THE FLAVOUR OF NEW ZEALAND WHOLE MILK POWDER

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RUSSELL DOUGLAS WILSON

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ABSTRACT

Results of this investigation indicate that there are certain fundamental differences in the flavour of New Zealand WMP as opposed to European (Danish) WMP. Sensory analysis has highlighted that this difference is evident in the scores which panellists give for the lactone attribute.

This difference in sensory evaluation can be directly linked to differences in the lactone profiles from New Zealand and Danish WMP. Danish WMP consistently contains the two gamma lactones γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone at levels greater than or equal to their flavour threshold values. While these two lactones are generally absent from New Zealand WMP.

The presence of γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone in WMP has been demonstrated to be related to the diet of the cow. By the addition of a grain concentrate consisting of 85% oats, 10% sunflower seeds and 5% barley it was possible to increase the levels of γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone to the point where the sensory panel was able to differentiate WMP's in respect to the presence or absence of these compounds.

There is the inference that the presence of the gamma lactones in WMP is also a function of dairy breed with Friesian cows showing a greater capacity than Jersey or mixed Jersey/Friesian cows to produce these flavour compounds. Also diet may be an important factor with the lipid content and fatty acid composition having an influencing the level of gamma lactones produced.

Analysis of the flavour volatiles from fresh New Zealand milkfat has indicated a possible causative role for terpenoid compounds in the distinctive "green/grassy" flavours often present. In particular such compounds as D-Limonene have been shown to be present in samples of New Zealand milkfat and when added to New Zealand milkfat has a tendency to increase the "green/grassy" flavour score. However this does not discount the contribution of compounds such as hexanal which was also detected in New Zealand milkfat.

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CHAPTER I: INTRODUCTION

The New Zealand Dairy Industry currently manufactures approximately 165 000 tonne of whole milk powder (WMP) annually, most of which is exported to a large number of markets around the world (NZDB Report, 1988). In some of these markets, particularly South East Asia, WMP from New Zealand is being sold in competition with WMP from European countries. With any food product a major factor in consumer acceptance is flavour, for it is flavour that will ultimately determine if the product is purchased again. However little is known about the chemical nature of the flavour of NZ WMP especially in comparison to WMP manufactured in Europe. It is known through sensory evaluation profiling that differences between New Zealand and European WMP's occur in certain attributes, notably in the area of lactone and feed related flavours.

In order to understand what constitutes the flavour of NZ WMP the present investigation was undertaken to study the chemical nature of the difference in the lactone flavour between NZ and European WMP. An attempt was also made to characterise the compounds responsible for the "grassy/feedy" flavour of New Zealand WMP.

CHAPTER II: REVIEW OF LITERATURE

2.0 Introduction.

The flavour of whole milk powder (WMP) is dependent on several factors:

(a) Flavour of the raw material (milk).

(b) Modifications due to processing as influenced by hygiene, physical disruption, heat of evaporation etc.

(c) Storage conditions of the WMP, in particular, temperature, humidity, length of storage etc.

Within each of these contributing factors are a myriad of factors that can influence flavour either to a lesser or greater degree.

The nature of milk flavour has been extensively researched and reviewed over the years resulting in a wealth of information on the flavour of milk and the products that can be manufactured from milk (Price and Manning, 1983), (Forss, 1970), (Badings and Neeter, 1980). In conjunction with studies into the nature of milk flavour there has also been extensive research into the causes and control of off-flavours in milk and dairy products (Bassette, et al.,1986).

In contrast to the large number of papers published on the flavour of milk and milkfat products the amount of research carried out on the flavour of WMP and dried milk products in general has been comparatively small (Badings and Neeter, 1980) (Hall and Lingnert, 1984) (Hall et al., 1985) (Hall and Andersson, 1985). Therefore in the absence of any definitive studies on the flavour of New Zealand WMP a certain degree of translation from studies on other dairy products is necessary.

2.1 The Chemical Composition of Flavour.

Flavour is normally judged as a total physiological response to the complex interrelationship between texture, taste and aroma. In order to measure flavour physicochemical analysis has concentrated on the identification of volatile aroma compounds. Classification of the aroma compounds which have been identified from

various dairy products reveals compounds from a wide range of chemical groups. Some of the most important groups of flavour compounds found in dairy products include lactones (γ and δ lactones derived from (4) and (5) hydroxy alkanoic acids), methyl ketones (2-alkanones), fatty acids (alkanoic acids), hydrocarbons, aldehydes, alcohols, sulphur compounds etc.

2.1.1 Lactones.

Freshly secreted milkfat contains small amounts of δ(5)-hydroxy and γ(4) hydroxy fatty acids esterified as part of the milk triglyceride. δ and γ lactones are formed respectively from these precursors by hydrolysis of the hydroxy acid ester bond to release the hydroxy acids which then spontaneously lactonise (Kinsella et al., 1967) (Figure 2.1).
Hydroxy fatty acids are formed in the mammary gland during the synthesis of fatty acids from acetate (Dimick et al., 1969). The δ lactones predominate in milkfat with γ lactones present in much lower concentrations (Table 2.1). Lactones are an integral part of the flavour of milkfat based dairy products and feature prominently in reviews on milkfat flavour (Kinsella et al., 1967) (Urbach, 1989).

Free lactones are considered to be important components of the unique flavour of butter (Boldingh and Taylor, 1962). The presence of lactones is not confined to milkfat but they are also present in a vast array of other foods (Maga, 1976). They are also believed to make an important contribution to the rich baked flavour of confectionery and bakery products which contain heated milkfat and are also major components of synthetic butter flavours used in margarine manufacture. This is because the levels and character of the lactone profiles in vegetable oils do not match that of milkfat (Maga, 1976) and the deodourisation of vegetables oils prior to margarine manufacture effectively eliminates any lactones that may have been present.
The level of lactones in milkfat is influenced by such factors as seasonal variation, stage of lactation, dairy breed and ketosis (Tables 2.2, 2.3, 2.4 and 2.5) (Dimick and Harner, 1968).

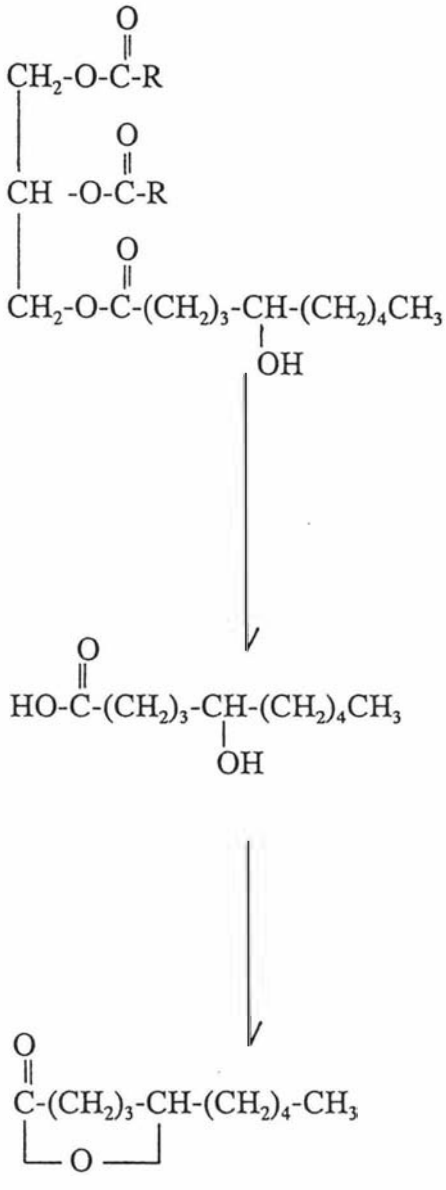


Figure 2.1 Mechanism of Lactone Formation in Milkfat.

Table 2.1 Level of δ and γ Lactones Isolated from Butterfat (ppm).

Carbon No.	δ-Lactones	γ-Lactones
6	2.0	trace
7	(0.2)*	
8	2.6	0.5
9	(0.4)*	0.2
10	15.0	1.2
11	0.7	0.5
12	35.0	1.6
13	1.5	0.5
14	34.0	1.4
15	6.4	1.3
16	23.2	1.3
18	(2.3)*	

* Semiquantitative
Kinsella (1969)

Table 2.2 Seasonal Variation of Lactone Levels in Bovine Milkfat.

Month	Average lactone level (ppm)
January	105
February	85
March	95
April	75
May	70
June	65
July	75
August	80
September	90
October	105
November	110
December	110

Table 2.3 Influence of Stage of Lactation on Lactone Levels in Bovine Milkfat.

Days of lactation	Lactone concentration (ppm)
25	40
50	70
75	90
100	120
125	140
150	150
175	160
200	160
225	150
250	145
275	140
300	120

NB. Tables 2.2 and 2.3 represents Northern Hemisphere data.

Table 2.4 Effect of Dairy Breed on Lactone Concentration.

Lactone	Holstein	Swiss Brown	Jersey-Guernsey
		Concentration (ppm)	
δ-Decalactone	23.8	18.3	20.5
δ-Dodecalactone	42.9	34.8	39.6
δ-Tetradecalactone	53.7	38.4	47.5
Total	120.4	92.5	107.6

Table 2.5 Influence of Ketosis on the Lactone Levels of Milkfat.

Cow	Condition	Lactone concentration (ppm)
1	Ketotic	22
1	Normal	60
2	Ketotic	66
2	Normal	125
3	Ketotic	70
3	Normal	110
4	Ketotic	90
4	Normal	110

2.1.1.1 Effect of diet on the level of lactones in milkfat.

Urbach and Stark (1978) carried out a comprehensive study of the effects of different diets on the level of lactones in milkfat . This study developed from attempts at elevating the levels of di- and polyunsaturated fatty acids in milkfat. By feeding an oil rich supplement (crushed sunflower seeds) it was possible to increase the level of di- and polyunsaturated fatty acids in the fat from 2 - 4% up to 20%.

Milkfat and meat from animals fed this diet was characterised by a sweet fruity raspberry like flavour due to the presence of high levels of γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone (Stark and Urbach, 1974). A diet of chopped lucerne hay and crushed oats increased the level of γ-Dodecalactone from 5 to 182 ppm. The addition of a protected oil seed supplement to the diet increased the level of γ-Dodec-*cis*-6-enolactone from 1 - 2 ppm to 24 ppm (Urbach and Stark, 1978). The flavour threshold for γ-Dodecalactone has been determined at 1 ppm. The flavour threshold of γ-Dodec-*cis*-6-enolactone has not been determined but it is likely to be 1 ppm or less (Urbach et al., 1972).

Total lactone content of milkfat (comprising mainly δ-lactones) has also been shown to be influenced by diet. In a study involving 276 animals the pasture fed group had a lower total lactone output than the group on the feed-lot regime. Also feeds that resulted in decreased milkfat output caused a corresponding decrease in lactone output (Dimick and Harner, 1968).

2.1.1.2 Mechanisms for the formation of γ-Lactones.

The mechanisms put forward for the formation of the γ-lactones have mainly been derived from the research carried out at the C.S.I.R.O.Laboratories, Highett, Victoria, Australia.

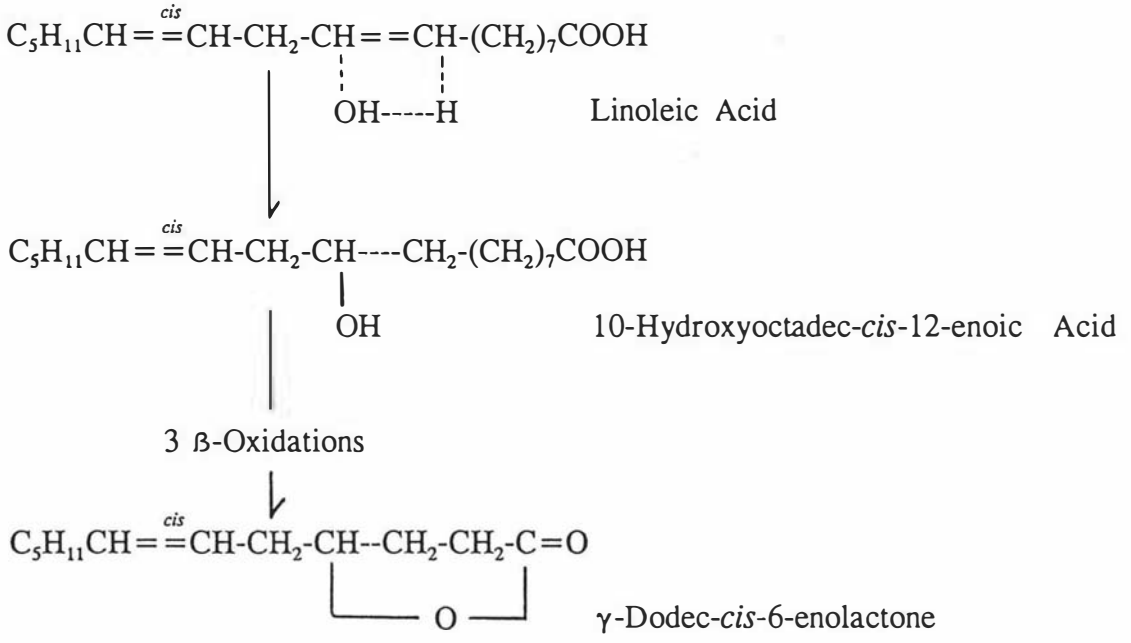


Figure 2.2 Suggested Mechanism for the Formation of γ-Dodec-*cis*-6-enolactone.

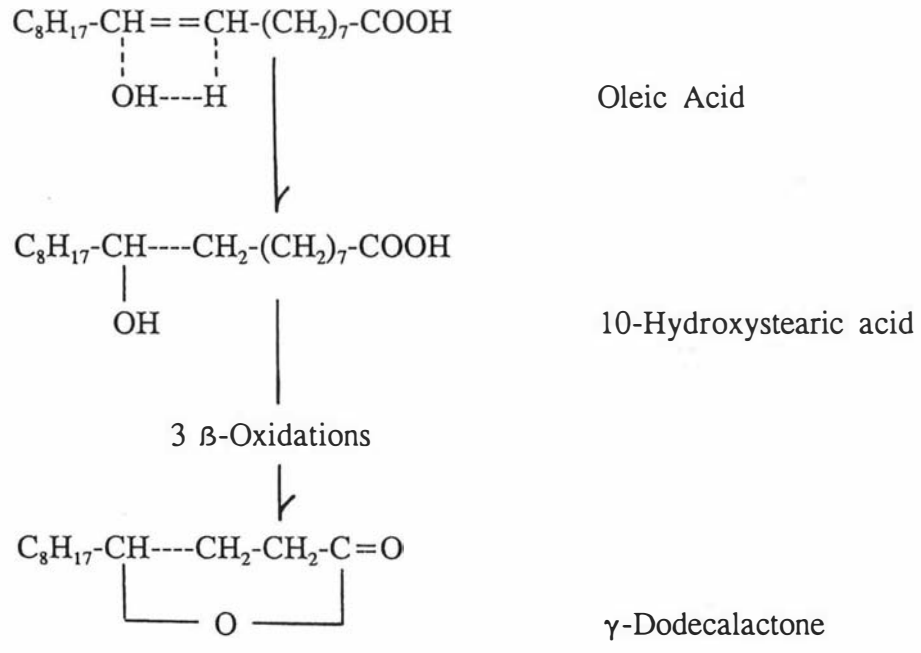


Figure 2.3 Suggested Mechanism for the Formation of γ-Dodecalactone.

The suggested mechanism for the formation of γ-Dodec-*cis*-6-enolactone is from linoleic acid which is converted to 10-hydroxyoctadec-*cis*-12-enoic acid by rumen micro-flora. This is then converted to 4-hydroxydodec-*cis*-6-enoic acid by three ß-oxidation steps (Figure 2.2). It is also suggested that γ-Dodecalactone is formed by a similar process from Octadecenoic acid (oleic acid) (Anet, 1975) (Figure 2.3).

This mechanism for the formation of γ-lactones taking place in the rumen differs from that of the δ-lactones whose origins have been demonstrated in the mammary gland (Walker, 1967) (Walker, et al., 1968). The rumen as the site of origin for γ-lactone formation was postulated to explain the presence of a strong γ-lactone odour in the gastrointestinal tract at slaughter (Anet, 1975).

It was concluded that in order to achieve an increase in the level of γ-Dodecalactones in milkfat several criteria had to be met. There was a need for a readily available source of starch (such as in crushed rather than whole oats) and a source of the fatty acid precursor; oleic acid for γ-Dodecalactone and linoleic acid for γ-Dodec-*cis*-6-enolactone (Urbach, 1982).

Although the presence of these two compounds have not been reported in New Zealand milkfat, McDowall et al., (1957) reported a sweet off-flavour in milkfat from cows grazing on ryegrass/clover pasture which had been drenched with 300 ml of linseed or peanut oil. It is likely that this could have been caused by elevated levels of γ-lactones.

In relation to microbial systems γ-Dodec-*cis*-6-enolactone is the most abundant lactone in malt broth cultures of *Fusarium poae*, a parasitic imperfect fungus which has been isolated from New Zealand oats (**Avena sativa L.**), and is responsible for the presence of a dominant canned peach-like aroma (Sarris and Latrasse, 1985).

This unsaturated γ-lactone is also the major component of the male tarsal scent in black-tailed deer (Brownlee, et al., 1969).

2.1.1.3 Lactones in Milkfat.

Walker et al., (1977) studied the distribution of lactones in milkfat fractions produced by solvent crystallisation. They found that the level of free lactones in spreadable butter made from 3:1 w/w blends of low and high melting fractions were higher (56.6 - 154 ppm) than the free lactone content of conventional butters (25.8 - 64.4 ppm). It was concluded that the increase was due to enrichment of the low melting fraction by the fractionation process and by further lactone formation during processing..

Apart from the homologous series of aliphatic γ and δ lactones there are also trace amounts of other lactones in milkfat. These include bovolide, dihydrobovolide and hydroxybovolide and are present in trace amounts in heated milkfat (Boldingh and Taylor, 1962). It is also thought that these compounds exist in the body fat of sheep with higher concentrations in forage fed animals compared to animals finished on grain (Bailey,1988, private communication). Dihydrobovolide has been identified in the essential oil of alfalfa (Kami, 1983). These compounds possess a celery like odour (Boldingh and Taylor, 1962).

2.1.2 Methyl Ketones.

Like the lactones this class of compound can exert a strong influence on the flavour of dairy products and like lactones, methyl ketones are not present in freshly secreted milk but are bound in milk triglyceride in a precursor form as ß-keto alkanoic acids. Methyl ketones were first identified in dairy products, containing heated milkfat, as a homologous series of odd carbon numbered compounds (Kinsella, 1969). Again like the lactones methyl ketones are released from the milk triglyceride by the action of heat in the presence of water (Van der Ven et al., 1963). However the conditions for release of methyl ketones is more severe than for the lactones with it being possible at certain conditions to liberate lactones but not methyl ketones (Stark et al., 1976).

The precursors of the odd (n) carbon numbered methyl ketones are the corresponding even (n+1) carbon numbered ß-keto alkanoic acids with generation of the methyl

ketones by hydrolysis and decarboxylation (Figure 2.4). The ß-keto alkanoic acids are synthesised in the mammary gland from acetate which again is similar to the synthesis of δ-hydroxy alkanoic acids the precursors for lactones (Lawrence and Hawke, 1966).

The flavour thresholds of methyl ketones are in the range 0.1 - 1.0 ppm depending on the chain length (Forss, 1972) and tend to be associated with stale flavours particularly in stored whole milk (Parks and Patton, 1961) and non fat powders (Bassette and Keeney, 1960) and also evaporated milks (Muck, et al., 1963).
Also like lactones the methyl ketone potential of milkfat is dependent upon such factors as diet (Urbach and Stark, 1978) and stage of lactation (Dimick and Walker, 1968).
Like lactones the levels of methyl ketones or more importantly the methyl ketone potential is influenced by many of the factors that influence the lactone potential. These include stage of lactation where levels are low initially and increase as lactation proceeds (Dimick and Walker, 1968). Methyl ketone levels can be suppressed by the feeding of oil supplements similar to the effects on the levels of δ-lactones (Urbach and Stark, 1978).

The contribution of methyl ketones to the flavour of dairy products is not always deleterious as they have been shown to be important flavour components of good quality butter (Winter et al., 1963) and are important in the flavour of blue-vein cheese. The mould Penicillium roqueforti has been shown to oxidise saturated fatty acids to ß-keto acids which form methyl ketones (Kinsella and Hwang, 1976).

