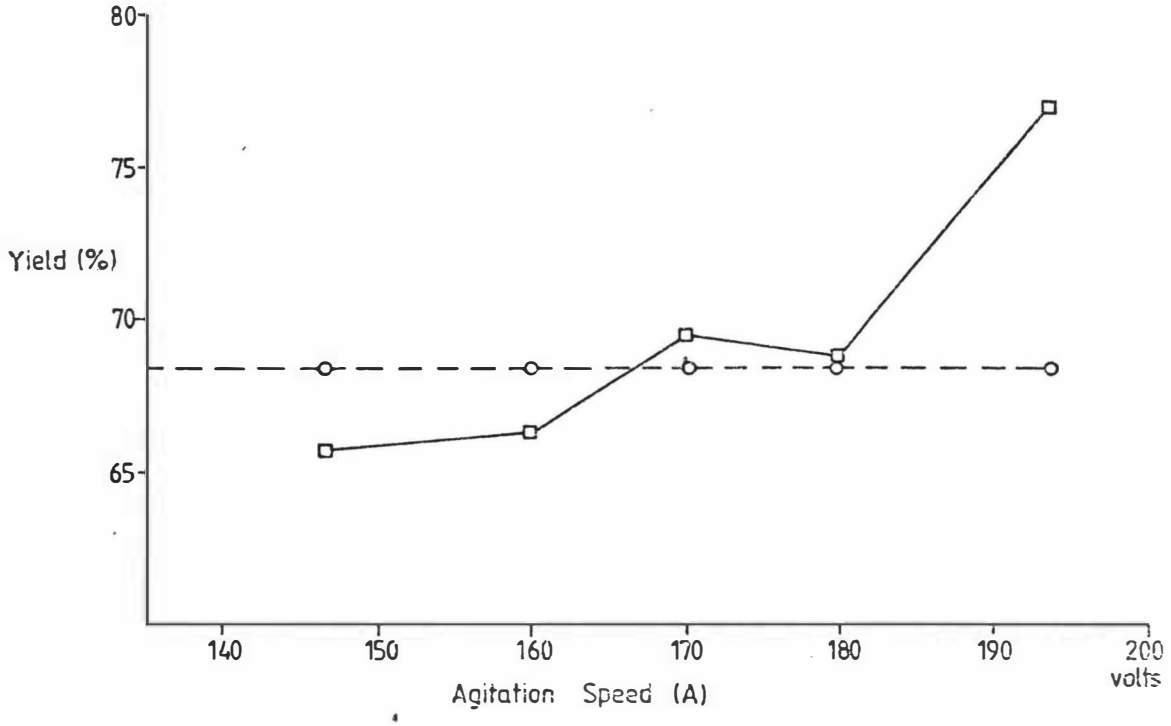


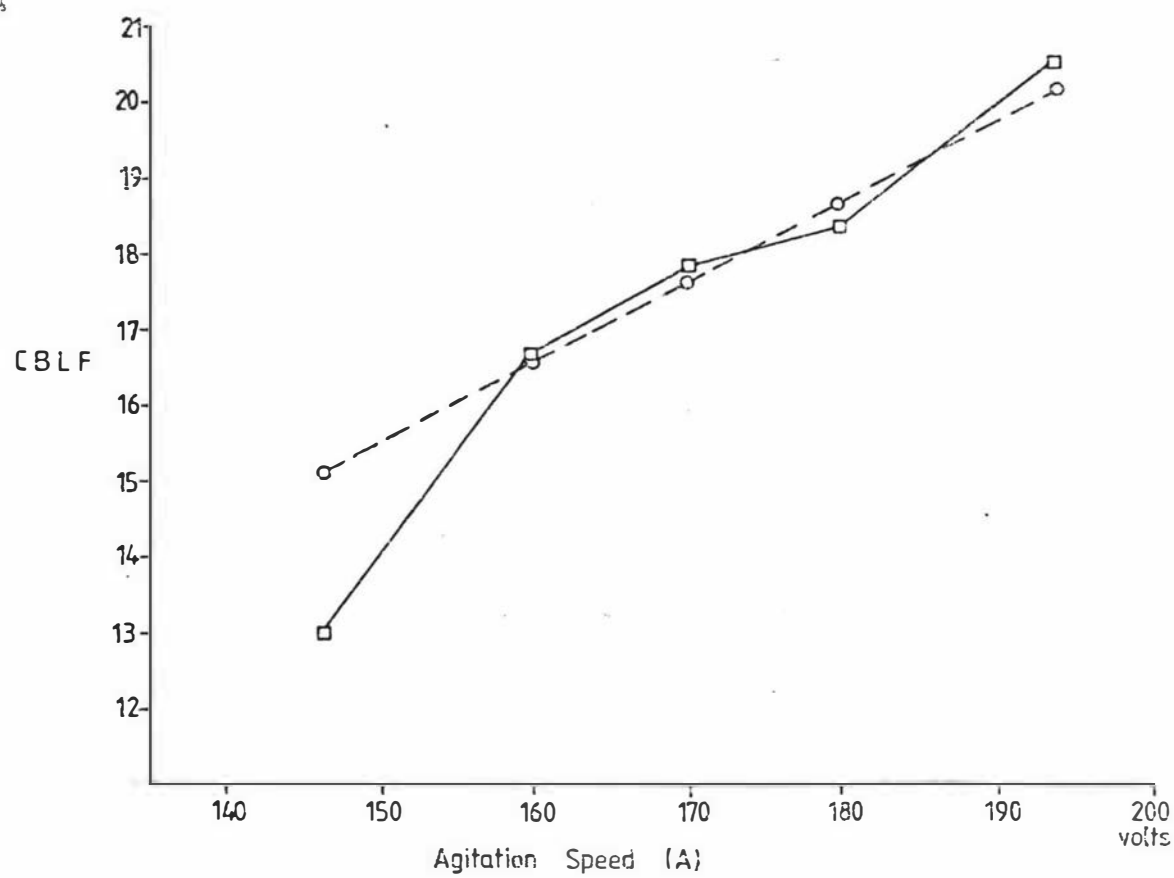
Figure 6·10 : The isolated effect of A on the dependent variables.
(a) Yield of the hard fraction.



(b) Yield of the intermediate fraction.



(c) Yield of the soft fraction.



(d) C B L F of the intermediate fraction.

models have shown some of these to be significant. Such interactions will affect the predicted optimum conditions, and hence to find the overall process optimum it was necessary to consider the combined effect of all six variables.

This was achieved by finding the overall optimum point on the response surface as predicted by the fitted empirical equations. The optimum area was deduced using a sequential search tehcnique for solving non-linear objective functions with the variables constrained (Rosenbrock and Storey, 1966; Box, Davies and Swann, 1969; Kuester and Mize, 1973).

The major aim of these experiments was to produce an intermediate fraction with a low CBLF value (i.e. with melting properties similar to those of cocoa butter). Thus the model for the CBLF of the intermediate fraction was minimised with each of the independent variables constrained within the extreme experimental limits used in these experiments. This procedure predicted the following optimum levels for the six independent variables:

$T_1 = 9.2^{o}C$, $T_2 = 5.2^{o}C$, $S_1 = 1.6:1$

$S_2 = 6.4:1$, $W = 0.6\%$, $A = 146.2$ volts

The optimum levels of T_1, S_1, S_2 and A are at the extreme values studied in these experiments, and have the same optimum values as was predicted by consideration of their isolated effects. However, the optimum T_2 value predicted above was $5.2^{o}C$, whereas the predicted optimum from consideration of the isolated effect of T_2 was $5.6^{o}C$. Similarly, study of the isolated effect of W predicted an optimum for the CBLF of the intermediate fraction with W set at 1.25%. However, consideration of interactive effects predicted an optimum CBLF value with W set at 0.6%.

A fractionation was performed under these predicted optimum conditions. This produced an intermediate fraction which melted at a slightly lower temperature and over a wider temperature range than cocoa butter (see Figure 6.11).

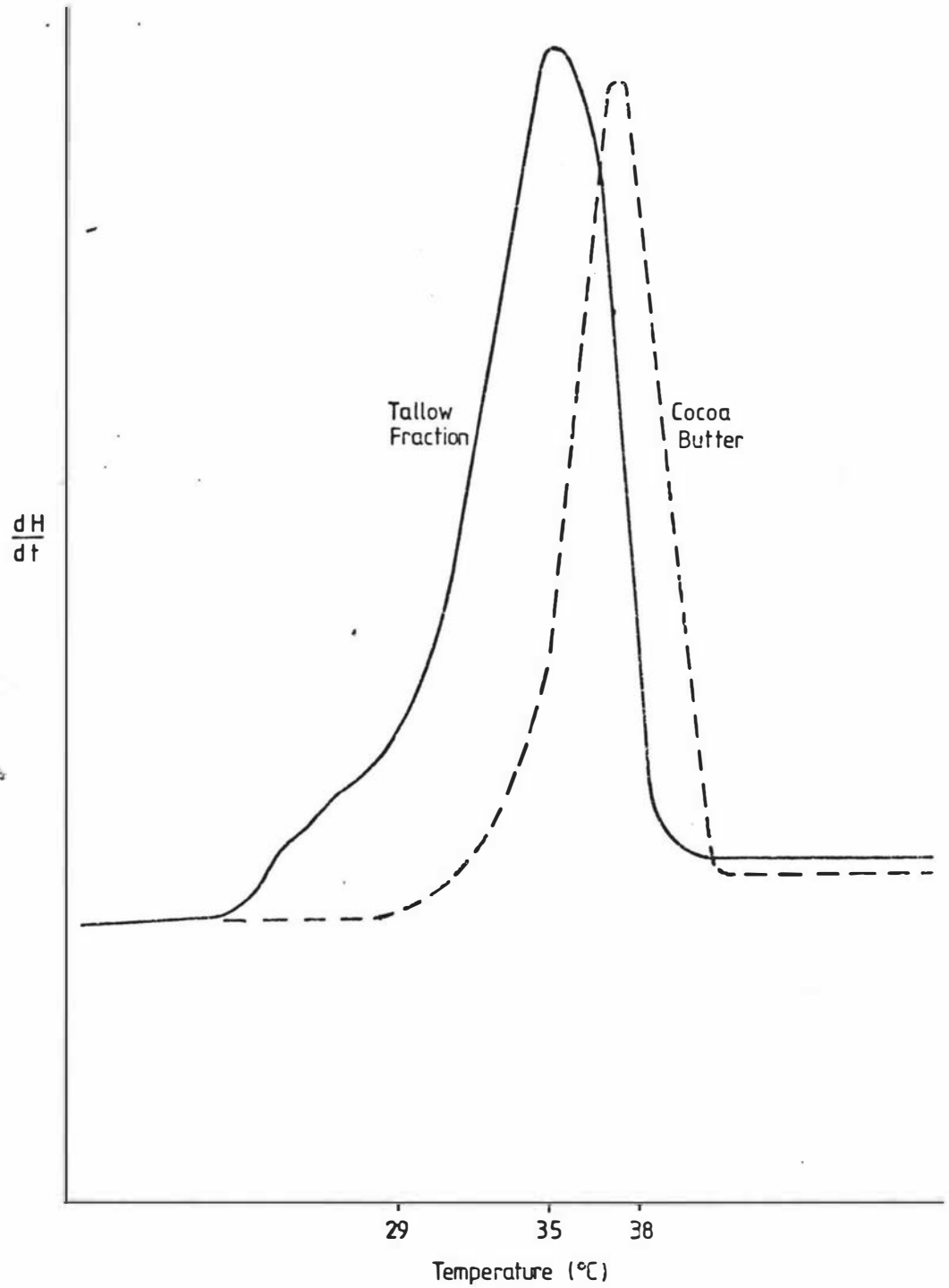


Figure 6·11: DSC profile of the intermediate fraction produced under the predicted optimum conditions and the DSC profile of a cocoa butter sample.

The measured yields of the three fractions produced in this fraction, and their predicted yields from the coded equations, as well as the measured and predicted CBLF of the intermediate fraction, are presented in Table 6.5.

Though the models for the yields of the intermediate and soft fractions do appear to give reasonable estimates of the measured yields of these two fractions, the models for the yield of the hard fraction and CBLF of the intermediate fraction do not. The predicted CBLF is a negative value, but it is obviously impossible in practice to have a fraction with a negative CBLF value. Similarly, the sum of the predicted yields of the three fractions is only 86.6%, whereas in practice they must obviously add to 100%.

Table 6.5: Predicted and measured values of the four response variables at the predicted optimum of the CBLF model

Variable	Measured Value	Predicted Value
CBLF of intermediate fraction	10.5	-3.1
Yield of hard fraction	37.0%	26.1%
Yield of intermediate fraction	6.6%	5.1%
Yield of soft fraction	56.4%	55.4%

However, the optimum conditions predicted by the CBLF model placed four of the six variables at the extreme levels at which they were studied in these experiments. Thus if the model is used to predict in this area, it is working at an extreme corner of the experimental area. And because of the nature of the central composite design, when a variable is examined at its extreme levels ($\pm$ 2.378 in this case) all of the other variables are set at their centre-points, so the interactive effect with more than one variable set at the extreme level is not examined, but is predicted from the interactive effects observed from the cube part of the experimental design.

6.9 COMPARISON OF RESULTS OF THE SCREENING EXPERIMENT AND THIS EXPERIMENT

In Table 6.6, the significant terms in the models fitted to predict each of the four response variables for both the screening experiment and this experiment are presented.

Table 6.6: Comparison of the significant terms in the fitted empirical models for the screening experiment and the central composite design.

Model		Significant Variables
Yield hard fraction	Screening Experiment	T_1S_1W $(T_1S_1 + T_2t + WM + S_2A)$ $(T_1M + T_2S_2 + S_1W + At)(T_1W + S_1M + T_2A + S_2t)$
	Central composite Design	T_1 S_1 W (T_1W)
Yield intermediate fraction	Screening Experiment	T_1 T_2 S_2 W $t(T_1S_2 + T_2M + S_1A + Wt)$ $(T_1W + S_1M + T_2A + S_2t)$
	Central composite Design	T_1 T_2 S_1 S_2 $(W^2)(T_1S_2)$ (T_2S_1) (S_1S_2) (S_2W)
Yield soft fraction	Screening Experiment	S_1 W
	Central composite Design	T_1 T_2W (W^2) (T_1S_2) (S_1W) (S_2W) (S_2A)
CBLF of intermediate fraction	Screening Experiment	T_1 T_2 S_1 S_2 W A $(T_1A + tM + S_1S_2 + T_2W)(T_1t + T_2S_1 + AM + S_2W)$ $(T_1M + T_2S_2 + S_1W + At)$
	Central composite design	T_1 T_2 S_1 S_2 W A $(T_1^2)(T_2^2)(W^2)$ $(T_1T_2)(T_1W)(T_1A)(T_2S_1)(T_2W)$

For each of the response variables, one or other of the models contains terms not shown to be significant in the other model. This is probably because the levels at which the independent variables were studied were different for the two experiments. The screening experiment has shown some chains of two-factor interactions to be significant which do not contain a corresponding significant two-factor interaction term in the model from the central composite design. It is possible that in the screening experiment, two or more non-significant interaction terms in each chain may have added to make the chain as a whole appear significant. It is also possible that the apparent significance of such a chain was due to an interactive effect with one of the two variables studied in the screening experiment but not in the central composite design (e.g. T_2t or T_1M).

Similarly, some of the models from the central composite design contained two-factor interactions which were not shown to be significant in the screening experiment. This may have been at least partly due to cancelling of coefficients in the two-factor interaction chains in the screening experiment. For instance, in the model from the central composite design for the yield of the intermediate fraction, both the T_2S_1 and S_2W interactions were shown to be significant, but not so with the screening experiment. In the central composite design model, these two terms had coefficients of -1.92 and 2.64. These are approximately equal in magnitude but opposite in sign, and thus if they appeared in the screening experiment in similar form they would tend to cancel each other and make the chain as a whole appear not significant. It is possible that some other significant two-factor interactions in the screening experiment may have been similarly masked by interactions containing t or M terms.

However, there is generally good agreement between the models. For the models for the yield of hard fraction and CBLF of the intermediate fraction the same main effects were shown to be significant from each set of experiments. And similarly with the yield of intermediate fraction, the main effects in each of the models are the same except that W has appeared as a quadratic term in the central composite design model and the screening experiment has failed to show the significance of S_1.

6.10 EXTRAPOLATION OF SOME OF THE INDEPENDENT VARIABLES

In an attempt to improve the CBLF value of the intermediate fraction, S_1 and S_2 were extrapolated outside their previous experimental limits to 1:1 and 10:1 respectively. The other variables were set at their levels predicted from the optimisation of the CBLF model. The fractionation conditions were thus:

$T_1 = 9.2^{o}C$, $T_2 = 5.2^{o}C$, $S_1 = 1:1$, $S_2 = 10:1$,
$W = 0.6\%$ $A = 146.22$ volts

After holding in the constant temperature room at the second crystallisation temperature for 60 minutes, no crystals had formed. It was necessary to hold for three days at this temperature to obtain crystal formation.

The intermediate fraction produced melted very similarly to cocoa butter (see Figure 6.12).

The yield of this intermediate fraction was 2.5% of the total tallow. The yields of the hard and soft fractions were 34.5% and 63.0% respectively.

No attempt was made to compare these results with the predicted values of the response variables at this point because the fitted models cannot be expected to predict accurately outside the experimental area over which they were determined.

6.11 FURTHER STUDY OF THE EFFECT OF ALTERING S_1 AND S_2

Because of the success of the fractionations with S_1 and S_2 extrapolated to 1:1 and 10:1 respectively, it was decided to investigate the effect of further varying S_1 and S_2. As an S_1 ratio of 1:1 was considered to be the lowest feasible without incurring handling problems, the effect of S_1 at lower limits was not considered. However, the effect of raising S_1 to 2.5:1, and of raising S_2 to 30:1, was considered. The levels of S_1 and S_2, and the yields of each of the fractions obtained, are presented in Table 6.7. The intermediate fraction produced in each case melted identically to the intermediate fraction produced in Section 6.10, the DSC profile of which is presented in Figure 6.12.

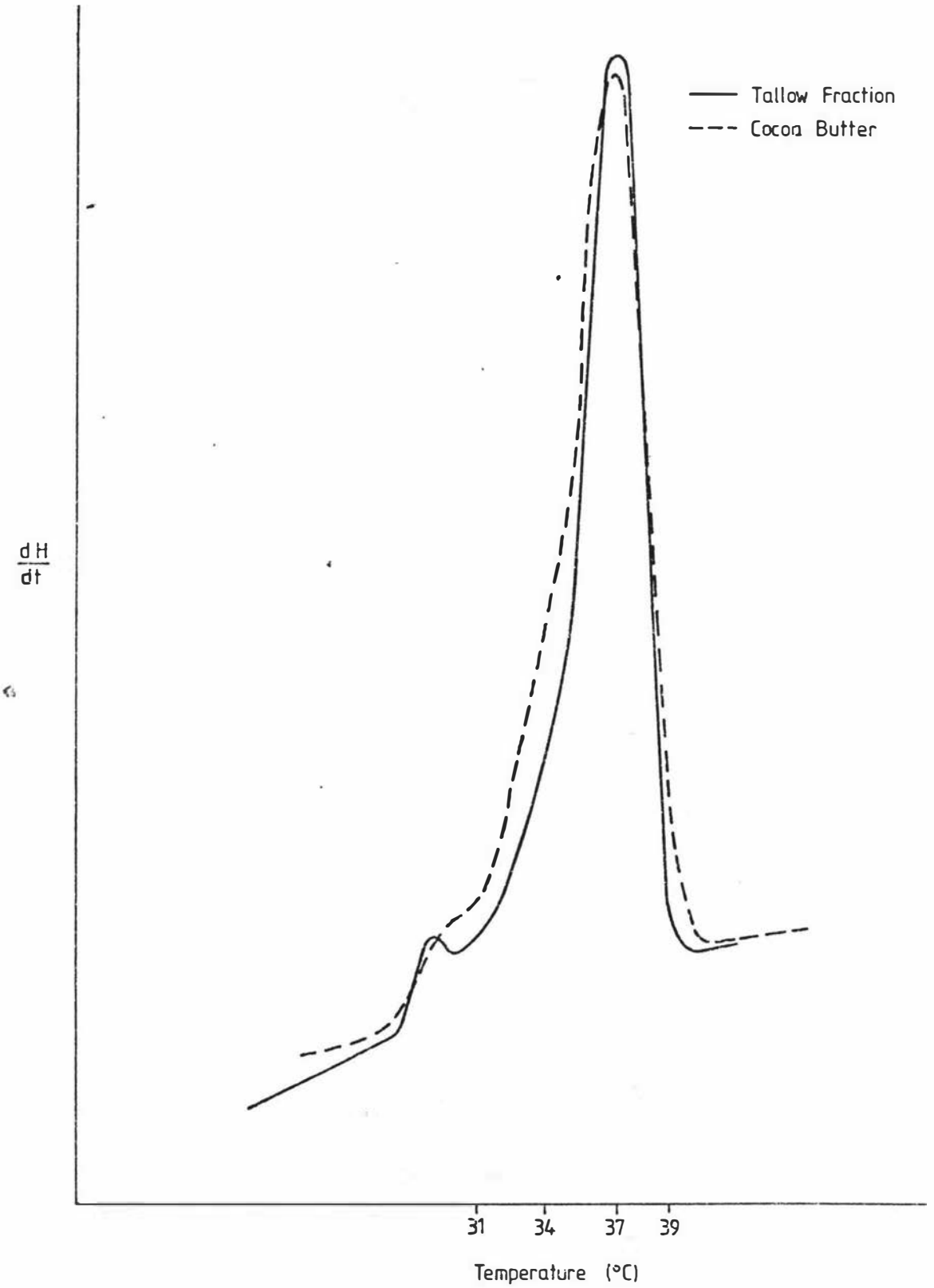


Figure 6·12 : Comparison of the DSC profiles of the intermediate fraction produced under the conditions of section 6·10 and cocoa butter.

Table 6.7: Levels of S_1 and S_2 and yields of fractions produced

Run	S_1	S_2	Yield of hard fraction (wt % of tallow)	Yield of intermediate fraction (wt % of tallow)	Yield of soft fraction (wt % of tallow)
1	1:1	20:1	34.5	2.5	63.0
2	2.5:1	10:1	36.5	5.8	57.7
3	1:1	30:1	37.0	3.2	59.8
4	2.5:1	30:1	34.5	5.0	60.5

Increasing S_2 has not affected the yield of the hard fraction produced (an overall average of 35.8% at the high level, and 35.5% at the low level) nor the yield of the intermediate fraction produced (4.1% at high level, 4.2% at low level).

Similarly, increasing S_1 has not affected the yield of the hard fraction (35.5% at the high level, 35.8% at the low level) but has increased the yield of the intermediate fraction. With S_1 at 1:1, the average yield of intermediate fraction was 2.9%, while with S_1 at 2.5:1, the average yield was 5.4%.

No interactive effect between S_1 and S_2, while set at these levels, is apparent.

6.12 STAGES OF FRACTIONATION

In the successful fractionation of Section 6.11, the average yield of the hard fraction was 35.6%.

It thus appears that to produce an intermediate fraction with good melting properties, it is necessary to remove about 36% of the total tallow in the first crystallisation.

In the screening experiment, no intermediate fraction was produced with melting properties close to those of cocoa butter, and similarly none of the hard fractions produced in any of the screening experiment fractionations had a yield close to 36%.

6.13 CONSIDERATION OF FURTHER EXPERIMENTS

Though optimisation of the model for CBLF appears to have successfully predicted the best region, within the range of independent variables studied, for producing an intermediate

fraction with good melting properties, the prediction of the actual value of the response variables at the predicted optimum was not good.

Also, extrapolation of two of the variables to levels beyond which they were studied in the central composite design experiments produced an intermediate fraction with better melting properties than was produced at the predicted optimum.

For these two reasons, it was decided to further study the system within an experimental area surrounding the optimum conditions predicted by this experiment.

6.14 CONCLUSIONS

(1) A central composite design was performed and empirical response equations for the yields of the three fractions and the CBLF of the intermediate fractions were fitted to the observed data. By the ratio of MS_{lof} to MS_{pe} these were shown to adequately represent the data.

(2) Examination of the isolated effects of the independent variables showed that the best-melting intermediate fraction was obtained with the first crystallisation temperature (T_1) at its low level (9.2°C), the second crystallisation temperature (T_2) at 4 to 6°C, the solvent : fat ratio at the first crystallisation (S_1) at its low level (1.6:1), the solvent : fat ratio at the second crystallisation (S_2) at its high level (6.4:1), the water content of the acetone (W) at 1.1% and with slow agitation (A).

(3) The CBLF model was optimised and the following optimum conditions predicted:

T_1 = 9.2°C
T_2 = 5.2°C
S_1 = 1.6:1
S_2 = 6.4:1
W = 0.6%
A = 146.2 volts

A fractionation performed under these conditions produced an intermediate fraction which melted at a lower temperature and over a slightly greater temperature range than cocoa butter. A further fractionation under these conditions, but with S_1 and S_2 extrapolated outside the experimental area to 1:1 and 10:1 respectively, produced an intermediate fraction which melted on the DSC identically to cocoa butter. The yields of the hard, intermediate and soft fractions were 34.5%, 2.5% and 63.0% respectively.

(4) Increasing S_2 to 30:1 appeared to have no significant effect upon the yields of the three fractions nor the melting properties of the intermediate fraction.

(5) Increasing S_1 to 2.5:1 significantly increased the yield of the intermediate fraction, but appeared to have no effect upon the yield of the hard fraction nor the melting properties of the intermediate fraction.

(6) It was decided to further study the response surface about the area of the optimum predicted from the CBLF model.

CHAPTER 7.

FURTHER STUDY OF THE INDEPENDENT VARIABLES AROUND THE PREVIOUS PREDICTED OPTIMUM

7.1 INTRODUCTION

In Chapter 6 it was shown that there are fractionation conditions which can produce an intermediate fraction melting very similarly to cocoa butter. It was decided to investigate more closely the effect of altering the levels of some of the independent variables, around the experimental region where this fraction was produced, upon the yield and properties of the fractions.

Four independent variables were chosen for further study - the two crystallisation temperatures and the solvent : fat ratio at each crystallisation. The water content of the acetone and the agitation speed were set at the optimum levels found in the previous experiment (0.6% and 146.2 volts input to the stirrer, respectively).

The intention was to fit a mathematical model describing the influence of each of the independent variables upon the values of the dependent variables within the experimental range of the independent variables studied.

7.2 METHODS

The fractionations were carried out as in Chapter 6 except that the fat solutions were held in the constant temperature room at the second crystallisation for three days to ensure complete crystallisation.

7.3 EXPERIMENTAL DESIGN

A design which would allow second order models to be fitted was chosen because the results of Chapter 6 showed that second order effects were important.

This design was based on an incomplete 3^4 design by Box and Behnken (1960), consisting of three blocks, each of nine runs, with one centre-point per block but no replication within blocks (Bacon, 1970). This lack of replication within blocks made it impossible to estimate the pure error to test for lack-of-fit of the fitted models. However, by running the whole experiment randomly (i.e. by ignoring blocks) three centre-points were obtained. By adding two more centre-point runs, a 29-run design with 4 degrees of freedom for estimating pure error was obtained. This design was chosen because:

(1) it required fewer runs than a four-factor central composite design;

(2) it can fit a second order model;

(3) each factor only needs to be set at three levels - this was advantageous for this experiment where the levels of the independent variables needed to be set close to each other as the approximate optimum area had already been ascertained.

The design is presented in Table 7.2.

7.4 LEVELS OF THE INDEPENDENT VARIABLES

Following the results of Chapter 6, the independent variables were studied at the levels shown in Table 7.1.

Table 7.1: Levels of the independent variables

VARIABLE	CODED LEVEL -1	0	1
T_1 °C	8.2	9.2	10.2
T_2 °C	4.0	5.2	6.4
S_1 (acetone : fat, v/w)	1.0	2.5	4.0
S_2 (acetone : fat, v/w)	6.0	9.0	12.0

7.5 RESULTS

The yields of the three fractions and the CBLF of the intermediate fractions are presented in Table 7.2.

7.6 EMPIRICAL EQUATIONS RELATING DEPENDENT TO INDEPENDENT VARIABLES

Each of the response variables was regressed, using Minitab, against each of the main effects, two-factor interactions and quadratic terms. Coefficients with t-statistics not significant at the 5% level were discarded, and only those with significant t-statistics contributed to the final model.

The models thus fitted for the yields of the hard and intermediate fractions, and the CBLF of the intermediate fractions are presented in Table 7.3. No terms were found to be significant at the 5% level for the yield of the soft fraction.

The adequacy of fit of each of the models was tested by the ratio of MS_{lof} to MS_{pe}. The tests for lack-of-fit, and the regression statistics for each model, are presented in Tables A6.1 to A6.3, Appendix 6. In each case, there was not shown to be significant lack-of-fit of the model at the 5% level.

7.6.1 Residuals from the Fitted Models

The residuals from each of the fitted models are presented in Table 7.4 in the same order as the experimental design order of the runs (even though the runs were performed in random order). Inspection of this table shows that residuals of opposite sign do not appear to be randomly distributed throughout the experiments -i.e. residuals of similar sign appear to be grouped together. This indicates that higher-order interaction terms are likely to be significant and would improve the fit of the models (Chatfield, 1970).

Table 7.2: The experimental design and the values of the response variables obtained

Run	T_1	T_2	S_1	S_2	DEPENDENT VARIABLES: Yield of hard fraction (wt % of tallow)	Yield of intermediate fraction (wt % of tallow)	Yield of soft fraction (wt % of tallow)	CBLF of intermediate fraction
1	-1	-1	0	0	39.7	5.6	54.7	9
2	1	-1	0	0	32.7	4.0	63.3	10.5
3	-1	1	0	0	38.0	7.0	55.0	15
4	1	1	0	0	34.4	10.8	54.8	9
5	0	0	-1	-1	32.5	3.0	64.5	9
6	0	0	1	-1	27.4	17.2	55.4	13
7	0	0	-1	1	34.4	3.8	61.8	6.5
8	0	0	1	1	29.2	8.3	62.5	10
9	0	0	0	0	26.9	11.5	61.6	11
10	-1	0	0	-1	35.9	8.1	56.0	14
11	1	0	0	-1	31.8	9.6	58.6	12
12	-1	0	0	1	36.4	4.9	58.7	14
13	1	0	0	1	34.8	6.3	58.9	13
14	0	-1	-1	0	35.6	9.8	54.6	13
15	0	1	-1	0	30.7	6.7	62.6	11
16	0	-1	1	0	26.6	12.8	60.6	13
17	0	1	1	0	27.1	12.6	60.3	14
18	0	0	0	0	29.9	15.7	54.4	14
19	0	-1	0	-1	24.1	12.9	63.0	14
20	0	1	0	-1	24.7	7.3	68.0	16
21	0	-1	0	1	25.9	19.0	55.1	18
22	0	1	0	1	25.5	11.3	63.2	19
23	-1	0	-1	0	33.2	6.8	60.0	12
24	1	0	-1	0	35.5	4.4	60.1	14
25	-1	0	1	0	26.0	14.4	59.6	17
26	1	0	1	0	19.2	19.5	61.3	24
27	0	0	0	0	30.4	13.8	55.8	11
28	0	0	0	0	30.3	10.7	59.0	13
29	0	0	0	0	27.0	11.1	61.9	14

Table 7.3: Empirical models for the response variables

Yield of hard fraction:

$$Y_H \text{ (wt \% of tallow)} = 30.07 - 1.83\,(T_1) - 3.75\,(S_1) + 4.43\,(T_1^2)$$

Yield of intermediate fraction:

$$Y_I \text{ (wt \% of tallow)} = 9.48 + 4.20\,(S_1) - 2.56\,(T_1^2)$$

CBLF of the intermediate fraction:

$$CBLF = 13.41 + 2.13\,(S_1)$$

Table 7.4: Residuals from the fitted models

Run No.	Fitted Model		
	Yield of hard fraction (% of tallow)	Yield of intermediate fraction (% of tallow)	CBLF of intermediate fraction
1	4.5	-3.0	-4.4
2	1.3	-3.9	-2.9
3	3.5	-0.8	1.6
4	3.3	2.0	1.6
5	0.0	-3.3	-2.2
6	2.5	2.3	-2.5
7	2.0	-2.5	-4.8
8	4.5	-6.4	-5.5
9	-2.2	0.5	-2.4
10	0.5	0.0	0.6
11	0.3	1.0	-1.4
12	1.5	-4.2	0.6
13	3.3	-2.2	-0.4
14	3.0	2.3	1.7
15	-2.0	-0.3	-0.3
16	1.5	-3.2	-2.5
17	2.5	-2.7	-1.5
18	0.8	4.5	0.6
19	-4.2	1.5	0.6
20	-4.2	-3.5	2.6
21	-3.2	8.5	4.6
22	-3.2	0.5	5.6
23	-5.3	2.2	0.7
24	0.4	0.2	2.7
25	-4.8	1.5	1.5
26	-8.1	7.1	8.5
27	1.8	2.5	-2.4
28	1.8	-0.5	-0.4
29	-1.2	0.5	0.6

Higher-order terms could not be fitted using this experimental design because of confounding.

7.6.2 Isolated Effect of the Variables

The observed response of each of the dependent variables to different levels of each of the independent variables considered one at a time are presented in Figures 7.1 to 7.4.

The predicted values of each of the response variables at the different levels of the independent variables considered one at a time are also presented in Figures 7.1 to 7.4. In each case, the predicted response curves appear to adequately represent the observed data.

The greatest yield of hard fraction was shown, from consideration of the isolated effects of the independent variables, to occur with both T_1 and S_1 at their low levels (8.2°C and 1:1 respectively). The lowest yield was shown to occur with T_1 at its centre-point level (9.2°C) and S_1 at its high level (4:1). However these results failed to account for the fact that the lowest yield of hard fraction in the experimental runs (19%) was obtained with both T_1 and S_1 at their high levels. It is possible that the inclusion of higher-order interaction terms in the model would have allowed this trend to be more accurately followed by the model.

T_2 and S_2 were not shown to have a significant effect upon the yield of the intermediate fraction over this range of conditions. The isolated effects of S_1 and T_1 show that the highest yield of intermediate fraction occurs with T_1 at its centre-point (9.2°C) and S_1 at its high level (4:1).

None of the four independent variables were shown to significantly affect the yield of the soft fraction. This appears reasonable, as under the conditions where a high yield of hard fraction was produced, a low yield of intermediate fraction was obtained - i.e. the sum of hard and intermediate fractions produced remained approximately constant, and thus the yield of soft fraction produced must remain constant.

