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THE GLC ANALYSIS OF THREE-COMPONENT FAT/OIL MIXTURES  
IN THE DETERMINATION OF COMPONENT  
PROPORTIONS

A thesis presented in fulfilment of the requirements  
for Master in Technology degree at  
Massey University

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ABSTRACT

By making use of the fatty acid composition of mixture components and the three-fat(s)/oil(s) mixture, obtained from the gas-liquid chromatographic analysis, the relationship between mixture components and mixture was expressed by the general mixture equation:

$$(A_i - C_i) X_1 + (B_i - C_i) X_2 = (M_i - C_i)$$

where  $A_i$  is the fatty acid percent value of the first mixture component;  $B_i$  is the fatty acid percent value of the second mixture component;  $C_i$  is the fatty acid percent value of the third mixture component;  $M_i$  is the fatty acid percent value of the mixture;  $X_1$  is the fraction of the first component in the mixture; and  $X_2$  is the fraction of the second component in the mixture.

The fraction of first two mixture component can then be estimated by solving simultaneous mixture equations. This can be done either by employing the Cramer's Rule method (McCracken and Dorn, 1964) or by the Gauss-Jordan Elimination method (Dodges, 1978). The third mixture component proportion (i.e.  $X_3$ ) can be obtained by substituting  $X_1$  and  $X_2$  into the following equation:

$$X_3 = 1 - X_1 - X_2$$

The F77 FREQC program, using the Gauss-Jordan Elimination Method to estimate the proportion of each mixture component and the Frequency Method to calculate the weighted mean proportion of each mixture component, gave the best results with almost all of the calculated weighted mean mixture component proportions to be within two calculated standard deviations.

The accuracy of the calculated estimations was dependent upon the accuracy of the experimental and literature component data. In most cases, literature data did not produce as good results as those calculated from experimental data.

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PREFACE

The prohibition of the import of New Zealand pure milk fat into Japan has compelled New Zealand Dairy Board to export milk fat with other fats or oils in the form of a mixture. Nevertheless, for custom declaration purposes, the proportion of each mixture constituent must be stated and determined by a reliable and accurate method. The method must be simple and rapid; and made available to the Japanese authority.

For two components mixture, a computer aided method had been developed by Jebson and Curtis (1981) which proved reliable. However, there was no published article regarding the analysis of three fat(s)/oil(s) mixture available. Hence the analysis, with the aid of computer programs and the gas-liquid chromatograph instrument, was carried out.

The aim of this work was to determine a simple, rapid and accurate method, capable of automation for routine use, for the analysis of three fat(s)/oil(s) mixture.

This research, the analysis of three fat(s)/oil(s) mixture, involved (1) the setting up of mathematical model for three fat(s)/oil(s) mixture; (2) the writing of computer programs using the mathematical model; (3) the Gas Chromatography in analysing the fatty acid compositions of fats, oils and mixtures; (4) the testing of computer programs with data collected from (3); (5) the analysing of results to determine which computer program gave the best results; and (6) the use of the best computer program to test the literature fatty acid values of components to determine the accuracy of the results.

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# chapter 1

## 1 LITERATURE REVIEW

### 1.1 Reichert-Meissl, Polenske and Kirschner Methods

Long before the existence of the Gas Liquid Chromatograph instrument and other modern techniques, attempts to determine the mixture component proportions were made by several research workers. Some of the interesting methods are Reichert-Meissl, Polenske, and Kirschner methods (Cocks and Rede, 1966).

In the Reichert-Meissl method, Reichert-Meissl value is calculated, which is the number of ml. of 0.1N alkali required to neutralize the steam-volatile, water-soluble fatty acids. The fatty acids are prepared by first saponifying 5gm of fat and acidifying the soap solution with dilute sulphuric acid, then followed by the distillation of a known volume of distillate under well controlled conditions. After filtering the insoluble acids from the distillate, the soluble fatty acids are titrated with standard alkali.

In the Polenske method, Polenske value, which is the number of ml. of 0.1N alkali required to neutralize the steam-volatile, water-insoluble fatty acids, is calculated. The insoluble acids filtered off from the distillate in the Reichert-Meissl method are dissolved in ethanol and titrated with 0.1N sodium or potassium hydroxide.