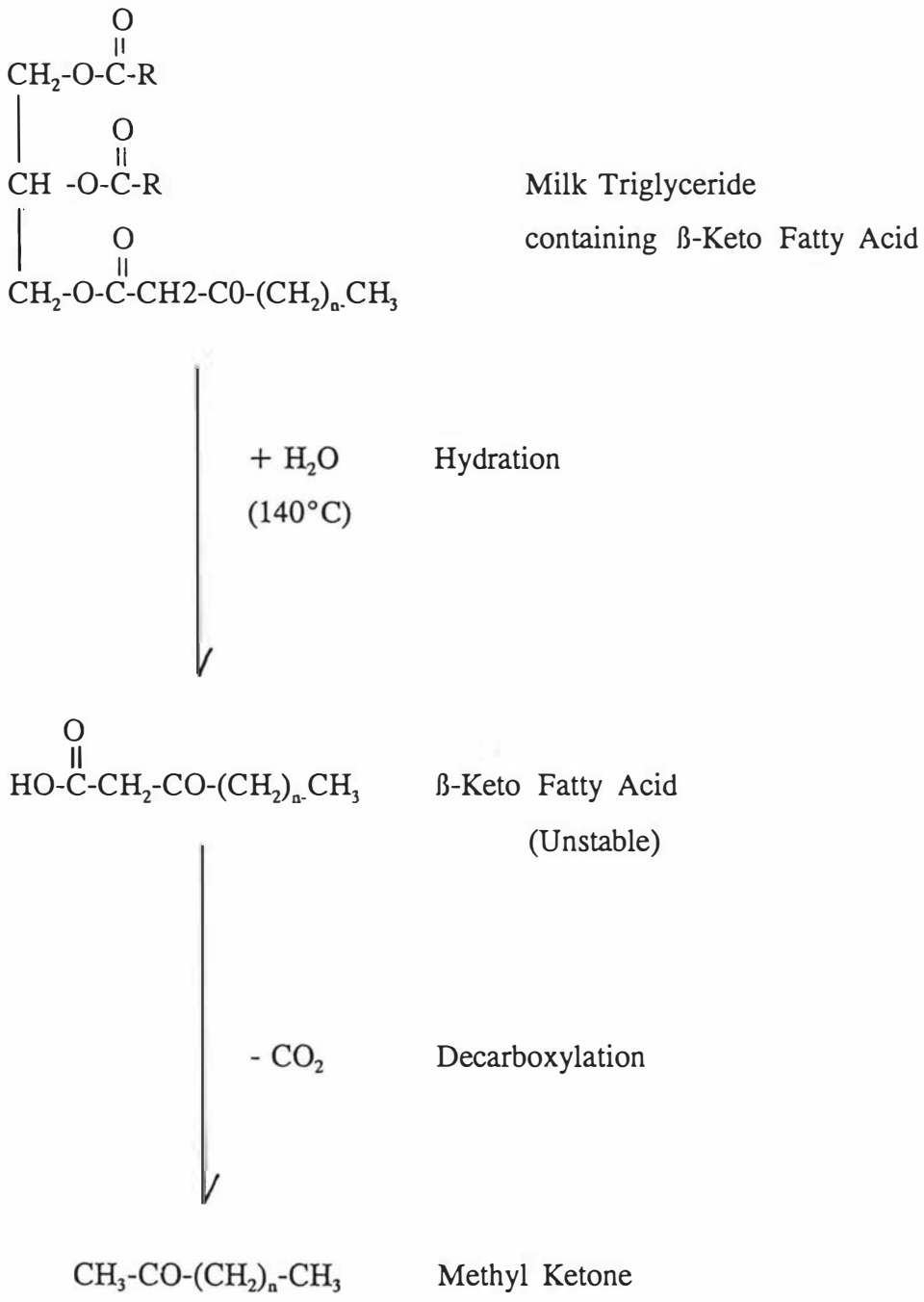


Figure 2.4 Mechanism for the Formation in Methyl Ketones in Milkfat (Kinsella, et al., 1967).

2.1.3 Fatty acids.

Free fatty acids have long been recognized as important contributors to rancid flavour defects in dairy products, however less attention has been paid to their contribution to the normal flavour of dairy products. Generally levels of free fatty acids have been used as a measure of milk quality with excessive levels indicating absent or inadequate pasteurisation not destroying natural lipases or microbial lipase activity.

Milkfat, unlike most other fats and oils, contains low molecular weight fatty acids (C4, C6, C8, C10) which may be released from the milk triglyceride by heat or lipolysis. The flavour threshold for the short chain fatty acids in milk range from 25 ppm for butanoic acid (C4) to 7 ppm for decanoic acid (C10) (Patton, 1964) (Kinsella, 1969). These fatty acids when present at levels in the order of 100 ppm will produce off flavours commonly referred to as lipolytic rancidity (Urbach et al., 1972). At lower levels fatty acids probably make a contribution to the desirable flavour of milkfat, although free fatty acids are of greater significance to the desirable flavour of cheese. Some idea of the importance of free fatty acids to dairy flavours can be gained in the commercial success of lipolysed butteroils (eg LBO 1100, Dairyland Food Laboratories, U.S.A.) for use as food ingredients to impart creamy/dairy type flavours. What seems to be important is the level of free fatty acids.

Branched medium chain fatty acids, in particular the 4-methyl branched Nonanoic (C9) and decanoic (C10) acids, have been implicated in the "sweaty" odour note (described in Chinese as "soo") of cooked mutton and lamb (Wong et al., 1975). New Zealand WMP is often referred to as having a "soo" flavour by some Chinese consumers.

(E)-6-Decenoic acid has been reported to give rise to an intense milky flavour (Shirakawa et al., 1988). Although a number of isomers of Decenoic acid (C10:1) have been found in milk (E)-6-Decenoic acid has yet to be found in dairy products (Forss et al., 1967).

Probably of greater importance to flavour is the contribution from the unsaturated fatty acids in the form of the oxidation products which they produce. The most important unsaturated fatty acids in milkfat are oleic (18:1), linoleic (18:2), linolenic (18:3) and

arachidonic (20:4) acids. These acids are susceptible to autoxidation through free radical chain reactions through to hydroperoxides which in turn form secondary products such as aldehydes (predominantly) and ketones (Dumont and Adda, 1979). It is these secondary products that can be detected organoleptically at levels as low as parts-per-billion (ppb) (Table 2.6). The rate of lipid oxidation is increased by a number of catalysts such as Cu^{2+} and Fe^{2+} (Allen, 1986). An example of this is the formation of oct-1-en-3-one, which is responsible for a metallic flavour in dairy products, from either linoleic or arachidonic acids (Wilkinson and Stark, 1967).

Table 2.6 Flavour Character and Threshold Levels of Selected Aliphatic Aldehydes.

Aldehyde (ppm)	Threshold in paraffin oil	Character
cis-3-Hexenal	0.09	'Green bean'
trans-2-Hexenal	0.60	'Green'
cis-4-Heptenal	0.000 5	Cream to tallow
trans-6-Nonenal	0.000 35	'Hydrogenation'
trans-2-*cis*-6-Nonadienal	0.001 5	Beany
trans-2-*trans*-6-Nonadienal	0.02	Cucumber
trans-2-*trans*-4-Decadienal	0.10	Stale frying oil

2.1.4 Hydrocarbons.

The presence of hydrocarbons in milk and dairy products has not been reported as extensively as some of the other chemical classes. The assumption is often made that hydrocarbons, in particular long chain hydrocarbons, are odourless and therefore make

little contribution to flavour. The exception to this may be the compounds phyt-1-ene, phyt-2-ene and neophytadiene thought to contribute to the flavour of grass fed meat and milkfat (Urbach and Stark, 1975). The level of these C-20 hydrocarbons have been shown to be influenced by diet with phyt-1-ene the predominant C-20 hydrocarbon in the milkfat of pasture fed cows whereas phyt-2-ene was dominant with stall fed cows.

2.2 Effect of Feed on the Flavour of Dairy Products.

In spite of the absence of scientific evidence identifying the flavour compounds responsible, it has long been known that the flavour of milk and dairy products can vary greatly depending on the type of feed that the cows have been consuming.
Gordon and Morgan (1972) identified twenty two compounds that were regularly present in feed flavoured milk. Many of the compounds present in feed flavoured milk are also present in volatile extracts from grass and corn silage (Morgan and Pereira, 1962, 1963). Through the addition of formulations containing combinations of these compounds to normal milk it was possible to reproduce a feed-like flavour. The formulation that most closely simulated feed flavour contained: methyl sulphide, 10 ppb; acetone, 16.2 ppm; butanone,1.6 ppm; isopropanol, 2.6 ppm; ethanol, 33 ppm; and propanol, 3.3 ppm. Feed flavour could be restored to feedy flavoured milk that had been vacuum distilled by simply adding back the distillate. This would indicate that the compounds responsible for feedy flavour in milk are present in the volatile fraction, but it was not known what contribution to feedy flavour was being made by minor components present including those that had been identified and those as yet unidentified.

In contrast to the relative lack of research into feed flavour in dairy products the influence of forage feeding on the flavour of meat has received more attention. Larick et al., (1987) investigated the effects of grain versus grass (three types: tall fescue, smooth broomegrass-red clover and orchard grass-red clover) diets on the flavour components of beef. Their results indicated that the greatest sensory difference between beef from grass-fed and grain-fed beef was in the fat fraction. The grass-fed beef

possessed a less desirable "grassy" flavour in comparison to the grain-fed beef. Their analysis, by direct sampling GC/MS (Suzuki and Bailey, 1985), of the volatile fraction from the grass-fed and grain-fed beef indicated higher concentrations of 2,3 Octanedione and various diterpenoids (C20 hydrocarbons: phytol, phytane, phyt-1-ene, phyt-2-ene, neophytadiene, and dihydrophytol). They inferred that the presence of diterpenoids in grass-fed beef was due to microbial fermentation of phytol, from chlorophyll, and deposition of diterpenoids present in the forage into adipose tissue. Urbach and Stark (1975) identified phyt-1-ene, neophytadiene and phytane in butterfat and also neophytadiene in the ryegrass that the cows had been grazing on.
Suzuki and Bailey,(1985) also identified 2,3,Octanedione as the predominant volatile from clover-fed lamb which they assigned as a good marker for forage-fed lamb.

2.3 Off-flavours in milk.

This area of the flavour chemistry of dairy products has probably received more attention than any other mainly because the off-flavours are often due to a single compound or class of compound.
Off-flavours in milk and dairy products can be grouped according to the mechanisms of how they arise (Shipe, et al., 1962).

2.3.1 Transmitted flavours.

This is the transfer of substances from the feed or environment into the milk while still in the udder. The mechanisms of flavour transfer have been demonstrated through a series of classic experiments using tracheal and ruminal fistulae (Dougherty et al., 1962). Detectable off-flavours appeared in the milk 15 min after the substances were introduced into the lungs compared to 30 min for substances introduced to the rumen. It was demonstrated that certain odours could be carried from the rumen to the lungs in the eruciated gases and thus were transmitted to the milk.

2.3.2 Feed Flavours.

There is the general conclusion that ingestion of certain feeds or the inhalation of strong odours are the primary cause of off-flavours in freshly drawn raw milk (Dougherty et al., 1962). Gordon and Morgan (1972) identified 10 compounds regularly found in feed flavoured milk and also determined a range of concentrations for some of these compounds in milk with strong feedy flavour (Table 2.7).

Table 2.7 Volatile Compounds Regularly Detected in Feed Flavoured Milk.

Compound	Range in concentration in milk with strong feedy flavour
Diethyl ether	
Acetaldehyde	
Methyl sulphide	25 - 45 ppb
Acetone	3.6 - 4.8 ppm
Ethyl acetate	
Butan-2-one	0.5 - 1.0 ppm
Isopropanol	0.3 - 0.4 ppm
Ethanol	10 - 20 ppm
Chloroform	
Propanol	1.5 - 2.5 ppm

2.3.2.1 Silage.

In certain countries silage is an important part of the cows diet. Compounds identified in silage and implicated in silage flavour of milk include methyl sulphide and methyl, ethyl and propyl esters (Morgan and Periera, 1962). It was found that freshly cut alfalfa hay contained high concentrations of *trans*-2-hexenal, *cis*-3-hexenals and *cis*-3-hexenols which give rise to a green grassy flavour (Morgan and Periera, 1963). Shipe et al., (1962) looked at the transmission of a series of volatile compounds commonly found in silage and the effect on milk flavour (Table 2.8).

2.3.2.2 Weed Flavours.

Certain weeds have the potential to impart serious off-flavours to milk. Of particular importance to the NZ dairy industry is the off-flavour produced by cows consuming landcress (*Coronopus didymus*) a cruciferous weed which is prevalent in pastures particularly during spring. Consumption of even small amounts of landcress imparts an intense burnt flavour to milk. The compounds primarily responsible for this flavour have been identified as benzyl methyl sulphide and benzyl mercaptan (Walker and Gray, 1970). These compounds arise from benzyl thiocyanate which is released from benzyl glucosinolate by enzyme action when the plant is crushed. This off-flavour can be controlled by farm management to restrict the intake of landcress in the period prior to milking but cannot be reduced to an acceptable level by commercial steam stripping (vacreation).

Table 2.8 Transmission of Flavour Substances to Milk.

Flavour Substance	Description of Flavour
Ethanol[a]	Sweet, vanilla-like, ester-like.
Propanol[a]	Alcohol-like, vanilla-like, duco cement.
Butan-2-ol	Sweet, ester-like, xylene.
Acetone[a]	Feedy, cowy, sweet, silage.
Butan-2-one[a]	Hay-like, sweet, aromatic, cowy.
Propanal	No detectable effect.
Butanal[a]	Malty, chemical, butanal.
Pentanal	No detectable effect.
Butanoic acid	No detectable effect.
Propionic acid	No detectable effect.
Methyl acetate	Sweet, ester, grassy.
Ethyl acetate	Sweet, fruity, ester.
Propyl acetate	Sweet, vanilla-like, feedy.
Butyl acetate	Banana, ester, malty.
Dimethyl sulphide[a]	Weedy, cowy, unclean, onion.
Cis-3-hexenol[a]	Grassy, weedy, musty, grass.
Green pasture	Grassy, cowy, barny, feedy.
Green corn silage	Barny, cowy, feedy, sweet.
Grass silage distillate[b]	Fruity, sweet, ketone.
Corn silage distillate[b]	Fruity, fermented, aromatic.

[a] Substances introduced by both the lung and rumen routes. The pasture and silage consumed in the normal manner. The other substances introduced by the lung route only.

[b] The silage distillates represent the neutral carbonyl-free fraction boiled between 36 and 100°C.

Shipe et al. (1962).

2.3.3 Microbial.

Microbial contamination of milk can produce a variety of flavours depending upon the identity of the organism present. These flavours are considered separate from the desirable flavours produced in fermented dairy products. Examples of specific off-flavours attributable to microbial action include malty flavour due to the presence of 3-methyl butanal formed by the action of *Streptococcus lactis* var. *maltigenes* on leucine and fruity flavour attributable to the presence of ethyl butyrate and ethyl hexanoate formed by the action of *Pseudomonas fragi* (Morgan, 1970).

2.4 Off-flavours of whole milk powder.

One of the main functional properties of WMP is its ability to be stored for long periods of time and still be reconstituted into liquid milk. For this reason a great deal of scientific interest has focused on the mechanisms that occur during storage and how the storage life of WMP can be extended with emphasis being placed on the identification of flavour compounds that contribute to storage flavours. Early studies focused on the problems of staling and oxidation of WMP's (Parks and Patton, (1961), (Nawar, et al, 1959). Through the identification of 2,4 dinitrophenylhydrazone derivatives it was established that poor quality WMP's could be distinguished from other WMP's by the type of aldehydes present and by their concentration. Furthermore it was suggested that the storage life of WMP could be extended by using deodourised milkfat in the manufacture of WMP. This resulted in an absence of methyl ketones in the WMP. Parks et al. (1969) identified 6-trans nonenal as a major contributor to the off-flavour of foam spray dried milk powder manufactured during warm summer months. It was thought that the 6-trans nonenal was formed by reactions in the drier involving ozone. This could be corrected by filtering the drier air through charcoal.

Boon et al. (1976) separated and identified individual monocarbonyl compounds from a variety of aged and fresh WMP's as Dinitrophenol (DNP) hydrazones. A range of

methyl ketones (C_3 - C_{15}) and saturated aldehydes (C_1 - C_{10}) were identified. Also the unsaturated aldehydes: undeca-2,4-dienal, pent-2-enal, hept-2-enal, non-2-enal, hept-2,4-dienal and nona-2,4-dienal. Analysis of fresh (1 h), oxidised (16 month) and old oxidised (5 year) WMP's showed an accumulation of monocarbonyls, predominantly saturated aldehydes, with storage time (Keen, et al.,1976). It was concluded from these results that saturated aldehydes are the predominant carbonyl products of lipid oxidation rather than unsaturated aldehydes which can be oxidised further.

Also in a series of reports Hall and Lingnert (1984), Hall et al. (1985) and Hall and Andersson (1985) analysed the flavour changes in WMP during storage profiling techniques. They also identified a range of volatiles derived from the oxidation of milkfat. The concentration of volatiles was measured with respect to storage time. Finally they used multiple linear regression analysis in order to generate predictive equations relating the intensity of flavour from the profiling study with the volatile compounds identified from the WMP. For such descriptors as 'cardboard like', which is a common indicator of oxidation in WMP, predictive equations involving hexanal, either as a single or in a multi-component equation, accounted for a high percentage of variance (R^2). In conclusion it was reported that flavour changes due to the storage of WMP was a complex issue and a prediction of the whole flavour profile could not be simply achieved by the derivation of any single predictive equation.

2.4.1 Effect of Lipid Oxidation on the Flavour of Whole Milk Powder.

Because WMP contains on average 28% milkfat, flavours arising from lipid oxidation play a major role in the flavour profile during storage and ultimately the intensity of the oxidation flavours determine acceptability.

The characteristics of lipid oxidation include:

1. a low activation energy for autoxidation of polyunsaturated fatty acids which negates any benefits of low temperature storage.

2. oxidation is accelerated by transition metal ions such as Fe^{2+} and Cu^{2+}. Both these ions are present in dairy products.

3. some volatile aldehydes and ketones which arise as secondary products of lipid oxidation are organoleptically detectable at the parts per billion (ppb) level (Table 2.9) (Allen, 1986).

Milk is generally stable to oxidation however processing may initiate the conditions favouring oxidation through such things as:

1. contamination by Cu^{2+} and other metal ions from processing equipment.
2. entrainment of oxygen.
3. intermixing of pro-oxidative catalysts.
4. destruction of natural antioxidants during processing.

Table 2.9 Compounds Contributing to Typical Oxidation Flavours.

Compounds	Flavours
Alkanals C_{6-11}	green to tallowy
Alk-2-enals C_{6-10}	green to fatty
Alka-2,4-dienals C_{7-10}	oily to deep fried
Deca-2,4,7-trienal	fishy, beany
Oct-1-en-3-one	metallic
Octa-1,*cis*-5-dien-3-one	metallic
Oct-1-en-3-ol	mushroom

2.5 Heat Induced Flavours in Milk.

It is recognised that heat alters the flavour of milk with the type and intensity of the flavour changes being dependent on the temperature and the duration of the heat treatment. Therefore the flavour of whole milk powder will be influenced by the degree of heat treatment that the milk receives during the evaporative and drying stages of the manufacture of whole milk powder. Scanlan et al. (1968) examined the effects of heat treatment on the volatile compounds from milk and found a variety of volatile compounds to be heat induced: $C_{3\text{-}5,7\text{-}11,13}$ *n*-methyl ketones, $C_{8,10,12}$ δ-lactones, benzaldehyde, furfural, phenylacetaldehyde, vanillin, oct-1-en-3-ol, heptanol, 2-butoxy-ethanol, maltol, acetophenone, benzonitrile, benzothiazole and diacetyl. It was also suggested that because of the increase in the level of diacetyl from 5 ppb (raw milk) to 38 ppb (heated milk), which is above the average flavour threshold for diacetyl in milk, that this could be contributing to the rich heated flavour of heat treated milk.

Badings (1977) demonstrated a direct relationship between the level of hydrogen sulphide and the intensity of cooked flavour. Addition of L-cystine (30 - 70 mg/Kg) after heat treatment was found to be effective in reducing the cooked flavour caused by hydrogen sulphide. Badings et al. (1981) attempted to distinguish between different levels of heat treatment by looking at the flavour volatiles. They showed that the difference in flavour between low pasteurised milk and ultra high temperature (UHT) milk is strongly influenced by heptan-2-one and nonan-2-one. They also found that the difference was moderately influenced by seven compounds and slightly influenced by thirty compounds. This out of a total of some 400 volatile compounds as demonstrated by GC/MS of extracts produced by the classical procedures of vacuum distillation, freeze concentration of distillate, solvent extraction and concentration of the extract by micro-distillation. Milk subjected to higher heat treatments such as sterilisation (15 min at 110°C) developed a "caramelization" flavour which along with contributions from methyl ketones and lactones is predominantly due to such compounds as maltol, iso-maltol, and furanones arising from Maillard reactions and thermal conversion of sugars (caramelization). It was proposed that the amount of volatile carbonyl compounds is

indicative of heat treatment and can be related to the level of heat treatment although this will be influenced by the fat content of the milk and also by oxidative deterioration. Thomas et al., (1975) using a GLC headspace analytical method found a correlation between hydrogen sulphide in the headspace and the degree of organoleptic cooked flavour. The degree of heat treatment also effects the astringency flavour of milk with an increase in astringency related to an increased heat treatment (Joglekar and Gupta, 1981). They concluded that sulphur compounds were derived from milk proteins while non sulphur compounds were derived from milkfat and the production of volatile sulphides and the onset of cooked flavour in heated milk closely parallels the activation of sulphydrl groups.