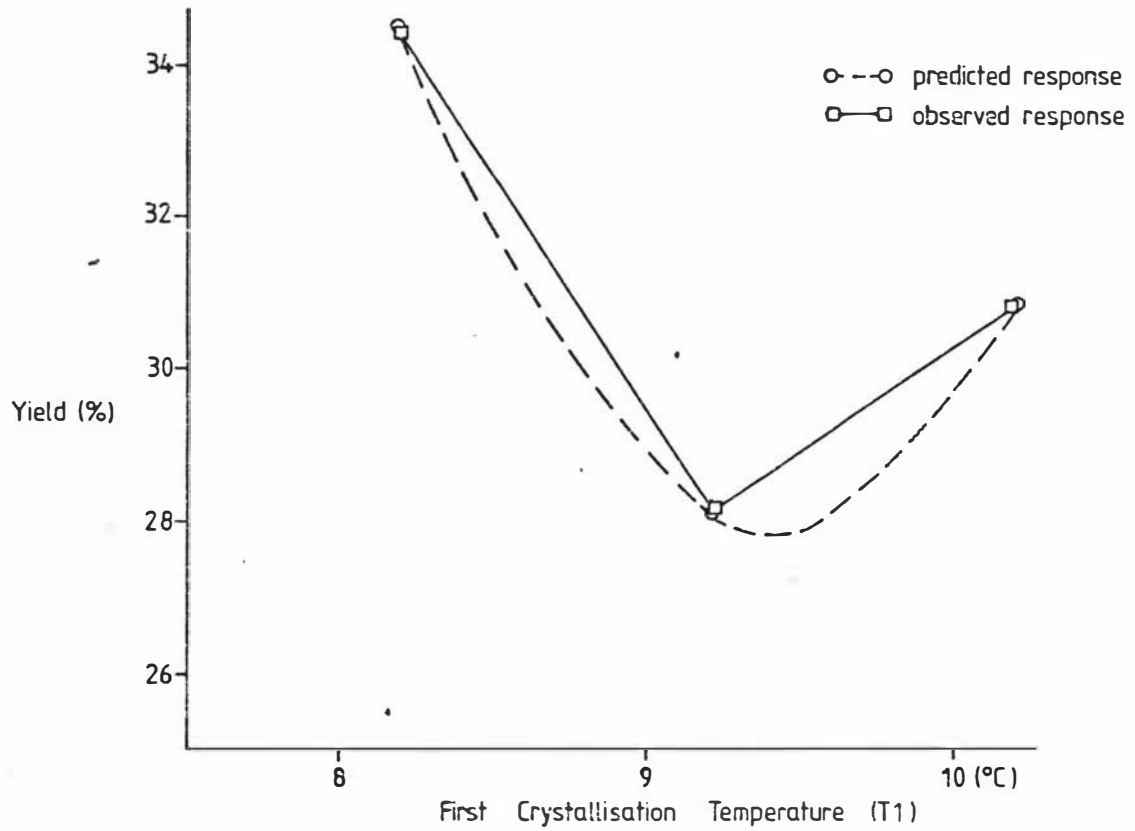
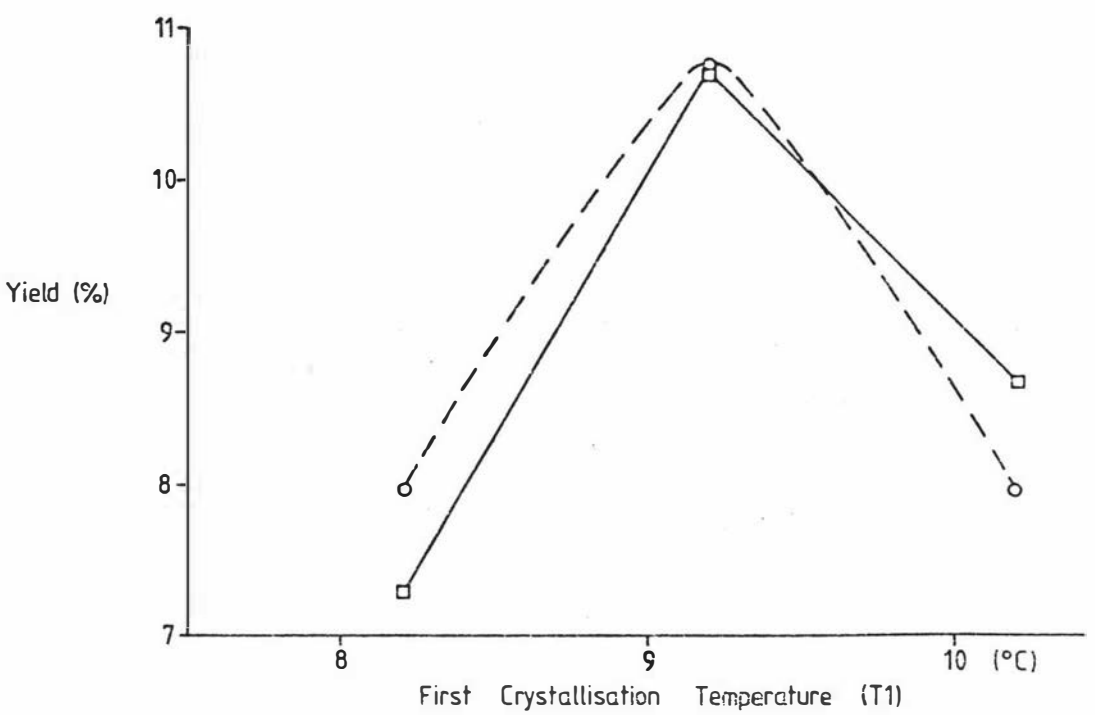
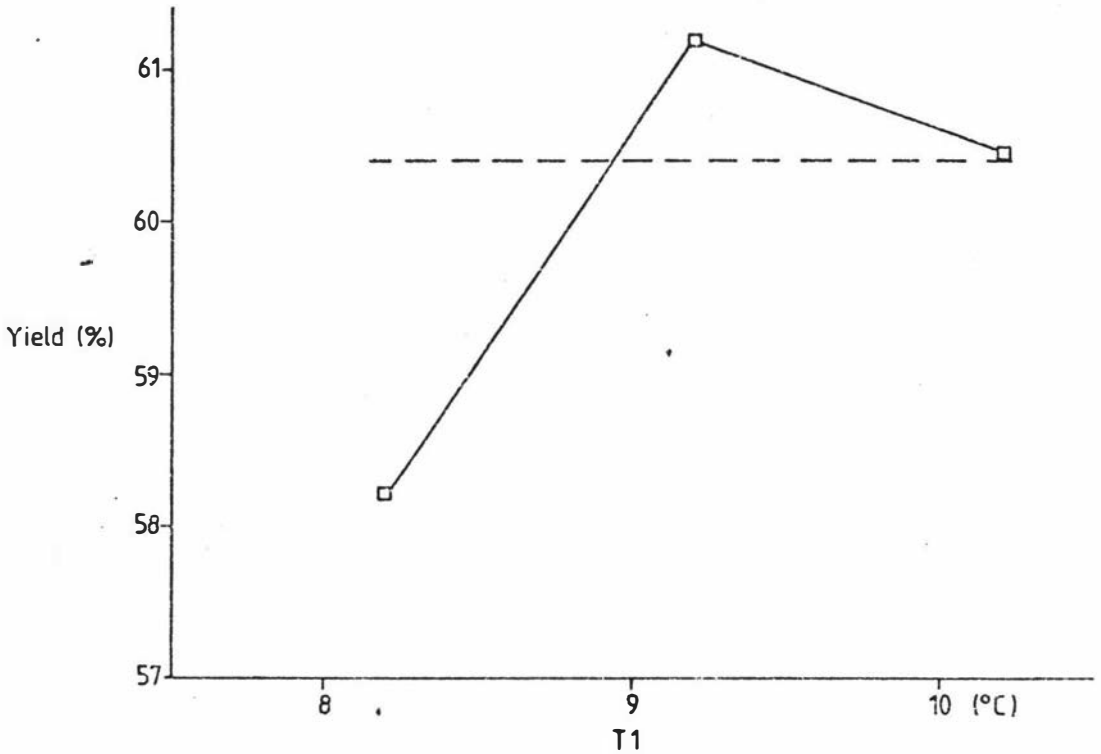


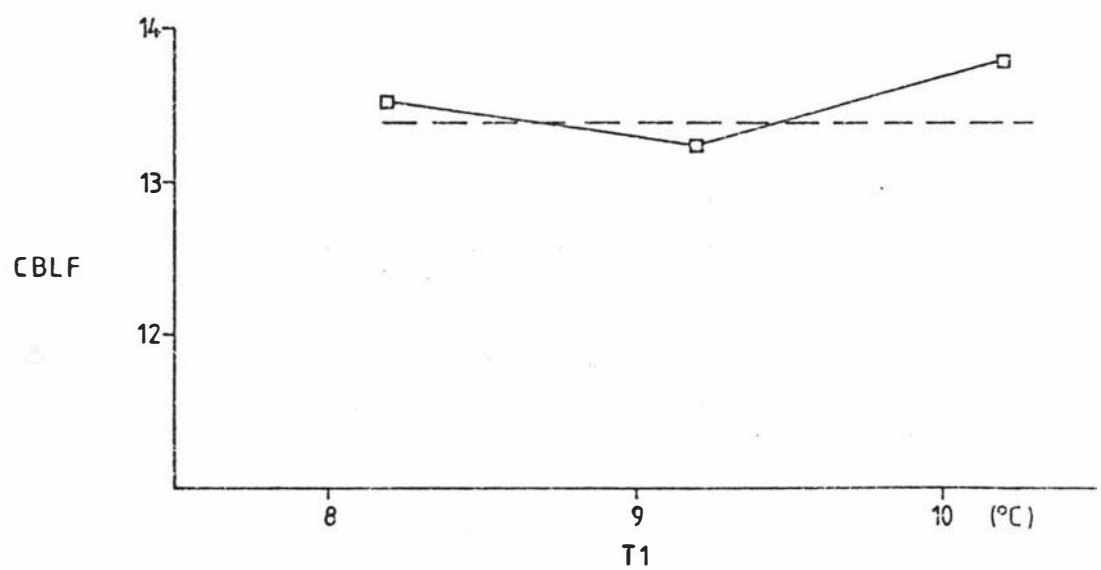
Figure 7·1 : The isolated effect of T1 on the dependent variables.
(a) Yield of hard fraction.



(b) Yield of intermediate fraction.



(c) Yield of the soft fraction.



(d) CBLF of the intermediate fraction.

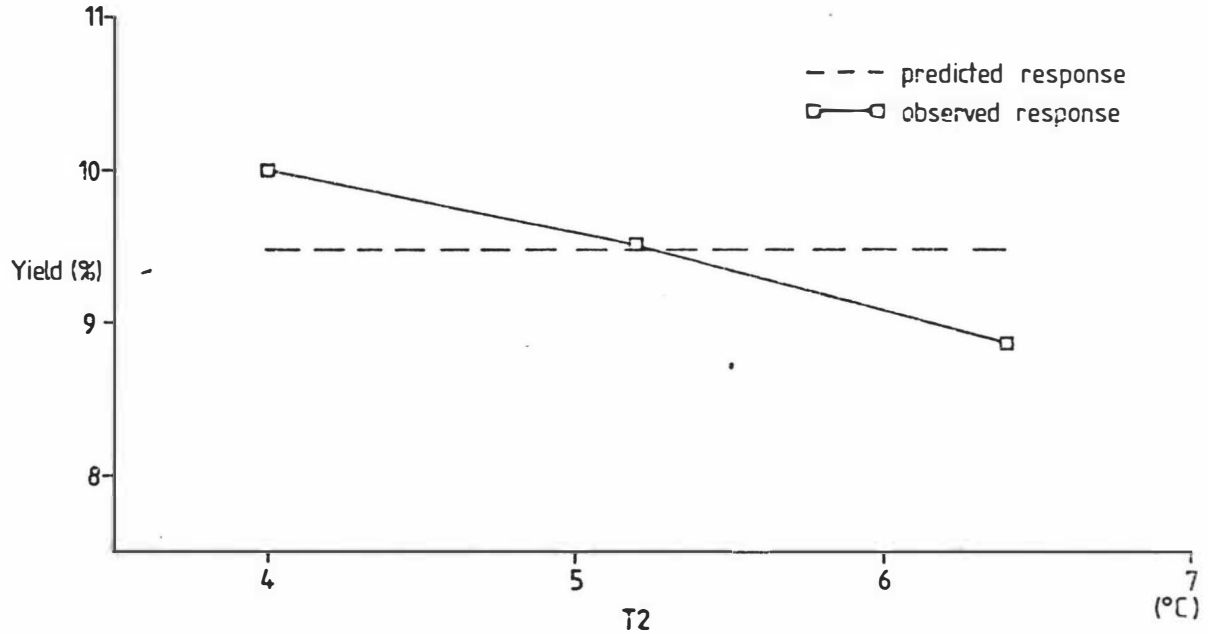
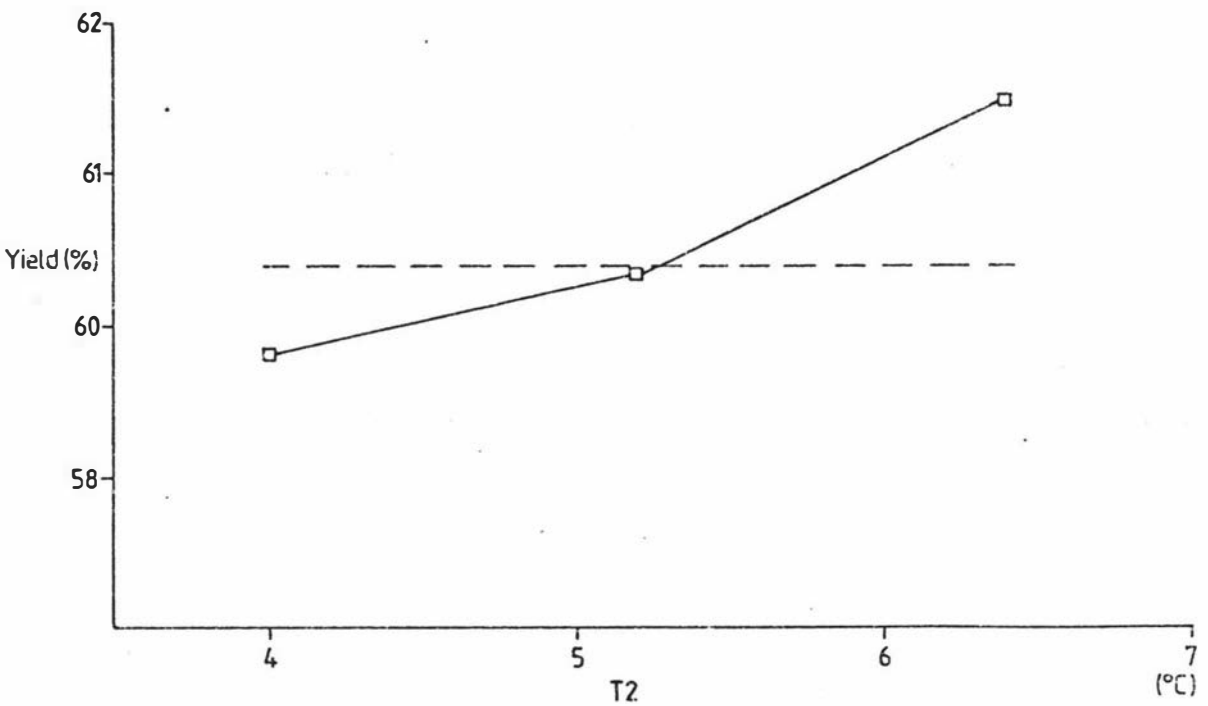
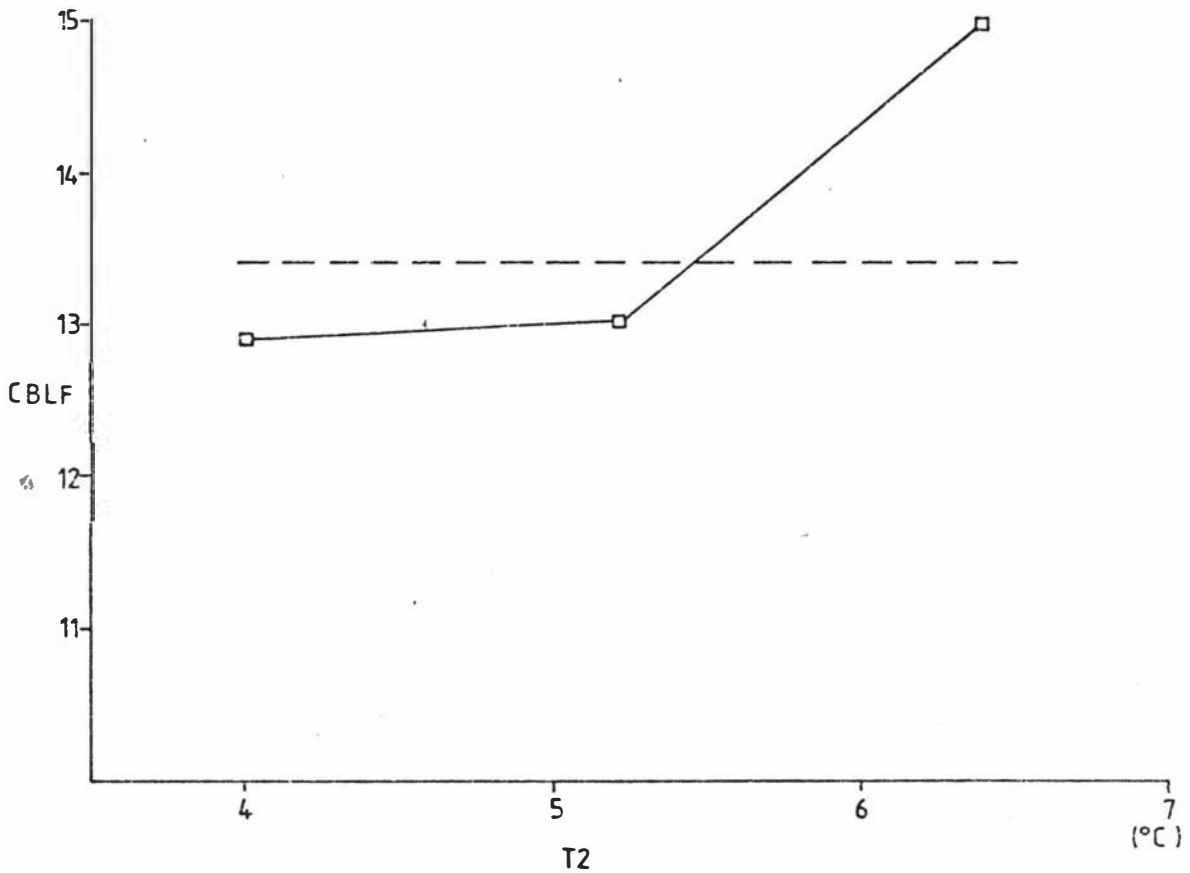


Figure 7.2 : The isolated effect of T2 on the dependent variables.
(a) Yield of intermediate fraction.



(b) Yield of soft fraction.



(c) CBLF of the intermediate fraction.

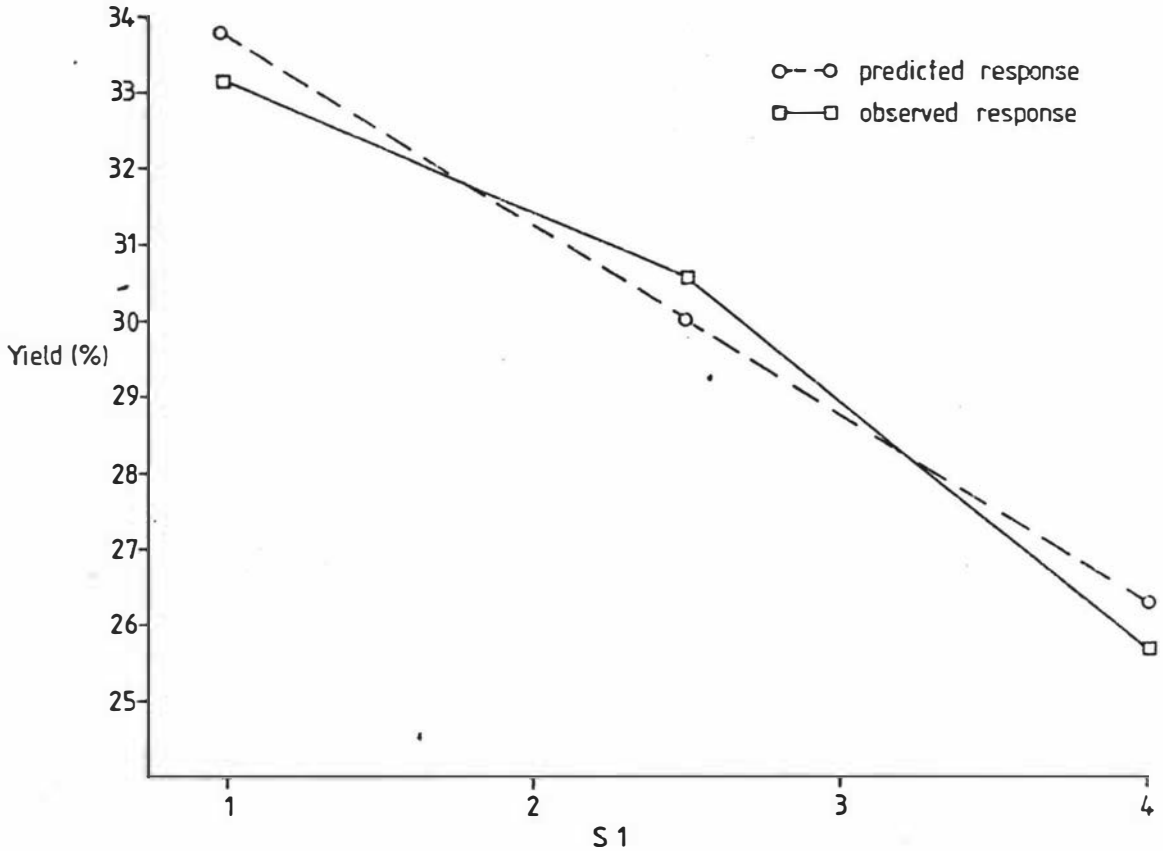
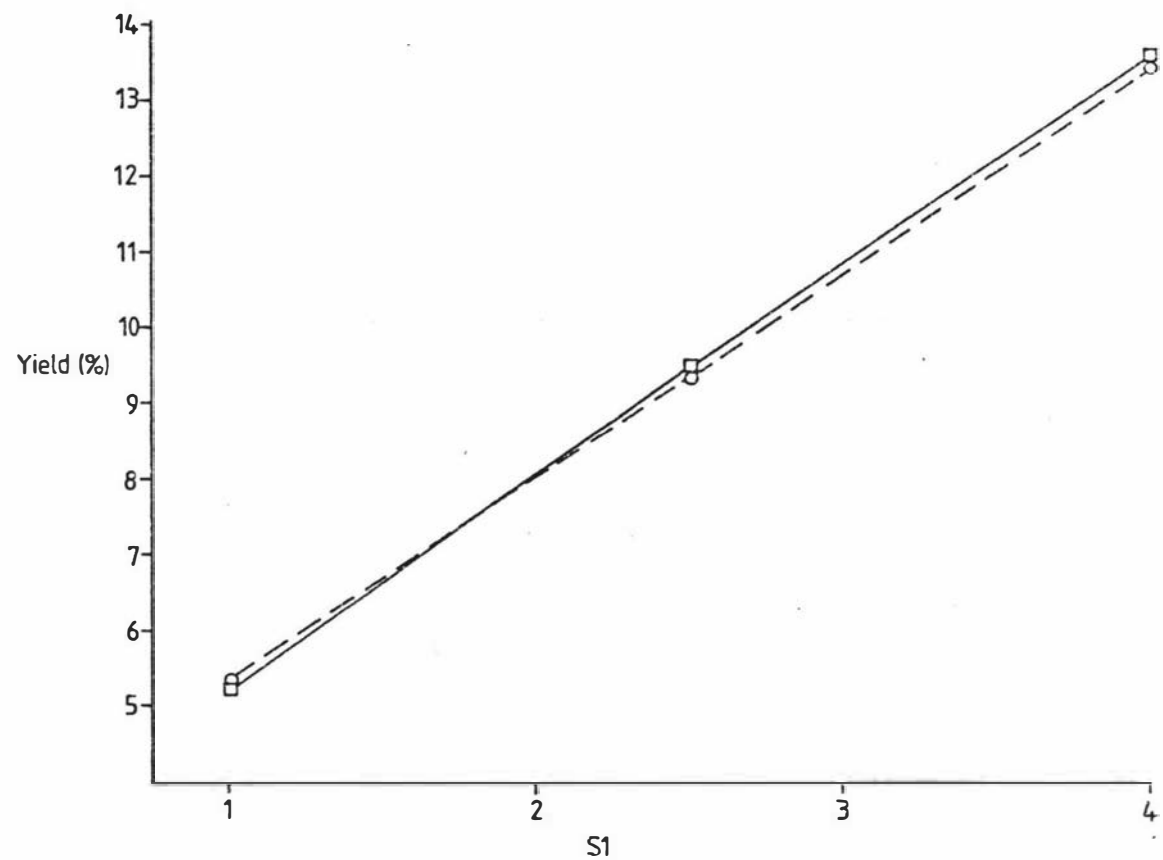
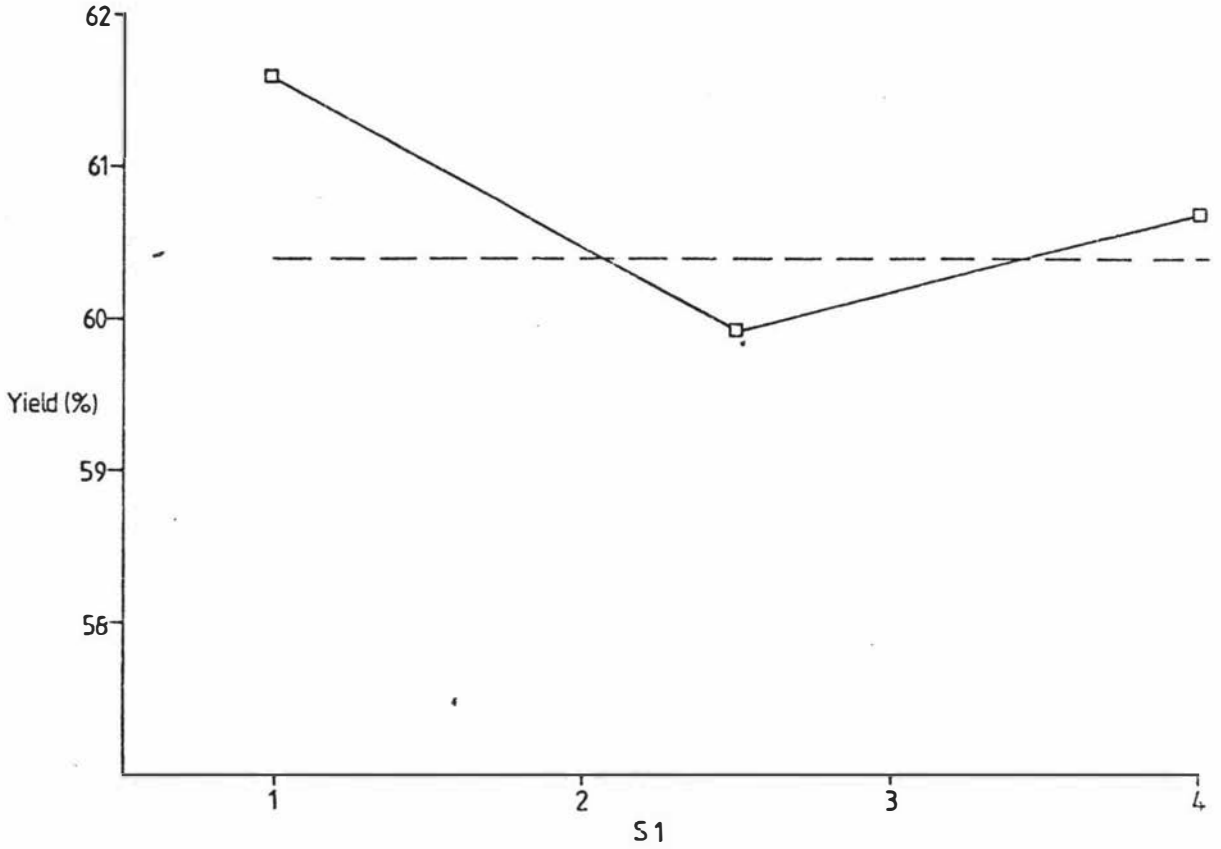


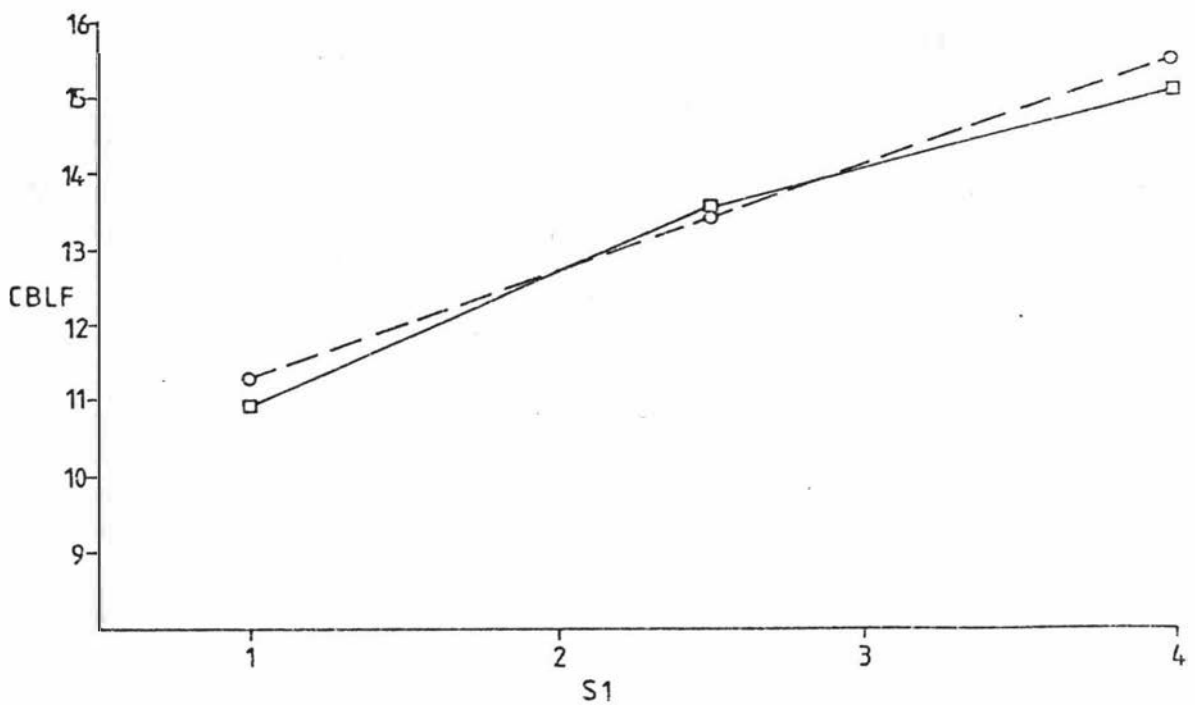
Figure 7·3 : The isolated effect of S1 on the dependent variables (a) Yield of the hard fraction.



(b) Yield of the intermediate fraction.



(c) Yield of the soft fraction.



(d) CBLF of the intermediate fraction.

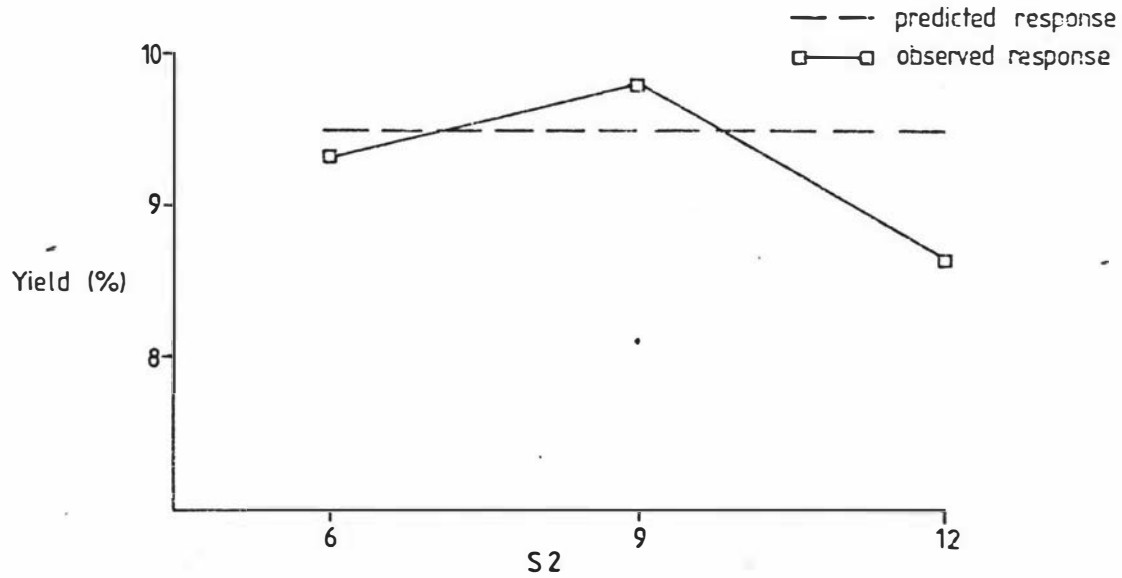
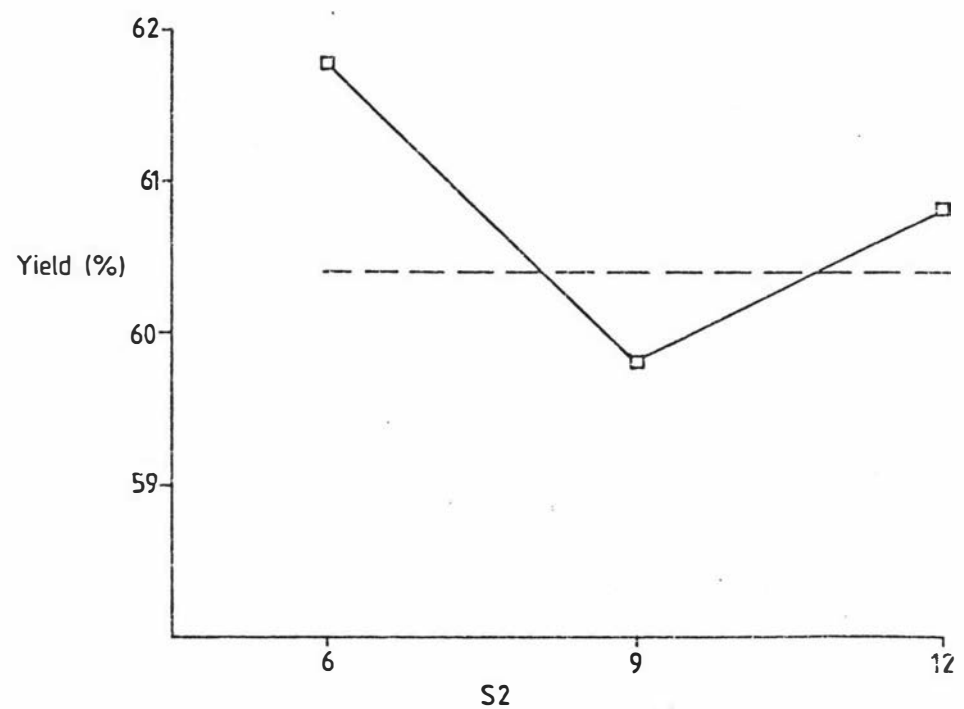
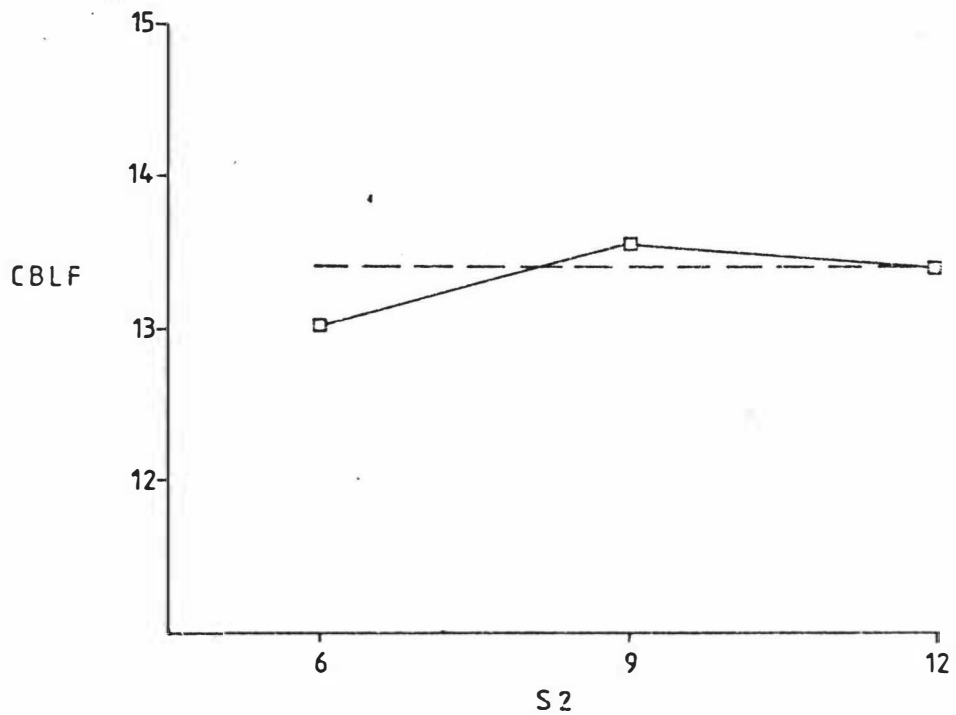


Figure 7·4 : The isolated effect of S2 on the dependent variables

(a) Yield of the intermediate fraction.



(b) Yield of the soft fraction.



(c) CBLF of the intermediate fraction.