Similarly, in the Kirschner method the Kirschner value, which is the number of ml. of 0.1N alkali solution required to neutralize the water-soluble volatile fatty acids which form water-soluble silver salts, is calculated. The fatty acids are prepared by adding silver sulphate to the solution remaining after determination of steam-volatile, water-soluble acids in Reichert-Meissl method.

For a mixture that has milk fat as one of its components, the proportion of milk fat is estimated as follows:

$$\text{Milk fat \%} = \frac{K - (0.1 P + 0.24)}{0.244}$$

where K is the Kirschner value ; and  
P is the Polenske value.

Based on Kirschner and Polenske values of milk fat, coconut oil and palm kernel oil mixture; and Reichert-Meissl and Polenske values of coconut oil and palm kernel oil mixture, two graphs were drawn by Williams (refer to "Laboratory Handbook For Oil And Fat Analyst" page 364). The graphs provide a convenient means of determining the proportions of mixture components in question.

These three methods give a measure respectively of the volatile fatty acids, soluble in water, insoluble in water, and those soluble in water whose silver salts are soluble in water. They do not provide an exact determination of chemical individuals but a quick means of obtaining a reasonable approximation of the contents in a mixture. The accuracy of estimations is dependent upon variations in the component materials. Also errors can occur from overheating during distillation and, in the case of calculating Kirschner value, the impurities in silver sulphate. There is no available method of estimating errors associated with the estimation of mixture components. The accuracy of the estimated values is therefore questionable.

#### 1.2 Timms Triglyceride Method (Timms, 1980)

This method makes use of the triglyceride compositions of mixture and its components in determining the proportion of each mixture component. Mixture components were classified into milk fat and non-milk fats. The non-milk fats involved were cocoa butter, palm kernel oil, coconut oil, beef tallow, lard, sunflower oil and soya bean oil. Seventy six samples of butter were obtained from three Australian factories between September 1975 - September 1976 and their triglyceride compositions were analysed by gas-liquid chromatography. Also the triglyceride compositions of milk and non-milk fats mixtures

and the non-milk fats were determined by gas-liquid chromatography.

As non-milk fats have substantial amounts of diglycerides with carbon number 32-38 and milk fat has substantial amounts of triglycerides with carbon number below 46; by ignoring those triglycerides with carbon numbers less than 32, Timms (1980) was able to use those triglycerides with carbon numbers 40, 42 and 44 to discriminate between milk fat and most non-milk fats. Therefore, when determining a function to discriminate between milk fat and non-milk fats, the non-milk fats equations disappear.

The triglyceride data were fitted to the following equation:

$$\sum ( a_i C_i ) = R + e$$

where  $i$  is the carbon number;  $C_i$  is the percentage of triglyceride with carbon number  $C_i$ ;  $a_i$  is a coefficient to be estimated;  $R$  is a constant defined to be 100 for pure milk and  $e$  is a random error.

Using only triglycerides with carbon numbers 40, 42 and 44 and by multiple linear regression, the equation becomes:

$$100 = 14.197 C_{40} - 36.396 C_{42} + 32.364 C_{44} - e \quad \text{----- (1)}$$

The errors, estimated by Timms, were approximately normally distributed with a standard deviation of 0.7088.

For any mixture of milk fat and non-milk fats, the following equations were used:

$$C_i^{\text{MIX}} = x C_i^{\text{NMF}} + (1 - x) C_i^{\text{MF}}$$

where the superscript MIX, NMF and MF refer to the mixture, non-milk fat and milk fat; and  $x$  is the proportion of non-milk fat in the mixture.

And

$$R = x \left[ \begin{array}{ccc} \text{NMF} & & \text{NMF} \\ a_{40} C_{40} & + a_{42} C_{42} & + a_{44} C_{44} \end{array} \right] + (1-x) \left[ \begin{array}{ccc} \text{MF} & & \text{MF} \\ a_{40} C_{40} & + a_{42} C_{42} & + a_{44} C_{44} \end{array} \right]$$

which reduces to the following form:

$$x = \frac{\begin{array}{c} \text{MF} \quad \text{MIX} \\ R - R \end{array}}{\begin{array}{c} \text{MF} \quad \text{NMF} \\ R - R \end{array}} \quad (2)$$

As  $R(\text{MF}) = 100$  by definition, an estimate of  $x$  can therefore be calculated by the following equation

$$x' = \frac{\begin{array}{c} \text{MIX} \\ 100 - R' \end{array}}{\begin{array}{c} \text{NMF} \\ 100 - R' \end{array}} \quad (3)$$

where  $R'(\text{NMF})$  and  $R'(\text{MIX})$  are estimated from equation (1) when  $C_{40}$ ,  $C_{42}$  and  $C_{44}$  for the non-milk fat and the mixture are substituted.