2.6 Isolation of Volatiles from Dairy Products.

WMP is a complex food system containing carbohydrate, lipid, protein and a small percentage of moisture (approx. 3%). Dispersed within this system are the volatile flavour compounds which are present in a range of concentrations from parts per billion (ppb) to parts per thousand (0.1%). In order to research the composition of this volatile fraction the problem of extracting the flavour volatiles from the food matrix must be overcome. There are numerous methods and combined procedures for the extraction and analysis of volatiles from foods (Weurman, 1969) (Maarse and Betz, 1981).

Sample preparation techniques available include: headspace sampling, headspace concentration, vacuum distillation and solvent extraction. For aqueous solutions there is the possibility of direct analysis, direct adsorption and direct extraction by organic solvents.

Because of the composition of dairy products in general and WMP in particular the direct methods are not really practicable.

Aroma compounds are present in milk and dairy products in low concentrations so that any analysis of flavour usually requires an initial isolation and concentration procedure to yield sufficient material for analysis.

The most commonly used procedures for the isolation of volatiles from dairy products

have included:

1. isolation of volatiles from non-volatile material by vacuum distillation.
2. concentration of the aqueous distillate by freeze concentration.
3. extraction of the volatiles from the aqueous distillate by liquid-liquid extraction.
4. concentration of the organic extract.

In conjunction with these methods there are the more specialised techniques of sample preparation such as headspace analysis, direct solvent extraction (eg. soxhlet extraction), Nickersen-Likens extraction (combined distillation and extraction) and the use of adsorbents.

2.6.1 Vacuum Distillation.

Probably the largest single constraint with sample preparation is that no one procedure will result in the complete isolation of all flavour volatiles from a particular food systems. Therefore selection of the procedure to be used is very much dependent on the objectives of the analysis and on the type of food system. Forss et al., (1967) studied the efficiency of three methods for the extraction of alcohols and ketones from butter oil and found a combination of high vacuum degassing and cold-finger molecular distillation to be the most appropriate with reduced pressure steam distillation the least favourable. Badings and Neeter (1985) described the use of the purge and trap injector which was directly coupled to a capillary gas chromatograph. This is a vapour phase sampling system and was used to study aroma compounds from milk and dairy products. Using this methodology it was possible to isolate more than 350 compounds.

Suzuki and Bailey (1985) described a direct sampling capillary GLC method for the rapid quantitative analysis of volatiles from small amounts of ovine fat (330 mg) by heating at 200°C for 30 min. The efficiency of recovery of selected compounds was very much dependent on the volatility of the compound and the associated temperature of the heated chamber. The percentage recovery for nonan-2-one was 92% compared to 18% for δ-tetradecalactone. The obvious advantages of these systems are the avoidance

of any labourious extraction procedures and both are rapid and relatively reproducible.

In a number of publications equipment used has been developed 'in house" which creates difficulties in attempting to reproduce results. Often compromises have to be made with regard to the choice of methodology and a major constraint is often availability of equipment which is often restricted because of economics.

Irrespective of which methods are used for analysis it is important that the formation of artifacts should be avoided and also the loss of compounds should be kept to a minimum in order to achieve an extraction and analysis that is representative of the original material.

2.6.2 Analysis of Flavour Volatiles from Dairy Products.

The analysis of flavour volatiles has been greatly aided by advances in analytical procedures especially by the continued development of combined gas chromatography and mass spectrometry instrumentation which permits the separation and identification of a large number of flavour compounds from complex mixtures. This is well illustrated by the identification of over 400 compounds from dairy products using GC/MS (Badings and Neeter (1980). The advancement of computer based data systems, with mass spectral library search, single ion monitoring and MS/MS systems have made the task of identifying flavour compounds much easier and quicker (Message, 1984). However the skill of the researcher still lies in the interpretation of the analytical information.

2.6.3 Analysis of Volatiles using the Gas Chromatographic Sniffing Technique.

This procedure is used to characterise the odours of single components from complex flavour mixtures in the effluent of the gas chromatograph and in many instances allows a greater sensitivity than a FID detector. Often it is this initial recognition of an important component that through enrichment techniques of the particular component

has led to the identification of important aroma compounds that previously were beyond the levels of detection (Drawert and Christoph, 1984).

2.7 Summary.

In summary therefore the flavour quality of whole milk powder is likely to be dependent on several factors:

A. The flavour quality of the milk used for whole milk powder manufacture which in turn is dependent on several factors:

(1) Stage of lactation.

(2) Diet and animal health.

(3) On farm hygiene.

(4) Storage and transport of raw milk.

B. The effects of the processes of whole milk powder production.

(1) Pasteurisation and storage (time and temperature).

(2) Heat of evaporation.

(3) Spray drying.

C. Storage conditions.

(1) Temperature and humidity.

(2) Length of storage.

(3) Storage atmosphere (air or inert gas).

All these factors have the ability to contribute to a greater or lesser degree to the final flavour of the WMP and while the dominant factors should be related to the milk being produced by the cow insufficient care to the subsequent steps of manufacture can quickly detract from the quality of the resultant WMP.

CHAPTER III: MATERIALS AND METHODS

3.1 MATERIALS.

New Zealand WMP was manufactured at Dairy Products Development Centre, N.Z.D.R.I. according to the procedures outlined in Section 3.2 European samples of WMP were supplied by the New Zealand Dairy Board (Wellington, New Zealand) and were mainly of Danish origin. New Zealand milkfat was obtained from Bay Milk Products Ltd (Te Puke) as Fresh Frozen Milkfat for Recombining (FFMR). Samples of European milkfat were also obtained through the New Zealand Dairy Board.

The oats, sunflower seeds and barley used in the grain feeding trials were obtained from local producers while the dairy meal used in Trial 1 was obtained from Harvey Farms Ltd (Wanganui, New Zealand).

3.2 METHODS.

3.2.1 WMP Specification.

Medium heat WMP samples were manufactured to meet the NZ Dairy Board specification 8100. This specification contains no specified preheat treatment unlike specification 8000 which requires a minimum preheat treatment of 95°C for 20 s.

3.2.2 WMP Manufacture.

Standardised milk (typically 3.5% fat to produce a 28% fat WMP) was heated to 95°C $\pm$ 2°C by direct steam injection and held for approximately 15 s in holding tubes leading to the evaporator.

3.2.2.1 Evaporation.

Standardised milk was evaporated in a pilot scale Weigand three-effect falling film

evaporator (Weigand Apparatebau GmbH, Karlsruhe, West Germany) with an evaporative capacity of 1200 Kg water/h. The evaporator had no vapour recompression system and its third effect was a triple pass type. A magnetic flow meter (Foxboro-Magflow, Model No. 2801-DTCC-SS with 696A converter, (The Foxboro Company, Massachusetts, USA) was used to monitor the milk flow to the evaporator.

3.2.2.2 Homogenisation.

The milk concentrate (46 $\pm$ 2% solids) was collected, mixed well and then homogenised using an APV Manton Gaulin two-stage homogeniser (Model No, KF 3-3PS, APV Ltd., Sussex, England) under pressures of approximately 1500 and 500 psi at the first and second stages respectively.

3.2.2.3 Spray Drying.

The whole milk concentrate was dried in a modified Anhydro (Anhydro A/S, Copenhagen, Denmark) Type II AK No. 6 pilot scale spray drier fitted with a static fluid bed with an evaporative capacity of 80 Kg/h. It had a total cyclone discharge configuration, and was equipped with a variable speed centrifugal disc atomizer (159 mm diameter), the speed of which was set to 15000 rpm. The drier inlet was heated by indirect gas firing and the temperature was set at 160°C. The concentrate feed rate was regulated using a Kent Veriflux controller (Kent Instruments (Australia) Pty Ltd., NSW, Australia) to maintain the drier outlet air temperature at 95°C. The spray drier had no powder cooling system.

3.2.2.4 Packaging and Storage.

WMP was packed into polythene lined multi-wall bags to 25 Kg and stored at ambient temperature. Samples for sensory evaluation were sub sampled (500 g) into aluminium lined sachets and heat sealed and stored at either 37°C or ambient temperature

(approximately 20°C).

3.2.3 Analysis of WMP.

3.2.3.1 Total Solids of Whole Milk Concentrate.

The milk concentrate used for the manufacture of WMP was tested for total solids according to the procedure of Mojonnier and Troy, (1925).

3.2.3.2 Moisture.

WMP samples were tested for moisture content using the toluene distillation method specified by the Ministry of Agriculture and Fisheries (1979).

3.2.3.3 Fat Content.

WMP samples were tested for fat content using the modified Rose-Gottlieb method (International Dairy Federation, 1969).

3.2.3.4 Solubility Index.

WMP samples were tested for ADMI solubility index as specified by the Ministry of Agriculture and Fisheries (1979).

3.2.3.5 Whey Protein Nitrogen Index (WPNI).

WMP samples were tested for WPNI as specified by the Ministry of Agriculture and Fisheries (1979).

3.2.3.6 Titratable Acidity.

Reconstituted milk (20 ml) was diluted with distilled water (20 ml) and titrated with "deci-normal" sodium hydroxide to pH 8.35 (Ministry of Agriculture and Fisheries, 1979).

3.2.4 Flavour Evaluation.

3.2.4.1 Sample Preparation.

WMP was reconstituted by mixing 50 g WMP with 350 g water at 50°C using a Jiffy mixer (Autocrat Radio Ltd, Auckland, New Zealand). Prior to flavour evaluation the reconstituted milk was allowed to hydrate for 2 h in a beaker kept at ambient temperature in the dark. Approximately 30 min before the flavour evaluation session, the reconstituted milk was cooled to 25°C. Approximately 30 ml reconstituted milk in a 50 ml beaker was provided to each panellist. A maximum of four reconstituted milk samples were evaluated in any one session.

3.2.4.2 Evaluation.

Flavour evaluations were conducted by the Product Use and Evaluation Section of the N.Z.D.R.I. using descriptive sensory analysis. Eight or more panellists, especially trained for the evaluation of reconstituted WMP, participated in the flavour evaluation sessions. Each sample of reconstituted WMP was evaluated for the flavour characteristics of 'sweetness', 'creaminess', 'cooked/caramelised', 'lactone', 'oxidised', 'feedy', 'taint' and 'age-related' and the textural attribute of 'astringency'. Each flavour attribute was scored on a 0 - 10 scale (0 = absent, 2 = threshold, 4 = weak, 6 = moderate, 8 = strong and 10 = intense) (see Appendix 1 and 2).

Data was analyzed by analysis of variance using a Microvax computer (Digital Equipment Corporation, Maynard, Massachusetts, U.S.A.), incorporating a statistical package (Fletcher, Department of Scientific and Industrial Research, Palmerston North, New Zealand).

3.2.4.3 Informal Flavour Evaluation.

Non statistical flavour evaluations of WMP and milkfat products were carried out using an "expert" panel of up to five people skilled in the evaluation of dairy flavours particularly WMP and milkfat. Members of the "expert" were personnel from the Flavour Chemistry Section, N.Z.D.R.I.and where possible the composition of the panel was the same throughout the course of this study.

3.2.5 Extraction of Lipid from WMP.

WMP (100 g) was mixed with distilled water (100 ml) using a mortar and pestle. This slurry was then added to 50 g of Microcel T-38 (Manville Corp., Denver, U.S.A.) and ground together using a mortar and pestle to an even consistency to ensure release of the lipid. The WMP and Microcel mixture was then placed in a 2.25 l Mason jar and 450 ml of carbon tetrachloride (CCl_4) added. This was then blended for 3 min at speed 4 using a Sorval Omni-mixer (DuPont Instruments, Newtown, Connectticutt, USA). This mixture was then filtered through Whatmann No.1 filter paper and the residue was twice re-extracted with CCl_4. The CCl_4 extracts were then bulked and the CCl_4 removed using a rotary evaporator (Buchi) to yield the milkfat fraction of the WMP.

3.2.5.1 Isolation of Lactones from Milkfat.

Milkfat (10 g) was ground onto 35 g Celite 545 using a mortar and pestle according to the procedure of Ellis and Wong, (1975). This mixture was then added to a Wong column containing (in ascending order): a glass wool plug, 6 g deactivated alumina and 20 g anhydrous sodium sulphate. Acetonitrile was then added to the column and allowed to percolate through until 20 ml of extract had been collected. The acetonitrile extract was concentrated to 1 - 2 ml using a rotary evaporator (waterbath $\leq$30 °C) and then percolated through a Pasteur pipette containing 1 g of deactivated alumina and washed

through with 2 x 1 ml of acetonitrile. Finally the effluent was concentrated to 1 ml under a stream of nitrogen.

3.2.5.2 Determination of Lactone Potential.

Milkfat (15 g) was placed in a vial, flushed with nitrogen, sealed by flame, and heated in an oven at 120 °C for 16 h. After cooling to 40 °C the vial was opened and 10 g of milkfat was ground onto Celite 545 as above.

3.2.5.3 Gas Liquid Chromatography of Lactones.

Acetonitrile extracts (1 μl) were injected onto a Varian Vista Model 5000 gas chromatograph fitted with a 12 m X 0.25 mm (I.D.) BP-1 fused silica column (Scientific Glass Engineering, Sydney, Australia) and temperature programmed from 50°C to 230°C at 5°C/min, then to 300°C at 15°C/min, and held for 20 min. Injector and detector temperatures were 250°C and 300°C respectively. Detection was by flame ionization, with hydrogen as the carrier gas.

3.2.5.4 Mass Spectral Analysis of Lactones.

Low resolution electron impact (70 eV) GC/MS analyses were carried out on a Shimadzu Model QP-1000 mass spectrometer directly coupled to a Shimadzu Model GC 9A gas chromatograph. The GC was fitted with a 30 m X 0.25 i.d. fused silica DB-1 column (J & W Scientific Ltd). The column was temperature programmed from 50°C to 230°C at 10°C and held for 10 min with a helium pressure of 0.3 Kg/cm^2 at a vacuum of 1 X 10^{-6} Torr. The QP-1000 was also fitted with the Shimadzu LSS-20 library search system which incorporated a modified version of the NBS/NIH/EPA mass spectral data base (National Bureau of Standards, U.S. Department of Commerce, Washington D.C., U.S.A.).

3.2.5.5 Quantification of Lactones.

Positive identification of lactones was by mass spectral and GC retention time comparison with authentic standards, except for γ-Hexadecalactone which was deduced from Kovats indices and mass spectral interpretation.
A standard solution of lactones (δC8, C10, C12, C14 and γC8, C10, C12; Grindsted Products A/S, Denmark) was prepared in pentane (10 mg/100 ml). Integrated peak areas were recorded using a Varian CDS 401 integrator and used to quantify the level of lactones.

3.2.6 Grain Concentrate Feeding of Dairy Cows.

3.2.6.1 Trial 1 - (September - December 1987).

Identical Twin dairy cows from the Massey University Dairy Research Unit were separated into two groups one of which was maintained as a control group on pasture only while the experimental group received a grain supplement during milking. The cows (15) in the experimental group were fed, ad libitum, a grain concentrate consisting of commercial meal (Harvey Farm dairy meal) and ground oats in a ratio of approximately 2:1 after morning and evening milking. Milk from these cows, along with the milk from the pasture fed group was collected and bulked together over a period of 4 days (8 milking) prior to spray drying.

3.2.6.2 Trial 2 - (September - November 1988).

A pedigree Friesian herd (Mr G Udy, 'Matipo', R D 5, Fielding, New Zealand) was used for this feeding trial. Twenty five cows, chosen at random, were fed approximately 4 Kg of grain concentrate twice a day during morning and evening milking. The rest of the herd served as the control group and were fed pasture only. The grain concentrate consisted of 85% oats (Avena sativa L.), 10% sunflower seeds and 5% barley (Hordeum

distichon L.). This mixture was milled and pelletised to a size of 15 mm (Massey University, Feed Centre).

3.2.7 Determination of the Lipid Content of the Feed Grains.

The oil content of the oats and sunflower seeds was determined according to the standard method (I.U.P.A.C.,1979). Seeds (10 g weighed to within 0.01 g) were ground thoroughly and transferred quantitatively to a thimble which was then placed in a soxhlet extractor. Redistilled hexane (150 ml) in a pre-weighed round bottom flask was then fitted to the soxhlet extractor. After extracting for 4 h the thimble was removed and the seeds ground again and extracted for a second time. The solvent was then removed on a rotary evaporator and any traces of solvent were removed by heating the flask for 10 min at 100°C. The weight of the flask was then recorded and the amount of oil calculated. The above extraction was then repeated until the difference in the weight of flask between extractions was less than 0.01 g.
The final weight of oil was calculated and the oil content recorded as a percentage based on the wet weight of the seeds.

3.2.7.1 Fatty Acid Analysis of Lipid from Grains.

Fatty acid analysis was carried out according to the method described by MacGibbon (1988). Fatty acid methyl esters (FAME) were formed by methoxide-catalysed methylation and analyzed on a Hewlett Packard 5890A gas chromatograph fitted with a Hewlett Packard 7673A autoinjector and a 12 m X 0.53 mm i.d. DB-WAX (J & W Scientific, Folsom, California, USA) fused silica capillary column with hydrogen as the carrier gas. Samples were injected at 50°C and the oven temperature programmed at 15°C/min to 150°C, then at 6°C/min to 220°C with a final holding time of 3 min.

3.2.8 Vacuum Distillation.

3.2.8.1 Vacuum Distillation of Whole Milk Powder.

WMP (2 Kg) was reconstituted with 5 l of Milli Q water using a Sorval Omni-mixer. This was then placed in a flange topped 10 l round bottom flask. Connected in series to the 10 l flask was a chilled water spiral condenser, a solid dry ice/isopropanol cold trap a liquid nitrogen cold trap and a vacuum pump (Speedivac ED 50, Edwards High Vacuum, England). This was then distilled for 2 h at 40°C.
After vacuum distillation the combined contents of the two cold traps were extracted with either diethyl ether or pentane in a ascending liquid/liquid extractor for 4 h.
The organic solvent was then dried overnight with anhydrous sodium sulphate and then concentrated to 1 - 2 ml using a Kuderna-Danish concentrator. Extracts were concentrated to approximately 100 μl for GC and GC/MS analysis by micro distillation using a nitrogen purge.

3.2.8.2 Vacuum Distillation of Milkfat.

Four Kg of freshly melted milkfat (40°C) was placed in a flange topped 10 l round bottom flask connected to a liquid nitrogen cold trap and a vacuum pump. Milli Q water (1.5 l) and internal standards (nonane 0.5 mg/Kg and octyl acetate 0.5 mg/Kg) were added and the milkfat was then vacuum distilled at 40°C for 2 h.
After vacuum distillation the combined contents of the two cold traps were extracted with either diethyl ether or pentane in a ascending liquid/liquid extractor for 4 h. The pentane extract was then concentrated using a Kuderna-Danish concentrator followed by a nitrogen purge to a final volume of 100 μl prior to GC and GC/MS analysis.

3.2.8.3 Effectiveness of the Vacuum Distillation of Dairy Products.

A simple method was used to determine the effectiveness of the vacuum distillation to extract a fraction representative of the flavour of the initial product. In the case of the

vacuum distillation of New Zealand milkfat this involved the addition of the vacuum distillate, in various proportions, to either fresh milkfat or deodourised milkfat. Evaluation was then carried out by the informal panel.

3.2.9 Mass Spectral Analysis of Vacuum Distillates.

Low resolution (resolution 1000) electron impact (70 eV) mass spectral analysis of vacuum distillates from WMP and milkfat was carried out essentially as described in Section 2.5.4. The difference being in the temperature programming of the gas chromatograph which was from 35°C to 230° at 5°C/min with a final holding time of 10 min.

High resolution (resolution 5000) electron impact (70 eV) GC/MS was carried out using a VG 70-250S double focusing magnetic sector mass spectrometer (VG Analytical, Manchester, England) directly coupled to a HP5890A capillary GC. GC separations were preformed on a 30 m X 0.25 mm (I.D.) fused silica DB-1 column (J & W Scientific Ltd) temperature programmed from 40°C (5 min) to 280°C at 5°C/min with a final holding time of 20 min. The VG 70-250S was fitted with the VG 11-250J data system which incorporated the NBS/NIH/EPA mass spectral data base.

CHAPTER IV: RESULTS

4.1 Manufacture of Whole Milk Powder.

All WMP, unless otherwise stated, was manufactured at NZDRI according to the New Zealand Dairy Board Specification 8000. Only WMP which conformed to the compositional and microbiological constraints of the specification were used in this study.

4.2 Sensory Analysis of New Zealand and European Whole Milk Powder.

Results of the descriptive sensory evaluation of New Zealand and equivalent European (Danish) WMP's are presented in Table 4.1. The mean sensory scores for the various attributes indicate that there was a significant difference between the two types of WMP in the lactone attribute. The mean sensory score for lactone flavour in the New Zealand WMP was 1.8 compared to the Danish WMP where the mean sensory score was 5.1. This resulted in a F-ratio of 13.24. No other significant differences for the other flavour attributes were found between the samples.