This is also verified by the fitted models for the yields of the hard and soft fractions:

$$\text{Yield of the soft fraction (wt \% of tallow)} = 100 - [\text{yield of hard fraction (\% of tallow)} + \text{yield of intermediate fraction (\% of tallow)}]$$

$$= 100 - [30.1 - 3.8(S_1) + 4.4(T_1^2) - 1.8(T_1)) + (9.5 + 4.2(S_1) - 2.6(T_1^2))]$$

The coefficients of S_1 almost cancel out, and the coefficients of T_1^2 partially cancel. The main and quadratic effects of T_1 also tend to cancel at some levels of T_1.

S_1 is the only independent variable which has been shown to significantly affect the CBLF of the intermediate fraction. The fraction with the best melting properties was produced at the lowest S_1 value (1:1). This was in contrast to the S_1 value necessary to obtain a high yield of the intermediate fraction.

As no two-factor interaction effects have been shown to be important over this range of conditions, study of the isolated effect of the variables above should predict the optimum value of each of the independent variables. However, there is the possibility that higher-order interaction terms may be important.

7.7 INTERPRETATION OF THE RESULTS

The intermediate fraction with the best melting characteristics in these runs (CBLF = 6.5) was obtained with $T_1 = 9.2^oC$, $T_2 = 5.2^oC$, $S_1 = 1:1$ and $S_2 = 12:1$. These are the same conditions as were used in Section 6.10 to produce a satisfactory-melting intermediate fraction except that S_2 is increased from 10:1 to 12:1 - however, in Section 6.11 it was shown that increasing S_2 to above 10:1 had no effect upon the intermediate fraction, and this appears to be the case from these experiments. However, the yield of the intermediate fraction was only 3.8% (2.5% in Section 6.10). The fitted model from these experiments predicted a yield of the intermediate fraction of 6.3%.

The model for the yield of intermediate fraction from these experiments showed that a greater yield of intermediate fraction would be obtained if S_1 was increased (it also predicts that T_1 should be set at 9.2°C for the best yield of intermediate fraction, and this was the condition used above). From the models, increasing S_1 increases the CBLF of the intermediate fraction as well as increasing its yield.

During execution of the experimental design, a run was performed under the above conditions but with S_1 increased to 4:1. The yield of the intermediate fraction was 8.3% (14.7% predicted) and the yields of the hard and soft fractions were 29.2% and 62.5% respectively. The predicted yield of hard fraction from the fitted model was 24.5%. The DSC profile of the intermediate fraction in this run is presented in Figure 7.5. While the peak maximum occurs at the same temperature as that of cocoa butter, the melting range is greater, with a significant portion of the triglycerides melting at a lower temperature. However, the yield of the intermediate fraction has been increased, and the melting properties of this intermediate fraction may be sufficiently similar to those of cocoa butter for it to be useful.

In the above fractionation, S_2 was set at a high level. As these experiments have failed to show that S_2 significantly affected either the yield or melting properties of the intermediate fraction over the range of levels studied, it was decided to investigate the effect of decreasing S_2. During execution of these experiments, a fractionation was run with S_2 decreased to 6:1, but with all other conditions as above (i.e. with T_1 = 9.2°C, T_2 = 5.2°C, and S_1 = 4:1). This greatly affected the fractionation. The yield of the intermediate fraction was 17.2%, and the yields of the hard and soft fractions were 27.4% and 55.4% respectively. The predicted yields of the hard and intermediate fractions were 24.5% and 14.7% respectively. The DSC profile of the intermediate fraction is presented in Figure 7.6. This shows that the maximum point of the melting peak was 4°C lower than that for cocoa butter. Examination of the yields of the fractions for the last two runs shows that decreasing S_2 has forced more of the lower-melting fraction into the intermediate fraction, and while this has increased the yield of the intermediate fraction it has reduced

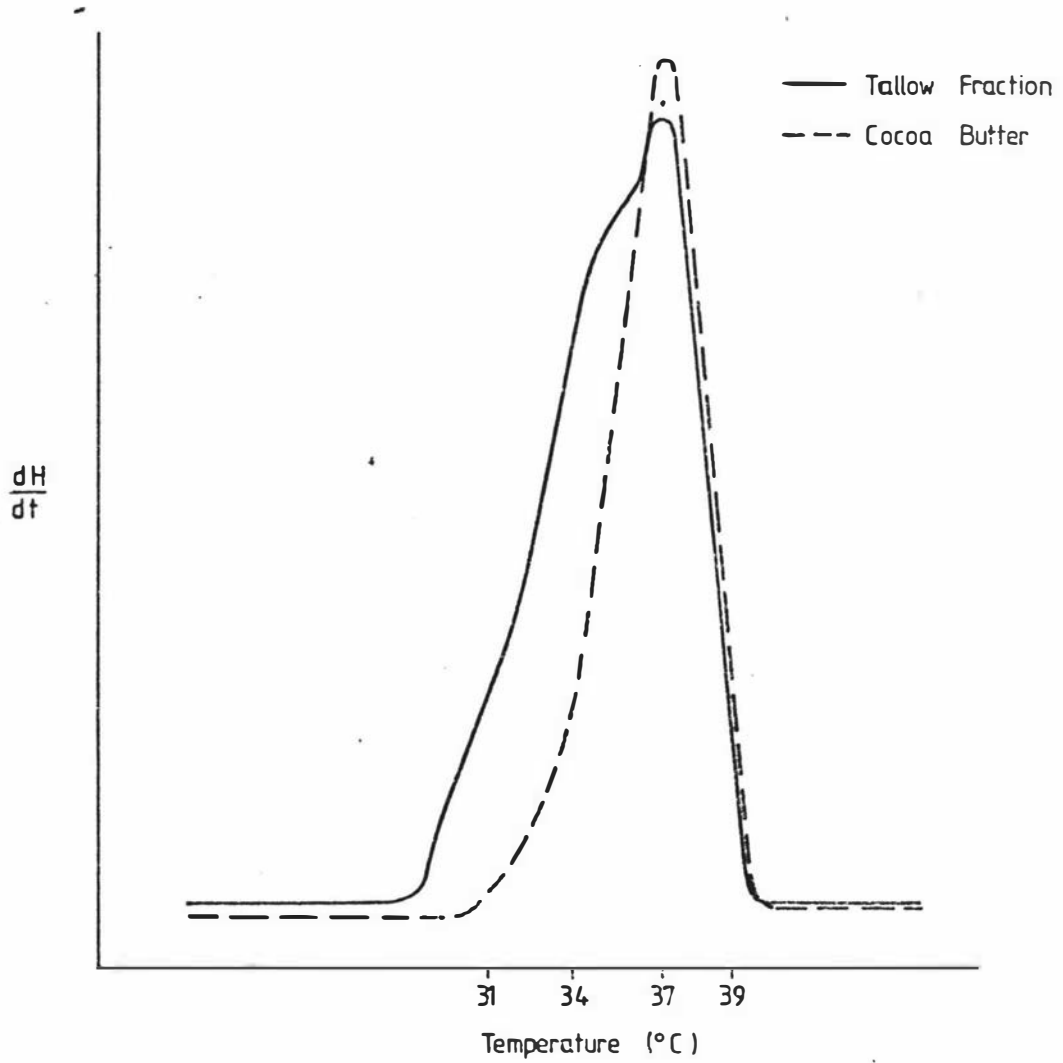


Figure 7·5 : DSC Profile of the intermediate tallow fraction produced with T1 = 9·2°C , T2 = 5·2°C, S1 = 4:1, S2 = 12:1; and the DSC profile of a cocoa butter sample.

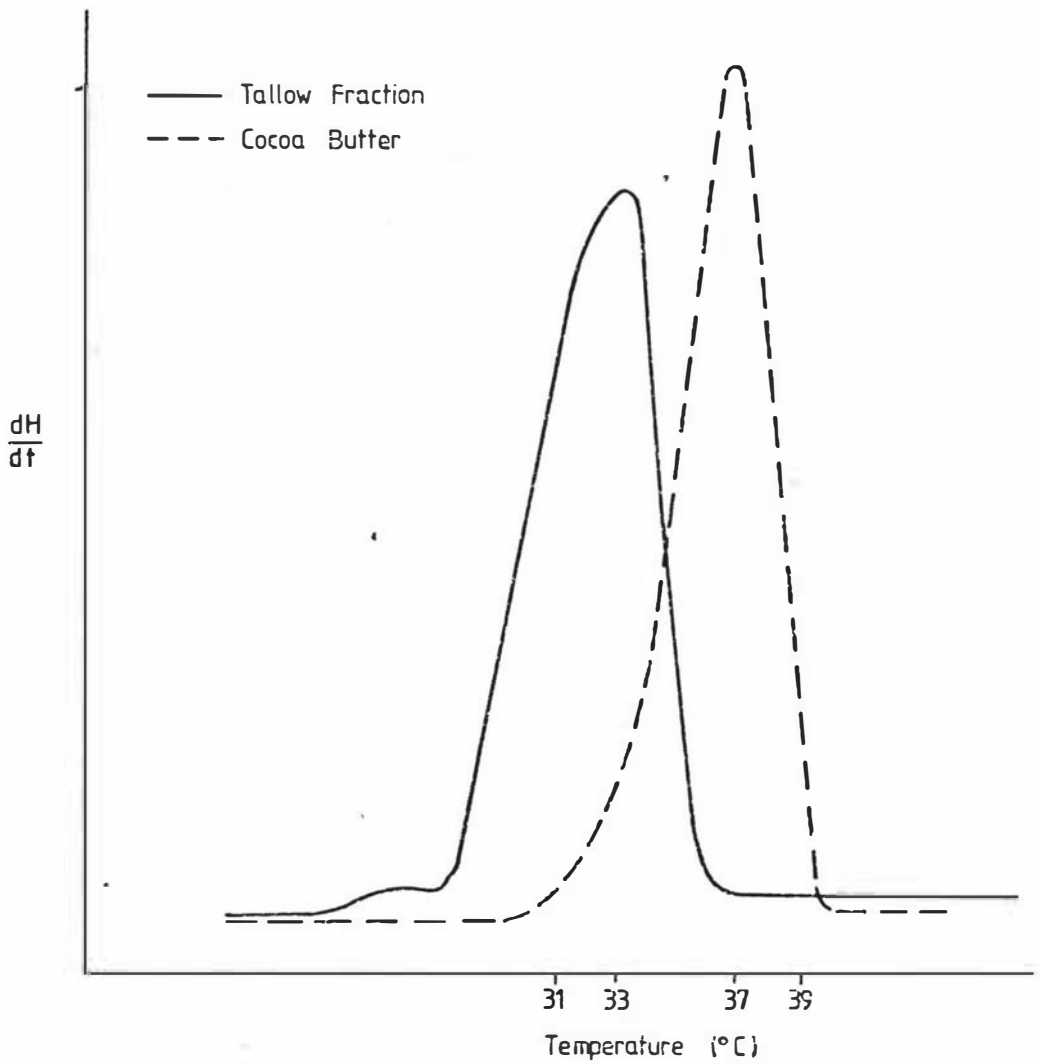


Figure 7·6 : DSC profile of the intermediate tallow fraction produced with T1=9·2°C, T2=5·2° C, S1=4:1, S2=6:1; and the DSC profile of a cocoa butter sample.

the "melting point" of the fraction. However, the model for CBLF of the intermediate fraction failed to predict that altering S_2 would affect the melting properties of the intermediate fraction.

Thus, while the fitted models have not shown S_2 nor interactive effects to be important, the above results suggest otherwise. It therefore seems likely, especially with consideration of the residuals, that higher-order interactive effects are important.

These results are summarised in Table 7.5.

These results confirm the trend found in Section 6.11, where increasing S_1 to 2.5:1 significantly increased the yield of the intermediate fraction. However, S_2 was still held at a high level, so the good melting properties of the intermediate fraction were maintained. These experiments have shown that further increasing S_1 to 4:1 increases the yield of the intermediate fraction still further, but there is broadening of the melting range, even with S_2 maintained at a high level.

Table 7.5: Summary of the effect of altering S_1 and S_2 around the optimum (with $T_1 = 9.2^{\circ}C$, $T_2 = 5.2^{\circ}C$)

S_1	S_2	Yield hard fraction (% of tallow)	Yield intermediate fraction (% of tallow)	Yield soft fraction (% of tallow)	DSC of intermediate fraction
1:1	12:1	34.4	3.8	61.8	Very similar to cocoa butter
4:1	12:1	29.2	8.3	62.5	Greater melting range than cocoa butter (see Figure 7.5)
4:1	6:1	27.4	17.2	55.4	Softer than cocoa butter (see Figure 7.6)

7.8 STAGES OF THE FRACTIONATION

The intermediate fraction with the best melting characteristics from the Box and Behnken design was produced in a

fraction where the yield of hard fraction was 34%. This confirms the results of Chapter 6 where it was found that to produce an intermediate fraction with satisfactory melting properties about 36% of the tallow had to be removed at the first crystallisation.

From observation of the isolated effects of the independent variables and the fitted models for the yields of the hard and intemediate fractions, it was shown that within the conditions used in fractionations of this chapter only the proportions of the two harder fractionations and not of the soft fraction were altered.

This was also the case in a fractionation where S_1 = 4:1 and S_2 = 12:1. This increased the yield of the intermediate fraction to 8.3%, and decreased the yield of hard fraction to 29%, while the yield of the soft fraction remained approximately the same as when S_1 was set at 1:1. In a fractionation with S_1 = 4:1 and S_2 = 6:1, the yield of intermediate fraction was 17%. This extra proportion of intermediate fraction was removed from the soft fraction (yield = 56%), and the intermediate fraction was predictably softer than in the previous fractionation.

7.9 TEMPERATURE HISTORY OF THE FAT SOLUTION DURING CRYSTALLISATION

During all of the previous crystallisations, agitation speed, crystallisation temperature and the volume of the fat solution will have affected the cooling rate of the fat solution, but no attempt was made to control the cooling rate as an independent variable.

Hence in the crystallisation of Section 7.7, with S_1 = 4:1 and S_2 = 12:1, the temperature history of the fat solution during the two crystallisations was studied. The cooling curves are presented in Figure 7.7.

It is apparent that the temperature of the fat solution does not reach the air temperature of the constant temperature room. This is probably due to heat input from the stirrer.

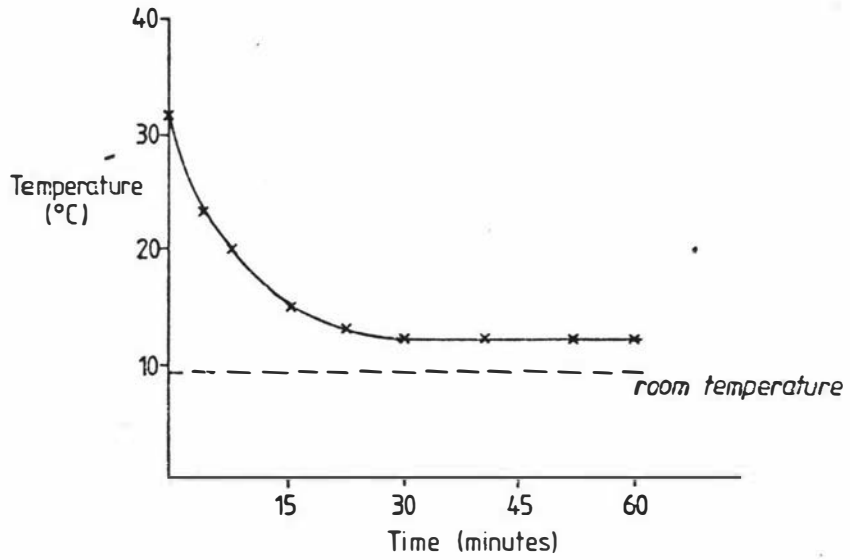
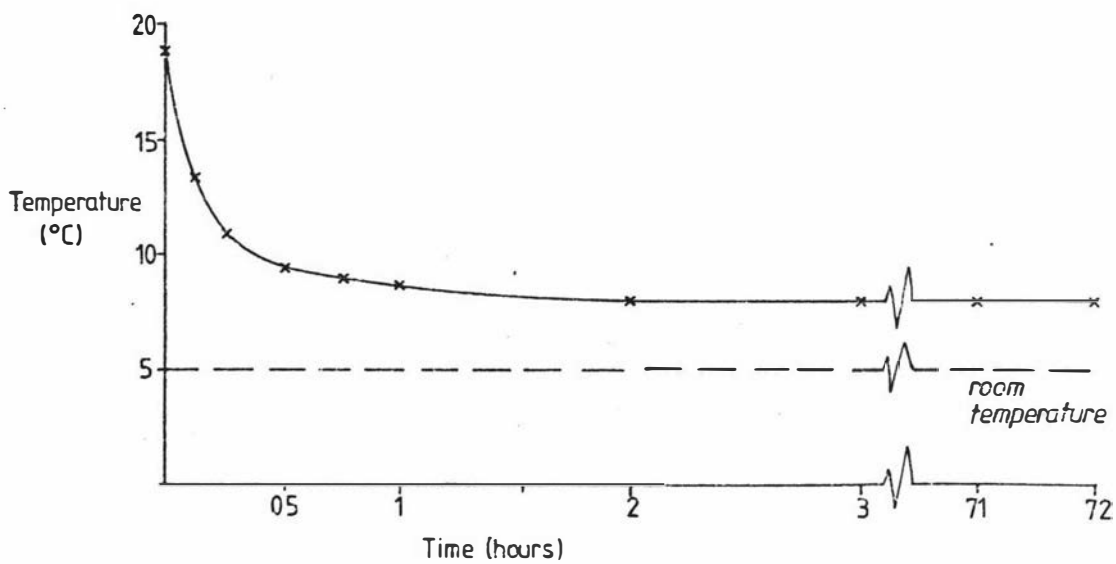


Figure 7·7 : Temperature history of the fat solution during crystallisation.
(a) First crystallisation.



(b) Second crystallisation.

7.10 CONCLUSIONS

(1) An incomplete 3^4 design was performed and empirical response equations for the yields of the hard and intermediate fractions and the CBLF of the intermediate fraction were fitted to the observed data. By the ratio of MS_{lof} to MS_{pe} these were shown to adequately represent the data.

(2) It was found that altering the temperatures and solvent : fat ratios at the two crystallisations, within the limits of the design, only affected the relative proportion of the hard and intermediate fractions, and did not significantly affect the yield of the soft fraction.

(3) The intermediate fraction with the best melting characteristics (CBLF = 6.5) was obtained with a first crystallisation temperature (T_2) of 5.2°C, a solvent to fat ratio at the first crystallisation (S_1) of 1:1 and a solvent to fat ratio at the second crystallisation (S_2) of 12:1. The yields of the hard, intermediate and soft fractions were 54%, 4% and 62% respectively.

(4) Increasing the solvent to fat ratio at the first crystallisation (S_1) to 4:1 increased the yield of the intermediate fraction to 8.3%, decreased the yield of the hard fraction to 29%, and caused the melting range of the intermediate fraction to increase at the lower end.

(5) With S_1 = 4:1 and S_2 decreased to 6:1, the melting point of the intermediate fraction was lowered but its yield was increased to 17%. The yield of the hard fraction was 27%.

(6) A further increase in the yield of the intermediate fraction could only be obtained, within the conditions studied, with a corresponding deviation of the melting properties of the intermediate fraction from those of cocoa butter.

CHAPTER 8
SCALE-UP OF THE FRACTIONATION

8.1 INTRODUCTION

Fractionation conditions have been described in Chapter 7 to give an intermediate fraction, with a yield of 8.3% of the total tallow, which melts over a slightly wider temperature range than cocoa butter. Intermediate fractions which melted over the same range as cocoa butter were obtained in an average of 4.1% yield. It was decided to scale-up using the conditions which gave the higher yield of intermediate fraction, in the hope that the properties of the intermediate fraction would be sufficiently similar to those of cocoa butter for it to be useful.

There were two main reasons for scaling-up the process:

(1) to produce a greater quantity of each of the fractions so that their properties and suitability for specific uses could be determined, and

(2) to elucidate the critical scale-up criteria for this process.

The fractionation was first attempted with 200 g of tallow, i.e. a ten fold increase in scale, and then with 1 kg of tallow.

8.2 FRACTIONATION OF TWO HUNDRED GRAMS OF TALLOW

8.2.1 Methods

The fractionations were performed in a sealed, 2.5 l, conical flask.

Cooling was achieved by holding in a constant temperature room or by partially immersing the flask in a refrigerated water-bath.

Agitation was provided by a magnetic stirrer when crystallisations were carried out in the constant temperature room. When a refrigerated water-bath was used, mixing was by an overhead Caframo electric stirrer. In each case, the

impeller dimensions were adjusted in proportion to the scale of the equipment in an attempt to maintain overall geometric similarity between the 20 g and 200 g scale equipment.

However, the nature of the agitation achieved with each type of stirrer used on the 200 g scale was quite different. The impeller with the magnetic stirrer was simply a flat rod lying on the bottom of the flask. This gave a predominantly radial flow pattern in the flask with some vortexing. The propeller of the overhead stirrer was held at a distance above the bottom of the flask and gave much more vertical mixing and less vortexing.

The fat solutions at the second crystallisation were seeded, when cooled to the final crystallisation temperature, with 0.1 g l^{-1} of crystals produced in an earlier tallow fractionation and which melted similarly to cocoa butter. This allowed the total holding time at the second crystallisation to be reduced from 72 to 24 hours.

The final solution temperatures for the first and second crystallisation were 12.5°C and 8°C respectively when 20 g had been fractionated. Therefore two hundred g of tallow dissolved in acetone (4:1(v/w), acetone : tallow) were cooled in the constant temperature room to 12.5°C before filtering. Because of the lower surface area : volume ratio of the larger vessel, the cooling rate of the fat solution was much slower. Similarly, the fat/acetone solution at the second crystallisation (12:1(v/w), acetone : fat) was cooled to 8°C, but at a slower rate than for the smaller scale. The cooling curves for this fractionation of 200 g are presented in Figure 8.1.

Agitation was provided by a magnetic stirrer. It was difficult to duplicate the stirring conditions for the 20 g fractionations exactly because no quantitative data was obtained for the stirrer speed on the 20 g scale, and the stirring rate changed with changing viscosity of the fat solution. However, visual assessment of the conditions used on the smaller scale showed that the agitation was not quite sufficient to keep all of the crystals in suspension at the end of the crystallisation, which resulted in some settling. Thus, the stirrer speed for the 200 g fractionation was adjusted to provide similar conditions.

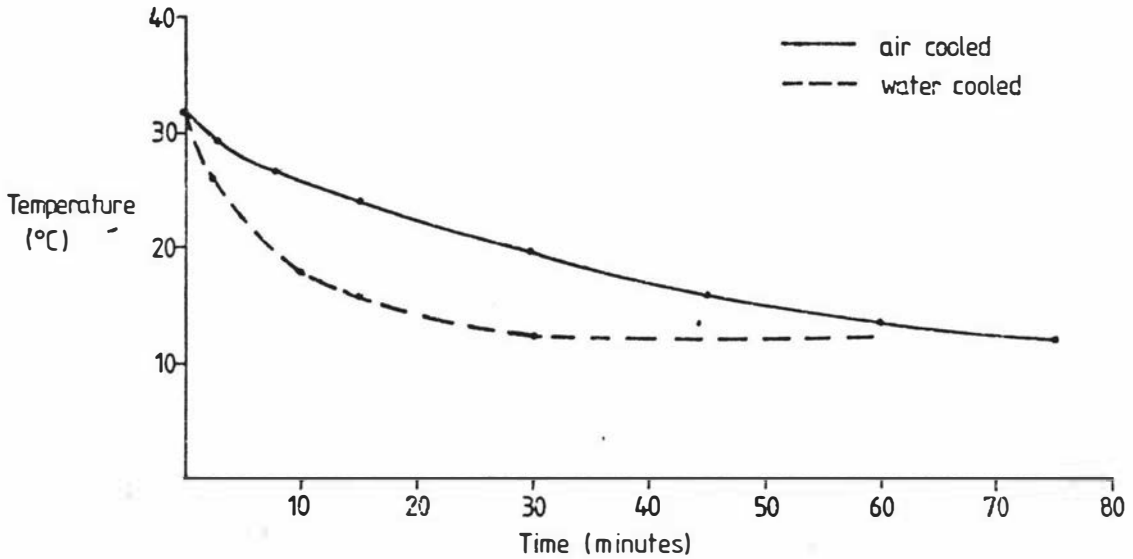
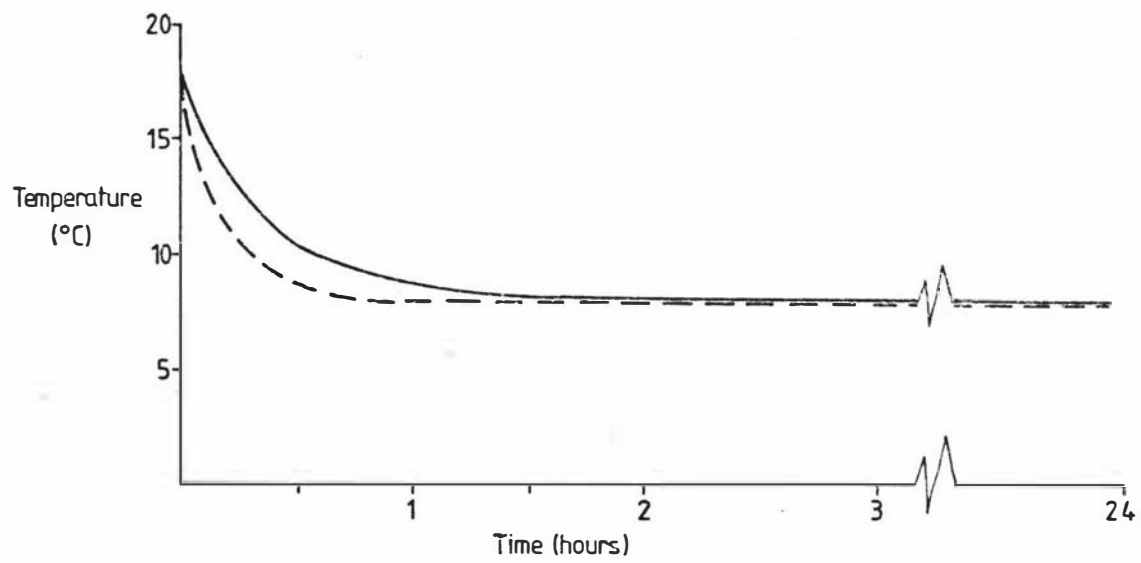


Figure 8·1: Cooling curves for the fractionation of 200g of tallow.
(a) First crystallisation.



(b) Second crystallisation.

8.2.2 Results

The yield of hard fraction was 38.0%, and the yields of the intermediate and soft fractions were 4.8% and 57.2% respectively; cf. 29.2%, 8.3% and 62.5% obtained from 20 g tallow. The DSC profile of the intermediate fraction produced on the 200 g scale is presented in Figure 8.2. It can be seen that it melts similarly to cocoa butter, but with a proportion of the fraction melting at a slightly lower temperature.

The above fractionation was repeated with the fat solution cooled in a water bath. This allowed the fat solution to be cooled at a rate similar to that achieved with the 20 g fractionation - see Figure 8.1. Stirring speed was set to 130 rpm to give a degree of agitation similar to that achieved with the magnetic stirrer. However, as mentioned above the flow regime with this stirrer was quite different from that obtained with the magnetic stirrer. Fractionation under these conditions gave yields of the hard, intermediate and soft fractions of 30.5%, 10.5% and 59.0% respectively. The DSC of the intermediate fraction was very similar to that of the intermediate fraction produced on the 200 g scale with air cooling.

As both cooling rate and the type of stirrer were altered for the above run, a further run was performed with slow cooling (i.e. cooled in the constant temperature room) but with the overhead stirrer at a speed of 130 rpm. The cooling curves for the fat solutions were identical to those for the first air-cooled run using 200 g tallow. The yields of the hard, intermediate and soft fractions obtained were 27.3%, 12.5% and 60.2% respectively.

The DSC profile of the intermediate fraction obtained was virutally identical to that of the intermediate fractions produced in the previous 200 g fractionations. The results of the 200 g runs, and the 20 g run using similar temperatures and solvent : fat ratios, are presented in Table 8.1.

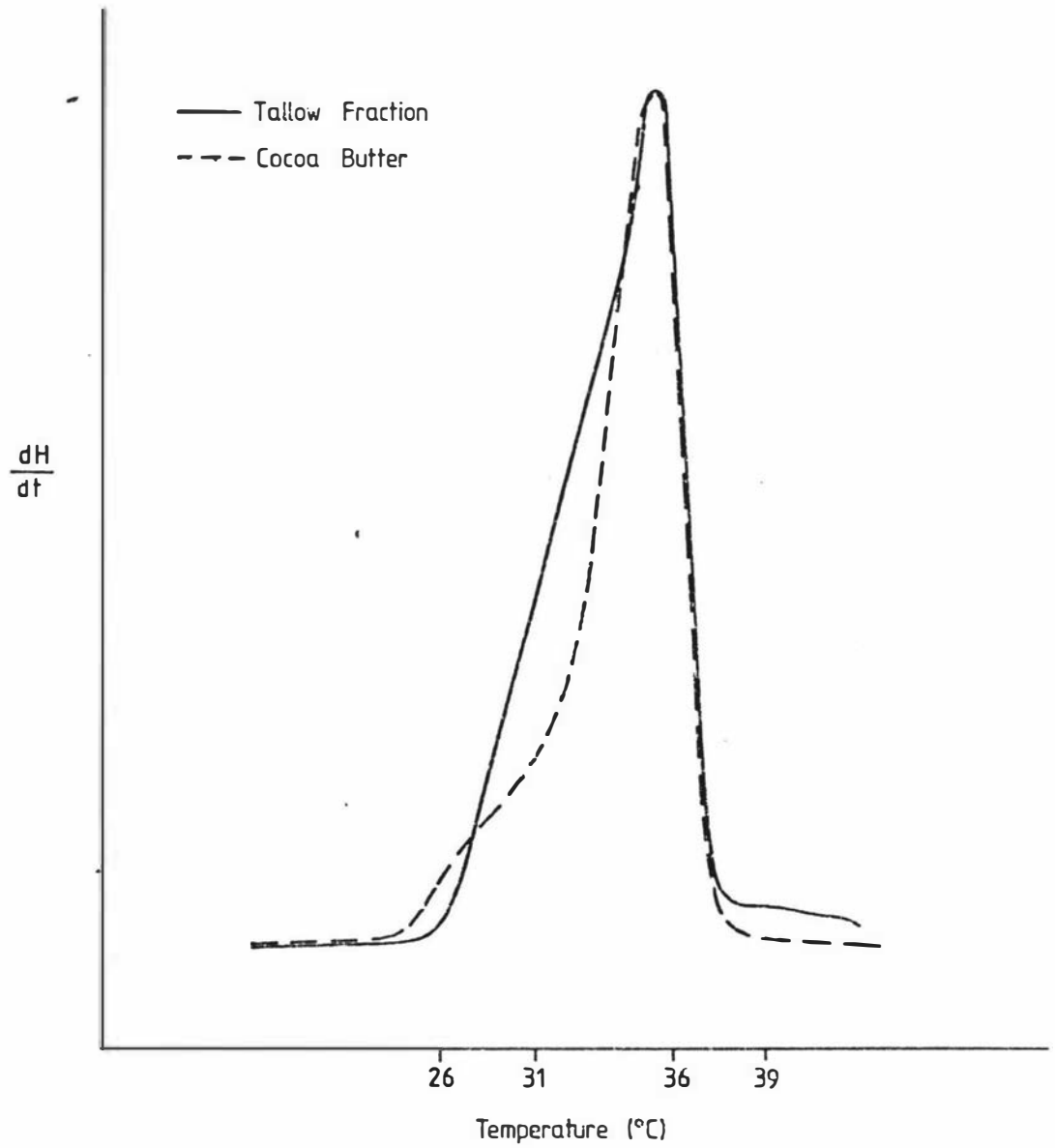


Figure 8.2 : DSC profile of the intermediate tallow fraction produced by fractionating 200g of tallow with air cooling; and the DSC profile of a cocoa butter sample.

Table 8.1: Yields and characteristics of fractions obtained from 20 g and 200 g tallow using similar temperatures and solvent : fat ratios (T_1 = 12.5°C, T_2 = 8°C, S_1 = 4:1, S_2 = 12:1)

Weight of tallow (g)	Cooling rate	Stirrer	Hard fraction (wt % of tallow)	Intermediate fraction (wt % of tallow)	Soft fraction (wt % of tallow)	DSC of intermediate fraction
20	Fast	Magnetic	29.2	8.3	62.5	Similar to cocoa butter, but a small amount of the fraction melts at a lower temp. (Fig. 7.5)
200	Slow	Magnetic	38.0	4.8	57.2	Slightly sharper than the above fraction (Fig. 8.2)
200	Fast	Overhead	30.5	10.5	59.0	"
*200	Slow	Overhead	27.3	12.5	60.2	"

* The products from this fractionation were analysed in Chapter 9.

8.2.3 Yields of the Three Fractions

In Chapter 7, it was found that altering the fractionation conditions over the range studied was only influencing the relative proportion of the hard and intermediate fractions, and not of the soft fraction. Similar results were obtained with the 200 g fractionations, i.e. about 40% of tallow went into the hard and intermediate fractions, and about 60% of the tallow into the soft fraction.

An increase in the yield of the intermediate fraction, while maintaining similar melting properties, was accompanied by a corresponding decrease in the yield of the hard fraction.

8.3 FRACTIONATION OF ONE KILOGRAM OF TALLOW

8.3.1 Methods

The fractionations were performed in a jacketed, stainless steel, stirred crystalliser. A built-in filter allowed

filtration of the crystals to be performed at the crystallisation temperature. Agitation was provided by a close-clearance anchor impeller driven by a variable-speed electric motor, to reduce the amount of crystal encrustation upon the cooling surface.

Cooling was achieved by pumping chilled water through the external jacket and the cooling rate controlled by varying the temperature and flow-rate of the cooling water. A solid state controller maintained the water temperature within a range of $\pm$ 0.1°C of the set point.

Seeding at the second crystallisation was performed as in Section 8.2.1. A diagram of the apparatus is presented in Figure 8.3.

The temperatures and solvent : fat ratios were T_1 = 12.5°C, T_2 = 8°C, S_1 = 4:1 and S_2 = 12:1 as in the smaller scale fractionations. The cooling rate was adjusted to approximate that attained with the air-cooled 200 g fractionations (see Figure 8.4), although the shape of the cooling curves differed slightly. Maintenance of an impeller tip speed in the large crystalliser similar to the impeller tip speed of the overhead stirrer in the 200 g fractionations required a stirrer speed of 20 rpm in the large crystalliser. However, the minimum speed achievable with the stirrer motor on the large crystalliser was 25 rpm, and the different types of stirrers and the different shapes of the crystallisers meant that the flow regimes within each crystalliser were very different.

The response variables were the yields, by weight, of the three fractions and the DSC profile of the intermediate fraction. Because of the close similarity in the DSC profiles of the intermediate fractions produced on the 1 kg scale, and in the DSC profile of cocoa butter, the CBLF was not a valid indication of the relative melting properties of the fractions. Fractions were produced which had similar melting ranges and similar DSC peak maximum temperatures, but which had DSC peaks that differed in shape within these limits. Thus a descriptive comparison of the DSC profiles of the intermediate fractions was used.