The lower 99% confidence limit for  $R'(\text{MF})$  is  $100 - 2.65 * 0.7088 = 98.12$  (73 DF) and the upper 99% confidence limit for  $R'(\text{MIX})$  when the mixture has a fraction  $x(D)$  of non-milk fat with  $R(\text{NMF}) = 0$  is  $100(1 - x(D)) + 1.88$ . When  $100(1 - x(D)) + 1.88 > 98.12$  or  $x(D)$  greater than or equal to 0.0376, at least 0.5% of such mixture, at the 1% significant level, is detectable. In general:

$$x(D) \geq \frac{101.88 - 98.12}{\begin{array}{c} \text{NMF} \\ 100 - R \end{array}} \quad (4)$$

Although lauric oils and animal fats had significant amounts of triglycerides with carbon numbers 40-44, the distribution among the three triglycerides was different from that of milk fat, therefore in Timms calculation it showed that the level of detectability of non-milk fat was not affected by the variations.

As little as 5% of any non-milk fat can be detected by this method with better than 99% confidence. Also, when a non-milk fat is involved, its amount can be quantified with 95% confidence limits to better than 2% absolute.

According to Timms (1980), the triglyceride analyses by GLC were more effective than fatty acid analyses for detecting non-milk fat in milk fat. However, the research carried out by Jebson and Curtis had proved otherwise. The levels of contamination down to 5% can be detected by Jebson and Curtis (1981) method (refer to "Computer Aided Gas Liquid Chromatographic Analysis Of Two Component Mixtures").

This method depended entirely on the experimental data obtained from the gas-liquid chromatographic analysis of triglyceride compositions of mixtures and mixture constituents. The accuracy of this method has therefore not been tested by data obtained from literature. Since this method based on the triglyceride percentage of C40, C42 and C44 instead of using all the triglyceride percent distribution, it is doubtful that this method will give as good estimation of mixture component proportions when literature component data are used. Moreover, when three mixture components were involved, this method was not able to estimate the proportion of each component in the mixture. For example, in the case of milk fat, soya bean oil and palm oil mixture, this method only gave the general milk fat and non-milk fat (i.e. soya bean oil and palm oil) proportion estimation.

### 1.3 Computer Aided Gas Liquid Chromatographic Analysis Of Two Component Mixtures

The evolution of new technology has made the development of new analytical techniques in determination of mixture component proportions possible. For instance, since its introduction in 1952, gas chromatography has become one of the most widely used modern analytical techniques. It provides rapid qualitative and quantitative analysis of complex mixture with precision and sensitivity. Also, the falling in price of computer, owing to the invention of cheaper silicon chip, has made the introduction of computer to research affordable. Long, tedious and accurate calculation can now be performed by computer, leaving more time to research workers to concentrate on their research.

The computer aided gas liquid chromatographic analysis of two component mixtures, of which one of the components was milk fat, was carried out by Jebson and Curtis (1981). Based on the mathematical model derived by Jebson and Curtis, a BASIC language program was written. By making use of the results of a gas liquid chromatographic analysis of the proportions of fatty acids present in the mixture and mixture components, the determination of each mixture component was performed by Cromemco computer.

In the analysis, the relation between the proportions of a number of fats in a mixture and their fatty acid composition was expressed as follows:

$$\sum (A_{ij} * X_j) = M_i \quad \text{----- (i)}$$

where  $i = 1$ , to number of fatty acids in the mixture.

$j = 1$ , to number of mixture components.

$A_{ij}$  is the fraction of acid  $i$  in fat  $j$ .

$M_i$  is the proportion of acid  $i$  in the mixture.

$X_j$  is the proportion of fat  $j$  in the mixture.

and 
$$\sum (X_j) = 1$$

For two mixture components case, it becomes:

$$A_{i1} X_1 + A_{i2} (1 - X_1) = M_i \quad \text{----- (ii)}$$

Re-arrange the equation (ii), it gives:

$$X_1 = \frac{M_i - A_{i2}}{A_{i1} - A_{i2}} \quad \text{----- (iii)}$$

where  $X_1$  is the proportion of the first fat in the mixture.