4.3 Identification of lactones.

Positive identification of the lactones was achieved by the comparison of gas chromatographic retention times of authentic standards and also by mass spectral analysis. The presence of both gamma and delta lactones were easily identified using ion chromatography of m/e 85 and m/e 99, the base peak ions of gamma and delta lactones respectively.
Electron impact mass spectra of γ-Dodecalactone and δ-Dodecalactone are given in Figure 4.1.

4.3.1 Lactone Content of New Zealand Whole Milk Powder.

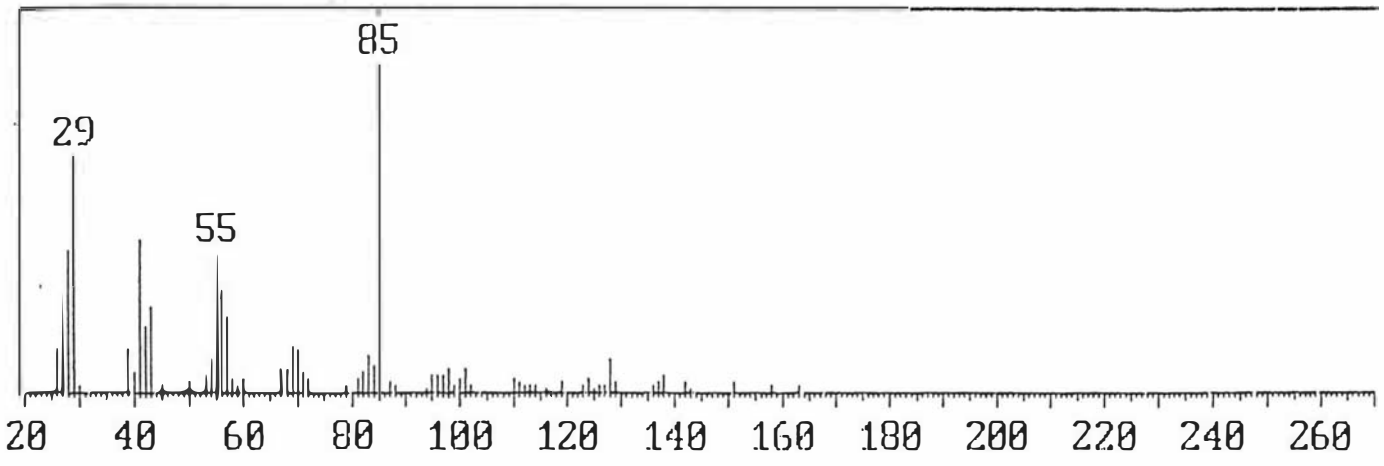
The typical lactone content of New Zealand WMP is given in Table 4.2 and Figure 4.2 and is characterised by a homologous series of delta lactones in particular C8, C10, C12,

C14 and C16 saturated delta lactones. The most abundant lactone present was δ-Dodecalactone with an average of 13.8 ppm as the free lactone and an average potential level of 35.8 ppm. Then in decreasing order of abundance was δ-C14, δ-C16, δ-C10 with some small amounts of δ-C8. The free lactone levels for the NZ WMP were routinely carried out on fresh powder of an average age of 3 to 8 weeks of age. The average total lactone potential was 116.1 ppm. Gamma lactones were absent except in trace amounts and were only identified using mass chromatography analysis of the GC\MS data (Figure 4.3).

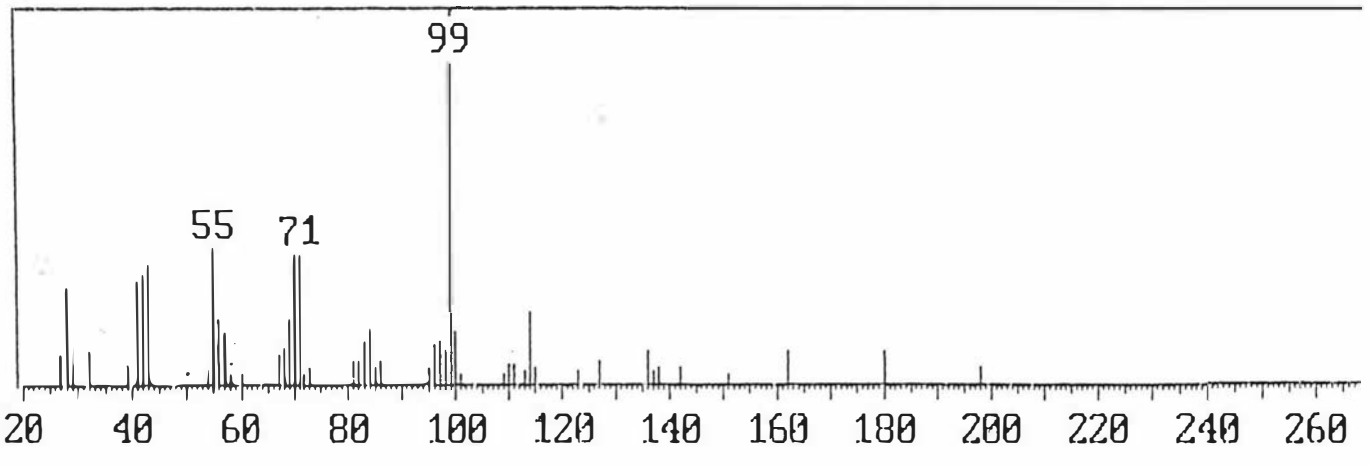
Table 4.1 Summary of Mean Sensory Scores and F-Ratios of New Zealand and Danish Whole Milk Powder.

Attribute	New Zealand	Danish	F-Ratios
Sweetness	5.4	5.4	0.00 ns
Creaminess	5.4	4.6	3.46 ns
Cooked/Caramelised	5.3	5.0	0.57 ns
Lactone	1.8	5.1	13.24 **
Oxidised	0.0	0.0	0.00 ns
Feedy	2.4	1.4	0.85 ns
Taint	0.0	0.2	1.00 ns
Age-related	0.0	0.2	1.00 ns
Astringency	4.3	4.6	0.20 ns

Figure 4.1 Electron Impact (70 eV) mass spectrum of γ-Dodecalactone and δ-Dodecalactone.



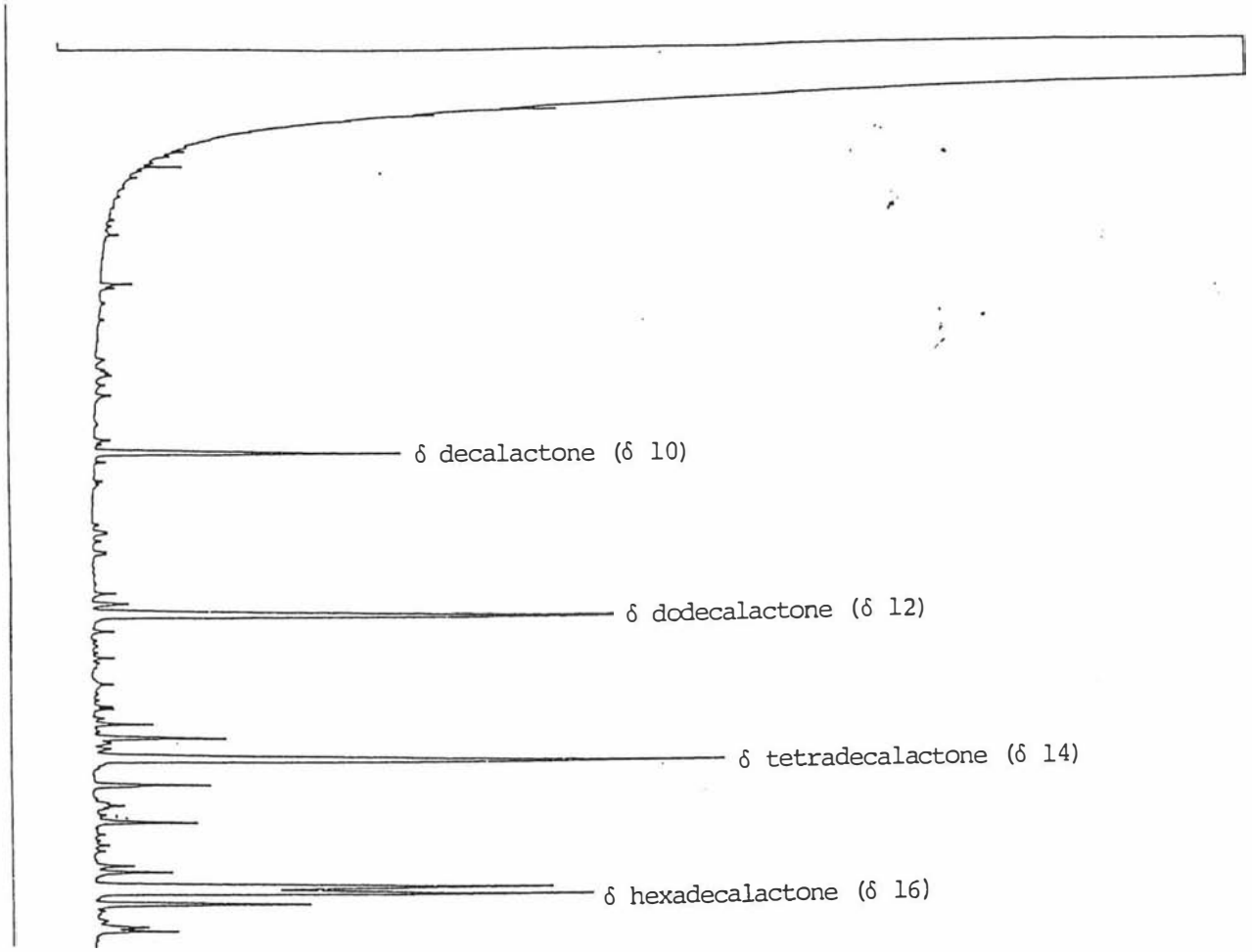
Gamma-dodecalactone



Delta-dodecalactone

Shimadzu QP-1000 GC/MS

Figure 4.2 Gas chromatographic separation of lactone extracts from New Zealand whole milk powder.



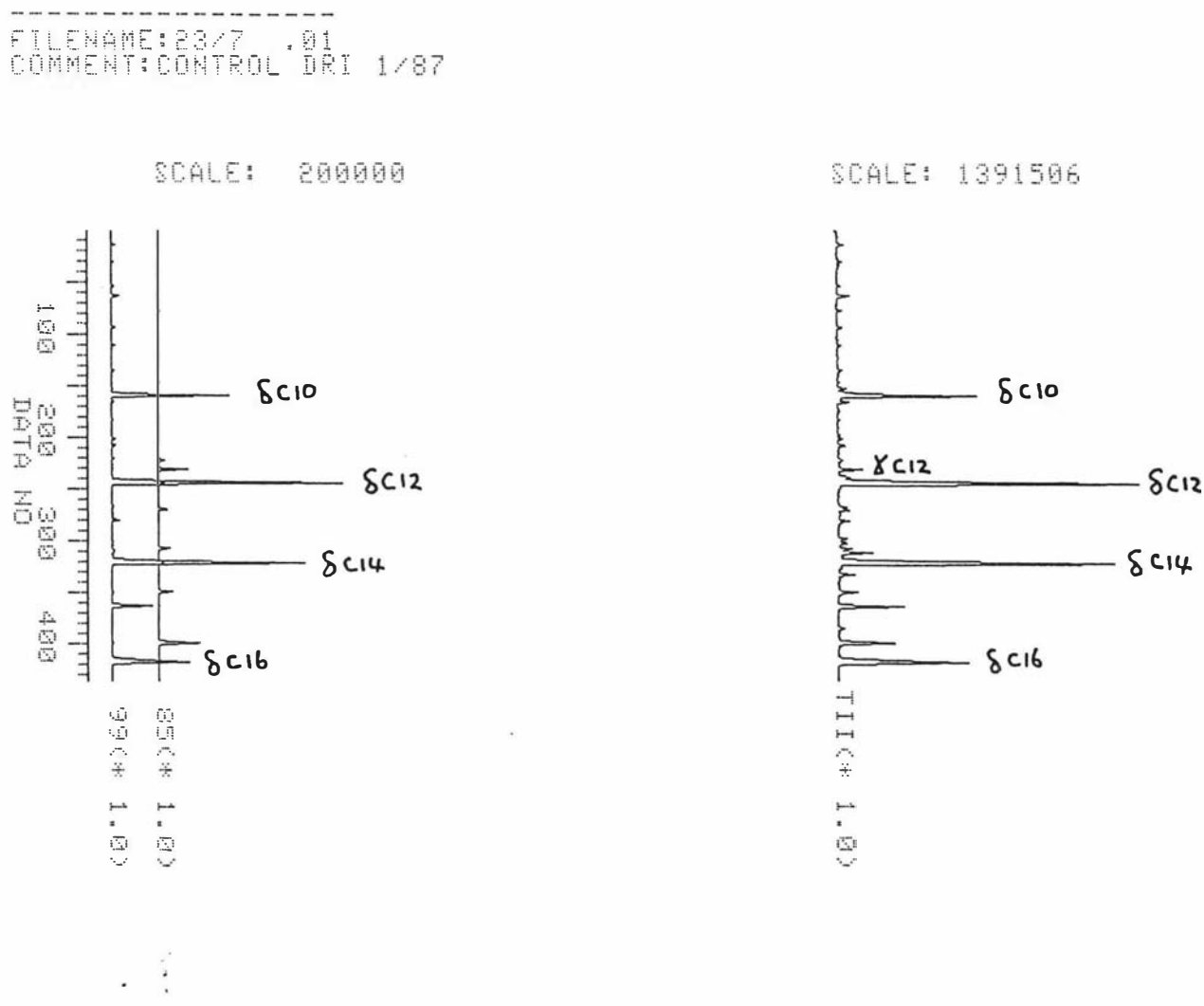
Gas chromatographic conditions:

12 m X 0.25 mm (I.D.) BP-1 fused silica column

50°C to 230°C at 5°C/min, 230°C to 300°C at 15°C/min (20 min).

Injector and detector temperatures were 250°C and 300°C respectively. Detection was by flame ionization, with hydrogen as the carrier gas.

Figure 4.3 Gas chromatography/mass spectrometry and mass chromatography analysis of lactone extracts from New Zealand whole milk powder.



GC/MS conditions:

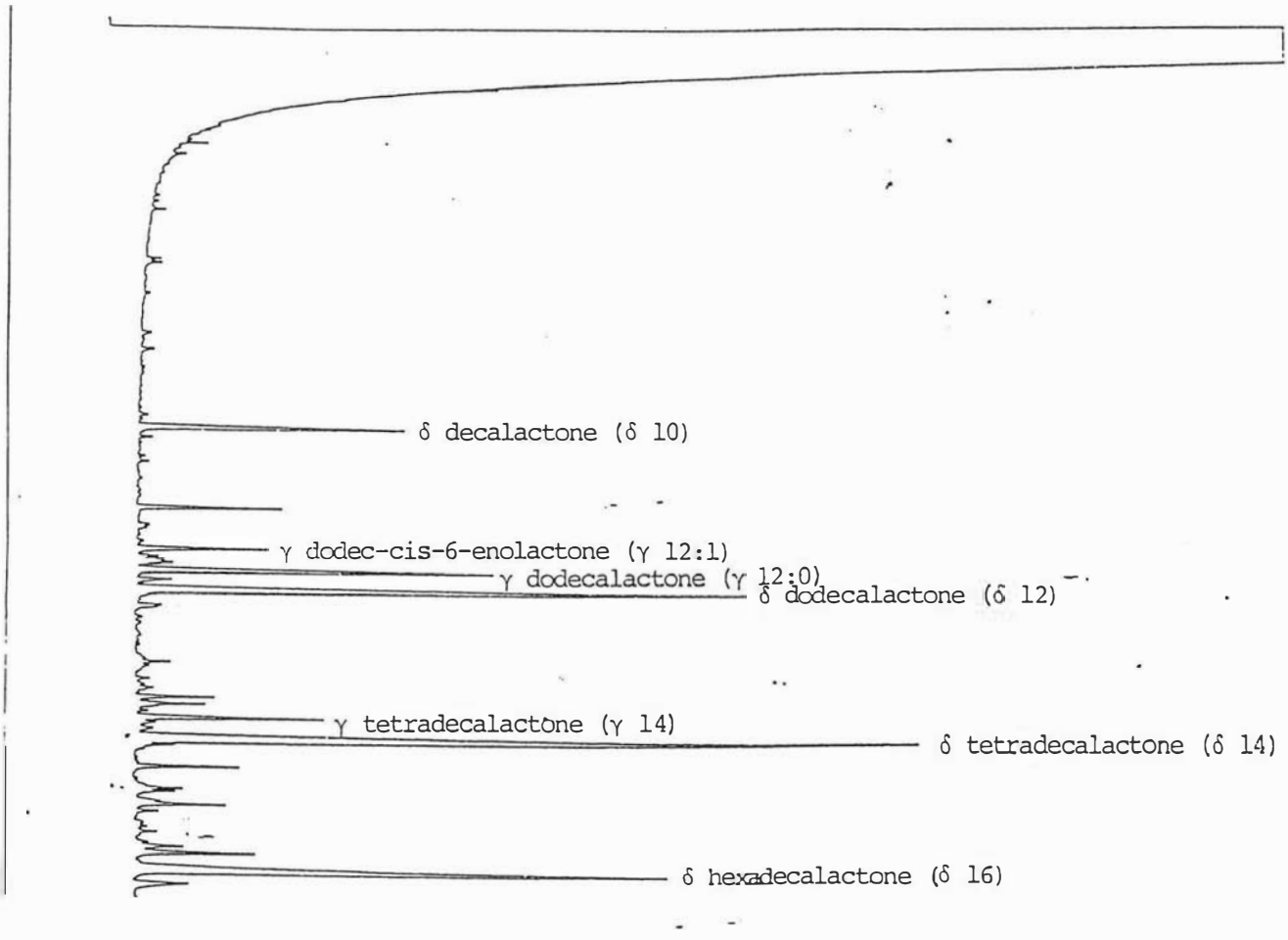
Shimadzù Model QP-1000 mass spectrometer

Electron impact (70 eV), 30 m X 0.25 i.d. fused silica DB-1 column (J & W Scientific Ltd), temperature programmed from 50°C to 230°C at 10°C/min and held for 10 min.

Mass chromatography:

m/e 85, γ-lactones; m/e 99, δ-lactones.

Figure 4.4 Gas chromatographic separation of lactone extracts from Danish whole milk powder.



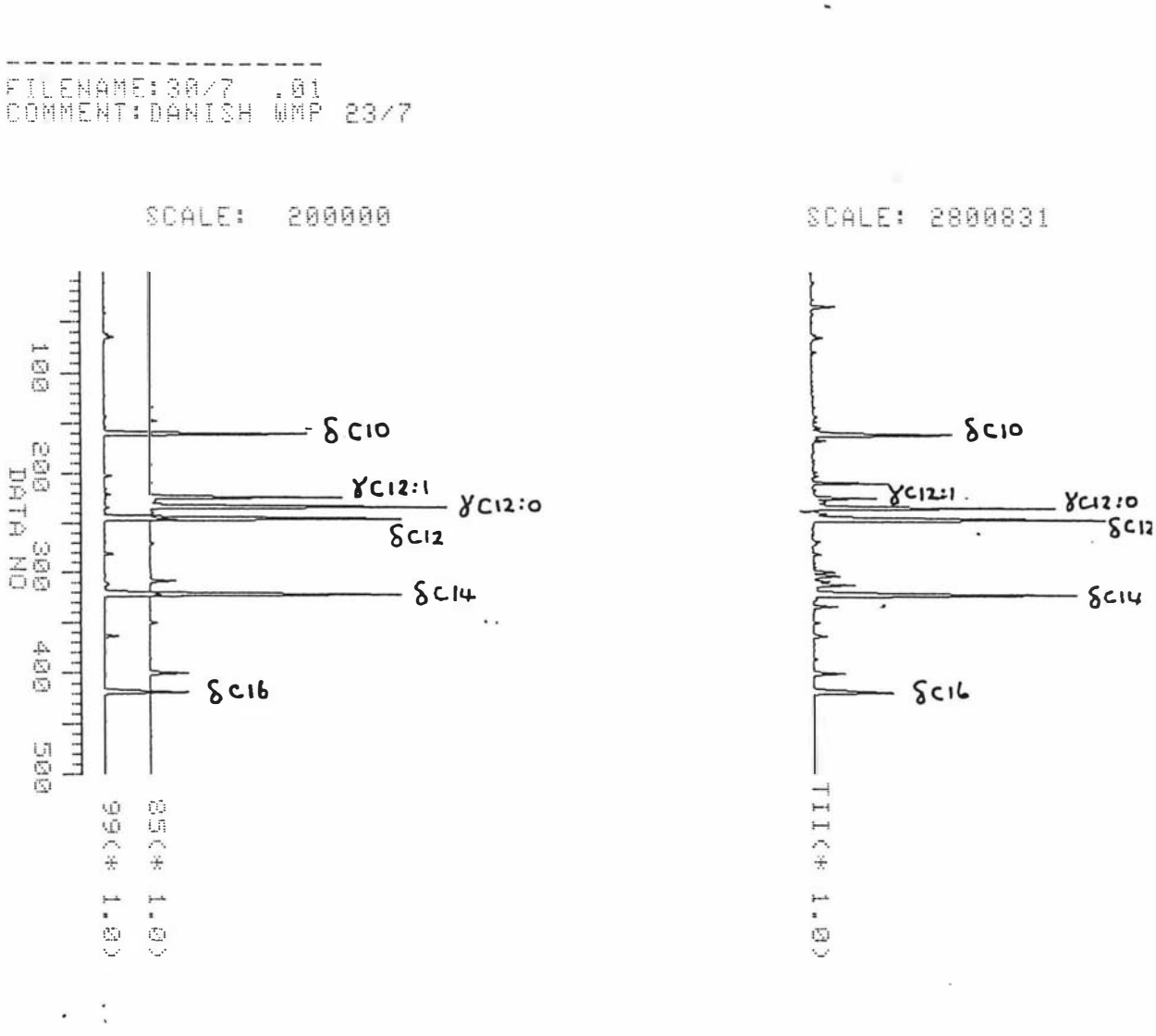
Gas chromatographic conditions:

12 m X 0.25 mm (I.D.) BP-1 fused silica column

50°C to 230°C at 5°C/min, 230°C to 300°C at 15°C/min (20 min).