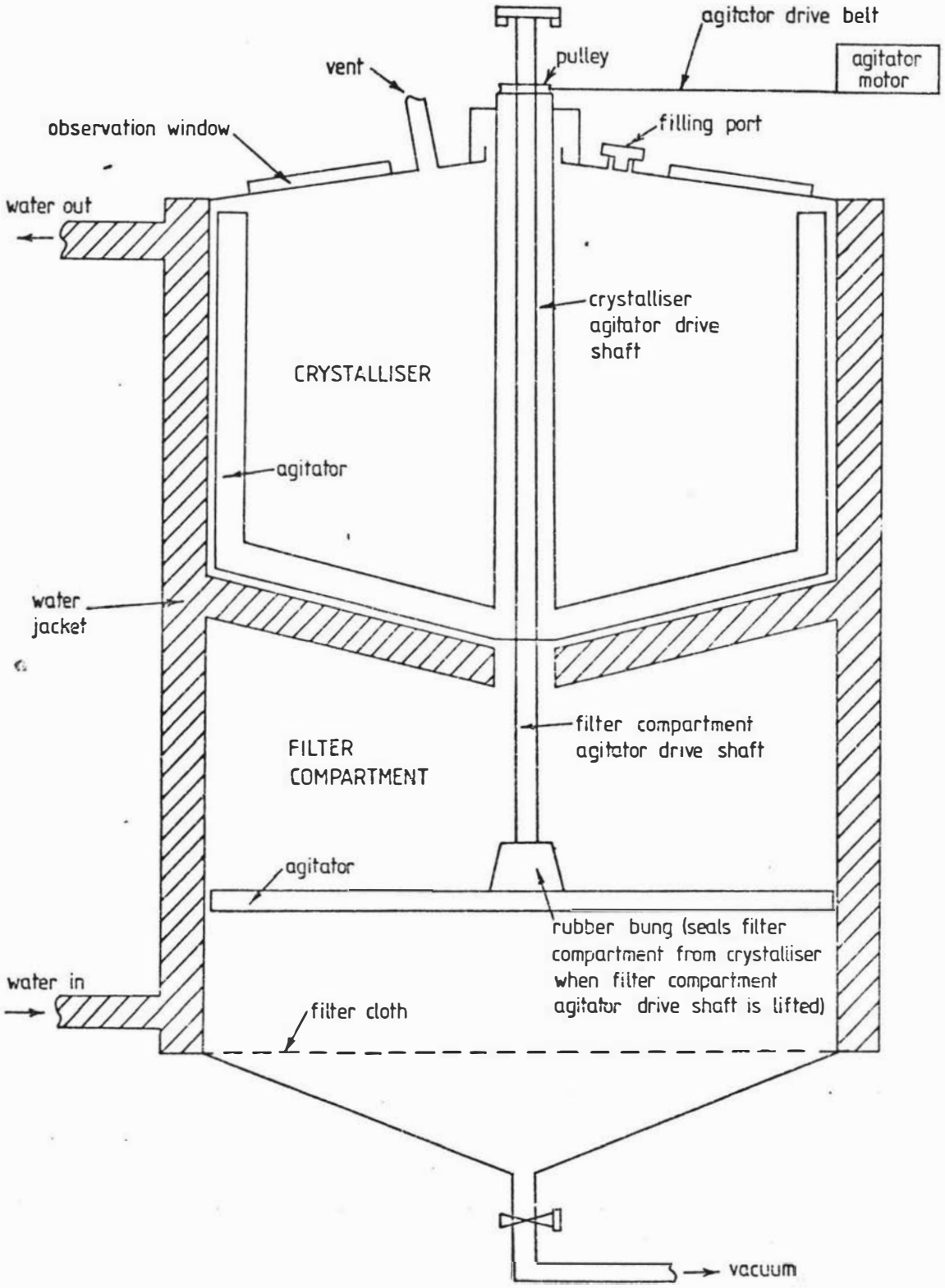


Figure 8·3 : Apparatus used for the fractionation of 1kg of tallow.

Scale 1:3

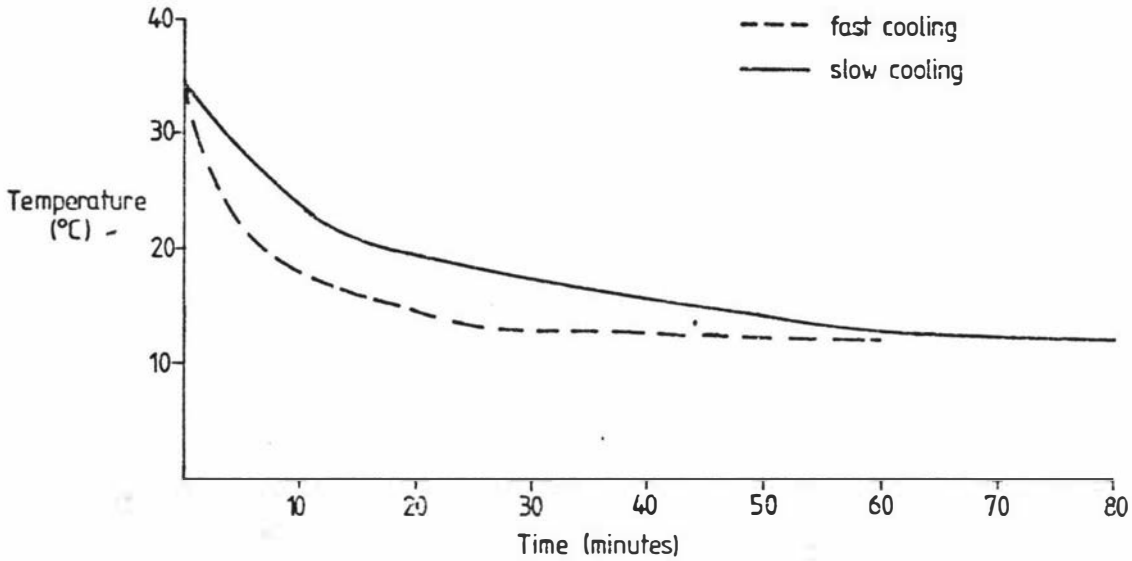
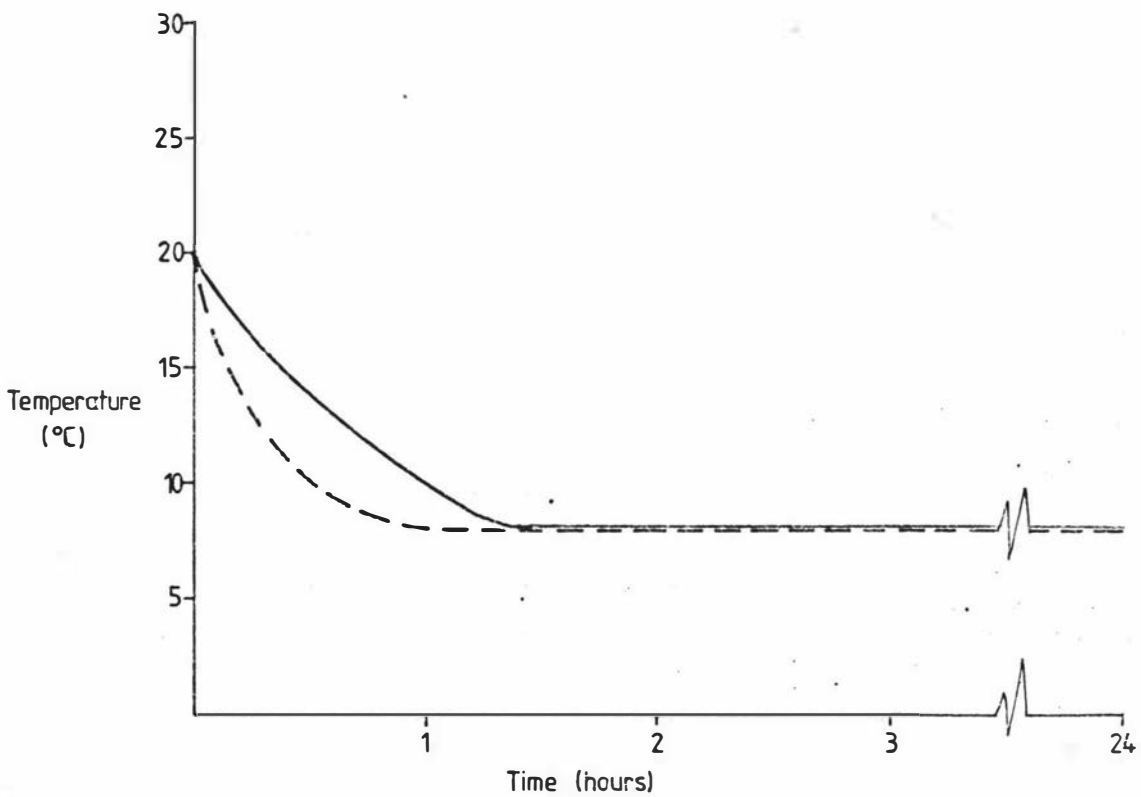


Figure 8·4 : Cooling curves for the fractionation of 1kg of tallow.
(a) First crystallisation.



(b) Second crystallisation.

8.3.2 Results

The yields of the hard, intermediate and soft fractions produced were 24.0%, 10.1% and 65.9% respectively. The DSC profile of the intermediate fraction is shown in Figure 8.5. It can be seen that while the DSC peak maximum for this fraction occurred at the same temperature as for cocoa butter, the melting range was greater than for cocoa butter, but similar to that of the intermediate fractions produced on the 200 g scale. The yields of the hard and intermediate fractions were less than in the most desirable 200 g fractionation.

When the stirring speed was increased from 25 to 50 rpm yields of the hard, intermediate and soft fractions were 23.0%, 9.8% and 67.2% respectively. In addition the DSC profile of the intermediate fraction was very similar to that produced using the slower agitation speed.

Since the cooling rate was lower than used in the fractionation of 200 g tallow, a further fractionation using a more comparable cooling rate (see Figure 8.4) was carried out with a stirrer speed of 25 rpm. This produced hard, intermediate and soft fractions in yields of 24.0%, 9.8% and 66.2% respectively. The DSC profile of the intermediate fraction was very similar to that of the previous intermediate fractions produced by fractionating 1 kg of tallow.

Increasing the agitation speed to 50 rpm and using the faster cooling rate gave 23.6%, 10.2% and 66.2% of the hard, intermediate and soft fractions respectively. The DSC profile of the intermediate fraction was very similar to that of the other intermediate fractions produced by fractionating 1 kg of tallow.

These results are summarised in Table 8.2.

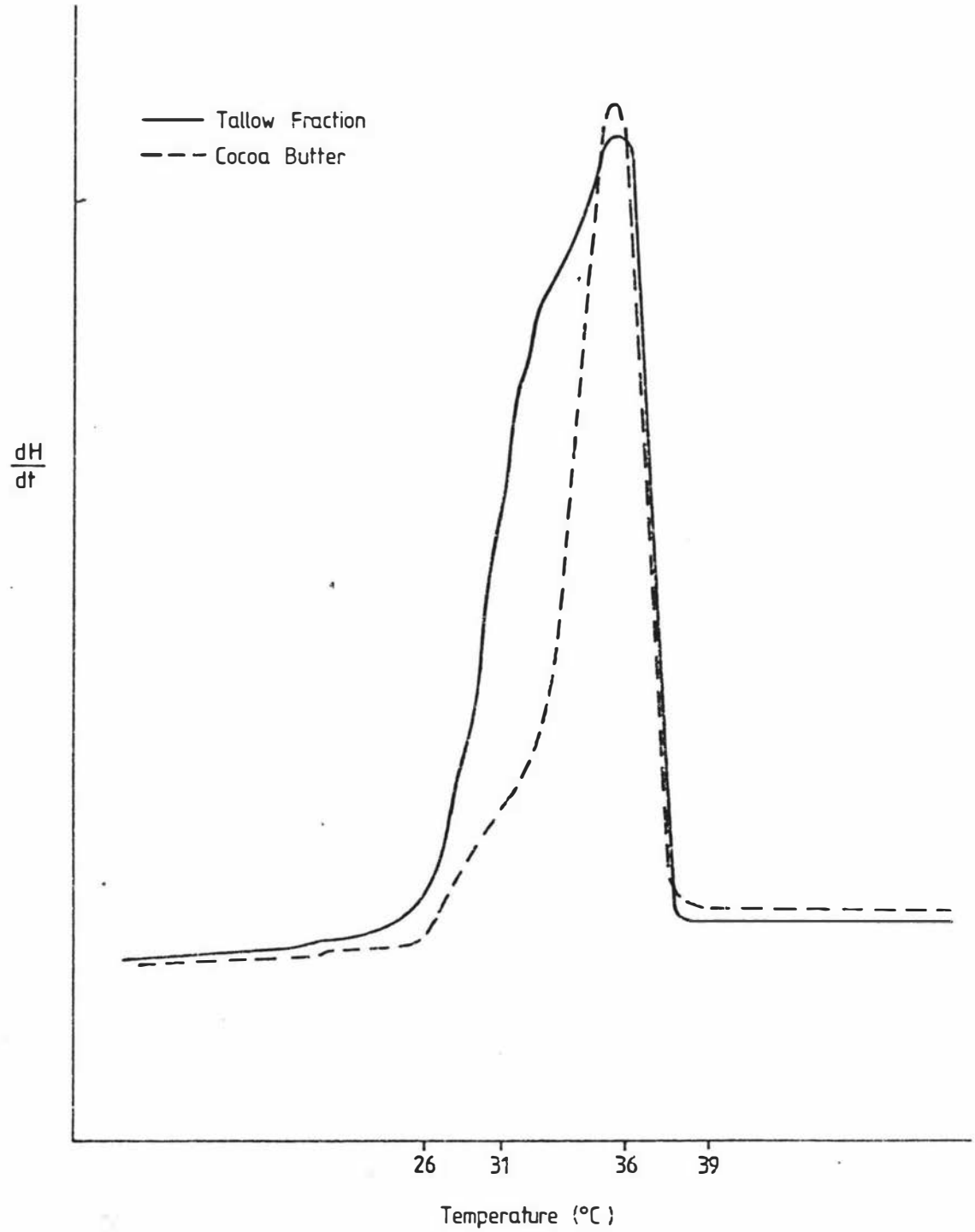


Figure 8·5: DSC profile of the intermediate fraction produced by fractionating 1kg of tallow with stirrer speed of 25rpm; and the DSC profile of a cocoa butter sample.

Table 8.2: The effect of agitation speed and cooling rate upon the fractionation of 1 kg of tallow

Cooling rate	Stirrer speed (rpm)	Hard fraction (wt % of tallow)	Intermediate fraction (wt % of tallow)	Soft fraction (wt % of tallow)	DSC of the intermediate fraction
Slow	25	24.0	10.1	65.9	DSC peak maximum occurs at the same temperature as cocoa butter, but a proportion of the fraction melts at a lower temperature (Figure 8.5)
Slow	50	23.0	9.8	67.2	"
Fast	25	24.0	9.8	66.2	"
Fast	50	23.6	10.2	66.2	"

Thus overall, varying the stirrer speed and/or the cooling rate between the above levels did not significantly affect the fractionation.

8.3.3 Comparison of the one kilogram fractions with those of the smaller scale fractionations

Each of the fractionations performed on 1 kg of tallow gave an intermediate fraction with a melting curve which was very similar to that of the intermediate fraction produced by fractionating 20 g of tallow using similar crystallisation temperatures and solvent : fat ratios (see Figure 7.5). However, the sizes of the intermediate fractions obtained from 1 kg tallow were slightly greater than from 20 g tallow (average of 10.0% compared to 8.3%). The average yield of hard fraction from 1 kg tallow was 23.7% compared to 29.2% from 20 g tallow. More of the hard and intermediate fractions, and less of the soft fraction, were produced on the 200 g scale than the 1 kg scale.

A comparison of the yields of the three fractions obtained on the different scales, using T_1 = 12.5°C, T_2 = 8°C, S_1 = 4:1 and S_2 = 12:1, is presented in Table 8.3. The 1 kg fractionation using a slow cooling rate and stirrer speed of 50 rpm was repeated, at a later time, 15 times to produce sufficient of each of the three fractions for further testing. From the

standard deviation of the yields of each of the fractions obtained from the 15 runs, the differences in yields obtained on each of the three scales (as shown in Table 8.3) were found to be significant at the 5% level.

Table 8.3: A comparison of the yields of the three fractions produced from the fractionations of different amounts of tallow (with T_1 = 12.5°C, T_2 = 8°C, S_1 = 4:1, S_2 = 12:1) using a slow cooling rate.

Weight tallow (g)	Hard fraction (wt % of tallow)	Intermediate fraction (wt % of tallow)	Soft fraction (wt % of tallow)
20	29.2	8.3	62.5
200	27.3	12.5	60.2
1000*	23.0	9.8	67.2

* average of 19 fractionations

In the fractionation of 1 kg tallow, variation of cooling rate and/or the agitation speed did not significantly affect either the yields of the three fractions or the melting properties of the intermediate fraction. Similarly, changing the cooling rate over a comparable range did not influence the fractionation of 200 g tallow. However, with 20 g tallow varying the agitation speed affected the melting properties of the intermediate fraction but not the proportion of the three fractions (see Figure 6.10). However, the range of stirrer speeds used on the 20 g scale was much greater than for the 1 kg scale, and the nature of the agitation quite different.

8.3.4 Further Attempts to Fractionate 1 kg of Tallow

The intermediate fractions produced from 200 g and 1 kg tallow melted less sharply than cocoa butter, but similarly to the 20 g intermediate fraction produced using similar solvent to fat ratios and crystallisation temperatures. Intermediate fractions melting similarly to cocoa butter were obtained by fractionating 20 g of tallow, but these were obtained in lower yields (see Section 6.11). The yield of the hard fractions from the 20 g fractionations where the sharpest-melting intermediate fractions were produced averaged 35.6%, compared to only 23% hard fraction when 1 kg of tallow was fractionated.

Thus, it was probably necessary to remove more of the tallow in the first crystallisation if the melting properties of the intermediate fraction produced from 1 kg of tallow were to be made more similar to those of cocoa butter.

Altering the cooling rate and/or the stirring speed did not alter the yield of the hard fraction, so the effect of altering the first crystallisation temperature (T_1), the solvent to fat ratio at the first crystallisation (S_1) and the water content of the acetone (W) were studied to determine their effect upon the yield of the hard fraction and the melting properties of the intermediate fraction. The stirrer speed was set at the previous high level of 50 rpm because, even though agitation speed had not been shown to significantly affect the 1 kg fractionations performed previously, it may have had a significant effect at different levels of T_1 and S_1.

Two fractionations were performed with S_1 = 4:1, T_1 = 12.5°C and the fast cooling rate used in the previous 1 kg fractionations. Changing the water content of the acetone from 0.6% to 0.5% and 0.7% did not significantly affect the fractionations.

A summary of the fractionations where T_1 or S_1 were altered is given in Table 8.4. Two 1 kg fractionations were performed, as above, but with T_1 decreased by 1°C to 11.5°C. The cooling rate was set at the slow and fast rates used in the previous fractionations upon 1 kg of tallow. Five further fractionations were performed with T_1 = 12.5°C, a stirrer speed of 50 rpm, and cooling at the fast rate used previously. S_1 was set at different levels for each of these fractionations. The values of S_1 were 1.0:1, 1.5:1, 1.7:1, 2.0:1 and 2.5:1.

The overall effect of lowering T_1 was to soften the intermediate fraction, (see Figure 8.6), even though by also using a fast cooling rate the yield of the hard fraction was similar to that obtained with the best fractionations on the 20 g scale.

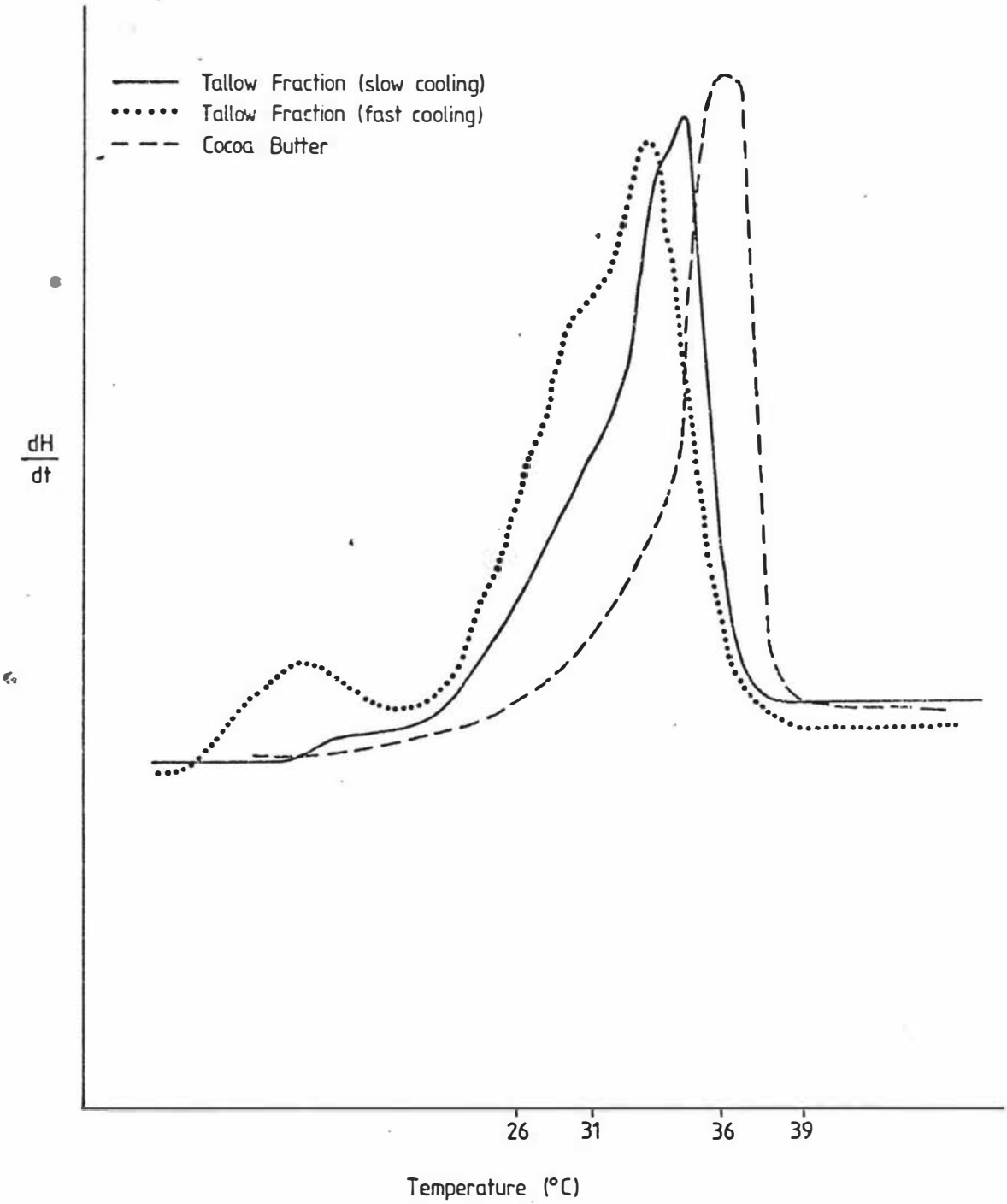


Figure 8·6 : DSC profiles of the intermediate fractions produced by fractionating 1kg of tallow with T1 = 11·5°C and slow and fast cooling rates; and the DSC profile of a cocoa butter sample.

With T_1 set at 12.5°C, altering S_1 had a great effect upon the yield of the hard fraction, and a slight effect upon the yield of the intermediate fraction. See Figure 8.7 for a plot of the yield of hard fraction against S_1. It can be seen that up to a critical ratio of approximately 2:1, the yield of hard fraction was very sensitive to the level of S_1.

8.4 COMPARISON OF ALL FRACTIONATIONS

The effect of S_1 upon the yield of the hard fraction from fractionations upon 20 g of tallow with T_1 = 12.5°C are also plotted in Figure 8.7. Overall there is close similarity between the two curves except at low S_1 values. The difference between the 1.0:1, 1.6:1 and 2.3:1 values for the 20 g fractionations cannot be said to be significant as there was only one run performed with S_1 = 1.6:1. The discrepancy between the 20 g and 1 kg fractionations at low S_1 values is important as the intermediate fractions with the best melting properties from 20 g were obtained with S_1 = 1:1. Obviously, the system using 1 kg was responding quite differently at low levels of S_1. It was necessary to increase S_1 to 1.5:1 or 1.7:1 to obtain similar yields of the hard fraction. Although this adjustment did not produce intermediate fractions which melted as sharply as those of the best 20 g fractions, they melted more like cocoa butter than the fraction of Figure 8.8 which was produced in a fractionation where the yield of hard fraction was 44.0%. This was obtained by setting S_1 = 1:1, the same as was used for the best 20 g fractionations.

The intermediate fractions produced on the 1 kg scale with T_1 = 12.5°C and S_1 values of 1.5:1 or greater, all melted similarly to the fraction analysed in Figure 8.9. This was in contrast to the 20 g scale fractionations, where altering S_1 over a similar range altered the melting properties of the intermediate fraction (see Figure 6.7d).

There was close similarity between the melting of the intermediate fractions produced by fractionating 200 g of tallow with S_1 = 4:1 and those produced on the 1 kg scale with S_1 = 1.5:1 or greater. The yields obtained on the 200 g scale were similar to those obtained on the 1 kg scale with S_1 = 2.5:1.

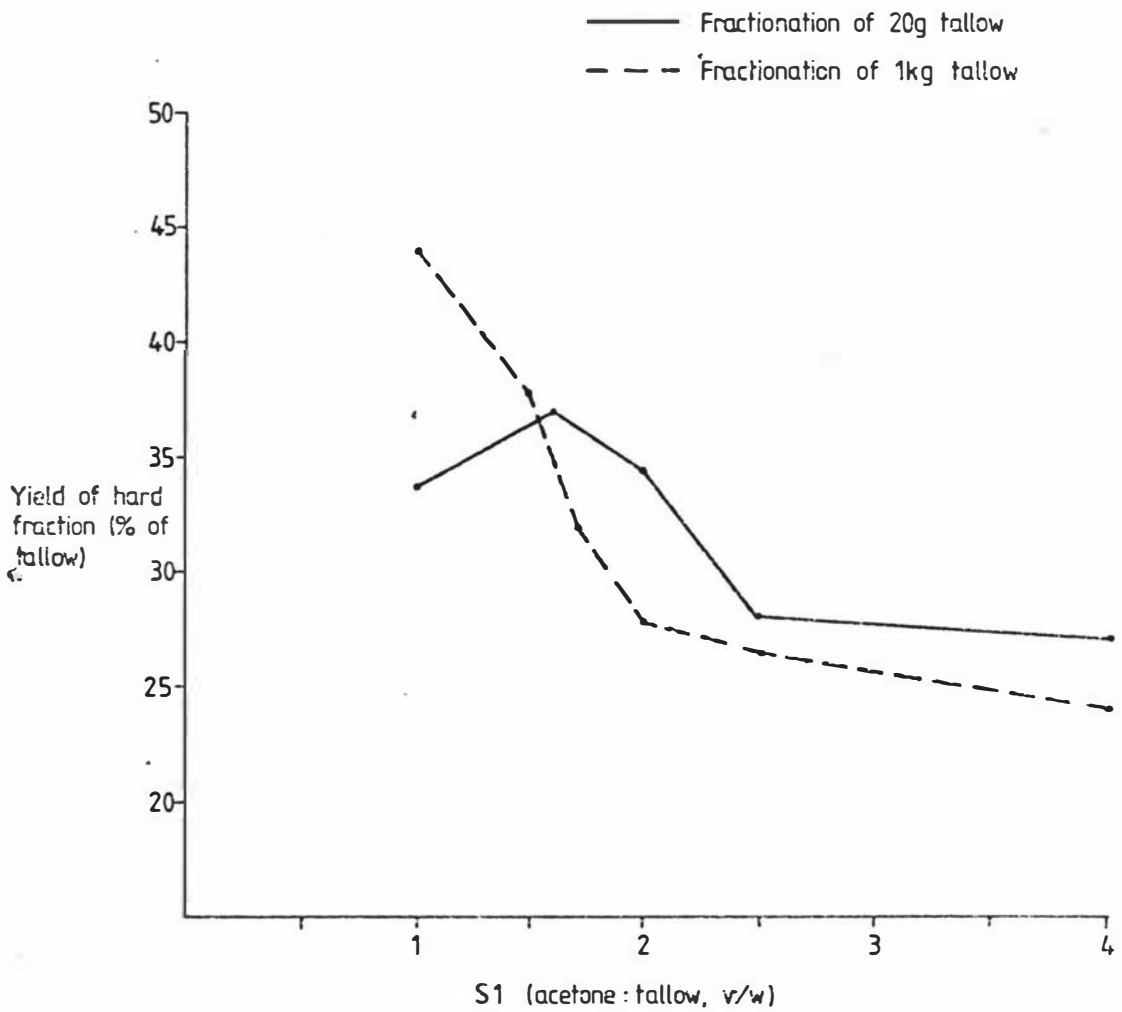


Figure 8·7: The effect of varying S1 upon the yield of hard fraction.

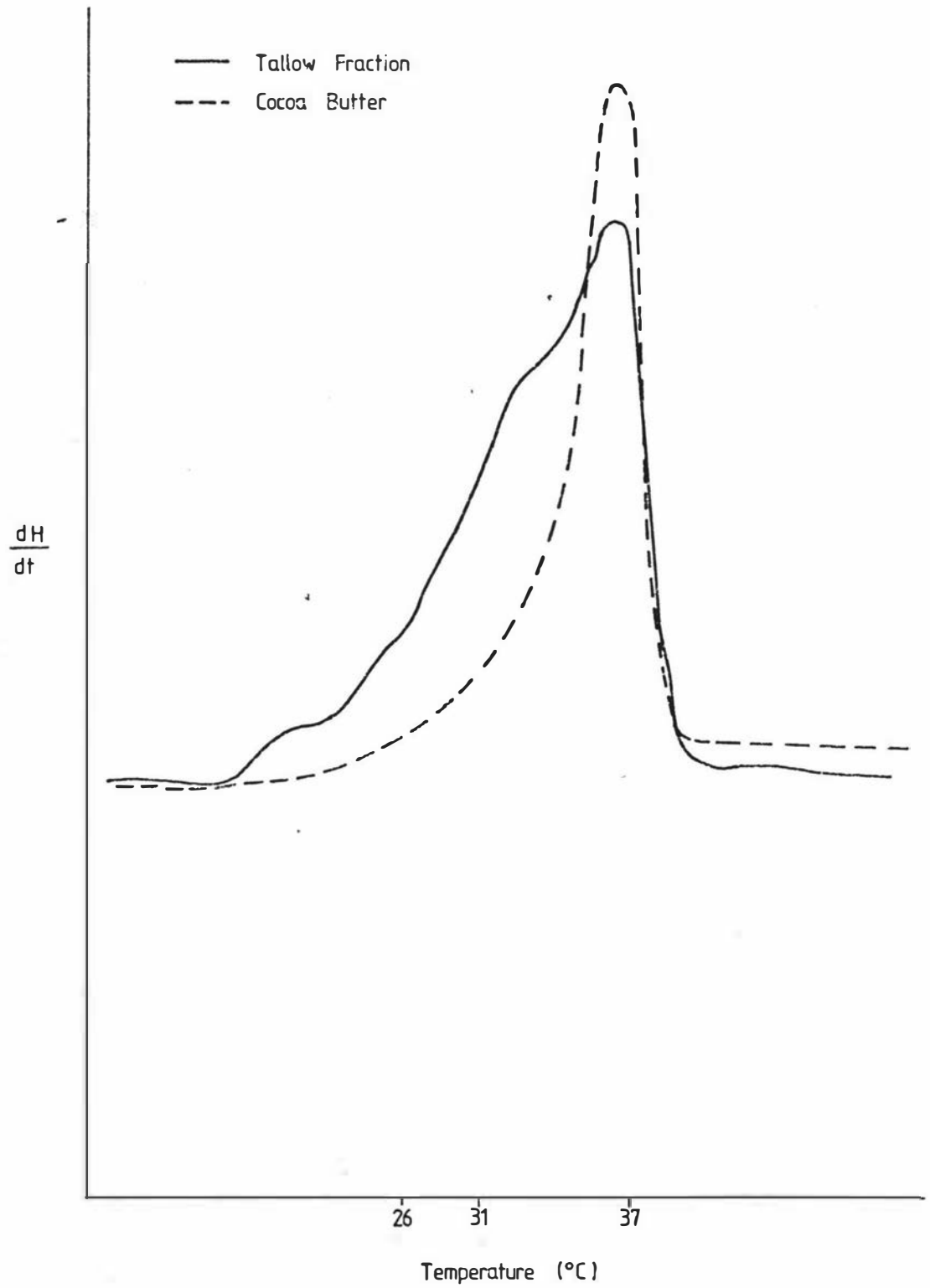


Figure 6·6 : DSC profile of the intermediate fraction produced by fractionating 1kg of tallow with S1 =1:1; and the DSC profile of a cocoa butter sample.

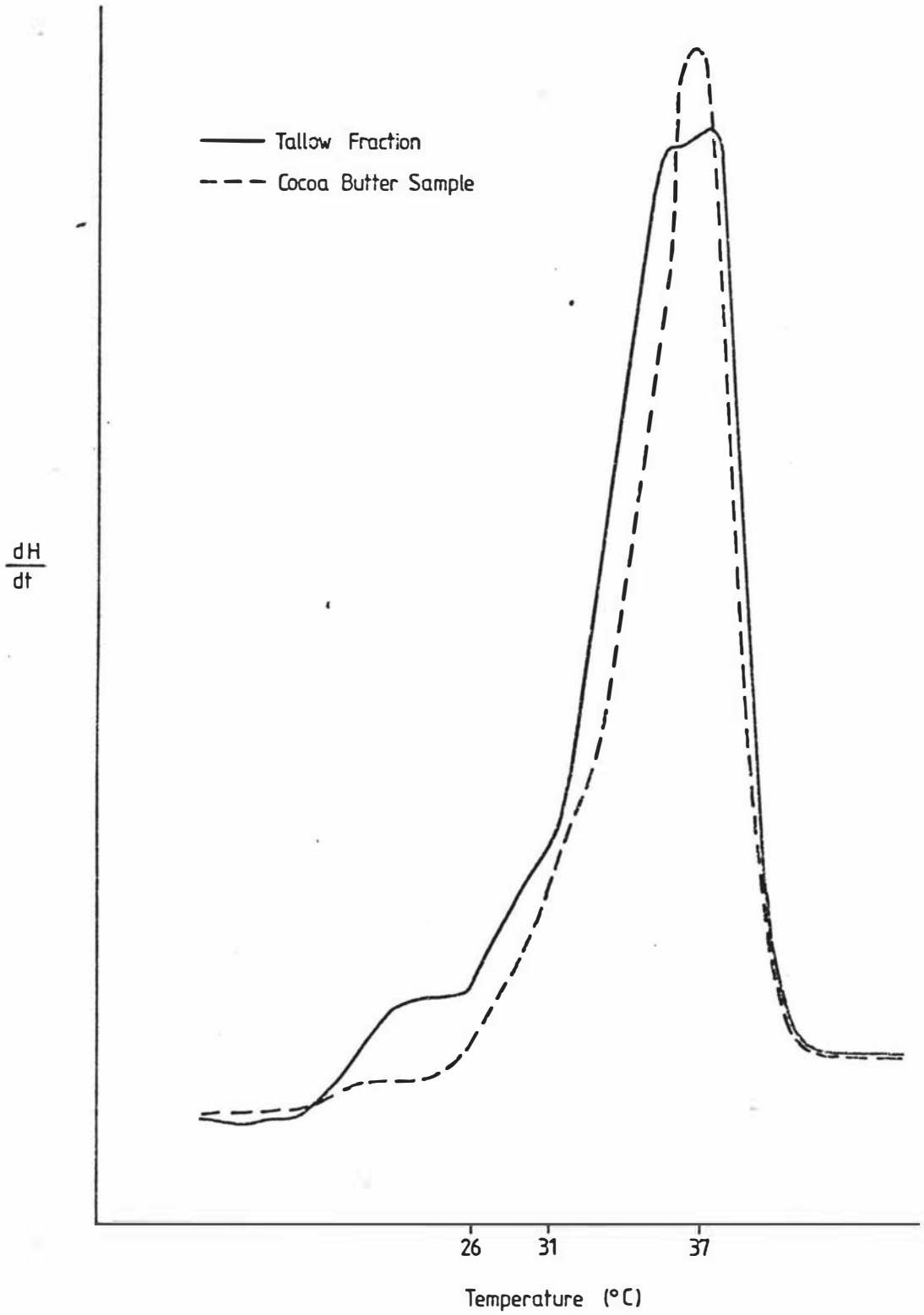


Figure 8·9 : DSC profile of the intermediate tallow fraction produced by fractionating 1kg of tallow with S1 = 1·5 : 1; and the DSC profile of a cocoa butter sample.

Table 8.4: The fractionation of 1 kg of tallow with different levels of T_1 or S_1

T_1 (°C)	S_1	Cooling rate	Stirrer speed (rpm)	Hard fraction (wt % of tallow)	Intermediate fraction (wt % of tallow)	Soft fraction (wt % of tallow)	DSC profile of the intermediate fraction
12.5	1.0:1	Fast	50	44.0	7.2	48.8	Peak maximum occurred at the same temperature as cocoa butter, but a proportion of the fraction melted at a lower temperature (Fig. 8.8)
12.5	1.5:1	Fast	50	37.3	8.6	54.1	Similar to above, but a smaller (Fig. 8.9) proportion of the fraction melted at a lower temperature.
12.5	1.7:1	Fast	50	32.0	7.9	60.1	"
12.5	2.0:1	Fast	50	28.0	-	-	"
12.5	2.5:1	Fast	50	26.6	9.4	64.0	"
11.5	4:1	Slow	50	22.0	7.0	71.0	DSC peak slightly broader than for cocoa butter, and peak maximum at a temperature 2°C below that of cocoa butter (Figure 8.6)
11.5	4:1	Fast	50	34.0	11.0	55.0	DSC peak slightly broader than above, and peak maximum at a temperature 4°C below that of cocoa butter (figure 8.6).