The error in  $X_1$  was determined by the errors in the variables used to determine  $X_1$  as below:

$$x = \frac{1}{(A_{i1} - A_{i2})^2} \left[ \text{abs}\{a_{i1}(A_{i2} - M_i)\} + \text{abs}\{a_{i2}(A_{i1} - M_i)\} + \text{abs}\{m_i(A_{i1} - A_{i2})\} \right] \quad \text{----- (iv)}$$

where  $x$  is the error in  $X_1$ ;  
 $a_{i1}$  is the error of acid  $i$  in the first fat;  
 $a_{i2}$  is the error of acid  $i$  in the second fat;  
 $m_i$  is the error of acid  $i$  in the mixture.

The best overall estimate of the proportion of the two fats was obtained by calculating a weighted average of the estimates from the individual fatty acid analysis. This was done by using following relation:

$$X' = \frac{\sum_2 (X_i/x_i)}{\sum_2 (1/x_i)} \quad \text{----- (v)}$$

where  $X'$  is the weighted average;  
 $X_i$  is the estimate of  $X_1$  from fatty acid  $i$  analysis;  
 $x_i$  is the estimate of error in  $X_i$ .

An estimate of error in X' was given by:

$$x = \frac{\sum_{i=1}^n (1/x_i)}{n} \quad \text{--- (vi)}$$

where n is number of estimates; and  
i varies from 1 to n

The error in the determination of proportion of each fatty acid was assessed either

1. from a standard deviation estimated from the difference in duplicate results; or
2. from a one-way analysis of variance of the results of a number of fatty acids analysis done in duplicate.

In order not to under-estimate the size of the errors, the larger of two estimates of variability was used in the calculation by Jebson and Curtis (1981).

### 1.3.1 Gas-liquid Chromatographic Analysis Of Methyl Esters

Methyl esters of fatty acids were prepared by the method of Shehata, de Man and Alexander (1970). They were then analysed by gas-liquid chromatography employing similar conditions as used by Gray (1973), except that the samples were injected at 40 degrees C and programmed up to 180 degrees C at a rate of 6 degrees C/min. The proportion of methyl esters of fatty acid was determined by Varian model 480 digital integrator and weight response factors of standard methyl esters were determined experimentally. Components and mixtures were analysed in duplicate.

### 1.3.2 Analysis Of Results

The analytical error of the GLC technique was estimated by a one-way analysis of variance, using the BASIC language program, for each fatty acid estimated from analytical results for a number of fats. The analysis of variance was also carried out for each fatty acid present in the mixture. The standard deviations and coefficients of variation for each fatty acid were calculated from the within-sample variances and the mean concentrations.

Calculation of the percentage of milk fat present in the mixtures were made under two sets of condition:

- 1) using the fatty acid compositions of the individual fats obtained experimentally; and
- 2) using the fatty acid compositions of the individual fats obtained from literature (Hildith and Williams, 1964; Codex Committee on Fats and Oils, 1978; Gray, 1973; and Taylor and Hawke, 1975).

There was good agreement between calculated milk fat percentage and the actual percentage of milkfat in the mixtures, and the deviations were generally consistent with the error estimates.

It was pointed out that when using literature fatty acid compositions to determine the milk fat percentage in the mixtures, care must be taken as the composition of oils can vary widely with the variety and with the climate in which the seeds are grown. Also, the composition of milk fat depends upon the stage of lactation, the feeds, and the genetics of the cows. Therefore, it is likely under some circumstances that the fraction of minor components will vary considerably from normal. Hence, when unreasonable results are obtained, that is when the estimated proportion of milk fat is less than 0% or greater than 100%, they are excluded or ignored.

Comparison was made by Jebson and Curtis (1981) between the methods of Cavitt and Weenink (1977) and theirs. It showed that their methods gave results of equivalent precision to the iodine value method, and better than the C14 or C18 methods proposed by Cavitt and Weenink.

The method is suitable for detecting small quantities of other oils in milkfat, and may be used as a means for detecting moderate contamination (i.e. the levels of contamination down to 5% may be detectable by this method).

However, the mathematical model derived by Jebson and Curtis (1981) becomes insufficient and too complicated when dealing with three components mixtures. Therefore, a new mathematical model, which may be similar to the one derived by Jebson and Curtis, was required.