Injector and detector temperatures were 250°C and 300°C respectively. Detection was by flame ionization, with hydrogen as the carrier gas.

Figure 4.5 Gas chromatography/mass spectrometry and mass chromatography analysis of lactone extracts from Danish whole milk powder.



GC/MS conditions

Shimadzu Model QP-1000 mass spectrometer

Electron impact (70 eV), 30 m X 0.25 i.d. fused silica DB-1 column (J & W Scientific Ltd), temperature programmed from 50°C to 230°C at 10°C/min and held for 10 min.

Mass chromatography:

m/e 85, γ-lactones; m/e 99, δ-lactones.

4.3.2 Lactone Content of European (Danish) Whole Milk Powder.

The lactone content of Danish WMP is given in Table 4.3 and Figure 4.4 and is characterised by a homologous series of delta lactones in particular C8, C10, C12, C14 and C16 saturated delta lactones. But unlike the lactone profile from New Zealand milkfat the two gamma lactones, γ-Dodec-cis-6-enolactone and γ-Dodecalactone, were present in measurable quantities. The levels of γ-Dodec-cis-6-enolactone and γ-Dodecalactone were recorded at 0.9 and 2.6 ppm respectively as free lactones and 2.6 and 5.8 ppm respectively as a lactone potential. The presence of the gamma lactones is highlighted using mass chromatographic analysis of the GC/MS data (Figure 4.5) and by the comparison of the GC retention time of authentic standards.
The average total lactone potential of the Danish WMP was determined at 84.6 ppm.

4.4 Effect of Diet on the Flavour of New Zealand Whole Milk Powder.

An initial study of the effect of diet on the lactone content of New Zealand WMP was determined by carrying out Feeding Trial 1 during September - December 1987 as previously outlined in Materials and Methods 3.2.6.1. The aim of this trial was to determine any effects an "European type" diet may have on the sensory profile and lactone content of the resultant WMP produced.
WMP was manufactured, according to specification, at three week intervals throughout the early part of the 1987/1988 dairy season (September, October, early November, late November and December). Samples of WMP were submitted for sensory analysis to evaluate the effect of season, feed type (pasture only and pasture supplemented with grain concentrate), storage temperature (ambient and 37°C) and storage time (3 and 6 months). Samples were also submitted for lactone analysis. Other analysis were determined by the outcome of the sensory analysis.

Table 4.2 Lactone Content of New Zealand Whole Milk Powder (Specification 8000).

Lactone	Free	Potential
	μg/g milkfat	
δ-Octalactone	trace	1.4 ± 1.1
δ-Decalactone	8.6 ± 1.5	18.1 ± 5.0
γ-Dodec-cis-6-enolactone	-	-
γ-Dodecalactone	trace	0.6 ± 0.3
δ-Dodecalactone	13.8 ± 2.8	35.8 ± 4.2
δ-Tetradecalactone	12.7 ± 3.2	32.3 ± 4.0
δ-Hexadecalactone	10.2 ± 2.9	27.9 ± 4.0
Total Potential		116.1 ± 18.6

Average of six WMP's

Table 4.3 Lactone Content of European Whole Milk Powder (Danish).

Lactone	Free	Potential
	μg/g milkfat	
δ-Octalactone	trace	0.8 ± 0.3
δ-Decalactone	6.4 ± 0.9	14.8 ± 2.1
γ-Dodec-cis-6-enolactone	0.9 ± 0.7	2.6 ± 0.7
γ-Dodecalactone	2.6 ± 1.5	5.8 ± 2.6
δ-Dodecalactone	9.6 ± 1.0	18.5 ± 1.7
δ-Tetradecalactone	14.3 ± 2.3	24.9 ± 3.3
δ-Hexadecalactone	8.0 ± 3.7	17.2 ± 5.3
Total Potential		84.6 ± 18.6

Average of three WMP's

4.4.1 The effect of concentrate feeding on the sensory evaluation of New Zealand WMP (Trial 1, 1987).

The mean sensory scores from the evaluation of WMP manufactured on five separate occasions during Trial 1 (1987/1988 season) are shown in Appendices II, III, IV, V and VI. The effects on the flavour of WMP, as determined by sensory analysis, produced by this trial can be summarised under the effects of the main variables and the interactions of these variables.

4.4.1.1 Effect of Feed Type.

The treatment means showing the effect of feed type, either pasture or pasture supplemented with grain concentrates as undertaken in feeding Trial 1 are presented in Table 4.4. Over the duration of the trial the feed type did not have any effect on any of the flavour attributes (Table 4.5). There was also no significant effect of feed type in interaction with season and time and temperature of storage (Table 4.6). However results indicated that on two occasions during the trial feed type (pasture or pasture and grain concentrates) did have an effect on the lactone flavour attributes. The first was from the WMP manufactured in November where there was a significant effect on the flavour attribute "Age-related". WMP from the concentrate feed group was significantly more age-related in flavour than WMP from the pasture fed group. Treatment means for this attribute were all below threshold indicating this result may not be of major significance (Table 4.7).

Table 4.4 Treatment means showing the effect of feed type (pasture or pasture supplemented with grain concentrates) on the flavour attributes of New Zealand WMP

	Feed Type	
Attribute	Pasture	Pasture and Grain Concentrate
Sweetness	5.1	5.1
Creaminess	4.8	4.8
Cooked/Caramelised	5.3	5.1
Lactone	1.6	2.0
Oxidised	1.0	1.2
Feedy	2.6	2.4
Taint	0.2	0.3
Age-related	0.9	1.2
Astringency	4.2	4.3

Table 4.5 Summary of F-ratios showing the effect of season, feed type, temperature and time on New Zealand WMP (Trial 1).

Attribute	Season	Feed Type	Temperature	Time
Sweetness	2.43 *	0.86 ns	3.90 *	0.09 ns
Creaminess	1.14 ns	0.22 ns	0.06 ns	0.00 ns
Cooked/Caramelised	1.21 ns	2.18 ns	3.59 ns	0.99 ns
Lactone	3.63 **	3.57 ns	0.00 ns	4.46 *
Oxidised	5.24 ***	0.87 ns	21.53 ***	15.18 ***
Feedy	4.94 ***	1.41 ns	10.65 **	35.11 ***
Taint	1.78 ns	0.14 ns	2.91 ns	0.87 ns
Age-related	1.84 ns	3.74 ns	15.62 ***	68 54 ***
Astringency	1.56 ns	0.74 ns	4.55 *	4.14 *

Levels of Significance

* = 5.0%

** = 1.0%

*** = 0.1%

Table 4.6 Summary of F-ratios showing interactions between season, feed type, temperature and time on New Zealand WMP (Trial 1).

Attribute	Season* Feed Type	Season* Temp	Feed Type* Temp	Season* Time	Feed Type* Time	Temp* Time
Sweetness	0.04 ns	0.67 ns	0.85 ns	2.27 ns	0.20 ns	0.36 ns
Creaminess	1.17 ns	1.02 ns	0.00 ns	2.27 ns	0.35 ns	0.15 ns
Cooked/ Caramelised	0.80 ns	0.13 ns	0.00 ns	1.51 ns	0.02 ns	0.16 ns
Lactone	1.71 ns	0.17 ns	0.69 ns	3.34 *	0.42 ns	0.00 ns
Oxidised	0.65 ns	1.39 ns	0.19 ns	2.84 *	1.38 ns	0.18 ns
Feedy	0.82 ns	0.54 ns	0.37 ns	2.96 *	0.02 ns	0.06 ns
Taint	0.44 ns	0.07 ns	3.62 ns	1.94 ns	0.16 ns	0.00 ns
Age-related	1.08 ns	2.81 *	0.13 ns	1.03 ns	0.74 ns	12.03 ***
Astringency	0.31 ns	0.45 ns	0.09 ns	2.93 ns	1.30 ns	1.68 ns

Levels of Significance

* = 5.0%

** = 1.0%

*** = 0.1%

Table 4.7 Treatment means showing the effect of feed type (pasture or pasture supplemented with grain concentrates) on the flavour attribute "Age-related" of New Zealand WMP (November 1987).

	Feed Type	
Attribute	Pasture	Pasture and Grain Concentrate
Age-related	0.7	1.5

The second was from the WMP manufactured in December where there was a significant effect on the flavour attribute "Lactone". WMP from the concentrate feed group was significantly more lactone in flavour than WMP from the pasture fed group. Treatment means for this attribute were above threshold for the concentrate fed group indicating this result may be of real significance (Table 4.8).

Table 4.8 Treatment means showing the effect of feed type (pasture or pasture supplemented with grain concentrates) on the flavour attributes of New Zealand WMP (December 1987).

	Feed Type	
Attribute	Pasture	Pasture and Grain Concentrate
Lactone	1.6	2.9

4.4.1.2 The Effect of Season.

Significant effects were noted for "Lactone", "Oxidised" and "Feedy" flavour attributes (Table 4.5) however these effects were all involved in interactions with time and temperature of storage (Table 4.6) and will be presented later.

4.4.1.3 The Effect of Storage Temperature.

Samples stored at 37°C were found to be significantly more "oxidised" than samples stored at ambient temperatures. The treatment means were 1.5 and 0.7 for storage at 37°C and ambient temperatures respectively. Both these values are below threshold.

4.4.1.4 Interaction effects.

A summary of the interaction effects from the sensory analysis of WMP produced during Trial 1 are presented in Table 4.6.

There were no significant interactions between feed type and the other factors of season, storage time and storage temperature. The only interaction related to the specific aims of this trial was the interaction between season and storage time with respect to the "lactone" flavour attribute. Flavour scores for the "lactone"attribute increased significantly after 6 months storage for WMP produced in late November and December. The treatment means for the "lactone" attribute at 6 months storage increased from 0.4 for WMP manufactured in September to 2.2 and 2.5 for WMP manufactured during late November and December respectively (Table 4.9). Both these values are above threshold.

Table 4.9 Treatment means showing interaction between season and time of storage for the attribute "lactone" (Trial 1, 1987).

	Storage time	
Season	3 months	6 months
September	1.8	0.4
October	1.9	1.4
Early November	2.6	1.4
Late November	1.9	2.2
December	2.0	2.5

4.4.2 The effect of concentrate feeding on the lactone content of New Zealand WMP (Trial 1, 1987).

The levels of the four lactones of interest (δ-Decalactone, γ-Dodec-*cis*-6-enolactone, γ-Dodecalactone and δ-Dodecalactone) in New Zealand WMP manufactured from the concentrate feeding trial 1 are presented in Table 4.10. These results indicate no differences in the lactone levels in WMP manufactured in September and October. For WMP manufactured during early and late November there was a quantifiable amount of γ-Dodecalactone potential of 0.2 and 0.6 ppm respectively with the grain supplemented group. For the pasture fed only group during this period there was only a trace of γ-Dodecalactone present, as determined by GC/MS mass chromatography. In the WMP manufactured during December there was a further increase in the levels of gamma lactones present. WMP manufactured from the grain supplemented group contained free and potential levels for γ-Dodecalactone of 0.2 and 0.9 ppm respectively while WMP from the pasture only group had only a trace of free γ-Dodecalactone and

Table 4.10 Effect of supplementing pasture feeding with grain concentrates on the level of free lactones and the level of lactone potential of NZ WMP (Trial 1, 1987).

	Level of lactone (μg/g milkfat)							
	δC-10		γC12:1		γC12		δC12	
	Free	Potential	Free	Potential	Free	Potential	Free	Potential
September 1987								
Pasture	6.7	14.2	-	-	-	-	11.5	28.5
Pasture/Grain	7.3	16.2	-	-	-	-	9.9	26.8
October 1987								
Pasture	5.6	17.4	-	-	-	-	13.6	29.3
Pasture/Grain	8.3	16.8	-	-	-	-	12.1	25.9
November 1987*								
Pasture	9.4	20.5	-	-	-	trace	10.5	34.6
Pasture/Grain	7.9	16.7	-	-	-	0.2	14.2	30.4
November 1987**								
Pasture	7.3	15.8	-	-	-	trace	12.7	27.8
Pasture/Grain	6.0	16.3	-	-	-	0.6	10.4	26.1
December 1987								
Pasture	9.2	21.4	-	-	trace	0.3	11.7	36.5
Pasture/Grain	8.4	17.6	-	-	0.2	0.9	11.2	25.8

* Early November ** Late November

Average standard deviation of lactone analysis ± 25 %.

0.3 ppm of γ-Dodecalactone potential.

For all WMP manufactured during the course of this feeding trial (September - December 1987) there was no γ-Dodec-*cis*-6-enolactone either as the free lactone or as lactone potential detected. The only other difference in lactone levels determined during this study was that on each occasion that WMP was manufactured the potential level of δ-Dodecalactone was greater in the pasture fed group than with the grain supplemented group. The potential levels of δ-Decalactone were more comparable with neither feed regime causing an increase or decrease in relation to the other group.

4.5 Effect of herd type on the flavour of New Zealand WMP.

In conjunction with the grain feeding trial that was carried out using the Massey University Identical Twin herd (Trial 1, 1987) a concurrent experiment was carried out to determine the effect of herd type on the flavour of New Zealand WMP. WMP was manufactured from milk taken from the factory supply of the Manawatu Co-op Dairy Co. and was deemed to be representative of normal supply from herds which were essentially of mixed breeding (ie. approximately 50:50 ratio of Jersey and Friesian cows). WMP was also manufactured from a pedigree Friesian herd (Mr G Udy, Fielding). The summary of F-ratios showing the effect of herd type, storage temperature and storage time on the sensory evaluation of these samples of WMP are given in Table 4.11.

Herd type (mixed Jersey/Friesian and Pedigree Friesian) was found to have a significant effect on the 'lactone' and 'sweetness' flavour attributes. Mean sensory scores for the lactone attribute, although significantly higher in the pedigree Friesian WMP (1.2 compared to 0.7 with a 5% level of significance), were below threshold (Table 4.12).

Table 4.11 Summary of F-ratios showing effect of herd type, storage temperature and storage time on New Zealand WMP (Udy Trial, August 1987).

Attribute	Herd Type	Temp	Herd Type* Temp	Time	Herd Type* Time	Temp* Time
Sweetness	4.23 *	2.25 ns	0.48 ns	2.45 ns	0.68 ns	0.66 ns
Creaminess	1.38 ns	0.15 ns	0.80 ns	0.68 ns	0.03 ns	0.49 ns
Cooked/ Caramelised	3.45 ns	0.83 ns	1.11 ns	4.04 **	0.27 ns	1.49 ns
Lactone	4.56 *	0.11 ns	0.35 ns	8.14 ***	2.39 ns	1.51 ns
Oxidised	1.06 ns	20.28 ***	2.98 ns	27.15 ***	1.54 ns	1.15 ns
Feedy	0.01 ns	0.55 ns	0.05 ns	7.40 ***	2.38 ns	0.70 ns
Taint	0.00 ns	0.04 ns	0.04 ns	2.09 ns	0.85 ns	0.37 ns
Age-related	0.73 ns	3.63 ns	0.06 ns	13.19 ***	0.92 ns	2.17 ns
Astringency	0.28 ns	0.61 ns	0.01 ns	2.08 ns	0.51 ns	0.31 ns

Table 4.12 Mean sensory scores showing the effect of herd type on the flavour attributes of New Zealand WMP (August 1987).

	Herd Type	
Attribute	Pedigree Friesian	Mixed Jersey/Friesian
Sweetness	5.1	4.7
Creaminess	4.8	4.6
Cooked/Caramelised	5.2	4.8
Lactone	1.2	0.7
Oxidised	3.7	4.1
Feedy	1.9	1.9
Taint	0.6	0.6
Age-related	2.0	2.4
Astringency	4.4	4.5

Other effects included the significant effect of storage temperature on the 'oxidised' attribute with mean sensory scores of 4.9 for samples stored at 37°C compared to 2.9 for samples stored at ambient temperature. The significance of this effect is illustrated by an F-ratio of 20.28 with a level of confidence of 0.1%.

However when this experiment was repeated with WMP manufactured during November 1987 analysis showed that herd type (mixed Jersey/Friesian and Pedigree Friesian) did not have any significant effect on the flavour attributes (Table 4.13). In particular herd type had no effect on the mean sensory scores for the various flavour attribute (Table 4.14).

Table 4.13 Summary of F-ratios from the Sensory evaluation of WMP manufactured from milk obtained from mixed breed herds (Manawatu Co-op) and pedigree Friesian herd (Udy) showing the effect of herd type, temperature and storage time and the interaction of these effects (November 1987).

Attribute	Herd	Temp	Herd* Time	Time	Herd* Time	Temp* Time
Sweetness	0.08 ns	3.22 ns	1.23 ns	2.27 ns	0.34 ns	0.28 ns
Creaminess	0.06 ns	0.06 ns	0.01 ns	9.19 ***	0.09 ns	0.05 ns
Cooked/ Caramelised	0.00 ns	1.79 ns	0.80 ns	2.77 *	0.23 ns	0.24 ns
Lactone	0.00 ns	9.74 **	0.01 ns	7.78 ***	0.41 ns	0.59 ns
Oxidised	0.00 ns	36.27 ***	0.13 ns	10.88 ***	0.07 ns	2.45 ns
Feedy	0.00 ns	0.24 ns	0.38 ns	12.49 ***	0.27 ns	1.83 ns
Taint	0.07 ns	0.00 ns	2.25 ns	0.86 ns	1.95 ns	1.72 ns
Age-related	0.55 ns	3.19 ns	0.16 ns	6.96 ***	0.35 ns	1.17 ns
Astringency	0.10 ns	0.65 ns	0.00 ns	3.46 *	0.19 ns	0.09 ns

Levels of Significance

* = 5.0%

** = 1.0%

*** = 0.1%

Table 4.14 Mean sensory scores showing the effect of herd type on the flavour attributes of New Zealand WMP (Udy Trial, November 1987).

	Herd Type	
Attribute	Pedigree Friesian	Mixed Jersey/Friesian
Sweetness	4.6	4.5
Creaminess	4.5	4.5
Cooked/Caramelised	4.6	4.6
Lactone	1.0	1.0
Oxidised	4.1	4.1
Feedy	1.3	1.3
Taint	0.2	0.2
Age-related	2.1	1.8
Astringency	4.1	4.2

Other effects that were evident in this experiment was the effect of time on the 'lactone' flavour attribute which decreased significantly with increased storage time. The mean sensory scores for 'lactone' flavour decreasing from 2.3 at 3 months to 0.4 at 12 months (Table 4.15)

Table 4.15 Mean sensory scores showing the effect of time on the Lactone flavour attribute.

	Storage Time			
	3 months	9 months	6 months	12 months
	Mean sensory score			
Lactone	2.3	0.8	0.5	0.4

Shading joins those samples not significantly different.

Because the aim of this trial was to establish if there were any significant difference in the sensory properties of WMP manufactured from Friesian cows verses cows of mixed breeding no chemical characterisation (ie lactone analysis) was carried out.

4.6 The Effect of Supplementing pasture feeding with grain concentrates on the flavour of New Zealand Whole Milk Powder (Trial 2, 1988).

4.6.1 Sensory analysis of WMP

WMP was manufactured from cows which had received grain supplements in addition to normal pasture feeding as described in Section 3.2.6.1.
Sensory evaluation was carried out at 3, 6, 9 and 12 months with samples stored at both ambient temperatures and 37°C. The mean sensory scores for each evaluation are give in Appendices 8, 9, 10 and 11. The F-ratios from the analysis of variance are presented in Table 4.16. and the summary of mean sensory scores showing the effect of feed type on the sensory attributes of New Zealand WMP is presented in Table 4.17. with the effect of feed type and storage time on the 'lactone' flavour attribute are presented in Table 4.18.

Feed type (pasture or pasture/grain concentrate) had a significant effect on the "lactone" flavour attribute with a F-ratio of 32.47 (5% level of confidence). This was reflected in higher mean sensory scores for the "lactone" attribute in WMP manufactured from the group receiving the grain supplement along with pasture compared to the pasture only group which was in turn higher than the second control group (MCDC). The magnitude of the mean sensory scores were 2.9, 1.5 and 0.9 respectively.