Increasing S_1 on the 1 kg scale slightly, but significantly, increased the yield of the intermediate fraction. Increasing S_1 on the 20 g scale also significantly increased the yield of the intermediate fraction (see Figure 6.7b). These results are summarised in Table 8.5.

Overall;

(i) The intermediate fractions produced which melted most like cocoa butter were those from the 20 g scale which had small yields (2.5% to 5.8%). A higher yield of an intermediate fraction (8.3%) was obtained on the 20 g scale, but the melting range of this fraction was slightly greater than that of cocoa butter. The process was scaled up for this latter fraction, because of its greater yield, in the hope that the melting properties of the fraction were sufficiently similar to those of cocoa butter for it to be useful.

(ii) An intermediate fraction was produced from 200 g tallow, with a yield of 4.8%, which melted similarly to the 8.3% yield 20 g intermediate fraction mentioned above - i.e. which melted over a slightly greater temperature range than cocoa butter. The yield of this fraction was increased to 12.5%, without affecting its melting properties, by adjusting agitation conditions.

(iii) A similar intermediate fraction was produced from 1 kg tallow, but it had a slightly lower yield (9.8%). Attempts on the 1 kg scale to produce an intermediate fraction which melted more like cocoa butter were unsuccsssful.

There are several possible reasons why fractionations on the different scales behaved differently:

(1) a different surface area to volume ratio on each scale - it is probable that the cooling surface influences nucleation, and hence changes in the surface area to volume ratio with scale may alter the nature of the products precipitated;

(2) different materials of construction of the crystalliser - this may also have an effect upon nucleation and hence alter the nature of the products precipitated.

Table 8.5: Comparison of the results of selected fractions from each of the three scales (with T_1 = 12.5°C, T_2 = 8°C, S_2 = 12:1 or 10:1)

Weight of tallow (g)	S_1	Hard fraction (wt % of tallow)	Intermediate fraction (wt % of tallow)	Soft fraction (wt % of tallow)	DSC profile of the intermediate fraction
20	1:1	34.5	2.5	63.0	Very similar to cocoa butter (see Figure 6.12)
20	2.5:1	36.5	5.8	57.7	"
20	4:1	29.2	8.3	62.5	A higher proportion of the fraction melts at a temperature lower than the temperature of maximum melting than it does with cocoa butter (Figure 7.5)
200 (magnetic stirrer)	4:1	38.0	4.8	57.2	" (Figure 8.2)
* 200 (propeller agitator)	4:1	27.3	12.5	60.2	"
*1000	4:1	23.0	9.8	67.2	" (Figure 8.5)
1000	2.5:1	26.6	9.4	64.0	"
1000	1.7:1	32.0	7.9	60.1	"
*1000	1.5:1	37.3	8.6	54.1	" (Figure 8.9)
1000	1.0:1	44.0	7.2	48.8	Melting range greater than for any of the above fractions (Figure 8.8)

* The three fractions from each of these fractionations were analysed in Chapter 9.

(3) different flow regimes - it was shown on the 200 g scale that changes in the flow regime within the crystalliser (caused by using different impellers) had a vast effect upon the crystallisations. Thus, different flow regimes between the different scales are likely to similarly influence the crystallisations, especially with the crystalliser used for the fractionation of 1 kg of tallow where a completely different type of impeller was used. As this was a close-clearance impeller, crystal deposition upon the cooling surfaces was inhibited relative to the smaller scale fractionations.

Differences in shape of the various crystallisers would also influence the flow regime.

8.5 CONCLUSIONS

(1) Fractionation of 200 g tallow with a first crystallisation temperature (T_1) of 12.5°C, a second crystallisation temperature (T_2) of 8°C, a solvent to fat ratio at the first crystallisation (S_1) of 4:1 and a solvent to fat ratio at the second crystallisation (S_2) of 12:1 produced an intermediate fraction which melted similarly to the intermediate fraction produced under similar conditions on the 20 g scale, but not as sharply as the 20 g intermediate fractions with the melting properties most like those of cocoa butter. Replacing the magnetic stirrer with an overhead stirrer greatly increased the yield of the intermediate fraction without altering its melting properties, and decreased the yield of the hard fraction. Changing the cooling rate within the limits used did not affect the fractionations on the 200 g scale.

(2) Fractionation of 1 kg of tallow using the above temperatures and solvent to fat ratios produced an intermediate fraction which melted similarly to that produced in the 200 g fractionations. Altering the stirrer speed and/or the cooling rate did not affect the fractionations upon the 1 kg scale. Decreasing T_1 to 11.5°C produced an intermediate fraction which melted at a lower temperature than cocoa butter. The yield of the hard fraction was very sensitive to S_1 values less than 2.0:1. Altering S_1 to 1.5:1 or 1.7:1 produced intermediate fractions which melted similarly to those produced with S_1 = 4:1, but the yield of hard fraction was greater, and the yield of soft and intermediate fractions less.

(3) The intermediate fraction produced from 20 g tallow (see Chapter 6) which melted similarly to cocoa butter could not be reproduced on the larger scales.

(4) There was a great difference in the response of the fractionation system with changes in the scale (and hence equipment) of fractionation.

8.6 DISCUSSION AND CONCLUSIONS ON THE FRACTIONATION METHODS

The 2-stage solvent fractionation process used has been at least partially successful in producing a fraction with melting properties more similar to those of cocoa butter than the original tallow. While fractionating 20 g of tallow produced intermediate fractions with melting properties similar to those of cocoa butter, the yield of these averaged only 4.0 wt % of the tallow. A fractionation scheme involving a greater number of crystallisation steps may have achieved a greater yield of a similar fraction, however this would have increased the complexity and cost of the process. Any attempt to increase the yield of the intermediate fraction in this work was only achieved with a consequential reduction in the similarity of the melting properties of the fraction to those of cocoa butter. This suggests that the degree of separation being achieved was the maximum obtainable with this 2-stage fractionation process, at least within the range of levels of the independent variables which were studied.

The different scales of fractionation all responded differently to variations in the levels of the independent variables, and attempts on the 1 kg scale to produce an intermediate fraction with melting properties similar to those of the best 20 g intermediate fraction were unsuccessful.

Because of the difficulties in attempting to scale-up the process, it may have been advantageous to use crystallisation equipment for the 20 g fractionations which was similar to that used for the 1 kg fractionations as it is obviously not possible to reproduce on a large scale the equipment used for the 20 g fractionations. However even this may not have been successful as the 200 g fractionations used equipment similar to that used in the 20 g fractionations, but the smaller-scale fractionation could still not be duplicated exactly. However here it was not possible to copy the flow regime and cooling rate of the smaller scale fractionation simultaneously.

Because of the large differences in the behaviour of the crystallisations on the various scales, and the inability to determine the critical factor(s) causing this, it is not possible to accurately predict scale-up criteria for future work, and it may be very difficult to reproduce the fractionation on a larger scale.

A further difficulty is the extreme sensitivity of the system to some of the process variables. On the 1 kg scale, a change in the first crystallisation temperature (T_1) of $1^{o}C$ caused large changes in both the yields of the three fractions and the melting properties of the intermediate fraction. Similarly, slightly changing the solvent to fat ratio at the first crystallisation (S_1) over some levels on the 1 kg scale caused large changes in the yields. In this work it was necessary to carefully control each of these variables in order to obtain consistent results, and careful control will be required in any future work on a larger scale. The sensitivity of the system to changes in these variables may have been at least partly responsible for some of the difficulties in scale up.

Apart from the fractionations performed in the screening experiment, all fractions were upon Smithfield 1½ R November 1977 tallow. Thus the effect of variation in tallow composition upon the fractionation process has not been determined in the work. Before any larger scale work or commercial feasibility study can be implemented, it will be necessary to make a further study of this.

The crystals produced at each crystallisation were in a form suitable for filtration, and filtration rates were very high in every case.

CHAPTER 9

EVALUATION OF THE FRACTIONATION PRODUCTS

9.1 INTRODUCTION

The aims of this chapter are:

(i) to study the composition and melting properties of some of the fractionation products, and to determine how changes in various process parameters affect these characteristics.

(ii) to determine the suitability of some of the fractionation products for specific applications.

Samples chosen for further analysis were:

(i) the best-melting intermediate fraction produced on the 20 g scale (see section 6.10).

(ii) the three fractions produced in the 200 g scale fractionation where the highest yield of intermediate fraction was obtained (see section 8.2.2).

(iii) the three fractions from the 1 kg scale fractionation where a solvent : fat ratio at the first crystallisation (S_1) of 4:1 was used (see table 8.5).

(iv) the three fractions from the 1 kg scale fractionation where a solvent : fat ratio of 1.5:1 at the first crystallisation was used (see Table 8.5).

Each of the above fractions was analysed as follows, and the results compared with other fats:

(i) fatty acid composition

(ii) the proportion and fatty acid composition of each of the triglyceride groups separated by argentation TLC.

(iii) the fatty acid composition at the 2-position of the cis monounsaturated triglycerides.

Representative hard and soft fractions were also analysed by DSC.

Details of the chemical analysis of each of the fractions are given in appendix 7.

The hard and soft fractions from the 1 kg fractionation with S_1 = 4:1 were tested in specific applications. The hard fraction was assessed for performance as a baking fat, both in a blend with butter and in a blend with the soft tallow fraction. The hard tallow fraction was also tested as a pastry fat, and the soft tallow fraction was tested as a frying medium and in the manufacture of mayonnaise. The melting behaviour of the intermediate fraction from the 1 kg fractionation with S_1 = 4:1 was studied by nuclear magnetic resonance (NMR) and compared with cocoa butter, a cocoa butter substitute (Nucoa) and a blend of cocoa butter and the intermediate tallow fraction (90% cocoa butter/10% tallow intermediate fraction).

9.2 FATTY ACID COMPOSITION OF THE FRACTIONS

The overall fatty acid composition of each of the fractions, and of the original tallow, are presented in table 9.1.

9.2.1 Hard Fractions

The proportion of 14:0, 16:0 and 18:0 was higher in each of the hard fractions than in the original tallow, and the proportion of 16:1 and 18:1 less.

The fatty acid compositions of the two hard fractions produced from 1 kg of tallow were quite different. However the yields of these two fractions were also quite different (37.3% and 23.0%). The higher yield fraction contained a lower proportion of saturated fatty acids and a higher proportion of 18:1 than the lower yield fraction (27.7% of 18:1 in the higher yield fraction compared to 19.3%).

The hard fraction produced from 200 g of tallow had a yield of 27.3%, which was similar to the yield of the hard fraction produced from 1 kg of tallow with S_1 = 4:1 (23.0%). The fatty acid composition of these two fractions was very similar.

Table 9.1: Fatty acid composition of tallow and fractions obtained by solvent crystallisation

		Fatty Acid (mole % in the fraction)										
		14:0	14:1 + iso-15:0	15:0	iso-16:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2
Scale of fractionation	Fraction											
20 g (S_1 = 4:1)	Intermediate	5.1	0.4	0.5	0.1	22.4	1.6	1.9	0.4	36.8	32.3	0.5
200 g (S_1 = 4:1)	Hard	8.4	0.3	1.1	1.0	33.6	2.8	2.6	0.2	30.6	18.7	0.7
	Intermediate	6.0	0.5	0.7	0.2	25.2	2.9	2.4	0.6	30.3	30.7	0.5
	Soft	9.8	1.0	0.9	1.0	21.0	5.0	1.4	1.1	16.0	41.3	1.5
1 kg (with S_1 = 4:1)	Hard	10.8	0.7	1.1	0.5	33.5	3.1	2.6	0.4	27.6	19.3	0.4
	Intermediate	7.0	0.8	0.7	0.2	24.3	2.8	1.7	0.5	28.2	33.4	0.4
	Soft	7.8	1.4	1.9	0.6	19.8	5.4	1.5	1.0	12.9	45.7	2.0
1 kg (with S_1 = 1.5:1)	Hard	8.6	0.9	1.1	0.5	28.3	3.2	2.5	0.5	26.4	27.7	0.5
	Intermediate	6.4	1.1	0.8	0.3	22.9	3.2	2.3	1.0	29.0	32.4	0.6
	Soft	7.7	1.8	1.1	0.5	19.3	4.4	1.5	1.3	14.8	46.0	1.6
November 1977 Smithfield tallow		8.4	1.0	1.2	0.4	23.8	3.9	1.4	0.8	20.0	36.1	3.0

9.2.2 Intermediate Fractions

The fatty acid composition of each of the intermediate fractions was much more alike.

Fractionation decreased the proportions of 14:0 and 16:1 in the intermediate fractions compared to the original tallow, and increased the proportion of 18:0. The proportion of 16:0 appeared relatively unchanged, and the proportion of 18:1 was only slightly decreased.

9.2.3 Soft Fractions

The soft fractions all showed an increase in the proportion of unsaturated fatty acids relative to the original tallow, and a corresponding decrease in the proportions of 16:0 and 18:0.

9.3 PROPORTION OF EACH OF THE TRIGLYCERIDE GROUPS

Argentation TLC separated each of the fractions into five triglyceride groups. Fatty acid analysis of the groups, and comparison to triglyceride standards run simultaneously on the TLC plate, showed the five triglyceride groups to be the same as those separated by argentation TLC of the whole tallows (see section 4.4).

The analyses for each of the fractions are summarised in table 9.2.

The hard fractions showed an increase in the proportions of the trisaturated and trans monounsaturated triglyceride groups compared with the original tallow, and a decrease in the proportion of the two most unsaturated triglyceride groups.

The intermediate fractions showed an increase in the proportion of the cis monounsaturated triglyceride group with a decrease in the proportions of the trisaturated triglyceride group and the two most unsaturated triglyceride groups.

The soft fractions showed a decrease in the proportions of the trisaturated and trans monounsaturated triglyceride groups compared to the original tallow, and an increase in the proportions of the two most unsaturated groups.

Table 9.2: Proportions of the different triglyceride groups in each of the fractions and the original tallow

			Triglyceride Group (mole % of total)				
			1	2	3	4	5
Scale of fractionation	Fraction	(wt % of total tallow)	Trisaturated	Trans monounsaturated	Cis monounsaturated	Diunsaturated *1	Diunsaturated *2
20 g	Intermediate	2.5	9.5	8.8	72.2	6.4	3.1
200 g	Hard	27.3	33.5	28.5	23.5	4.3	10.2
	Intermediate	12.5	12.6	12.9	54.8	9.1	10.6
	Soft	60.2	4.0	4.1	32.2	15.0	44.7
1 kg (with S_1= 4:1)	Hard	23.0	36.3	20.3	28.1	6.8	8.5
	Intermediate	9.8	10.4	11.3	53.9	10.4	14.0
	Soft	67.2	3.2	1.6	30.3	15.6	49.3
1 kg (with S_1= 1.5:1)	Hard	37.3	26.5	11.7	27.9	9.4	24.5
	Intermediate	8.6	10.0	7.5	57.1	9.7	15.7
	Soft	54.1	3.5	6.5	28.0	14.5	47.5
November 1977 Smithfield tallow			14.6	11.1	29.9	13.1	31.3

*1 contain Trans double bonds

*2 contain only cis double bonds

The proportion of cis monounsaturated triglycerides in the intermediate fraction from 20 g tallow was 72.2%, compared to 29.9% in the original tallow. However the total proportion of cis monounsaturated triglycerides from the original tallow which entered the intermediate fraction was very low. Only 6 % of the total cis monounsaturated triglycerides in the original tallow were concentrated into the intermediate fraction because of its low yield (2.5%).

The proportions of each of the triglyceride groups in the intermediate fractions from 200 g and 1 kg of tallow were very similar, and these fractions also had similar yields. Compared with the 20 g intermediate fraction the cis monounsaturated group was consistently smaller (average of 55.3%).

In the 200 g scale fractionation, 21.0% of the cis monounsaturated triglycerides in the original tallow were concentrated into the intermediate fraction, while 19.6% entered the hard fraction and 59.4% the soft fraction. When 1 kg of tallow was fractionated with S_1 = 4:1, 16.5% of the cis monounsaturated triglycerides in the original tallow were precipitated in the intermediate fraction, 20.1% in the hard fraction, and 63.4% were left in the soft fraction. In the 1 kg scale fractionation with S_1 = 1:5, 16.1% of the cis monounsaturated triglycerides in the original tallow entered the intermediate fraction, 34.1% the hard fraction and 49.8% the soft fraction.

The yields of the hard and soft fractions from the 200 g and 1 kg scales varied, and similarly there were differences in the proportions of each of the triglyceride groups in these fractions.

In the two 1 kg scale hard fractions analysed, one had a yield of 37.3% of the total tallow, and the other 23.0%. The fraction with the highest yield contained the lowest proportions of trisaturated and trans monounsaturated triglycerides, and the highest proportions of the two most unsaturated triglyceride groups. The two fractions contained similar proportions of cis monounsaturated triglycerides.

The 200 g scale fractionation had a yield of hard fraction intermediate to that of the two 1 kg scale yields (27.3%) and had similarly ranked proportions of the trisaturated and the most unsaturated triglyceride groups.

Thus it appears that any increase in the yield of the hard fraction was accompanied by a corresponding increase in the heterogeneity of the triglyceride groups in the fraction, with an increase in the proportions of the two most unsaturated triglyceride groups, and a decrease in the proportion of the triglyceride group which was predominant in the low yield hard fraction - the trisaturated triglycerides. This was also reflected in the fatty acid composition of the hard fractions, with the highest yield hard fraction having the highest proportion of 18:1 and the lowest of 16:0 (see section 9.2.1).

9.4 FATTY ACID COMPOSITION OF THE TRIGLYCERIDE GROUPS

The proportion of 14:0 in the trisaturated triglycerides was higher in the soft fractions (average = 26.2%) than the intermediate fractions (23.1%) or the hard fractions (14.9%). The proportion of 16:0 in the trisaturated triglycerides of the hard fractions (average = 44.1%) was higher than in the intermediate fractions (39.7%) or the soft fractions (36.3%), and the concentration of 18:0 was also higher in the trisaturated triglycerides of the hard fractions (average = 38.0%) than in the intermediate fractions (29.9%) or the soft fractions (23.3%).

In the most unsaturated triglyceride group, the concentration of 18:0 was lower in each of the soft fractions analysed than in their corresponding hard and intermediate fractions. There was an average of 13% of 18:0 in the most unsaturated triglyceride group of the three soft fractions analysed, compared with an average of 21% in their corresponding intermediate fractions and 20% in their corresponding hard fractions.

Thus the nature of the triglycerides entering the different fractions during cyrstallisation was dependent upon the chain length of the constituent fatty acids as well as their degree of unsaturation.

9.5 PROPORTION OF 2-OLEO DISATURATED TRIGLYCERIDES IN THE INTERMEDIATE FRACTIONS

It has been shown that a high concentration of 2-oleo disaturated triglycerides is required in a tallow fraction for it to have composition and properties similar to those of cocoa butter. The proportions of cis monounsaturated and 2-oleo disaturated triglycerides in the intermediate fractions analysed, and the tallow, are presented in Table 9.3.

Table 9.3: Proportion of 2-oleo disaturated triglycerides in the intermediate fractions analysed, and the original tallow

Fat	Proportion of cis mono-unsaturated triglycerides in the fat (mole %)	Proportion of oleic acid at the 2-position of the cis mono-unsaturated triglycerides (mole % of total f.a.'s at 2-position)	2-Oleo disaturated triglycerides in the fat (mole %)
Tallow (Smithfield November 1976)	29.9	51.4	15.4
20 g intermediate fraction	72.2	70.5	51.0
200 g intermediate fraction	54.8	51.5	28.2
1 kg intermediate fraction (with S_1 = 4:1)	53.9	68.2	36.8
1 kg intermediate fraction (with S_1 = 1.5:1)	57.1	60.2	34.4

These results show that the proportion of 2-oleo disaturated triglycerides in the intermediate fractions was greater than in the original tallow. There was an increase in the proportion of cis monounsaturated triglycerides in all of the intermediate fractions, and also an enrichment of those cis monounsaturated triglycerides with oleate in the 2-position in the intermediate fractions produced from 20 g and 1 kg tallow, but not when 200 g was fractionated.

The intermediate fraction from the 20 g scale had a much higher concentration of 2-oleo disaturated triglycerides than any of the other intermediate fractions.

9.6 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

9.6.1 Hard Fractions

The DSC profiles of the hard fractions from the 1 kg scale fractionations are presented in figure 9.1. The melting properties of the two fractions were only slightly different from each other, but their compositions were very different (see tables 9.1 and 9.2). The fraction produced with S_1 = 4:1 had a slightly higher melting range, but contained 56.6% of tri-saturated and trans monounsaturated triglycerides compared to only 38.2% in the other fraction.

The DSC profile of the 200 g hard fraction was identical to that of the 1 kg hard fraction produced with S_1 = 4:1. These two fractions had very similar compositions (see tables 9.1 and 9.2).

9.6.2 Intermediate Fractions

The 200 g and 1 kg scale intermediate fractions all showed similar DSC profiles even though the 200 g scale intermediate fraction had a lower concentration of 2-oleo disaturated triglycerides. It appears that there was no selection between 2-oleo disaturated triglycerides and other cis monounsaturated triglycerides entering the 200 g scale intermediate fraction (see table 9.3). It is possible that the composition and proportion of the other triglycerides in the 200 g scale intermediate fraction may have acted to give the fraction a DSC profile similar to that of the 1 kg intermediate fractions, or that the melting properties of the fraction may not be very sensitive to changes in the concentration of 2-oleo disaturated triglycerides over the range between the 1 kg scale and 200 g scale intermediate fractions (35.6% and 28.2% 2-oleo disaturated triglycerides respectively).

9.6.3 Soft Fractions

The DSC profile of the soft fraction produced by fractionating 1 kg of tallow with S_1 = 4:1 is presented in figure 9.2.

9.7 COMPARISON WITH OTHER FATS

9.7.1 Hard Fractions

The fatty acid compositions of the 200 g scale hard fraction and the 1 kg scale hard fraction produced with S_1 = 4:1 were very similar to that of a fraction produced from North American beef tallow (fraction 2 in figure 2.9). The fatty acid composition of each of these fractions is presented in table 9.4. The DSC profile of each of these three fractions is presented in figure 9.1. There is close similarity between the melting properties of each of these fractions.

Table 9.4: Fatty acid composition of the 200 g hard fraction, the 1 kg hard fraction with S_1 = 4:1, and fraction 2 by Luddy et al (1977) from North American beef tallow (see figure 2.9)

Fraction	Fatty Acid (mole % of total)					
	14:0	16:0	16:1	18:0	18:1	Others
Hard fraction from 200 g tallow	8.4	33.6	2.8	30.6	18.7	5.9
Hard fraction from 1 kg tallow (S_1 = 4:1)	10.8	33.5	3.1	27.6	19.3	5.7
North American beef tallow fraction	6.0	36.0	1.0	34.0	21.0	2.0

The North American beef tallow fraction was successfully used to harden shortening and margarine stock, and was also combined with another North American beef tallow fraction, a fraction with properties similar to those of cocoa butter (Fraction 3 of figure 2.9) to produce a fat with a higher melting range than cocoa butter for certain confectionery uses (Luddy et al, 1977).

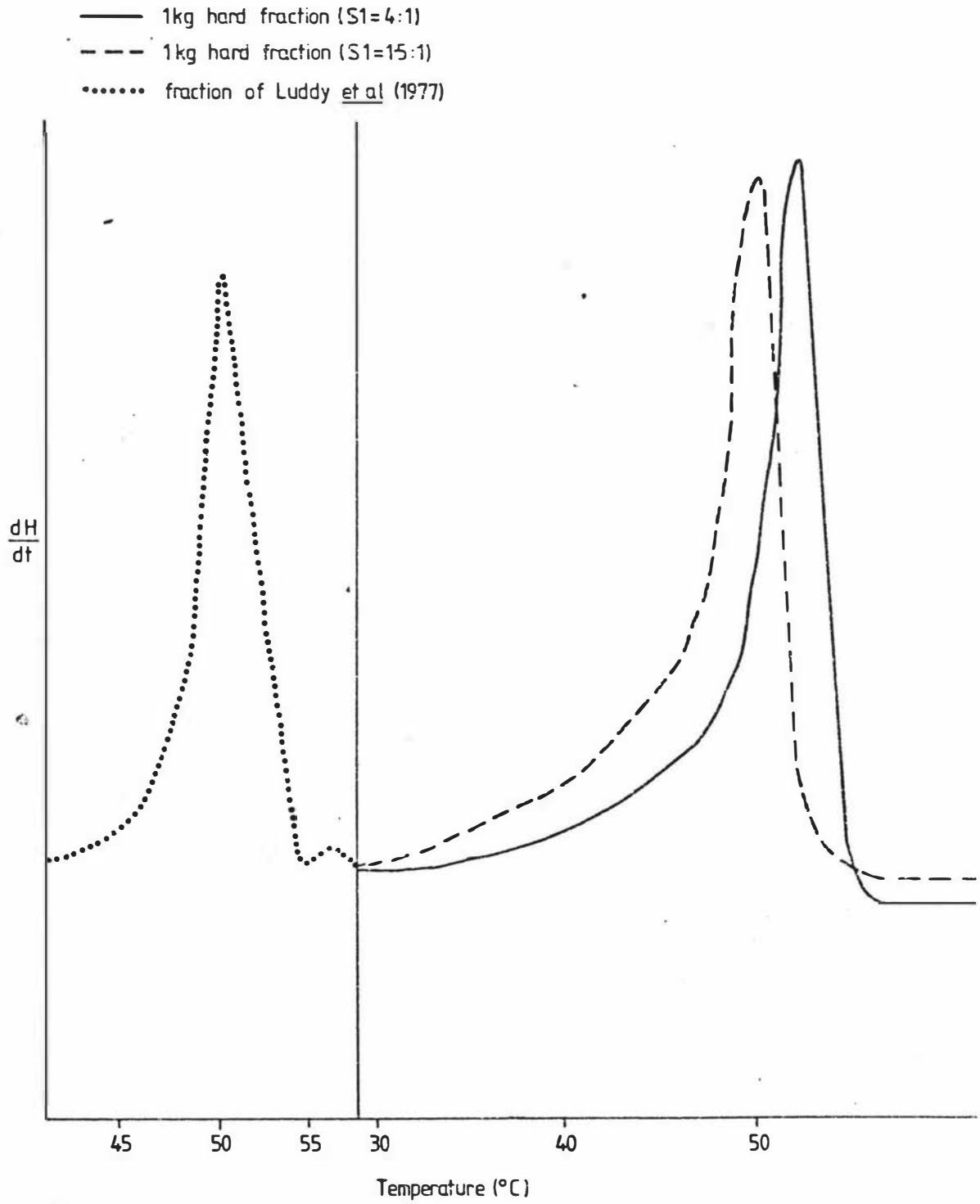


Figure 9·1 : DSC profiles of the hard fractions produced from 1kg of tallow, and of a fraction of Luddy et al, 1977

9.7.2 Intermediate Fractions

The fatty acid compositions of each of the intermediate fractions analysed, cocoa butter and several fats used to partially or completely replace cocoa butter in chocolate or chocolate-type products, are presented in table 9.5. Similar data for triglyceride composition is presented in table 9.6.

The intermediate fractions analysed all had a fatty acid composition much closer to that of cocoa butter than did the original tallow, though they all had less 18:1 and more 14:0 and 16:1 than cocoa butter. The intermediate fraction produced on the 20 g scale had the fatty acid composition most like that of cocoa butter.

Coberine is a commercial cocoa butter equivalent produced by the solvent fractionation of palm, illipe and shea fats with acetone (Wolf, 1975). Illipe butter is a natural fat which is very similar to cocoa butter and is perfectly miscible with cocoa butter in all proportions (Minifie, 1980). The beef tallow fraction (fraction 3 of figure 2.9) was produced by solvent fractionation (see section 2.6.3) and shows good compatability with cocoa butter (Luddy *et al*, 1973 , 1977, 1978). The intermediate fractions produced in this work had fatty acid compositions which were at least as similar to the fatty acid composition of cocoa butter as any of the cocoa butter replacer fats presented in table 9.5.

Table 9.5: Fatty acid compositions of each of the intermediate fractions analysed, cocoa butter and several fats which have been used to replace all or a portion of cocoa butter in chocolate or chocolate-type products

Fat	Palm kernel oil stearine *1	Illipe butter *2	Coberine *2	Cocoa butter (from section 2.2.3.1)	North American beef tallow fraction (fraction 3 of figure 2.9)	20 g intermediate tallow fraction	200 g intermediate tallow fraction	1 kg intermediate tallow fraction (S_1 = 1.5:1)	1 kg intermediate tallow fraction (S_1 = 4:1)
Fatty acid (mole % in fat)									
12:0	55.5	1.0	1.2	-	-	-	-	-	-
14:0	21.0	0.3	0.9	0.1	3.0	3.1	6.0	6.4	7.0
16:0	8.0	16.3	36.3	25.3	32.0	22.4	25.2	22.9	24.3
16:1	-	0.2	-	0.2	2.0	1.6	2.9	3.2	2.8
18:0	9.0	43.4	26.3	34.3	28.0	36.8	30.3	29.0	28.2
18:1	0.5	30.9	29.5	35.8	33.0	32.3	30.7	32.4	33.4
18:2	-	1.5	0.5	2.8	-	0.5	0.5	0.6	0.4
Others	6.0	6.4	5.3	1.3	2.0	3.3	4.4	5.5	3.9

*1 Gordon, Padley & Timms (1979)

*2 Sheppard, Iverson & Weihrauch (1978)

Cocoa butter has 79.4% of cis monounsaturated triglycerides(see section 2.2.3.1), so the intermediate fraction produced on the 20 g scale, which contained 72.2% of cis monounsaturated triglycerides, was the only intermediate tallow fraction produced which had a composition comparable to this (table 9.6). However this fraction had slightly different proportions of the other triglyceride groups, with 9.5% trisaturated triglycerides (compared to 2.2% in cocoa butter) and 9.5% polyunsaturated triglycerides (compared to 18.4% in cocoa butter). It also had 8.8% trans monounsaturated triglycerides whereas cocoa butter, like all vegetable fats, contains no trans unsaturated fatty acids (Eckey, 1954; Swern, 1964).

Table 9.6: Triglyceride composition of each of the intermediate fractions analysed, cocoa butter, and some cocoa butter replacer fats.

Triglyceride group	Triglyceride group (mole % in fat)		
	Trisaturated	Disaturated	More unsaturated
Fat			
Cocoa butter (from 2.2.3.1)	2.2	79.4(*0.0)	18.4
20 g intermediate fraction	9.5	81.0(*8.8)	9.5
200 g intermediate fraction	12.6	67.7(*12.9)	19.7
1 kg (S_1 = 4:1) intermediate fraction	10.4	65.2(*11.3)	24.4
1 kg (S_1 = 1.5:1) intermediate fraction	10.0	64.6(*7.5)	25.4
Illipe butter (Minifie, 1980)	1.0	83.0(*0.0)	16.0
North American beef tallow fraction (Ha pson, Luddy & Rothbart, 1975) *1	2.0	90.0	8.0
Fraction from Japanese mutton tallow (Kawada and Matsui, 1968)	0.8	75.6	23.6

* = percentage of disaturated triglycerides in the total fat which contain a trans double bond.

*1 = fraction 3 by the scheme of Luddy et al (1973, 1976, 1977, 1978) - see figure 2.9.

The proportion of disaturated triglycerides in the 20 g intermediate fraction was slightly greater than in the cocoa butter substitute produced from Japanese mutton tallow by Kawada and Matsui (1968), but slightly less than in Illipe butter or the North American beef tallow fraction (Hampson, Luddy & Rothbart, 1975).

In section 9.5 it was shown that 70.5% of the cis monounsaturated triglycerides in the 20 g intermediate fraction had oleic acid at the 2-position. The other intermediate tallow fractions from this project had less oleic acid in the 2-position of the cis monounsaturated triglycerides (see table 9.3).

Sampugna and Jensen (1969) analysed a cocoa butter sample and found it to contain 74.1% of cis monounsaturated triglycerides, and about 93% of these had oleic acid at the 2-position (see section 2.2.3.1, table 2.3). Thus there is about 69% of 2-oleo disaturated triglycerides in cocoa butter, compared to 51.0% in the best 20 g intermediate fraction.

Though the importance of 2-oleo disaturated triglycerides has been well-established, (see section 2.2.3.1), there is little information on their actual concentration in various cocoa butter replacer fats. In the best intermediate fraction of Kawada and Matsui (1968), 68.6% of the disaturated triglycerides had 18:1 at the 2-position. As there was 75.6% disaturated triglycerides in the fraction, this gave a total concentration of 51.9% of disaturated triglycerides in the fat with 18:1 at the 2-position. This was very similar to the concentration of 2-oleo disaturated triglycerides in the 20 g intermediate fraction (51.0%). However in the fraction of Kawada and Matsui, no allowance was made for the presence of trans 18:1 acids. As the fraction was made from mutton tallow, it is very likely that it contained a significant quantity of trans acids.

The higher concentration of 2-oleo disaturated triglycerides in the 20 g intermediate fraction compared to the other intermediate fractions analysed was reflected in the DSC profiles of the fractions. The 20 g intermediate fraction had the DSC profile most like that of cocoa butter.

9.7.3 Soft Fractions

The DSC profile of the soft fraction produced by fractionating 1 kg of tallow with S_1 = 4:1, the DSC profiles of two North American beef tallow fractions, and the DSC profile of a commercial salad oil (Luddy et al, 1973) are presented in figure 9.2. The North American beef tallow fractions were products 4 and 5 by the scheme shown in figure 2.9. They comprised 5 wt % and 60 wt % of the beef tallow respectively.

It can be seen from figure 9.2 that the soft fraction produced by fractionating 1 kg of New Zealand mutton tallow with S_1 = 4:1 melted over a much higher temperature range than the North American beef tallow fractions or the salad oil. The commercial salad oil was completely melted at 0°C. The North American beef tallow fractions still had a significant proportion of solid triglycerides at this temperature, and only a small proportion of the soft fraction produced in this project had melted at 0°C.

Similar results were obtained when a salad oil test was applied to the soft fraction produced in this work. The American Oil Chemist's Society (AOCS) salad oil test requires an oil to remain clear after holding at 0°C for 5½ hours (AOCS, 1973). The 60% yield beef tallow fraction remained clear for 3 hours at 0°C, but when mixed with a high linoleic commercial salad oil at the 40% level it remained clear for over 16 hours at 0°C. The unmodified 60% yield beef tallow fraction remained clear when held overnight at 6.7°C, but solidified when held overnight at 4.4°C. Random re-arrangement of the fatty acids altered the properties of the fraction such that it would not solidify when held overnight at 4.4°C (Luddy et al, 1977).

The soft fraction produced in this project from 1 kg of New Zealand mutton tallow (S_1 = 4:1) solidified completely after being held for 1 hour at 0°C, and also solidified after being held overnight at 7°C. Thus, it failed by a wide margin to reach salad oil specifications.

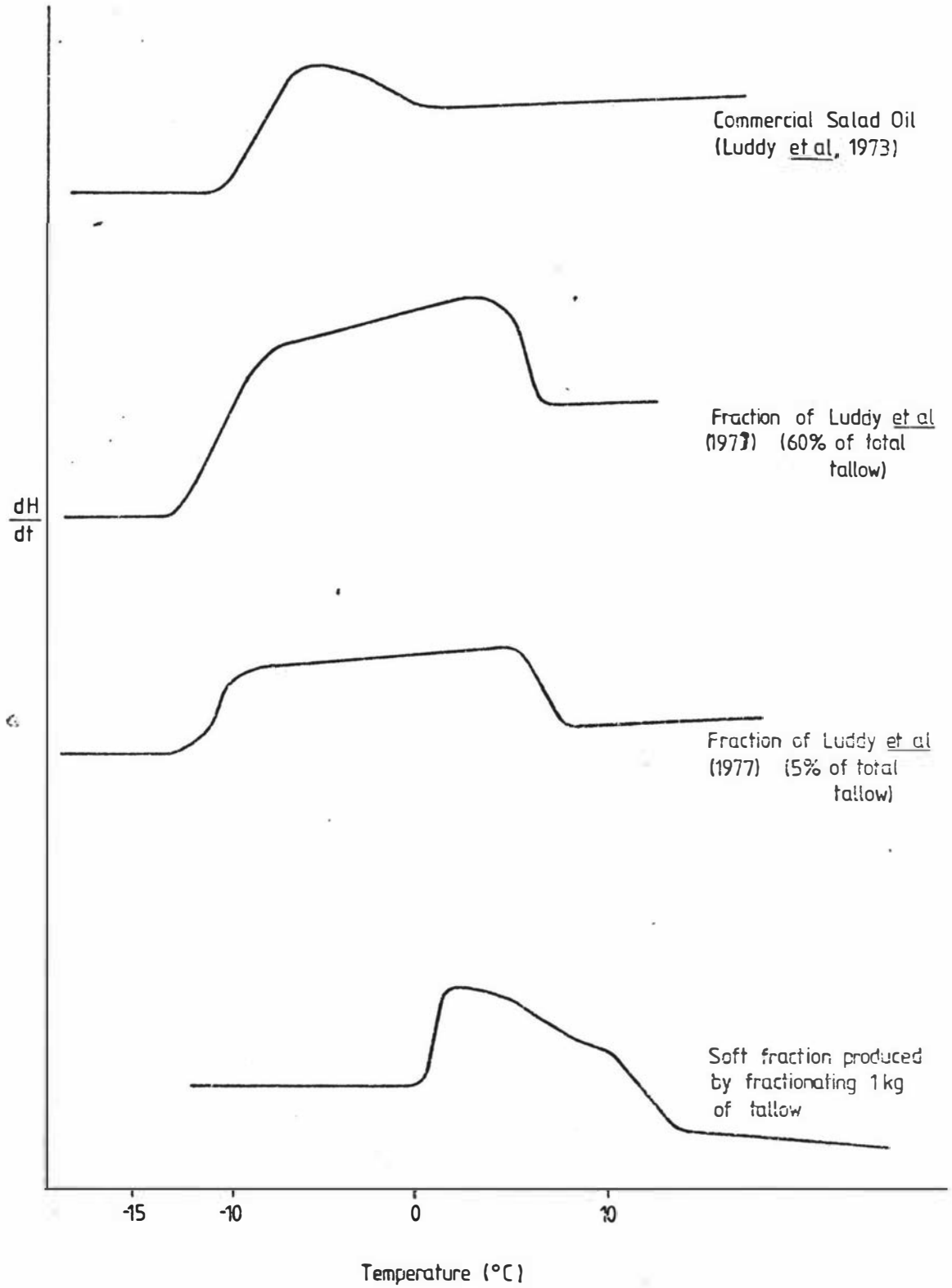


Figure 9·2 : DSC profile of the soft fraction produced by fractionating 1 kg of tallow with S1 = 4:1, the two softest fractions produced by Luddy et al (1977) and a commercial salad oil. (Luddy et al, 1977)

Comparison of the fatty acid composition of the soft fraction produced in this work (with S_1 = 4:1) and the fatty acid composition of a soyabean salad oil (see table 9.7) shows that the salad oil contained a much higher proportion of polyunsaturated fatty acids. The average concentration of 18:1 in five household and commerical cooking and salad oils (Sheppard et al, 1978) was 42.1%, compared to 44.3% in the soft fraction from this work presented above. However the average concentration of 18:2 in the published analyses was 34.4%, compared to only 2.0% in the soft fraction from this work.

The unmodified soft fraction produced in this work may be useful as a frying or baking shortening. Because of the low concentration of polyunsaturated fatty acids in the soft fraction it is likely to be relatively resistant to oxidation, and hence have a long life in frying. Soft fractions produced by solvent fractionation of North American beef tallow of similar yield of the total tallow have shown extremely high resistance to oxidation (Luddy et al, 1979). The fatty acid composition of a commercial shortening produced for heavy-duty frying is presented in table 9.7 (from Sheppard et al, 1978). It contains about 8% less 18:1 than the soft fraction produced in this work, about 8% more 18:0, and only slightly more polyunsaturated acids.

The soft fraction produced in this work may be modified by blending with other harder (possibly the hard fraction produced in this work) or softer fats, or possibly by fatty acid re-arrangement.

Table 9.7: Fatty acid composition of the soft fraction produced by fractionating 1 kg of tallow (S_1 = 4:1), two North American beef tallow fractions (fractions 4 & 5 from figure 2.9), a commercial soyabean salad oil, zero-erucic acid rapeseed oil and a commercial shortening for heavy-duty frying (Sheppard, Iverson and Weihrauch, 1978).

	Fatty Acid Composition (mole % in fat)							
Fatty acid	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
Fat								
1 kg scale soft fraction	7.8	19.8	5.4	12.9	45.7	2.0	Tr.	6.4
Beef tallow fraction 4	4.0	24.0	5.0	11.0	52.0	?	?	4.0
Beef tallow fraction 5	3.0	21.0	5.0	8.0	58.0	?	?	5.0
Soyabean salad oil	-	8.9	-	5.8	59.6	20.4	0.9	4.4
Zero-erucic acid rapeseed oil	-	4.8	0.5	1.5	55.2	22.2	11.0	6.8
Commercial shortening for heavy-duty frying	2.9	22.2	4.3	20.8	35.9	5.7	1.0	7.2

The combined yield of the two North American beef tallow fractions presented in table 9.7 was 65 wt % of the tallow. The concentration of 18:1 in a blend of these two fractions would be 57.5%, compared to 45.7% in the soft fraction produced in this project by fractionating 1 kg of tallow with S_1 = 4:1. The ratio of these two concentrations was very similar to the ratio of 18:1 concentrations in the two starting materials - 44% in the North American beef tallow (Luddy et al, 1977), and 36.1% in the New Zealand mutton tallow. Thus the fractionation of Luddy et al (1975, 1976, 1977, 1978), on a different tallow, has produced two soft fractions with a combined yield very similar to that of the soft fraction produced in this work, but with a higher proportion of 18:1 which is directly proportional to the higher quantity of 18:1 in the starting material. In both cases, solvent fractionation from acetone was used, but the fractionation schemes were quite different.

All of the soft fractions analysed in this chapter had very similar triglyceride and fatty acid compositions.

9.8 SUMMARY OF COMPOSITION OF THE FRACTIONS

(1) The hard fractions produced by solvent fractionation contained a greater proportion of trisaturated and trans monounsaturated triglyceride groups than the original tallow, and a smaller proportion of the two most unsaturated triglyceride groups. The soft fractions contained a smaller proportion of the trisaturated and trans monounsaturated triglyceride groups than the original tallow, and a greater proportion of the two most unsaturated triglyceride groups.

(2) The hard fractions contained more 16:0 and 18:0 than the original tallow, and less 16:1 and 18:1. The soft fractions contained more unsaturated fatty acids than the original tallow, and less 16:0 and 18:0. The intermediate fractions had less 14:0 and 16:1 than the original tallow, and more 18:0.

(3) The nature of the triglycerides entering the different fractions during crystallisation was dependent upon the chain length of the constituent fatty acids as well as their degree of unsaturation.

(4) The cis monounsaturated triglycerides present in the original tallow were concentrated in the intermediate fraction in each of the three fractionation procedures. The 20 g and 1 kg fractionations also selectively concentrated the cis monounsaturated triglycerides with oleate in the 2-position into the intermediate fractions.

(5) The highest proportion of 2-oleo disaturated triglycerides (51.0%) was obtained in the intermediate fraction from 20 g tallow and this had a DSC profile with the closest similarity to that of cocoa butter. The other intermediate fractions had lower proportions of 2-oleo disaturated triglycerides, and their DSC profiles showed less similarity to that of cocoa butter.

(6) The DSC profile and fatty acid composition of the 200 g scale hard fraction and the 1 kg scale hard fraction produced with S_1 = 4:1 were very similar to that of a fraction produced from North American beef tallow (Luddy et al, 1977) which was successfully used to harden shortenings and margarine stock, and these tallow hard fractions may be suitable for similar applications.

(7) The soft fraction produced in the fractionation of 1 kg of tallow with S_1 = 4:1 did not reach salad oil specifications (AOCS cold test). However the fatty acid composition of this fraction was very similar to the fatty acid composition of a heavy duty frying oil, and the tallow soft fraction may be suitable for this purpose.

9.9 FURTHER TESTING OF THE FRACTIONS

The fractionation performed upon 1 kg of tallow with S_1 = 4:1 was repeated 15 times to produce sufficient of each of the three fractions for further testing.

The fractions were deodorised prior to testing in an attempt to remove traces of acetone and other flavour and odour compounds. The deodorisation method was to distil the fraction in a current of steam (50% by wt of fat) under vacuum (29 ± 0.5 inHg vacuum) with the fat held at 220°C over a period of 5 hours (Shearon, Seestrom and Hughes, 1950; Andersen and Williams, 1962; Cocks and van Rede, 1966; Weiss, 1970; Gavin, 1977; Young, 1978).

The soft fraction was tested as a frying medium, in mayonnaise and as a base stock in a baking fat. The hard fraction was tested, in blends with various base stocks, as a baking fat. It was also tested, without other fats added, as a pastry fat. The melting properties of the intermediate fraction were compared, using N.M.R., with the melting properties of cocoa butter, a cocoa butter substitute and a cocoa butter/intermediate tallow fraction blend.

The tests on the hard and soft fractions were carried out by the Food Technology Research Centre, Massey University

(Food Technology Research Centre, 1981), and the NMR tests by Cadbury Schweppes Hudson Limited, Dunedin, New Zealand.

9.9.1 Evaluation of the Soft Tallow Fraction as a Frying Medium

The suitability of the soft fraction for deep frying was evaluated using a method similar to that of Defouw, Zabik and Gray, 1981.

One kg of the soft tallow fraction, rapeseed oil (Sunfield Vegetable Oil, Fletcher Industries Ltd) and Chefade, a commercial frying shortening (Abels (N.Z.) Ltd) were each used to fry 500 g of raw sulphited potato chips. The cooking time was 7-10 minutes, and the cooking temperature 125-130^{o}C. The frying was repeated on 24 batches of chips, and the chips from every fourth fry were presented to a trained taste panel.

The soft tallow fraction was found to be an acceptable frying medium for up to 24 fries, but the fat did have strong odour and flavour characteristics. It was thus recommended that the fraction be subjected to further deodorisation treatment. The fraction showed acceptable but not outstanding stability during frying. However the fraction had no anti-oxidants added during or after deodorisation.

9.9.2 Evaluation of the Soft Tallow Fraction for Mayonnaise Manufacture

Samples of mayonnaise were prepared from both the soft tallow fraction and rapeseed oil. These were compared by a panel.

The soft tallow fraction mayonnaise was deemed to be acceptable in terms of both pourability and texture, and was judged better in these two characteristics than the mayonnaise made from rapeseed oil. However the colour, odour and flavour of the fraction were considered to be unacceptable in mayonnaise.

9.9.3 Evaluation of Tallow Fractions for Use in Baking

Baking tests were performed using a blend of 25% tallow hard fraction/75% tallow soft fraction and a blend of 20% tallow hard fraction/80% butter. These ratios were calculated from the proportion of trisaturated and trans monounsaturated triglycerides measured in the fats. The two fat blends were used to make both cakes and biscuits, and were compared by a taste panel to similar cakes and biscuits made using butter. The fat blends were prepared by finely flaking the hard fraction and blending it through the base fat. Heating was not used for blending because of the marked alteration in the properties of butter on melting.

The biscuits and cakes made with the hard tallow fraction/soft tallow fraction blend were of very poor quality compared to when butter alone was used. The hard tallow fraction/butter blend produced biscuits of much better quality than when the all tallow fat blend was used, and the biscuits made with this blend were judged acceptable by the taste panel. However the cake made with the hard tallow fraction/butter blend was much denser and heavier than that made with butter alone.

The results of the hard tallow fraction/butter blend, especially in biscuit manufacture, suggest that the hard tallow fraction has some potential as a component in baking fats. These results are especially encouraging when it is considered that the hard tallow fraction was simply grated and mixed through the fat in its solid state. An improved blending process, and the addition of emulsifiers, may give a better product for baking (Food Technology Research Centre, 1981).

Similarly, while the soft fraction was not suitable in baking fat at a 75% concentration, it may be useful at lower concentrations and/or when blended with fats other than the tallow hard fraction.

9.9.4 Evaluation of the Hard Tallow Fraction as a Pastry Fat

The hard tallow fraction was used to make pastry, and this was compared by a taste panel to pastry produced using a commercial pastry margarine (Abels (N.Z.) Ltd).

The tallow hard fraction proved very difficult to use. It was too hard to be blended directly with the flour during pastry manufacture, and it had to be grated and then sieved to produce fines which were mixed with the flour. The tallow hard fraction pastry gave an unacceptable result on baking, with very little rising occurring, and the baked products had very poor eating qualities. They produced a waxy coating on the inside of the mouth due to the high melting range of the tallow hard fraction.

Thus the melting range of the tallow hard fraction is too high for the fraction to be useful in pastry. The hardness of the fat creates problems in both the food preparation and eating stages.

9.9.5 Nuclear Magnetic Resonance (NMR) of the Intermediate Fraction

The melting properties of the intermediate fraction were compared, using NMR, with cocoa butter, a cocoa butter substitute (Nucoa) and a blend of 90% cocoa butter/10% tallow intermediate fraction. These tests were performed by Cadbury Schweppes Hudson Limited, Dunedin, New Zealand, and the exact methods are not known.

The cocoa butter/tallow intermediate fraction blend gave NMR results, under the conditions of the tests, identical to the results of the cocoa butter sample. The intermediate tallow fraction alone gave slightly different results, but gave results much closer to those of cocoa butter than did Nucoa.

Nucoa is a cocoa butter substitute produced by solvent fractionation and hydrogenation of palm kernel oil (Wolf, 1975) and is used in N.Z. as a cocoa butter substitute in non-chocolate coverings (Brown, 1981).

9.10 DISCUSSION AND CONCLUSIONS ON USES FOR THE FRACTIONS

The tests on the three fractions in this chapter have shown that they each have potential for some specific application. However further testing of each of the fractions is required before their absolute effectiveness in any situation can be determined.

The hard fraction showed promising results when blended with butter for baking applications, but the baking performance of this blend was still inferior to that when butter was used alone. The method used to blend the two fats, however, may have impaired the performance of the fat blend. Modifications in the blending procedure, and trials with the addition of emulsifiers, may result in improvements in the performance of the hard fraction in this application.

The hard fraction was also tested as a pastry fat, but its high melting range made it totally unsuitable for this application. There seems to be little point in further testing of the unmodified hard fraction for this purpose. However blends of the tallow hard fraction and other softer fats may produce a more useful pastry fat.

The soft fraction proved very successful as a deep frying medium. It has excellent stability when it is considered that no anti-oxidants were added to the fat. The suitability of this fraction for deep frying was predicted in section 9.7.3 from consideration of its fatty acid composition. However the flavour and odour of the fraction were quite strong, despite the deodorisation procedure, and this detracted from its overall performance. The soft tallow fraction was also successful in mayonnaise. This is an encouraging result because it is convenient, particularly to the household consumer, if one fat can be used for both frying and mayonnaise manufacture. However in these trials the mayonnaise was not refrigerated. If the oil in mayonnaise crystallises, then the mayonnaise emulsion breaks. Thus the commercial mayonnaise manufacturer normally chooses an oil which meets AOCS salad oil specifications (Weiss, 1970) so that it does not crystallise in the refrigerator. However an oil

with a higher melting range may be satisfactory in mayonnaise that does not need to be kept for a long period.

Testing of the mayonnaise made with the tallow soft fraction was largely thwarted by the strong odour and flavour of the fat. This precluded mouth testing of the mayonnaise, and the samples were judged on appearance (texture and colour) and pourability only. Thus it is probably desirable to carry out taste testing of the mayonnaise, as well as trials on the effect of refrigeration, once the method of deodorising the fraction has been improved. The colour of the soft fraction was also considered by the testers to be unsuitable for mayonnaise, so bleaching may also be required.

The odour and flavour problems with the soft fraction may have been due to inadequate processing conditions, air leaks in the deodorisation equipment or poor storage conditions after deodorisation. Even very minute air leaks in deodorisation equipment can cause serious flavour problems (Williams, 1950), so this cannot be overlooked as a possible cause of flavour deterioration. However when the soft fraction was used for frying, the odour and flavour of the fat improved considerably after the fourth fry. As the frying conditions were far less severe than those used for deodorisation, this suggests that at least some of the flavour and odour compounds formed after deodorisation. The flavour and odour of the soft fraction deteriorated again after the 20th fry, as they also did in the other two frying fats tested. It may be possible to improve the stability of the soft fraction by adding anti-oxidants to the fat either during or after deodorisation (Weiss, 1970).

The soft fraction gave very poor performance as a baking fat when blended with the tallow hard fraction (25% hard fraction/75% soft fraction). However altering the ratio of the two fats may improve the properties of the blend for baking purposes.

The chemical composition and DSC profile of the intermediate fraction produced from 1 kg of tallow with S_1 = 4:1 suggest that this fraction is probably only suitable as a cocoa butter

substitute rather than as a cocoa butter extender or equivalent. However the NMR results indicate that cocoa butter can accommodate at least 10% of the tallow fraction in admixture without affecting its melting behaviour. No other cocoa butter/tallow intermediate fraction blends were examined, so the degree of compatability between the two fats at higher concentrations is unknown. It is necessary to determine this before the potential of the fraction can be assessed. In order to fully determine the comptability, both the solid/liquid phase behaviour and the phase behaviour below the solidus line must be examined (Paulicka, 1973). Legal and market requirements largely affect the possible uses of this fraction irrespective of its physical properties. It is illegal to use such a fat in chocolate in New Zealand (N.Z. Dept. of Health, 1973), so the product is limited to use in chocolate-type products. The market demand for such a fat in New Zealand must be carefully assessed, probably in conjunction with further physical testing of the fraction.

Thus while each of the three fractions produced in this work does show promise in certain applications, further work is necessary before the full potential of any of the fractions can be determined.

A fraction containing a higher proportion of 2-oleo disaturated triglycerides than the intermediate fractions produced in this work and/or with a greater yield may be able to be produced if a fractionation scheme involving a greater number of crystallisations was employed. However any increase in the complexity of the process would increase the processing costs, and this must be balanced against any increase in the value of the products.

All of the work in this thesis has been with inedible mutton tallow. An edible mutton tallow source is necessary before any commercial application of this work can be considered. Consideration of some aspects of this, as well as some discussion on the effects which variation in tallow composition may have upon the fractions, is presented in Appendix 8.

CHAPTER 10

GENERAL CONCLUSIONS AND CONSIDERATIONS FOR FUTURE WORK

The inedible New Zealand mutton tallow analysed contained four main fatty acids (myristic, palmitic, stearic and oleic); trisaturated, disaturated and more unsaturated triglycerides, and a significant proportion of 2-oleo disaturated triglycerides. The variation in composition over each season, and in some cases between seasons, was significant, with the most dramatic variation being the decrease in the proportion of 2-oleo disaturated triglycerides from November to June in each season. This variation in composition may cause raw material problems for industrial fractionation as the yields and/or the composition of the fractions, and the process conditions required to produce consistent fractions, are likely to be affected by any large variations in tallow composition. This is particularly important if one of the objectives is to produce a cocoa butter replacer. The variation in composition of tallows from different meat killing plants was not studied. Differences between plants may lead to even greater overall variation in the composition of tallow available throughout New Zealand. Only inedible mutton tallow was studied in this work, but edible grade tallow must be used for any commercial application and this could introduce further variations.

Acetone fractionation was used to separate the tallows into three fractions with widely different properties: a hard, a soft and an intermediate fraction. However it was not found possible to concentrate all of any specific triglyceride type from the tallow into any one fraction.

The 20 g intermediate fraction contained a high proportion (51.0%) of 2-oleo disaturated triglycerides, which are of primary importance in cocoa butter, but the yield of this fraction was very low (2.5 wt % of the tallow) and only a small proportion (8.3%) of the 2-oleo disaturated triglycerides in the original tallow ended up in this fraction. Most of the 2-oleo disaturated triglycerides in the tallow still remained in the hard and soft fractions in low concentrations where their melting

properties were not utilised in the unique way they are in cocoa butter or some cocoa butter replacer fats. Any large increase in the yield of the intermediate fraction was only achieved with a corresponding decrease in the proportion of 2-oleo disaturated triglycerides in the fraction and with a decrease in the similarity of the melting properties of the fraction to those of cocoa butter.

The purely empirical approach, using different experimental designs in sequence to study how the various process variables affected the properties and yields of the fractions, was successful in that an intermediate fraction with melting properties very similar to those of cocoa butter was obtained. Thus the model developed to compare the melting properties of the intermediate fractions with those of cocoa butter adequately represented the fractionation scheme, and the cocoa butter likeness factor (CBLF), which was based on the differential scanning calorimeter profiles of cocoa butter and the tallow intermediate fractions, was shown to be a satisfactory dependent variable in this model.

The fractionation scheme was especially sensitive to changes in the temperature and solvent to fat ratio at the first crystallisation.

It was not found possible to predict scale-up criteria which will ensure that the fractions can be reproduced on a larger scale. Variations were found on scale-up from 20 g to 1 kg which could not be explained, and there may be other unknown factors in further scale-up.

It appears that three commercial fats can be produced from tallow using this fractionation method. Of the fractionation products, the composition and melting properties of the 1 kg scale hard fraction were very similar to a fat used as a plasticiser in shortenings and margarines. A blend of the tallow hard fraction with butter (20% tallow hard fraction/80% butter) was adequate as a shortening in biscuits, but less successful in cakes. The melting range of the hard fraction was too high for use as a pastry shortening. Further study on the compatability

of cocoa butter and the intermediate tallow fraction is required before the potential of this fraction as an alternative to cocoa butter in chocolate-type products can be assessed. The soft tallow fraction performed very well as a deep-frying medium, and mayonnaise made with it had near-ideal pourability and texture. It was unsuitable as a base-stock in baking shortening.

There are a number of topics which require further study before commercial application of this work can be considered. These include:

(1) more intensive study of each of the fractions in specific applications.

(2) market demand for these products.

(3) a cost estimate of the process.

(4) the effect upon the process of variability in tallow composition.

(5) the problems of further scale-up.

(6) . improved refining and storage procedures for the fractions.

Appendix 1: Polymorphism of Triglycerides

In monoacid saturated triglycerides, three main polymorphic states occur. These are α, β' and β in increasing order of melting point.

The cross-sectional structure of each of these different types of packing is shown in Figure A1.1, where each of the ellipses represents the orientation of the zig-zag plane of the hydrocarbon chains in a triglyceride.

In the β state the zig-zag planes of the hydrocarbon chains in different molecules are parallel. The subcell structure of this, where the subcell is the smallest repetition unit within the chain structure, is triclinic parallel (T $\parallel$). In the β' state the zig-zag planes of the hydrocarbon chains in alternate rows of triglycerides are oppositely orientated. The subcell structure of this is orthorhombic perpendicular (O $\perp$) as every chain plane is approximately perpendicular to its four nearest neighbours (Norris, 1977).

In the α state, the hydrocarbon chain planes are randomly orientated. The chain packing is hexagonal (H) (Lutton, 1972).

Trilaurin molecules in the β form have a "tuning fork" conformation, with chains 1 and 2 being extended in a straight line, and chain 3 being packed close to chain 1. The molecules are arranged in double chain layers with the glycerol residues at the centre and the methyl groups at the layer boundaries. See Figure A1.2 (Larsson, 1963). It is probable that the "tuning fork" configuration also occurs in the α and β' forms of simple triglycerides. Cooling the α form to a sufficiently low temperature results in the formation of an O $\perp$ subcell known as the sub-α form. At least one other polymorphic form for simple triglycerides has also been proposed (Norris, 1977).

Unsaturated triglycerides show similar polymorphism to saturated triglycerides except that the double bond causes

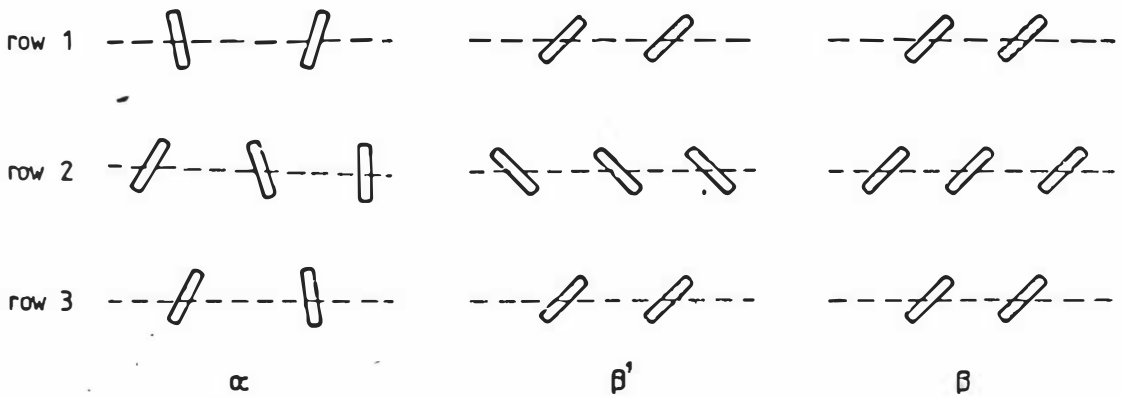


Figure A1·1 : Cross-sectional structure of different polymorphic forms.
(Lutton, 1972; Jacobsberg and Jacqmain, 1976)

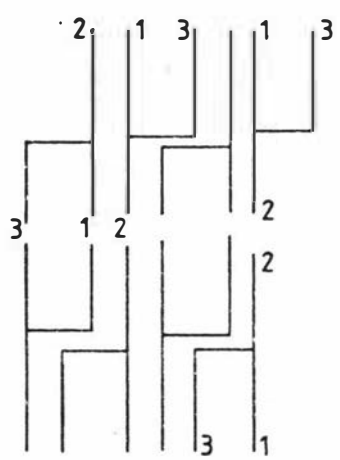


Figure A1·2 : Double chain length "tuning fork" structure of trilaurin.
(Larsson, 1963)

chain distortion which decreases the chain packing density and melting point. Trans double bonds can be more easily accommodated in the zig-zag structure than cis double bonds. The only major difference between a fully saturated triglyceride and a trans unsaturated triglyceride is that the β' form frequently does not occur in the trans triglyceride.

The greater degree of chain disruption caused by cis double bonds causes additional polymorphic complexity. Triple chain length structures (denoted by the suffix "-3" following the chain packing designation - Lutton, 1950) occur in 2-oleo disaturated triglycerides. The triple chain length structure for the β-3 form of SOS is shown in Figure A1.3 (Larsson, 1972).

SOS is isomorphous with the stable forms of POS, POP and cocoa butter (Willie and Lutton, 1966).

The distinguishing feature of this structure is that the chains are sorted into two equivalent saturated layers with the unsaturated layer between.

Disaturated triglycerides with oleic acid at the 1- or 3-positions also show triple chain length structures, but their stable form is β' rather than β. These molecules do not have the tuning fork conformation. The saturated chains pack alongside each other leaving the oleic acid chain in the 1 or 3- position extended in the opposite direction. This is the "chair" conformation.

Although the mono-oleo disaturated triglycerides predominantly form triple chain length structures, double chain length structures may also occur (donated by the suffix "-2") with the β' subcell.

In triglycerides containing two oleic acid molecules, chain sorting occurs to produce a layer of saturated chains between two equivalent layers of unsaturated chains. If both of the oleic acid molecules are at the terminal positions, α, β'-2 and stable β-3 forms occur. If one of the oleic acid molecules is at the 2-position, only α and stable β'-3-forms

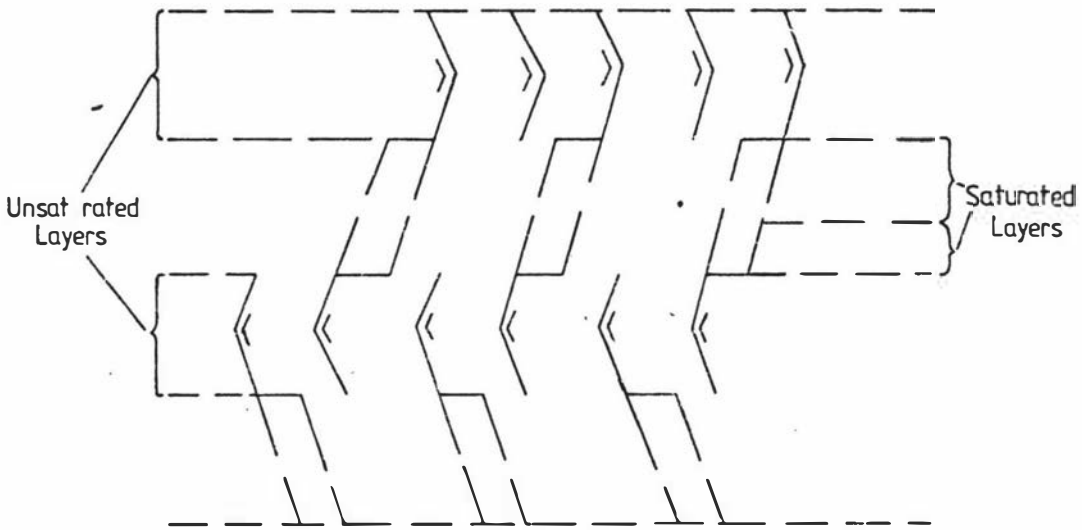


Figure A1·3 : Triple chain length structure for the β-3 form of SOS.

(Larsson, 1972)

occur (Norris, 1977).

Di-acid saturated triglycerides normally show polymorphism similar to the simple saturated triglycerides, but this may vary quite considerably depending upon the relative chain lengths of the acids concerned and upon the symmetrical or unsymmetrical nature of the triglyceride molecule (Chapman, 1962).

Polymorphism in substances may be either monotropic or enantiotropic. In the latter, inter-conversion between the different forms is possible at certain temperatures. For monotropic substances, the same sequence of stability in the different forms is maintained throughout the range of temperatures in which they are solid, and transformation can only occur to produce stable from unstable forms. Triglycerides are virtually always monotropic. (Bailey, 1950; Maron and Prutton, 1969).

Appendix 2: Solvents which have been used for Solvent Fractionation of Fat Substances

Acetates: Farr, 1954; Rubin, Teasdale and Mertens, 1959; Akiya, 1970.

Acetone: Kistler, Muckerheide and Myers, 1946; Riemenschneider and Luddy, 1946; Riemenschneider, Luddy, Swain and Ault, 1946; Demmerle, 1947; Towne, 1949; Skau, Dopp, Burleigh and Banowetz, 1950; Boucher and Skau, 1952; Morris, Gordon, Brenner, Meyers, Riemenschneider and Ault, 1952; Texaco Development Co., 1952; Cording, Willard, Edwards and Eskew, 1953; Pramuk, Whiting and McLaren, 1953; Farr, 1954; Luddy, Fertsch and Riemenschneider, 1954; Riemenschneider, 1954; Ayres, 1957; McGuine, 1960; Best, Soeters, Davies and Paul, 1961; Spadaro, Lovegren, Feuge and Patton, 1961; Sinnema, 1962; Aarhus Oliefabrik, 1964; Loders and Nucoline, 1964; Chen and de Man, 1966; Akiya, 1970; Beresford and Rossell, 1971; Unilever, 1971; Kawada, Suzuki and Matsui, 1972; Luddy, Hampson, Herb and Rothbart, 1973, 1976, 1978; Viarengo and Pasculli, 1973; Sherbon and Dolby, 1973; Taylor, 1973; Martinenghi, 1974; Zondek, 1974; Baliga and Shitole, 1981.

Ethanol: Demmerle, 1947; Subrahmanyam and Achaya, 1958; Krishnamurthy, Ramalingaswamy, Banerjee and Achaya, 1965.

Hexane: Pramuk, Whiting and McLaren, 1953; Ayres, 1957; Bernardini, 1968; Akiya, 1970; Errboe, Braemer-Madsen and Anderson, 1971; Lovegren, Gray and Feuge, 1973; Martinenghi, 1974; Fuji Oil Co., 1975 & 1976; Bernardini and Bernardini, 1976.

Isopropanol: Ayres, 1957; Koslowsky, 1972, 1973, 1975; Kowlowsky and Letan, 1975.

Ketones - other low molecular weight ketones: Texaco Development Co., 1952; Cording, Willard, Edwards and Eskew, 1953; Ayres, 1957; Farr, 1954; Rubin, Teasdale and Mertens, 1959.

Methanol: Kistler, Muckerheide and Myers, 1946; Demmerle, 1947; Feldpush, 1950; Zondek, 1973 , 1974.

Methyl formate: Blaney, 1972.

Mixtures - certain solvent mixtures have also been used: Boucher and Skau, 1951, 1952; Texaco Development Co., 1952; Pramuk, Whiting and McLaren, 1953; Ayres, 1957; Akiya, 1970; Kassabian, 1971.

Naptha: Bailey, Feuge and Kraemer, 1943.

Nitropropane (1- and 2-): Zilch, 1967; Kawada and Matsui, 1968; Kao Soap Co., 1971.

Petroleum ether: James, 1943; Hilditch and Williams, 1964; Kundu, 1970; Litchfield, 1972.

Propane: Rayshich, 1953.

Appendix 3: Results of the Triglyceride and Fatty Acid Analyses of Selected New Zealand Mutton Tallows

For each tallow, the following analyses are presented:

(1) Total fatty acid composition.

(2) The proportion of each of the five triglyceride groups separated by argentation TLC.

(3) The fatty acid composition of each of these triglyceride groups.

(4) The fatty acid composition of the total tallow as calculated from the proportion and composition of each of the triglyceride groups.

(5) The fatty acid composition at position 2 of the cis monounsaturated triglycerides.

(6) The fatty acid composition at the 2-position of each of the triglyceride groups and of the whole tallow are given for the May 1977 tallow sample obtained from Ocean Beach, as well as the fatty acid composition at the 2-position of the whole tallow as calculated from the proportion, and fatty acid composition at the 2-position, of each of the triglyceride groups.