The effect of feed type on the "lactone" flavour attribute was most pronounced at the 3 month evaluation (Appendix 8). The mean sensory scores for the grain fed WMP was 4.1 and 4.0 for WMP stored at ambient and 37°C respectively. This compared to 1.4 fore the pasture only group (both ambient and 37°C) and 0.6 and 0.8 for the MCDC control WMP at ambient and 37°C respectively.

Other effects of feed type on the flavour attributes of WMP included a significant effect on the "age-related" flavour attribute where this effect had a F-ratio of 66.89 (5% level of significance). The mean sensory scores for the "age-related" attribute were 1.6 for the grain supplemented group, and 2.2 for both the pasture only control and the MCDC control.

Other interactions which occurred other than the effect of feed type but which concerned the "lactone" flavour attribute was the effect of storage time (F-ratio 8.63, 5% level of significance) and the interaction between storage time and storage temperature (F-ratio 7.65, 5% level of confidence).

4.6.2 The effect of concentrate feeding on the lactone content of New Zealand WMP (Trial 2, 1988).

The results of lactone analysis of the WMP produced during the second concentrate feeding trial (1988/1989 season) are presented in Table 4.19. These results indicate significant differences, depending on the feeding regime, in the lactone profiles of the WMP's. WMP produced from the grain fed Friesian group contained the highest levels of γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone at 1.9 and 3.6 ppm free lactone respectively and 2.8 and 4.4 ppm lactone potential respectively. In contrast the grass fed

Friesian group produced WMP with 0.7 and 1.0 ppm for free and potential γ-Dodec-*cis*-6-enolactone respectively and 1.0 and 1.3 for free and potential γ-Dodecalactone respectively. The lactone levels determined for the second control group which was produced from milk from the local factory supply (MCDC group) contained no quantifiable levels of the two γ-lactones but did contain potential levels for γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone of 0.9 and 1.0 respectively. The only other result of note was the lower potential for δ-Dodecalactone in WMP from the two Friesian groups at 23.6 ppm compared to the result for the MCDC group at 33.5 ppm.

Table 4.16 Summary of F-ratios showing the effect of feed type, storage time and storage time on New Zealand Whole Milk Powder (Trial 2, 1988).

	Main Effects			Interactions	
Attribute	Feed	Temp	Time	Feed* Time	Temp* Time
Sweetness	17.77 ns	3.58 ns	1.99 ns	2.14 ns	1.25 ns
Creaminess	3.36 ns	0.61 ns	2.08 ns	0.75 ns	5.46 *
Cooked/ Caramelised	2.00 ns	2.32 ns	11.43 **	3.21 ns	0.48 ns
Lactone	32.47 *	0.29 ns	8.63 *	6.95 *	7.65 *
Oxidised	7.61 ns	17.12 ns	65.74 ***	7.01 *	15.80 **
Feedy	0.90 ns	0.76 ns	19.51 **	0.90 ns	6.51 *
Taint	3.96 ns	1.12 ns	11.05 **	8.88 **	4.21 ns
Age-related	66.89 *	45.96 *	6.67 *	1.38 ns	4.92 *
Astringency	3.07 ns	0.52 ns	1.02 ns	1.83 ns	0.21 ns

* = 5.0% significance

** = 1.0% significance

*** = 0.1 significance

Table 4.17 Summary of mean sensory scores showing the effect of feed type on the sensory attributes of New Zealand Whole Milk Powder (Trial 2, 1988).

	Feed Type		
Attribute	MCDC Control	Pasture Only	Pasture/Grain
Sweetness	4.8	5.0	5.2
Creaminess	4.7	5.0	4.8
Cooked/ Caramelised	5.0	5.2	5.1
Lactone	0.9	1.5	2.9
Oxidised	2.5	2.3	1.1
Feedy	1.6	1.6	1.3
Taint	0.5	0.0	0.1
Age-related	2.2	2.2	1.6
Astringency	4.2	4.6	4.4

Table 4.18 Interaction means showing the effect of feed type and storage time on the "lactone" flavour attribute of New Zealand Whole Milk Powder (Trial 2, 1988).

	Storage Time			
Feed Type	3 months	6 months	9 months	12 months
MCDC Control	0.7	1.2	0.7	1.0
Pasture Only	1.4	2.1	1.6	1.0
Pasture/Grain	4.1	3.0	2.8	1.9

Table 4.19 Effect of grain feeding on the level of lactones in NZ WMP (Trial 2).

	Level of Lactone (μg/g milkfat			
	δC-10	γC-12:1	γc12	δC-12
MCDC				
Free	10.2	-	-	22.5
Potential	11.7	0.9	1.0	33.5
Friesian Grass Fed				
Free	10.4	0.7	1.0	23.2
Potential	11.4	1.0	1.3	23.6
Friesian Grain Fed				
Free	10.9	1.9	3.6	20.9
Potential	12.4	2.8	4.4	23.6

Manufactured 23.11.88 NZDRI Average standard deviation of lactone analysis $\pm$ 25 %.

4.7 Lipid content of grains used in the Feeding Trials.

The average total lipid content of the oats and sunflower seeds used in Feeding Trial 2 were measured at 3.6% and 52%. The fatty acid profiles of the oats, sunflower seeds and the barley component from Trial 2 are given in Table 4.20 along with the fatty acid profile of maize oil similar to the likely profile of the maize component in Feeding Trial 1. The two elements of interest are the oleic acid (18:1) content of oats at 36.2 g/100 g, which is higher than the other grains, and the linoleic acid (18:2) content of sunflower seeds at 62.4 g/100 g. This too is higher than the other grains although maize at 62.0 g/100 g is similar but has a higher palmitic acid (16:0) content at 10.2 g/100 g compared to sunflower seeds at 6.9 g/100 g.

Table 4.20 Fatty acid compositions obtained from the grains used in grain feeding trials 1 and 2.

	Fatty acid composition of grains			
	(g/100g)			
Fatty acid	Maize	Barley	Oats	Sunflower seeds
4:0	0.0	0.0	0.0	0.0
6:0	0.0	0.0	0.0	0.0
8:0	0.0	0.0	0.0	0.0
10:0	0.0	0.0	0.0	0.0
12:0	0.0	0.0	0.0	0.1
14:0	0.1	0.6	0.0	0.2
14:1	0.0	0.0	0.0	0.0
15:0	0.0	0.0	0.0	0.0
16:0	10.2	23.9	17.2	6.9
16:1	0.1	0.0	0.3	0.1
17:0	0.0	0.0	0.0	0.0
17:1	0.0	0.3	0.0	0.0
18:0	1.4	1.9	1.1	3.9
18:1	24.0	12.9	36.2	24.7
18:2	62.0	52.5	41.8	62.4
18:3	1.4	6.1	1.7	0.4

4.8 Flavour volatiles from New Zealand milkfat.

Because no single sample of New Zealand WMP was identified by the sensory panel as possessing a distinct "feedy" flavour it was decided that analysis of milkfat samples which had been identified in previous instances, particularly in early lactation as being very "green/grassy" would be carried out.

The compounds identified from the vacuum distillation of a sample of New Zealand milkfat (Fresh Frozen Milkfat for Recombining - FFMR) are presented in Table 4.21. A total of 57 compounds were positively identified in the extract from the vacuum distillate from the sample of New Zealand FFMR. The most abundant class of compound were the hydrocarbons with 33 compounds identified. Other classes of compounds identified were aldehydes (4 compounds), ketones (5), acids (5), alcohols (1), lactones (3) and terpenoids (6). Compounds were identified either by mass spectral comparison with the mass spectral library or also by comparison of mass spectrums and gas chromatographic retention times of authentic compounds.

4.8.1 Effectiveness of the vacuum distillation of New Zealand milkfat.

In order to evaluate the effectiveness of the vacuum distillation of New Zealand milkfat (FFMR) the distillate from 4 Kg of milkfat was added back to 1 Kg of fresh milkfat which was then evaluated by the informal panel with emphasis on the level of the "green/grassy" flavour attribute. The results of this evaluation, which are presented in Table 4.22, show that the addition of the vacuum distillate to good quality milkfat had the effect of increasing the level of "green/grassy" flavour, as perceived by the informal panel. The mean value for "green/grassy" flavour increased from 1.6 (on the 0 - 5 scale where 0 = absent and 5 = intense) for the control sample to 3.6 for the control plus the vacuum distillate.

Table 4.21 Compounds identified by GC/MS analysis of the extract from the vacuum distillation of New Zealand milkfat (FFMR).

Data No.	Compound	Identification
	Hydrocarbons	
15	Benzene	MS
34	Cyclohexane	MS
77	3-methyl hexane	MS
91	1,3-dimethyl cyclopentane	MS
146	Heptane	MS,RT
206	methyl cyclohexane	MS
338	toluene	MS
433	dimethyl cyclohexane	MS
482	2,2,5 trimethyl hexane	MS
529	tetrachloroethane	MS
544	3-octene	MS
555	octane	MS,RT
574	2-octene (E)	MS
609	2-octene (Z)	MS,RT
730	2,5 dimethyl heptane	MS
764	ethyl benzene	MS
808	1,3 dimethyl benzene	MS
837	3,4 dimethyl heptane	MS
914	dimethyl benzene	MS
1047	nonane (int. std.)	MS,RT
1100	3,4 dimethyl octane	MS (cont)

52	3-methyl-butan-2-one	MS

1190	2,2,6 trimethyl octane	MS
1212	2,5 dimethyl octane	MS
1223	propyl benzene	MS
1261	1-ethyl-4-methyl benzene	MS
1271	1-ethyl-3-methyl benzene	MS
1415	1,2,4 trimethyl benzene	MS
1703	3,7 dimethyl nonane	MS
2230	naphthalene	MS
3238	tetradecane	MS,RT
3603	pentadecane	MS,RT
	Aldehydes	
72	pentanal	MS,RT
447	hexanal	MS,RT
934	heptanal	MS,RT
1156	benzaldehyde	MS,RT
	Ketones	
409	hexan-2-one	MS,RT
890	heptan-2-one	MS,RT
1864	nonan-2-one	MS,RT
2738	undecan-2-one	MS,RT
3514	tridecan-2-one	MS,RT
	Acids	
627	butanoic acid	MS
1616	hexanoic acid	MS
2386	octanoic acid	MS
3049	γ hydroxy acid	MS
3094	decanoic acid	MS (cont)

	Alcohols	
827	hexan-1-ol	MS
	Lactones	
2572	δ-Decalactone	MS,RT
3403	δ-Dodecalactone	MS,RT
4144	δ-Tetradecalactone	MS,RT
	Terpenoids	
1173	α-Pinene	MS,RT
1363	ß-Pinene	MS,RT
1616	D-Limonene	MS,RT
1893	Linalool	MS,RT
2303	α-Terpineol	MS,RT
3279	Carophyllene	MS
	Others	
2413	Octyl acetate (int. std.)	MS,RT
25	Tetrachloromethane	MS

Table 4.22 The effect on the level of "green/grassy" flavour in New Zealand milkfat by the addition of the aqueous vacuum distillate from 4 Kg of New Zealand milkfat. Mean scores using the informal panel (three panellists).

Sample	"green/grassy" flavour score
Control	1.6
Control + vacuum distillate	3.6

Scale: 0 = absent, 1 = threshold, 2 = weak, 3 = moderate, 4 = strong, 5 = intense.

4.9 The effect of terpenes on the flavour of New Zealand milkfat.

4.9.1 D-Limonene.

Extracts from New Zealand have indicated the presence of a number of terpenoids. Of these D-limonene when added to deodourised milkfat gave a flavour reminiscent of the "green/grassy" flavour present in some samples of New Zealand milkfat. When added to deodourised milkfat at a level of 1 ppm the flavour scores from the informal panel for "green/grassy" flavour increased from 0 to 3 - 4 (Table 4.23).

Table 4.23 The effect of the addition of D-limonene on the flavour of New Zealand milkfat.

Sample	"green/grassy" flavour score
Deodourised milkfat	0
Deodourised milkfat + 1 ppm D-Limonene	3 - 4

Scale: 0 = absent, 1 = threshold, 2 = weak, 3 = moderate, 4 = strong, 5 = Intense

4.9.2 Other Terpenoids.

The effect on milkfat flavour of the other terpenoids found in New Zealand milkfat are presented in Table 4.24. Each compound was added at the arbitrary level of 1 ppm to deodourised NZ milkfat and was evaluated by an informal panel. Comments on flavour rather than specific scores for "green/grassy" flavour were recorded.

Table 4.24 The effect of the addition of selected terpenoids on the flavour of New Zealand milkfat.

Sample	Comments on Flavour
Deodourised milkfat	bland, slight milkfat flavour
+ α-pinene	antiseptic, pine oil
+ ß-pinene	turpentine, antiseptic
+ Linalool	green, citrus, fruity
+ α-terpineol	floral, antiseptic
+ Carophyllene	spicy, herbaceous

CHAPTER V: DISCUSSION

5.1 Manufacture of Whole Milk Powder.

All New Zealand WMP used during the course of this study was manufactured at NZDRI according to the strict conditions for the production of specification 8000 WMP. This specification is for an agglomerated, non instantised, medium heat WMP. No vitaminised WMP's were considered during this study.

Denmark was chosen as the source of European WMP because of its competition to New Zealand WMP in many world markets and also because the Danish farming system is very different to the New Zealand farming system. In general terms, the Danish dairy farming industry is based primarily on the feeding of high energy grain supplements to predominantly Friesian dairy cattle whereas New Zealand dairy production, due to economic factors, is based on an all pasture feeding system using a number of breeds (Friesian, Jersey etc.) with also a proportion of cows of mixed breeding.

Unlike the sample of New Zealand WMP, very little information was available about the Danish WMP. In particular nothing was known about the specific diet, the breed of cow or the exact conditions of manufacture. However all the Danish WMP samples were subjected to an initial sensory analysis and microbiological analysis before sensory analysis by the trained panel and subsequent chemical analysis. Only one sample of Danish WMP was rejected as being inconsistent with the other Danish samples.

5.2 Sensory Analysis of Whole Milk Powder.

Most of the methodology and prior understanding of the sensory evaluation of WMP has been derived from the study of Cooper (1981) which outlined the use of various profiling methods to study the effect of seasonal changes and processing variables on the sensory properties of WMP's. It was this study that established, through systematic group discussions, the sensory profile for New Zealand WMP and also the use of the 0 -10 linear scale which offers both ease of use and sensitivity to sample differences. In this study only the descriptors for WMP were used, factors such as the colour and aroma of WMP were not considered.

5.2.1 Sensory Analysis of New Zealand and European WMP.

Initial analysis of New Zealand and Danish WMP sample indicated that the most significant difference between these samples was in the area of the lactone attribute. In all instances the Danish WMP samples scored consistently higher mean sensory scores for the lactone flavour attribute as determined by the trained panel. This difference in lactone flavour was consistent with all comparisons between New Zealand and Danish WMP's. The difference in mean sensory scores were, as would be expected, often variable but the differences were always statistically significant. The mean sensory values for lactone flavour in the New Zealand WMP were in general agreement with those reported previously (Cooper, 1981), though that study indicated that lactone-like flavours could be attributed to the addition of vitamins A and D.

In contrast nothing has been previously reported on the descriptive analysis of Danish WMP. The only report that may have relevance was that of Hall and Lingnert, (1984) who reported on the odour and flavour profiles of Swedish WMP however the lactone descriptor was not used and emphasis was on aspects of the oxidation of WMP.

Evaluation of the Danish WMP by an informal panel skilled in the discrimination of dairy flavours indicated that not only was the Danish WMP higher in lactone flavour but also that the nature of the lactone flavour was different from New Zealand WMP. Based on these results the chemical characterisation of the lactone profiles of New Zealand and Danish WMP's was carried out.

It is interesting to note that in the initial sensory analysis of New Zealand and Danish WMP that no significant differences were detected in the "feedy" flavour attribute. At the outset of this study one of the objectives was to determine the cause of feedy flavour in New Zealand WMP.

5.3 Lactone Analysis of Whole Milk Powder.

In order to determine the lactone content of WMP a prior extraction of the milkfat fraction is necessary. The method used in this study has been shown to recover 85 - 96%

Addendum:

Ion chromatography is the selection of ions of a specific molecular weight (eg. 85 and 99) from the total ion current (TIC) in order to produce a chromatogram which reflects the abundance of the specific ions.

of the total lipid in the powder (Keen et al., 1976). In order to determine the lactone potential of the milkfat the bound γ- and δ-hydroxyacids need to be converted to their corresponding lactones. The established method to achieve this is to heat the milkfat at 120°C for 16 h (Walker et al., 1977). The complete generation of lactones can also be completed within 2 h but at the much higher temperature of 180°C (Stark and Urbach, 1976).

Analysis of the lactone content was achieved using combined gas chromatography/mass spectrometry in particular the use of ion chromatography. This allowed the monitoring of the base peak of δ-lactones (m/z 99) and the base peak of γ-lactones (m/z 85). Positive identification of lactones was also confirmed and quantified by the comparison of the gas chromatographic retention time of authentic standards.

5.3.1 Lactone Analysis of New Zealand and Danish WMP.

The lactone profile of New Zealand WMP was characterised by the predominance of the delta lactones, δ-Octalactone (δ-C8), δ-Decalactone (δ-C10), δ-Dodecalactone (δ-C12), δ-Tetradecalactone (δ-C14) and δ-Hexadecalactone (δ-C16). There was only a trace of the gamma lactone γ-Dodecalactone, as determined by mass spectral analysis using selective ion monitoring, but levels were generally too low to quantify by gas chromatography. The levels of delta lactones, calculated on a milkfat basis, in the New Zealand WMP were within the range of results reported previously for New Zealand milkfat (Walker, et al., 1977).

Nothing has been reported previously about the lactone content of Danish WMP, or indeed Danish milkfat in general, therefore there is no published data on which to compare the results of this study, specially on the presence or absence of gamma lactones.

After the initial identification of δ-C14 and δ-C16 lactones the concentration of these was not routinely determined because of the belief that they do not make a significant

contribution to flavour. This is based on their presence in milkfat at concentrations around their flavour threshold unlike the other shorter chain delta lactones (δ-C8, δ-C10 and δ-C12) which are present at concentrations well in excess of their flavour thresholds. The potential levels of these three lactones in milkfat are approximately 10 times their flavour threshold (Urbach et al., 1972).

Walker, et al., (1977) also reported the general absence of gamma lactones in New Zealand milkfat although one of the three milkfat's they used for fractionation did contain levels of γ-Dodecalactone at 2.1 and 3.6 ppm for free lactone and lactone potential respectively. The presence of gamma lactones were attributed to the possible influence of feed type, seasonal effects or stage of lactation (Dimick and Harner, 1968).

By contrast the lactone profile of the Danish WMP samples is characterised not only by the same series of delta lactones as present in the New Zealand WMP but also by the presence of two gamma lactones; in particular γ-Dodec-cis-6-enolactone and γ-Dodecalactone. Again positive identification of these two gamma-lactones was by a combination of mass spectral comparison from the computer based mass spectrum library (NBS/NIH/EPA) of the VG mass spectrometer and also of authentic standards and also comparison of gas chromatographic retention times.

Some of the differences in the levels of free lactones between New Zealand and Danish WMP and hence lactone flavour may be linked to the age of the WMP. Generally lactone levels in New Zealand WMP were recorded with fresh powder unlike the Danish WMP where a lot less was known about the history of the product, although it would be fair to assume that the Danish WMP was significantly older than the New Zealand WMP at time of analysis. Free lactone levels, as distinct from lactone potential, are influenced by such factors as length of storage and storage temperature (Dimick, et al., 1969).

It is quite likely that the presence of γ-Dodec-cis-6-enolactone was not detected by many of the previous researchers and it has only been the development of more sophisticated equipment such as capillary chromatography with an increased ability to separate closely eluting compounds that has allowed it to be separated from its saturated analogue. Indeed Badings and Neeter (1980) in their comprehensive study of aroma compounds

from milk failed to identify γ-Dodec-cis-6-enolactone, although they did designate an unknown peak eluting prior to γ-Dodecalactone as a γ-lactone. Based on its gas chromatographic retention time relative to γ-Dodecalactone it is likely that this peak was in fact γ-Dodec-cis-6-enolactone.

γ-Dodec-cis-6-enolactone is not the only unsaturated γ-lactone that has been identified in bovine milkfat, others include bovolide (Boldingh and Taylor, 1962), dihydrobovolide and a diunsaturated C-14 lactone.

5.4 Possible Origins of Gamma Lactones in Danish Whole Milk Powder.

The two gamma lactones identified in Danish WMP are the same compounds reported in milkfat and meat samples produced in trials carried out in Australia in the 1970's in an attempt to increase the level of polyunsaturated fatty acids in milkfat and meat. However as a consequence of the feeding of protected lipid supplements to dairy cows there was a resultant off-flavour in the butter. This was described as a strong 'sweet raspberry' flavour and was due to high levels of γ-dodec-cis-6-enolactone and γ-Dodecalactone. Both these compounds were present at levels well in excess of their flavour thresholds. γ-Dodecalactone has a flavour threshold of approximately 1 ppm and though the flavour threshold of γ-Dodec-cis-6-enolactone has not been determined it is thought to be of the same order or in fact lower than that of γ-dodecalactone. Flavour thresholds were determined by addition to butter made from deodourised milkfat which had been reimulsified with distilled water (Urbach et al., 1972).