Table A3.1

Tallow: Smithfield Mutton Tallow, 1½R, November 1976

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	15.8	7.9	32.0	10.4	33.9		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	14.8	8.3	6.1	7.0	4.6	2.6	5.9	6.3
iso - 15:0 + 14:1	3.1	0.7	0.5	1.2	-	0.9	0.7	1.0
15:0	2.6	1.1	1.2	0.7	0.7	0.7	0.9	1.2
iso - 16:0	0.4	0.2	0.2	0.2	-	0.3	0.2	0.2
16:0	39.7	28.6	26.0	13.2	17.5	15.3	22.2	23.9
16:1		3.3	3.6	4.2	3.3	5.9	4.9	3.8
17:0				1.0			2.5	
17:1			1.0	0.9	1.7	2.1	1.0	1.2
18:0	39.4	26.1	25.7	8.3	16.1	10.7	20.2	21.8
18:1		31.7	35.7	63.3	56.2	57.1	40.6	39.1
18:2						4.4	0.9	1.5

Table A3.2

Tallow: Smithfield Mutton Tallow, 1½R, November 1976

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of total tallow	17.0	5.8	33.5	10.2	33.5		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	12.7	7.8	5.8	7.0	4.2	3.2	4.8	6.1
iso - 15:0 + 14:1	1.4	0.8	0.4	1.2		0.8	0.7	0.7
15:0	2.1	1.4	1.2	1.0	0.7	0.8	0.9	1.2
iso - 16:0	0.3	0.2	0.2	0.3		0.2	0.2	0.2
16:0	41.0	26.6	26.3	14.0	17.6	16.6	20.8	24.7
16:1	-	2.2	4.6	4.1	4.5	5.9	6.1	4.1
17:0				1.4			3.4	
17:1			0.8	1.6	1.0	1.2	1.4	0.8
18:0	42.5	26.6	25.4	8.2	15.5	9.9	21.0	22.2
18:1	-	34.4	35.3	61.2	56.5	55.9	38.8	38.3
18:2						5.5	1.9	1.8

Table A3.3

Tallow: Smithfield Mutton Tallow, 1½R, December 1976

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of total tallow	14.0	12.1	28.4	11.9	33.6		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	20.7	7.7	8.4	7.1	6.3	3.0	7.6	8.0
iso - 15:0 + 14:1	3.3	0.7	0.4	0.7	0.4	0.3	0.8	0.8
15:0	2.3	1.4	0.2	0.3	0.7	1.3	1.2	1.1
iso - 16:0	0.6	0.5	0.2	0.4	0.3	-	0.2	0.2
16:0	38.8	21.5	29.9	15.0	15.8	14.3	22.8	23.2
16:1	-	4.6	3.1	5.2	6.0	5.4	5.1	4.0
17:0	-	-	-	1.0	-	-	1.6	-
17:1	-	-	1.5	1.0	1.1	1.8	0.9	1.2
18:0	34.3	32.3	23.1	8.4	12.5	9.8	18.5	20.1
18:1	-	31.3	33.2	60.9	56.9	57.6	39.0	39.3
18:2	-	-	-	-	-	6.5	2.3	2.2

Table A3.4

Tallow: Smithfield Mutton Tallow (1½R, January 1977)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of total tallow	13.3	7.4	27.2	15.4	36.7		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	9.0	5.7	4.2	5.3	2.4	2.1	3.4	3.9
iso - 15:0 + 14:1	1.9	0.7	0.5	2.7		0.6	0.5	0.7
15:0	2.9	1.0	1.1	0.3	0.5	0.7	0.8	1.1
iso - 16:0	0.2	0.4	0.1	0.1		0.3	0.1	0.2
16:0	38.8	27.0	27.2	13.3	16.5	14.4	20.5	22.4
16:1		1.7	1.8	5.3	2.4	3.8	2.5	2.4
17:0				0.8			2.7	
17:1			0.6	0.9	0.6	2.3	0.9	1.0
18:0	47.2	32.1	29.1	10.9	14.9	11.7	24.0	23.2
18:1		31.4	35.4	60.4	59.1	56.3	40.6	41.7
18:2					3.6	7.8	4.0	3.4

Table A3.5

Tallow: Smithfield Mutton Tallow (½ R, March, 1977)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	16.9	7.1	22.8	10.5	42.7		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	8.0	5.1	4.8	4.3	2.7	1.8	2.7	3.9
iso - 15:0 + 14:1	1.3	1.1	0.6	0.7	0.5	1.0	0.7	0.9
15:0	2.7	1.6	1.1	0.6	1.2	1.2	1.2	1.5
iso - 16:0	0.6	0.8	0.3	0.3	0.8	0.4	0.6	0.5
16:0	35.9	24.8	26.3	15.1	16.9	13.7	20.6	21.4
16:1		2.2	3.0	3.3	4.4	5.2	4.5	3.5
17:0				1.4			2.1	
17:1		1.3	1.4	0.5	0.7	2.2	0.7	1.4
18:0	51.5	3.42	29.2	24.7	16.5	13.7	26.1	25.4
18:1		28.9	33.3	49.1	52.1	50.9	37.3	36.8
18:2					4.2	9.9	3.5	4.7

Table A3.6

Tallow: Ocean Beach Mutton Tallow (May, 1977)

	Triglyceride Group										Fatty Acid (mole % of tallow)			
	1		2		3		4		5		Whole Tallow		Calculated from triglyceride groups	
% of total tallow	14.9		6.7		26.5		14.6		37.3					
Fatty Acid	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position	Mole %	Mole % at 2-position
14:0	7.6	20.0	3.6	7.8	4.4	5.0	1.9	2.5	1.5		3.0	5.0	3.4	5.2
iso - 15:0 + 14:1	1.9	2.4	0.8	1.8	1.1	1.2			0.2	2.1	0.5	2.1	0.7	1.6
15:0	2.4	2.9	1.2		1.3	0.9	0.5	1.0	0.4		0.8	1.3	1.0	0.8
iso - 16:0	0.4	0.9	0.1		0.5	0.4			0.1		0.1	0.3	0.2	0.2
16:0	39.1	30.9	23.6	20.3	25.7	13.1	16.4	7.9	14.0	6.3	20.4	12.0	21.8	12.9
16:1			5.2	8.0	1.8	4.0	3.3	4.0	6.0	5.2	4.1	4.4	3.5	4.1
17:0		5.6		3.7		1.1		1.7			2.1	2.2		1.6
17:1			1.2	0.7	0.5	2.2	2.0	1.5	1.5	1.3	0.4	0.8	0.9	1.3
18:0	48.6	37.3	29.6	17.3	32.6	12.7	17.1	9.0	13.4	6.1	26.3	14.6	25.4	13.7
18:1			34.7	40.4	32.1	59.4	56.8	69.4	53.6	63.1	39.1	52.0	39.1	52.1
18:2							2.0	3.0	9.3	15.9	3.2	5.3	3.8	6.4

Table A3.7

Tallow: Smithfield Mutton Tallow (1½R, November 1977)

	Triglyceride Group						Fatty Acid (mole % of tallow)	
	1	2	3		4	5	Determined	Calculated from triglyceride groups
% of tallow	14.6	11.1	29.9		13.1	31.3		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	13.4	9.0	8.1	9.7	4.9	3.1	8.4	7.0
iso - 15:0 + 14:1	2.0	1.4	0.3	0.5	0.4	0.6	1.0	0.8
15:0	1.7	0.5	1.6	1.5	0.6	0.9	1.2	1.1
iso - 16:0	0.5	0.6	0.4	0.5	0.1	0.4	0.4	0.4
16:0	43.8	28.9	25.2	18.8	17.4	17.8	23.8	25.0
16:1		4.4	3.8	4.5	4.4	5.3	3.9	3.9
17:0				0.8			1.4	
17:1		0.8	0.9	0.9	1.1	1.9	0.8	1.1
18:0	38.6	26.0	28.1	11.4	15.1	14.0	20.0	23.3
18:1		28.4	31.6	51.4	54.5	53.7	36.1	36.5
18:2					1.5	2.3	3.0	0.9

Table A3.8

Tallow: Smithfield Mutton Tallow (1½R January 1978)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	15.9	8.5	32.3	12.7	30.6		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	11.6	4.5	6.0	7.1	3.3	2.4	5.3	5.3
iso - 15:0 + 14:1	1.9	1.3	0.4	0.3	0.1	0.4	0.5	0.7
15:0	1.7	0.6	1.0	1.2	0.2	0.7	1.2	0.9
iso - 16:0	0.3	0.9	0.4	0.9	0.1	0.1	0.3	0.3
16:0	41.3	24.9	28.4	19.5	20.7	18.5	26.3	26.1
16:1		7.2	3.7	5.9	6.8	4.7	3.4	4.1
17:0				0.6			2.2	
17:1		4.5	0.8	0.9	5.6	1.2	1.7	1.7
18:0	43.2	22.9	27.4	13.0	17.0	11.3	23.1	23.3
18:1		33.2	31.9	50.6	44.5	55.2	34.4	35.7
18:2					1.7	5.5	1.6	1.9

Table A3.9

Tallow: Smithfield Mutton Tallow (1½R, February 1978)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	17.2	10.2	26.6	11.7	34.3		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	
14:0	13.4	5.0	5.5	5.0	1.8	2.5	5.6	5.3
iso - 15:0 + 14:1	3.5	0.4	0.3	0.8	0.2	0.1	0.8	0.8
15:0	1.7	0.7	0.9	2.6	0.7	0.4	1.2	0.8
iso - 16:0	0.6	1.3	0.8	0.5	0.6	0.6	0.5	0.7
16:0	48.4	30.1	26.1	20.1	25.7	18.7	26.0	27.8
16:1		3.7	3.5	2.4	3.7	3.4	2.6	2.9
17:0				0.8			0.2	
17:1		1.4	1.6	1.3	0.6	1.9	0.7	1.3
18:0	32.4	29.8	28.5	15.9	21.1	15.6	22.5	22.0
18:1		27.6	32.8	50.6	45.6	50.3	37.8	36.2
18:2						6.5	2.1	2.2

Table A3.10

Tallow: Smithfield Mutton Tallow (1½R, March 1978)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	19.0	11.6	29.8	12.4	27.2		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	
14:0	6.6	4.9	3.0	3.6	2.7	2.4	3.3	3.7
iso - 15:0 + 14:1	1.2	0.6	0.4	0.7	0.3	0.2	0.5	0.5
15:0	1.3	1.1	1.0	0.6	0.7	0.8	0.7	1.0
iso - 16:0	0.4	0.8	0.5	1.4	0.3	0.1	0.4	0.4
16:0	40.7	35.0	27.2	23.2	18.5	19.2	23.8	27.1
16:1		0.8	2.5	3.8	3.3	3.1	3.5	2.1
17:0				0.6			2.0	
17:1		1.1	1.2	1.3	0.4	1.3	1.0	0.9
18:0	49.8	36.4	29.2	12.2	18.5	15.7	28.8	29.0
18:1		19.3	35.0	52.5	53.1	50.9	33.1	33.0
18:2					2.2	7.3	2.9	2.3

Table A3.11

Tallow: Smithfield Mutton Tallow (1½R, May, 1978)

	Triglyceride Group						Fatty Acid (mole % of tallow)	
	1	2	3		4	5	Determined	Calculated from triglyceride groups
% of tallow	15.9	8.7	25.3		11.2	38.9		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	7.1	5.0	4.0	4.5	2.0	1.3	3.1	3.3
iso - 15:0 + 14:1	0.4	0.6	0.9	1.8	0.4	0.3	0.4	0.5
15:0	1.2	1.0	0.5	0.7	0.7	0.6	0.8	0.7
iso - 16:0	1.5	0.7	0.3	2.4	0.3	0.2	0.5	0.5
16:0	40.0	30.0	26.9	24.2	20.0	15.3	22.6	24.0
16:1		3.9	1.9	5.8	3.5	4.0	3.6	2.8
17:0				1.2			1.8	
17:1		0.9	0.7	0.5	1.0	1.7	1.0	0.9
18:0	49.8	34.9	32.1	19.4	18.0	15.7	26.7	27.2
18:1		23.0	32.7	39.5	51.0	52.6	36.3	36.4
18:2					3.1	8.3	3.3	3.6

Table A3.12

Tallow: Smithfield Mutton Tallow (1½R, April 1978)

	Triglyceride Group						Fatty Acid (mole % of tallow)	
	1	2	3		4	5	Determined	Calculated from triglyceride groups
% of tallow	15.8	8.5	31.0		9.7	35.0		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	11.9	5.4	4.0	1.4	3.2	2.2	4.5	4.7
iso - 15:0 + 14:1	3.8	1.1	0.6	0.2	0.5	0.5	0.8	1.1
15:0	2.5	1.0	0.9	0.8	0.9	0.5	0.8	1.0
iso - 16:0	0.6	0.7	0.3	2.5	0.3	0.4	0.5	0.4
16:0	41.8	29.3	26.6	18.5	17.3	17.9	23.4	25.3
16:1		2.0	2.3	6.7	4.1	5.3	3.2	3.1
17:0				2.2			1.7	
17:1		1.4	0.8	1.6	1.6	1.2	0.8	0.9
18:0	39.4	34.8	30.2	15.7	17.9	14.8	24.9	25.5
18:1		24.3	34.3	50.4	51.8	49.5	36.6	35.0
18:2					2.4	7.8	2.8	3.0

Table A3.13

Tallow: Smithfield Mutton Tallow (1½R, June 1978)

	Triglyceride Group					Fatty Acid (mole % of tallow)	
	1	2	3	4	5	Determined	Calculated from triglyceride groups
% of tallow	19.7	8.5	27.1	9.7	35.0		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	10.0	1.9	5.0	3.9	1.8	1.6	2.9	3.7
iso - 15:0 + 14:1	2.5	0.4	0.6	1.4	0.3	0.2	0.7	0.8
15:0	2.4	0.4	0.8	0.4	0.8	0.7	0.8	1.0
iso - 16:0	0.9	0.5	0.4	2.8	0.3	0.5	0.4	0.5
16:0	35.4	29.5	27.3	24.3	21.4	16.9	25.5	25.0
16:1		2.5	3.4	7.2	3.2	5.3	4.0	3.3
17:0				2.8			2.4	
17:1		0.2	0.8	0.3	1.9	1.2	0.8	0.8
18:0	48.8	32.9	32.6	17.2	24.8	15.5	26.9	29.1
18:1		31.7	31.1	39.7	44.1	52.5	33.7	33.7
18:2					1.4	5.6	1.9	2.1

Appendix 4: Regression Equations and Regression Statistics from the Screening Experiment (Chapter 5)

Table A4.1: Regression Equation for the Yield of the Hard Fraction

$$\text{Yield of Hard Fraction (\%)} = 19.4 - 11.0\,(T_1) + 0.43\,(T_2) - 4.90\,(S_1) + 0.11\,(S_2) + 5.01\,(W) - 0.42\,(A) - 0.80\,(t) + 0.23\,(M) - 0.13\,(T_1T_2 + S_1t + S_2M + WA) + 4.09\,(T_1S_1 + T_2t + WM + S_2A) - 1.38\,(T_1S_2 + T_2M + S_1A + Wt) - 2.05\,(T_1W + S_1M + T_2A + S_2t) - 1.15\,(T_1A + tM + S_1S_2 + T_2W) + 0.55\,(T_1t + T_2S_1 + AM + S_2W) + 3.58\,(T_1M + T_2S_2 + S_1W + At)$$

Simplified Equation for the Yield of the Hard Fraction and Regression Statistics

$$\text{Yield of the Hard Fraction (\%)} = 19.4 - 11.0\,(T_1) - 4.90\,(S_1) + 5.01\,(W) + 4.09\,(T_1S_1 + T_2t + WM + S_2A) - 2.05\,(T_1W + S_1M + T_2A + S_2t) + 3.61\,(T_1M + T_2S_2 + S_1W + At)$$

Correlation Coefficient:

$R^2 = 97.8\%$

$R^2 = 96.3\%$, adjusted for d.o.f.

Analysis of Variance Summary:

Source	d.o.f.	SS	MS
Regression	6	3256.10	542.7
Residual	9	73.42	8.2
Total	15	3329.52	

Table A4.2: Regression Equation for the Yield of the Intermediate Fraction

Yield of Intermediate Fraction (%) = 26.1 + 11.7 (T_1)

- 5.01 (T_2) - 1.19 (S_1) - 3.51 (S_2) + 7.76 (W) + 0.36 (A)
+ 3.36 (t) + 0.99 (M) - 1.86 (T_1T_2 + S_1t + S_2M + WA) - 1.54
(T_1S_1 + T_2t + WM + S_2A) + 2.69 (T_1S_2 + T_2M + S_1A + Wt)
+ 4.26 (T_1W + S_1M + T_2A + S_2t) + 0.46 (T_1A + tM + S_1S_2 + T_2W)
+ 1.31 (T_1t + T_2S_1 + AM + S_2W) + 1.69 (T_1M + T_2S_2 + S_1W + At)

Simplified Equation for the Yield of the Intermediate Fraction and Regression Statistics

Yield of Intermediate Fraction (%) = 26.1

+ 11.7 (T_1) - 5.01 (T_2) - 3.51 (S_2) + 7.76 (W) + 3.36 (t)
+ 2.69 (T_1S_2 + T_2M + S_1A + Wt) + 4.26 (T_1W + S_1M + T_2A + S_2t)

Correlation Coefficient: R-squared = 95.4 per cent
R-squared = 91.4 per cent, adjusted for degrees of freedom.

Analysis of Variance:

Source	d.o.f.	SS	MS
Regression	7	4355.0	622.1
Residual	8	210.1	26.3
Total	15	4565.1	

Table A4.3: Regression Equation for the Yield of the Soft Fraction

$$\text{Yield of Soft Fraction (\%)} = 54.55 - 0.76\,(T_1) + 4.59(T_2) + 6.09\,(S_1) + 3.70\,(S_2) - 12.78\,(W) + 0.18\,(A) - 2.96\,(t) - 2.13\,(M) + 1.74\,(T_1T_2 + S_1t + S_2M + WA) - 2.55\,(T_1S_1 + T_2t + WM + S_2A) - 1.31\,(T_1S_2 + T_2M + S_1A + Wt) - 2.21\,(T_1W + S_1M + T_2A + S_2t) + 0.69\,(T_1A + tM + S_1S_2 + T_2W) - 2.19\,(T_1t + T_2S_1 + AM + S_2W) - 1.55\,(T_1M + T_2S_2 + S_1W + At)$$

Simplified Equation for the Yield of the Soft Fraction and Regression Statistics

$$\text{Yield of Soft Fraction (\%)} = 54.55 + 6.09\,(S_1) - 12.78\,(W)$$

Correlation Coefficient: R-squared = 71.6%
R-squared = 67.2%, adjusted for d.o.f.

Analysis of Variance:

Source	d.o.f.	SS	MS
Regression	2	3204.1	1602.1
Residual	13	1270.2	97.7
Total	15	4474.3	

Table A4.4: Regression Equation for the CBLF of the Intermediate Fraction

$$CBLF = 18.67 + 4.31\,(T_1) - 0.89\,(T_2) + 2.09\,(S_1) - 1.17\,(S_2) + 1.39\,(W) + 1.72\,(A) + 0.03\,(t) + 0.06\,(M) + 0.15\,(T_1T_2 + S_1t + S_2M + WA) + 0.23\,(T_1S_1 + T_2t + WM + S_2A) + 0.14\,(T_1S_2 + T_2M + S_1A + Wt) - 0.12\,(T_1W + S_1M + T_2A + S_2t) - 0.49\,(T_1A + tM + S_1S_2 + T_2W) - 1.24\,(T_1t + T_2S_1 + AM + S_2W) - 1.23\,(T_1M + T_2S_2 + S_1W + At)$$

Simplified Equation for the CBLF of the Intermediate Fraction and Regression Statistics

$$CBLF = 18.67 + 4.31\,(T_1) - 0.89\,(T_2) + 2.09\,(S_1) - 1.16\,(S_2) + 1.39\,(W) + 1.72\,(A) - 0.49\,(T_1A + tM + S_1S_2 + T_2W) - 1.25\,(T_1t + T_2S_1 + AM + S_2W) - 1.23\,(T_1M + T_2S_2 + S_1W + At)$$

Correlation Coefficient: R-squared = 99.7%

R^2 = 99.2%, adjusted for d.o.f.

Analysis of Variance:

Source	d.o.f.	SS	MS
Regression	9	536.06	59.6
Residual	6	1.78	0.3
Total	15	537.84	

Appendix 5: Regression Equations and Regression Statistics from the Central Composite Design (Chapter 6); and an Example of the Method used to compare Observed Responses with Responses Predicted from the Fitted Equations

Table A5.1: Empirical Model and Regression Statistics for the Yield of the Hard Fraction

Empirical Model:

Yield of hard fraction (%) = $21.37 - 5.51\ (T_1) - 2.01\ (S_1) + 3.63\ (W) - 2.91\ (T_1W)$

Correlation Coefficient:

R-squared = 59.3%

R-squared = 56.3%, adjusted for degrees of freedom

Analysis of Variance:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Regression	4	2332.1	583.0
Residual	54	1600.7	29.6
TOTAL	58	3932.8	

Test for Goodness of Fit:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Residual	54	1600.7	
Pure error (from one-way analysis of variance)	14	288.9	20.6
Lack of fit	40	1311.8	32.8

$\frac{MS_{lof}}{MS_{pe}} = \frac{32.8}{20.6} = 1.59.$ As $F_{40,14,.05} = 2.27$, cannot accept the hypothesis that there is significant lack of fit of the model at the 5% level.

Table A5.2: Empirical Model and Regression Statistics for the Yield of the Intermediate Fraction

Empirical Model:

Yield of intermediate fraction (%) = 10.20
+ 3.46 (T_1) - 4.48 (T_2) + 1.32 (S_1) - 1.25 (S_2) + 1.27 (w^2)
- 1.48 (T_1S_2) - 1.92 (T_2S_1) - 1.36 (S_1S_2) + 2.64 (S_2W)

Correlation Coefficient:

R^2 = 72.0%

R^2 = 66.8%, adjusted for degrees of freedom.

Analysis of Variance:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
Regression	9	2102.1	233.6
Residual	49	818.4	16.7
Total	58	2920.5	

Test for Goodness of Fit:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Residual	49	818.4	
Pure error (from one-way analysis of variance)	14	234.1	16.7
Lack of fit	35	584.3	16.7

$$\frac{MS_{lof}}{MS_{pe}} = \frac{16.7}{16.7} = 1.00$$

As $F_{35,14,.05} = 2.28$, cannot accept the hypothesis that there is significant lack of fit of the model at the 5% level.

Table A5.3: Empirical Model for the Yield of the Soft Fraction

Empirical Model:

Yield of soft fraction (%) = $68.43 + 2.06\ (T_1) + 4.22\ (T_2)$
$- 4.32\ (W) - 2.41\ (W^2) + 2.07\ (T_1S_2) + 3.05\ (S_1W) - 2.36\ (S_2W)$
$+ 2.52\ (S_2A)$

Correlation Coefficient:

$R^2 = 60.0\%$

$R^2 = 53.6\%$, adjusted for degrees of freedom.

Analysis of Variance:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Regression	8	2950.8	368.9
Residual	50	1970.4	39.4
Total	58	4921.2	

Test for Goodness of Fit:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Residual	50	1970.4	
Pure error (from one-way analysis of variance)	14	430.4	30.7
Lack of fit	36	1540.0	42.8

$$\frac{MS_{lof}}{MS_{pe}} = \frac{42.8}{30.7} = 1.39$$

As $F_{36,14,.05} = 2.28$, cannot accept the hypothesis that there is significant lack of fit of the model at the 5% level.

Table A5.4: Empirical Model for the CBLF of the Intermediate Fraction

Empirical Model:

$$CBLF = 17.69 + 1.31\,(T_1) + 0.51\,(T_2) + 0.94\,(S_1) - 0.58\,(S_2) - 0.76\,(W) + 1.07\,(A) - 0.54\,(T_1^2) + 1.36(T_2^2) + 1.01\,(W^2) + 0.77\,(T_1T_2) - 1.23\,(T_1W) - 1.02\,(T_1A) - 0.55\,(T_2S_1) - 0.92\,(T_2W)$$

Correlation Coefficient:

$R^2 = 76.7\%$

$R^2 = 69.4\%$, adjusted for degrees of freedom

Analysis of Variance:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Regression	14	551.7	39.4
Residual	44	167.3	3.8
Total	58	719.0	

Test for Goodness of Fit:

Source of Variation	Degrees of freedom	Sum of Squares	Mean Sum of Squares
Residual	44	167.3	
Pure Error (from one-way analysis of variance)	14	56.9	4.1
Lack of fit	30	110.4	3.7

$$\frac{MS_{lof}}{MS_{pe}} = \frac{3.7}{4.1} = 0.90$$

As $F_{30,14,.05} = 2.31$, cannot accept the hypothesis that there is significant lack of fit at the 5% level.

Table A5.5: Example Calculation of the Data Used to Compare the Observed and Predicted Responses of the CBLF of the Intermediate Fraction to the Isolated Effect of W

(1) Predicted Response

The empirical model that best predicts the CBLF of the intermediate fraction from the levels of the six independent variables is given in Table A5.4. From this, the isolated effect of W is:

$$Y = 17.69 - 0.76W + 1.01\ (W^2 - \text{average coded level of } W^2)$$
$$= 17.69 - 0.76W + 1.01\ (W^2 - 0.73406)$$

By substituting coded values of W into this equation, the predicted Y was calculated. The results of this are:

W	-2.378	-1	0	1	2.378
Y	24.5	18.7	16.9	17.2	20.9

Y is the predicted CBLF of the intermediate fraction and is plotted in Figure 6.9.

As an aid to plotting the predicted response curve, the minimum point of the function was calculated:

$$\frac{dY}{dW} = -0.76 + 2.02W = 0 \text{ at the minimum}$$
$$W = 0.38 \text{ at minimum (in coded terms)}$$
$$= 1.25\% \text{ of water in the acetone.}$$

(2) Observed Response

The observed response of the yield of the hard fraction to the isolated effect of W was calculated by averaging the yields observed at each of the coded levels of W.

Appendix 6: Regression Equations and Regression Statistics from the Box and Behnken Design (Chapter 7)

Table A6.1: Empirical Model for the Yield of the Hard Fraction and Regression Statistics

$$Y = 30.07 - 1.83\ (T_1) - 3.75\ (S_1) + 4.43\ (T_1^2)$$

Correlation Coefficients:

$R^2 = 54.1\%$

$R^2 = 48.6\%$, adjusted for d.o.f.

Analysis of Variance:

Source	d.o.f.	SS	MS
Regression	3	347.2	115.7
Residual	25	294.6	11.8
TOTAL	28	641.8	

Test for Lack of Fit:

Source	d.o.f.	SS	MS
Residual	25	294.6	
Pure Error	4	13.2	3.3
Lack of fit	21	281.4	13.4

$$\frac{MS_{lof}}{MS_{pe}} = \frac{13.4}{3.3} = 4.1$$

As $F_{21,4,.05} = 5.79$, cannot accept the null hypothesis that there is significant lack of fit of the model at the 5% level.

Table A6.2: Empirical Model for the Yield of the Intermediate Fraction and Regression Statistics

$$Y = 9.48 + 4.20\ (S_1) - 2.56\ (T_1^2)$$

Correlation Coefficient:

$R^2 = 45.5\%$

$R^2 = 41.1\%$, adjusted for d.o.f.

Analysis of Variance:

Source	d.o.f.	SS	MS
Regression	2	257.7	128.9
Residual	26	311.2	12.0
TOTAL	28	568.9	

Test for Lack of Fit:

Source	d.o.f.	SS	MS
Residual	26	311.2	
Pure error	4	16.0	4.0
TOTAL	22	295.2	13.4

$$\frac{MS_{lof}}{MS_{pe}} = \frac{13.4}{4.0} = 3.4$$

As $F_{27,4,.05} = 5.78$, cannot accept the null hypothesis that there is significant lack of fit of the model at the 5% level.

Table A6.3: Empirical Model for the CBLF of the Intermediate Fraction and Regression Statistics

$$Y = 13.41 + 2.13\ (S_1)$$

Correlation Coefficients:

$R^2 = 16.9\%$
$R^2 = 13.8\%$, adjusted for degrees of freedom.

Analysis of Variance:

Source	d.o.f.	MS	SS
Regression	1	54.19	54.2
Residual	27	266.35	9.9
TOTAL	28	320.54	

Test for Lack of Fit:

Source	d.o.f.	MS	SS
Residual	27	266.4	
Pure Error	4	9.2	2.3
Lack of fit	23	257.2	11.2

$$\frac{MS_{lof}}{MS_{pe}} = \frac{11.2}{2.3} = 4.9$$

As $F_{23,4,.05} = 5.78$, cannot accept the null hypothesis that there is significant lack of fit of the model at the 5% level.

Appendix 7: Results of the Triglyceride and Fatty Acid Analyses of Some Selected Tallow Fractions

For each fraction, the following analyses are presented:

(1) Total fatty acid composition.

(2) The proportion of each of the five triglyceride groups separated by argentation TLC.

(3) The fatty acid composition of each of these triglyceride groups.

(4) The fatty acid composition of the total tallow as calculated from the proportion and composition of each of the triglyceride groups.

(5) The fatty acid composition at position 2 of the cis-monounsaturated triglycerides.

Fraction: The soft fraction produced by fractionating 1 kg of tallow with S_1=1.5:1 (see 8.3.3 (54.1 wt % of total tallow))

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	3.5%	6.5%	28.0%		14.4%	47.6%	Determined	Calculated from triglyceride groups
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	28.1	12.6	11.9	6.7	4.2	4.0	7.7	7.6
iso-15:0+14:1	7.4	2.7	1.8	2.4	0.7	0.4	1.8	1.2
15:0	2.4	1.5	1.4	1.0	0.6	0.4	1.1	0.9
iso-16:0	1.2	1.0	0.6	1.5	0.2	0.1	0.5	0.4
16:0	37.9	26.3	27.2	20.7	16.4	18.1	19.3	21.6
16:1		6.1	4.9	6.7	4.8	6.1	4.4	5.4
17:0				2.0			1.5	
17:1		1.3	1.1	1.9	0.8	1.9	1.3	1.4
18:0	23.0	20.1	17.2	8.7	15.2	13.2	14.8	15.4
18:1		28.4	33.9	48.4	55.4	53.0	46.0	44.5
18:2					0.7	2.8	1.6	1.4

Fraction: The hard fraction produced by fractionating 1 kg of tallow with S_1=1.5:1 ((see 8.3.3) - (37.3 wt % of tallow))

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	26.5%	11.7%	27.9%		9.4%	24.5%	Determined	Calculated from triglyceride groups
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	14.0	10.4	7.7	9.7	4.2	4.3	8.6	8.5
iso-15:0+14:1	1.5	0.6	0.8	1.9	0.5	0.2	0.9	0.8
15:0	1.7	1.5	1.1	1.0	0.5	0.3	1.1	1.1
iso-16:0	0.4	0.4	0.3	1.0	0.3	0.2	0.3	0.3
16:0	43.3	29.5	25.4	22.5	17.3	19.6	28.3	28.4
16:1		2.9	3.5	5.9	4.4	4.4	3.2	2.8
17:0				2.2			2.5	
17:1		0.4	0.9	1.6	1.5	1.3	0.5	0.8
18:0	39.1	26.3	29.3	12.9	16.3	15.2	26.4	26.9
18:1		28.0	31.0	41.3	53.6	52.9	27.7	29.9
18:2					1.4	1.6	0.5	0.5

FRACTION: The intermediate fraction produced by fractionating 1 kg of tallow with S_1=1.5:1 ((see 8.3.3) - (8.6 wt % of tallow))

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	10.0	7.5	57.1		9.7	15.7	Determined	Calculated from triglyceride groups
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	21.0	9.4	5.2	8.3	0.4	3.4	6.4	5.2
iso-15:0+14:1	4.3	0.8	0.7	0.1	0.5	0.3	1.1	1.0
15:0	2.1	1.6	0.7	0.3	0.6	0.6	0.8	0.9
iso-16:0	0.6	0.5	0.1	0.2	0.4	0.4	0.3	0.3
16:0	42.4	30.7	23.0	13.9	17.4	18.8	22.9	24.3
16:1		2.7	1.9	1.6	3.0	3.6	3.2	2.1
17:0				1.2			2.3	
17:1		0.7	0.3	0.8	1.2	1.4	1.0	0.6
18:0	29.6	27.7	36.3	13.4	21.9	20.4	29.0	31.1
18:1		25.9	33.8	60.2	52.4	47.9	32.4	33.8
18:2					2.2	3.2	0.6	0.7

FRACTION: The hard fraction produced in the fractionation of 200 g of tallow with the propeller agitator (see 8.2.2) - (27.3 wt % of tallow))

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	33.5%	28.5%	23.5%		4.3%	10.2%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	15.9	6.4	4.1	7.5	4.8	4.7	8.4	8.8
iso-15:0+14:1	0.3	1.1	0.4	0.7	0.2	0.9	0.3	0.6
15:0	1.8	2.9	1.0	0.8	0.9	1.1	1.1	1.8
iso-16:0	0.5	3.0	0.4	0.7	0.3	0.3	1.0	1.2
16:0	43.4	29.7	29.0	24.9	21.8	24.9	33.6	33.3
16:1		2.4	2.3	3.1	4.0	3.0	2.8	1.7
17:0				3.2			2.6	
17:1		0.4	0.7	0.7	2.1	1.3	0.2	0.5
18:0	38.1	27.1	29.5	19.1	18.3	26.2	30.6	30.9
18:1		27.0	32.6	39.3	43.0	36.0	18.7	20.9
18:2					4.6	1.7	0.7	0.4

FRACTION: The intermediate fraction produced in the fractionation of 200 g of tallow with the propellor agitator (see 8.2.2) - (12.5 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	12.6%	12.9%	54.8%		9.1%	10.6%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	24.6	11.6	2.9	11.4	2.3	3.4	6.0	6.8
iso-15:0+14:1	6.6	1.0	0.4	0.3	0.2	0.3	0.5	1.2
15:0	2.2	1.7	0.8	0.8	0.4	0.5	0.7	1.0
iso-16:0	1.0	0.6	0.4	0.6	0.1	0.2	0.2	0,5
16:0	37.4	24.5	23.8	17.9	16.8	21.9	25.2	24.8
16:1		3.8	1.9	1.9	2.6	3.8	2.9	2.2
17:0				1.3			2.4	
17:1		0.9	0.5	0.9	1.0	1.3	0.6	0.6
18:0	28.2	27.4	34.8	13.4	20.4	24.0	30.3	30.6
18:1		28.5	34.5	51.5	54.5	43.7	30.7	32.2
18:2					1.7	0.9	0.5	0.3

FRACTION: The soft fraction produced by fractionating 1 kg of tallow with S_1=4.0:1 (see 8.3.2) - (67.2 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	3.2%	1.6%	30.3%		15.6%	49.3%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	19.4	17.8	11.4	10.4	5.8	4.2	7.8	7.7
iso-15:0+14:1	7.6	3.9	2.0	0.8	1.0	0.8	1.4	1.5
15:0	2.1	2.3	1.7	0.5	0.6	0.7	1.9	1.1
iso-16:0	0.8	1.4	0.6	0.6	0.3	0.3	0.6	0.4
16:0	36.7	26.0	27.4	18.5	17.8	14.4	19.8	19.8
16:1		5.2	3.7	5.1	5.2	4.5	5.4	4.2
17:0				0.8			1.5	
17:1		1.2	1.1	1.4	0.9	1.4	1.0	1.2
18:0	23.4	17.8	19.3	10.8	13.5	11.8	12.9	14.8
18:1		24.4	32.8	51.1	53.6	58.3	45.7	47.4
18:2					1.3	3.6	2.0	2.0

FRACTION: The soft fraction produced in the fractionation of 200 g of tallow with the propellor agitator (see 8.2.2) - (60.2 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	4.0%	4.1%	32.2%		15.0%	44.7%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	31.2	8.0	11.7	10.7	6.1	5.6	9.8	8.8
iso-15:0+14:1	7.8	3.7	1.3	0.6	0.7	1.0	1.0	1.4
15:0	2.0	1.6	1.3	2.5	0.7	0.8	0.9	1.0
iso-16:0	1.0	1.2	0.7	1.7	0.2	0.3	1.0	0.5
16:0	34.4	30.8	27.8	16.1	20.9	18.1	21.0	22.8
16:1		5.5	4.8	13.0	5.2	4.9	5.0	4.7
17:0				3.4			1.4	
17:1		0.6	0.9	2.3	1.3	1.3	1.1	1.1
18:0	23.6	20.9	18.0	7.1	16.0	13.5	16.0	16.0
18:1		27.7	33.5	42.6	48.1	51.0	41.3	41.9
18:2					0.8	3.5	1.5	1.7

FRACTION: The hard fraction produced by fractionating 1 kg of tallow with S_1=4.0:1 (see 8.3.2) - (23.0 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	36.3%	20.3%	28.1%		6.8%	8.5%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	14.9	8.1	6.0	8.2	5.4	4.0	10.8	9.4
iso-15:0+14:1	0.8	0.2	0.4	0.4	0.4	0.3	0.7	0.5
15:0	1.5	0.9	0.8	0.7	0.8	0.8	1.1	1.1
iso-16:0	0.3	0.5	0.3	0.4	0.3	0.2	0.5	0.3
16:0	45.7	32.7	32.0	25.1	27.8	29.6	33.5	36.6
16:1		3.4	2.9	3.2	3.9	3.6	3.1	2.1
17:0				3.2			2.6	
17:1		0.3	0.8	1.5	1.7	1.7	0.4	0.5
18:0	36.8	27.7	28.9	15.5	23.4	20.6	27.6	30.4
18:1		26.2	27.9	41.8	34.7	37.5	19.3	18.7
18:2					1.6	1.7	0.4	0.3

FRACTION: The intermediate fraction produced by fractionating 1 kg of tallow with S_1=4.0:1 (see 8.3.2) - (9.8 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	10.4%	11.3%	53.9%		10.4%	14.0%	Determined	Calculated from triglyceride groups
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %		
14:0	25.3	10.9	3.4	4.7	3.8	3.8	7.0	6.6
iso-15:0+14:1	2.3	0.7	0.4		0.4	0.3	0.8	0.6
15:0	1.8	1.3	1.3		0.8	0.7	0.7	1.2
iso-16:0	0.9	0.6	0.2		0.4	0.3	0.2	0.4
16:0	42.1	32.2	25.5	14.6	20.2	20.5	24.3	26.7
16:1		3.0	1.9	2.4	3.8	4.0	2.8	2.3
17:0				1.3			1.7	
17:1		0.5	0.8	1.2	1.1	1.6	0.5	0.8
18:0	27.6	26.8	33.9	7.6	21.1	20.5	28.2	29.2
18:1		24.0	32.6	68.2	46.6	45.4	33.4	31.5
18:2					1.8	2.9	0.4	0.6

FRACTION: The intermediate fraction produced in the fractionation of 20 g of tallow (see 6.10) - (4.1 wt % of tallow)

	Triglyceride Group						Fatty acid composition (mole % of fraction)	
	1	2	3		4	5		
% of total fraction	9.5%	8.8%	72.2%		6.4%	3.1%		
Fatty Acid	Mole %	Mole %	Mole %	Mole % of F.A.'s at the 2-position	Mole %	Mole %	Determined	Calculated from triglyceride groups
14:0	21.5	8.6	1.3	8.0	0.1	4.0	3.1	3.9
iso-15:0+14:1	4.7	0.5			0.1	0.1	0.4	0.5
15:0	2.2	1.2	0.5	0.2	0.7	0.7	0.5	0.7
iso-16:0	0.6	0.4			0.2	0.3	0.1	0.1
16:0	37.0	29.6	21.6	9.1	16.3	25.1	22.4	23.5
16:1		3.1	0.9	1.2	1.0	3.5	1.6	1.1
17:0				0.7			1.9	
17:1		0.6	0.3	0.7	1.0	1.6	0.4	0.4
18:0	34.0	33.9	40.9	9.6	26.2	33.2	36.8	38.4
18:1		22.1	34.5	70.5	49.9	30.8	32.3	31.0
18:2					4.5	0.6	0.5	0.3

Appendix 8:

Further Aspects of Tallow Quality

a) Edible Quality

A survey of New Zealand meat killing plants was carried out to determine their tallow production statistics. Unfortunately, approximately 50% of the meat killing plants did not reply. However, of the 21 plants from which replies were received, none were producing edible mutton tallow (or margarine grade mutton tallow) apart from a very small amount (approximately 230 tonnes/year) of a special edible mutton tallow produced from caul and kidney fats, and this would not be representative of the overall mutton tallow composition. Thus the studies in this thesis were made upon inedible mutton tallow. However before this tallow can be used commercially for purposes such as those suggested in this work, it is necessary that it be made suitable for edible purposes. Edible grade tallow must be produced from clean, sound, wholesome fat, usually trimmings, but excluding mesenteric and intestinal fats, and it must be produced in facilities up to full edible standards (Tulloch, 1975). Thus to improve the type of tallow studied in this work to edible standards, further selection of the raw materials may be required and this may alter the composition of the tallows. In New Zealand a further tallow type, margarine fat, is produced. This is an inedible fat when produced, but it can be used in margarine after re-sterilisation. It may be possible to use a similar tallow grade for commercial fractionation because of the sterilising effect of the acetone recovery and deodcrisation processes. Some raw materials are not permitted in New Zealand margarine fat (Tulloch, 1975) - condemned materials, trotters, pelt or hide by-products and save-all and trap grease - but these products are all likely to be minor constituents in any tallow anyway, so their exclusion from inedible tallow to produce a margarine grade tallow would probably have little effect upon the composition of the tallow. It has been estimated that approximately half of the total production of tallow in New Zealand could be either edible fat or margarine fat if the raw material was properly selected (Department of Biotechnology, 1975).