The diet used in this study consisted of crushed oats and protected sunflower seeds and the link was established between oats in the diet and the level of γ-Dodecalactone and the oil supplement with the level of γ-Dodec-cis-6-enolactone (Urbach, 1982).

Therefore it can be postulated that the presence of the two gamma lactones in the Danish WMP could well be related to the feeding regime of the cows. It is known that the feeding of high energy grain concentrates is a common practice in some areas of Europe, in particular Denmark, especially during the winter months.

In order to test the hypothesis that the presence of these gamma lactones in the Danish WMP can be related to the type of feed two feeding trials using NZ dairy cows were organised.

5.5 The Effect of Concentrate Feeding on the Flavour of New Zealand WMP (Trial 1).

5.5.1 Sensory Analysis of WMP.

The results of the sensory analysis of WMP manufactured on five separate occasions during Trial 1 which used the Identical Twin cows at Massey University indicated that, over the course of the trial, the feed type, either pasture or pasture supplemented with grain concentrates had no significant effect on the flavour attributes of the WMP. However there were some small differences in the mean sensory scores for the lactone attributes between pasture feeding and the concentrate feeding for WMP manufactured at the end of the trial -December. These differences were found to be significant with mean sensory scores above threshold. It had been expected that as a result of supplementing the pasture feeding with grain concentrates that significant differences would have been reflected in some of the flavour attributes.

There may be several explanations of why greater differences were not detected. It was noted that during this trial there was a certain degree of refusal of feed amongst the group receiving the grain concentrate. In particular during the early part of the trial (September - October) there were a number of individual cows who refused the grain concentrate totally. This would probably go some way in explaining the lack of effect, at least in the early part of the trial, seen with the supplement feeding. This refusal of the grain supplement may have been linked to the composition of the grain concentrate which was a commercially prepared supplement which was mixed with milled oats. This combination was chosen because it incorporated the elements thought necessary to result in changes to the lactone profile (Urbach and Stark, 1978).

Oats, which formed the basis of the Australian research, and feed intake were considered to be the two crucial elements necessary in the feeding trial to influence changes in the lactone profile (Urbach, private communication, 1988).

Another possible reason for the lack of differences could lie in the duration of the trial. In the absence of information from similar trials it was assumed that 4 months should have been of sufficient duration to effect any changes that were likely to happen. This sort of time frame is commonly used with feedlot beef where animals are removed from grass and grain feed from 100 - 120 days in order to decrease the level of "grassy"flavour in beef (Larick, et al., 1987). Therefore the changes seen with the December WMP may have just been an indication of greater changes that may have come about if the trial had been allowed to continue further. Although no specific times are mentioned with the Australian feeding trials it can be inferred from their results that the effects of interest were demonstrated well within the nominal 100 day time period (Urbach and Stark, 1978).
Therefore the view could be taken that more emphasis should be placed on results from the end of the trial rather than results arrived at by averaging results through out the trial.

5.5.2 Lactone Analysis of WMP.

Lactone analysis of WMP produced during feeding trial 1 showed no differences in the lactone profiles of WMP produced from grass fed versus grain supplemented cows on four out of the five occasions that WMP was manufactured. Only in the samples of WMP manufactured during December was there an appreciable difference in the lactone content of WMP from the grain fed group in comparison to the grass fed group. This difference was in the form of increased concentrations in the free and potential levels of γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone in the grain fed group. This corresponded to the sensory results which also indicated an increase in the mean sensory

scores for the lactone attribute for the grain fed group in WMP manufactured during December.

Although there was good agreement between both sensory and analytical methods in both cases the levels recorded were low with sensory scores slightly above threshold and the analytical levels below or just equal to the flavour threshold levels of these compounds (Urbach, et al., 1972).

5.5.3 The Effect of Concentrate Feeding on Milk Production and Gross Milk Composition.

The effects of the initial grain feeding trial on milk production and milk composition has been reported separately to this study by Suksombat (1988). Although this study was not concerned with the gross effects on milk production and composition it may be appropriate to discuss some key points as an indicator of the effectiveness of the trial. This is important in determining if this trial was representative of animal husbandry practices occurring in other countries, in particular Europe (Denmark).

Generally concentrate supplementation increased the yield of milk and milk components which was consistent with other studies (Reviewed: Suksombat, 1988).

5.6 Other Factors which may have Influenced the Initial Trial and Reasons for the Changes Incorporated in the Second Trial.

At the end of the first trial between grass and grain feeding the results indicated that the magnitude of the desired effects on the sensory and chemical nature (lactone composition) of the flavour of the WMP did not duplicate the original differences between NZ and Danish WMP. Therefore an attempt was made to identify areas that may have influenced the results and to look at changes that would enhance the small differences detected in Trial 1.

5.6.1 Breed of Cow.

Probably the most important factor in planning a repeat of the concentrate feeding trial was the choice of breed of cow. The herd used in Trial 1 was a mixed breed herd containing both Jersey and Friesian cows as well as other cows of mixed breeding. The influence that this mixture of breeds will have is not known however there is evidence that breed does have an effect on lactone concentration (Dimick and Harner, 1968).
It was decided that as the trial was an attempt to duplicate feeding regimes that take place in Europe, in order to study the effects on the flavour of WMP, then it would seem appropriate that where possible a similar breed of cow should be used. This effectively meant the use of Friesian cows only and also because some Friesians in New Zealand are derived from a Friesian/Jersey cross, care was also taken in selecting which Friesian herd to use (Keen:private communication, 1989). Keen and Udy (1980) had earlier identified differences in herd type based on milkfat colour and postulated a link between colour and purity of breed. Thus some animals classified as pure-breed Friesian could have some Jersey influence in their pedigree, this would result in milkfat of a higher colour compared to more "purebred" Friesians.
A herd (Mr G Udy, Matipo, RD 5, Feilding) had been previously identified on the basis of milkfat colour as possessing these more defined "purebred" Friesian characteristics.

This herd had been used previously to show a significant effect on the lactone flavour attribute in WMP between Pedigree Friesian and a mixed breed factory supply herd. Though the difference was significant the scores were less than threshold. So in summary there was a significant though small difference in the lactone flavour attribute of interest on similar feed type.

5.6.2 Lactational Effects.

Lactational effects could have exerted an influence on the lactone levels in the WMP during the first grain feeding trial. Stage of lactation has previously been shown to

influence the total lactone concentration with levels peaking at 175 - 200 days of lactation with the concentration at 200 days being 4 times greater than the concentration at 25 days (160 ppm cf. 40 ppm) (Dimick and Harner, 1968). The Massey Identical Twin herd used for the initial study was classified as a "factory supply herd" with all individuals commencing lactation at approximately the same time and hence by the end of the trial (December) the herd would have been lactating for at maximum 150 days still 25 - 50 days short of peak lactone concentration.

As opposed to this the pedigree Friesian herd at Fielding was classified as a "town milk supply" herd with individual lactations commencing at different times of the year. Therefore in contrast to the Massey herd there would be a significant number of individuals with a greater number of lactation days (greater than 150 days) at the outset of the feeding trial. Common dairy practice in Europe (Denmark) has lactation commencing on a year round basis and in general does not use the same degree of coordinated calving as that which occurs in New Zealand.

5.6.3 Composition of Feed.

The most important requirement in the grain feeding trial was that the animals should consume the grain concentrate, this clearly was a problem area in the first feeding trial. The feed used in the second Trial consisted of 85% oats, 10% sunflower seeds and 5% barley. This combination was chosen firstly due to its precursor fatty acid composition and secondly on its palatability.

The choice of oats as the primary grain was influenced by the Australian feeding trials and the fatty acid composition of the oats, 36.2% oleic acid, fitted the requirements postulated as being necessary to influence the lactone content of milkfat (Stark, et al., 1978). The sunflower seeds at 62.4% linoleic acid were included as a good source of linoleic acid while the barley was included solely to aid pelletising.

5.7 The Effect of Supplementing Pasture Feeding with Grain Concentrates on the Flavour of New Zealand WMP (Trial 2).

5.7.1 Sensory Analysis of WMP.

In the repeated trial it was found that feed type did have a significant effect on the flavour of WMP, in particular there was an increase in the mean sensory scores for the lactone attribute with grain supplementation compared to the pasture control group. This effect was most pronounced at 3 months of age. With both the initial and 3 month evaluations the mean sensory scores for the lactone attribute were greater than threshold level (2 +) indicating that at these levels there is likely to be a significant impact on flavour.

Although not equal to the sensory scores recorded for the samples of Danish WMP this second trial has demonstrated a significant effect of feed type and that the difference is in the area that distinguishes Danish WMP from New Zealand WMP.

The scores for lactone flavour decreased with storage time and were generally less than threshold at 12 months of storage.

As this study encompasses the only detailed work involving the sensory and chemical characterisation of WMP produced from grain supplemented and grass fed cows little comparison with other relevant studies can be made. Cooper (1981) had associated "lactone-like" flavours in WMP with powders that had been fortified with vitamins A and D and although the levels of these vitamins were not determined in these experimental powders it is unlikely that any changes in the levels of these vitamins that this trial may have produced would have been detected by the panel and recorded under the lactone attribute. The composition of the panel used in this study was completely different to the group used by Cooper (1981). The Australian study on the effect of diet on the lactone levels of milkfat did not contain any sensory analysis information and only dealt with the chemical analysis of the milkfat. Some comment was made however on the likely impact that the increased lactone levels may have on the suitability of the milkfat for applications such as baking (Urbach and Stark, 1978).

The only other effect attributable to feed type was decreased scores for "age-related" flavour with the grain fed WMP in comparison to the other two types of WMP. It may be that the decreased scores for "age-related" flavour with the grain fed WMP may be due to the increased lactone flavour partially masking the aged flavours. If this were the case then it may be possible for WMP with increased lactone flavour to have a greater shelf life than low lactone flavoured WMP. This aspect of desirable flavours such as lactone, creaminess etc competing and effectively masking the undesirable flavours such as age-related and oxidised flavours has not been addressed before. With a product such as WMP which is designed to be stored for extended periods of time any mechanism that delays the onset of storage flavours is likely to be of major economic benefit.

5.7.2 Lactone Analysis of WMP (Trial 2).

The result of lactone analysis of the WMP produced during the second concentrate feeding trial indicated significant qualitative and quantitative differences in the lactone profile between the three groups of animals. After 3 months the grain supplemented group contained significant levels of γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone in the WMP. The WMP, which was analysed at between 3 and 6 weeks of age contained both free and potential levels of the two lactones in excess of their flavour thresholds. Based on the threshold data for γ-Dodecalactone at 1 ppm and the estimated threshold for γ-Dodec-*cis*-6-enolactone at less than 1 ppm the levels recorded for the grain fed WMP would be likely to contribute to the flavour (Urbach, et al., 1972) of the WMP. The free and potential of the two gamma lactones recorded in the Friesian grass fed WMP are around the threshold level and would be expected to make less of a contribution. While in the MCDC control group there were no free levels of the gamma lactones recorded and hence a lactone flavour would not be expected. However this WMP did contain some potential for the two gamma lactones at around threshold levels and depending on storage factors etc. may upon release make some contribution to flavour.

In the case of the grain fed group the level of γ-Dodec-*cis*-6-enolactone and γ-Dodecalactone did not reach the levels recorded in the Australian study (Urbach and Stark, 1978). In their study with bovine milkfat, diets containing oats only routinely produced γ-Dodecalactone levels of between 20 and 180 ppm depending on individual animals while the inclusion of protected sunflower seed supplements resulted in levels of γ-Dodec-*cis*-6-enolactone between 10 and 25 ppm. All these levels were greatly in excess of their respective flavour threshold values (approximately 1 ppm) and likely to give rise to dominant flavours in the milkfat. Indeed the starting point of their research was to identify the cause of the "sweet raspberry" off-flavour in the meat and milkfat of animals receiving protected lipid supplements. In the present study the aim was not to repeat the level of gamma lactones achieved by the Australians but through the use of grain supplements, as commonly used in Europe, to produce WMP with similar flavour characteristics to Danish WMP. Chemical analysis of Danish WMP had previously highlighted the fact that the presence of the two gamma lactones was the single greatest contributor to the difference in flavour between samples of New Zealand and Danish WMP.

5.8 The Effect of Diet on the Flavour of New Zealand WMP.

In summary the results of the present study indicate that several factors, including diet, can influence the flavour of WMP.

5.8.1 Influence of Breed of Dairy Cow.

It would appear that different breeds (Jersey and Friesian) on similar diets can produce WMP of different flavour. In particular Friesian cows appear to produce greater levels of gamma lactones on grain supplemented diets than Jersey cows. Little information is available concerning the effects that breed differences can have on dairy products other than the research of Dimick and Harner (1968) who reported higher level of lactones with Holstein cows compared to Jersey-Guernsey cows. Also the work of Keen and Udy

(1980) who indicated differences in the colour of milkfat produced by different dairy breeds.

5.8.2 The Influence of Time.

It would appear from both trials that there is a lag time involved to effect changes due to grain supplementation. In both trials changes were at their greatest after 90 - 120 days on the grain diet and may be linked to other factors such as stage of lactation or other seasonal variations. Dimick and Hamer (1968) have shown that time of season and stage of lactation will both influence the lactone levels in milkfat.

5.9 The Effects of Grain Feeding on Rumen Fermentation.

Although it was not an objective of this study to look at the effect of varying diet on rumen fermentation it should be remembered that increasing amounts of grain concentrates can have a significant effect on rumen fermentation patterns.

Two aspects that have prompted this type of research has been the attempts to produce a more spreadable butter and also a so-called "healthier" fatty acid profile in the milkfat by the increase in the levels of polyunsaturated fatty acids (18:2, 18:3 etc.) (Banks, 1987).

A high starch diet also has the effect of changing the rumen microflora with a corresponding change in the products of rumen fermentation. The ratio of propionate to acetate increases with a high starch diet compared to all grass feeding with the acetate:propionate:butyrate ratio in the rumen changing from 70:30:10to 50:50:10(Balch and Rowland, 1957).

5.9.1 The Effect of Ruminal Microfloral Changes on the Production of Lactones.

These changes in the ruminal fermentation have been postulated as being the cause of the increased levels of the gamma lactones in milkfat from cows being feed a high starch diet. It has been postulated that the propionate metabolism as favoured by the high starch diet favours microorganisms with the capacity to hydrate oleic and linoleic acids to the corresponding 10-hydroxy acids. This has been validated by the presence of 10-hydroxyoctadecanoic acid in the rumen of sheep receiving a diet of chopped lucerne and crushed oats and 10-hydroxyoctadec-*cis*-12-enoic acid in the caecum of sheep receiving the same diet but with the addition of protected sunflowers seeds. In both cases when the oats were withdrawn from the diet the amount of these compounds was very much lower (Anet, 1975). The focus of the present study was on the flavour of WMP with reference to the effect that diet may have on the flavour of the resulting WMP, so that there was no incentive to study the biochemical changes that may have occurred due to the grain feeding. However from the present study a comparison with previous grain feeding trials can be made.

5.10 The Effect of Grain Feeding on the Flavour Composition of New Zealand WMP.

The second aspect of this study was the identification of compounds which may contribute to the "green/grassy" flavours that are regularly identified in samples of New Zealand WMP by customers of certain ethnic origins (Asian). To study this aspect of WMP flavour effectively requires samples that possess these flavour characteristics and that differ significantly from the standard product. Although the sensory analysis of New Zealand and Danish WMP indicated differences in the scores for the "feedy" flavour attribute these differences were not statistically different.

During the course of the two grain concentrate feeding trials there were no effects on "feedy" flavour due to feed type identified. It was expected that if feedy flavour was related to pasture intake then these feeding trials would have highlighted this. It may be that the trained WMP panel may not have been proficient enough in the identification

of this attribute or the pasture intake in conjunction with the grain concentrate was still sufficient to contribute feed or pasture flavour.

5.11 Green/grassy Flavour of New Zealand.

5.11.1 Sensory Analysis of Milkfat Products.

Although at the time of this study there was a panel at N.Z.D.R.I. trained to evaluate WMP there was no panel trained to evaluate milkfat products, therefore an informal panel was used to evaluate samples of milkfat on a casual basis. Personnel (3 - 4 people) for this panel were drawn from the members of the Flavour Section who had previous experience in evaluating milkfat products and were familiar with the flavour nuances of New Zealand milkfat when compared to European product. Because of the small number of panellists it was not possible to perform any statistical analysis on any of the results obtained using the informal panel. However the lack of statistical analysis was compensated for by a high degree of commitment from the panel to the task of evaluating the milkfat samples and at the same time remain subjective.

5.12 Effectiveness of the Vacuum Distillation of New Zealand Milkfat.

The criteria for any method aiming to extract a flavour fraction from a food product should be that the extract is indeed representative of the flavour of the food from whence it was derived. In this study one of the primary aims was to determine the compound or compounds that contribute to the "green/grassy" flavour commonly reported in New Zealand milkfat. As vacuum distillation was the preferred method of isolation of flavour volatiles it was important to gain some knowledge on the effectiveness of this method. This was measured organoleptically by the addition of the vacuum distillate, collected from the distillation of 4 Kg of New Zealand milkfat, to New Zealand milkfat. Because the "green/grassy" flavour was the attribute of interest the addition of the distillate should increase the level of this attribute and this was indeed

the case with the mean scores for "green/grassy" flavour increasing from 1.6 to 3.6 on the 0 - 5 scale. From this it was assumed that the key components of the "green/grassy" flavour nuance could be isolated by vacuum distillation.

Although the use of this technique has not been commonly reported it would seem to be a logical and very simple test to evaluate that an extract isolated from a product is representative of the flavour of the original product. Weurman (1969) in a review of the isolation and concentration techniques of volatiles from food systems stresses this point also.

5.13 Volatile Compounds in New Zealand Milkfat (FFMR).

The identification of compounds present in the diethyl ether extract from the vacuum distillation of New Zealand milkfat was the first occasion that a qualitative study had been carried out on the flavour volatiles present in New Zealand milkfat. Quantification of the flavour volatiles was not attempted due to the difficulties in determining the volatility of each individual compound and the efficiency of extraction from the aqueous phase of the distillate into the organic phase of each compound.

Previous studies carried out in New Zealand tended to focus on specific areas of milkfat flavour such as the composition of the lactone fraction and the effects of milkfat fractionation on the distribution of flavour precursors (Walker, 1972). In the past there has been the tendency to directly extrapolate results from overseas investigations to draw conclusions about New Zealand milkfat. This of course is a valid assumption but ignores some of the unique aspects of the New Zealand dairy industry that do not occur overseas. In particular the seasonal nature of lactation, an all grass feeding regime made up of differing pasture grass species and the mixed nature of the dairy breeds are all likely to exert influences on the flavour profile of New Zealand milkfat in comparison to overseas samples of milkfat.

5.14 Classes of Compounds Present in New Zealand Milkfat (FFMR).

5.14.1 Hydrocarbons.

Hydrocarbons made up the most abundant (33 compounds) class of compounds identified in New Zealand milkfat. A lot of these compounds were present in relatively small amounts, based on peak area, and like other investigators the assumption was made that the contribution to flavour by this group of hydrocarbons would be minimal, particularly the longer chain hydrocarbons which are often accepted as odourless. Exceptions to this may be the contribution to flavour of such compounds as naphthalene but again the major consideration would be the concentration at which it is present. Badings and Neeter, (1980) also identified several hydrocarbons, including naphthalene, in their isolation of compounds from milk.

The presence of the C20 hydrocarbons (phyt-1-ene, phyt-2-ene and neophytadiene) as reported by Urbach and Stark, (1975) and implicated in the flavour of grass fed meat were not identified.

5.14.2 Aldehydes.

The presence of aldehydes in milkfat is generally an indication that lipid oxidation has taken place and their contribution to flavour is in the main undesirable with descriptors for the flavour of individual aldehydes ranging from green and tallowy (C6 - C11 alkanals) to green oily flavours (alkenals) (Badings and Neeter, 1980).

The presence of the alkanals, pentanal, hexanal and heptanal, in the sample of New Zealand milkfat would tend to indicate that some lipid oxidation has taken place. But due to the milkfat being relatively fresh and the lack of any distinct oxidised flavours being recorded by sensory evaluation the assumption is made that aldehydes do not make a major contribution to fresh milkfat flavour.

Hexanal has been described as giving rise to "green" flavours and therefore the presence of hexanal in New Zealand milkfat could make a contribution to the "green/grassy" flavour nuance detected in this sample of milkfat (Badings and Neeter, 1980). The

flavour threshold values for hexanal in milk and oil have been determined at 50 ppb and 150 ppb respectively (Kinsella, 1969). It is likely that in this particular sample of New Zealand milkfat that hexanal was present in excess of its FTV for oil. Hex-*cis*-3-enal another aldehyde with a distinct "green/grass" flavour character was not detected in the New Zealand milkfat.