Thus before any commercial application of this work can be considered, it is necessary to determine if and how present inedible mutton tallow production can be modified to meet edible requirements.

b) Variation in Composition

The main factor affecting tallow composition is the make-up of the body fats on the animals entering the meat killing plant. However the practices followed within the plant can also affect the composition of the tallow. Tallow from only one meat killing plant was extensively studied in this work, so the effect of different plants upon tallow composition in New Zealand has not been studied.

Variation in tallow composition means that different yields of the three fractions may be obtained from different batches of tallow. For instance, the proportion of 2-oleo disaturated triglycerides was found to decrease significantly throughout the killing seasons (November to June) with an overall range from 10.0% (May, 1978) to 20.5% (November, 1976). Thus if a fraction containing a specified proportion of 2-oleo disaturated triglycerides is required from these tallows, the November tallow has the potential to produce twice as much of such a fraction. However it seems likely that fractionation of these two tallows under identical conditions would not produce fractions with similar compositions, irrespective of their relative yields. The characteristics of the tallow/solvent system may be altered to such an extent by changes in the composition of the tallow that different fractionation conditions are required to give products with similar compositions.

If the variation in tallow composition is found to seriously affect the fractionation process, it may be necessary for any large scale operation to bulk sufficient mutton tallow, from one period of the year, to supply the fractionation plant over an entire operating season. However this presupposes that a sufficient quantity of suitable tallow would be available at any one period, it would require large storage facilities and it would not overcome the problem of variation in composition between

seasons. Alternatively, the process conditions may be able to be varied to allow for changes in the composition of the tallow. This would require further experimentation to firstly determine how and if the process variables can be manipulated to compensate for changes in composition, it would necessitate constant and detailed monitoring of the incoming tallow, and it would probably lead to variation in the yields of the various fractions with consequential economic considerations.

ABBREVIATIONS AND NOMENCLATURE

A	agitation speed
A_i	area of DSC peak
A_T	sum of areas of DSC peaks for one sample
AOCS	American Oil Chemist's Society
CBLF	cocoa butter likeness factor
DSC	differential scanning calorimetry
E	elaidic acid
GLC	gas-liquid chromatography
H	heat content
i.d.	internal diameter
I.V.	iodine value
MS_{lof}	mean sum of squares due to lack of fit
MS_{pe}	mean sum of squares due to pure error
M	month in which a tallow is produced
n	number of separate peaks on the DSC curve for one sample
n_a	number of axial points in central composite design
n_c	number of cube points in central composite design
n_o	number of centre points in central composite design
N_T	total number of points in central composite design
NMR	nuclear magnetic resonance
O	oleic acid
P	palmitic acid
S	stearic acid
S_1	solvent to fat ratio at the first crystallisation
S_2	solvent to fat ratio at the second crystallisation
sn	stereospecific number
t	time
T_1	first crystallisation temperature
T_2	second crystallisation temperature
T_{i1}	temperature of the final deviation of DSC peak from base-line
T_{i2}	temperature of the initial deviation of DSC peak from base-line
T_{im}	temperature of DSC peak maximum
TG	triglyceride

TLC	thin-layer chromatography
Tris	tris (hydroxymethyl) aminomethane
W	water content of the acetone
X	independent variable in experimental design
Y	dependent variable in experimental design
Y_{CBLF}	CBLF of the intermediate fraction
Y_H	yield of the hard fraction
Y_I	yield of the intermediate fraction
Y_S	yield of the soft fraction
α	distance of axial points from the origin in a central composite design
α, β', β	polymorphic states of triglycerides

Pure stereospecific triglycerides are abbreviated as by Litchfield (1972) e.g. sn- glycerol -1- palmitate -2- oleate -3-stearate = sn - POS

Fatty acids are designated by the number of carbon atoms: number of double bonds, e.g. octadecanoic acid (stearic acid) = 18:0.

REFERENCES

Aarhus Cliefabrik, Brit. Pat. 1,006,041 (1964).

Akiya, T., Yukagaku 19, 577 (1970).

American Oil Chemist's Society, Official and Tentative Methods of the American Oil Chemist's Society, 3rd ed., Champaign, Illinois, 1973.

Andersen, A.J. and Williams, P.N., Refining of Oils and Fats for Edible Purposes, Pergamon Press, Oxford, London, New York, Paris, 1962.

Anonymous, J. Amer. Oil Chem. Soc. 54, 70A (1977).

Arnold, J.M., U.S. Pat. 3,095,480 (1963).

Ashton, J., The Market from a Seller's Viewpoint, p.34. In Proceedings of the Seminar on Rendering, Biotechnology Dept, Massey University, 1975.

Ayres, A.U., U.S. Pat. 2,807, 411 (1957).

Babayan, V.K., Cereal Sci. Today 15, No.7, 214 (1970).

Bacon, D.W. Ind. Eng. Chem. 62, 7 (1970).

Bailey, A.E., Melting and Solidification of Fats, Interscience Publishers, New York, 1950.

Bailey, A.E., Feuge, R.O. and Kraemer, E.A., J. Amer. Oil Chem. Soc. 20, 129 (1943).

Baliga, B.P. and Shitole, A.D., J. Amer. Oil Chem. Soc. 58, 110 (1981).

Bednarcyk, N.E., Edible Oils and Fats, Noyles Development Co., Park Ridge, New Jersey, 1969.

Belz, M.H., Statistical Methods for the Process Industries, McMillan, London, 1973.

Bentz, A.P. and Breidenbach, B.G., J. Amer. Oil Chem. Soc. 46, 60 (1969).

Beresford, M.W. and Rossell, J.B., Ger. Pat. 2,126,429 (1971).

Berger, K.G., Oil Palm News 22, 10 (1977).

Bernardini, E., Riv. Ital. Sostanze Grasse 45, 739 (1968).

Bernardini, E. and Bernardini, M., Riv. Ital. Sostanze Grasse 46, 607 (1969).

Bernardini, E. and Bernardini, M., Oleagineux 25, 99 (1970).

Bernardini, E. and Bernardini, M., Le Fractionnement par Solvant des Huiles, Graisses et Acides Gras. p.211. In Filtration in the Refining and Fractionation of Oils and Fats. International Symposium, Belgian Filtration Society, Brussels, 1976.

Best, R.L., Soeters, J., Davies, A.C. and Paul, S., U.S. Pat. 3,012,891 (1961).

Bhambani, T.R., Shitole, A.A. and Kane, J.G., J. Oil Technol. Ass. India 4, No.1, 3 (1972).

Blaney, T.L., Ger. Pat. 2,163,742 (1972).

Blank, M.L., Verdino, E. and Privett, O.S., J. Amer. Oil Chem. Soc. 42, 87 (1965).

Boucher, R.E. and Skau, E.L., J. Amer. Oil Chem. Soc. 28, 483 (1951).

Boucher, R.E. and Skau, E.L., J. Amer. Oil Chem. Soc. 29, 582 (1952).

Box, G.E.P. and Behnken, D.W., Technometrics 2, 455 (1960).

Box, M.J., Davies, D. and Swann, W.H., Non-linear Optimisation Techniques, Oliver and Boyd, Edinburgh, 1969 (Mathematical and Statistical Techniques for Industry, Imperial Chemical Industries Ltd, Monograph No.5).

Box, G.E.P. and Hunter, J.S., Ann. Math. Stat. 28, 195 (1957).

Box, G.E.P. and Hunter, J.S., Technometrics 3,333 (1961).

Box, G.E.P. and Hunter, J.S., Technometrics 3,449 (1961).

Box, G.E.P. and Wilson, K.B., J. Roy. Stat. Soc. B13, 1 (1951).

Braae, B., Detergent Fractionation of Fatty Oils, p.189. In Filtration in the Refining of Oils and Fats, International Symposium, Belgian Filtration Society, Brussels, 1976.

Brockerhoff, H., Comp. Biochem. Physiol. 19, 1 (1966).

Brockerhoff, H., Hoyle, R.J. and Wolmark, N., Biochim. Biophys. Acta 116, 67 (1966).

Brockerhoff, H. and Yurkowski, M., J. Lipid Res. 7, 62 (1966).

Brown, C., personal communication, 1981.

Brown, J.B., J. Amer. Oil Chem. Soc. 32, 646 (1955).

Brown, J.B. and Kolb, D.K., Progr. Chem. Fats Other Lipids 3, 57 57 (1955).

Bussey, D.M., Ryan T.C., Gray J.I. and Zabik, M.E., J. Fd. Sci. 46, 526 (1981).

Callow, E.H., J. Agr. Sci. 51, 361 (1958).

Cavanagh, G.C., Brit. Pat. 801,102 (1958) and U.S. Pat. 2,883,405 (1959).

Cavanagh, G.C., personal communication, 1976 via. Bissell, T., personal communication, 1977.

Chapman, D., Chem. Rev. 62, 433 (1962).

Chapman, G.M., Akehurst, E.E. and Wright, W.B., J. Amer. Oil Chem. Soc. 48, 824 (1971).

Chatfield, C., Statistics for Technology, Penguin Books, 1970.

Chen, P.C. and deMan, J.M., J. Dairy Sci. 49, 612 (1966).

Christie, W.W., Lipid Analysis, Pergamon Press, 1973.

Christie, W.W. and Moore, J.H., J. Sci. Fd. Agr. 22, 120 (1971).

Cocks, L.V. and van Rede, C., Laboratory Handbook for Oil and Fat Analysts, p.335, Academic Press, London and New York, 1966.

Coenen, J.W.E., Rev. Fr. Corps Gras 21, 343 (1974).

Construzioni Meccaniche Bernardini (C.M.B.), Palm Oil Fractionation and Refining using the C.M.B. Process, Tipolitografia Eurprint, Rome.

Contreras, O.R., Migliaro, A.O. and Raffo A.R., J. Amer. Oil Chem. Soc. 48, 98 (1971).

Coppa-Zucarri, G., Chem. Processing 17, No.3, 13 (1971).

Cording, J., Willard, M.J., Edwards P.W. and Eskew R.K., J. Amer. Oil Chem. Soc. 30, 66 and 111 (1953).

Cramer, D.A. and Marchello, J.A., J. Anim. Sci. 23, 1002 (1964).

Craske, J.D. and Edwards, R.A., J. Chromatogr. 53, 253 (1970).

Crossley, A., Paul, S., Pardun, H. and Soeters, C.J., Brit. Pat. 841,316 (1960) and U.S. Pat. 2,975,061 (1961) - A.

Crossley, A., Paul, S., Pardun, H. and Soeters, C.J., Brit. Pat. 841,317 (1960) and U.S. Pat. 2,975,060 (1961) - B.

Dallow, P.J., Mfring. Confect. 54, No.2, 26 (1974).

Daniel, C., Technometrics 1, 311 (1959).

Daniels, T., Thermal Analysis, John Wiley and Sons, New York, 1973.

Davies, O.L., The Design and Analysis of Industrial Experiments, Longman Group Ltd., London, for Imperial Chemical Industries Ltd., 1978.

Decossas, K.M., Koltun, S.P., Spadaro, J.J., Feuge, R.O., Pollard, E.F. and Patton, E.L., J. Amer. Oil Chem. Soc. 39, 146 (1962).

Defouw, C.L., Zabik, M.E. and Gray, J.I., J. Fd. Sci. 46, 452 (1981).

Demmerle, R.L., Ind. Eng. Chem. 39, 126 (1947).

Department of Biotechnology, Proceedings of the Seminar on Rendering, Massey University, Palmerston North, 1975.

Deroanne, C., Fd. Sci. Technol. 10, 251 (1977).

Dobson, R.D., U.S. Pat. 3,360,376 (1967).

Driscoll, G.L., Duling, I.N. and Magnotta, F., Purity Determinations Using a Differential Scanning Calorimeter. p.271. In Porter, R.S. and Johnson, J.F., Analytical Calorimetry, Plenum Press, New York, 1968. (From proceedings of the Am. Chem. Soc. Symposium, San Francisco, 1968).

Dugan, L., Lipids. p.139. In Fennema, O.R., Principles of Food Science, Part I - Food Chemistry, Marcel Dekker, New York and Basel, 1976.

Duncan, W.R.H. and Garton, G.A., J. Sci. Fd. Agr. 18, 99 (1967).

Eckey, E.W., Vegetable Fats and Oils, Reinhold Publishing Co., New York, 1954 (American Chem. Soc. Monograph Series No.123).

Elias, S., Fd. Eng. 4, No.9, 115 (1979).

Errboe, J.T., Braemer-Madsen, J. and Andersen, B., Brit. Pat. 1,219,245 (1971).

Farr, G.W., U.S. Pat. 2,903,363 (1954).

Feldpush, N.V., U.S. Pat. 2,510,015 (1950).

Feuge, R.O., J. Amer. Oil Chem. Soc. 41, No.4, 4 (1964).

Feuge, R.O., Gajee, B.B. and Lovegren, N.V., J. Amer. Oil Chem. Soc. 50, 50 (1973).

Feuge, R.O. and Lovegren, N.V., J. Amer. Oil Chem. Soc. 35, 194 (1958).

Fisher, R.A. and Yates, F., Statistical Tables for Biological, Agricultural and Medical Research, 6th ed., Oliver and Boyd, Edinburgh, 1963.

Food Technology Research Centre, Project 80-40, Massey University, Palmerston North, 1981.

Fuji Oil Co. Ltd, Jap. Pat. 75/47,253 (1975) and Netherl. Pat. 76 04, 177, 19 (1976).

Garton, G.A. and Duncan, W.R.H., Biochim. Biophys. Acta 98, 436 (1965).

Garton, G.A. and Duncan, W.R.H., J. Sci. Fd. Agr. 20, 39 (1969).

Gavin, M.A., J. Amer. Oil Chem. Soc. 54, 528 (1977).

Gee, W.P., U.S. Pat. 2,450,235 (1948).

Gillies, M.T., Shortenings, Margarines and Food Oils, Noyes Data Corporation, Park Ridge, New Jersey, 1974 (Fd. Tech. Review No.10).

Gloyer, S.W., Ind. Eng. Chem. 40, 228 (1948).

Gloyer, S.W. and Stewart, W., J. Amer. Oil Chem. Soc. 27, 462 (1950).

Gordon, M.H., Padley, F.B. and Timms, R.E., Fette, Seifen, Anstrichm. 81, 116 (1979).

Goss, W.H., J. Amer. Oil Chem. Soc. 26, 584 (1949).

Gray, A.P., A Simple Generalised Theory for the Analysis of Dynamic Thermal Measurement. p.209. In Porter, R.S. and Johnson, J.F., Analytical Calorimetry, Plenum Press, New York, 1968 (from proceedings of the Am. Chem. Soc. Symposium, San Francisco, 1968).

Gray, M.S., Lovegren, N.V. and Feuge, R.O., J. Amer. Oil Chem. Soc. 53, 727 (1973).

Haighton, A.J. and Vermaas, L., Fette, Seifen, Anstrichm. 71, 614 (1969).

Hampson, J.W., Luddy, F.E. and Rothbart, H.L., Candy and Snack Ind. 140, No.12, 37 (1975).

Hannewijk, J., Haighton, A.J. and Hendrikse, P.W., Dilatometry of Fats p.119. In Boekenoogen, H.A., Analysis and Characterisation of Oils, Fats and Fat Products, vol.I, Interscience Publishers, New York and Sydney, 1964.

Hansen, R.P. and Czochanska, Z., N.Z. J. Sci. 19, 413 (1976).

Hasman, J.M. and Zielinski, R.J., U.S. Pat. 3,796,816 (1974).

Hawke, J.C., Morrison, I.M. and Wood, P.R., J. Sci. Fd. Agr. 28, 293 (1977).

Herbert, L.S. and Kearney, K.J., J. Fd. Technol. 10, 55 (1975).

Hilditch, T.P., The Chemical Constitution of Natural Fats, 3rd ed., Chapman and Hall, London, 1956.

Hilditch, T.P. and Williams, P.N., The Chemical Constitution of Natural Fats, 4th ed., Chapman and Hall, London, 1964.

Hilditch, T.P. and Shrivastava, R.K., J. Amer. Oil Chem. Soc. 26, 1 (1949).

Hill, E.E., Husbands, D.R. and Lands, W.E.M., J. Biol. Chem. 243, 4440 (1968).

Hinnekens, H., Fat Fractionation Without Solvent. p.145. In Filtration in the Refining and Fractionation of Oils and Fats, International Symposium, Belgian Filtration Society, Brussels, 1976.

Hixson, A.W. and Bockelmann, J.B., Trans. Am. Inst. Chem. Eng. 38, 891 (1942).

Huyghebaert, A. and Hendrickx, H., Lebensmittel-Wissenschaft und Technologie. 4, 59 (1971).

Jacobsberg, B. and Jacqmain, D., Objectifs et Modes du Fractionnement des Corps Gras. p.119. In Filtration in the Refining of Oils and Fats, International Symposium, Belgian Filtration Society, Brussels, 1976.

James, E.M., U.S. Pat. 2,459,820 (1943).

Jamieson, G.R., Topics Lipid Chem. 1, 107 (1970).

Johnston, G.M., J. Amer. Oil Chem. Soc. 49, 462 (1972).

Jurriens, G. and Kroesen, A.C.J., J. Amer. Oil Chem. Soc. 42, 9 (1965).

Kao Soap Co. Ltd, Jap. Pat. 16, 510/71 (1971).

Kassabian, R., Brit. Pat. 1,239,288 (1971).

Kassabian, R., U.S. Pat. 3,632,616 (1972).

Kassabian, R., The Fractionation of Palm Oil. Paper presented at the Fall Meeting of the American Oil Chemist's Society, Philadelphia, 1974.

Kawada, T. and Matsui, N., Brit. Pat. 1,203,885 (1968).

Kawada, T., Suzuki, S. and Matsui, N., Ger. Pat. 1,958,792 (1972).

Kawamura, K., J. Amer. Oil Chem. Soc. 56, 753 (1979).

Kistler, D.R., Muckerheide, V.J. and Myers, L.D., Oil and Soap 23, 146 (1946).

Kooyker, W. and Gustafson, D., Fd. Prod. Develop. 11, No.7, 24 (1977).

Koslowsky, L., Oleagineux 27, 557 (1972).

Koslowsky, L., Oil Palm News 15, 24 (1973).

Koslowsky, L., Grasas Aceites 26, 95 (1975).

Koslowsky, L., U.S. Pat. 4,004,041 (1977).

Koslowsky, L. and Letan, A., Riv. Ital. Sostanze Grasse. 52, 217 (1975).

Kreulen, H.P., J. Amer. Oil Chem. Soc. 53, 393 (1976).

Krishnamurthy, A., Ramalingaswamy, P.A., Banerjee, P.K. and Achaya, K.T., Res. Ind. (New Delhi) 10, No.3, 72 (1965).

Kuester, J.L. and Mize, J.H., Optimisation Techniques with Fortran, McGraw-Hill, New York, 1973.

Kundu, M.K., Fette, Seifen, Anstrichm. 72, 1029 (1970).

Lacey, E.F. and Leaders, W.M., U.S. Pat. 2,552,797 (1951).

Landmann, W., Lovegren,N.V. and Feuge, R.O., J. Amer. Oil Chem. Soc. 38, 466 (1961).

Larsson, K., Proc. Chem. Soc. p.87 (1963).

Larsson, K. Fette, Seifen, Anstrichm. 74, 136 (1972).

Lav, J., Smegal, H. and Giancarlo, R., Ger. Pat. 2,142,134 (1973).

L'estrange, J.L. and Mulvihill, T.A., J. Agr. Sci. 84, 281 (1975).

Litchfield, C., Analysis of Triglycerides, Academic Press, London and New York, 1972.

Loders and Nucoline Ltd, Fd. Proc. and Packag. 33, No.388, 9 (1964).

Loncin, M., Filtration in the Refining and Fractionation of Oils and Fats, International Symposium, Belgian Filtration Society, Brussels, 1976.

Lovegren, N.V., Gajee, B.B., Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 50, 53 (1973).

Lovegren, N.V., Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 48, 519 (1971).

Lovegren, N.V., Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 50, 129 (1973).

Lovegren, N.V.,Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 53, 83 (1976).

Lovegren, N.V., Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 53, 108 (1976).

Lovegren, N.V., Gray, M.S. and Feuge, R.O., J. Amer. Oil Chem. Soc. 53, 727 (1976).

Luddy, F.E., Fertsch, G. and Riemenschneider, R.W., J. Amer. Oil Chem. Soc. 31, 266 (1954).

Luddy, F.E., Hampson, J.W. and Herb, S.F., U.S. Pat. 4,049,839 (1977).

Luddy, F.E., Hampson, J.W., Herb, S.F. and Rothbart, H.L. J. Amer. Oil Chem. Soc. 50, 240 (1973).

Luddy, F.E., Hampson, J.W., Herb, S.F. and Rothbart, H.L., U.S. Pat. 3,994,585 (1976).

Luddy, F.E., Hampson, J.W., Herb, S.F. and Rothbart, H.L., U.S. Pat. 4,072,766 (1978).

Luddy, F.E., Hampson, J.W. and Koos, R.E., Abstract 73 for the AOCS 70th annual meeting, San Francisco, 1979. In J. Amer. Oil Chem. Soc. 56, 185A (1979).

Luddy, F.E., Rothbart, H.L., Hampson, J.W., Miller, R.L. and Alt, E.F., Fd. Eng. Int'l. 1, No.7, 8 (1976).

Lutton, E.S., J. Amer. Oil Chem. Soc. 27, 276 (1950).

Lutton, E.S., J. Amer. Oil Chem. Soc. 49, 1, (1972).

Magnusson, J.R. and Hammond, E.G., J. Amer. Oil Chem. Soc. 36, 339 (1959).

Marcus, J.G., Fd Prod. Develop. 8, No.7, 74 (1974).

Maron, S.H. and Prutton, C.F., Principles of Physical Chemistry, Collier-Macmillan Ltd, London, 1969.

Martinenghi, G.B., Oleagineux 27, 267 (1972).

Martinenghi, G.B., Oleagineux 29, 315 (1974).

Martinenghi, G.B., Riv. Ital. Sostanze Grasse 52, 311 (1975).

McFarlane, P., The Occurrence of Chromatiaceae in Waste Treatment Lagoons and their Utilisation to Treat Fellmongery Effluent, Ph.D. thesis, Biotechnology Dept, Massey University, 1979.

McGuine, T.H., U.S. Pat. 2,952,696 (1960).

McGuine, T.H. and Moss, W.E., U.S. Pat. 2,952,696 (1960).

McNair, H.M. and Bonelli, E.J., Basic Gas Chromatography, Varian Aerograph, Walnut Creek, California, 1968.

Melnick, D. and Gooding, C.M., U.S. Pat. 2,921,855 (1960).

Mertens, W.G., Rubin, L.J. and Teasdale, B.F., J. Amer. Oil Chem. Soc. 38, 286 (1961).

Minifie, B.W., Chocolate, Cocoa and Confectionery: Science and Technology, J. and A. Churchill, London, 1970.

Minifie, B.W., Chocolate, Cocoa and Confectionery: Science and Technology, 2nd ed., AVI Publishing Co., Westport, Connecticut, 1980.

Minowa, Masyoshi, Toyoshima, Yasuo, Yasuda and Nozomi, Jap. Pat. 77/61,263 (1977).

Mishkel, M.A. and Spritz, N., Advances in Experimental Medical Biology, Vol.4, Plenum Press, New York, 1969.

Morris, S.G., Gordon, C.F., Brenner, N., Meyers, J.S., Riemenschneider, R.W. and Ault, W.C., J. Amer. Oil Chem. Soc. 29, 441 (1952).

Morrison, I.M., Modifications in the Structure of the Triacylglycerols of Bovine Milk Fat, Ph.D. thesis, Biochemistry Dept., Massey University, 1976.

Muckerheide, V.J., U.S. Pat. 2,514,608 (1950).

Mullin, J.W., Crystallisation, Butterworths, London, 1961.

Myers, R.H., Response Surface Methodology, Allyn and Bacon, Boston, 1971.

Naudet, M. Rev. Fr. Corps Gras 11, 326 (1964).

New Zealand Department of Health, Food and Drug Regulations, Government Printer, Wellington, 1973 .

New Zealand Department of Statistics, New Zealand Official Yearbook, Government Printer, Wellington, 1980.

Norris, R., The Physical Properties of Triacylglycerols of Milk Fat, Ph.D. thesis, Chemistry Dept., Massey University, 1977.

O'Neill, M.J., Analyt. Chem. 36, 1238 (1964).

Padley, F.B., Paulussen, C.N., Soeters, C.J. and Tresser, D., Rev. Int. Chocolaterie 27, 226 (1972).

Parsons, A.M., U.S. Pat. 3,892,789 (1975).

Patterson, H.B.W., Chem. Ind. p.771 (1976).

Pattinson, M., Edible Tallow Specifications. p.20. In Proceedings of the Seminar on Rendering, Biotechnology Dept., Massey University, 1975.

Paulicka, F.R., Candy Ind. and Confect. J. 134, No.12, 5 (1970).

Paulicka, F.R., Chem. Ind., p.835 (1973).

Perkin-Elmer Corporation, Instructions - Differential Scanning Calorimeter, Norwalk, Connecticut, U.S.A., 1969.

Poot, C., Dijkshoorn, W., Haighton, A.J. and Verburg, C.C., J. Amer. Oil Chem. Soc. 52, 69 (1975).

Pramuk, M.B., Whiting, McLaren, F.H., U.S. Pat. 2,645,652 (1953).

Pratt, T.W., J. Amer. Oil Chem. Soc. 30, 497 (1953).

Pratt, T.W., J. Amer. Oil Chem. Soc. 33, 549 (1956).

Rayshich, A., J. Amer. Oil Chem. Soc. 30, 575 (1953).

Rek, J.H.M., Oils, Fats, Soaps and Waxes. p.473. In Mackenzie, R.C., Differential Thermal Analysis, Vol.II, Applications, Academic Press, London and New York, 1972.

Rek, J.H.M., U.S. Pat. 4,049,687 (1977).

Riel, R.R. and Paquet, R., J. Inst. Can. Sci. Technol. Aliment. 5, 210 (1972).

Riemenschneider, R.W., J. Amer. Oil Chem. Soc. 31, 517 (1954).

Riemenschneider, R.W. and Luddy, F.E., J. Amer. Oil Chem. Soc. 23, 385 (1946).

Riemenschneider, R.W., Luddy, F.E., Barford, R.A., Herb, S.F. and Magidman, P., J. Amer. Oil Chem. Soc. 41, 693 (1964).

Riemenschneider, R.W., Luddy, F.E., Swain, M.L. and Ault, W.C., J. Amer. Oil Chem. Soc. 23, 276 (1946).

Röbbeling, K.H., Fette, Seifen, Anstrichm. 72, 1037 (1970).

Rosenbrock, H.H. and Storey, C., Computational Techniques for Chemical Engineers, Pergamon Press, 1966.

Rossell, J.B., Advan. Lipid Res. 5, 355 (1967).

Rossell, J.B., Chem. Ind. p.832 (1973).

Rubin, L.J., Teasdale, B.F. and Mertens, W.G., U.S. Pat. 2,910,363 (1959).

Ruddick, R.B., U.S. Pat. 2,934,547 (1960) and Brit. Pat. 886,138 (1960).

Ryberg, J.R., Cereal Sci. Today 15, No.1, 16 (1970).

Ryberg, J.R., Baker's Dig. 45, No.4, 41 (1971).

Sambuc, E., Rev. Fr. Corps Gras 11, 319 (1964).

Sampugna, J. and Jensen, R.G., Lipids 4, 444 (1969).

Sampugna, J., Jensen, R.G., Parry, R.M., Jr., and Krewson, C.F., J. Amer. Oil Chem. Soc. 41, 132 (1964).

Scott, T.W., Setchell, B.P. and Bassett, J.M., Biochem. J. 104, 1040 (1967).

Seugé, J.P. and Vinconneau, H.F., Oleagineux 30, 25 (1975).

Shearon, W.H., Seestrom, H.E. and Hughes, J.P., Ind. Eng. Chem. 42, 1266 (1950).

Sheppard, A.J., Iverson, J.L. and Weihrauch, Composition of Selected Dietary Fats, Oils, Margarines and Butter, p.341. In Kuksis, A., Fatty Acids and Glycerides, Plenum Press, New York and London, 1978 (Handbook of Lipid Research, Volume 1).

Sherbon, J.W. and Dolby, R.M., J. Dairy Sci. 56, 52 (1973).

Shorland, F.B., J. N.Z. Inst. Chem. 12, No.1, 10 (1948).

Shorland, F.B., J. Sci Fd. Agr. 4, 497 (1953)

Shorland, F.B., Barton, R.A., Cramer, D.A. and Czochanska, Z., J. Agr. Sci. 69, 367 (1967).

Sink, J.D., Watkins, J.L., Ziegler, J.H. and Miller, R.C., J. Anim. Sci. 23, 121 (1964).

Sinnema, Y.A., U.S. Pat. 3,070,445 (1962).

Sinnema, Y.A., U.S. Pat. 3,084,049 (1963).

Sinnema, Y.A., Ger. Pats. 1,492,932 (1967) and 1,492,933 (1967).

Skau, E.L., Dopp, W.N., Burleigh, E.G. and Banowetz, L.F. J. Amer. Oil Chem. Soc. 27, 556 (1950).

Smorenburg, J.J., Netherl. Pat. 7,103,763 (1972).

Societe Pour L'Equipment des Industries Chimiques Speichim, Brit. Pat. 1,387,531 (1975).

Soeters, C.J., Fette, Seifen, Anstrichm. 72, 711 (1970).

Spadaro, J.J., Lovegren, N.V., Feuge, R.O. and Patton, E.L., J. Amer. Oil Chem. Soc. 38, 461 (1961).

Staker, D.D., Plantholt, R.H. and Kriege, D.J., Ger. Pat. 1,917,453 (1969).

Stedman, B., personal communication, 1981.

Stein, W. and Hartmann, H., U.S. Pat. 3,870,735 (1975).

Stevens, A.H., Brit. Pat. 667,365 (1952).

Subrahmanyam, V.V.R. and Achaya, K.T., J. Amer. Oil Chem. Soc. 35, 467 (1958).

Swern, D., Bailey's Industrial Oil and Fat Products, Interscience Publishers, New York, London and Sydney, 1964.

Tatsumi, C., Hashimoto, Y., Terashima, M. and Matsuo, T., U.S. Pat. 4,032,405 (1977).

Taylor, A.M., Oil Palm News. 15, 18 (1973).

Taylor, M.W. and Hawke, J.C., N.Z. J. Dairy Sci. Technol. 10, 49 (1975).

Taylor, H.H., Luddy, F.E., Hampson, J.W. and Rothbart, H.L., Substitutability of Fractionated Beef Tallow for Other Fats and Oils in the Food and Confectionery Industries - an Economic Evaluation. Paper presented at the Fall meeting of the American Oil Chemist's Society, Cincinnati, Ohio, 1975.

Texaco Development Corporation, Brit. Pat. 667,365 (1952).

Thomas, A.E. and Paulicka, F.R., Chem. Ind. p.774 (1976).

Thompson, S.W., U.S. Pat. 3,102,814 (1963).

Tirtiaux, F., Belg. Pat. 713,430 (1968).

Tirtiaux, F., Oleagineux 31, 279 (1976).

Towne, C.C., U.S. Pat. 2,459,054 (1949).

Tulloch, J.D., Meat Regulations Governing Rendering Plants. p.8. In Proceedings of the Seminar on Rendering, Biotechnology Dept., Massey University, Palmerston North, 1975

Unilever Ltd, Brit. Pat. 1,147,475 (1966).

Unilever Ltd, Netherl. Pat. 7,107,447 (1971).

Unilever Ltd, Netherl. Pat. 7,314,265 (1974).

Van Beers, G.J., The Determination of Linoleic Acid with Special Reference to the Isotopic Dilution Technique. p.72. In Devine, J. and Williams, P.N., The Chemistry and Technology of Edible Oils and Fats - Proceedings of a Conference Arranged by Unilever Ltd, Pergamon Press, 1961.

Van Wijngaarden, D., Analyt. Chem. 39, 848 (1967).

Viarengo, A. and Pasculli, R., U.S. Pat. 3,755,390 (1973).

Walker, R.C. and Bosin, W.A., J. Amer. Oil Chem. Soc. 48, 50 (1971)

Watson, E.S., O'Neill, M.J., Justin, J. and Brenner, N., Analyt. Chem. 36, 1233 (1964).

Weiss, T.J., J. Oil and Oilseeds 20, No.1, 12 (1967).

Weiss, T.J., Food Oils and their Uses, AVI Publishing Co., Westport, Connecticut, 1970.

Wendlandt, W., Thermal Methods of Analysis, 2nd ed., Wiley, New York, 1974.

Williams, K.A., Oils, Fats and Fatty Foods, J. and A. Churchill Ltd., London, 1950.

Willie, R.L. and Lutton, E.S., J. Amer. Oil Chem. Soc. 43, 491 (1966).

Wisniak, J. and Barrientos, J., J. Amer. Oil Chem. Soc. 44, 143 (1967).

Witzel, H. and Becker, K., Fette, Seifen, Anstrichm. 71, 507 (1969).

Wolf, A.J., Mfring. Confect. 55, No.6, 61 (1975).

Young, V., Chem. Ind. p.692 (1978).
Zilch, K.T., U.S. Pat. 3,345,389 (1967).
Zondek, K., Fr. Pats. 2,141,466 (1973) and 2,149,257 (1973) and Ger. Pat. 2,312,812 (1974).