Another unsaturated aldehyde hept-*cis*-4-enal was been previously identified in contributing a "creamy" flavour at certain concentrations and creamy flavour is viewed as being an important flavour attribute of milkfat (Kinsella, et al., 1967). However this compound was not identified in the extract from New Zealand milkfat but with a FTV of 1.5 ppb in butteroil in may still contribute to flavour but be at too low a concentration for positive identification by GC/MS analysis (Kinsella, 1969).

5.14.3 Methyl Ketones.

Milkfat is characterised as containing a potential to produce a range of methyl ketones from the release of ß-keto fatty acids esterified in milkfat triglycerides. The release of the methyl ketones occurs slowly with storage but can be accelerated by heat treatment. Analysis of the New Zealand milkfat identified a series of methyl ketones including C6, C7, C9, C11 and C13 methyl ketones. The presence of these compounds would be expected in even freshly manufactured anhydrous milkfat due to the heat of processing. Walker (1972) showed that up to 20% of the total methyl ketone potential could be lost during the preparation of anhydrous milkfat with the loss greatest in respect of the short chain (C3 and C5) methyl ketones.

The levels of methyl ketones were not determined but based on the organoleptic evaluation of the milkfat indicating a lack of stall or "blue cheese" type flavours this would tend to indicate that the levels of methyl ketones are only a small percentage of their full potential.

5.14.4 Fatty Acids.

The presence of free fatty acids in New Zealand milkfat is expected based on the amount of processing that is required to manufacture anhydrous milkfat with numerous opportunities for the release of fatty acids from the milk triglyceride. The fatty acids identified were butanoic, hexanoic, octanoic and decanoic acids.

5.14.5 Alcohols.

The only alcohol identified in the New Zealand milkfat was hexanol. Generally alcohols are present in milkfat as oxidation products of unsaturated fatty acids. Mick et al. (1982) identified a range of primary, secondary and tertiary alcohols in sour cream butter but the indication was that most of these were formed during the fermentation of the cream. Hex-*cis*,2-enol, a well known contributor of "green/grassy" flavours was identified in the sour cream butter.

5.14.6 Lactones.

As expected from the results of the lactone analysis of New Zealand WMP a series of δ-lactones was identified in the sample of New Zealand milkfat. δ-Decalactone, δ-Dodecalactone and δ-Tetradecalactone were the only lactones identified and as in New Zealand WMP no γ-lactones were identified. The reasons for the presence or absence of the various lactones have been discussed previously and also the factors that can influence the levels and composition of the lactone profile of New Zealand milkfat.

5.14.7 Terpenoids.

Several terpene compounds were identified in the sample of New Zealand milkfat, many have not been previously identified in milkfat. Mick et al. (1982) also identified a

number of terpenes in their study of sour cream butter. They inferred that the terpenes were most likely derived from the feed and although they did not mention any direct contribution to the flavour of the sour cream butter they did suggest that they may make a significant contribution to the final flavour of the butter. Linalool was identified as the most abundant terpene present at 1 - 10 μg/Kg.

The methods used for the analysis of the sour cream butter were very similar to those used in this study although only 500 g of butter was used in comparison to 4 Kg of milkfat. Vacuum distillation at approximately the same temperature , time and vacuum level were used.

5.15 Flavour Properties of the Terpenoids Identified in New Zealand Milkfat (FFMR).

The flavour properties of the six terpenoid compounds identified in New Zealand milkfat are presented in Table 5.1. In general these compounds, depending on the levels at which they are present could give rise to pine, citrus or floral type flavours.

5.16 The Effect of Terpenes on the Flavour of New Zealand Milkfat.

The effect of terpenes on the flavour of New Zealand milkfat was investigated by adding defined amounts of standard terpenes to deodourised milkfat, of these compounds D-limonene was the most effective in producing a flavour reminiscent to the "green/grassy" flavour present in some samples of New Zealand milkfat. This was achieved at a level of only 1 ppm which produced a "green/grassy" flavour in the moderate to strong range.

Table 5.1. Flavour properties of the terpenoids from New Zealand milkfat (FFMR).

Compound	Flavour properties
α-pinene	Turpentine-like
ß-pinene	Turpentine-like
D-Limonene	Sweetly fresh, lemon-like
Linalool	Citrus, floral
α-terpineol	Sweetly floral, lilac-like
Carophyllene	Spicy

From: Flavour Technology - Profiles, Products and Applications.

5.17 Origins of Terpenes in New Zealand Milkfat.

The most likely source of the terpenes identified in New Zealand milkfat is from the feed. The particular milkfat sample used for this study was produced at a time of year (mid summer) when the cows would have been fed nothing but pasture. Terpenes are known to be components of grass thus making it possible for these compounds to be transferred directly from the feed or may have been formed from other terpenes by chemical reactions occurring in the rumen.

CHAPTER VI: CONCLUSIONS

It is evident from this study that there are certain fundamental differences in the flavour of New Zealand WMP as opposed to European (Danish) WMP. Sensory analysis has highlighted that the major difference is in the scores which panellists give for the lactone attribute. This difference in sensory properties can be directly linked to differences in the lactone profiles from New Zealand and Danish WMP. Danish WMP consistently contains the two gamma lactones γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone at levels greater than or equal to their flavour threshold values. However these two lactones are generally absent in New Zealand WMP. The presence or absence of γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone in WMP has been demonstrated to be primarily related to the diet of the cow. By the addition of a grain concentrate consisting of 85% oats, 10% sunflower seeds and 5% barley, in conjunction with pasture feeding, it was possible to increase the levels of γ-Dodecalactone and γ-Dodec-*cis*-6-enolactone to the point where the sensory panel was able to differentiate WMP's in respect to the presence or absence of these compounds.

Secondary to this is the inference that the presence of the gamma lactones in WMP is also a function of dairy breed with Friesian cows showing a greater capacity than Jersey or mixed Jersey/Friesian cows to produce these compounds. Also diet composition may be an important factor with the lipid content and fatty acid composition having an influencing the level of gamma lactones produced. Grains high in oleic acid (eg. oats) and linoleic acid (eg. sunflower seeds) have been reported in giving rise to high levels of gamma lactones in milkfat, this has been substantiated by this study.

Analysis of the flavour volatiles from fresh New Zealand milkfat has indicated a possible causative role for terpenoid compounds in the distinctive "green/grassy" flavours often present. In particular such compounds as D-Limonene have been shown to be present in samples of New Zealand milkfat and when added to New Zealand milkfat has a tendency to increase the "green/grassy" flavour score. However this does not discount the contribution of compounds such as hexanal which was also detected in New Zealand milkfat.

In conclusion this study has identified the major sensory and flavour chemistry differences between New Zealand and European (Danish) WMP. This has been linked primarily to diet and secondly to the breed of cow. This implies that with due consideration to the type of feed and the breed of cow it would be possible to produce milk which has similar flavour characteristics to European (Danish) WMP.

Also the presence of terpenoid compounds in samples of New Zealand milkfat may be contributing to a green/grassy flavour attribute. Once again the presence or absence of these compounds in milkfat are probably linked to diet.

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APPENDIX 1

Questionnaire Used in Whole Milk Powder Evaluations

Name _____ Date _____

MILK POWDER PANEL

In front of you are a number of milk powder samples. Please evaluate them for the following characteristics using a 0 - 10 scale where:

0 = Absent
2 = Threshold
4 = Weak
6 = Moderate
8 = Strong
10 = Intense

	Sample Nos.			
FLAVOUR:	_____	_____	_____	_____
Sweetness	_____	_____	_____	_____
Creaminess	_____	_____	_____	_____
Cooked/Caramelised	_____	_____	_____	_____
Lactone	_____	_____	_____	_____
Oxidised	_____	_____	_____	_____
Feedy	_____	_____	_____	_____
Taint	_____	_____	_____	_____
Age-related	_____	_____	_____	_____
TEXTURE:				
Astringency	_____	_____	_____	_____

COMMENTS:

APPENDIX 2

Definitions of Sensory Terms Used in the Evaluation of Whole Milk Powder.

WHOLE MILK POWDER DEFINITIONS

FLAVOUR:

Sweetness tongue	- The intensity of one of the basic tastes perceived at the tip of the and exemplified by sucrose.
Creaminess	- The intensity of a flavour associated with fresh, vacreated cream.
Cooked/ Caramelised	- The intensity of a flavour typical of milk heated to 74°C or higher.
Lactone	- The intensity of a flavour associated with the formation of lactone compounds from the milkfat, variously described as 'perfumy', 'coconut', 'fruity', 'estery' etc.
Oxidised	- The intensity of a group of flavours found when hydrolytic oxidation of the milkfat has taken place, variously described as 'oxidised', 'oily', 'tallowy', 'waxy', 'painty', 'metallic' etc.
Feedy	- The intensity of a group of flavours related to the diet of the cow, typically found in early season milk or cream.
Taint	- The intensity of a number of flavours, commonly caused by the inadvertent addition of a substance during processing - this includes flavours such as 'chemical', 'plasticky', 'metallic', 'burning', 'soapy', 'synthetic', etc. This group also includes flavours which appear to have come from outside sources through absorption into the powder etc. rather than something which occurs during processing.
Age-related	- The intensity of a flavour associated with prolonged storage of whole milk powder variously described as 'stale', 'cereal', 'mealy', etc.

APPENDIX 3

The effect of grain Feeding on the flavour of New Zealand WMP (Trial 1 (1987).

Summary of mean sensory scores for experimental WMP (September Production).

Time	Initial		3 Month				6 Month			
Feed Type	Pasture	Conc.	Pasture		Conc.		Pasture		Conc.	
Storage Temp.			Amb.	37°C	Amb.	37°C	Amb.	37°C	Amb.	37°C
Sweetness	5.0	5.3	5.1	4.9	5.5	4.5	5.0	5.0	5.1	4.6
Creaminess	4.6	5.4	4.1	4.4	4.8	5.0	4.8	4.8	4.9	5.0
Cooked/ Caramelised	5.2	5.3	5.0	4.9	5.4	4.9	5.1	4.9	5.0	4.9
Lactone	2.2	2.6	1.3	2.4	1.8	1.9	0.9	0.3	0.4	0.0
Oxidised	0.0	0.2	0.9	2.9	1.1	2.5	1.8	1.5	1.4	2.1
Feedy	3.8	3.0	4.0	2.1	2.8	2.5	2.6	2.3	3.3	1.8
Taint	0.0	0.0	0.4	0.0	1.1	0.4	0.0	1.3	0.5	0.8
Age-related	0.0	0.0	0.9	0.3	0.5	0.5	1.3	1.3	1.4	3.1
Astringency	4.4	4.3	3.9	4.5	4.5	4.8	4.9	5.3	4.4	4.6

APPENDIX 4

The effect of grain Feeding on the flavour of New Zealand WMP (Trial 1 (1987).

Summary of mean sensory scores for experimental WMP (October Production).

Time	Initial		3 Month				6 Month			
Feed Type	Pasture	Conc.	Pasture		Conc.		Pasture		Conc.	
Storage Temp.			Amb.	37°C	Amb.	37°C	Amb.	37°C	Amb.	37°C
Sweetness	4.8	4.8	5.1	5.5	5.2	5.3	5.3	4.8	4.6	5.0
Creaminess	4.9	4.6	4.9	4.9	5.0	5.1	4.8	4.1	4.5	4.5
Cooked/ Caramelised	5.0	5.1	5.6	5.4	5.4	5.1	5.4	4.9	4.9	5.0
Lactone	2.0	2.1	2.0	2.1	2.0	1.6	1.4	1.4	1.1	1.9
Oxidised	0.0	0.0	0.3	0.3	0.1	1.0	0.6	0.8	0.9	0.4
Feedy	4.6	3.9	3.8	3.3	3.3	3.1	3.5	2.6	2.5	3.5
Taint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.6	0.4
Age-related	0.0	0.0	0.0	0.0	0.7	0.4	0.8	2.3	0.8	3.1
Astringency	3.8	3.4	3.4	3.5	3.4	4.4	4.9	4.4	5.3	4.4

APPENDIX 5

The effect of grain Feeding on the flavour of New Zealand WMP (Trial 1 (1987).

Summary of mean sensory scores for experimental WMP (early November Production).

Time	Initial		3 Month				6 Month			
Feed Type	Pasture	Conc.	Pasture		Conc.		Pasture		Conc.	
Storage Temp.			Amb.	37°C	Amb.	37°C	Amb.	37°C	Amb.	37°C
Sweetness	5.3	5.1	5.3	5.4	5.2	5.0	5.1	4.9	5.3	5.0
Creaminess	5.1	4.6	4.9	5.0	4.7	4.6	4.5	4.8	4.8	4.6
Cooked/ Caramelised	5.4	5.3	5.7	5.5	5.2	5.1	5.3	5.1	5.0	4.6
Lactone	4.0	3.0	2.3	2.3	2.8	3.0	1.3	1.1	2.0	1.4
Oxidised	0.0	0.0	0.0	0.8	0.0	0.4	0.5	2.4	0.9	2.0
Feedy	4.8	4.3	4.6	2.8	3.2	3.3	2.1	1.8	1.8	1.0
Taint	0.0	0.0	0.0	0.8	0.0	0.3	0.0	0.0	0.0	0.0
Age-related	0.0	0.4	0.4	0.5	0.2	0.0	1.4	1.1	1.9	1.4
Astringency	3.6	3.6	3.2	4.8	3.9	4.5	4.0	4.6	4.5	3.9

APPENDIX 6

The effect of grain Feeding on the flavour of New Zealand WMP (Trial 1 (1987).

Summary of mean sensory scores for experimental WMP (late November Production).

Time	Initial		3 Month				6 Month			
Feed Type	Pasture	Conc.	Pasture		Conc.		Pasture		Conc.	
Storage Temp.			Amb.	37°C	Amb.	37°C	Amb.	37°C	Amb.	37°C
Sweetness	5.5	5.0	5.0	4.5	5.0	4.6	5.1	5.1	5.1	4.8
Creaminess	4.5	4.6	4.9	4.4	5.0	4.6	5.0	4.9	4.8	4.8
Cooked/ Caramelised	5.3	5.3	4.8	4.9	5.4	5.0	5.8	5.3	5.4	5.1
Lactone	2.8	3.6	2.1	2.0	1.8	1.6	1.4	2.0	2.8	2.8
Oxidised	0.0	0.3	0.0	2.0	0.0	0.6	0.6	1.4	0.6	3.0
Feedy	3.8	2.9	2.9	2.0	3.5	3.0	1.8	0.8	1.6	0.8
Taint	0.0	0.0	0.0	0.9	0.0	0.4	0.0	0.0	0.3	0.0
Age-related	0.9	1.1	0.0	1.1	0.5	1.0	0.3	1.4	1.0	3.4
Astringency	4.0	4.8	4.0	3.9	4.3	4.1	4.4	4.4	3.8	4.5

APPENDIX 7

The effect of grain Feeding on the flavour of New Zealand WMP (Trial 1 (1987).

Summary of mean sensory scores for experimental WMP (December Production).

Time	Initial		3 Month				6 Month			
Feed Type	Pasture	Conc.	Pasture		Conc.		Pasture		Conc.	
Storage Temp.			Amb.	37°C	Amb.	37°C	Amb.	37°C	Amb.	37°C
Sweetness	5.7	5.0	5.2	5.0	5.3	4.9	5.6	5.9	5.1	5.8
Creaminess	5.0	4.1	4.6	5.3	4.8	4.8	5.1	5.3	4.9	5.1
Cooked/ Caramelised	6.1	4.8	5.9	5.7	5.0	5.2	5.4	5.3	5.4	5.3
Lactone	2.0	3.0	1.1	1.6	3.3	2.0	1.6	2.0	2.4	4.0
Oxidised	0.0	0.6	0.0	0.6	0.4	1.0	1.6	1.4	2.3	2.8
Feedy	4.2	3.6	3.3	3.4	2.6	2.3	1.4	1.1	0.9	0.8
Taint	0.0	0.0	0.0	1.1	0.7	0.3	0.0	0.0	0.0	0.0
Age-related	0.0	0.0	0.0	0.0	0.4	0.6	1.3	1.5	1.3	1.9
Astringency	3.1	3.6	4.2	4.4	4.1	4.7	3.9	3.5	4.4	4.1

Evaluated for the above characteristics using a 0 - 10 scale where:
0 = Absent, 2 = Threshold, 4 = Weak, 6 = Moderate, 8 = Strong and 10 = Intense.

APPENDIX 8

The effect of grain supplements on the flavour of New Zealand WMP. Summary of mean sensory scores of experimental WMP (Trial 2, 1988) - 3 month evaluation.

	Treatment					
	MCDC Control		Pasture Only		Grain/Pasture	
Attribute	Ambient	37°C	Ambient	37°C	Ambient	37°C
Sweetness	4.5	4.8	5.0	5.0	5.3	5.3
Creaminess	4.5	4.5	4.9	4.8	4.6	4.9
Cooked/ Caramelised	3.9	4.5	4.9	5.0	4.9	5.0
Lactone	0.6	0.8	1.4	1.4	4.1	4.0
Oxidised	1.8	2.3	0.5	0.3	0.0	0.0
Feedy	2.0	1.9	2.3	2.6	0.8	2.4
Taint	1.4	0.6	0.0	0.0	0.4	0.0
Age-related	2.5	1.5	0.5	1.4	1.5	0.8
Astringency	3.5	3.8	4.0	4.5	4.8	4.8

Evaluated for the above characteristics using a 0 - 10 scale where:

0 = Absent, 2 = Threshold, 4 = Weak, 6 = Moderate, 8 = Strong and 10 = Intense.

APPENDIX 9

The effect of grain supplements on the flavour of New Zealand WMP. Summary of mean sensory scores of experimental WMP (Trial 2, 1988) - 6 month evaluation.

	Treatment					
	MCDC Control		Pasture Only		Grain/Pasture	
Attribute	Ambient	37°C	Ambient	37°C	Ambient	37°C
Sweetness	4.8	4.9	5.2	4.9	5.1	5.0
Creaminess	4.8	4.7	5.2	5.3	4.5	5.2
Cooked/ Caramelised	5.2	5.3	5.0	5.3	4.7	5.0
Lactone	1.1	1.3	1.5	2.7	3.0	2.9
Oxidised	0.9	1.8	1.3	1.4	0.2	0.7
Feedy	1.0	1.1	1.0	1.0	0.8	0.7
Taint	0.3	0.0	0.0	0.2	0.0	0.3
Age-related	1.6	1.7	2.4	1.9	0.9	1.5
Astringency	4.6	4.3	4.8	4.5	3.4	4.4

Evaluated for the above characteristics using a 0 - 10 scale where:

0 = Absent, 2 = Threshold, 4 = Weak, 6 = Moderate, 8 = Strong and 10 = Intense.

APPENDIX 10

The effect of grain supplements on the flavour of New Zealand WMP. Summary of mean sensory scores of experimental WMP (Trial 2, 1988) - 9 month evaluation.

	Treatment					
	MCDC Control		Pasture Only		Grain/Pasture	
Attribute	Ambient	37°C	Ambient	37°C	Ambient	37°C
Sweetness	4.8	4.9	5.1	5.1	5.8	5.3
Creaminess	4.3	5.0	5.0	5.1	4.6	5.0
Cooked/ Caramelised	4.6	5.3	5.5	5.4	5.3	5.0
Lactone	0.3	1.0	1.5	1.6	3.1	2.6
Oxidised	2.6	4.1	2.3	4.0	0.2	1.1
Feedy	1.3	1.0	1.4	0.8	1.1	1.3
Taint	0.5	0.0	0.0	0.0	0.0	0.0
Age-related	2.8	2.7	3.3	2.0	1.3	1.6
Astringency	4.5	4.4	4.8	5.0	4.7	4.2

Evaluated for the above characteristics using a 0 - 10 scale where:

0 = Absent, 2 = Threshold, 4 = Weak, 6 = Moderate, 8 = Strong and 10 = Intense.

APPENDIX 11

The effect of grain supplements on the flavour of New Zealand WMP. Summary of mean sensory scores of experimental WMP (Trial 2, 1988) - 12 month evaluation.

	Treatment					
	MCDC Control		Pasture Only		Grain/Pasture	
Attribute	Ambient	37°C	Ambient	37°C	Ambient	37°C
Sweetness	5.3	4.8	5.1	4.9	5.1	5.0
Creaminess	5.1	4.5	5.1	4.8	4.8	4.5
Cooked/ Caramelised	5.3	5.4	5.4	5.4	5.5	5.5
Lactone	1.6	0.4	1.4	0.6	2.6	1.3
Oxidised	1.0	5.8	3.3	6.3	2.6	4.8
Feedy	3.1	1.5	2.6	1.8	2.4	1.5
Taint	0.0	0.0	0.0	0.0	0.0	0.0
Age-related	1.3	3.6	2.1	4.1	2.0	3.5
Astringency	4.3	4.1	4.6	4.3	4.1	4.8

Evaluated for the above characteristics using a 0 - 10 scale where:

0 = Absent, 2 = Threshold, 4 = Weak, 6 = Moderate, 8 = Strong and 10 = Intense.